COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

VOLUME 2

Joint FAO/WHO Expert Committee on Food Additives (JECFA)

Combined Specifications from 1st through the 37th Meetings 1956 - 1990

Food and Agriculture Organization of the United Nations
Rome, 1992
CORRIGENDUM

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Joint FAO/WHO Expert Committee on Food Additives (JECFA)

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INTRODUCTION

A Joint FAO/WHO Conference on Food Additives met in Geneva, Switzerland, in September 1955 and recommended that the two international organizations collect and disseminate information on food additives. Since that time, over 500 substances have been evaluated and provided with specifications for purity and identity by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

JECFA specifications of food additives are intended to serve as a guide for manufacturers and users of the additives, as well as the basis for new or revised national legislation or regulations of member countries of FAO and WHO. The specifications were published variously in FAO Nutrition Meetings Reports Series (NMRS), within WHO Technical Report Series (TRS) or as FAO Food and Nutrition Papers (FNP) comprising 35 separate volumes, published over many years and most of them now out of print.

During its more than 30 years of activity, the Committee has reevaluated many food additives in light of changing requirements or new technical and scientific information. Some have been reevaluated more than once. As a result, the specifications for 50 substances were withdrawn over the years because the use of these additives was no longer technically justified or because of safety considerations based on newer scientific information.

This present Compendium was prepared in order to consolidate in one reference source, all of the JECFA food additive specifications which are currently applicable. This includes specifications from all JECFA meetings dealing with food additives up to and including the 37th meeting in 1990. The Compendium consists of general notices; remarks applying to the standards, tests and assays; individual specifications listed in alphabetical order by substance title; and seven annexes plus an index.

There are many general tests for identity and purity which are applicable to a wide range of substances in the Compendium. In 1978, these were assembled along with other general reference material and were published as FNP number 5, "Guide to Specifications - JECFA". FNP 5 was revised in 1983 and a second revision was published in 1991 to coincide with and complement this Compendium. The test procedures and other information from FNP 5, revision 2, are referenced in the specifications throughout the Compendium.

Users of this Compendium are encouraged to submit their comments or suggestions to the Chief, Food Quality and Standards Service, Food Policy and Nutrition Division, FAO, via delle Terme di Caracalla, 00100 Rome, Italy.
ACKNOWLEDGEMENTS

This Compendium was prepared by Dr. Kenji Ishii of the Japan Food Additives Association (JFAA) over a period of 18 months at the FAO Headquarters in Rome, Italy. Dr. Ishii was assisted in this massive undertaking by several colleagues in Japan. These were Dr. Kunitoshi Yoshihira and Dr. Toshio Itoh, both of the National Institute of Hygienic Science, as well as the following scientists from the JFAA: Mr. Koh Murai, Dr. Takashi Akiyama, Mr. Rikio Goto, Mr. Nobuyoshi Nosaka, Dr. Tetsuya Kato and Dr. Izumi Kumashiro. FAO gratefully acknowledges the considerable effort of all these experts and especially the outstanding work done by Dr. Ishii. FAO further expresses its appreciation to Mr. Saburosuke Suzuki, Chairman, and Dr. Teruo Shiro, Senior Managing Director, of the Japan Food Additives Association and to the National Institute of Hygienic Science of Japan, for their generous support in providing the services of Dr. Ishii and of the other experts to prepare this document.

The final draft of this Compendium was reviewed and edited by Mr. D.F. Dodgen, Review Chemist, Division of Food Chemistry and Technology, Centre for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, D.C., USA, and Dr. Juhani Paakkanen, Senior Advisor, Ministry of Trade and Industry, Division for Food Affairs, Helsinki, Finland. Their review was invaluable.

FAO acknowledges with gratitude the generous contribution of the International Life Sciences Institute, Washington, D.C., USA, to the publication of this Compendium.
SCOPE OF THIS COMPREHENDIUM

This Compendium presents in a consolidated and up-dated form, all of the specifications for identity and purity of over 500 food additives, prepared by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and published in about 35 separate volumes since the first session of the Committee in 1956. Each volume previously published represented the results of a given meeting.

The format of individual additive specifications varied from meeting to meeting and ranged from relatively uncomplicated earlier specifications to the more complex recent issuances. The consolidation and up-dating for this Compendium necessitated a uniform format of presentation for the specifications. This format was agreed to by JECFA experts after being proposed by the consultant preparing the draft Compendium (Dr. Kenji Ishii - see Acknowledgements). Early specifications tended to be quite simple in presentation. These were edited to generally conform to the agreed format without changing the substance or meaning of the specification requirements. Typographical errors were also corrected where found.

Many of the older specifications do not meet modern requirements for identity and purity. These specifications will be reviewed at future JECFA meetings and revised accordingly.

The user of this Compendium will need to become acquainted with both the index and the various annexes. To assist in this, the following summary and explanation of each is offered:

- **Annex 1** details the General Specifications for Enzyme Preparations Used in Food Processing, with two appendices. Appendix A is "Determination of Antibiotic Activity" and Appendix B is "General Considerations and Specifications for Enzymes from Genetically Manipulated Microorganisms". All enzyme substances must meet the requirements detailed in Annex 1, in addition to the individual specification for the enzyme. Enzymes from microbial sources must also meet the requirements regarding antibiotic activity in Appendix A. Lastly, any enzyme from a genetically manipulated microorganism should further include specification requirements as summarized in Appendix B.

- **Annex 2** was included to provide general guidance on specifications for colour lakes prepared by the reaction of alumina and appropriate colouring agents.

- **Annex 3** is designed to clarify the nomenclature to be used in describing chlorophyll and its derivatives.

- **Annex 4** provides a listing of former titles of specifications which have been changed in the present Compendium, along with a listing of their current titles. This permits cross-checking of older JECFA references, if desired.

- **Annex 5** is a cross-index of Codex functional classes and JECFA defined functional uses.

- **Annex 6** represents a listing of the individual substances by JECFA functional uses.

- **Annex 7** identifies those substances which previously had specifications, but which are not included in this Compendium, for reasons stated in the Annex.

- The **Index** at the end of this Compendium will assist the user in finding specifications as it cross references the various synonyms used for each substance.
SPECIAL NOTE

The methods and analytical procedures described in this Compendium are designed to be carried out by properly trained personnel in a suitably equipped laboratory. In common with many laboratory procedures, the methods quoted frequently involve hazardous materials.

For the correct and safe execution of these methods it is essential that laboratory personnel follow standard safety procedures for the handling of hazardous materials.

While the greatest care has been exercised in the preparation of this information, FAO expressly disclaims any liability to users of these procedures for consequential damages of any kind arising out of, or connected with their use.
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ANNEXES

1. General Specifications for Enzyme Preparations used in Food Processing
   
   Appendix A: Determination of Antibiotic Activity
   
   Appendix B: General Considerations and Specifications for Enzymes from Genetically Manipulated Microorganisms

2. General Specifications for Aluminum Lakes of Colouring Matters

3. Nomenclature of Chlorophyll and Chlorophyll Derived Products

4. Former and Current Titles of JECFA Specifications

5. Cross Index of Codex Functional Classes and JECFA Defined Uses

6. Substances Listed by JECFA Functional Uses

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INDEX
**INDIGOTINE**

SYNONYMS
CI Food Blue 1, FD&C Blue No. 2, Indigo Carmine
INS No. 132, EEC No. E132

DEFINITION
Indigotine consists essentially of a mixture of disodium 3,3'-dioxo-[Δ2Z-biindolines]-5,5'-isomer, and disodium 3,3'-dioxo-[Δ2Z-biindolines]-5,7'-isomer, and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

Indigotine may be converted to the corresponding aluminium lake in which case only the General Specifications for Aluminium Lakes of Colouring Matters shall apply.**

Class
Indigoid

Code numbers
CI (1975) No. 73015
CAS No. 860-22-0 (5,5' isomer)

Chemical name
Disodium 3,3'-dioxo-[Δ2Z-biindolines]-5,5'-disulfonate

Chemical formula
$C_{16}H_{12}N_{2}Na_{2}O_{5}S_{2}$

Structural formula

![Structural formula of Indigotine](image)

Molecular weight
466.36

Assay
Content not less than 85% total colouring matters. Disodium 3,3'-dioxo-[Δ2Z-biindolines]-5,7'-disulfonate: Not more than 18%.

DESCRIPTION
Blue powder or granules

FUNCTIONAL USE
Food colour

---

* These specifications were prepared at the 28th session of JECFA (1984) and published in FNP 31/1 (1984).

** See Annex 2 at end of this Compendium.
CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility
   Soluble in water
   Sparingly soluble in ethanol

** B. Identification of colouring matters
   Passes test

PURITY TESTS

** Loss on drying at 135°C
   Not more than 15%

** Chloride and sulfate calculated as sodium salts
   Not more than 0.2%
   Weigh accurately about 2 g sample instead of the 4.5-5 g stated in the General Methods.

*** Arsenic
   Not more than 3 mg/kg

*** Lead
   Not more than 10 mg/kg

*** Mercury
   Not more than 1 mg/kg

* Heavy Metals
   Proceed as directed in the Limit Test for Heavy Metals

** Subsidiary colouring matters
   Excluding disodium 3,3'-dioxy-2,2'-bi-indolyline-5,7'-disulfonate: Not more than 1%
   See description under TESTS

Organic compounds other than colouring matters

Isatin-5-sulfonic acid
5-sulfoanthranilic acid
Anthranilic acid
   Total not more than 0.5%
   See description under TESTS

** Unsulfonated primary aromatic amines
   Not more than 0.01% calculated as aniline

** Ether extractable matter
   Not more than 0.2%
   Weigh accurately about 2 g sample instead of the 5 g stated in the General Methods.


TESTS

PURITY TESTS

* Subsidiary colouring matters

Use the following conditions:
- Developing solvent: No. 3
- Height of ascent of solvent front: approximately 17 cm

Note 1. The 5,7' isomer is separated as a wide blue zone just in front of the main blue band. Do not include this zone in the subsidiary colouring matter zones which are cut out and measured.

Note 2. The 15 ml sodium hydrogen carbonate solution used in the general procedure is replaced by 15 ml 0.05 N hydrochloric acid in order to avoid the decomposition which the sulfonated indigo undergoes in alkaline solution.

* Organic compounds other than colouring matters

HPLC elution gradient:
2 to 100% gradient followed by elution at 100%.

METHOD OF ASSAY

Determination of Total Colouring Matters by Titration with Titanous Chloride *

Weight of sample: 1.0-1.1 g
Buffer: 15 g sodium hydrogen tartrate
Weight (D) of colouring matters equivalent to 1.00 ml of 0.1 N TiCl₃: 0.02332 g

Isomer content by paper chromatography

Refer to the conditions for the determination of subsidiary colouring matters (above). Cut the isomer band from the chromatogram in the manner detailed for the subsidiary bands, extract into solvent and measure the absorbance at its λmax. Measure the absorbance of the corresponding blank at the same wavelength. As a standard use 0.1 ml of an 0.20% solution of the sample applied to the 18 cm x 0.7 cm rectangle.

Isomer expressed as a percentage of the sample =

$$\frac{A}{A_s} \times 20\% \times \frac{D}{100}$$

where A and As are the net absorbances of the isomer and standard, respectively, and D is the total colouring matters content of the sample.

Isomer content by HPLC

The 5,7' isomer separates under the HPLC conditions detailed above for the separation of subsidiary colouring matters, and the amount present can be quantified.

5'-INOSINIC ACID*

SYNONYMS
Inosinic acid, IMP
INS No. 630, EEC No. 630

DEFINITION

Chemical name
Inosine-5'-monophosphoric acid
C.A.S. number
131-99-7
Chemical formula
C_{10}H_{13}N_{5}O_{5}P
Structural formula

Molecular weight
348.21
Assay
Content not less than 97.0% and not more than 102.0% of C_{10}H_{13}N_{5}O_{5}P on the dried basis

DESCRIPTION
Odourless, colourless or white crystals, or a white crystalline powder, having a characteristic taste

FUNCTIONAL USE
Flavour enhancer

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Freely soluble in water.
Slightly soluble in ethanol

** B. UV absorbance
Passes test;
See description under TESTS

** C. Positive test for ribose
Passes test;
See description under TESTS

---

* These specifications were prepared at the 29th session of JECFA (1985) and published in FNP 34 (1986).

IDENTIFICATION TESTS (continued)

D. Positive test for organic phosphate  
   Passes test  
   See description under TESTS

PURITY TESTS

Loss on drying  
Not more than 3% (120°C, 4 h)

* pH  
1.0 - 2.0 (1 in 20 soln)

* Arsenic  
Not more than 3 mg/kg  
Test as directed in Limit Tests (Method II)

* Lead  
Not more than 10 mg/kg  
Proceed as directed in the specifications for Glutamic Acid

* Heavy metals  
Not more than 20 mg/kg  
Test a solution of 1 g of the sample in 25 ml of water as directed in the Limit Test (Method I)

Related foreign substances  
Chromatographically not detectable  
See description under TESTS

TESTS

IDENTIFICATION TESTS

B. UV absorbance  
A 1 in 50,000 solution of the sample in 0.01 N hydrochloric acid exhibits an absorbance maximum at 250 ± 2 nm. The ratio A250/A260 is between 1.55 and 1.65, and the ratio A280/260 is between 0.20 and 0.30.

C. Positive test for ribose  
To 3 ml of a 3 in 10,000 solution of the sample in water, add 0.2 ml of a 1 in 10 solution of orcinol in ethanol and subsequently 3 ml of a 1 in 1,000 solution of ferric ammonium sulfate in hydrochloric acid. Then, heat in a boiling water bath for 10 min. A green colour is produced.

D. Positive test for organic phosphate  
To 5 ml of a 1 in 20 solution of the sample add 2 ml of magnesia mixture TS. No precipitate is formed. To this mixture add 7 ml of nitric acid, boil for 10 min, neutralize with sodium hydroxide TS, add ammonium molybdate TS, and warm. A yellow precipitate, which dissolves in sodium hydroxide TS or ammonia TS, is formed.

PURITY TESTS

Related foreign substances

Proceed as directed under the test of Chromatography (Thin-layer chromatography)*, using 1 μl of a 1 in 200 solution of the sample as the sample solution, a mixture of 80 volumes of a saturated solution of ammonium sulfate, 18 volumes of a 13.6% w/v solution of sodium acetate and 2 volumes of isopropanol as the developing solvent, and microcrystalline cellulose as the absorbent. Stop the development when the solvent front has advanced about 10 cm from the point of the application, dry the plate in air, and observe under ultraviolet light (about 254 nm) in a dark place. Only a spot of 5'-inosinic acid is detected.

METHOD OF ASSAY

Weigh accurately about 0.5 g of the sample, dissolve in and make to 1,000 ml with 0.01 N hydrochloric acid. Take 10 ml of this solution and dilute with 0.01 N hydrochloric acid to 250 ml. Determine the absorbance A of the solution in a 1-cm cell at the wavelength of 250 nm using 0.01 N hydrochloric acid as the reference. Calculate the content of \( \text{C}_{10}\text{H}_{13}\text{N}_{4}\text{O}_{11} \) in the sample by the formula:

\[
\text{Content (\%) = } \frac{A \times 250,000 \times 100 \times 100}{349 \times \text{weight of sample (mg)} \times (100 - \text{Loss on drying (\%)})}
\]

INSOLUBLE POLYVINYLPyRROLIDONE

SYNONYMS
Crosapidone, cross linked polyvidone, insoluble PVP, polyvinylpolypyrrolidone, cross linked homopolymer of 1-ethenyl-2-pyrrolidone, insoluble cross linked homopolymer of N-vinyl-1-pyrrolidone, INS No. 1202

DEFINITION
Insoluble Polyvinylpyrrolidone is a poly-[1-(2-oxo-1-pyrrolidinyl)-ethylene], cross linked in a random fashion. It is produced by the polymerization of N-vinyl-2-pyrrolidone in the presence of either caustic catalyst or N,N'-divinylimidazolidone. Due to its insolubility in all common solvents the molecular weight range is not amenable to analytical determination.

Assay
Products of commerce contain not less than 11.0% and not more than 12.8% nitrogen calculated on the anhydrous basis

DESCRIPTION
A white hygroscopic powder with a faint, non-objectionable odour

FUNCTIONAL USES
Colour stabilizer, colloidal stabilizer, clarifying agent

CHARACTERISTICS

IDENTIFICATION TESTS
** A. Solubility
Insoluble in water, ethanol and ether

B. Absorption of iodine
Passes test
See description under TESTS

PURITY TESTS
** Water content
Not more than 6% (Karl Fischer Method)

** pH
5 - 8 in an aqueous suspension (1% w/v aqueous solution)

** Sulfated ash
Not more than 0.4%
Proceed as directed under the test of Ash (Sulfated ash, Method I) using 2 g of the sample.

** Arsenic
Not more than 3 mg/kg (Method II)

*** Zinc
Not more than 25 mg/kg
Test 2 g of the sample, accurately weighed.

* These specifications were prepared at the 33rd session of JECFA (1988) and published in FNP 38 (1988).


PURITY TESTS (continued)

* Heavy metals

Not more than 10 mg/kg
Test 2 g of the sample as directed in the Heavy Metals Limit Test (Method II).

Water-soluble matter
Not more than 1.5%
See description under TESTS

Free N-vinylpyrrolidone
Not more than 0.1%
See description under TESTS

Free N,N'-divinylimidazolidone
Not more than 2 mg/kg
See description under TESTS

TESTS

IDENTIFICATION TESTS

B. Absorption of iodine
Add 0.1 ml of 0.1 N iodine to a suspension of 1 g of the sample in 10 ml of water and shake for 30 sec. There should be no blue colouration on shaking up with 1 ml of starch TS.

PURITY TESTS

Water-soluble matter
Transfer about 25 g, accurately weighed, to a 500-ml flat bottom flask, add 225 ml of water and a 5 cm magnetic stirring bar, and place on a combination heater-stirrer. Attach a water-cooled condenser, and reflux gently, with stirring, for 20 h. Allow the slurry to cool, transfer to a 250-ml volumetric flask, with the aid of 25 ml of water, add water to volume, and mix. Allow the bulk of the solids to settle for about 15 min, decant the liquid into centrifuge tubes, and centrifuge until clear. Typically, at least 1 h at 12,000 rpm is required. Transfer 50.0 ml of the clear supernatant liquid to a tared 250 ml beaker, evaporate, and dry to constant weight in a forced air oven at about 90°. Calculate the percentage of water soluble substances by the formula

$$\frac{W}{Q} \times 100$$

where \(W\) is the weight, in g, of the residue and \(Q\) is the weight, in g, of the test specimen taken.

Free N-vinylpyrrolidone
Suspend 4.0 g of the sample with 30 ml of water, stir 15 min., pass through a sintered glass filter of 9-15 μm (= type G 4) in a 250-ml conical flask. Wash the residue with 100 ml of water, add 500 mg of sodium acetate to the combined filtrates, and titrate with 0.1 N iodine until the colour of iodine no longer fades. Add an additional 3.0 ml of 0.1 N iodine, allow to stand for 10 min, and titrate the excess iodine with 0.1 N sodium thiosulfate, adding 3 ml of starch TS as the end-point is approached. Perform a blank determination. Not more than 0.72 ml of iodine is consumed, corresponding to not more than 0.1% vinlypyrrolidone.

Free N,N'-divinylimidazolidone

**Principle**
Free N,N'-divinylimidazolidone migrating from insoluble PVP into a solvent (acetone) is determined by capillary column gas chromatography.

**Internal standard solution**
Dissolve 100 mg of heptanoic nitrile (oenanthic acid nitrile) weighed out to within an accuracy of 0.1 mg in 500 ml of acetone.

**Preparation of the specimen**
Weigh out about 2-2.5 g of the polymer to within an accuracy of 0.2 mg into a 50 ml Erlenmeyer flask. By means of a pipette, add 5 ml of internal standard solution. Subsequently, run in about 20 ml of acetone. Shake the mixture for 4 h or let it equilibrate for at least 15 h and analyze the supernatant solution by gas chromatography.

**Calibration solution**
Weigh out about 25 mg of N,N'-Divinylimidazolidone with an accuracy of 0.2 mg into a flask and make up to 100 ml with acetone. By means of a pipette, transfer 2.0 ml of this solution into another 50 ml calibration flask, make up to 50 ml with acetone. Transfer 2 ml of this solution to another flask, add 5 ml of the internal standard solution (see above) and make up to 25 ml with acetone.

**Gas chromatography condition**
- Column: Capillary (fused silica) "DB-Wax" (cross-linked Carbowax 20 ml), length 30 m, i. d. 0.25 mm, film thickness 0.5 µm.
- Column oven temperature: Programmed, 140° - 240°, 4°/min
- Injector: Split injector, 220°; split effluent 30 ml
- Detector: Thermionic detector (optimized according to manufacturers instructions), 250°.
- Carrier gas: Helium, 1 bar (overpressure)
- Amount injected: 1 µl of supernatant solution of specimen or calibration solution.

**Procedure**
Obtain a reliable determination of the calibration factor for the specific conditions of analysis by means of repetitive injections of the calibration solution. Analyze the sample. The content of N,N'-divinylimidazolidone in insoluble PVP shall be not more than 2 mg/kg.

**Calculation of the calibration factor**

\[
f = \frac{W_D \times A_s}{W_s \times A_D}
\]

\(W_D = \) Amount of N,N'-divinylimidazolidone taken (mg)
\(W_s = \) Amount of internal standard (mg)
\(A_s = \) Area of peak of internal standard
\(A_D = \) Area of peak for N,N'-divinylimidazolidone
Calculation of the content of N,N'-divinylimidazolidone

\[ C_D = \frac{1000 \times f \times A_D \times W_s}{A_0 \times W_s} \text{ (mg/kg)} \]

- \( C_D \) = Concentration of N,N'-divinylimidazolidone (mg/kg)
- \( f \) = Calibration factor
- \( A_D \) = Area of peak for N,N'-divinylimidazolidone
- \( W_s \) = Amount of internal standard added to the sample (mg)
- \( A_0 \) = Area of peak of internal standard
- \( W_s \) = Amount of specimen taken (g)

**METHOD OF ASSAY**

The nitrogen content is determined according to Kjeldahl with a modified digestion. Place about 0.1 g, accurately weighed, in the digestion flask of the apparatus. Add 1 g of a powdered mixture of 10 parts of potassium sulfate and 1 part of cupric sulfate, and wash down any adhering material from the neck of the flask with a fine jet of water. Add 7 ml of sulfuric acid, rinsed down the wall of the flask, then, while swirling the flask, add 1 ml of 30% hydrogen peroxide cautiously down the side of the flask (Do not add hydrogen peroxide during the digestion). Repeat the addition of 1 ml of 30% hydrogen peroxide (usually 3 to 6 times) until a clear, light green solution is obtained on heating the mixture. Heat for an additional 4 h. Cautiously add to the digestion mixture 20 ml of water and proceed to the steam distillation as directed in the Kjeldahl method*.

---

α-IONONE*

SYNONYM
4-(2,6,6-Trimethyl-2-cyclohexenyl)-3-buten-2-one

DEFINITION

Chemical name 4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-2-one
C.A.S. number 127-41-3
Chemical formula C₁₅H₂₀O
Structural formula

Molecular weight 192.30
Assay Content not less than 98% of total ionones expressed as C₁₅H₂₀O of which not less than 85% is α-IONONE.

DESCRIPTION
Colourless to pale yellow liquid, with a woody violet odour

FUNCTIONAL USE
Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility Insoluble in water and glycerol. Soluble in propane-1,2-diol, most fixed oils and mineral oils

** B. Refractive index  n₀²⁰ : 1.497 - 1.502

** C. Specific gravity  d₀²⁰ : 0.930 - 0.936;  d₂₀ : 0.927 - 0.933

 d₁₀²⁰ : 0.928 - 0.934

** D. Infrared spectrum See Appendix at the end of these specifications

* These specifications were prepared at the 28th session of JECFA (1984) and published in FNP 31/2 (1984). The product of commerce used in food under this name may contain varying proportions of isomeric ionones.

PURITY TEST

* Solubility in ethanol 1 ml dissolves in 10 ml of 60% ethanol

METHOD OF ASSAY

Proceed as directed under the method for Gas-Liquid Chromatography in the General Methods*, using the conditions as given in the Method of Assay for Cinnamaldehyde and calculate the content by the method of area normalization.

APPENDIX

** Infrared spectrum: α-Ionone


** Infra-red spectra through the courtesy of the International Organization of the Flavour Industry (IOFI), Geneva, Switzerland, and of the Sadlier Research Laboratories, Inc., Philadelphia, USA.
β-ionone

SYNONYM
4-(2,6,6-Trimethyl-1-cyclohexenyl)-3-buten-2-one

DEFINITION
Chemical name
4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one
C.A.S. number
79-77-6
Chemical formula
C_{13}H_{21}O
Structural formula

Molecular weight
192.30
Assay
Content not less than 97% of total ionones expressed as C_{13}H_{21}O of which not less than 90% is β-Ionone

DESCRIPTION
Colourless to pale yellow liquid with a woody violet odour

FUNCTIONAL USE
Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Insoluble in water and glycerol
Soluble in propane-1,2-diol, most fixed oils mineral oils

** B. Refractive index
n_20^o: 1.517 - 1.522

** C. Specific gravity
d_20^0: 0.942 - 0.948;  
d_20^3: 0.947 - 0.945

d_20^3: 0.941 - 0.945

PURITY TEST
Infrared spectrum
See Appendix at the end of these specifications

* These specifications were prepared at the 28th session of JECFA (1980) and published in FNP 31/2 (1984) superseding the earlier specifications published in FNP 25 (1982). The product of commerce used in food under this name may contain varying proportions of isomeric ionones.

METHOD OF ASSAY

Proceed as directed under the Method for Gas-Liquid Chromatography in the General Methods*, using the conditions as given in the Method of Assay for Cinnamaldehyde, and calculate the content by the method of area normalization.

APPENDIX

** Infrared Spectrum: β-Ionone

---


** Infrared spectra through the courtesy of the Norda Essential Oil & Chemical Co., Inc., and the Sadtler Research Laboratories, Inc., Philadelphia, Pa., USA.
### IRON OXIDES*

**SYNONYMS**

<table>
<thead>
<tr>
<th>Name</th>
<th>CI Pigment Yellow 42 and 43; INS No. 172(iii), EEC No. E172</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron Oxide Yellow</td>
<td>INS No. 172(iii), EEC No. E172</td>
</tr>
<tr>
<td>Iron Oxide Red</td>
<td>CI Pigment Red 101 and 102; INS No. 172(ii), EEC No. E172</td>
</tr>
<tr>
<td>Iron Oxide Black</td>
<td>CI Pigment Black 11; INS No. 172(i), EEC No. E172</td>
</tr>
</tbody>
</table>

**DEFINITION**

Iron Oxides are produced synthetically and consist essentially of anhydrous and/or hydrated iron oxides. The range of hues includes yellows, reds, browns and blacks. Food quality iron oxides are primarily distinguished from technical grades by the comparatively low levels of contamination by other metals. This is achieved by the selection and control of the source of the iron and/or by the extent of chemical purification during the manufacturing process.

**Class**

Inorganic pigment

**Code numbers**

<table>
<thead>
<tr>
<th>Name</th>
<th>CI (1975) No. 77492; CAS No. 51274-00-1</th>
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</thead>
<tbody>
<tr>
<td>Iron Oxide Yellow</td>
<td>CI (1975) No. 77491; CAS No. 1300-37-1</td>
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<tr>
<td>Iron Oxide Red</td>
<td>CI (1975) No. 77499; CAS No. 1317-61-9</td>
</tr>
<tr>
<td>Iron Oxide Black</td>
<td></td>
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</tbody>
</table>

**Chemical name**

<table>
<thead>
<tr>
<th>Name</th>
<th>Hydrated ferric oxide, hydrated iron (III) oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron Oxide Yellow</td>
<td>Anhydrous ferric oxide, anhydrous iron (III) oxide</td>
</tr>
<tr>
<td>Iron Oxide Red</td>
<td>Ferroso ferric oxide, iron (II,III) oxide</td>
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<tr>
<td>Iron Oxide Black</td>
<td></td>
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</table>

**Chemical formula**

<table>
<thead>
<tr>
<th>Name</th>
<th>FeO(OH) · xH₂O</th>
<th>Fe₂O₃</th>
<th>FeO.Fe₂O₃</th>
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<tbody>
<tr>
<td>Iron Oxide Yellow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron Oxide Red</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron Oxide Black</td>
<td></td>
<td></td>
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</tbody>
</table>

**Formula weight**

<table>
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<tr>
<th>Name</th>
<th>88.85 FeO(OH)</th>
<th>159.70 Fe₂O₃</th>
<th>231.55 FeO.Fe₂O₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron Oxide Yellow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron Oxide Red</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron Oxide Black</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Assay**

Not less than 60% of iron (Fe)

**DESCRIPTION**

Powder; yellow, red, brown or black in hue.

**FUNCTIONAL USE**

Food colour

---

*These specifications were prepared at the 35th session of JECFA (1989) and published in FNP 49 (1990).
CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility
  Insoluble in water. Insoluble in organic solvents.
  Soluble in concentrated mineral acids.

PURITY TESTS

Water soluble matter
Not more than 1.0%

Arsenic
Not more than 3 mg/kg

Barium
Not more than 50 mg/kg

Cadmium
Not more than 10 mg/kg

Chromium
Not more than 100 mg/kg

Copper
Not more than 50 mg/kg

See description under TESTS

Lead
Not more than 10 mg/kg

Mercury
Not more than 1 mg/kg

Nickel
Not more than 100 mg/kg

Zinc
Not more than 100 mg/kg

TESTS

PURITY TESTS

** Arsenic, barium, cadmium, chromium, copper, lead, mercury, nickel, zinc

Weigh 5 g of the sample and transfer to a beaker. Add 50 ml concentrated hydrochloric acid and heat on a hot plate until dissolved. Dilute with water to 100 ml in a volumetric flask. Determine the trace metals content by Atomic Absorption Spectrophotometry.

METHOD OF ASSAY

Weigh accurately about 0.2 g of the sample, add 10 ml of 5 N hydrochloric acid and heat cautiously to boiling in a 200-ml conical flask until the sample has dissolved. Allow to cool, add 6 to 7 drops of 30% hydrogen peroxide solution and again heat cautiously to boiling until all the excess hydrogen peroxide has decomposed (about 2-3 min). Allow to cool, add 30 ml of water and about 2 g of potassium iodide and allow to stand for 5 min. Add 30 ml of water and titrate with 0.1 N sodium thiosulfate adding starch TS as the indicator towards the end of the titration. Each ml of 0.1 N sodium thiosulfate is equivalent to 5.585 mg of Fe (III).


SYNONYM

α-Isomethyl ionone

DEFINITION

Chemical name 4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-methyl-3-buten-2-one
C.A.S. number 127-51-5
Chemical formula C₁₄H₂₀O
Structural formula

Molecular weight 206.33
Assay Content not less than 98% of total methyl ionone and not less than 90% of α-isomethyl ionone

DESCRIPTION

Yellowish liquid with a woody-violet odour

FUNCTIONAL USE

Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility Soluble in ethanol, in fixed oils, in propane-1,2-diol. Insoluble in glycerol and in water.

*** B. Refractive index nD: 1.499 - 1.503

*** C. Specific gravity dD: 0.929 - 0.934; dG: 0.932 - 0.937

D. Infrared spectrum Information required**

METHOD OF ASSAY

Method A (For total methyl ionone). Weigh accurately about 1.8 g of the sample and proceed as directed under the method or Aldehyde and Ketone Determination in the General Methods***, using 103.2 as the equivalence factor (e) in the calculation.

Method B (For α-isomethyl ionone). Determine by gas-liquid chromatography as directed in the Method of Assay for Allyl-α-ionone.

* These specifications were prepared at the 24th session of JECFA (1980) and published in FNP 17 (1980).

** The references to identity, purity and methods of analysis were felt to require further confirmation. Information on infrared spectrum is required.

ISOAMYL BUTYRATE*

SYNONYMS
Isoamyl butanoate, butanoic acid 3-methylbutyl ester

DEFINITION

- **Chemical names**: Isoamyl butyrate, isopentyl butanoate
- **C.A.S. number**: 106-27-4
- **Chemical formula**: C₉H₁₈O₂
- **Structural formula**:

\[
\text{CH₃CH₂CH₂COOCH₂CH₂CH} \quad \text{CH₃}
\]

- **Molecular weight**: 158.24
- **Assay**: Content not less than 98% of C₉H₁₈O₂

DESCRIPTION
Colourless liquid with a characteristic fruity odour

FUNCTIONAL USE
Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

** ** A. Solubility
Practically insoluble in water.
Insoluble in glycerol and propane-1,2-diol.
Soluble in most fixed oils and mineral oils.

** ** B. Refractive index

\[ n_D^20 : 1.409 - 1.414 \]

** ** C. Specific gravity

\[ d_5^20 : 0.863 - 0.867, \quad d_20^2 : 0.860 - 0.864 \]

PURITY TESTS

** ** Solubility in ethanol
1 ml dissolves in 4 ml of 70% ethanol.

** ** Acid value
Not more than 1.0

METHOD OF ASSAY

Weigh accurately about 1 g of the sample, and proceed as directed under the method for Ester Determination in the General Methods**, using 79.12 as the equivalence factor (e) in the calculation.

* These specifications were prepared at the 23rd session of JECFA (1979) and published in FNP 12 (1979) superseding the earlier specifications published in NMRS 44B (1969).

ISOAMYL GALLATE*  
(Tentative)

DEFINITION

Chemical names 2-Methylbutyl gallate, iso-amyl ester of 3,4,5-trihydroxy-benzoic acid, isoamyl ester of gallic acid
Chemical formula C₃H₁₄O₅
Structural formula

\[
HO-\overset{\text{COO}}{\text{O}}(\text{CH}_2)_2\text{CH(CH}_3)_2
\]

Molecular weight 240.24

DESCRIPTION

Isoamyl Gallate is a white to pale brownish yellow crystalline solid, odourless, with a slightly bitter taste

FUNCTIONAL USES

Antioxidant

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility  
Insoluble in water; soluble in ethanol

** B. Melting range  
140°-145°

C. Positive test for isoamyl alcohol  
Dissolve 0.5 g of the sample in 10 ml of sodium hydroxide TS, distill, and take 4 ml of the distilled portion. This solution is separated in two layers and emits a strong isoamyl alcohol odour

D. Colour reaction  
Dissolve 0.1 g of the sample in 5 ml of ethanol, and add 1 drop of ferric chloride TS. A violet colour is produced

PURITY TESTS

** Loss on drying  
Not more than 0.5% after drying for 2 h at 105°

** Sulfated ash  
Not more than 0.05%

** Arsenic  
Not more than 3 mg/kg (Method II)

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

PURITY TESTS (continued)

* Lead

Not more than 10 mg/kg
Test 1 g of the sample using 10 μg lead ion (Pb) in the control as directed in the Limit Test

* Heavy metals

Not more than 30 mg/kg
Test 0.67 g of the sample as directed in the Limit Test

Chlorinated organic compounds

Not more than 100 mg/kg as chlorine
See description under TESTS

Free acid

Not more than 0.5% calculated as gallic acid (8.506 mg of gallic acid is equivalent to 1 ml of 0.05 N sodium hydroxide)
See description under TESTS

PURITY TESTS

Chlorinated organic compound

Dissolve 1 g of the sample in 10 ml of 0.1 N sodium hydroxide. Acidify with nitric acid solution and filter off the precipitate. Mix the precipitate with 2 g of calcium carbonate, dry the mixture and then ignite. Take up the ignition residue in 20 ml of dilute nitric acid TS and filter. Mix the solution with 0.5 ml of 0.1 N silver nitrate. The turbidity should not be more than that obtained in a similar volume by addition of 0.5 ml of 0.1 N silver nitrate and 0.3 ml of 0.01 N hydrochloric acid.

Free acid

To a mixture of 50 ml of carbon dioxide-free water and 50 ml of acetone, add 5 drops of bromcresol green TS and titrate with 0.005 N hydrochloric acid to match a buffer (pH 5.0) TS containing the same amount of indicator. Dissolve about 0.400 g of the sample in 50 ml of acetone and add 50 ml of carbon dioxide-free water, 5 drops of bromcresol green TS and the amount of 0.005 N hydrochloric acid found in the preliminary test to bring the solvent to pH 5.0. Titrate the solution back to pH 5.0 with 0.05 N sodium hydroxide, matching against the buffer (pH 5.0) TS.

ISOBUTANOL*  

**SYNONYMS**  
Isobutyl alcohol, IBA, isopropyl carbinol

**DEFINITION**

Chemical names  
2-Methyl-1-propanol, 2-methyl-propan-1-ol

C.A.S. number  
78-83-1

Chemical formula  
C₆H₁₂O

Structural formula  
\[
\text{CH}_3\text{-CH-CH}_2\text{OH} \\
\text{CH}_3
\]

Molecular weight  
74.12

Assay  
Content not less than 99% of C₆H₁₂O

**DESCRIPTION**

Clear, colourless, flammable liquid with a characteristic odour

**FUNCTIONAL USE**

Flavouring agent, extraction solvent

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

**A. Solubility**

Soluble in water. Miscible with ethanol and ether.

**B. Specific gravity**

\[
\begin{align*}
d^{20} & : 0.802 - 0.804 \\
d^{25} & : 0.799 - 0.801
\end{align*}
\]

C. Infra-red spectrum  
See next page

**PURITY TESTS**

**Distillation range**

106° - 109°

**Colour**

Not more than Colour Standard No. 10

**Non-volatile residue**

Not more than 1 mg/100 ml

**Water-content**

Not more than 0.2% (Karl Fischer Method)

*These specifications were prepared at the 28th session of JECFA (1984) and published in FNP 31/2 (1984).

PURITY TESTS (continued)

Acidity

Not more than 0.003% (as acetic acid)
See description under TESTS

Aldehydes and Ketones

Not more than 0.2% (as butanal)
See description under TESTS

TESTS

PURITY TESTS

Acidity

To 60 g of the sample add a few drops of phenolphthalein TS and titrate with 0.1 N ethanolic potassium hydroxide to a pink end-point which persists for at least 15 sec. Not more than 0.3 ml is required.

Aldehydes and Ketones

Proceed as directed under the General Method for Determination of Aldehydes and Ketones using 10 g of the sample and 36.06 as the equivalent factor (e) in the calculation.

METHOD OF ASSAY

Using the procedures for Gas Chromatography described in the General Methods*, establish the following conditions:

- Column: 2.4 m length, 6 mm diameter copper column packed with 23% Carbowax 1500 on Chromosorb W (60/80 mesh), or equivalent.
- Carrier gas: Helium, at flow rate of 150 ml/min.
- Detector: Flame ionization type
- Temperatures: Injection - 150°
  Column - 70°
  Detector - 150°

Inject 1 to 5 μl of sample, obtain chromatogram and determine content of each constituent by the method of area normalization.

Infra-red Spectrum: Isobutanol**

---


**ISOMALT**

**SYNONYMS**
Isomaltitol, hydrogenated isomaltulose, hydrogenated palatinose, INS No. 953

**DEFINITION**

Chemical name  
Isomalt is an approximate equimolar mixture of  
6-O-α-D-Glucopyranosyl-D-glucitol (GPG) and  
1-O-α-D-Glucopyranosyl-D-mannitol (GPM) dihydrate

C.A.S. number  
64519-82-0

Chemical formula
GPG - C₁₂H₂₀O₁₁  
GPM - C₁₇H₂₄O₁₁·2H₂O

**Structural formula**

![Structural formula of GPG and GPM](image)

6-O-α-D-Glucopyranosyl-D-glucitol  1-O-α-D-Glucopyranosyl-D-mannitol dihydrate

Molecular weight
GPG: 344.32  
GPM: 380.32

Assay
Content not less than 98% of the mixture of GPG and GPM determined on the anhydrous basis as follows:

GPG: 43 - 57%  
GPM: 43 - 57%

**DESCRIPTION**
Odourless, white, sweet tasting, crystalline slightly hygroscopic substance

**FUNCTIONAL USE**
Sweetening agent

---

* These specifications were prepared at the 33rd session of JECFA (1988) and published in FNP 38 (1990).

CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility
   Soluble in water. Insoluble in ethanol.

* B. Specific rotation
   \( [\alpha]_D^\circ \): Not less than +90-92° (4% w/v soln)

* C. Melting range
   Between 145° and 150°

PURITY TESTS

* Water
   Not more than 7% (Karl Fischer Method)

* Sulfated ash
   Not more than 0.05%
   Proceed as directed in the test of Ash (Sulfated ash, Method I) using 5 g of the sample.

Nickel
   Not more than 2 mg/kg
   Determine by atomic absorption according to the method described for Nickel in Polyols in FNP 37, A.11.

* Arsenic
   Not more than 3 mg/kg (Method II)

* Lead
   Not more than 1 mg/kg
   Prepare a sample solution as directed under Limit Test for Lead for organic compounds and determine the lead content by atomic absorption.

* Heavy metals
   Not more than 10 mg/kg
   Test 2 g of the sample as directed in the Limit Test (Method II).

D-Mannitol
   Not more than 0.5%
   See Method of Assay

D-Sorbitol
   Not more than 0.5%
   See Method of Assay

* Reducing sugars
   Not more than 1.5%
   Test 7 g of sample by the General Method for Reducing Substances (as glucose) Method II. The weight of cuprous oxide shall not exceed 250 mg.

---

METHOD OF ASSAY

High performance liquid chromatography method

Prepare a 10% aqueous solution of the sample.

Conditions of HPLC:

- Apparatus: High pressure liquid chromatograph
- Column packing: Cation exchanger Aminex A 7 in Ca\(^{2+}\) form
- Column: Length: 25 cm, Interior diameter (ID): 7.8 mm
- Solvent: Acetonitrile - water 20 : 80
- Pressure: 60 bars
- Flow rate: 0.4 ml/min
- Temperature: 45\(^\circ\)
- Detector: Differential refractometer
- Sensitivity: x 16
- Recorder: 50 mV, speed: 2 mm/min.
- Injection volume: 20 \(\mu\)l

Retention times and limits of detection

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention times</th>
<th>Limits of detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>9.7 min</td>
<td>0.10 mg/ml</td>
</tr>
<tr>
<td>Isomalulose</td>
<td>10.7 min</td>
<td>0.10 mg/ml</td>
</tr>
<tr>
<td>Glucose</td>
<td>14.0 min</td>
<td>0.10 mg/ml</td>
</tr>
<tr>
<td>GPM</td>
<td>18.1 min</td>
<td>0.10 mg/ml</td>
</tr>
<tr>
<td>Fructose</td>
<td>21.6 min</td>
<td>0.15 mg/ml</td>
</tr>
<tr>
<td>GPG</td>
<td>25.6 min</td>
<td>0.15 mg/ml</td>
</tr>
<tr>
<td>Mannitol</td>
<td>34.6 min</td>
<td>0.15 mg/ml</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>49.8 min</td>
<td>0.30 mg/ml</td>
</tr>
</tbody>
</table>
ISOPROPYL ACETATE*

** DEFINITION **

** Chemical names **
Isopropyl acetate, propan-2-ol acetate

** C.A.S. number **
108-22-5

** Chemical Formula **
$C_7H_{10}O_2$

** Structural formula **
\[
\begin{array}{c}
\text{C H}_3 \\
\text{C H}_3 \\
\text{C O O} \\
\text{C H} \\
\text{C H}_3
\end{array}
\]

** Molecular weight **
102.13

** Assay **
Content not less than 99% of $C_7H_{10}O_2$

** DESCRIPTION **
Clear colourless liquid having a characteristic odour

** FUNCTIONAL USES **
Flavouring agent, extraction solvent

** CHARACTERISTICS **

** IDENTIFICATION TESTS **

** A. Solubility **
Sparingly soluble in water. Miscible with ethanol and ether.

** B. Specific gravity **
$d_{20}^o: 0.872 - 0.874$; $d_{25}^o: 0.866 - 0.869$

** C. Boiling point **
About 88°

** D. Infrared spectrum **
See Appendix at the end of these specifications.

** PURITY TESTS **

** Water content **
Not more than 0.2% (Karl Fischer Method)

** Non-volatile residue **
Not more than 5 mg/100 ml

** Arsenic **
Not more than 3 mg/kg (Method II)

---

* These specifications were prepared at the 26th session of JECFA (1982) and published in FNP 25 (1982) superseding the earlier specifications published in FNP 19 (1981).

PURITY TESTS (continued)

**Heavy metals**

Not more than 10 mg/kg.

See description under TESTS

**Acidity**

Not more than 0.01% (as acetic acid).

See description under TESTS.

TESTS

**PURITY TESTS**

* **Heavy metals**

Evaporate 2 g of the sample to dryness on a steam bath in a glass evaporating dish. Cool, add 2 ml of hydrochloric acid TS, and slowly evaporate to dryness again on the steam bath. Moisten the residue with 1 drop of hydrochloric acid TS, add 10 ml of hot water, and digest for 2 min. Cool, and dilute to 25 ml with water. Test this solution as directed in the Limit Test (Method II).

**Acidity**

Transfer 69 ml (60 g) into a 250-ml Erlenmeyer flask, add phenolphthalein TS and titrate with 0.1 N ethanolic potassium hydroxide to a pink end-point that persists for at least 15 sec.

Not more than 1 ml is required.

**METHOD OF ASSAY**

Transfer 25.0 ml of 1 N potassium hydroxide TS into a suitable heat-resistant pressure bottle provided with a tight closure that can be securely fastened, and then add 10 ml of isopropanol and a few pieces of glass rod. To the mixture in the pressure bottle add about 1.3 g of the sample contained in a sealed glass ampoule and accurately weighed. Cap the bottle, shake it vigorously to break the ampoule, and allow it to stand at room temperature for 30 min. Uncap the bottle, add phenolphthalein TS, and titrate with 0.5 N sulfuric acid to the disappearance of the pink colour. Perform a residual blank titration. Each ml of 0.5 N sulfuric acid is equivalent to 51.07 mg of C₅H₁₀O₂.

**APPENDIX**

Infrared spectrum: Isopropyl Acetate**

 Condition: Between salts


** Infrared spectrum through the courtesy of the International Organization of the Flavour Industry (IOFI), Geneva, Switzerland, and of the SADTLER RESEARCH LABORATORIES, Inc. Philadelphia, USA
ISOPROPYL CITRATE MIXTURE*

SYNONYM

INS No.384

DEFINITION

Chemical names

Citric acid mixed ester of 2-propanol. The article of commerce, monoisopropyl citrate mixture, is composed of approximately 38 parts by weight of isopropyl citrate in 62 parts by weight of mono- and diglycerides

Structural formula and approximate composition

\[ \text{CH}_2\text{-COO-}[\text{R or H}] \]
\[ \text{HO-C-COO-}[\text{R or H}] \]
\[ \text{CH}_2\text{-COO-}[\text{R or H}] \]

where \( R \) is the isopropyl group. The major component of the 38 parts of isopropyl citrate mixture is monoisopropyl citrate (approximately 25 parts), the remainder being diisopropyl citrate (approximately 9 parts) and triisopropyl citrate (approximately 4 parts)

Approximate composition:

Monoisopropyl citrate - 27 parts by weight
Diisopropyl citrate - 9 parts by weight
Triisopropyl citrate - 2 parts by weight

DESCRIPTION

Isopropyl Citrate Mixture is an oil miscible semi-solid material. The commercial product, monoisopropyl citrate mixture, is a viscous, colourless syrup exhibiting some crystallization upon standing, and may be further specified as to saponification value, acid value, citric acid and isopropyl content.

FUNCTIONAL USES

Antioxidant, sequestrant

CHARACTERISTICS

IDENTIFICATION TESTS

A. Solubility
Soluble in water and ethanol

B. Positive test for citrate
Passes test
See description under TESTS

C. Positive test for isopropanol
Passes test
See description under TESTS

* These specifications were prepared at the 17th meeting of JECFA (1973) and published in FNP 4 (1978).

PURITY TESTS

* Sulfated ash
Not more than 0.3%

* Arsenic
Not more than 1 mg/kg (Method II) using a 3 g sample

* Lead
Not more than 10 mg/kg
Test 1 g of the sample using 10 µg lead ion (Pb) in the control as directed in the Limit Test

* Heavy metals
Not more than 30 mg/kg
Test 0.67 g of the sample as directed in the Limit Test

Acids other than citric acid
Should be absent

Alcohols other than isopropanol
Should be absent

TESTS

IDENTIFICATION TESTS

B. Positive test for citrate
Reflux 3 g of sample with 50 ml of sodium hydroxide TS for 1 h, and let stand to cool. This solution is used for the following tests:

(1) Neutralize the solution with a (1 in 20) sulfuric acid solution, add an excess of mercuric sulfate TS, heat to boil, and add potassium permanganate TS. The permanganate colour of the solution disappears, and a white precipitate forms.

(2) Neutralize the solution with hydrochloric acid, add an excess of calcium chloride TS, and boil. A white crystalline precipitate is formed which is insoluble in sodium hydroxide TS, but soluble in dilute hydrochloric acid TS.

C. Positive test for isopropanol
Reflux 2 g of sample with 50 ml of sodium hydroxide TS for 1 h. Distill off 20 ml. Place 8 g of chromic oxide in a flask, add 15 ml water and 2 ml concentrated sulfuric acid. Provide the flask with a reflux condenser and add 5 ml distillate slowly through the condenser. Reflux for 30 min, then cool and distill off 2 ml. Add 3 ml water and 10 ml mercuric sulfate TS to the distillate. Heat in a boiling water bath for 3 min. A white or yellow precipitate within 3 min indicates the presence of isopropanol.

ISOPROPYL MYRISTATE

SYNONYMS
Isopropyl tetradecanoate, tetradecanoic acid isopropyl ester

DEFINITION
Chemical name: Tetradecanoic acid propan-2-ol ester
C.A.S. number: 110-27-0
Chemical formula: C_{17}H_{34}O_2
Structural formula: \( \text{CH}_3(\text{CH}_2)_{12}\text{COOCH} (\text{CH}_3)_2 \)
Molecular weight: 270.46
Assay: Content not less than 99% of C_{17}H_{34}O_2

DESCRIPTION
A colourless, mobile, odourless or almost odourless liquid

FUNCTIONAL USE
Carrier solvent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Insoluble in water, glycerol and propan-1,2-diol
Soluble in ethanol and isopropanol

** B. Specific gravity
0.847 - 0.854

** C. Refractive index
n^20_0: 1.432 - 1.434

PURITY TESTS

** Arsenic
Not more than 3 mg/kg (Method II)

** Heavy metals
Not more than 10 mg/kg
Test 2 g of the sample as directed in the Limit Test (Method II)

** Acid value
Not more than 0.5

* These specifications were prepared at the 25th of JECFA (1981) and published in FNP 19 (1981).

PURITY TESTS (continued)

* Saponification value 205 - 211

Iodine value  
Not more than 1
See description under TESTS

TEST

PURITY TEST

* Iodine value Wijs solution

All materials and glassware must be dry. Dissolve 19.0 g of iodine monochloride in 1 litre of a mixture of 700 ml of acetic acid TS and 300 ml of carbon tetrachloride. Filter if the solution is not clear. Store in a cool, dark place.

Procedure

Weigh in a glass thimble accurately about 0.5 g of the sample. Place it in a 300-ml glass-stoppered bottle, add 10 ml of carbon tetrachloride, and then 25 ml of the Wijs solution by pipette. Swirl the liquid to mix well. Stopper tightly, place in a cool dark place (20°-25°), and allow to stand for 30 min. Add 15 ml of 10% potassium iodide solution and 100 ml of water. Titrate with 0.1 N sodium thiosulfate, shaking the mixture vigorously, until the yellow colour has almost disappeared. Add 1 to 2 ml of starch TS as the indicator and continue the titration until the blue colour has just disappeared. Carry out a blank determination under the same conditions at the same time.

Calculation

\[
\text{Iodine value} = 12.69 \times \frac{N(v - V)}{w}
\]

where:

\[
N = \text{normality of } \text{Na}_2\text{S}_2\text{O}_3 \text{ solution}
\]

\[
v = \text{volume of } \text{Na}_2\text{S}_2\text{O}_3 \text{ solution used for blank test, in ml}
\]

\[
V = \text{volume of } \text{Na}_2\text{S}_2\text{O}_3 \text{ solution used for the sample, in ml}
\]

\[
w = \text{sample weight, in g}
\]

METHOD OF ASSAY

Weigh accurately about 1.5 g of the sample and proceed as directed under the method for Determination of Esters in the General Methods*, using 135.2 as the equivalence factor (e) in the calculation.

ISOQUINOLINE*
(Tentative)**

DEFINITION

Chemical name

Isoquinoline

C.A.S. number

119-65-3

Chemical formula

C₇H₆N

Structural formula

![Chemical Structure]

Molecular weight

129.16

Assay

Content not less than 98% of C₇H₆N

DESCRIPTION

Colourless crystals liquid with an odour reminiscent of bitter almond

FUNCTIONAL USE

Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility

Very soluble in ethanol and fixed oils, soluble in most organic solvents, slightly soluble in water

*** B. Refractive index

nD²: about 1.621

*** C. Specific gravity

dD²: about 1.091

D. Infrared spectrum

See Appendix at the end of these specifications.

PURITY TEST

*** Solidification point

Not lower than 24.5°.

---

* These specifications were prepared at the 24th session of JECFA (1980) and published in FNP 17 (1980).

** The references to identity, purity and methods of analysis were felt to require further confirmation.

METHOD OF ASSAY

Determine by gas-liquid chromatography as directed in the Method of Assay for Allyl-α-ionone.

APPENDIX

* Infrared spectrum: Isoquinoline

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* Infrared spectrum through the courtesy of the International Organization of the Flavour Industry (IOFI), Geneva, Switzerland, and of the SADTLER RESEARCH LABORATORIES, Inc. Philadelphia, USA.
KARAYA GUM*

SYNONYMS
Karaya, gum karaya, Sterculia, gum sterculia, Kadaya, Katilo, Kullo, Kuterra; INS No. 416, EEC No. E416

DEFINITION
Karaya Gum is a dried exudation from the stems and branches of Sterculia urens Roxburgh and other species of Sterculia (Fam. Sterculiaceae) or from Cochlospermum gossypium A.P. De Candolle or other species of Cochlospermum (Fam. Bixaceae). It consists mainly of high molecular-weight acetylated polysaccharides, which on hydrolysis yield galactose, rhamnose, and galacturonic acid, together with minor amounts of glucuronic acid.

C.A.S. number 9000-36-6

DESCRIPTION
Unground Karaya Gum occurs in tears of variable size and in broken irregular pieces having a characteristic semi-crystalline appearance. It is pale yellow to pinkish brown in colour, translucent and horny. Powdered Karaya Gum is a pale grey to pinkish brown. The gum has a distinctive odour of acetic acid and a mucilaginous, slightly acetous taste.

FUNCTIONAL USES
Emulsifier, stabilizer, thickening agent

CHARACTERISTICS**

IDENTIFICATION TESTS

*** A. Solubility
2 g added to 50 ml of water swells to form a granular, stiff, slightly opalescent gel which is acid to litmus. Insoluble to ethanol.

B. Swelling by ethanol solution
Karaya gum swells in 60% ethanol distinguishing it from other gums.

C. Colour reaction
Passes test
See description under TESTS

D. Colour reaction
Passes test
Warm 0.5 g of the sample with 2 ml of 5 M sodium hydroxide; a brown colour is produced.

E. Precipitate formation
Passes test
See description under TESTS

F. Gum constituents
Passes test
See description under TESTS

* These specifications were prepared at the 33rd session of JECFA (1988) and published in FNP 38 (1988).

Items of commerce may contain extraneous materials such as pieces of bark which must be removed before use in food.

** Unground samples should be powdered to pass a standard ISO sieve of 355 µm (USA No. 45) and mixed well before performing any of the following tests.

PURITY TESTS

* **Loss on drying**
  Not more than 20% after drying (105°, 5 h).

* **Total ash**
  Not more than 8%

**Acid insoluble ash**
Not more than 1%
See description under TESTS

**Acid insoluble matter**
Not more than 3%
See description under TESTS

* **Arsenic**
  Not more than 3 mg/kg
  A sample solution prepared as directed for organic compounds will meet the requirements of the Limit Test for Arsenic (Method II).

* **Lead**
  Not more than 10 mg/kg
  A sample solution prepared as directed for organic compounds will meet the requirements of the Limit Test for Lead.

* **Heavy metals**
  Not more than 40 mg/kg
  Test 0.5 g of the sample as directed in Method II under the Limit Test for Heavy Metals using 20 μg of lead ion (Pb) in the control (Solution A).

**Volatile acid**
Not less than 10%, calculated as acetic acid.
See description under TESTS

**Starch**
Not detectable
To a 1 in 10 solution of the sample add a few drops of iodine TS. No blue colour should be produced.

* **Microbiological criteria**
  *Salmonella* spp.: Negative in 1 g
  *E. Coli*: Negative in 1 g

TESTS

IDENTIFICATION TESTS

C. **Colour reaction**
Boil 1 g of the sample with 20 ml of water until a mucilage is formed. Add 5 ml of hydrochloric acid and boil the mixture for 5 min. A permanent red or pink colour develops.

E. **Precipitate formation**
Shake 1 g of the sample with 80 ml of water for 24 h. Boil 4 ml of the resulting mucilage with 0.5 ml of concentrated hydrochloric acid, add 1 ml of 5 M sodium hydroxide and filter. To the filtrate add 3 ml of potassium cupric tartrate solution and heat. A red precipitate is formed.

IDENTIFICATION TESTS (continued)

F. Gum constituents

Proceed as directed under Identification of gum constituents in the General Methods using the following as reference standards: galactose, rhamnose, galacturonic acid, glucuronic acid, mannose, arabinose and xylose. Galactose, rhamnose galacturonic acid, and glucuronic acid should be present and mannose, arabinose and xylose should be absent.

PURITY TESTS

Acid-insoluble ash

Weigh 3 g of the sample to the nearest 0.1 mg in a tared crucible. Ignite at a low temperature (about 550°), not to exceed a very dull redness, until free from carbon, cool in a desiccator, and weigh. If a carbon-free ash is not obtained, wet the charred mass with hot water, collect the insoluble residue on an ashless filter paper, and ignite, in the crucible, the residue and filter paper until the ash is white or nearly so. Add the filtrate, evaporate it to dryness, and heat the whole to a dull redness. If a carbon-free ash is still not obtained, cool the crucible, add 15 ml of ethanol, break up the ash with a glass rod, burn off the ethanol, again heat the whole to a dull redness and cool. Boil this ash with 25 ml of dilute hydrochloric acid TS for 5 min. Collect the insoluble matter on a tared Gooch crucible or ashless filter, wash with hot water, ignite, cool and weigh. Calculate the percentage of acid-insoluble ash from the weight of the sample.

Acid-insoluble matter

Weigh about 5 g of the sample, to the nearest 0.1 mg and transfer into a 250 ml beaker or Erlenmeyer containing 100 ml of 5% weight/volume hydrochloric acid. Cover with a watch glass or attach the flask to a condenser having cold water running through it. Boil gently until the gum is completely dissolved (about 3 h.). Filter the solution through a tared porcelain or glass fritted crucible 10 to 20 μm porosity. Wash the residue several times with hot water until the washings are free from acid (pH paper). Dry the crucible to constant weight at 105°, cool to room temperature in a dessicator and weigh. Calculate as percentage.

Volatile acid

To 1 g contained in a 700 ml long necked flask add 100 ml of water and 5 ml of syrupy orthophosphoric acid, allow to stand for several h., or until the gum is completely swollen, and boil gently for two h. under a reflux condenser; steam-distil until 800 ml of distillate is obtained and the acid residue measures about 20 ml, and titrate the distillate with 0.1 M sodium hydroxide using phenolphthalein TS as indicator. Repeat the procedure without gum. The difference between the titrations represents the amount of alkali required to neutralise the volatile acid. Each ml of 0.1 M sodium hydroxide is equivalent to 0.00600 g of volatile acid, calculated as acetic acid.

LACTIC ACID*

SYNONYMS

INS No. 270, EEC No. E270

DEFINITION**

Chemical names
Lactic acid, 2-hydroxy propionic acid, 1-hydroxyethane-1-carboxylic acid.

C.A.S. number
50-21-5 (L-: 79-33-4; D-: 10326-41-7; DL-: 598-82-3)

Chemical formula
C₃H₆O₃

Structural formula
\[
\text{CH}_3\text{CHOHCOOH}
\]

Molecular weight
90.08

Assay**
Lactic Acid contains not less than 95.0% and not more than 105.0% of the labelled concentration of C₃H₆O₃.

DESCRIPTION

Colourless or yellowish, nearly odourless, syrupy liquid with an acid taste, consisting of a mixture of lactic acid (C₃H₆O₃) and lactic acid lactate (C₆H₁₀O₅). It is obtained by the lactic fermentation of sugars or is prepared synthetically. Common products of commerce are 50-90% solutions.

(Note. Lactic acid is hygroscopic and when concentrated by boiling, it condenses to form lactic acid lactate, which on dilution and heating hydrolyzes to lactic acid).

FUNCTIONAL USE

Acid

CHARACTERISTICS

IDENTIFICATION TESTS

A. Solubility
Miscible with water and with ethanol

*** B. Positive test for acid
A 1 in 10 solution of the sample is acid to litmus paper.

*** C. Positive test for lactate
Passes test

* These specifications were prepared at the 21st session of JECFA (1977) and published in NMRS No.57 (1977).

** Lactic acid contains a portion of lactic acid lactate; see "DESCRIPTION". The data given consider the product as lactic acid only.

PURITY TESTS

* Sulfated ash
  Not more than 0.1%
  Test 2 g of the sample by Method I under the test for Ash (Sulfated ash).

Chloride
  Not more than 0.2%
  See description under TESTS

Sulfate
  Not more than 0.25%
  See description under TESTS

* Arsenic
  Not more than 3 mg/kg (Method II)

* Heavy metals
  Not more than 10 mg/kg
  A solution of 2 g of the sample in 25 ml of water meets the requirements of the Limit Test for Heavy Metals (Method I) using 20 µg of lead ion (Pb) in the control (Solution A).

Iron
  Not more than 10 mg/kg
  See description under TESTS

Cyanide
  passes test
  See description under TESTS

Citric, oxalic, phosphoric or tartaric acid
  Dilute 1 g of the sample to 10 ml with water, add 40 ml of calcium hydroxide TS, and boil for 2 min. No turbidity is produced.

Sugars
  Add 5 drops of the sample to 10 ml of hot alkaline cupric tartrate TS. No red precipitate is formed.

Readily carbonizable substances
  Superimpose carefully 5 ml of the sample kept at 15° on 5 ml of conc. sulfuric acid kept at 15°. No deep grey colour is produced within 15 min. at the contact zone of the two liquids.

Volatile fatty acid
  Heat 5 ml of the sample on a water bath. No strong fatty acid-like odour is evolved.

Methanol
  Not more than 0.2%
  See description under TESTS

TESTS

PURITY TESTS

* Chloride

Weigh accurately a portion of the sample equivalent to about 5 g of lactic acid, dissolve in 50 ml of water, and neutralize to litmus with sodium hydroxide solution (1 in 4). Add 2 ml of potassium chromate TS and titrate with 0.1 N silver nitrate to the first appearance of a red tinge. Each ml of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl.

* Sulfate

Weigh accurately a portion of the sample equivalent to about 50 g of lactic acid, transfer into a 600-ml beaker, dissolve in 200 ml of water, and neutralize to between pH 4.5 and 6.5 with sodium hydroxide solution (1 in 2), making the final adjustment with a more dilute alkali solution. Filter, if necessary, and heat the filtrate or clear solution to just below the boiling point. Add 10 ml of barium chloride TS, stirring vigorously, boil the mixture gently for 5 min., and allow to stand for at least 2 hours, or preferably overnight. Collect the precipitate of barium sulfate in a tared Gooch crucible, wash until free from chloride, dry, and ignite at 600° to constant weight. The weight of barium sulfate so obtained, multiplied by 0.412, represents the weight of SO₄ in the sample taken.

Iron

To the ash obtained in the test for Sulfated ash add 2 ml of dilute hydrochloric acid (1 in 2), and evaporate to dryness on a steam bath. Dissolve the residue in 1 ml of hydrochloric acid, dilute to 40 ml with water, and add 40 mg of ammonium persulfate crystals and 10 ml of ammonium thiocyanate TS. Any red or pink colour does not exceed that produced by 20 ml of Iron Standard Solution (20 μg Fe) in an equal volume of solution containing the quantities of reagents used in the test.

Cyanide

Reagents

- Chloramine-T TS: Dissolve 1 g of chloramine-T (C₅H₇NNaO₂SCl·3H₂O) in water to make 100 ml. Prepare freshly before use.
- Pyridine-pyrazolone TS: Dissolve 0.5 g of 1-phenyl-3-methyl-5-pyrazolone in 100 ml of hot water at 75° and cool to room temperature. Mix with 20 ml of pyridine containing 0.025 g of bis-(1-phenyl-3-methyl-5-pyrazolone). Prepare freshly before use.

Procedure

To 0.1 g of the sample add 3 ml of 20% of sodium hydroxide solution and heat for 10 min on a water bath. After cooling, add 1 drop of phenolphthalein TS and add dropwise dilute acetic acid TS until the pink colour has disappeared. Add 3 drops of dilute acetic acid TS and water to make 40 ml.

Cyanide
(contin'd)

Add 0.6 ml of chloramine-T TS and allow to stand for 3 min. Add 10 ml of pyridine-pyrazolone TS and allow to stand for 25 min. No blue colour is produced (limit approx. 1 mg/kg).

Methanol

To 5 ml of the sample add 8 ml of water and 5 g of calcium carbonate, and distill. Dilute 5 ml of the initial distillate with water to 100 ml. To a 1 ml portion of the solution, add 0.1 ml of dilute phosphoric acid (1 in 20) and 0.2 ml of potassium permanganate TS and allow to stand for 10 min. Add 0.3 ml of sodium sulfite solution (1 in 4) and 3 ml of sulfuric acid, and add 0.2 ml of chromotropic acid TS. For the control, prepare in the same manner as the sample, using a 1 ml portion of the solution which is prepared by adding water to 1 ml of methyl alcohol to 100 ml and subsequently adding water to a 1 ml portion of this solution to 100 ml. The colour of the sample solution is not darker than that of a control.

METHOD OF ASSAY

Weigh accurately a portion of the sample equivalent to about 3 g of lactic acid, transfer to a 250-ml flask, add 50 ml of 1 N sodium hydroxide, mix, and boil for 20 min. Add phenolphthalein TS, titrate the excess alkali in the hot solution with 1 N sulfuric acid, and perform a blank determination. Each ml of 1 N sodium hydroxide is equivalent to 90.08 mg of C₃H₆O₃.
LACTIC AND FATTY ACID ESTERS OF GLYCEROL*

SYNONYMS
Lactic acid esters of mono- and diglycerides; lactoglycerides
INS No. 472b, EEC No. E472b

DEFINITION
'Lactic Acid and Fatty Acid Esters of Glycerol' consists of mixed glycerol esters of lactic acid and fatty acids of food fats.

The article of commerce may be further specified as to monoglyceride content, total lactic acid, acid value, saponification value, free fatty acid content, solidification point of the free fatty acids, iodine value, free glycerol content and water content.

Structural formula

\[
\begin{align*}
\text{CH}_2 & \quad \text{OR}_1 \\
\text{CH} & \quad \text{OR}_2 \\
\text{CH}_2 & \quad \text{OR}_3
\end{align*}
\]

where R₁, R₂, and R₃ each may be a fatty acid moiety, a lactic acid moiety, or hydrogen (approximate composition)

DESCRIPTION
'Lactic and Fatty Acid Esters of Glycerol' are waxy solids of variable consistency and conforms to the following specifications

FUNCTIONAL USE
Emulsifier

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Insoluble in cold water but dispersible in hot water

** B. Positive test for fatty acids
Passes test

** C. Positive test for lactic acid
Passes test

** D. Positive test for glycerol
Passes test

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

PURITY TESTS

* Arsenic  
   Not more than 3 mg/kg (Method II)

* Heavy metals  
   Not more than 10 mg/kg  
   Test 2.0 g of the sample as directed in Method II under the Limit Test for Heavy Metals

* Acids  
   Acids other than lactic and fatty acids shall not be detectable

** LACTITOL*  
(Tentative)**

SYNONYMS
Lactit, lactositol, lactobiosit  
INS No. 966

DEFINITION
Chemical name 4-O-β-D-Galactopyranosyl-D-glucitol  
C.A.S. number 585-86-4  
Chemical formula C_{12}H_{24}O_{11}  
Structural formula

Molecular weight 344.32  
Assay Not less than 95% and not more than 102% lactitol on the anhydrous basis

DESCRIPTION
Sweet tasting crystalline powders or colourless solutions. Crystalline products occur in both monohydrate and dihydrate forms.

FUNCTIONAL USES
Sweetening agent, texturiser

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility  
Very soluble in water

*** B. Specific rotation  
\([\alpha]_D^2 = +13^\circ \text{ to } +15^\circ \) calculated on the anhydrous basis (10% w/v aqueous solution)

* These specifications were prepared at the 33rd session of JECFA (1988) and published in FNP 38 (1988).

** Information is required on the maximum water content of solutions of lactitol, a method for determining water content of solutions and the applicability of the General Methods for Reducing Substances as alternatives to the Luff-Schoorl Method.

C. Main peak in HPLC

The main HPLC peak exhibited by the sample in the assay has the same elution time as that of the lactitol standard. See Method of Assay

PURITY TESTS

* Water content

Crystalline products: not more than 10.5% (Karl Fisher Method) Solutions: Information required**

* Sulfated ash

Not more than 0.1% on the anhydrous basis Test an amount of the sample equivalent to 2 g of the anhydrous substance as directed under the test for Ash (Sulfated ash) Method I.

* Chlorides

Not more than 100 mg/kg of the anhydrous basis. Test an amount of sample equivalent of 10 g of the anhydrous substance by the Chlorides Limit Test using 3.0 ml of 0.01 N hydrochloric acid in the standard.

* Sulfates

Not more than 200 mg/kg on the anhydrous basis. Test an amount of sample equivalent to 10 g of the anhydrous substance by the Sulfates Limit Test using 4.0 ml of 0.01 N sulfuric acid in the standard.

 Nickel

Not more than 2 mg/kg on the anhydrous basis Proceed as directed in the specifications for SORBITOL.

* Arsenic

Not more than 2 mg/kg on the anhydrous basis. Test an amount of sample equivalent to 1.0 g of the anhydrous substance as directed in the Limit Test for Arsenic, Method II.

* Lead

Not more than 1 mg/kg on the anhydrous basis. Prepare a sample solution using an amount of material equivalent to 1.0 g of the anhydrous substance as directed under Limit Test for Lead for organic compounds and determine the lead content by atomic absorption.

* Heavy metals

Not more than 10 mg/kg on the anhydrous basis. Test an amount of sample equivalent to 2.0 g of the anhydrous substance in 25 ml of water as directed in the Heavy Metals Limit Test (Method I).

 Other polyols

Not more than 2.5% on the anhydrous basis See Method of Assay

 Reducing sugars

Not more than 0.2% on the anhydrous basis as lactose See description under TESTS

TESTS

PURITY TESTS

Reducing sugars

Modified Luff-Schoorl Method**

Prepare Copper reagent as follows: 338 g of crystallized sodium carbonate (Na₂CO₃, 10H₂O) is dissolved in 300 to 400 ml of


** Information required - see footnote in the previous page.
Reducing sugars
(continued)

Lukewarm water. A solution of 50 g citric acid in 50 ml of water is added, and then a solution of 25 g crystallized copper sulfate, free from iron, in about 100 ml of water. The mixture is allowed to cool and make up to 1 l. After a few days’ standing, the clear solution is decanted or siphoned off. It keeps indefinitely and shows no auto-reduction even upon boiling.

Place 25 ml of this reagent and 15 g of Lactitol, dissolved in 25 ml water in a 300 ml Erlenmeyer flask, add a few pieces of pumice stone, and heat over a free flame, holding the flask by the hand, so that the solution begins to boil in about 2 min. Place the flask on a wire gauze covered with an asbestos screen, connect with a reflux condenser, and boil for exactly 10 min. longer. Cool at once in running water and after 5 min. add 3 g of potassium iodide. Acidify with 20 ml of 25% hydrochloric acid and shake until the evolution of gas stops. The remaining foam may be removed by a few drops of ether. Titrate the liberated iodine with 0.1 N thiosulfate, using 1 ml of 2% starch solution toward the end, until the blue colour disappears and the precipitate is cream coloured. Run a blank with 25 ml of the copper reagent and 25 ml of water. The difference between the two titrations is equivalent to the reduced copper and to the quantity of reducing sugar present which is calculated as lactose from the following table:

<table>
<thead>
<tr>
<th>0.1 N Thiosulfate</th>
<th>Lactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml</td>
<td>mg</td>
</tr>
<tr>
<td>1</td>
<td>3.6</td>
</tr>
<tr>
<td>2</td>
<td>7.3</td>
</tr>
<tr>
<td>3</td>
<td>11.0</td>
</tr>
<tr>
<td>4</td>
<td>14.7</td>
</tr>
<tr>
<td>5</td>
<td>18.4</td>
</tr>
<tr>
<td>6</td>
<td>22.1</td>
</tr>
<tr>
<td>7</td>
<td>25.8</td>
</tr>
<tr>
<td>8</td>
<td>29.5</td>
</tr>
<tr>
<td>9</td>
<td>33.2</td>
</tr>
<tr>
<td>10</td>
<td>37.0</td>
</tr>
<tr>
<td>11</td>
<td>40.8</td>
</tr>
<tr>
<td>12</td>
<td>44.6</td>
</tr>
<tr>
<td>13</td>
<td>48.4</td>
</tr>
<tr>
<td>14</td>
<td>52.2</td>
</tr>
<tr>
<td>15</td>
<td>56.0</td>
</tr>
<tr>
<td>16</td>
<td>59.9</td>
</tr>
<tr>
<td>17</td>
<td>63.8</td>
</tr>
<tr>
<td>18</td>
<td>67.7</td>
</tr>
<tr>
<td>19</td>
<td>71.7</td>
</tr>
<tr>
<td>20</td>
<td>75.7</td>
</tr>
<tr>
<td>21</td>
<td>79.8</td>
</tr>
<tr>
<td>22</td>
<td>83.9</td>
</tr>
<tr>
<td>23</td>
<td>88.0</td>
</tr>
</tbody>
</table>
METHOD OF ASSAY

Principle
Lactitol and other by-product polyols from the manufacture of Lactitol are determined by High performance liquid chromatography. Principal by-product polyols are the hexitols: sorbitol, mannitol, galactitol (dulcitol), and lower polyols such as glucitols.

Apparatus
High performance liquid chromatograph with elevated temperature capability, differential refractometric detector and 0.45 μm membrane filter before column.

- Column: Aminex HPX 87 (Calcium form) with dimensions 300 x 7.8 mm, or equivalent column designed for carbohydrate analyses.
- Standards: Lactitol, sorbitol, mannitol
- Eluent: Water (degassed)

Procedure
Equilibrate chromatography column to 85°C. Adjust eluent flow rate through column to 0.6 ml/min. Accurately prepare an aqueous solution of sample about 40% by weight. Inject 10 μl of the 40% sample solution on the column. Record the chromatogram for peaks occurring at the retention-time of Lactitol and thereafter. Approximate retention times for Lactitol and other polyols using the recommended column are:

<table>
<thead>
<tr>
<th>Polyol</th>
<th>Retention Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactitol</td>
<td>12 min</td>
</tr>
<tr>
<td>Ribitol</td>
<td>15 min</td>
</tr>
<tr>
<td>Erythritol</td>
<td>16 min</td>
</tr>
<tr>
<td>Mannitol</td>
<td>18 min</td>
</tr>
<tr>
<td>Galactitol</td>
<td>20 min</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>21 min</td>
</tr>
</tbody>
</table>

For Assay, compare sample response relative to standard sample of Lactitol which has a known purity.

For Other polyols, measure the area of all peaks occurring after Lactitol through Sorbitol. The weight corresponding the sum of the areas of these peaks, calculated as Lactitol, is not greater than 2.5% of the dry weight of the sample.
LECITHIN*

SYNONYMS  Phosphatides, phospholipids;INS No. 322, EEC No. E322

DEFINITION  Food grade lecithin is usually prepared from oil-bearing seeds used for food especially soybeans. It may also be prepared from animal sources. It is a complex mixture of acetone-insoluble phosphatides which consists chiefly of phosphatidyl-choline, phosphatidyl-ethanolamine, and phosphatidyl-inositol, combined with various amounts of other substances such as triglycerides, fatty acids, and carbohydrates. Refined grades of lecithin may contain any of these components in varying proportions and combinations depending on the type of fractionation used. In its oil-free form, the preponderance of triglycerides and fatty acids is removed and the product contains 90% or more of phosphatides representing all or certain fractions of the total phosphatide complex.

C.A.S. number 8002-43-5

Assay  Content not less than 60% of acetone-insoluble matter (phosphatides)

DESCRIPTION  The consistency of both natural grades and refined grades of lecithin may vary from plastic to fluid, depending upon free fatty acid and oil content, and upon the presence or absence of other diluents. Its colour varies from light yellow to brown, depending on the source, on crop variations, and on whether it is bleached or unbleached. It is odourless or has a characteristic, slight nutlike odor and a bland taste. Edible diluents, such as cocoa butter and vegetable oils, often replace soybean oil to improve functional and flavor characteristics.

FUNCTIONAL USES  Emulsifier, antioxidant

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility  Lecithin is only partially soluble in water, but it readily hydrates to form emulsions. The oil-free phosphatides are soluble in fatty acids, but are practically insoluble in fixed oils.

B. Phosphorus  See description under TESTS

* These specifications which apply to both bleached and unbleached lecithins were prepared at the 37th session of JECFA (1990) superseding the earlier specifications published in FNP 37 (1986). These substances were evaluated for specifications only.

IDENTIFICATION TESTS (continued)

C. Choline  See description under TESTS

D. Fatty acids  See description under TESTS

E. Test for hydrolysis  See description under TESTS

PURITY TESTS

* Loss on drying  Not more than 2% (105°, 1 h)
* Arsenic  Not more than 3 mg/kg (Method II)
* Lead  Not more than 10 mg/kg
* Heavy metals  Not more than 40 mg/kg  See description under TESTS

Acid value  Not more than 36  See description under TESTS

Peroxide value  Not more than 10  See description under TESTS

Toluene-insoluble matter  Not more than 0.3%  See description under TESTS

TESTS

IDENTIFICATION TESTS

B. Phosphorus  Ignite 1 g of the sample with 2 g of anhydrous sodium carbonate. Cool and dissolve the residue in 5 ml of water and 5 ml of nitric acid. Add 5 ml of ammonium molybdate TS and heat to boiling. A yellow precipitate is obtained.

C. Choline  To 0.5 g of the sample, add 5 ml of diluted hydrochloric acid (1+1), heat in a water bath for 2 hours, and filter. Use this solution as the test solution. Perform Paper Chromatography* with 10 μl of the test solution, using choline chloride solution (1+200) as the control solution and n-butanol-water-acetic acid mixture (4:2:1) as the developing solvent. A red-orange spot corresponding to the spot obtained from the control solution is observed. For the filter paper, use a No. 2 filter paper for chromatography. Stop the development when the developing solvent rises about 25 cm, air-dry, spray with Dragendorff TS to develop a colour, and observe in daylight.

D. Fatty acids

Reflex 1 g of the sample for 1 h with 25 ml of 0.5 N ethanolic potassium hydroxide. When cooled to 0°, a precipitate of potassium soap is obtained.

E. Test for hydrolysis

To a 800 ml beaker add 500 ml of water (30-35°). Then slowly add 50 ml of the sample with constant stirring. Hydrolyzed lecithin will form a homogeneous emulsion. Non-hydrolyzed lecithin will form a distinct mass of about 50 g.

PURITY TESTS

* Acid value

Weigh accurately about 2 g of the well-mixed sample into a 250-ml Erlenmeyer flask. Dissolve in 50 ml of petroleum ether by shaking gently. Then add 50 ml of ethanol, previously neutralized to phenolphthalein with 0.1 N sodium hydroxide, and shake to mix. Add 4 drops of phenolphthalein TS and titrate while shaking with 0.1 N sodium hydroxide until the pink colour persists for 5 sec.

Acid value = ml 0.1 N NaOH x 2.8

Reagents

- Acetic acid-chloroform solution: Mix 3 volumes of acetic acid with 2 volumes of chloroform.
- Potassium iodide solution, saturated: Dissolve excess potassium iodide in freshly boiled water. Excess solid must remain. Store in the dark. Test daily by adding 0.5 ml to 30 ml of the acetic acid-chloroform solution, then add 2 drops of starch TS. If the solution turns blue, requiring more than 1 drop of 0.1 N sodium thiosulfate to discharge the colour, prepare a fresh solution.

Procedure

Weigh accurately about 5 g of the sample into a 250-ml Erlenmeyer flask. Add 30 ml of the acetic acid-chloroform solution and swirl to dissolve. Add 0.5 ml of the saturated potassium iodide solution, allow to stand with occasional shaking for 1 min. and add 30 ml of water. Slowly titrate with 0.01 N sodium thiosulfate with vigorous shaking until the yellow colour is almost gone. Add about 0.5 ml of starch TS, and continue the titration, shaking vigorously to release all the iodine from the chloroform layer, until the blue colour has just disappeared.

Perform a blank determination and make any necessary correction.

\[
\text{Peroxide value} = \frac{S \times 10}{\text{Weight of sample (g)}}
\]

\[S = \text{ml of 0.01 N sodium thiosulfate}\]
TESTS (continued)

Toluene-insoluble matter

Weigh 10 g of the well-mixed sample into a 250-ml flask. Add 100 ml of toluene and shake until dissolved. Filter through a tared filter funnel G3 or equivalent with a porosity of 16-40 μm. Wash the flask with 25-ml portions of toluene and pour the washings through the funnel. Place the funnel in a forced-draft oven and dry at 105° for 1 h. Weigh dried funnel and subtract tare to determine weight of toluene insoluble residue:

\[
\text{Weight of residue (g)} = \frac{\text{Weight of sample (g)}}{\times 100 \%}
\]

Heavy metals

Test 0.5 g of the sample as directed in the Limit Test (Method II), using 20 μg of lead ion (Pb) in the control (Solution A).

METHOD OF ASSAY

Purification of phosphatides

Wash about 10 g of the sample 3 times well with each 100 ml of acetone and the insoluble residue (phosphatides) is used. Residues (phosphatides) obtained from assays carried out previously can also be used. Dissolve 5 g of these phosphatides in 10 ml of petroleum ether, and add 25 ml of acetone to the solution. Transfer approximately equal portions of the precipitate to each of two 40-ml centrifuge tubes using additional portions of acetone to facilitate the transfer. Stir thoroughly, dilute to 40 ml with acetone, stir again, chill for 15 min. in an ice bath, stir again, and then centrifuge for 5 min. Decant the acetone, stir, centrifuge, and decant as before. The solids after the second centrifugation require no further purification and may be used for preparing the phosphatide-acetone solution. 5 g of the purified phosphatides are required to saturate about 16 litres of acetone.

Phosphatide-acetone solution

Add a quantity of purified phosphatides to sufficient acetone, previously cooled to a temperature of about 5°, to form a saturated solution, and maintain the mixture at this temperature for 2 h., shaking it vigorously at 15-min. intervals. Decant the solution through a rapid filter paper, avoiding the transfer of any undissolved solids to the paper and conducting the filtration under refrigerated conditions (not above 5°).

Procedure

If it is plastic or semisolid, soften a portion of the sample by warming it in a water bath at a temperature not exceeding 60° and then mixing it thoroughly. Transfer about 2 g of a well-mixed sample, accurately weighed, into a previously tared 40-ml centrifuge tube, that contains a glass stirring rod, and add 15 ml of Phosphatide-Acetone Solution from a buret. Warm the mixture in a water bath until the lecithin melts, but avoid evaporation of the acetone. Stir until the sample is completely disintegrated and dispersed, and then transfer the tube into an ice bath, chill for 5 min, remove from the ice bath, and add about one half of the required volume of Phosphatide-Acetone Solution, previously chilled for 5 min in an ice bath. Stir the mixture to complete dispersion of the sample, dilute to 40 ml with chilled Phosphatide-Acetone Solution (5°), again stir, and return the tube and contents to
METHOD OF ASSAY (continued)

the ice bath for 15 min. At the end of the 15-min chilling period, stir again while still in the ice bath, remove the stirring rod, temporarily supporting it in a vertical upside-down position, and centrifuge the mixture immediately at about 2000 rpm for 5 min. Decant the supernatant liquid from the centrifuge tube, crush the centrifuged solids with the same stirring rod previously used, and refill the tube to the 40-ml mark with chilled (5°) Phosphatide-Acetone Solution and repeat the chilling, stirring, centrifugation, and decantation procedure previously followed. After the second centrifugation and decantation of the supernatant acetone, again crush the solids with the assigned stirring rod, and place the tube and its contents in a horizontal position at room temperature until the excess acetone has evaporated. Mix the residue again, dry the centrifuge tube and its contents at 105° for 45 min. in a forced-draft oven, cool, and weigh. Calculate the percentage of acetone-insoluble matter by the formula \((100R/S) - B\), in which \(R\) is the weight of residue, \(S\) is the weight of the sample, and \(B\) is the percentage of toluene-insoluble matter (see Purity Tests).
**LECTHIN, PARTIALLY HYDROLYZED**

**SYNONYMS**

Phosphatides, phospholipids
INS No 322, EEC No E322

**DEFINITION**

Partially hydrolyzed lecithin is prepared by partial hydrolysis of lecithin by the use of a suitable lipase. When the desired degree of hydrolysis is attained, the product is heated in order to inactivate the residual enzyme.

**C.A.S. number**

8002-43-5

**Assay**

Content not less than 56% of acetone-insoluble matter (phosphatides)

**DESCRIPTION**

The consistency of hydrolyzed lecithin may vary from plastic to fluid, depending upon free fatty acid and oil content, and upon the presence or absence of other diluents. Its colour varies from light yellow to brown, depending on the source, on crop variations, and on whether it is bleached or unbleached. It is odourless or has a characteristic, slight nutlike odour and a bland taste. Edible diluents, such as cocoa butter and vegetable oils, often replace soybean oil to improve functional and flavour characteristics.

**FUNCTIONAL USES**

Emulsifier, antioxidant synergist

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

**A. Solubility**

Partially hydrolyzed lecithin is only partially soluble in water, but it readily hydrates to form emulsions. The oil-free phosphatides are soluble in fatty acids, but are practically insoluble in fixed oils.

**B. Phosphorus**

See description under TESTS

**C. Choline**

See description under TESTS

**D. Fatty acids**

See description under TESTS

**E. Test for hydrolysis**

See description under TESTS

* These specifications were prepared at the 37th session of JECFA (1990) superseding the earlier specifications published in FNP 37 (1986). These specifications apply to both bleached and unbleached lecithins.

PURITY TESTS

* Loss on drying Not more than 2% (105°, 1 h)
* Arsenic Not more than 3 mg/kg (Method II)
* Lead Not more than 10 mg/kg
* Heavy metals Not more than 40 mg/kg
  Test 0.5 g of the sample as directed in the Limit Test (Method II).
* Acid value Not more than 45
  Proceed as directed in the Test for Acid value under Lecithin.

Peroxide value Not more than 10
Proceed as directed in the Test for Peroxide value under Lecithin.

Toluene-insoluble matter Not more than 0.3%
Proceed as directed in the Test for Toluene-insoluble matter under Lecithin.

IDENTIFICATION TESTS

B. Phosphorus Ignite 1 g of the sample with 2 g of anhydrous sodium carbonate. Cool and dissolve the residue in 5 ml of water and 5 ml of nitric acid. Add 5 ml of ammonium molybdate TS and heat to boiling. A yellow precipitate is obtained.

C. Choline To 0.5 g of the sample, add 5 ml of diluted hydrochloric acid (1 + 1), heat in a water bath for 2 hours, and filter. Use this solution as the test solution. Perform Paper Chromatography* with 10 μl of the test solution, using choline chloride solution (1 + 200) as the control solution and n-butanol-water-acetic acid mixture (4:2:1) as the developing solvent. A red-orange spot corresponding to the spot obtained from the control solution is observed. For the filter paper, use a No. 2 filter paper for chromatography. Stop the development when the developing solvent rises about 25 cm, air-dry, spray with Dragendorff TS to develop a colour, and observe in daylight.

* D. Fatty acids Reflux 1 g of the sample for 1 h with 25 ml of 0.5 N ethanolic potassium hydroxide. When cooled to 0°, a precipitate of potassium soap is obtained.

E. Test for hydrolysis To a 800 ml beaker add 500 ml of water (30°-35°). Then slowly add 50 ml of the sample with constant stirring. Hydrolyzed lecithin will form a homogeneous emulsion. Non-hydrolyzed lecithin will form a distinct mass of about 50 g.

METHOD OF ASSAY Proceed as directed in the Method of Assay under Lecithin.

**SYNONYMS**

Petroleum spirits, petroleum ether

**DEFINITION**

Light petroleum fractions boiling between 25° and 105°. Mixed paraffinic (normal and iso) and cycloparaffinic hydrocarbons

**DESCRIPTION**

A clear, colourless, mobile, highly flammable liquid with a characteristic petroleum-like odour

**FUNCTIONAL USE**

Extraction solvent

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

** A. Solubility**

Insoluble in water. Soluble in ethanol

**PURITY TESTS**

** Non-volatile residue**

Not more than 2 mg/100 ml

**Sulfur**

Not more than 10 mg/kg

Proceed as directed under the Determination of Sulphur for HEPTANES

** Arsenic**

Not more than 3 mg/kg (Method II)

** Heavy metals**

Not more than 10 mg/kg

See description under TESTS

** Benzene**

Not more than 0.05% v/v

Proceed as directed under the Determination of Aromatic Hydrocarbons in the General Methods

** Aromatic hydrocarbons**

Not more than 0.3% v/v

**Polycyclic aromatic hydrocarbons**

The following absorbance limits per cm of optical path length shall not be exceeded:**

<table>
<thead>
<tr>
<th>Wave length (nm)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>280 - 289</td>
<td>0.15</td>
</tr>
<tr>
<td>290 - 299</td>
<td>0.12</td>
</tr>
<tr>
<td>300 - 359</td>
<td>0.08</td>
</tr>
<tr>
<td>360 - 400</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* These specifications were prepared at the 25th session of JECFA (1981) and published in FNP 19 (1981).

**PURITY TESTS (continued)**

**Bromine index**

Not more than 200
See description under TESTS

**TESTS**

**PURITY TESTS**

- **Heavy metals**

Evaporate 2 g of the sample to dryness on a steam bath in a glass evaporating dish. Cool, add 2 ml of hydrochloric acid TS, and slowly evaporate to dryness again on the steam bath. Moisten the residue with 1 drop of hydrochloric acid TS, add 10 ml of hot water, and digest for 2 min. Cool, and dilute to 25 ml with water. Test this solution as directed in the Limit Test.

**Bromine index**

**Principle**

A known mass of the sample dissolved in a specified solvent is titrated with standard bromide-bromate solution. The end point is indicated by a dead stop electrometric titration apparatus, when the presence of free bromine causes a sudden change in the electrical conductivity of the system. The bromine index is the number of mg of bromine that will react with 100 g of the sample under the conditions of the test.

**Apparatus**

- Dead-Stop Electrometric Titration Apparatus: Any dead-stop apparatus may be used incorporating a high-resistance polarizing current supply capable of maintaining approximately 0.8 V across two platinum electrodes and with a sensitivity such that a voltage change of approximately 50 mV at these electrodes is sufficient to indicate the end point.

- Titration Vessel: A jacketed glass vessel of approximately 150-ml capacity of such a form that can be conveniently maintained at 1° to 5°. A pair of platinum electrodes spaced not more than 5 mm apart, shall be mounted to extend well below the liquid level. Stirring shall be by a mechanical or electromagnetic stirrer and shall be rapid, but not so vigorous as to draw air bubbles down to the electrodes.

- Burettes: 10 and 50-ml capacity.

- Iodine Number Flasks: Glass-stoppered, 500 ml capacity.

Reagents
- Bromide+Bromate Solution (0.05 N): Dissolve 5.1 g of potassium bromide and 1.4 g of potassium bromate in water and dilute to 1,000 ml. Standardize to four significant figures as follows: Place 50 ml of glacial acetic acid and 1 ml of hydrochloric acid TS in a 500 ml iodine number flask. Chill the solution in an ice bath for approximately 10 min and with constant swirling of the flask, add from a 50 ml burette 40 to 45 ml of bromide-bromate solution, estimated to the nearest 0.01 ml, at a rate such that the addition takes between 90 and 120 sec. Stopper the flask immediately, shake the contents, place it again in the ice bath, and add 5 ml of 15% potassium iodide solution in the lip of the flask.

After 5 min remove the flask from the ice bath and allow the 15% potassium iodide solution to flow into the flask by slowly removing the stopper. Shake vigorously, add 100 ml of water in such a manner as to rinse the stopper, lip, and walls of the flask, and titrate promptly with 0.05 N sodium thiosulfate. Near the end of the titration add starch TS and titrate slowly to the disappearance of the blue colour. Calculate the normality of the bromide-bromate solution as follows:

\[ N_1 = \frac{A_1 N_2}{A_1} \]

where:

- \( N_1 \) = normality of the bromide-bromate solution;
- \( A_1 \) = ml of the bromide-bromate solution;
- \( N_2 \) = normality of the \( \text{Na}_2\text{S}_2\text{O}_3 \) solution; and
- \( A_2 \) = ml of the \( \text{Na}_2\text{S}_2\text{O}_3 \) solution required for titration of the bromide-bromate

- Titration Solvent: prepare 1,000 ml of titration solvent by mixing the following volumes of materials: glacial acetic acid (714 ml), carbon tetrachloride (134 ml), methanol (134 ml), and sulfuric acid (18 ml of 1 + 5).

Procedure
Switch on the titrimeter and allow the electrical circuit to stabilise according to the manufacturer's instructions. Cool the titration vessel to 0° - 5° by circulating a suitable coolant through the jacketed titration vessel. Add 110 ml of the titration solvent and 8 to 10 g of the sample.

Switch on the stirrer and adjust to a rapid stirring rate, but not so rapid as to draw air bubbles into the solution. Allow the contents to cool to 0° - 5° and maintain this temperature throughout the titration. Add the bromide-bromate solution in small increments from a 10 ml burette until the end point detector (magic eye or potentiometric) indicates that the end point has nearly been reached. Continue adding 0.1 ml of the reagent at a time until the detector indicates a stable end point has been reached (end point lasting more than 30 sec). Repeat the determination but without the addition of the sample; less than 0.1 ml of the bromide-bromate solution should be required.
The bromine index is calculated as follows:

\[
\text{bromine index} = \frac{(T_1 - T_2)N \times 7.990}{W}
\]

where:

- \( T_1 \) = number of ml of the bromide-bromate solution required for the titration of the sample;
- \( T_2 \) = number of ml of the bromide-bromate solution required for the blank titration;
- \( N \) = normality of the bromide-bromate solution; and
- \( W \) = weight of the sample in g.
SYNONYM  
Linalol

DEFINITION

- Chemical names: 3,7-Dimethyl-1,6-octadien-3-ol, linalool
- C.A.S. number: 78-70-6
- Chemical formula: $C_{10}H_{16}O$
- Structural formula:

```
     H3C
    /   \  
   /     \ 
  H2C--CH2-CH2-CH3
```

- Molecular weight: 154.25
- Assay: Content not less than 91% of $C_{10}H_{16}O$

DESCRIPTION

Colourless liquid with a characteristic floral-woody odour

FUNCTIONAL USE

Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
   Practically insoluble in water. Insoluble in glycerol. Soluble in propane-1,2-diol, fixed oils and mineral oils.

** B. Refractive index
   $n_D^0: 1.461 - 1.465$

** C. Specific gravity
   $d_D^0: 0.858 - 0.867$

D. Infrared spectrum
   See Appendix at the end of these specifications.

---

* These specifications were prepared at the 26th session of JECFA (1982) and published in FNP 25 (1982) superseding the earlier specifications published in FNP 12 (1979).

Some commercial grades of linalool may be composed almost entirely of the specific chemicals whose structural formula is shown above, while other commercial grades, that conform to the limits of these specifications, and of satisfactory purity may contain other isomeric and closely related terpenic esters.

PURITY TESTS

* Solubility in ethanol
  1 ml dissolves in 2 ml of 70% of ethanol

* Ester
  Not more than 1% w/w.
  See description under TEST.

TEST

PURITY TEST

Ester

Weigh accurately about 10 ml of the sample and proceed as directed under the Method for Ester Determination in the General Methods*, using 98.15 as the equivalence factor (e) in the calculation.

METHOD OF ASSAY

Transfer 10 ml of the sample, previously dried with sodium sulfate, into a 125-ml glass-stoppered Erlenmeyer flask previously cooled in an ice bath. Add to the cooled oil 20 ml of dimethyl aniline (monomethyl-free) and mix thoroughly. To the mixture add 8 ml of acetyl chloride and 5 ml of acetic anhydride, cool for several min, permit it to stand at room temperature for another 30 min, then immerse the flask in an water bath maintained at $40^\circ \pm 1^\circ$ for 16 h. Wash the acetylated oil with three 75 ml portions of ice water, followed by successive washes with 25 ml portions of 5% sulfuric acid, until the separated acid layer no longer becomes cloudy or emits an odour of dimethyl aniline when made alkaline. After removal of the dimethyl aniline, wash the acetylated oil first with 10 ml of 10% sodium carbonate solution and then with successive portions of water until the washings are neutral to litmus. Finally dry the acetylated oil with anhydrous sodium sulfate. Weigh accurately about 1.2 g of the acetylated oil and proceed as directed under the Method for Ester Determination in the General Methods*.

Calculate the percent of linalool ($C_{10}H_{11}O$) by the formula:

$$L = \frac{7.707 (b - S)}{W - 0.021 (b - S)}$$

in which $L =$ percent of linalool, $b =$ the number of ml of 0.5 N hydrochloric acid consumed in the residual blank titration, $S =$ the number of ml of 0.5 N hydrochloric acid consumed in the titration of the sample, and $W =$ the weight of the sample in grams.

METHOD OF ASSAY (continued)  

Note: When this method is applied to essential oils containing appreciable amounts of esters perform and Ester Determination on a sample of the original oil and calculate the percent of total linalool by the formula:

\[
L = \frac{7.707 (b - S) (1 - 0.0021E)}{W - 0.021 (b - S)}
\]

in which \(L\) = percent of linalool, \(E\) = the percent of esters, calculated as linalyl acetate (\(C_{12}H_{20}O_2\)) in the sample of the original oil, and \(b, S,\) and \(W\) are as defined in the preceding paragraph.

The entire procedure is applicable only to linalool and linalool-containing oils. It is not intended for the determination of other tertiary alcohols.

APPENDIX

* Infrared spectrum: Laevorotatory Linalool F. B.

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* Infrared spectrum through the courtesy of the International Organization of the Flavour Industry (IOFI), Geneva, Switzerland, and of the SADTLER RESEARCH LABORATORIES, Inc. Philadelphia, USA.
LINALYL ACETATE*

SYNONYMS
Linalool acetate, bergamol

DEFINITION
Chemical names 3,7-Dimethyl-1,6-octadien-3-yl acetate, linalyl acetate
C.A.S. number 115-95-7
Chemical formula C_{12}H_{20}O_{2}
Structural formula

![Structural formula of Linalyl Acetate]

Molecular weight 196.29
Assay Content not less than 90% of esters expressed as linalyl acetate (C_{12}H_{20}O_{2})

DESCRIPTION Colourless liquid with a characteristic ‘bergamot-lavender odour’

FUNCTIONAL USE Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility Insoluble in water and glycerol. Soluble in fixed oils and mineral oils. Miscible with ethanol.

** B. Refractive index \( n^2_0 \): 1.448 - 1.460

** C. Specific gravity \( d_{20}^2 \): 0.899 - 0.917; \( d_{15}^2 \): 0.895 - 0.914

D. Infrared spectrum See Appendix at the end of these specifications.

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* These specifications were prepared at the 26th session of JECFA (1982) and published in FNP 25 (1982) superseding the earlier specifications published in FNP 12 (1979).

Some commercial grades of linalyl acetate may be composed almost entirely of the specific chemical whose structural formula is shown above, while other commercial grades, that conform to the limits of these specifications, and of satisfactory purity may contain other isomeric and closely related terpenic esters.

PURITY TESTS

* Solubility in ethanol
  1 ml dissolves in 5 ml of 70% ethanol

* Acid value
  Not more than 2

METHOD OF ASSAY

Weigh accurately about 1 g of the sample, and proceed as directed under the method for Ester Determination in the General Methods* using 98.15 as the equivalence factor (e) in the calculation. The volume of 0.5 N ethanolic potassium hydroxide consumed by the sample should be corrected for the Acid value.

APPENDIX

** Infrared spectrum:

Linalyl Acetate

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** Infrared spectrum trough the courtesy of the International Organization of the Flavour Industry (IOFI), Geneva, Switzerland, and of the SADTLER RESEARCH LABORATORIES, Inc. Philadelphia, USA.
LIPASE FROM ANIMAL*

SYNONYMS
Lipase, triglycerin lipase, tributyrase;
INS No. 1104

SOURCES
Commercial enzyme preparations of animal lipase are obtained from two primary
sources, 1) edible forestomach of calves, kids and lambs and 2) animal pancreatic
tissue. These preparations may be partially purified edible tissue preparations or they
may be aqueous extracts.

ACTIVE PRINCIPLE
Triacylglycerol lipase

SYSTEMATIC NAME AND NUMBER
Triacylglycerol acylhydrolase - EC 3.1.1.3

REACTION CATALYZED
The enzyme preparations hydrolyze triglycerides or simple fatty acid esters yielding di­
or monoglycerides plus free fatty acids.

DESCRIPTION
The enzyme preparations are dispersible in water and insoluble in ethanol.

FUNCTIONAL USES
Cheese making and modifications of lipids.

GENERAL SPECIFICATIONS
Must conform to the "General Specifications for Enzyme Preparations used in
Food Processing"**

CHARACTERISTICS

IDENTIFICATION TESTS

1. Pregastric
esterase activity

The sample shows Pregastric esterase activity
See description under TESTS

2. Esterase activity

The sample shows Esterase activity
See description under TESTS

TESTS

ASSAY AND CHARACTERIZATION OF PREGASTRIC ESTERASE***

Principle of Method
The action of an enzyme inoculum on the assay substrate is measured by a recording
pH stat.

Definition of Unit
One lipase unit is defined as 1/250th of chart width or 0.01 ml of base.

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* These specifications were prepared at the 15th session of JECFA (1971) and published in NMRS 50B (1972).

** See Annex 1 at the end of this compendium.

1065, (1967).
**Procedure**

**Instrument**
A sargent recording pH stat* is equipped with a 2.5 ml burette and 7 ml reaction vessels. It is set at v/5 vol with stirrer at 8 throughout the work. Temperature and pH are adjusted as indicated. The burette is charged with 0.025 N NaOH. One ml of a 1/100th dilution of enzyme is used as the inoculum.

**Preparation of Enzyme Inoculum**
The enzyme dilution is prepared by adding 0.200-1,000 g powder into a dry 100 ml volumetric flask. Approximately 75-80 ml 0.5 M NaCl solution is added and the flask is placed upon a rotary shaker (120 rpm) for 19.0 min. The flask is removed, made up to 100 ml with 0.5 M NaCl, mixed well, the sample removed and injected so that inoculation occurs after a rehydration time of exactly 20 min.

**Preparation of Substrate**
Ninety-five ml distilled water are placed in a half-pint glass freezer jar suitable for attachment to an osterizer; 2,600 g casein**, 0.5 ml 10% lecithin solution and 5.0 ml n-tributyrin are added. An osterizer head is tightened atop the jar. The substrate mixture is homogenized at low speed for 1 min. Spare substrate is covered and tempered to 42° in a water bath until used.

**Assay Procedure**
The small pH stat reaction vessel is charged with 5 ml substrate and a magnetic stirring bar.
To begin an assay, a reaction vessel is transferred to the pH stat, the temperature control and stirring control are turned on. The pH stat function control is turned to Run. The unit adjusts to the control pH of 6.20 and then 1 ml of enzyme dilution is inoculated.
The slope developed over the first 5 min following instrument pH adjustment is used to calculate activity. Standard curves are prepared and compared with colorimetric assay for standardization of esterase activity.

**Procedure**
(continued)

**Relative Precision**
A comparison of the precision of the colorimetric vs. the pH stat method favoured the latter. Between substrates, as prepared from one day to the next and using the same source of enzyme powder, the pH stat method had a coefficient of variability of 8.4% compared with 17.2% for the colorimetric assay.

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** Sheftone C 2 Soluble Casein Flour, Sheffield Chemical Co., Norwich, N.Y.
PHOTOMETRIC PROCEDURE FOR DETERMINING ESTERASE ACTIVITY*

**Principle of Method**
Fatty acid esters of 2-naphthol are employed as substrates. The 2-naphthol liberated by hydrolysis is coupled with a diazonium salt to form an azo dye, the concentration of which is estimated photometrically.

**Reagents**
Stock solution of 2-naphthyl ester, $2.96 \times 10^{-2}$ M.
To a 10 ml volumetric flask add successively 3.75 g of melted Brij 35*, 0.296 M of a 2-naphthylester and approximately 5 ml of redistilled 1,4-dioxane, reagent grade. Mix the contents of the flask, warming slightly if necessary to obtain solution, and dilute to volume with additional dioxane. The ester should contain no free 2-naphthol or, at the most, only traces. Stock solutions of the caprylate and the palmitate esters are stable for at least several months if refrigerated.

- Phosphate or citrate buffer, 0.067 M
  The choice of buffer will depend upon the pH optimum of the esterase.
- Phosphate buffer, 1.0 M, pH 6.8
- 4-Sulfamoylbenzenediazonium chloride solution
  Prepare this reagent by mixing equal volumes of a solution of sulfanilamide, 0.035M in 0.48 N hydrochloric acid, and a solution of sodium nitrite, 0.042 M. Let this mixture stand for several minutes at room temperature, and then place it in an ice bath. When kept ice-cold, this reagent is stable for at least 6 to 8 h.
- Hydrochloric acid, 1.6 N
- Sodium hydroxide, 3.3 N

**Note:** 2-naphthyl caprylate and 2-naphthyl palmitate may be synthesized according to the general procedure of Nachlas and Seligman (1) or of Gomori (2); they possess melting points at 43.0-44.0° and 69.0-70.5°, respectively.

**Assay Procedure**
Prepare a buffered solution of substrate in the following manner immediately prior to its use. All reagents should be at room temperature. Into a gently agitated mixture of 40 ml of 0.067 M phosphate or citrate buffer and approximately 50 ml of water, slowly add 1 ml of the stock solution of ester with a pipette, the tip of which is held beneath the surface of the mixture. Dilute to 100 ml with additional water.

Transfer 5 ml of the buffered solution of substrate to a 25 x 200 mm Pyrex test tube calibrated to contain 25 ml. Place the tube in a constant temperature water bath. After equalization of temperature, add 1 ml of an esterase preparation, and mix the contents of the tube thoroughly. Following incubation, the length of which is determined primarily by the rate at which the substrate is hydrolyzed, add 5 ml of 1.0 M phosphate

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**Brij 35, a polyoxyethylene lauryl alcohol, is a colourless, nonionic surfactant having a melting range of approximately 40-44° and is manufactured by Atlas Powder Company, Wilmington 99, Del.**
Assay procedure (contd) buffer, pH 6.8. Immediately add 0.5 ml of 4-sulfamoylbenzenediazonium chloride solution. Wait exactly 1 min for the development of colour, and add 5 ml of 1.6 N hydrochloric acid. Place the tube in water which is kept at a rolling boil for 20 min. Cool the contents of the tube to room temperature. Add 5 ml of 3.3 N sodium hydroxide, letting it flow down the wall of the tube. Without undue agitation, dilute the contents of the tube to 25 ml with water and mix thoroughly. Measure the optical density of this solution at 460 nm using as a reference blank the solution from a concomitant control in which the esterase was inactivated prior to incubation by heating.

Many esterase preparations may be assayed simultaneously by staggering the addition of enzyme to the buffered solution of substrate at 2 min intervals. In order that all samples will be incubated for the same length of time, the sequential addition of 1.0 M phosphate buffer, 4-sulfamoylbenzenediazonium chloride solution, and 1.6 N hydrochloric acid, all of which requires approximately 1.5 min for a single tube, is staggered also at 2 min intervals. At this stage, i.e., after addition of the hydrochloric acid, subsequent steps do not need be performed immediately because the colour is stable if the mixture is not exposed to sunlight.

Standard Curve

From stock solutions of 2-naphthol in 1,4-dioxane and Brij 35, prepare buffered solutions of this compound in the same way that the buffered solution of substrate is prepared.

Take 5 ml samples of these buffered solutions, and, except for incubating them with esterase, subject them to the same procedure outlined in the assay technique. When measuring the optical densities of the final solutions, use as a reference blank the solution of a concomitant control without the 2-naphthol. The stock solutions of 2-naphthol are somewhat less stable under refrigeration than are those of the 2-naphthyl esters and, therefore, should be prepared immediately prior to their use. The standard curve is reproducible, thus obviating the preparation of standards for every series of assays.

References

LIPASE FROM *ASPERGILLUS ORYZAE, VAR* *(Tentative)*

**SYNONYMS**
Lipase, triglycerine lipase, tributyrase

**INS No. 1104**

**SOURCES**
Commercial enzyme preparations of Lipase (*Aspergillus*) are produced by the controlled fermentation of *Aspergillus oryzae, var.*

**ACTIVE PRINCIPLE**
Triacylglycerol lipase

**SYSTEMATIC NAME AND NUMBER**
Triacylglycerol acylhydrolase - EC 3.1.1.3

**REACTION CATALYZED**
The enzyme preparations hydrolyze triglycerides or simple fatty acid esters yielding di- or monoglycerides plus free fatty acids preferentially in an oil-water interface in an insoluble or heterogeneous system.

**DESCRIPTION**
The enzyme preparations occur as off-white to tan amorphous powders or liquids. They are soluble in water, the solutions usually being light yellow in colour. They are practically insoluble in alcohol, chloroform and ether.

**FUNCTIONAL USE**
Manufacture of cheese

**GENERAL SPECIFICATIONS**
Must conform to the "General Specifications for Enzyme Preparations used in Food Processing"**

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

Lipase activity

The sample shows lipase activity

See description under TESTS

**TESTS**

**LIPASE ACTIVITY ASSAY***

**Principle of Method**
Lipase is incubated with an olive oil emulsion at pH 6.5 and 30°. The acid that is liberated in the reaction mixture during 5 min is titrated to pH 8.0. The results reflect a practically linear relationship between reaction rate and enzyme concentration within recommended range.

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* These specifications were prepared at the 15th session of JECFA (1971) and published in NMRS 50B (1972).

** See Annex 1 at the end of this compendium.

Reagent
- Olive oil, N.F.
- Amerchol L-101, Am. Cholesterol Products, Edison, N.J.
- Sodium barbital
- Gum Arabic, U.S.P.
- Sodium acetate $\text{H}_2\text{O}$
- Sodium chloride
- 0.5 N NaOH
- 0.02 N NaOH
- 0.1 N HCl
- Ethyl Alcohol, denatured No. 23A is suitable.

Apparatus
- Waring Blender
- Two magnetic stirrers: One of which is suitable for submersion in water bath (Model MS-7, Tri-R Instruments, Jamaica, New York)
- pH meter for titrations: Equipped with a small combination electrode (such as No. 4858-L15, A.H. Thomas)
- Microburette: 10 ml with 0.02 ml divisions (Kimble, No. 17107F with Luer type syringe needle 20C, 1-1/2")

Solutions
Substrate Emulsion
Stir 30 g gum arabic and 270 ml dist. water magnetically for 30 min at room temperature. Break possible lumps with a glass rod. Cool to 5°. Weigh 6 g Amerchol into a 400 ml beaker, add 72 g olive oil and 222 g of the chilled gum arabic solution. Stir with a glass rod and pour into Waring Blender. Blend 5 min. Adjust to pH 6.3 with 0.5 N NaOH, cool to 5° and blend again for 7 min. The temperature may have increased to a maximum of 47° and the pH to 6.5. If not, adjust to pH 6.5. The emulsion is stable for at least 8 days if stored in the refrigerator. Be sure, however that the temperature does not drop below 1°.

Buffer
- Stock solution: Dissolve 9.7 g sodium acetate (3H$_2$O) and 14.7 g sodium barbital with dist. water to 500 ml. This solution is 0.14 N barbital and 0.14 N acetate, pH 9.9. Store in refrigerator.

- Working solution: Dilute 40 ml stock solution, 16 ml 8.5% NaCl solution and 53 ml 0.1N HCl with dist. water to 200 ml, pH 6.5. For minor adjustments use 0.1N HCl or 0.1N NaOH. Store in refrigerator. Discard if crystals appear during storage.

Enzyme Solutions
For the assay of solid preparations, prepare first a stock solution by stirring the sample magnetically in a suitable amount of water for about 30 min before preparing the final dilution by further dilution with water. The stock solution is stable for several hours at 5° but the highly diluted final dilution should be used within 5 min. For the assay of unknowns it is necessary to find the suitable dilution by trial. Preparations whose activity is roughly known should be diluted to 1 to 3.6 lipase units/ml. The terms "lipase units" will be defined below.
Emulsion and buffer are premixed to weight ratio of 3:1 respectively to provide the substrate. 8.0 ml portions of the substrate are introduced into 100 ml beakers containing magnetic stirring bars. Stir the substrate solution magnetically. Run each sample, replicate (blank and sample) containing substrates as follows:

**Blank**
- Add 40 ml 23A alcohol
- Add 2.0 ml sample of enzyme solution

**Sample**
- Equilibrate substrate in a 30° water bath
- Add 2.0 ml sample of enzyme solution
- Incubate at 30° for 5.0 min
- Add 40 ml 23A alcohol

Titrate both blank and sample with 0.02N NaOH to pH 8.0.

**Calculations**

1 Lipase Unit (LU) = quantity of lipase required to yield 1 μmole H/min

Lipase Activity (LA) = number of LU/g of preparation

\[
LA = \frac{ml\ NaOH \times N\ NaOH \times 10^5/\text{mg enzyme preparation added} \times 10^3}{x\ min}
\]

When 0.02 N NaOH and a 5 min reaction time are used.

\[
LA = \frac{ml\ NaOH \times 0.004/\text{mg enzyme}}{}
\]
LITHOL RUBINE BK*

SYNONYMS
CI Pigment Red 57: FD & C Red No. 7, Brilliant Carmine 6B
Rubinpigment, Carmine 6B, Litholrubintoner BKL,
Permanent Rubin L6B
INS No. 180. EEC No. E180

DEFINITION
Lithol Rubine BK consists essentially of calcium 3-hydroxy-4-(4-methyl-2-
sulfonatophenylazo)-2-naphthalene-carboxylate and subsidiary colouring matters
together with water, calcium chloride and/or calcium sulfate as the principal
uncoloured components.

Class
Monoazo

Code numbers
CI (1975) No. 15850: 1
CAS No. 5284-04-9

Chemical name
Calcium 3-hydroxy-4-(4-methyl-2-sulfonatophenylazo)-2-
naphthalene-carboxylate

Chemical formula
C_{19}H_{18}CaN_{3}O_{8}S

Structural formula

\[
\begin{array}{c}
\text{HO} \\
\text{COOCa/2} \\
\text{SO_{3}Ca/2} \\
\text{H_{3}C} \\
\end{array}
\]

Molecular weight
424.45

Assay
Content not less than 90% total colouring matters

DESCRIPTION
Red powder

FUNCTIONAL USE
Food Colour

* These specifications were prepared at the 30th session of JECFA (1986) and published in FNP 37 (1986).
CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility Slightly soluble in hot water (90°). Insoluble in cold water. Insoluble in ethanol

** B. Absorbance Maximum at about 442 nm (in dimethylformamide)

PURITY TESTS

** Loss on drying at 135° Not more than 10%

** Chloride and sulfate calculated as calcium salts See description under TESTS for determination of chloride and sulfate

*** Arsenic Not more than 3 mg/kg

*** Lead Not more than 10 mg/kg

* Heavy metals Not more than 40 mg/kg Proceed as directed in the Limit Test for Heavy Metals.

Subsidiary colouring matters Not more than 0.5% See description under TESTS

Organic compounds other than colouring matters

2-Amino-5-methylbenzenesulfonic acid, calcium salt Not more than 0.2% See description under TESTS

3-hydroxy-2-naphthalenecarboxylic acid, calcium salt Not more than 0.4% See description under TESTS

Unsulfonated primary aromatic amines Not more than 0.01% See description under TESTS

Ether extractable matter Not more than 0.2% See description under TESTS


PURITY TESTS

Chloride

Mix 1 g of the sample with 100 ml of water and let stand for 30 min, shaking occasionally. Filter and wash the residue with a small amount of water. Combine the washings with the filtrate. Acidify with 5 ml of 1.5 N nitric acid and titrate with 0.1 N silver nitrate solution as directed under the method for the Determination of Chloride as Sodium Chloride. Each ml of 0.1 N silver nitrate solution is equivalent to 0.00555 g of calcium chloride.

Sulfate

Weigh accurately about 1 g of the sample, mix 100 ml of water and heat on a water bath for 10 min. Cool, filter and wash the residue with a small amount of water. Combine the washings with the filtrate. Dilute to 150 ml with water and acidify with hydrochloric acid, adding 1 ml in excess. Heat the solution to boiling and add an excess of 0.25 N barium chloride drop by drop, with stirring. Allow the mixture to stand on a hot plate for 4 h, or leave it overnight at room temperature and then bring it to about 80° and allow the precipitate to settle. Filter off the precipitated barium sulfate, wash with hot water and ignite at a dull red heat in a tared crucible until a constant weight is obtained. Carry out a blank determination and apply any necessary correction.

% of sulfate as calcium sulfate = \( \frac{\text{Weight of } \text{BaSO}_4 (g) \times 0.583}{\text{Weight of sample}} \times 100 \)

Subsidiary colouring matters

Apparatus

- Spectrophotometer, suitable for use in the visible range
- Separatory funnels

Reagents

- Glacial acetic acid
- Diethyl ether
- Hydrochloric acid 8 N
- 2% Sodium hydroxide
- Standard solution of 2-hydroxy-1-(4-tolylazo)-3-naphthalene carboxylic acid

Procedure

Boil 0.10 g of sample gently with 100 ml of glacial acetic acid and with 75 ml of hydrochloric acid 8 N until the colour has dissolved. Cool and transfer the solution to a 1000 ml separatory funnel, washing any residual solution into the funnel with small portions of acetic acid. Extract the acidic solution with 150 ml of ether and separate the miscible solution formed by adding about 150 ml of water. Transfer the lower layer to a second funnel and extract with another 100 ml of ether.
Subsidiary colouring matters (continued)

Combine the ether extracts and wash with 100 ml portions of water until the washings are colourless, and twice more after the last colourless washings. Remove the subsidiary colour from the ether layer by extraction with 20 ml portions of 2% sodium hydroxide. Warm to expel the ether. Determine the colour concentration using the spectrophotometer.

Standard

3-Hydroxy-4-(4-methylphenylazo)-2-naphthalene-carboxylic acid has an absorptivity of 0.032 L mg⁻¹ cm⁻¹ at 505 nm.

* Organic compounds other than colouring matters

Use ammonium sulfate 10% in place of the ammonium sulfate 25% listed in the General Methods.

Add 100 ml eluant to 5 g cellulose, stir, allow to settle and decant. Place 0.100 g of the colour sample in a beaker and add 5 ml ethanol. Stir to ensure complete wetting of the sample. Transfer the 5 g cellulose to the beaker containing the sample. Add 10 g ammonium sulfate and stir thoroughly. Transfer the mixture to the column. Rinse the beaker with 25 ml eluant, adding the rinse to the column. Then follow the procedure detailed in the general column chromatography method.

* Unsulfonated primary aromatic amines

Mix 2 of the sample well with 150 ml of toluene and boil gently for 5 min. Filter after cooling and wash the residue with a small amount of toluene. Combine the washing with the filtrate and extract with three 10-ml portions of N hydrochloric acid and dilute the combined extracts to 100 ml with water. Follow the procedure detailed in the general method.

* Ether-extractable matter

Dry 2 g of the sample in a desiccator (sulfuric acid) for 24 h and extract using Method II.

METHOD OF ASSAY

Titration with Titanous Chloride

Place about 0.2 g of the sample, accurately weighed, in a 500-ml Erlenmeyer flask and add 5 ml of sulfuric acid. Mix well and add 100 ml of ethanol. Shake well, heat on a water bath. Add a solution made by dissolving 20 g of sodium hydrogen tartrate in 100 ml of boiling water and mix with 20 ml of 30% sodium hydroxide solution, shaking vigorously. Titrate with 0.1 N titanous chloride.

Each ml of 0.1 N titanous chloride is equivalent to 10.61 mg of C₁₄H₁₂CaN₂O₆S.

MAGNESIUM CARBONATE*

SYNONYMS

INS No.504(i), EEC No.504

DEFINITION

Magnesium Carbonate is a basic hydrated or a normal hydrated magnesium carbonate or a mixture of the two.

Chemical name

Magnesium carbonate

C.A.S. number

546-93-0

Assay

Magnesium Carbonate contains the equivalent of not less than 24.0% and not more than 26.4% of Mg.

DESCRIPTION

Magnesium Carbonate occurs as odourless, light, white friable masses or as a bulky white powder.

FUNCTIONAL USES

Anticaking and antibleaching agent.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility

Practically insoluble in water; insoluble in ethanol.

** B. Positive test for carbonate

The sample dissolves with effervescence when treated with dilute acetic acid TS.

** C. Positive test for magnesium

To a solution of the sample in dilute acetic acid TS add ammonium chloride TS and ammonium carbonate TS. No precipitate is formed. Add sodium phosphate TS. A white crystalline precipitate is formed which is insoluble in ammonia TS.

PURITY TESTS

Acid insoluble substances

Not more than 0.05%.

See description under TESTS.

Water soluble substances

Not more than 1%.

See description under TESTS.

Calcium

Not more than 0.4%.

See description under TESTS.

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNS 4 (1978).

**Heavy metals**

Not more than 30 mg/kg.

See description under TESTS.

**TESTS**

**PURITY TESTS**

**Arsenic**

Not more than 3 mg/kg.

A solution of 1 g of the sample, weighed to the nearest mg, in 10 ml of dilute hydrochloric acid TS and 25 ml of water meets the requirements of the Limit Test for Arsenic (Method II).

**Lead**

Not more than 10 mg/kg.

See description under TESTS.

**Heavy metals**

Not more than 30 mg/kg.

See description under TESTS.

**TESTS**

**Acid insoluble substances**

Weigh 5 g of the sample to the nearest mg, and mix with 75 ml of water. Add hydrochloric acid in small portion, with agitation until no more of the sample dissolves, and boil for 5 min. If an insoluble residue remains, filter, wash well with water until the last washing is free from chloride, ignite, cool and weigh. Calculate as percentage.

**Water soluble substances**

Weigh 2 g of the sample to the nearest mg. Add 100 ml of freshly boiled and cooled water, boil while stirring, cool and filter. Evaporate a 50 ml portion of the filtrate to dryness on a water bath, and dry the residue at 120°C for 3 h. Cool, weigh and calculate as percentage. (The weight of the residue should not exceed 10 mg).

**Calcium**

Weigh 1 g of the sample to the nearest 0.1 mg and dissolve in a mixture of 3 ml of sulfuric acid and 22 ml of water. Add 50 ml of ethanol, and allow the mixture to stand overnight. If crystals of magnesium sulfate separate, warm the mixture to about 50°C to dissolve. Filter through a tared previously ignited porcelain filter crucible and wash the precipitate several times with a mixture of 2 volumes of ethanol and 1 volume of dilute sulfuric acid TS. Ignite the crucible and contents at a dull red heat, cool and weigh. The weight of calcium sulfate obtained, multiplied by 0.294, gives the equivalent of calcium in the sample taken for the test.

**Lead**

Dissolve 1 g of the sample, weighed to the nearest mg, in 10 ml of dilute hydrochloric acid TS. Neutralize with ammonia TS, using phenolphthalein as indicator and dilute to 20 ml with water. This solution meets the requirements of the Limit Test for Lead, using 10 μg of lead ion (Pb) in the control.

**Method of Assay**

Dissolve 0.5 g of the sample, weighed to the nearest mg, in 10 ml of dilute hydrochloric acid TS, and evaporate the solution to dryness on a steam bath. Towards the end of the evaporation, stir frequently to desintegrate the residue so that finally a dry powder is obtained. Dissolve the powder in 20 ml of water, and evaporate to dryness in the same manner as before. Redissolve the residue in 25 ml of water, and filter if necessary. This solution meets the requirements of the Limit Test for Heavy Metals (Method I). Using 15 µg of lead ion (Pb) in the control (Solution A).

* Heavy metals

Weigh 1 g of the sample to the nearest 0.1 mg, and transfer to a 250 ml conical flask. Pipette into the flask 50 ml of N sulfuric acid and swirl to dissolve. Titrate the excess acid with N sodium hydroxide solution, using methyl orange TS as indicator. Subtract from the volume of N sulfuric acid consumed the number of ml of N sulfuric acid corresponding to the weight of Ca in the sample taken, using as a factor 20.04 mg of Ca for each ml of N sulfuric acid. The difference is the volume of N sulfuric acid used to neutralize the magnesium carbonate and each ml is equivalent to 12.16 of Mg.

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MAGNESIUM CHLORIDE*

SYNONYMS
INS No. 511, EEC No. 511

DEFINITION
Chemical name: Magnesium chloride hexahydrate
C.A.S. number: 7786-30-3
Chemical formula: $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$
Formula weight: 203.30
Assay: Content not less than 99.0% and not more than the equivalent of 105.0% of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$

DESCRIPTION
Colourless, odourless flakes, granules, lumps or crystals. It is very deliquescent.

FUNCTIONAL USE
Firming agent, colour retention agent

CHARACTERISTICS

IDENTIFICATION TESTS
** A. Solubility
Very soluble in water. Freely soluble in ethanol.

** B. Positive test for magnesium
Passes test

** C. Positive test for chloride
Passes test

PURITY TESTS

Ammonium
Not more than 50 mg/kg
See description under TESTS

** Arsenic
Not more than 3 mg/kg
Test a solution of 1 g of the sample in 35 ml of water as directed in the Limit Test (Method II).

** Lead
Not more than 10 mg/kg
Test a solution of 1 g of the sample in 10 ml of water as directed in the Limit Test.

** Heavy metals
Not more than 30 mg/kg
Test a solution of 0.67 g of the sample in 25 ml of water as directed in the Limit Test.

* These specifications were prepared at the 27th session of JECFA (1983) and published in FNP 28 (1983).

TESTS

PURITY TESTS

**Ammonium**

Dissolve 1 g of the sample in 90 ml of water, and slowly add 10 ml of a freshly boiled and cooled solution of sodium hydroxide (1 in 10 soln). Allow to settle, then decant 20 ml of the supernatant liquid into a colour comparison tube, dilute to 50 ml with water, and add 2 ml of Nessler's TS. Any colour does not exceed that produced by 10 μg of ammonium (NH₄) ion in 48 ml of water and 2 ml of the sodium hydroxide solution.

METHOD OF ASSAY

**Chelatometry**

Dissolve about 450 mg of the sample, accurately weighed, in 25 ml of water, add 5 ml of ammonia/ammonium chloride buffer TS and 0.1 ml of eriochrome black TS and titrate with 0.05 M disodium ethylenediaminetetraacetate until the solution is blue in colour. Each ml of 0.05 M disodium ethylenediaminetetra-acetate is equivalent to 10.16 mg of MgCl₂·6H₂O.
MAGNESIUM DI-L-GLUTAMATE*

SYNONYMS         Magnesium glutamate
                   INS No.625, EEC No.625

DEFINITION

   Chemical name    Monomagnesium di-L-glutamate tetrahydrate
   C.A.S. number    18543-68-5
   Chemical formula C_{10}H_{16}MgN_{2}O_{6}·4H_{2}O
   Structural formula \left[ \begin{array}{c} \text{HOOC-CH-CH}_{2}\text{-CH}_{2}\text{-COO} \\ \text{NH}_{2} \end{array} \right] \text{Mg} \cdot 4\text{H}_{2}\text{O} \quad 2
   Molecular weight  388.62
   Assay            Content not less than 95.0% and not more than 105.0 % of C_{10}H_{16}MgN_{2}O_{6} on the anhydrous basis

DESCRIPTION

   Odourless, white or off-white crystals or powder, having a characteristic taste

FUNCTIONAL USE

   Flavour enhancer, salt substitute

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
   Very soluble in water. Insoluble in ethanol

** B. Positive test for glutamic acid
   Passes test
   Proceed as directed in the Positive test for glutamic acid under Monoammonium L-Glutamate.

** C. Positive test for magnesium
   Passes test

PURITY TESTS

** Specific rotation
   [\alpha]_{D}^{20} : +23.8 to +24.4°
   Test a solution of 10 g of sample (dried basis) in 100 ml of 2N hydrochloric acid, using a 200-mm tube.

** pH
   6.4 - 7.5 (1 in 10 soln)

* These specifications were prepared at the 31st session of JECFA (1987) and published in FNP 38 (1988).

PURITY TESTS (continued)

* Water content
  Not more than 24% (Karl Fisher Method)

* Chlorides
  Not more than 0.2%.
  Test 0.07 g of the sample as directed in the Chlorides Limit Test using 0.4 ml of 0.01 N hydrochloric acid in the control.

* Sulfates
  Not more than 0.2%.
  Test 0.12 g of the sample as directed in the Sulfates Limit Test using 0.5 ml of 0.01 N sulfuric acid in the control.

* Arsenic
  Not more than 3 mg/kg.
  Test 1 g of the sample as directed in the Arsenic Limit Test (Method II).

* Lead
  Not more than 10 mg/kg.
  Test 1 g of the sample as directed in the Lead Limit Test using 10 µg of lead ion (Pb) in the control.

* Heavy metals
  Not more than 20 mg/kg.
  Test a solution of 1 g of the sample in 25 ml of water as directed in the Heavy Metals Limit Test (Method I).

Pyrrolidone carboxylic acid
  Passes test
  Proceed as directed in the Purity Test for Pyrrolidone carboxylic acid under Monosodium L-Glutamate.

METHOD OF ASSAY

Dissolve about 250 mg of the sample, previously dried and weighed accurately, in 6 ml of formic acid, and add 100 ml of glacial acetic acid. Titrate with 0.1 N perchloric acid* determining the end-point potentiometrically. Run a blank determination in the same manner and correct for the blank.

Each ml of 0.1 N perchloric acid is equivalent to 7.914 mg of $\text{C}_{10}\text{H}_{16}\text{MgN}_2\text{O}_8$.
Calculate the content on the anhydrous basis.

MAGNESIUM GLUCONATE*

SYNONYMS
Magnesium gluconate dihydrate;
INS No. 580

DEFINITION
Chemical name
Magnesium di-D-glucurate dihydrate

C.A.S. number
17140-79-3

Chemical formula
C₁₂H₂₂MgO₁₂·2H₂O

Structural formula

\[
\begin{array}{c}
\text{COO} \\
\text{H–C–OH} \\
\text{OH–C–H} \\
\text{H–C–OH} \\
\text{H–C–OH} \\
\text{CH₂OH}
\end{array}
\]

Mg·H₂O

2

Molecular weight
450.66

Assay
Content not less than 98.0% and not more than the equivalent of 102.0% of C₁₂H₂₂MgO₁₂·2H₂O

DESCRIPTION
White to off white, odourless, fine powder

FUNCTIONAL USES
Buffering agent, firming agent, yast food

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Soluble in water. Sparingly soluble in ethanol.

** B. Positive test for magnesium
Passes test

C. Derivation to phenylhydrazide of gluconic acid
Passes test
Proceed as directed in the specifications for Sodium Gluconate

* These specifications were prepared at the 27th session of JECFA (1983) and published in FNP 28 (1983).

PURITY TESTS

* Arsenic
  Not more than 3 mg/kg (Method II)

* Lead
  Not more than 10 mg/kg

* Heavy metals
  Not more than 20 mg/kg
  Test a solution of 1 g of the sample in 25 ml of water as directed in the Limit Test (Method II)

* Reducing substances
  Not more than 0.5% calculated as D-glucose (Method I)

* Microbiological criteria
  Total aerobic microbial: Max 1000 in 1 g
  Total yeasts and moulds: Max 100 in 1 g
  E. coli: Absent in 1 g

METHOD OF ASSAY

Chelatometry
Dissolve about 0.6 g of the sample, accurately weighed in 50 ml of water, add 10 ml of ammonia/ammonium chloride buffer solution and 5 drops of eriochrome black TS. Titrate with 0.05 M disodium ethylenediaminetetraacetate to a deep blue colour.

\[
\text{ml of disodium EDTA} \times 0.05 \times 45.07 = \frac{\text{ml of disodium EDTA} \times 0.05 \times 45.07}{\text{sample wt (g)}}
\]

where 45.07 = equivalence factor for magnesium gluconate, dihydrate.

MAGNESIUM HYDROXIDE CARBONATE*

SYNONYMS
Magnesium subcarbonate (light or heavy), hydrated basic magnesium carbonate, magnesium carbonate hydroxide; INS No.504(ii), EEC No.504

DEFINITION
Chemical name
Magnesium carbonate hydroxide hydrated.
Assay
Content not less than 40.0% and not more than 45.0% of MgO.

DESCRIPTION
Odourless, light, white, friable masses, or a bulky-white powder.

FUNCTIONAL USES
Alkali, drying agent, colour-retention agent, carrier, anti-caking agent.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Practically insoluble in water.
Insoluble in ethanol.

B. Alkalinity
Slurry shows slight alkalinity.

** C. Positive test for magnesium
When treated with dilute hydrochloric acid TS, it dissolves with effervescence and the resulting solution gives positive test for magnesium.

PURITY TESTS

Soluble salts
Not more than 1.0%.
See description under TESTS.

Calcium
Not more than 1.0%.
See description under TESTS.

Acid insoluble matter
Not more than 0.05%.
See description under TESTS.

** Arsenic
Not more than 3 mg/kg.
Test a solution of 1 g of the sample in 10 ml of dilute hydrochloric acid TS as directed in the Limit Test (Method II).

Lead
Not more than 10 mg/kg.
See description under TESTS.

* These specifications were prepared at the 27th session of JECFA (1983) and published in FNP 28 (1983).

PURITY TESTS (continued)

**Heavy metals**
Not more than 30 mg/kg.
See description under TESTS.

TESTS

IDENTIFICATION TESTS

PURITY TESTS

**Soluble salts**
Mix 2 g of the sample with 100 ml of a mixture of equal volumes of n-propanol and water. Heat the mixture to the boiling point with constant stirring, cool to room temperature, add water to make 100 ml and filter. Evaporate 50 ml of the filtrate on a steam bath to dryness, and dry at 105° for 1 h. The weight of the residue does not exceed 10 mg.

**Calcium**
Dissolve about 1 g of the sample, accurately weighed, in a mixture of 3 ml of sulfuric acid and 22 ml of water. Add 50 ml of ethanol and allow the mixture to stand overnight. If crystals of magnesium sulfate separate, warm the mixture to about 50° to dissolve them. Filter through a Gooch crucible containing an asbestos mat that previously has been washed with dilute sulfuric acid TS, water, and ethanol, and ignited and weighed. Wash the crystals on the mat several times with a mixture of 2 volumes of ethanol and 1 volume of dilute sulfuric acid TS. Ignite the crucible and contents to a dull red heat, cool, and weigh. The weight of calcium sulfate so obtained, multiplied by 0.2944 gives the equivalent of calcium in the sample taken for the test.

**Acid insoluble matter**
Mix 5 g of the sample with 75 ml of water, add hydrochloric acid in small portions, with agitation, until no more of the sample dissolves, and boil for 5 min. If an insoluble residue remains, filter, wash well with water until the last washing is free from chloride, ignite, cool, and weigh.

**Lead**
Dissolve 1 g of the sample in 10 ml of dilute hydrochloric acid TS and neutralize with ammonia TS using phenolphthalein TS. Test this solution using 10 μg of lead ion (Pb) in the control as directed in the Limit Test.

**Heavy metals**
Dissolve 0.5 g of the sample in 10 ml of dilute hydrochloric acid TS and evaporate the solution to dryness on a steam bath. Toward the end of the evaporation stir frequently to disintegrate the residue so that finally a dry powder is obtained. Dissolve the residue in 20 ml of water, and evaporate to dryness in the same manner as before. Redissolve the residue in 25 ml of water, and filter if necessary. Test this solution using 15 μg of lead ion (Pb) in the control as directed in the Limit Test.

---

METHOD OF ASSAY

Dissolve about 1 g of the sample, accurately weighed, in 30 ml of 1 N sulfuric acid, add methyl orange TS, and titrate the excess acid with 1 N sodium hydroxide. From the volume of 1 N sulfuric acid consumed, deduct the volume of 1 N sulfuric acid corresponding to the content of calcium oxide in the weight of the sample taken for the assay. The difference is the volume of 1 N sulfuric acid equivalent to the magnesium oxide present. Each ml of 1 N sulfuric acid is equivalent to 20.15 mg of MgO.
MAGNESIUM HYDROGEN PHOSPHATE*

SYNONYMS
Magnesium phosphate, dibasic; secondary magnesium phosphate; dimagnesium phosphate; INS No.343(ii).

DEFINITION
Chemical names
Magnesium hydrogen orthophosphate trihydrate, magnesium salt of phosphoric acid (1:1)

C.A.S. number
7757-86-0

Chemical formula
MgHPO₄ · 3H₂O

Formula weight
174.33

Assay
Not less than 96.0% of Mg₂P₂O₇, calculated on the ignited basis.

DESCRIPTION
Odourless white crystalline powder.

FUNCTIONAL USES
Nutrient adjunct, dietary supplement, nutrient agent.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Slightly soluble in water, soluble in dilute acids, but insoluble in ethanol.

** B. Positive test for phosphate
Dissolve about 200 mg in 10 ml of diluted nitric acid TS, and add, dropwise, ammonium molybdate TS. A greenish yellow precipitate of ammonium phosphomolybdate forms that is soluble in ammonia TS.

C. Positive test for magnesium
Dissolve 100 mg in 0.5 ml of diluted acetic acid TS and 20 ml of water. Add 1 ml of ferric chloride TS, let stand for 5 min and filter. The filtrate gives a positive test for Magnesium.

PURITY TESTS

** Loss on ignition
Not less than 29% and not more than 36%.
Weigh accurately about 1 g, and ignite, preferably in a muffle furnace, at 800° ± 25° to constant weight.

** Fluoride
Not more than 10 mg/kg (Method III).
Ten ml of N hydrochloric acid are used to dissolve the sample.

* These specifications were prepared at the 26th session of JECFA (1982) and published in FNP 25 (1982).

PURITY TESTS (continued)

* Arsenic
Not more than 3 mg/kg.
Use 1 g of the sample dissolved in 5 ml of dilute hydrochloric acid TS and proceed as directed in the Limit Test (Method II).

Lead
Not more than 5 mg/kg.
See description under TESTS.

Heavy metals
Not more than 30 mg/kg.
See description under TESTS.

METHOD OF ASSAY

Dissolve 1 g in 20 ml of dilute hydrochloric acid TS, evaporate the solution to a volume of about 10 ml on a steam bath, dilute to about 20 ml with water, and cool. This solution meets the requirements of the Limit Test for Lead using 5 µg of lead ion (Pb) in the control.

Suspend 1.33 g in 20 ml of water, and add hydrochloric acid, dropwise, until the sample just dissolves. Adjust the pH to between 3 and 4, filter, and dilute the filtrate to 40 ml with water. For the control (Solution A), add 20 µg of lead ion (Pb) to 10 ml of the filtrate, and dilute to 40 ml. For the sample (Solution B), dilute the remaining 30 ml of the filtrate to 40 ml. Add 10 ml of hydrogen sulfide TS to each solution, and allow to stand for 5 m. Solution B is no darker than Solution A.

Weigh accurately about 500 mg of the residue obtained in the test for Loss on Ignition, and dissolve it by heating in a mixture of 50 ml of water and 2 ml of hydrochloric acid. Cool, dilute to 100.0 ml with water, and mix. Transfer 50.0 ml of this solution into a 400-ml beaker, add 100 ml of water, and heat to 55° to 60°. From a buret add 15 ml of 0.1 M disodium EDTA, add a magnetic stirring bar, and adjust with sodium hydroxide TS to pH 10 while stirring. Add 10 ml of ammonia-ammonium chloride buffer TS and 12 drops of eriochrome black TS, and continue the titration with 0.1 M disodium EDTA until the wine-red colour changes to pure blue. Calculate the weight, in mg, of Mg₃(PO₄)₂ in the residue taken by the formula:

\[ 2 \times 11.13 \times V, \]

in which V is the volume, in ml of 0.1 M disodium EDTA required in the titration of the 50.0-ml aliquot.

MAGNESIUM HYDROXIDE*

SYNONYMS

INS No. 528, EEC No. E528

DEFINITION

Chemical name: Magnesium hydroxide
C.A.S. number: 1309-42-8
Chemical formula: Mg(OH)₂
Formula weight: 58.32
Assay: Magnesium Hydroxide, after drying at 105° for 2 h, contains not less than 95.0% of Mg(OH)₂

DESCRIPTION

Odourless, white bulky powder with a slightly alkaline taste

FUNCTIONAL USE

Alkali, colour adjunct

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility  Practically insoluble in water and in ethanol
B. Positive test for alkali  Magnesium hydroxide is alkaline to moistened litmus paper
C. Positive test for magnesium  Passes test  See description under TESTS

PURITY TESTS

** Loss on dring  Not more than 2% (105°, 2 h)
** Loss on ignition  Not more than 30 - 33% (approx. 800° to constant weight)
Alkalies (free) and soluble salts  Passes test  See description under TESTS
Calcium oxide  Not more than 1.5%  See description under TESTS
Arsenic  Not more than 3 mg/kg  See description under TESTS

* These specifications were prepared at the 19th session of JECFA (1975) and published in NMRS 55B (1976).
PURITY TESTS (continued)

**Lead**
Not more than 10 mg/kg
See description under TESTS

**Heavy metals**
Not more than 40 mg/kg
See description under TESTS

TESTS

IDENTIFICATION TESTS

* C. Positive test for magnesium
To a solution of the sample in dilute acetic acid add 2 N ammonium chloride TS and a solution of 20 g of ammonium carbonate TS in 20 ml ammonia TS made to 100 ml with water. No precipitate is formed. Add a solution of 12 g of disodium hydrogen phosphate (Na$_2$HPO$_4$·7H$_2$O) in 100 ml. A white crystalline precipitate is formed which is insoluble in ammonia TS.

PURITY TESTS

**Alkalies (free) and soluble salts**
Boil 2 g of the sample with 100 ml of water for 5 min in a covered beaker and filter while hot. Add methyl red TS and titrate 50 ml of the cooled filtrate with 0.1 N sulfuric acid. Not more than 2 ml of the acid is required to reach the endpoint. Evaporate 25 ml of the filtrate to dryness and dry at 105° for 3 h. Not more than 10 mg of residue remains.

**Calcium oxide**
Dissolve about 500 mg of the sample, accurately weighed, in a mixture of 3 ml of concentrated sulfuric acid and 22 ml of water. Add 50 ml of ethanol and allow the mixture to stand overnight. If crystals of magnesium sulfate separate, warm the mixture to about 50° to dissolve. Filter through a Gooch crucible containing an asbestos mat previously washed with dilute sulfuric acid TS, water, and ethanol and ignited and weighed. Wash the crystals on the mat several times with a mixture of 3 volumes of ethanol and 1 volume of water. Ignite the crucible and contents at a dull red heat, cool and weigh. The weight of calcium sulfate obtained, multiplied by 0.4119, gives the equivalent of calcium oxide in the sample taken for the test.

* **Arsenic**
A solution of 1 g of the sample in 15 ml of dilute hydrochloric acid TS and 20 ml of water meets the requirements of the Limit Test for Arsenic acid (Method II)

* **Lead**
Dissolve 1 g of the sample in 10 ml of dilute hydrochloric acid TS, neutralize to phenolphthalein TS. This solution meets the requirements of the Limit Test for Lead.

**PURITY TESTS (continued)**

* **Heavy metals**

Dissolve 1 g of the sample in 10 ml of dilute hydrochloric acid TS, and evaporate to dryness on a steam bath. Toward the end of the evaporation, stir the residue frequently, disintegrate to obtain a dry powder. Dissolve the powder in 20 ml of water and filter. A 10 ml portion of the filtrate meets the requirements of the Limit Test for Heavy Metals (Method I) using 20 μg of lead ion (Pb) in the control (Solution A).

**METHOD OF ASSAY**

Transfer about 400 mg of the sample, previously dried at 105° for 2 h and accurately weighed, into a conical flask. Add 25 ml of 1 N sulfuric acid and, after solution is complete, add methyl red TS and titrate the excess acid with 1 N sodium hydroxide. Subtract from the volume of 1 N sulfuric acid consumed in the assay the volume of 1 N sulfuric acid corresponding to the weight of CaO in the sample taken for the assay using as a factor 28.04 mg of CaO for each ml of 1 N sulfuric acid. Each ml of 1 N sulfuric acid used to neutralize the magnesium hydroxide is equivalent to 29.16 mg of Mg(OH)$_2$.

MAGNESIUM DL-LACTATE*

SYNONYMS
DL-Lactic acid magnesium salt, magnesium di-DL-lactate
INS No. 329

DEFINITION
Chemical name
Magnesium DL(-)-2-hydroxypropionate
C.A.S. number
18917-93-6
Chemical formula
Mg(C_{3}H_{5}O_{2})_{2} \cdot xH_{2}O \quad (x = 0 - 3)
Structural formula
\[\text{CH}_{3}\text{-CH-COO-Mg-OOC-CH-CH}_{2} \cdot x\text{H}_{2}\text{O}\]
Molecular weight
202.45 (anhydrous)
Assay
Content not less than 97.5% and not more than 101.5% of Mg(C_{3}H_{5}O_{2})_{2} on the dried basis

DESCRIPTION
White crystalline powder having a bitter taste

FUNCTIONAL USES
Buffering agent, dough conditioner, dietary supplement

CHARACTERISTICS

IDENTIFICATION TESTS
** A. Solubility
Soluble in water when shaking with water for 30 min
Practically insoluble in ethanol

** B. Specific rotation
\(\alpha_{D}^{25} = +2.0^\circ\) to \(-2.0^\circ\) on the dried basis (3 g/100 ml aqueous solution)

** C. Positive test for magnesium
Passes test

** D. Positive test for lactate
Passes test

PURITY TESTS
** Loss on drying
Not more than 23.0% (120°, 24 h)

* These specifications were prepared at the 27th session of JECFA (1983) and published in FNP 28 (1983).

PURITY TESTS (continued)

* Chlorides

Not more than 0.01%
Test 1 g of the sample as directed in the Limit Test using 0.3 ml of 0.01 N hydrochloric acid in the control.

* Arsenic

Not more than 3 mg/kg (Method II)

* Lead

Not more than 10 mg/kg
Test 1 g of the sample as directed in the Limit Test for Lead using 10 μg of lead ion (Pb) in the control.

* Heavy metals

Not more than 20 mg/kg
Test a solution of 1 g of the sample in 25 ml of water as directed in the Limit Test for Heavy Metals (Method I).

* Microbiological criteria

Total aerobic microbial count : Max 1000/g
Total yeast and moulds : Max 100/g
E. coli : Absent in 1 g

METHOD OF ASSAY

Proceed as directed in the Method of Assay for Magnesium L-Lactate.

MAGNESIUM L-LACTATE*

SYNONYMS

L-Lactic acid magnesium salt, magnesium di-L-lactate; INS No. 329

DEFINITION

Chemical name: Magnesium L(−)-2-hydroxypropionate dihydrate
C.A.S. number: 18917-93-6
Chemical formula: Mg(C₃H₇O₂)₂ · 2H₂O
Structural formula:

```
<table>
<thead>
<tr>
<th>OH</th>
<th>OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₃-CH-COO-Mg-OOCH-CH₂ -2H₂O</td>
<td></td>
</tr>
</tbody>
</table>
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Molecular weight: 238.48
Assay: Content not less than 97.5% and not more than 101.5% of Mg(C₃H₇O₂)₂ on the dried basis

DESCRIPTION

White crystalline powder having a bitter taste

FUNCTIONAL USES

Buffering agent, dough conditioner, dietary supplement

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Soluble in water when shaken with water for 30 min. Practically insoluble in ethanol

** B. Specific rotation
[α]D₂⁰: -7.5° to -8.8° on the dried basis (5 g/100 ml aqueous solution)

** C. Positive test for magnesium
Passes test

** D. Positive test for lactate
Passes test

PURITY TESTS

** Loss on drying
14.0 - 17.0% (120°, 24 h)

** Chlorides
Not more than 0.01%
Test 1 g of the sample as directed in the Limit Test using 0.3 ml of 0.01 N hydrochloric acid in the control

* These specifications were prepared at the 27th session of JECFA (1983) and published in FNP 28 (1983).

PURITY TESTS (continued)

* **Arsenic**
  Not more than 3 mg/kg (Method II)

* **Lead**
  Not more than 10 mg/kg
  Test 1 g of the sample as directed in the Limit Test for Lead using 10 μg of lead ion (Pb) in the control

* **Heavy metals**
  Not more than 20 mg/kg
  Test a solution of 1 g of the sample in 25 ml of water as directed in the Limit Test for Heavy Metals (Method I)

* **Microbiological criteria**
  Total aerobic microbial count : Max 1000/g
  Total yeast and moulds : Max 100/g
  *E. coli* : Absent in 1 g

**METHOD OF ASSAY**

**Cheletometry**

Dissolve about 0.5 g of the previously dried sample, accurately weighed, in 25 ml of water, add 5 ml of ammonia/ammonium chloride buffer TS and 0.1 ml of ethylenediaminetetraacetate until the solution is blue in colour. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 10.12 mg of Mg(C₃H₅O₃)₂.

MAGNESIUM OXIDE*

SYNONYMS
INS No.530, EEC No.530

DEFINITION

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Magnesium oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A.S. number</td>
<td>1309-48-4</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>MgO</td>
</tr>
<tr>
<td>Formula weight</td>
<td>40.31</td>
</tr>
<tr>
<td>Assay</td>
<td>Magnesium oxide contains not less than 96.0% of MgO after ignition at about 800°.</td>
</tr>
</tbody>
</table>

DESCRIPTION
Magnesium oxide occurs as a very bulky white powder, known as light magnesium oxide, or as a relatively dense, white powder, known as heavy magnesium oxide. 5 g of light magnesium oxide occupy a volume of 40 to 50 ml, while 5 g of heavy magnesium oxide occupy a volume of 10 to 20 ml.

FUNCTIONAL USE
Anticaking agent.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Practically insoluble in water; insoluble in ethanol.

B. Alkalinity
The sample is alkaline to moistened litmus paper.

C. Positive test for magnesium
Passes test.
See description under TESTS.

PURITY TESTS

** Loss on drying
Not more than 5% after ignition at 800° to 825° to constant weight.

Alkali (free) and soluble salts
Passes test.
See description under TESTS.

Calcium oxide
Not more than 1.5%.
See description under TESTS.

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* These specifications were prepared at the 17th session of JECFA (1973) and published in FNS 4 (1978).

PURITY TESTS (continued)

* Arsenic

Not more than 3 mg/kg (Method II).
A solution of 1 g of the sample, weighed to the nearest mg, in 20 ml of diluted hydrochloric acid TS and 15 ml of water meets the requirements of the Limit Test for Arsenic (Method II).

Lead

Not more than 10 mg/kg.
See description under TESTS.

Heavy metals

Not more than 40 mg/kg.
See description under TESTS.

TESTS

IDENTIFICATION TESTS

B. Positive test for magnesium

To a solution of the sample in dilute acetic acid TS add ammonium chloride TS and ammonium carbonate TS. No precipitate is formed. Add sodium phosphate TS. A white crystalline precipitate is formed which is insoluble in ammonia TS.

PURITY TESTS

Alkali (free) and soluble salts

Boil 2 g of the sample, weighed to the nearest mg, with 100 ml of water for 5 min in a covered beaker and filter while hot. Add methyl red TS and titrate 50 ml of the cooled filtrate with 0.1 N sulfuric acid. Not more than 2 ml of the acid should be consumed. Evaporate 25 ml of the filtrate to dryness and dry at 105° for 1 h. Not more than 10 mg of residue should remain.

Calcium oxide

Weigh 400 mg of the sample to the nearest 0.1 mg, and dissolve in a mixture of 3 ml of sulfuric acid and 22 ml of water. Add 50 ml of ethanol, and allow the mixture to stand overnight. If crystals of magnesium sulfate separate, warm the mixture to about 50° to dissolve. Filter through a tared, previously ignited, porcelain filter crucible, and wash the precipitate several times with a mixture of 2 volumes of ethanol and 1 volume of dilute sulfuric acid TS. Ignite the crucible and contents at a dull red heat, cool and weigh. The weight of calcium sulfate obtained, multiplied by 0.4119, gives the equivalent of calcium oxide in the sample taken for the test.

* Lead

Dissolve 1 g of the sample, weighed to the nearest mg, in 20 ml of dilute hydrochloric acid TS and neutralize with ammonia TS, using phenolphthalein as indicator. This solution meets the requirements of the Limit Test for Lead, using 10 μg of lead ion (Pb) in the control.

PURITY TESTS (continued)

* **Heavy metals**

Dissolve 500 mg of the sample in 20 ml of dilute hydrochloric acid TS, and evaporate the solution to dryness on a steam bath. Towards the end of the evaporation, stir frequently to disintegrate the residue so that finally a dry powder is obtained. Dissolve the powder in 20 ml of water and evaporate to dryness in the same manner as before. Redissolve the residue in 25 ml of water and filter if necessary. This solution meets the requirements of the Limit Test for Heavy Metals (Method I), using 20 μg of lead ion (Pb) in the control (Solution A).

**METHOD OF ASSAY**

Ignite about 400 mg of the sample to constant weight at 800° to 825° in a tared platinum crucible. Weigh the residue accurately, dissolve in 25.0 ml of N sulfuric acid, boil gently to remove any carbon dioxide and cool. Add methyl red TS and titrate the excess acid with N sodium hydroxide. Subtract from the volume of N sulfuric acid consumed the number of ml of N sulfuric acid corresponding to the weight of CaO in the sample taken, using as a factor 28.04 mg of CaO for each ml of N sulfuric acid. The difference is the volume of N sulfuric acid used to neutralize the magnesium oxide and each ml is equivalent to 20.16 mg MgO.

MAGNESIUM SILICATE (SYNTHETIC)*

SYNONYMS

INS No.553(i), EEC No.553a

DEFINITION

Synthetic magnesium silicate is of variable composition but the molar ratio of MgO to SiO₂ is approximately 2:5.

Assay

Content not less than 15% of MgO and not less than 67% of SiO₂, calculated on the ignited basis.

DESCRIPTION

Very fine, white, odourless, tasteless powder, free from grittiness.

FUNCTIONAL USE

Anticaking agent.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Insoluble in water and in ethanol.

B. Readily decomposed by mineral acids.

** C. pH
7.0 - 10.8 (1 in 10 slurry).

D. Positive test for magnesium
Passes test
See description under TESTS.

E. Positive test for silicate
Passes test
See description under TESTS

PURITY TESTS

** Loss on drying
Not more than 15% (105°, 2 h).
Retain the sample for determination of Loss on ignition.

Loss on ignition
Not more than 15%, determined on the dried basis.
See description under TESTS.

Free alkali
Not more than 1% (as NaOH).
See description under TESTS.

Soluble salts
Not more than 3%.
See description under TESTS.

* These specifications were prepared at the 25th session of JECFA (1981) and published in FNP No.25 (1981).

PURITY TESTS (continued)

* **Fluoride**
  Not more than 10 mg/kg.
  Weigh 2.5 g of the sample to the nearest mg; and proceed as directed in the Fluoride Limit Test (Method I or III).

**Silicon dioxide**
  Passes test.
  See description under TESTS.

**Arsenic**
  Not more than 3 mg/kg.
  See description under TESTS.

**Lead**
  Not more than 10 mg/kg.
  See description under TESTS.

**Heavy metals**
  Not more than 10 mg/kg.
  See description under TESTS.

TESTS

IDENTIFICATION TESTS

D. *Positive test for magnesium*
  Mix about 0.5 g of the sample with 10 ml of dilute hydrochloric acid TS, filter, and neutralize the filtrate to litmus paper with ammonia TS. The neutralized filtrate yields no precipitate with ammonium carbonate TS, but a white, crystalline precipitate, which is insoluble in ammonia TS, is formed upon the subsequent addition of sodium phosphate TS.

E. *Positive test for silicate*
  Prepare a bead by fusing a few crystals of sodium ammonium phosphate on a platinum loop in the flame of a Bunsen burner. Place the hot, transparent bead in contact with the sample, and again fuse. Silica floats about in the bead, producing, upon cooling, an opaque bead with a web-like structure.

PURITY TESTS

* **Loss on ignition**
  Weigh to the nearest 0.1 mg, 1 g of the sample in a tared platinum crucible provided with a cover. Gradually apply heat to the crucible at first, then strongly ignite at 900/1000° for 20 min. Cool, weigh and calculate as percentage.

**Free alkali**
  Add 2 drops of phenolphthalein TS to 20 ml of dilute filtrate prepared in the test for soluble salts (see below), representing 1 g of the sample. Not more than 2.5 ml of 0.1 N hydrochloric acid should be required to discharge the pink colour produced.

PURITY TESTS (cont'n)

**Soluble salts**

Boil 10 g of the sample with 150 ml of water for 15 min. Cool to room temperature, and add water to restore the original volume. Allow the mixture to stand for 15 min, and filter until clear. To 75 ml of the clear filtrate add 25 ml of water. Evaporate 50 ml of this solution, representing 2.5 g of the sample, in a tared platinum dish on a steam bath to dryness, and ignite gently to constant weight. Cool, weigh and calculate as percentage (The weight of the residue should not exceed 50 mg).

* Arsenic

A 10 ml sample portion of the solution (see below) diluted to 35 ml with water meets the requirements of the Limit Test for Arsenic (Method II).

* Lead

Neutralize a 10 ml portion of the sample solution (see below) with ammonia TS, using phenolphthalein as indicator, and dilute to 20 ml with water. This solution meets the requirements of the Limit Test for Lead, using 10 μg of lead ion (Pb) in the control.

* Heavy metals

A 5 ml portion of the sample solution (see below) diluted to 25 ml with water meets the requirements of the Limit Test for Heavy Metals (Method I), using 20 μg of lead ion (Pb) in the control (Solution A).

**Sample solution for the determination of arsenic, lead and heavy metals:**

Weigh 10 g of the sample to the nearest mg, and transfer into a 250 ml flask, and add 50 ml of 0.5 N hydrochloric acid. Attach a reflux condenser to the flask, heat on a steam bath for 30 min, cool, and let the undissolved material settle. Decant the supernatant liquid through a Whatman No.3 filter paper, or equivalent, into a 100 ml volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-ml portions of hot water, decanting each washing through the filter into the flask. Finally, wash the filter paper with 15 ml of hot water, cool the filtrate to room temperature, dilute to volume with water and mix.

**METHOD OF ASSAY**

**Magnesium oxide**

Weigh 1.5 g of the sample, to the nearest 0.1 mg, transfer into a 250 ml conical flask, add 50 ml of N sulfuric acid, and digest on a steam bath for 1 h. Cool to room temperature, add methyl orange TS, and titrate the excess acid with N sodium hydroxide. Each ml of N sulfuric acid is equivalent to 20.15 mg of MgO.

METHOD OF ASSAY (continued)

**Silicon dioxide**

Transfer about 0.7 g of the sample, weighed to the nearest 0.1 mg, into a 150 ml beaker and add 20 ml of N sulfuric acid, and heat on a steam bath for 1 h and 30 min. Decant the supernatant liquid through an ashless filter paper, and wash the residue, by decantation, three times with hot water. Treat the residue with 25 ml of water and digest on a steam bath for 15 min. Finally, transfer the residue to the filter paper and wash thoroughly with hot water. Transfer the filter paper and its contents to a platinum crucible. Heat to dryness, incinerate, then ignite strongly for 30 min, cool and weigh. Moisten the residue with water, and add 6 ml of hydrofluoric acid* and 3 drops of sulfuric acid. Evaporate to dryness, ignite for 5 min, cool and weigh. The loss in weight represents the weight of SiO₂.

---

* **Warning**: Toxic, corrosive, must not contact skin; work with fume hood.
** DL-MALIC ACID**

**SYNONYMS**

Pomalous acid; INS No. 296, EEC No. E296

**DEFINITION**

Chemical names

DL-Malic acid, hydroxybutanedioic acid, hydroxysuccinic acid

C.A.S. number

6915-15-7

Chemical formula

C₇H₆O₇

Structural formula

\[
\text{COOH} \\
\text{CHOH} \\
\text{CH₂} \\
\text{COOH}
\]

Molecular weight

134.09

Assay

DL-Malic Acid contains not less than 99.0% of C₇H₆O₇

**DESCRIPTION**

White or nearly white crystalline powder or granules having an acid taste.

**FUNCTIONAL USES**

Acidifier, flavouring agent

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

**A. Solubility**

Very soluble in water. Freely soluble in ethanol

**B. Melting range**

Between 127° and 132°.

**C. Positive test for malate**

Passes test

See description under TESTS

**PURITY TESTS**

**Sulphated ash**

Not more than 0.1%

Proceed as directed under the test for Ash (Sulphated ash, Method I) using 2 g of the sample.

---

* These specifications were prepared at the 19th session of JECFA (1975) and published in NMRS 55B (1976).

PURITY TESTS (continued)

* Arsenic

Not more than 3 mg/kg

A sample solution prepared as directed for organic compounds meets the requirements of the Limit Test for Arsenic (Method II).

* Lead

Not more than 10 mg/kg

A sample solution prepared as directed for organic compounds meets the requirements of the Lead Limit Test using 10 μg of lead ion (Pb) in the control.

* Heavy metals

Not more than 20 mg/kg

A solution of 1 g of the sample in 25 ml of water meets the requirements of the Limit Test for Heavy Metals (Method I) using 20 μg of lead ion (Pb) in the control (Solution A).

Fumaric and maleic acid

Not more than 1.0% of fumaric acid and not more than 0.05% of maleic acid.

See description under TESTS

IDENTIFICATION TESTS

C. Positive test for malate

Neutralize 5 ml of a 1 in 20 solution of the sample with ammonia TS and add 10 mg of sulfanilic acid. Heat the solution on a water bath for a few minutes, add 5 ml of a 1 in 5 solution of sodium nitrite and heat slightly. Make alkaline with sodium hydroxide TS. A red colour is produced.

PURITY TESTS

Fumaric and maleic acid

Preparation of solutions

Buffer solution A: In a 1000-ml volumetric flask dissolve 74.5 g of potassium chloride in 500 ml of water, add 100 ml of concentrated hydrochloric acid, and dilute to volume with water.

Buffer solution B: Dissolve 171.0 g of dipotassium hydrogen phosphate, K₂HPO₄·3H₂O, in 1000 ml of water, and add potassium dihydrogen phosphate, KH₂PO₄, until the pH is exactly 7.0.

Maxima suppressor: Dissolve, with the aid of a magnetic stirrer, 1 g of gelatin in 65 ml of hot, boiled water. After cooling, add 35 ml of ethanol as a preservative.

Standard solution: Weigh out accurately about 20 g of the sample, 200 mg of fumaric acid and 10 mg of maleic acid, both of the highest purity available, and transfer into a 500-ml volumetric flask. Add 300 ml of sodium hydroxide TS, a few drops of phenolphthalein TS and continue adding sodium hydroxide TS to a faint pink colour that persists for at least 30 sec. Dilute to volume with water, and mix.

Sample solution: Transfer about 4 g of the sample, accurately weighed, to a 100-ml volumetric flask and dissolve in 25 ml of water. Add phenolphthalein TS, and neutralize with sodium hydroxide TS as directed for standard solution. Dilute to volume with water, and mix.

Procedure

Transfer two 25-ml portions of the "Sample solution" into separate 100-ml volumetric flasks. Dilute one flask (Sample A) to volume with "Buffer solution A". To the other flask (Sample B) add 50 ml of "Buffer solution B" and dilute to volume with water.

Rinse a polarograph cell with a portion of "Sample A", add a suitable volume of the solution to the cell, immerse it in a water bath regulated at 24.5 - 25.5°, add 2 drops of the "Maxima suppressor", and then de-aerate by bubbling nitrogen through the solution for at least 5 minutes. Insert the dropping mercury electrode (negative polarity) of a suitable polarograph, adjust the current sensitivity as necessary, and record the polarogram from -0.1 to -0.8 volt at the rate of 0.2 volt per minute, using a saturated calomel electrode as the reference electrode. Transfer 25 ml of the "Standard solution" into a 100-ml volumetric flask, and dilute to volume with "Buffer solution A". Obtain the polarogram of this solution (Standard A) in the same manner as directed for "Sample A". In each polarogram, determine the height of the maleic acid plus fumaric acid wave occurring at the half-wave potential near -0.56 volt, recording that for the sample as \( i_s \), and that for the standard as \( i_r \).

In the same manner, obtain polarograms from "Sample B" and a "Standard B", except record the polarogram from -1.05 to -1.7 volts at the rate of 0.1 volt per minute. In each polarogram, determine the height of the maleic acid wave occurring at the half-wave potential near -1.33 volts, recording that for the sample as \( i_s' \), and that for the standard as \( i_r' \).

Calculation

Calculate the weight in mg, \( p \), of combined maleic acid and fumaric acid in the sample taken by the formula:

\[
500C \times \frac{i_s}{i_r - i_s}
\]

in which \( C \) is the concentration, in mg per ml, of combined maleic acid and fumaric acid in the Standard solution. Similarly, calculate the weight in mg, \( q \), of maleic acid in the sample taken by the formula:

\[
500C' \times \frac{i_s'}{i_r' - i_s'}
\]
METHOD OF ASSAY

Dissolve about 2 g of the sample, accurately weighed, in 40 ml of recently boiled and cooled water, add 2 drops of phenolphthalein TS and titrate with 1 N sodium hydroxide to the first appearance of a faint pink colour which persists for at least 30 seconds. Each ml of 1 N sodium hydroxide is equivalent to 67.04 mg of C_4H_6O_3.

in which C' is the concentration, in mg per ml of maleic acid in the Standard solution.

Calculate the weight of fumaric acid in mg, r, in the sample taken from the difference in these values, i.e. (r = p - q).

Finally, calculate the percentage of fumaric and maleic acids present by multiplying r and q, respectively, by 0.025.

Fumaric and maleic acid (cont'd)

in which C' is the concentration, in mg per ml of maleic acid in the Standard solution.
**MALT CARBOHYDRASE**

**SYNONYM**
Malt

**SOURCES**
Malt is the product of controlled germination of barley

**ACTIVE PRINCIPLES**
1. α-Amylase (glycogenase, diastase)
2. β-Amylase (glycogenase, diastase)

**SYSTEMATIC NAMES AND NUMBERS**
1. 1,4-α-D-Glucan glucanohydrolase - EC 3.2.1.1
2. 1,4-α-D-Glucan maltohydrolase - EC 3.2.1.2

**REACTIONS CATALYSED**
1. Hydrolysis of 1,4-α-glucosidic linkages in polysaccharides, (starch, glycogen) yielding dextrins and oligo- and monosaccharides.
2. Hydrolysis of 1,4-α-glucosidic linkages in polysaccharides, (starch, glycogen) yielding successively maltose units from the non-reducing ends of the chains.

**FUNCTIONAL USES**
Brewing, baking, manufacture of alcoholic beverages and manufacture of syrups.

**GENERAL SPECIFICATIONS**
Must conform to the "General Specifications for Enzyme Preparations used in Food Processing***

**CHARACTERISTICS**

**IDENTIFICATION TESTS**
1. α-Amylase activity
   The sample shows α-amylase activity***
2. α- and β-Amylase activity
   The sample shows Diastatic power
   See description under TESTS (next pages)

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* This specification was prepared at the 15th session of JECFA (1971) and published in NMRS 50B (1972).

** See Annex 1 at the end of this compendium.

Determination of a diastatic power of malt (combined activity of α- and β-amylase) is shown below.*

Reagents

For Digestion of Starch Solution

- Acetate buffer solution: Dissolve 68 g of sodium acetate (CH₃COONa · 3H₂O), reagent grade, in 500 ml of one N acetic acid and make the solution up to one litre with distilled water.
- Sodium hydroxide solution: 0.5 N
- Special starch: Starch manufactured specifically for diastatic power determination is available from Merck & Co., Rahway, New Jersey. It is designated, "Soluble Starch Merck," according to Lintner, "Special for diastatic power determination." No other manufacturer of this special starch is known to the society.

When purchasing new batches of starch, test them in parallel with the lot in use. Variations of more than ± 3° Diastatic Power in the averages of a series of parallel tests indicate an unsuitable batch of starch.

For Reducing Substances, Ferricyanide Procedure

- Acetic acid-salt solution: Dissolve 70 g of potassium chloride and 20 g of crystallized zinc sulfate (ZnSO₄ · 7H₂O) in distilled water, add 200 ml of glacial acetic acid and make up to one liter with distilled water.
- Alkaline ferricyanide solution: 0.05N. Dissolve 16.5 g of potassium ferricyanide (K₃Fe(CN)₆) and 22 g of anhydrous sodium carbonate (Na₂CO₃) in distilled water and make up to one liter. Keep the solution in the dark away from the light.
- Standardization of the Alkaline Ferricyanide Solution: With an accurate pipette add 10 ml (± 0.05 ml) of the alkaline ferricyanide solution to 25 ml of acetic acid salt solution. Add one ml of potassium iodide solution, and two ml of starch solution (see Preparation of Starch Solution, below). Titrate with 0.05N sodium thiosulfate solution, using a 10 ml of semi-micro burette, until the blue starch iodide colour is discharged. Calculate the normality of the alkaline ferricyanide solution, which should lie between 0.0495 and 0.0505.
- Potassium iodide solution: Dissolve 50 g of potassium iodide in 50 ml of distilled water and make up to 100 ml. Add one or two drops of concentrated sodium hydroxide solution. The final solution should be colourless.
- Sodium thiosulfate solution: 0.05N. Dissolve 12.41 g of dry, large clear crystals of sodium thiosulfate (Na₂S₂O₃ · 5H₂O) and 3.8 g of borax (Na₂B₄O₇ · 10H₂O) (used as a preservative) in 100 to 200 ml of distilled water and make up to one liter.

- **Standardization of Sodium Thiosulfate Solution**: Standardize the sodium thiosulfate solution with potassium dichromate. Dry pure $K_2Cr_2O_7$ (small crystals) for an hour or more at 105° and weigh $0.1000 \pm 0.0005$ g portions for the standardization into 250 ml Erlenmeyer flasks. Dissolve the crystals in 50 ml of water, add two ml of potassium iodide solution and eight ml of concentrated hydrochloric acid solution. Mix thoroughly and titrate with the thiosulfate solution, swirling the mixtures constantly until the brown colour changes to a yellowish green. Add one or two ml of starch solution and continue the titration until the colour changes from blue to light green. About 40 ml of 0.05$N$ thiosulfate solution will be used for the titration. Calculate the normality of the thiosulfate solution by use of the formula:

$$ N = \frac{1.000 w}{49.035 v} $$

in which:

- $N$ = the normality of the thiosulfate solution,
- $v$ = the volume of the thiosulfate solution used for the titration,
- $w$ = the weight of $K_2Cr_2O_7$ used,
- $49.035$ = the iodimetric equivalent weight of $K_2Cr_2O_7$.

The normality of the sodium thiosulfate solution should lie between 0.0495 and 0.0595.

- **For Reducing Substances**, **Fehling's Solution Procedure**

- Methylene blue, one per cent aqueous solution
- Soxhlet’s modification of Fehling’s Solution

Prepare by mixing immediately before use equal volumes of (A) and (B):

(A) Copper sulfate solution: Dissolve 34.639 g of copper sulfate ($CuSO_4 \cdot 5H_2O$) in water, dilute to 500 ml and filter through prepared asbestos.

(B) Alkaline tartrate solution: Dissolve 173 g of Rochelle salt (sodium potassium tartrate) and 50 g of sodium hydroxide in water, dilute to 500 ml, allow to stand for two days and filter through prepared asbestos.

Standardize the mixed Fehling’s solution by using an invert sugar solution, according to the LANE-EYNON Volumetric method, or by using pure dextrose according to the method described in Note 2, below.

- Sodium chloride. 0.5 per cent. Dissolve 5.0 g of sodium chloride reagent grade in freshly distilled water and dilute the solution to one liter.

- **Apparatus**

- Balance: See MALT-4,* EXTRACT, Apparatus (b)
- Electric hot plate
- Filter paper: See MALT-4,* EXTRACT, Apparatus (d)
- Funnels: 20 cm. See MALT-4,* EXTRACT, Apparatus (f)

Apparatus (continued)

- Glassware:
  Burettes, 20 ml semicro-micro; 50 ml
  Flasks, Erlenmeyer, 125 ml; 250 ml
  Flasks, volumetric, 100 ml; 250 ml; one liter
  Pipettes, 10 ml (precision); 20 ml; 200 ml (fast)
- Infusion flasks, 1 L Erlenmeyer flask or glass-stoppered bottle is suitable.
- Mash beaker: See MALT-4*, EXTRACT, Apparatus (I)
- Mill, fine Grind: See MALT-4*, EXTRACT, Apparatus (k)
- Stopwatch
- Water bath, 20° (± 0.2°)
- Water bath, boiling water.

Wash all glassware to be subsequently used in the determination first with chromic acid cleaning solution, then rinse with ordinary tap water not less than four times, and finally rinse with distilled water at least twice. Thoroughly drain the infusion flasks.

Digestion of the Starch Solution

Preparation of the Special Starch Solution

Prepare a 2% solution of special starch by weighing the equivalent of 2 g of starch dry basis, for each 100 ml of solution required, and macerating it with not over 5% of the final volume of cold freshly distilled water, stirring it into a smooth thin paste. Add the starch paste slowly with constant stirring to vigorously boiling freshly distilled water at such a rate that boiling does not cease. The volume of boiling water should be not less than 75% of the final volume of starch solution. Continue boiling the solution for 2 min after the last of the paste is added, then quickly add 10% of the final volume of cold freshly distilled water to the boiling solution and transfer it quantitatively to a glass-stoppered volumetric flask. Mix by inverting the flask, wash down the neck of the flask with a little distilled water and cool the solution to 20°. When cooled add 2 ml of acetate buffer solution for each 100 ml of final volume and make to the mark with distilled water. Mix again by inverting the flask. Keep the flask tightly stoppered and at 20° until used.

Preparation of the Malt Infusion

Grind separately not over 25.5 g of malt according to the method for fine grinding of malt for determination of extract. (MALT-4*, EXTRACT, Preparation of the Sample for Mashing, Fine Grinding.) Collect the finely ground malt in a mash beaker. Carefully brush malt particles remaining in the mill into the mash beaker. Without delay, place the mash beaker with its contents on the balance, adjust the weight of ground malt to 25 g (± 0.05 g) and transfer it to the infusion flask. Add 500 ml of 0.5% sodium chloride solution, close the flask, swirl, and note the time. Let the infusion stand for 2.5 h at 20° (± 0.2°) agitating it by rotation at 20 min intervals. The infusion flask must not be mixed by inverting it, and the quantity of grist left adhering to the inner walls of the flask as a result of agitation must be as small as possible. Gentle swirling of the contents of the flask without splashing it

Preparation of the Malt Infusion (continued)

against the walls will give sufficient mixing. At the end of 2.5 h, filter the infusion through a 32 cm fluted filter in a 20 cm funnel. Return the first 50 ml of filtrate to the filter. Collect the filtrate until 3 h have elapsed after the time the sodium chloride solution and the ground malt were first mixed. Place a watch glass over the funnel and a suitable cover around the stem and over the receiver to reduce evaporation losses during the filtration.

Diastasis

When the filtration of the infusion is completed, transfer immediately 20 ml of the filtrate to a 100 ml volumetric flask and dilute it to the mark with 0.5% sodium chloride solution. Mix well, and with a pipette, transfer 10 ml of the diluted extract to a 250 ml volumetric flask and bring it to 20°. Add 200 ml of buffered starch solution at 20° to the flask from a fast flowing pipette and start a stopwatch the instant the addition begins. Mix by rotating the flask during the addition. Maintain the "starch infusion" mixture at 20° (± 0.1°) for exactly 30 min from the time the addition of the starch solution was begun. At the end of 30 min add 20 ml of 0.5N sodium hydroxide rapidly and mix by inverting the flask. Make the volume up to 250 ml at 20° with distilled water and mix well.

Preparation of the Blank Correction Solution

Prepare a blank solution by adding 20 ml of 0.5 N sodium hydroxide to the 10 ml of diluted malt extract before adding the 200 ml of starch solution. Otherwise treat the blank solution in exactly the same way as the starch solution actually undergoing diastasis.

Determination of Reducing Substances

Ferricyanide Procedure

The Titration (A)

With a pipette add 5 ml (± 0.05 ml) of the digested starch solution to a 125 ml-Erlenmeyer flask. Add 10 ml (± 0.05 ml) of alkaline ferricyanide reagent, mix well, and immerse the flask in a vigorously boiling water bath for exactly 20 min by a stopwatch. The level of the boiling water should be slightly above the level of the mixture in the flask. After 20 min in the bath remove the flask and cool under running water to room temperature. Add 25 ml of acetic acid-salt solution and one ml of potassium iodide solution. Mix well and titrate with the 0.05 N sodium thiosulfate solution to the complete disappearance of the blue colour. Use a 10 ml-semimicrolitre for the titration and read it to 0.01 ml. Call the volume of sodium thiosulfate solution used for the titration, "A".

The Blank Correction (B)

Add with a pipette 5 ml of the blank correction solution to a 125 ml-Erlenmeyer flask, add 10 ml (± 0.05 ml) of 0.05N alkaline ferricyanide solution. Immerse the flask in boiling water for 20 min, cool, and titrate it in exactly the same way as for the direct titration above. Call the number of ml of 0.05 N sodium thiosulfate solution required for the blank titration, "B".
Calculation

Calculate the diastatic power of the malt in degrees by use of the formulas:

\[
\text{Diastatic Power}^\text{as is)} = (B-A) \times 23 \\
\text{Diastatic Power}^\text{dry basis)} = \frac{(\text{as is}) \times 100}{100 - M}
\]

in which,

- \(B\) = ml of sodium thiosulfate used for the blank correction titration
- \(A\) = ml of sodium thiosulfate used for the direct titration
- \(M\) = per cent moisture in the malt

Report Diastatic Power as Degrees to the nearest whole number.

Example

\(M = 4.1\%\)

\(B = 9.65\) ml of 0.05 N \(Na_2S_2O_3\) solution

\(A = 5.04\) ml of 0.05 N \(Na_2S_2O_3\) solution

\[
\text{Diastatic Power}^\text{as is)} = (9.65 - 5.04) \times 23 = 106 \\
\text{Diastatic Power}^\text{dry basis)} = \frac{106 \times 100}{100 - 4.1} = 111
\]

The Titration (A)

Mix 10.0 ml of Soxhlet solution and 10 ml of water in a 200 ml-Erlenmeyer flask and bring it to a boil, preferably on an electric hot plate, or over a small gas flame. Add, from a burette, about two-thirds of the volume of digested starch solution probably necessary for complete reduction of the copper, and boil for 15 to 20 sec, rotating the flask constantly. Remove from the heat, and, if the liquid is decidedly blue, add more digested starch solution, boil for about 10 sec, and again observe the colour. When the blue colour is almost discharged, and after boiling gently for about 2 min, add three drops of a 1% methylene blue solution. Continue boiling, and adding more diluted starch solution until 0.1 ml or even one drop completely discharges the blue colour. Near the end-point the colour is violet lavender.

After the methylene blue indicator is added do not interrupt the boiling. The flask must remain filled with steam to prevent entrance of air. Titrate* into the gently simmering solution in flask. On cooling the blue colour usually returns.

Repeat the titration, adding at once almost the whole amount of the digested starch solution required and finish off the titration drop by drop as given above. Call the volume of digested starch solution required to reach the end-point in the second titration "A".

The Blank Correction (B)

Add to 10 ml of Soxhlet solution and 10 ml of water a volume of blank correction solution equal to \(A\), the final value of digested starch solution required in the direct titration. Boil the mixture and again determine the end point by titrating with the digested starch solution as in The Titration (A), above. Call the volume of digested starch solution used to reach the end-point "B".

---

* A burette with an S- or Z-shaped extension to the tip that will extend over the boiling flask is an advantage in this titration.
Calculation

Calculate the diastatic power of malt in degrees by use of the formulas:

\[
\text{Diastatic Power }^* \text{ (as is)} = \frac{5.000}{A} \times B
\]

\[
\text{Diastatic Power }^* \text{ (dry basis)} = \frac{(\text{as is}) \times 100}{100 - M}
\]

in which,

- \(A\) = ml of digested starch solution used in the direct titration
- \(B\) = ml of digested starch solution used in the blank correction titration
- \(M\) = per cent moisture in the malt

In the formula for Diastatic Power \(^*\) (as is), \(\frac{5.000}{A}\) is the apparent diastatic power which must be corrected for the effect of the reducing substances in the starch solution and in the salt infusion prior to diastasis.

The fraction \(\frac{B}{A}\) is the correction factor for these effects.

Report Diastatic Power as Degrees to the nearest whole number.

Example

- \(A = 44.2\) ml of digested starch solution in the direct titration
- \(B = 41.3\) ml of digested starch solution in the blank correction titration
- Diastatic Power \(^*\) (as is) = \(\frac{5.000 \times 41.3}{44.2} = 106\)

\[
\text{Diastatic Power }^* \text{ (dry basis)} = \frac{106 \times 100}{100 - 4.1} = 111
\]

Notes

1. Ferricyanide Procedure

Collaborative work was established that when the conditions of the method are maintained, the volume of 0.05 Normal ferricyanide solution corrected for the blank and multiplied by 23 gives the diastatic power in Degrees "as is". See Reference below.

2. Standardization of Soxhlet Solution

Instead of preparing a solution of invert sugar, pure dextrose may be used to standardize the Soxhlet Solution. Dry pure, reagent grade, anhydrous dextrose \((C_6H_{12}O_6)\) for 1.5 hr at 103\(^\circ\)C weigh out 4.82 g, dissolve it in distilled water and make the volume up to one litre. 25 ml of the solution contain exactly 120.5 mg of dextrose and should exactly reduce 25 ml of Soxhlet Solution (Table 5, A.S.B.C. "Tables Related to Determinations on Wort, Beer, and Brewing Sugars and Syrups").

If the standardization is based on 10 ml of Soxhlet Solution and 10 ml of distilled water, prepare the dextrose solution to contain 1.265 g of dextrose in 40 ml which should exactly reduce 10 ml of Soxhlet Solution (Table of "Factors for 10 ml Soxhlet solution to be used in connection with Lane-Eynon general volumetric method", Official Methods of Analysis, A.O.A.C.).
3. Degrees Diastatic Power

A definition of "Degrees Diastatic Power" is implied in the foregoing instructions for determination of diastatic power. The original definition of "Degrees Lintner" is as follows: A malt has a diastatic power of 100°L, if 0.1 cc of a clear 5% infusion of the malt, acting on 100 cc of a 2% starch solution at 20° for one h, produces sufficient reducing sugars to reduce completely 5 cc of Fehling's solution. Since the original Lintner method used a malt infusion prepared with distilled water, while the method given above uses a 0.5% sodium chloride solution for extraction of the diastase, the "Lintner" of the diastatic power designation was dropped. The use of 0.5% sodium chloride solution and reporting of diastatic power in the form, "Diastatic Power-120" became effective January 1, 1957.

References


SYNONYMS  
D-Maltitol, hydrogenated maltose;  
INS No. 965

DEFINITION

Chemical names  
α-D-Glucopyranosyl-1,4-D-glucitol

C.A.S. number  
585-88-6

Chemical formula  
C₁₂H₂₄O₁₁

Structural formula

Molecular weight  
344.31

Assay  
Not less than 98.0% of D-maltitol, C₁₂H₂₄O₁₁

DESCRIPTION  
Sweet tasting, white crystalline powder

FUNCTIONAL USE  
Sweetening agent, humectant, stabilizer

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility  
Very soluble in water, slightly soluble in ethanol

** B. Melting range  
Between 148° and 151°

** C. Specific rotation  
[α]D²⁰ = +105.5° to +108.5°  
Determine the specific rotation for a solution of 5 g of sample in 100 ml of water

D. Thin layer chromatogram  
Passes test  
See description under TESTS

PURITY TESTS

** Water  
Not more than 1% (Karl Fisher method)

** Sulfated ash  
Not more than 0.1%  
Test 2 g of sample as directed under the test for Ash (Sulfated ash) Method I

* These specifications were prepared at the 33rd session of JECFA (1988) and published in FNP 38 (1988)

PURITY TESTS (continued)

* **Chlorides**
Not more than 50 mg/kg
Test 10 g of sample by the Chlorides Limit Test using 1.5 ml of 0.01 N hydrochloric acid in the standard

* **Sulfates**
Not more than 100 mg/kg
Test 10 g of sample by the Sulfates Limit Test using 2.0 ml of 0.01 N sulfuric acid in the standard

**Nickel**
Not more than 2 mg/kg
Proceed as directed in the specifications for Sorbitol

* **Lead**
Not more than 1 mg/kg
Prepare a sample solution as directed under Limit Test for Lead for organic compounds and determine the lead content by atomic absorption

* **Heavy metals**
Not more than 10 mg/kg
Test 2 g of the sample in 25 ml of water as directed in the Heavy Metals Limit Test (Method I)

* **Reducing sugar**
Not more than 0.1%
Test 7 g of sample by the General Method for Reducing Substances (as glucose) Method II. The weight of cuprous oxide shall not exceed 20 mg

TESTS

IDENTIFICATION TESTS

* **D. Thin layer chromatography.**
Examine by Thin layer chromatography using silica gel as the coating substance

**Reference solution**
Dissolve 50 mg maltitol standard in 20 ml water

**Test solution**
Dissolve 50 mg of sample in 20 ml of water

**Procedure**
Apply 2 μl of each of the Reference and Test solution to the bottom of the TLC plate. Develop the chromatogram over a path of 17 cm using as the mobile phase a mixture of 70 volumes of propanol, 20 volumes of ethyl acetate and 10 volumes of water. Allow the plate to dry in air and spray with a 0.2% w/v solution of sodium periodate. Allow the plate to dry in air for 15 min and spray with a 2% w/v solution of tetramethyl-diamino-phenylmethane in a mixture of 20 volumes of glacial acetic acid and 80 volumes of acetone. The principal spot in the chromatogram obtained from the test solution is similar in position, colour and size to the principal spot obtained from the Reference solution.

---

Determine by High performance liquid chromatography method

Chromatographic system
A high performance liquid chromatograph equipped with:

- Pump (Waters type 510)
- Heating oven
- Differential refractometer (Waters type R401 or equivalent)
- Integrating recorder
- Sampling valve with a 20 µl sampling loop
- Column 30 cm x 8 mm packed with AMINEX HPX 87C (calcium form) from BIORAD or equivalent
- Mobile phase: bidistilled, degassed water filtered through 0.45 µm Millipore filter or equivalent.

The chromatograph is operated at a temperature of 85° and a flow rate of 0.5 ml/min.

Calibration
Accurately weigh into four 100 ml volumetric flasks 0.5, 1.0, 1.5 and 2.0 g respectively of pure maltitol. Dissolve and make up to volume with bidistilled water. (NOTE: maintain constant stirring for 1 h to ensure complete dissolution). Filter the solutions through 0.45 µm Millipore filters or equivalent. Inject 20 µl portions of each of the standard solutions into the chromatograph and plot the peaks areas obtained as a function of concentration in g/100 ml.

Procedure
Accurately weigh about 1.5 g of the sample into a 100 ml volumetric flask. Dissolve and make up to volume with bidistilled water. Filter through a 0.45 µm Millipore filter or equivalent. Inject duplicate 20 µl portions of the solution into the chromatograph and determine the mean peak area. Read the concentration of maltitol in the sample solution from the calibration curve and calculate the maltitol content of the sample from the formula:

\[
\text{maltitol content (\%)} = \frac{A \times 100}{S}
\]

Where

\( A \) = concentration of maltitol in the sample solution (g/100 ml)
\( S \) = weight of sample taken (g)
MALTITOL SYRUP*

SYNONYMS  
Hydrogenated high maltose-glucose syrup, hydrogenated glucose syrup; INS No. 965

DEFINITION  
A mixture consisting of mainly maltitol with sorbitol and hydrogenated oligo- and polysaccharides. It is manufactured by the catalytic hydrogenation of high maltose-content glucose syrup. The article of commerce is supplied both as a syrup and as a solid product.

Assay  
The following ranges apply on the dried basis:

<table>
<thead>
<tr>
<th>Component</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltitol</td>
<td>50 - 90%</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>less than 8%</td>
</tr>
<tr>
<td>Maltotriitol</td>
<td>5 - 25%</td>
</tr>
<tr>
<td>Hydrogenated polysaccharides</td>
<td>less than 30%</td>
</tr>
<tr>
<td>containing more than three glucose or glucitol units</td>
<td></td>
</tr>
</tbody>
</table>

DESCRIPTION  
Sweet-tasting, colourless and odourless, clear viscous liquids or sweet-tasting white crystalline masses.

FUNCTIONAL USE  
Sweetening agent; humectant; stabiliser

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility  
Very soluble in water, and slightly soluble in ethanol

** B. Specific gravity  
Not less than 1.360  
(does not apply to the dried product)

** C. Refractive index  
$n^\circ: 1.476 - 1.482$

** D. Specific rotation  
$[\alpha]_D^\circ: +105^\circ$ to $+125^\circ$

Determine the specific rotation for a solution of 7 g dry weight of sample in 100 ml of water to which 2 drops of Ammonia TS, strong, has been added

PURITY TESTS

** Water content  
Not more than 26% (Karl Fisher method)

** Sulfated ash  
Not more than 0.1%  
Test 3 g of sample as directed under the test for Ash (Sulfated ash) Method I

** Chlorides  
Not more than 50 mg/kg  
Test 10 g of sample by the Chlorides Limit Test using 1.5 ml of 0.01 N hydrochloric acid in the standard

* These specifications were prepared at the 33rd session of JECFA (1988) and published in FNP 38 (1988).

PURITY TESTS (continued)

* Sulfates
Not more than 100 mg/kg
Test 10 g of sample by the Sulfates Limit Test using 2.0 ml of 0.01 N sulfuric acid in the standard

Nickel
Not more than 2 mg/kg
Proceed as directed in the specifications for Sorbitol

* Lead
Not more than 1 mg/kg
Prepare a sample solution as directed under Limit Test for Lead for organic compounds and determine the lead content by atomic absorption

* Heavy metals
Not more than 10 mg/kg
Test 2 g of the sample in 25 ml of water as directed in the Heavy Metals Limit Test (Method I)

* Reducing sugar
Not more than 0.3%
Test 7 g of sample by the General Method for Reducing Substances (as glucose) Method II. The weight of cuprous oxide shall not exceed 50 mg

METHOD OF ASSAY
The method is composed of two parts. Maltitol, the major component of Maltitol syrups is determined, together with sorbitol, using Gas liquid chromatography. Maltotriitol and hydrogenated polysaccharides are assayed using High performance liquid chromatography.

Determination of Sorbitol and Maltitol

Principle
Sorbitol and maltitol are analysed by Gas liquid chromatography after transformation into sitylated derivatives.

Apparatus
Gas chromatograph with a flame ionization detector
Column: 5% OV 101 on chromosorb G, AW-DMCS 80-100 mesh,
length: 65 cm, internal diameter: 1/8 inch (3.175 mm)
Chromatographic injection and detection temperature 280°.

Conditions
- Column temperature: Programmed temperature increase from 169° to 290° at 8°/min
- Carrier gas: Nitrogen flow 25 ml/min
- Detector gases: Air 400 ml/min; Hydrogen 30 ml/min
- Injection volume: 1 µl

Reagents
- Anhydrous pyridine
- Trimethylsilylimidazole
- Trimethylchlorosilane (TMCS)
- Pure xylitol (internal standard for maltitol)
- Pure sorbitol
- Maltitol of known purity
- Reagent TRI-SIL Z: 2.1 g of Trimethylsilylimidazole in 10 ml of pyridine

Determination of Sorbitol and Maltitol (continued)

**Determination of response coefficients**

Prepare 2 mixtures containing:

<table>
<thead>
<tr>
<th></th>
<th>No. 1</th>
<th>No. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylitol</td>
<td>150 mg</td>
<td>150 mg</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>100 mg</td>
<td>150 mg</td>
</tr>
<tr>
<td>Squalane</td>
<td>300 mg</td>
<td>300 mg</td>
</tr>
<tr>
<td>Maltitol (dry)</td>
<td>150 mg</td>
<td>250 mg</td>
</tr>
</tbody>
</table>

Dissolve and adjust to 100 ml with pyridine.

In separate septum equipped vials, introduce 0.2 ml of mixture 1 or 2, 0.2 ml of TRI-SIL Z. Cap and through septum inject 2 ml of TMCS. Stir. Place in drying oven at 60° for 15 min. Cool and inject.

The order of the elution is as follows:

Xylitol - Sorbitol - Squalane - Maltitol

**Analysis**

Prepare a solution containing the following internal standards:

- Xylitol 1500 mg
- Squalane 3000 mg

Dissolve and adjust to 100 ml with pyridine. In a 10 ml tare box, weigh exactly a sample of about 70 to 100 mg of the Maltitol Syrup (MS) being tested. Add 1 ml of internal standards solution and 9 ml of pyridine. Stir until total dissolution. In a septum equipped vial, take off 0.2 ml of the solution and add 0.2 ml of TRI-SIL Z. Cap and inject 2 ml of TMCS. Place in drying oven at 60° for 15 min. Cool and inject.

**Calculation**

Using the response coefficient mixture with chromatographic responses most similar to those of the sample, compute response coefficients (K) for the following pairs of analyte and standard: sorbitol - xylitol and maltitol - squalane, based on chromatographic peak areas.

\[
K = \frac{\text{analyte area}}{\text{standard area}} \times \frac{\text{standard weight}}{\text{analyte weight}}
\]

Calculate the percentage sorbitol and maltitol in Maltitol Syrup using the response coefficients and dry weight of MS sample:

\[
= \frac{\text{analyte area}}{\text{standard area}} \times \frac{1}{K} \times \frac{\text{standard weight}}{\text{MS weight}} \times 100
\]

**NOTE:** Standard solution may be stored for several weeks.
Determination of hydrogenated oligo- and polysaccharides

Apparatus
- High performance liquid chromatograph (HPLC)
- Detection: differential refractometer
- Integrator recorder
- Column: AMINEX 50 W-X4 20-30 μm (silver form)
  2 columns series length 30 cm
- Internal diameter 9.5 mm each
- Eluent: double distilled degassed water (filtered through Millipore membrane filter 1.2 μm)

Chromatographic conditions
- Column temperature: 85°C
- Eluent flow: 0.5 ml/min

Sample preparation
Adjust sample concentration to 20% dry matter in water. Inject a 25 ml sample of the 20% solution through a 0.65 μm filter. Usual analysis time is 40 min.

Calculation
Results are expressed as percentage of total peak area for eluents separated through sorbitol (about 40 min). The order of elution is: hydrogenated polysaccharides (in a broad band of closely spaced peaks), maltotriitol, maltitol (and other dimers) and sorbitol (and other monomers). If one is uncertain of the position of sorbitol in the chromatogram, a sorbitol standard should be run. Compute the percentage of maltotriitol and hydrogenated polysaccharides in the MS sample as percentage of total peak area for chromatographic peaks eluted through the sorbitol peak.
** MALTOL*  

** SYNONYM**  
INS No. 636

** DEFINITION**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical name</td>
<td>3-Hydroxy-2-methyl-4-pyrone</td>
</tr>
<tr>
<td>C.A.S. number</td>
<td>118-71-8</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>$\text{C}_6\text{H}_8\text{O}_3$</td>
</tr>
<tr>
<td>Structural formula</td>
<td><img src="attachment%D9%84%D9%89" alt="Structural formula" /></td>
</tr>
<tr>
<td>Molecular weight</td>
<td>126.11</td>
</tr>
<tr>
<td>Assay</td>
<td>Content not less than 99% of $\text{C}_6\text{H}_8\text{O}_3$</td>
</tr>
</tbody>
</table>

** DESCRIPTION**

White to off-white crystalline powder having a characteristic caramel-butterscotch odour.

** FUNCTIONAL USE**

Flavouring agent

** CHARACTERISTICS**

** IDENTIFICATION TESTS**

**A. Solubility**  
Soluble in water and ethanol.

**B. Melting range**  
160° - 164°

**C. Colour reaction for phenolic compounds**  
Passes test.  
See description under TESTS

**D.**  
Passes test.  
See description under TESTS.

**E. Iodoform reaction**  
Passes test.  
See description under TESTS.

**Sulfated Ash**  
Not more than 0.2%. Proceed as directed in the test of Ash (Sulfated ash, Method I), using 1 g of the sample.

**Arsenic**  
Not more than 3 mg/kg (Method II)

---

* These specifications were prepared at the 25th session of JECFA (1981) and published in FNP 19 (1981) superseding the earlier specifications published in NMRS 44B (1967).

PURITY TESTS

* Lead
   Not more than 10 mg/kg

* Heavy metals
   Not more than 20 mg/kg. Test 1 g of the sample as directed in the Limit Test (Method II).

TESTS

IDENTIFICATION TESTS

C. Colour reaction for phenolic compounds
   Dissolve 0.1 g of the sample in 10 ml of ethanol and add 3 drops of ferric chloride TS. A reddish violet colour is produced.

D. Iodoform reaction
   Dissolve 0.5 g of the sample in 10 ml of sodium hydroxide TS and pass carbon dioxide through the solution. White crystals are formed; collect and recrystallize from dilute ethanol. The crystals melt between 160° - 164°.

E. Iodoform reaction
   Dissolve 0.1 g of the sample in 5 ml dioxane, add 1 ml of sodium hydroxide TS, and add sufficient iodine-potassium iodide TS (Iodine TS) with shaking until the colour remains. Heat on a water bath for 5 min. Yellow crystals are formed.

METHOD OF ASSAY

Standard Solution:
Transfer about 50 mg of Maltol Reference Standard**, accurately weighed, into a 250-ml flask, dilute to volume with 0.1 N hydrochloric acid, and mix. Pipet 5 ml of this solution into a 100-ml volumetric flask, dilute to volume with 0.1 N hydrochloric acid, and mix.

Assay Solution:
Transfer about 50 mg of the sample, accurately weighed, into a 250-ml flask, dilute to volume with 0.1 N hydrochloric acid. Pipet 5 ml of this solution into a 100-ml volumetric flask, dilute to volume with 0.1 N hydrochloric acid, and mix.

Procedure:
Determine the absorbance of each solution in a 1-cm quartz cell at 274 nm using 0.1 N hydrochloric acid as the blank.

Calculation:
Calculate the percent of Maltol in the sample by the formula:

\[
\text{\% of Maltol} = \frac{W_s \times A_A \times 100}{A_s \times W_A}
\]

where:
- \( A_A \) = absorbance of the Assay Solution
- \( A_s \) = absorbance of the Reference Standard Solution
- \( W_A \) = weight in mg of the Assay solution (sample)
- \( W_s \) = weight in mg of the Reference Standard


** Available from the United States Pharmacopeia, 12601 Twinbrook Parkway, Rockeville, Md. 20852, USA.
MANNUITOL*

SYNONYMS

d-Mannitol, mannite
INS No. 421, EEC No. E421

DEFINITION

Chemical name
d-Mannitol
C.A.S. number 69-65-8
Chemical formula C₄H₇O₆
Structural formula

\[
\begin{align*}
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H} \\
\text{OH} & \quad \text{OH} \\
\text{OH} & \quad \text{OH}
\end{align*}
\]

Molecular weight 182.2
Assay Not less than 96.0% and not more than 102.0% d-mannitol on the anhydrous basis

DESCRIPTION
Sweet tasting, white, odourless, crystalline powder

FUNCTIONAL USES
Sweetening agent; humectant; stabilizer

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Soluble in water, very slightly soluble in ethanol, practically insoluble in chloroform and in ether

** B. Melting range
Between 165° and 169° with softening at a lower temperature

C. Main peak in GLC
The sample has the same GLC elution time as the mannitol standard used in the assay (see Method of Assay)

D. Ferric derivative
Passes test
See description under TESTS

PURITY TESTS

* Loss on drying
Not more than 0.3% (105°, 4 h)
Specific rotation
Passes test
See description under TESTS

* These specifications were prepared at the 33rd session of JECFA (1988) and published in FNP 38 (1988).

PURITY TESTS (continued)

* pH
Between 5 to 8
Add 0.5 ml of a saturated solution of potassium chloride to 10 ml of a 10% w/v solution of the sample then measure the pH.

* Sulfated ash
Not more than 0.1%
Test 2 g of the sample as directed under the test for Ash (Sulfated ash, Method I).

* Chlorides
Not more than 70 mg/kg
Test 10 g of sample by the Chlorides Limit Test using 2.0 ml of 0.01 N hydrochloric acid in the standard.

* Sulfates
Not more than 100 mg/kg
Test 10 g of sample by the Sulfates Limit Test using 2.0 ml of 0.01 N sulfuric acid in the standard.

**Nickel**
Not more than 2 mg/kg
Proceed as directed in the specifications for Sorbitol.

* *Arsenic*
Not more than 3 mg/kg (Method II)
Proceed as directed in the Limit Test for Arsenic, Method II.

* Lead
Not more than 1 mg/kg
Prepare a sample solution as directed under Limit Test for Lead for organic compounds and determine the lead content by atomic absorption.

* Heavy metals
Not more than 10 mg/kg
Test a solution of 2 g of the sample in 25 ml water as directed in the Limit Test (Method I).

* Reducing sugars
Not more than 0.3%
Test 7 g of the sample by the General Method for Reducing Substances (as glucose) Method II. The weight of cuprous oxide shall not exceed 50 mg.

**Total sugars**
Not more than 1.0% as glucose
See description under TESTS

TESTS

IDENTIFICATION TESTS

D. Ferric derivative
Add 5 drops of a saturated solution of the sample to 1 ml of ferric chloride TS (*") in a Nessler tube. Add 5 drops of water to a second Nessler tube containing 1 ml of ferric chloride TS. Add 5 drops of 5 N sodium hydroxide to each tube: a brown precipitate forms in the blank. A yellow precipitate in the sample tube indicates the presence of mannitol. On vigorous shaking a clear solution results in the sample tube but the brown precipitate in the blank tube persists. Additional 5 N sodium hydroxide causes no precipitation in the sample tube but further precipitation takes place in the blank.

PURITY TESTS

* Specific rotation

Accurately weigh and dissolve 2.0 g of sample and 2.6 g of disodium tetraborate in about 20 ml of water previously heated to about 30°C; shake continuously for 15-30 min without further heating. Dilute the resulting clear solution to 25 ml with water. The specific rotation, calculated with reference to the anhydrous substance, is between +23° and +25°.

* Total sugars

Transfer 2.1 g of the sample into a 250 ml flask fitted with a ground glass joint, add 40 ml of 0.1 N hydrochloric acid, attach a reflux condenser, and reflux for 4 h. Transfer the solution to a 400 ml beaker, rinsing the flask with about 10 ml of water, neutralize with 6 N sodium hydroxide and proceed as directed in the General Method for Reducing Substances (as glucose) Method II. The weight of the cuprous oxide shall not exceed 50 mg.

METHOD OF ASSAY

Gas-liquid chromatography method

Reagent-internal standard preparation
Dissolve a suitable quantity of n-hutyboronic acid in pyridine to obtain a solution having a concentration of about 20 mg per ml. This is the reagent solution. Add a suitable quantity of methyl nonadecanoate, the internal standard, to the reagent solution to obtain a solution having a concentration of about 2 mg per ml. This is the reagent-internal standard preparation.

Standard preparation
Dissolve an accurately weighed quantity of Mannitol reference standard (United States Pharmacopoeia) in water to obtain a solution having a concentration of about 1.5 mg per ml.

Assay preparation
Dissolve an accurately weighed quantity of the sample, equivalent to about 75 mg of anhydrous mannitol in water in a 100 ml volumetric flask, dilute to volume with water, and mix.

Procedure
Pipet 1 ml portions of the standard preparation and the test preparation into separate flat-bottom, wide-mouth vials, about 4 cm high and not less than 1.7 cm in internal diameter, so that the liquid forms a shallow, even pool. Transfer the vials to a vacuum oven, and heat at a temperature not higher than 50°C to dryness. Add 1.0 ml reagent internal standard preparation to each residue, mix, and allow to stand for 20 min. Inject a 1.0 µl portion of the solution from the standard preparation into a suitable gas chromatograph equipped with a hydrogen flame-ionization detector, and a 1.8 m x 4 mm column packed with 3% liquid phase G 7 on support SIA.

Gas-liquid chromatography method

Procedure (continued)
The carrier gas is nitrogen flowing at 50 ml per min. The temperature of the injection port is 245°, the column temperature is 205°, and the detector temperature is 260°. The retention time of the internal standard is about 4 min, and that of mannitol is about 6.5 min. In a suitable chromatogram, the resolution, $R_e$, is not less than 4.0 between the peaks for mannitol and the internal standard, and six replicate injections of the solution from the standard preparation show a relative standard deviation of not more than 1.5%. Similarly, inject a 1.0 µl portion of the solution from the test preparation. Calculate the chromatographic purity by the formula $100 \left( \frac{C_sR_u}{C_uR_s} \right)$, in which $C_s$ is the concentration, in mg per ml, of USP Mannitol RS in the standard preparation, $C_u$ is the concentration, in mg per ml, of specimen in the test preparation, and $R_u$ and $R_s$ are the ratios of the peak areas of mannitol to those of the internal standard of the test preparation and the standard preparation, respectively. The value is between 96 and 102.0.

High performance liquid chromatography (Alternative method)

See Method of Assay in the specifications for Sorbitol.
dl-MENTHOL*

SYNONYM
dl-3-p-Menthanol

DEFINITION

- Chemical names: dl-Menthol, (RS)-menthol, rac-menthol
- C.A.S. number: 89-78-1
- Chemical formula: C_{10}H_{18}O
- Structural formula:

\[
\begin{align*}
\text{CH}_3 & \\
\text{OH} & \\
\text{H}_3C & \text{CH}_3
\end{align*}
\]

- Molecular weight: 156.27

DESCRIPTION

Colourless prisms or needles, or a white crystalline powder having a peppermint-like odour.

FUNCTIONAL USE

Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Melting range

32° - 38°

** B. Solidification point

Passes test. See description under TESTS.

** C. Specific rotation

[\alpha]_D^2 : -2° to +2°

PURITY TEST

Readily oxidizable substances

Passes test. See description under TESTS.

* These specifications were prepared at the 11th session of JFCFA (1967) and published in NMRS 44B (1969).

TESTS

IDENTIFICATION TEST

B. Solidification point

Determine as directed in the General Methods*, using a sample previously dried in a desiccator over silica gel for 24 h and adjusting the temperature of the cooling bath to a temperature between 23° and 25°. dl-Menthol solidifies between 27° and 28°. Continue the stirring. After a few minutes the temperature quickly rises to 30.5° to 32°.

PURITY TEST

Readily oxidizable substances

Transfer 500 mg of dl-menthol into a clean, dry test tube, add 10 ml of potassium permanganate TS. Place the test tube in a beaker of water maintained between 45° and 50°. At 30 sec intervals, quickly remove the test tube from the bath and shake. The colour of potassium permanganate is still apparent after 5 min.

I-MENTHOL*

SYNONYM
1-3-p-Menthanol

DEFINITION

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>1-Menthol</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A.S. number</td>
<td>2216-51-5</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>C₁₀H₁₉O</td>
</tr>
<tr>
<td>Structural formula</td>
<td><img src="image" alt="Structural formula" /></td>
</tr>
</tbody>
</table>

Molecular weight 156.27

DESCRIPTION

Colourless prisms or needles, or a white crystalline powder having a peppermint-like odour.

FUNCTIONAL USE

Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Melting range 41° - 44°

** B. Specific rotation [α]D: -40° to -52°

---

* These specifications were prepared at the 11th session of JFCFA (1967) and published in NMRS 44B (1969).

METHANOL*

SYNONYMS

Carbinol

DEFINITION

Chemical name: Methanol, methyl alcohol
C.A.S. number: 67-56-1
Chemical formula: CH₄O
Structural formula: CH₂OH
Molecular weight: 32.04
Assay: Content not less than 99.5% of CH₄O

DESCRIPTION

Clear colourless, mobile liquid with a characteristic odour

FUNCTIONAL USE

Extraction solvent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Miscible with water, ether and ethanol

** B. Specific gravity
0.792 - 0.795

** C. Refractive index
nD: 1.328 - 1.330

D. Boiling point
About 65°

PURITY TESTS

** Distillation range
Between 64.5° and 65.5°

** Non-volatile residue
Not more than 3 mg/100 ml

** Water content
Not more than 0.1% (Karl Fisher Method)

Arsenic
Not more than 1 mg/kg (Method II)
See description under TESTS

Heavy metals
Not more than 1 mg/kg
See description under TESTS

Acidity
Not more than 15 mg/kg as formic acid
See description under TESTS

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* These specifications were prepared at the 28th session of JECFA (1984) and published in FNP 31/2 (1984).

PURITY TESTS (continued)

**Alkalinity**
Not more than 3 mg/kg as NH₃
See description under TESTS

**Aldehydes and ketones**
Not more than 0.015% w/v (as acetone)
See description under TESTS

TESTS

**PURITY TESTS**

* **Arsenic**
Transfer 3 g of the sample and 30 ml of water into the generator flask, and proceed as directed in the Limit Test (Method II).

* **Heavy metals**
Evaporate 20 g of the sample to dryness on a steam bath in a glass evaporating dish. Cool, add 2 ml of hydrochloric acid TS, and slowly evaporate to dryness again on the steam bath. Moisten the residue with 1 drop of hydrochloric acid TS, add 10 ml of hot water, and digest for 2 min. Cool, and dilute to 25 ml with water. Test this solution as directed in the Limit Test (Method I).

**Acidity**
To a mixture of 10 ml of ethanol and 25 ml of water add 0.5 ml of phenolphthalein TS, and titrate with 0.02 N sodium hydroxide to the first pink colour that persists for at least 30 sec. Add 19 ml (about 15 g) of the sample, mix and titrate with 0.02 N sodium hydroxide until the pink colour is restored. Not more than 0.25 ml is required.

**Alkalinity**
Add 1 drop of methyl red TS to 25 ml of water, add 0.02 N sulfuric acid until a red colour just appears, then add 29 ml (about 22.5 g) of the sample, mix. Not more than 0.2 ml of 0.02 N sulfuric acid is required to restore the red colour.

**Aldehydes and ketones**

**Principle**
The aldehydes and ketones present are converted with 2,4-dinitrophenylhydrazine into the corresponding 2,4-dinitrophenylhydrazones. In alkaline medium these have a red colour, which is determined spectrophotometrically or visually.

**Apparatus**
- Photoelectric absorptionmeter or spectrophotometer, with 0.5-cm cells.
Alternatively flat bottom tubes, capacity about 20 ml
- Water bath, controlled at 60° ± 1°

**Reagents**
The reagents used shall be of a recognized analytical reagent quality. Distilled water or water of at least equal purity shall be used throughout

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PURITY TESTS (continued)

Aldehyde and ketones

Reagents (continued)
- Carbonyl-free methanol: Reflux 1000 ml of methanol with 5 g of 2,4-dinitrophenylhydrazine and 5 drops of concentrated hydrochloric acid \((d = 1.18)\) for 2-3 h. Distil off the methanol using a 300 mm by 25 mm diameter Widmer or other suitable distillation column. Reject the first 100 ml and collect the next 800 ml, rejecting the remainder. If, in spite of the precautions taken, the distillate is found to be coloured, then it should be re-distilled.

- 2,4-Dinitrophenylhydrazine solution: Dissolve 0.03 g of 2,4-dinitrophenylhydrazine in 40 ml of the carbonyl-free methanol containing 0.3 ml of concentrated hydrochloric acid \((d = 1.18)\) and dilute to the mark in a 50-ml one-mark volumetric flask with the carbonyl-free methanol. Prepare this solution fresh each day.

- Potassium hydroxide solution: Dissolve 10 g of potassium hydroxide in 10 ml of water, cool and dilute to the mark in a 50-ml one-mark volumetric flask with carbonyl-free methanol. Prepare this solution fresh each day.

- Standard acetone solution: Weigh 1.00 g of acetone and dilute to the mark in a one-mark 100 ml volumetric flask with carbonyl-free methanol. Dilute 1.0 ml of this solution to 100 ml with the carbonyl-free methanol. 1 ml of the diluted solution contains 0.1 mg of acetone.

Procedure
Prepare five solutions by diluting 1.0, 2.0, 4.0, 8.0 and 10.0 ml portions of the standard acetone solution to 25.0 ml with carbonyl-free methanol. To 1.0 ml of each of the solutions thus obtained (containing 0.004 - 0.04 mg of acetone/ml), contained in a test tube fitted with a ground glass stopper, add 1.0 ml of the 2,4-dinitrophenylhydrazine solution. Stopper the tube and heat for 50 min in the water bath at 60°, cool, add 8.0 ml of the potassium hydroxide solution and after 5 to 15 min measure the optical density of each solution at a wavelength of 430 nm using as a blank 1.0 ml of the carbonyl-free methanol treated in the same way. Prepare a calibration chart by plotting weights (in mg) of acetone against corresponding values of optical density.

Dilute 5.0 ml of the sample to 25.0 ml with the carbonyl-free methanol. Transfer 1.0 ml of this solution to a test tube fitted with a ground glass stopper and add 1.0 ml of the 2,4-dinitrophenylhydrazine solution. Stopper the tube and heat for 50 min in the water bath at 60°, cool, add 8.0 ml of the potassium hydroxide solution and after 5 to 15 min measure the optical density of the solution at the wavelength of 430 nm using as a blank 1.0 ml of the carbonyl-free methanol treated in the same way. By reference to the calibration chart prepared as described above, read the acetone content (in mg) of the solution.

The content is not more than 0.03 mg.
METHOD OF ASSAY

Using the procedures for Gas chromatography described in the General Methods*, establish the following conditions:

- Column: 1.8 m length, 4 mm internal diameter packed with 120-150 mesh Porapak R, or equivalent
- Carrier gas: nitrogen, at flow rate of 25 ml/min
- Detector: flame ionization type
- Temperatures: injection port - 200°
  column - about 160°
  detector - 210°

Prepare standard solution of 0.4% (v/v) methanol in dioxane. Adjust column temperature and/or gas flow rate so that methanol retention time is about 5-7 min. Adjust detector so that 8 µl of standard solution provides at least one-half scale deflection. Inject 5-10 µl sample, obtain chromatogram and determine methanol content by the method of area normalization.

METHYL ANTHRANILATE*

SYNONYMS
Methyl $\alpha$-aminobenzoate, 2-aminobenzoic acid methyl ester

DEFINITION
Chemical names
Methyl 2-aminobenzoate, methyl anthranilate
C.A.S. number
134-20-3
Chemical formula
C$_7$H$_8$NO$_3$
Structural formula
\[
\begin{array}{c}
\text{HN} \\
\text{COOCH}_3
\end{array}
\]
Molecular weight
151.17
Assay
Content not less than 98% of C$_7$H$_8$NO$_3$

DESCRIPTION
Colourless to pale yellow liquid (above 24°C) with a characteristic bluish fluorescence and a Concord grape-like odour.

FUNCTIONAL USE
Flavouring agent

CHARACTERISTICS
IDENTIFICATION TESTS
** A. Solubility
Slightly soluble in water. Soluble in propane-1,2-diol and most fixed oils. Insoluble in glycerol.

** B. Refractive index
$n_P^P : 1.580 - 1.585$ (in the supercooled liquid form)

** C. Specific gravity
$d_T^P : 1.161 - 1.169$

PURITY TESTS
** Solubility in ethanol
1 ml dissolves in 5 ml of 60% ethanol at 25°C

** Acid value
Not more than 1.0

** Solidification point
Not less than 22°C
Determine as directed in the General Methods, supercooling the sample to 18°C, and seeding the melt to induce crystallization.

* These specifications were prepared at the 23rd session of JECFA (1979) and published in FNP 12 (1979) superseding the earlier specifications published in NMRS 44B (1969).

METHOD OF ASSAY

Weigh accurately about 1 g of the sample and proceed as directed under the method for Ester Determination in the General Methods*, using 75.59 as the equivalence factor (e) in the calculation.

** See General Methods (Guide to JECFA Specifications), FNP 5/Rev. 2 (1991)
METHYL CELLULOSE*

SYNONYMS

Cellulose methyl ether;
INS No. 461, EEC No. E461

DEFINITION

Methyl Cellulose is the methyl ether of cellulose. It is prepared from wood pulp or cotton by treatment with alkali and methylation of the alkali cellulose with methyl chloride. The article of commerce can be specified further by viscosity.

Chemical names

Methyl ether of cellulose
Cellulose methyl ether

C.A.S. number

9004-67-5

Chemical formula

\[ \{C_6H_{12}O_6(OH)_x(OCH_3)_y\}_n \]

where 

\[
\begin{align*}
x & = 1.00 \text{ to } 1.55 \\
y & = 2.00 \text{ to } 1.45 \\
x+y & = 3.00 \\
(y & = \text{degree of substitution})
\end{align*}
\]

Structural formula

![Structural formula of Methyl Cellulose](image)

where \( R = \text{H or CH}_3 \)

Molecular weight

Unsubstituted structural unit: 162.14
Structural unit with total degree of substitution of 1.45: 182
Structural unit with total degree of substitution of 2.00: 190
Macromolecules: from about 20 000 \((n \text{ about 100})\)
up to about 380 000 \((n \text{ about 2,000})\)

Assay

Content no less than 25% and not more than 33% of methoxyl groups**

---

* These specifications were prepared at the 28th session of JECFA (1984) and published in FNP 31/2 (1984).

** Some products of commerce designated "methyl cellulose" also contain components substituted with small amounts (max. 5%) of hydroxyethyl and/or hydroxypropyl groups. Development of separate specifications for these products should be considered.
DESCRIPTION

Hygroscopic white or off-white, odourless and tasteless fine granules, filaments or powder

FUNCTIONAL USES

Thickening agent, emulsifier, stabilizer

CHARACTERISTICS

IDENTIFICATION TESTS

A. Solubility
   Passes test
   See description under TESTS

B. Foam test
   Passes test
   See description under TESTS

C. Precipitate formation
   Passes test
   See description under TESTS

PURITY TESTS

A. Loss on drying
   Not more than 10% (105°, about 3 h)

B. pH
   5.0 - 8.0 (1 in 100 soln)

C. Sulfated ash
   Not more than 1.5%
   Proceed as directed under the test for Sulfated ash (Method I), using 1 g of the sample

D. Arsenic
   Not more than 3 mg/kg (Method II)

E. Lead
   Not more than 10 mg/kg

F. Heavy metals
   Not more than 40 mg/kg
   Test 0.5 g of the sample as directed in the Limit Test (Method II)

TESTS

IDENTIFICATION TESTS

A. Solubility
   Swelling in water, producing a clear to opalescent, viscous, colloidal solution
   Insoluble in ethanol, ether and chloroform
   Soluble in glacial acetic acid

B. Foam test
   An 0.1% solution of the sample is shaken vigorously. A layer of foam appears.
   (This test permits the distinction of sodium carboxymethyl cellulose from other cellulose ethers.)

C. Precipitate formation
   To 5 ml of an 0.5% solution of the sample, add 5 ml of a 5% solution of copper sulfate or of aluminium sulfate. No precipitate appears. (This test permits the distinction of sodium carboxymethyl cellulose from other cellulose ethers.)

METHOD OF ASSAY

Determine the content of methoxyl groups by the method for Ethoxyl and Methoxyl Group Determination in the General Methods.*

α-METHYL CINNAMIC ALDEHYDE*  
(Tentative)**

SYNONYM  
α-Methylcinnamaldehyde

DEFINITION

- Chemical name: 2-Methyl-3-phenyl-2-propenal
- C.A.S. number: 101-39-3
- Chemical formula: C_{10}H_{10}O
- Structural formula:

```
  C H |  C H |  O
     \  /   \  /
    H   H   O
```

- Molecular weight: 146.19
- Assay: Content not less than 97% of C_{10}H_{10}O

DESCRIPTION

Yellow liquid with a cinnamon-like odour

FUNCTIONAL USE

Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility  
Soluble in ethanol, in fixed oils and in propane-1,2-diol, insoluble in glycerol and water

*** B. Refractive index  
\( n_\beta ^2 : 1.602 - 1.607 \)

*** C. Specific gravity  
\( d_2^\circ : 1.037 - 1.040, d_4^\circ : 1.035 - 1.039 \)

D. Infrared spectrum  
See Appendix at the end of these specifications

---

* These specifications were prepared at the 24th session of JECFA (1980) and published in FNP 17 (1980).

** The references to identity, purity and methods of analysis were felt to require further confirmation. Information required on proportions of the cis- and trans- isomers.

PURITY TESTS

* Solubility in ethanol
  1 ml dissolves in 2 ml of 70% ethanol

* Acid value
  Not more than 3

METHOD OF ASSAY

Method A
Weigh accurately about 2.3 g of the sample and proceed as directed under the method for Aldehyde Determination in the General Methods*, using 73.10 as the equivalence factor (e) in the calculation.

Method B
Determine by gas-liquid chromatography, as directed in the Method of Assay for Allyl-α-ionone.

APPENDIX

** Infrared spectrum: α-Methyl Cinnamic Aldehyde

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** Infra-red spectra through the courtesy of the International Organization of the Flavour Industry (IOFI), Geneva, Switzerland, and of the Sudler Research Laboratories, Inc., Philadelphia, USA.
6-METHYLCOUMARIN*
(Tentative)**

SYNONYM
6-Methylbenzopyrone

DEFINITION
Chemical name 6-Methylcoumarin
C.A.S. number 92-48-8
Chemical formula C_{10}H_{8}O_{2}
Structural formula

Molecular weight 160.18
Assay Information required**

DESCRIPTION
White, crystalline solid, with a sweet, fruity hay-like odour.

FUNCTIONAL USE
Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility
Soluble in hot ethanol and in fixed oils, slightly soluble in hot water

B. Infrared spectrum
See Appendix at the end of these specifications.

PURITY TEST

*** Melting point
Not lower than 73.5°.

---

* These specifications were prepared at the 24th session of JECFA (1980) and published in FNP 17 (1980).

** The references to identity, purity and methods of analysis were felt to require further confirmation. Information on assay is required.

METHOD OF ASSAY

Determine by gas-liquid chromatography, using the following conditions.

- Column length: 1.60 m
- Column diameter: 3.0 mm
- Column material: glass
- Column packing: S.P. 1000 4%
- Column support: Chromosorb 100/120 mesh
- Carrier gas: Helium
- Flow rate: 50 ml/min
- Detector type: FID
- Detector temperature: 250°
- Temperature of inj. port: 250°
- Column temperature
  - Isothermal: 200°
  - Temperature programme: 5°/min
  - Final temp. isothermal: 250°

The above general conditions are to be applied together with the information provided under the General Methods.

Remark: Allow the chromatogram to develop until all compounds have been eluted.

APPENDIX

** Infrared spectrum: 6-Methyl Coumarin


** Infrared spectrum through the courtesy of the International Organization of the Flavour Industry (IOFI), Geneva, Switzerland, and of the SADTLER RESEARCH LABORATORIES, Inc. Philadelphia, USA.
METHYL ETHYL CELLULOSE*

SYNONYMS
MEC; INS No. 465, EEC No. E465

DEFINITION
Methyl Ethyl Cellulose is prepared from cellulose by treatment with alkali, dimethyl sulfate and ethyl chloride. It is a mixed ether of cellulose in which both the methyl and ethyl groups are attached to the anhydroglucose units by ether linkages. The article of commerce can be specified further by viscosity.

Chemical names
Ethyl methyl ether of cellulose

C.A.S. number
9004-69-7

Chemical formula
\{\text{C}_6\text{H}_{12}\text{O}_6\text{(OH)}_2\text{(OCH)}_3\text{(OC}_2\text{H}_5)_y\}_n

where 
\begin{align*}
z &= 0.57 \text{ to } 0.8 \\
y &= 0.2 \text{ to } 0.4 \\
x &= 3 - (x + y) \\
(y + z) &= \text{degree of substitution}
\end{align*}

Structural formula

\[\begin{array}{c}
\text{H} \\
\text{O} \\
\text{OR} \\
\text{H} \\
\text{OR} \\
\text{H} \\
\text{OR} \\
\text{CH}_2\text{OR}
\end{array}\]

Where \(R = \text{H or CH}_3\) or \(\text{C}_2\text{H}_5\)

Molecular weight
Unsubstituted structural unit: 162.14
Structural unit with a total degree of substitution of 0.77: 181
Structural unit with a total degree of substitution of 1.2: 190
Macromolecules: 30 000 - 40 000 (\(n\) about 200)

Assay
Methyl Ethyl Cellulose contains, on the dried basis, not less than 3.5% and not more than 6.5% of methoxyl groups (-OCH\(_3\)), not less than 14.5% and not more than 19.0% of ethoxyl groups (-OCH\(_2\)CH\(_3\)), and not less than 13.2% and not more than 19.6% of total alkoxy groups, calculated as methoxyl.

DESCRIPTION
Methyl ethyl cellulose is a hygroscopic and slightly yellowish odourless and tasteless fibre or powder

FUNCTIONAL USES
Emulsifier, stabilizer, thickening agent, foaming agent

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).
CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility  Swelling in water, producing a clear to opalescent, viscous, colloidal solution. Insoluble in ethanol.

B. Foam production  Passes test  See description under Tests

C. Precipitate formation  Passes test  See description under Tests

* D. Determine the substituents by Gas Chromatography

PURITY TESTS

* Loss on drying  Not more than 15% for the fibrous form, and not more than 10% for the powdered form, after drying to constant weight

* Sulfated ash  Not more than 0.6%  Proceed as directed under the test for Ash (Sulfated ash, Method I) using 1.0 g of the sample

* Arsenic  Not more than 3 mg/kg  A sample solution prepared as directed for organic compounds will meet the requirements of the Limit Test for Arsenic (Method II)

* Lead  Not more than 10 mg/kg  A sample solution prepared as directed for organic compounds will meet the requirements of the Limit Test for Lead

* Heavy metals  Not more than 20 mg/kg  Test 1.0 g of the sample as directed in Method II under the Limit Test for Heavy Metals using 20 μg of lead ion (Pb) in the control (Solution A)

TESTS

IDENTIFICATION TESTS

B. Foam production  A 0.1% solution of the sample is shaken vigorously. A layer of foam appears. (This test permits the distinction of sodium carboxymethyl cellulose from other cellulose ether and alginates and natural gums).

C. Precipitate formation  To 5 ml of an 0.5% solution of the sample add 5 ml of a 5% solution of copper sulfate or of aluminium sulfate. No precipitate appears. (This test permits the distinction of cellulose ethers from sodium carboxymethyl cellulose, gelatine, carob bean gum and tragacanth).

METHOD OF ASSAY

Determination of the Ethoxyl group*

Apparatus
The apparatus for ethoxyl group determination is shown in the accompanying diagram. The boiling flask, D, is fitted with an aluminium foil-covered Vigreaux column, E, on the sidearm and with a bleeder tube through the neck and to the bottom of the flask for the introduction of steam and nitrogen. A steam generator, B, is attached to the bleeder tube through tube C, and a condenser, F, is attached to the Vigreaux column. The boiling flask and steam generator are immersed in an oil bath, A, equipped with a thermoregulator such that a temperature of 155° and the desired heating rate may be maintained. The distillate is collected in a 150-ml beaker, G, or other suitable container.

Procedure
Transfer about 100 mg, weighed to the nearest 0.1 mg, of the sample, previously dried at 105° for 2 h, into the boiling flask, and add 10 ml of chromium trioxide solution (60 g in 140 ml of water). Immerse the steam generator and the boiling flask in the oil bath (at room temperature) to the level of the top of the chromium trioxide solution. Start cooling water through the condenser and pass nitrogen gas through the boiling flask at the rate of one bubble per second. Starting at room temperature, raise the temperature of the oil bath to 155° over a period of not less than 30 min, and maintain this temperature until the end of the determination. Distil until 50 ml of distillate is collected. Detach the condenser from the Vigreaux column, and wash it with water, collecting the washings in the distillate container. Titrate the combined washings and distillate with 0.02 N sodium hydroxide to a pH of 7.0, using a pH meter set at the expanded scale. (Note: Phenolphthalein TS may be used for this titration if it is also used for all standards and blanks.)

* Ethoxyl and methoxyl can be separately determined by gas chromatography (Cobler, Samsel and Beaver, Talanta, 2, 473, 1962).
**Procedure (continued)**

Record the volume, $V_1$, of the 0.02 N sodium hydroxide used. Add 500 mg of sodium bicarbonate and 10 ml of dilute sulfuric acid TS, and then, after evolution of carbon dioxide has ceased, add 1 g of potassium iodide. Stopper the flask, shake the mixture and allow it to stand in the dark for 5 min. Titrate the liberated iodine with 0.02 N sodium thiosulfate to the sharp disappearance of the yellow colour, confirming the end-point by the addition of a few drops of starch TS. Record the volume of 0.02 N sodium thiosulfate required as $Y_1$.

Make several reagent blank determinations, using only the chromium trioxide solution in the above procedure. The ratio of the sodium hydroxide titration ($V_b$), corrected for variation in normalities, will give the acidity-to-oxidizing ratio $V_b/Y_1 = K$, for the chromium trioxide carried over in the distillation. The factor $K$ should be constant for all determinations.

Make a series of blank determinations using 100 mg of methyl cellulose (containing no foreign material) in place of the sample, recording the average volume of 0.02 N sodium hydroxide required as $V_m$ and the average volume of 0.02 N sodium thiosulfate required as $Y_m$.

**Calculation**

Calculate the ethoxyl content of the sample, in mg, by the formula:

$$45.0 \times \{N_1(V_1 - V_m) - kN_2(Y_1 - Y_m)\},$$

in which:

- $N_1 = \text{exact normality of the } 0.02 \text{ N sodium hydroxide solution,}$
- $N_2 = \text{exact normality of the } 0.02 \text{ N sodium thiosulfate solution,}$
- $k = \frac{V_bN_1}{Y_1N_2}$

Record the percentage of ethoxyl as B%.

**Determination of the Methoxyl content**

Determine the Ethoxyl plus Methoxyl content (Total alkoxyl content) as directed under Ethoxyl and Methoxyl Group Determinations in General Methods*.

Then calculate the Methoxyl content as follows:

$$\% \text{ Methoxyl} = \frac{31}{45} \times (A - B)$$

where A is the total alkoxyl content expressed as % ethoxyl, B is the Ethoxyl content expressed as %, as determined above.

**Determination of Total Alkoxyl content (as Methoxyl)**

Each ml of 0.1 N sodium thiosulfate required in the determination of Total Alkoxyl content is equivalent to 0.517 mg of alkoxyl expressed as methoxyl.

METHYL p-HYDROXYBENZOATE*

SYNONYMS
Methylparaben, methyl p-oxybenzoate.

DEFINITION
Chemical names
Methyl p-hydroxybenzoate, methyl ester of p-hydroxybenzoic acid.

C.A.S. number
99-76-3

Chemical formula
C₇H₁₀O₃

Structural formula

\[
\text{COOCH}_3
\]

Molecular weight
152.15

Assay
After drying for 5 h over silica gel, contains not less than 99% of C₇H₁₀O₃

DESCRIPTION
Almost odourless, small colourless crystals of white crystalline powder.

FUNCTIONAL USE
Antimicrobial preservative.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Slightly soluble in water. Freely soluble in ethanol and propylene glycol. Soluble in ether.

** B. Melting range
125-128°

C. Positive test for
p-hydroxybenzoate
Melting range of p-hydroxybenzoic acid derived from the sample is between 213° and 217°.
Proceed as directed in the specifications for Ethyl p-hydroxybenzoate.

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* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

PURITY TESTS

* Loss on drying
Not more than 0.5% after drying for 5 h over silica gel.

* Sulfated ash
Not more than 0.05%.
Proceed as directed under the test of Ash (Sulfated ash, Method I) using 2.0 g of the sample.

Acidity
Passes test
See description under TESTS

* Arsenic
Not more than 3 mg/kg.
A sample solution prepared as directed for organic compounds meets the requirements of the Limit Test for Arsenic (Method II).

* Heavy metals
Not more than 10 mg/kg.
See description under TESTS

p-Hydroxybenzoic acid and salicylic acid
Passes tests
See description under TESTS.

TESTS

PURITY TESTS

Acidity
Heat 750 mg of the sample with 15 ml of water at 80° for 1 min, cool and filter. The filtrate should be acid or neutral to litmus. To 10 ml of the filtrate add 0.2 ml of 0.1 N sodium hydroxide and 2 drops of methyl red TS. The solution should be yellow, without even a light cast of pink.

* Heavy metals
Weigh 2 g of the sample to the nearest mg, and dissolve in 23 ml of acetone. Add 2 ml of dilute acetic acid TS, 2 ml of water, and 10 ml of hydrogen sulfide TS. Any colour produced does not exceed that produced in a control made with 23 ml of acetone, 2 ml of dilute acetic acid TS, 2 ml of Standard Lead Solution (20 μg Pb ion), and 10 ml of hydrogen sulfide TS.

p-Hydroxybenzoic acid and salicylic acid
Dissolve 0.5 g of the sample, weighed to the nearest mg, in 30 ml of ether, add 20 ml of a 1 in 100 sodium hydrogen carbonate solution, shake, and separate the water layer. Wash the water layer with two 20-ml portions of ether, add 5 ml of dilute sulfuric acid and 30 ml of ether, and shake. Separate the ether layer, and shake with about 10 ml of water. Filter the ether layer, and wash the vessel and the filter with a small amount of ether. Combine the washings and the filtrate, evaporate the ether on a water bath, and dry the residue over sulfuric acid to constant weight. The weight of the residue should not exceed 5 mg. Dissolve any residue in 25 ml of water, heat to about 70°, filter, and add a few drops of dilute ferric chloride TS. No violet to reddish violet colour should be produced.

METHOD OF ASSAY

Titrimetric method

Weigh, to the nearest mg, 2 g of the sample, previously dried for 5 h over silica gel, and transfer into a flask. Add 40 ml of N sodium hydroxide and rinse the sides of the flask with water. Cover with a watch glass, boil gently for 1 h and cool. Add 5 drops of bromothymol blue TS and titrate the excess sodium hydroxide with N sulfuric acid, matching the colour against a buffer solution TS (pH 6.5) containing the same proportion of indicator. Perform a blank determination with the reagents and make any necessary correction. Each ml of N sodium hydroxide is equivalent to 152.2 mg of C₆H₅O₂.

Alternate method

Weigh, to the nearest mg, 0.1 g of the sample, previously dried for 5 h over silica gel, and transfer into a 300-ml glass-stoppered flask. Add 10 ml of N sodium hydroxide and heat for 15 min on a water bath. After cooling, add 50.00 ml of 0.1 N potassium bromate, 5.0 g of potassium bromide and 30 ml of N hydrochloric acid. Let the mixture stand for 15 min in the dark in the closed flask. Add 1 g of potassium iodide, shake the flask vigorously, and titrate with 0.1 N sodium thiosulfate using starch TS as indicator. Each ml of 0.1 N potassium bromate is equivalent to 25.36 mg of C₆H₅O₂.
**SYNONYMS**

Dimethyl anthranilate, 2-methylbenzoic acid methyl ester

**DEFINITION**

- **Chemical names**: Methyl 2-methylaminobenzoate, methyl N-methylanthranilate
- **C.A.S. number**: 85-91-6
- **Chemical formula**: C₉H₈NO₂
- **Structural formula**:

![Structural formula]

**Molecular weight**: 165.20

**Assay**: Content not less than 98% and not more than the equivalent of 102% of C₉H₈NO₂

**DESCRIPTION**

Colourless to pale yellow oily liquid with a slight bluish fluorescence and an odour reminiscent of orange blossom and mandarin peel

**FUNCTIONAL USE**

Flavouring agent

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

**A. Solubility**: Practically insoluble in water and in glycerol. Soluble in most fixed oils.

**B. Refractive index**: nD²₀ : 1.578 - 1.581

**C. Specific gravity**: d₂₀¹ : 1.123 - 1.132

**PURITY TESTS**

**Solubility in ethanol**: 1 ml dissolves in 10 ml of 70% ethanol

**Solidification point**: Not less than 14°

**Acid value**: Not more than 1.0

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* These specifications were prepared at the 23rd session of JECFA (1979) and published in FNP 12 (1979).

METHOD OF ASSAY

Weigh accurately about 1.1 g of the sample and proceed as directed under the method for Ester Determination (Hydroxylamine Method) in the General Methods*, using 82.60 as the equivalence factor (e) in the calculation.

METHYL β-NAPHTHYL KETONE*

SYNONYMS
Naphthyl methyl ketone, 2-acetyl-naphthalene

DEFINITION
Chemical name: Methyl 2-naphthyl ketone
C.A.S. number: 93-08-3
Chemical formula: C_{12}H_{10}O
Structural formula:

\[
\begin{array}{c}
\text{\includegraphics[width=2cm]{structure.png}}
\end{array}
\]

Molecular weight: 170.21
Assay: Content not less than 99% of C_{12}H_{10}O

DESCRIPTION
White crystalline solid, with an odour suggestive of orange blossom.

FUNCTIONAL USE
Flavouring agent

CHARACTERISTICS
IDENTIFICATION TESTS

** A. Solubility
Practically insoluble in water. Insoluble in glycerol. Soluble in most fixed oils.

** B. Infrared spectrum
See Appendix at the end of these specifications.

PURITY TESTS

** Solubility in ethanol
1 g dissolves in 5 ml of 95% ethanol at 25°

** Solidification point
Not less than 52°

** Sulfated ash
Not more than 0.05%.
Test 10 g of the sample as directed in the test of Ash (Sulfated ash, Method I).

** Chlorinated compounds
Passes test

---


METHOD OF ASSAY

Weigh accurately about 1.5 g of the sample and proceed as directed under the method for Aldehyde and Ketone Determination (Hydroxylamine Method) in the General Methods*, using 85.10 as the equivalence factor (e) in the calculation.

APPENDIX

** Infrared spectrum: Methyl β-Naphyl Ketone


METHYL PHENYLACETATE*

SYNONYM

Phenylacetic acid methyl ester

DEFINITION

Chemical name: Methyl phenylacetate
C.A.S. number: 101-41-7
Chemical formula: C₉H₁₀O₂
Structural formula:

\[
\begin{array}{c}
\text{C} \\
\text{H₂} \\
\text{O} \\
\text{C} \\
\text{H₃}
\end{array}
\]

Molecular weight: 150.18
Assay: Content not less than 97% of C₉H₁₀O₂

DESCRIPTION

Colourless or nearly colourless liquid having intense odour suggestive of honey and jasmine.

FUNCTIONAL USE

Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Refractive index

\[ n_D^\circ : 1.5050 - 1.5090 \]

** B. Specific gravity

\[ d_D^\circ : 1.061 - 1.067 \]

PURITY TESTS

** Acid value

Not more than 1.

** Chlorinated compounds

Passes test

METHOD OF ASSAY

Weigh accurately about 1 g, and proceed as directed for Ester Determination in the General Methods**, using 75.09 as the equivalence factor \( e \) in the calculation.

---

* These specifications were prepared at the 11th session of JECFA (1967) and published in NMRS 44B (1969).

**METHYL SALICYLATE**

**SYNONYM** Wintergreen oil, artificial,

**DEFINITION**
- Chemical name: Methyl salicylate
- C.A.S. number: 119-36-8
- Chemical formula: C₉H₈O₃

**STRUCTURAL FORMULA**

[Chemical structure diagram]

- Molecular weight: 152.15
- Assay: Content not less than 98% of C₉H₈O₃

**DESCRIPTION** Colourless to light yellow, transparent liquid having a cool odour.

**FUNCTIONAL USE** Flavouring agent

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

**A.** Refractive index

\[ n_D^2 : 1.5340 - 1.5380 \]

**B.** Specific gravity

\[ d_3^\circ : 1.180 - 1.188 \]

**METHOD OF ASSAY** Proceed as directed under the method for Ester Determination in the General Methods**, using about 2 g, accurately weighed. Use 50 ml of 0.5 N alcoholic potassium hydroxide and reflux on the steam bath for 2 h. Each ml of 0.5 N potassium hydroxide consumed is equivalent to 76.08 mg of C₉H₈O₃.

---

* These specifications were prepared at the 11th session of JECFA (1967) and published in NMRS 44B (1969).

**MICROCRYSTALLINE CELLULOSE**

**SYNONYMS**
Cellulose gel;  
INS No. 460 (i), EEC No. E460

**DEFINITION**
Microcrystalline Cellulose is purified, partially depolymerized cellulose prepared by treating alpha cellulose, obtained as a pulp from fibrous plant material, with mineral acids.

- Chemical names: Cellulose
- C.A.S. number: 9004-34-6
- Chemical formula: \((C_6H_{10}O_5)_n\)

**DESCRIPTION**
Microcrystalline Cellulose occurs as a fine, white, odorless, free flowing, crystalline powder consisting of nonfibrous particles which may be compressed into self-binding tablets which disintegrate rapidly in water.

**FUNCTIONAL USES**
Emulsifier, stabilizer, anticaking and dispersing agent

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

**A. Solubility**
Insoluble in water, ethanol, ether and dilute mineral acids. Slightly soluble in sodium hydroxide solution

**B. Dispersion formation**
Passes test  
See description under TESTS

**C. Colour reaction**
Passes test  
See description under TESTS

**PURITY TESTS**

**A. Loss on drying**
Not more than 7% after drying at 105°C for 3 h

**B. pH**
5.5 - 7.0  
Shake 5 g of the sample with 40 ml of water for 20 min and centrifuge. Use the supernatant for pH determination

**Water soluble substances**
Not more than 0.16%  
See description under TESTS

---

**Footnotes:**
- **These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).**
- **See General Methods (Guide to JECFA Specifications), FNP 5/Rev.2 (1991).**
**PURITY TESTS (continued)**

* **Sulfated ash**
  Not more than 0.05%
  Proceed as directed under the test for Ash (Sulfated Ash, Method I) using 2.0 g of the sample.

* **Arsenic**
  Not more than 3 mg/kg
  A sample solution prepared as directed for organic compounds will meet the requirements of the Limit Test for Arsenic (Method II).

* **Heavy metals**
  Not more than 10 mg/kg
  Test 2.0 g of the sample as directed in Method II under the Limit Test for Heavy Metals.

**Starch**
  Not detectable
  See description under TESTS.

**TESTS**

**IDENTIFICATION TESTS**

B. **Dispersion test**
  Mix 30 g of the sample with 270 ml of water in a high-speed (18,000 rpm) blender for 5 min. Transfer 100 ml of the mixture to a 100-ml graduated cylinder, and allow to stand for 3 h. A white, opaque, bubble-free dispersion, which does not form a supernatant liquid at the surface, is obtained.

C. **Colour reaction**
  To 1 mg of the sample add 1 ml of phosphoric acid and heat on a water bath for 30 min. Add 4 ml of a 1 in 4 solution of pyrocatechol in phosphoric acid and heat for 30 min. A red colour is produced.

**PURITY TESTS**

**Water soluble substances**
  Shake 5 g of the sample with approximately 80 ml of water for 10 min, filter through Whatman No. 42 or equivalent filter paper into a tared beaker, evaporate to dryness on a steam bath and dry at 105° for 1 h. Cool, weigh and calculate as percentage.

**Starch**
  To 20 ml of the dispersion obtained in the Identification Test B add a few drops of iodine TS, and mix. No purplish to blue or blue colour should be produced.

MINERAL OIL*

** A. Solubility
Insoluble in water.
Sparingly soluble in ethanol. Soluble in ether.

B. Burning
Burns with bright flame and with paraffin-like characteristic smell

** Arsenic
Not more than 1 mg/kg (Method II) using a 3 g sample

** Lead
Not more than 1 mg/kg
Proceed as directed in the Limit Test for Lead using 1 μg lead ion (Pb) in the control

** Heavy metals
Not more than 10 mg/kg
Test a 2 g sample as directed in Method II in the Limit Test, using 20 μg of lead ion (Pb) in the control (Solution A).

** Readily carbonisable substances
Passes test
See description under TESTS

** Polycyclic aromatic hydrocarbons
Passes test
See description under TESTS

* These specifications were prepared at the 37th session JECFA (1990) superseding the earlier specifications published in FNP 38 (1988).

PURITY TESTS (continued)

Solid paraffins

Passes test

See description under TESTS

TESTS

PURITY TESTS

Acidity or alkalinity

To 10 ml of the sample add 20 ml of boiling water and shake vigorously for 1 min. Separate the aqueous layer and filter. To 10 ml of the filtrate, add 0.1 ml of phenolphthalein solution TS. The solution is colourless. Not more than 0.1 ml of 0.1 N sodium hydroxide is required to change the colour to pink.

Readily carbonisable substances

Place 5 ml of the sample in a glass-stoppered test tube that previously has been rinsed with chromic acid cleaning mixture. Add 5 ml of sulfuric acid TS, and heat in a boiling water bath for 10 min. After the test tube has been in the bath for 30 sec, remove it quickly, and while holding the stopper in place, give three vigorous vertical shakes over an amplitude of about 10 cm. Repeat every 30 sec. Do not keep the test tube out of the bath longer than 3 sec for each shaking period. At the end of 10 min from the time when first placed in the water bath, remove the test tube. The sample remains unchanged in colour, and the acid does not become darker than standard colour produced by mixing in a similar test tube 3 ml of ferric chloride TS, 1.5 ml of cobaltous chloride TS, and 0.5 ml of cupric sulfate TS, this mixture being overlaid with 5 ml of mineral oil.

Polycyclic aromatic hydrocarbons

Transfer 25.0 ml of sample to a 125 ml separator and add 25 ml of hexane. Mix and add 5.0 ml of dimethyl sulfoxide. Shake vigorously for 1 min and allow to stand until two clear layers are formed. Transfer the lower layer to a second separating funnel, add 2 ml of hexane and shake the mixture vigorously. Allow to stand until two clear layers are formed. Separate the lower layer and measure its absorbance over the range 260 nm to 420 nm using as reference liquid the clear lower layer obtained by vigorously shaking 5.0 ml of dimethyl sulfoxide with 25 ml of hexane for 1 min. Prepare a reference solution in trimethylpentane containing 7.0 mg of naphthalene per litre and measure the absorbance of that solution at the maximum at 275 nm using trimethylpentane as compensation liquid. At no wavelength in the range 260 nm to 420 nm does the absorbance of the test solution exceed one-third that of the reference solution at 275 nm.

Use hexane, dimethyl sulfoxide and trimethylpentane in quality specified for ultraviolet spectrophotometry.
Solid paraffin

Dry a suitable quantity of the substance to be examined by heating at 100° for 2 h and cool in a desiccator over concentrated sulfuric acid. Place in a glass tube with an internal diameter of about 25 mm, close the tube and immerse in a bath of iced water. After 4 h the liquid is sufficiently clear for a black line 0.5 mm wide against a white background held vertically behind the tube to be easily seen.
Mixed Microbial Carbohydrase and Protease from Bacillus Subtilis, var.

**Sources**
Commercial preparations of mixed carbohydrases and proteases are produced by the controlled fermentation of *Bacillus subtilis*, var.

**Active Principles**
1. Alpha-amylase
2. Proteases: usually contain following two enzymes
   2-a. Microbial serine proteinase
   2-b. Microbial metalloproteinases

**Systematic Names and Numbers**
1. 1,4-α-D-glucan glucanohydrolase - EC 3.2.1.1
   2-a. None - EC 3.4.21.14
   2-b. None - EC 3.4.24.4

**Reactions Catalysed**
1. Hydrolysis of 1,4-α-glucosidic linkages in polysaccharides, yielding primarily dextrins and oligosaccharides.
2. Hydrolysis of polypeptides yielding peptides of lower molecular weight. The neutral proteinase (2-b) cleavage preferentially bonds adjacent to a hydrophobic amino-acid residue.

**Description**
The enzyme preparations occur as off-white to tan amorphous powders. They are soluble in water, the solutions usually being light yellow to dark brown in colour. They are practically insoluble in alcohol, chloroform and ether. Preparations can vary in the relative concentrations of each of the active principles. Powdered and liquid products are available.

**Functional Uses**
Preparation of starch syrups, alcohol, beer, glucose, bakery products, fish meal, tenderizing meat, and the preparation of protein hydrolysates.

**General Specifications**
Must conform to the "General Specifications for Enzyme Preparations used in Food Processing"**

**Characteristics**

**Identification Tests**
1. Protease activity  The sample shows bacterial proteinase activity***
2. α-Amylase activity  The sample shows bacterial α-amylase activity***

---

* This specification was prepared at the 15th session of JECFA (1971) and published in NMRS 50B (1972).

** See Annex 1 at the end of this compendium.

MIXED CAROTENOIDS*
(Tentative)**

**DEFINITION**

Mixed Carotenoids (alfalfa) are obtained by solvent extraction of alfalfa, removal of chlorophyls through saponification and subsequent purification of the carotenoids by solvent extraction. The main colouring principle consists of carotenoids of which lutein accounts for the major part. Variable amounts of carotenes will also be present.

Mixed Carotenoids may contain fats, oils and waxes naturally occurring in the plant material. Articles of commerce may be added vegetable oils for standardizing purposes or approved food additives in order to formulate water soluble products. Only the following solvents may be used for the extraction: methanol, ethanol, propan-2-ol, hexane, acetone, dichloromethane and methyl ethyl ketone.

**Class**

Carotenoid

**Code numbers**

Lutein: CAS No. 127-40-2

**Chemical name**

Lutein: 3,3'-dihydroxy-d-carotene; 
β,ε-carotene-3,3'-diol

**Chemical formula**

Lutein: C₄₀H₇₀O₂

**Structural formula:**

![Structural formula image]

**Molecular weight**

Lutein: 568.88

**Assay**

Content of total colouring matter (calculated as lutein) not less than declared

**DESCRIPTION**

Dark, yellowish brown liquids with a weak hay-like colour

**FUNCTIONAL USES**

Food colour

---

**Footnotes:**

* These specifications were prepared at the 37th session of JECFA (1990) superseding the earlier specifications for MIXED CAROTENOIDS published in FNP 38 (1988).

** Information required on composition of commercial products and method to distinguish between mixed carotenoids and synthetic colours.
CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility
  Insoluble in water. Soluble in hexane.

** B. Spectrophotometry
  A chloroform solution of the sample shows maximum absorption at about 445 nm.

C. Carr-Price test
  A solution of the sample in chloroform turns blue on addition of an excess of Carr-Price TS.

PURITY TESTS

*** Heavy metals
  Test 0.5 g of the sample as directed in the Heavy Metals limit Test (method II), using 20 μg of lead ion (Pb) in the control (solution A).

Residual solvents

Acetone: Not more than 50 mg/kg, singly or in combination
Methanol: Not more than 10 mg/kg
Ethanol: Not more than 10 mg/kg
Propan-2-ol: Not more than 10 mg/kg
Hexane: Not more than 10 mg/kg
Dichloromethane: Not more than 10 mg/kg
Methyl ethyl ketone: Not more than 10 mg/kg

METHOD OF ASSAY

Proceed as directed in Method of Assay for Oil-Soluble Food Colours** using the following conditions:

\[
W = \text{amount (g) to obtain adequate absorbance}
V_1 = V_2 = V_3 = 100 \text{ ml}
V_4 = V_5 = 10 \text{ ml}
A_{\text{abs}} = 2500
\lambda_{\text{max}} = \text{app.} 445 \text{ nm}
\]


MODIFIED STARCHES*

SYNONYMS

See Table I for individual chemically modified starches.

DEFINITION

Modified Starches are food starches which have one or more of their original characteristics altered by treatment in accordance with good manufacturing practice by one of the procedures listed in Table I. In the case of starches treated with heat in the presence of acid or with alkali, the alteration is a minor fragmentation. When the starch is bleached, the change is essentially in the colour only. Oxidation involves the deliberate production of carboxyl groups. Treatment with reagents such as orthophosphoric acid results in partial substitution in the 2,6 or 3-position of the anhydroglucose unit unless the 6-position is occupied for branching. In cases of cross-bonding, where a poly-functional substituting agent, such as phosphorus oxychloride connects two chains, the structure can be represented by: Starch-O-R-O-Starch, where R = cross-bonding group and Starch refers to the linear and/or branched structure. The article of commerce can be specified by the parameter specific for the particular type of modification as indicated in Column 3 of Table I, and may also be further specified as to the loss on drying, sulfated ash, protein and fat.

DESCRIPTION

Most chemically treated starches are white or off-white, tasteless and odourless powders. According to the drying method these powders can consist of whole granules having the appearance of the original native starch, or aggregates consisting of a number of granules (pearl starch, starch-grits) or, if pregelatinized, of flakes, amorphous powder or coarse particles.

FUNCTIONAL USES

Thickening agent, stabilizer, binder.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility

Insoluble in cold water (if not pregelatinized); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol.

B. Microscopic examination

Passes test

See description under TESTS

C. Iodine stain

Passes test

See description under TESTS


IDENTIFICATION TESTS (continued)

D. Copper reduction
   Passes test
   See description under TESTS

E. Differentiation test
   Passes test for type of starch
   See description under TESTS for:
   1. Hypochlorite oxidised starch
   2. Specific reaction for acetyl groups
   3. Positive test for ester groups

PURITY TESTS

Sulfur dioxide
   Not more than 50 mg/kg for modified cereal starches
   Not more than 10 mg/kg for other modified starches unless otherwise specified in Table I
   See description under TESTS

* Arsenic
   Not more than 1 mg/kg
   See description under TESTS

* Lead
   Not more than 2 mg/kg
   See description under TESTS

* Heavy metals
   Not more than 40 mg/kg
   See description under TESTS

Additional purity specifications for individual chemically modified starches
   See column 3 of Table I
   See description under TESTS

TESTS

IDENTIFICATION TESTS

B. Microscopic examination
   Chemically treated starches which have not been pregelatinized retain their granular structure and can be identified as starches by microscopic observation. Shape, size and sometimes striations are characteristics of the botanical origin. In polarized light under crossed nicol prisms the typical polarization cross will be observed

C. Iodine stain
   Add a few drops of 0.1 N potassium tri-iodide to an aqueous suspension of the sample. These starches stain with iodine in the same way as native starches. The colour can range from dark blue to red

D. Copper reduction
   Place about 2.5 g of the sample previously washed with water, in a boiling flask, add 10 ml of dilute hydrochloric acid (3%) and 70 ml of water, mix, reflux for about three hours and cool. Add 0.5 ml of the resulting solution to 5 ml of hot alkaline cupric tartrate TS. (*)
   A copious red precipitate is produced

E. Differentiation test
   To differentiate between various treated starches perform the following tests:

E. Differentiation test (continued)

1. Test for hypochlorite-oxidized starch (not for slightly oxidized potato starch)

**Principle**
Because of the carboxyl group content, hypochlorite-oxidized starch has anionic properties. It can be dyed with positively charged dyes such as methylene blue.

**Procedure**
50 mg of the sample are kept in suspension for 5-10 min in 25 ml of a 1% aqueous dye solution and stirred occasionally. After decantation of the excess solution, the starch is washed with distilled water. Microscopic inspection clearly shows colouring, if the sample is hypochlorite-oxidized starch. By this test hypochlorite-oxidized starch is distinguished from native and acid modified starch of the same botanical origin.

2. Specific reaction of acetyl groups

**Principle**
Acetate is liberated upon saponification of acetylated starch. After concentration the acetate is converted to acetone by heating with calcium hydroxide. The acetone thus produced stains blue with o-nitrobenzaldehyde.

**Procedure**
About 10 g of the sample is suspended in 25 ml water to which is added 20 ml of 0.4 N NaOH. After shaking for 1 h, the starch is filtered off and the filtrate evaporated in an oven at 110°. The residue is dissolved in a few drops of water and transferred to the test tube. Calcium hydroxide is added and if the sample is an acetylated starch, the tube heated thereby gives off acetone vapours. These produce a blue colour on a paper strip soaked in a fresh saturated solution of o-nitrobenzaldehyde in 2 N NaOH. The blue colour is more distinct when the original yellow colour of the reagents is removed with 1 drop of a 1 in 10 solution of hydrochloric acid.

3. Positive test for ester groups

The infrared spectrum of a thin film gives a typical absorption band at about 1720 cm⁻¹ which is an indication for ester groups. The limit of detection is about 0.5% acetyl, adipyl or succinyl groups in the product.

**PURITY TESTS**

**Sulfur dioxide**

**Scope**
The method is applicable, with minor modifications, to liquid or solid samples even in the presence of other volatile sulfur compounds.

**Principle**
The sulfur dioxide is released from the sample in a boiling acid medium and is removed by a stream of carbon dioxide. The separated gas is collected in dilute hydrogen peroxide where it is oxidized to sulfuric acid and titrated with standard alkali. Alternatively, the sulfuric acid may be determined gravimetrically.
Sulfur dioxide (continued)

Apparatus

"Monier-Williams" apparatus for the determination of sulfuric acid, constructed with standard-taper glass connections, can be obtained from any reliable scientific glass apparatus store. It is customary, however, to construct the apparatus with regular laboratory glassware using stopper connections (see fig. 1).

The assembly consists of a 1000-ml two-neck round-bottom boiling flask to which a gas-inlet tube, a 60-ml dropping funnel having a 2-mm bore stopcock, and a sloping Allihn reflux condenser are attached. A delivery tube connects the upper end of the condenser to the bottom of a 250-ml conical receiving flask, which is followed by a Peligot tube.

In operation, carbon dioxide is passed through the scrubber and bubbled through the heated reaction mixture, sweeping sulfur dioxide through the condenser and into the receivers where it is absorbed quantitatively.

Fig. 1
Preparation of solutions

- Sodium carbonate solution: Dissolve approximately 15 g of Na₂CO₃ or 40 g of Na₂CO₃·10H₂O in distilled water, and dilute to 100 ml.

- Hydrogen peroxide, 3%: Dilute 10 ml of C.P. (Chemical Purity) neutral 30% hydrogen peroxide (H₂O₂) with distilled water to 100 ml.

Procedure

Pass carbon dioxide from a generator or cylinder through the sodium carbonate scrubber solution to remove chlorine, thence into the gas-inlet tube of the boiling flask. Place 15 ml of the 3% hydrogen peroxide in the receiving flask and 5 ml in the Peligot tube. Connect the apparatus and introduce into the boiling flask, by means of the dropping funnel, 300 ml of distilled water and 20 ml of concentrated hydrochloric acid. Boil the contents approximately 10 min. in a current of carbon dioxide. Weigh, to the nearest g, 100 g of the sample and disperse in approximately 300 ml of recently-boiled distilled water. Transfer the slurry to the boiling flask by means of dropping funnel, regulating the sample addition rate and the gas flow rate through the apparatus to prevent drawback of hydrogen peroxide, inclusion of air, or burning of sample. Boil the mixture gently for 1 h. in a slow current of carbon dioxide. Stop the flow of water in the condenser just before the end of the distillation. When the delivery tube just above the receiving flask becomes hot, remove the tube from the condenser immediately. Wash the delivery tube and the Peligot tube contents into the receiving flask, and titrate with 0.1 N sodium hydroxide, using bromphenol blue indicator (see Note).

Perform a blank determination on the reagents, and correct results accordingly.

\[
\% \text{sulfur dioxide} = \frac{(S-B) \times 0.0032}{W} \times 100
\]

in which

- \(S\) = ml of 0.1 N sodium hydroxide used for the sample
- \(B\) = ml of 0.1 N sodium hydroxide used for the blank
- \(W\) = the weight (in grams) of the sample.

Note. A gravimetric determination may be made after titration. Acidify with HCl, precipitate with BaCl₂, settle, filter, wash, ignite, and weigh as BaSO₄.

* Arsenic

A sample solution prepared as directed for organic compounds meets the requirements of the Limit Test for Arsenic (Method II) using 1 ml of standard arsenic solution.

PURITY TESTS (continued)

* Lead

Transfer 2.0 g of the sample to an evaporating dish, add 1 ml of sulfuric acid (1 in 4), distributing it evenly through the sample, and evaporate most of the water on a steam bath. Char and dehydrate the sample by heating on a hot plate, while heating at the same time with an infrared lamp from above, and then heat in a muffle furnace at 500° until the residue is free from carbon. Remove the dish from the furnace, cool, and cautiously wash down the inside of the dish with water. Add 1 ml of N hydrochloric acid, evaporate to dryness on a steam bath, then add 2 ml of N hydrochloric acid and heat briefly while stirring on a steam bath. Quantitatively transfer the solution into a separator with the aid of small quantities of water, and neutralize with N ammonium hydroxide. The sample solution meets the requirements of the Limit Test for Lead using 4 μg of lead ion in the control.

* Heavy metals

Prepare and test 500 mg of the sample as directed in Method II under the Limit Test for Heavy Metals using 20 μg of lead ion in the control (Solution A) and 500° as the ignition temperature.

### TABLE I

**ADDITIONAL PURITY SPECIFICATIONS FOR INDIVIDUAL CHEMICALLY MODIFIED STARCHES**

(all percentages calculated on dry substance)

<table>
<thead>
<tr>
<th>MODIFICATION</th>
<th>PROCESS LIMITATIONS</th>
<th>END-PRODUCT SPECIFICATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrin roasted starch</td>
<td>Dry heat treatment with hydrochloric acid max 0.15% or ortho-phosphoric acid max 0.17%</td>
<td>Final pH 2.5 - 7.0</td>
</tr>
<tr>
<td>(INS No. 1400)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid treated starch</td>
<td>Treatment with hydrochloric acid max 7.0% or ortho-phosphoric acid max 7.0% or sulfuric acid max 2.0%</td>
<td>Final pH 4.8 - 7.0</td>
</tr>
<tr>
<td>(INS No. 1401)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline treated starch</td>
<td>Treatment with sodium hydroxide max 1.0% or potassium hydroxide max 1.0%</td>
<td>Final pH 5.0 - 7.5</td>
</tr>
<tr>
<td>(INS No. 1402)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Bleached starch             | Treatment with peracetic acid and/or hydrogen peroxide max 0.45% of active oxygen, or sodium hypochlorite max 0.82% of available chlorine, or sodium chlorite max 0.5%, or sulfur dioxide or alternative permitted forms of sulfites, | Added carboxyl groups max 0.1%
<p>| (INS No. 1403)              |                                                                                      | No residual reagent        |
|                             |                                                                                      | Residual SO₂ max 50 mg/kg  |
|                             |                                                                                      | Residual manganese max 50 mg/kg |</p>
<table>
<thead>
<tr>
<th>MODIFICATION</th>
<th>PROCESS LIMITATIONS</th>
<th>END-PRODUCT SPECIFICATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidized starch (INS No. 1404)</td>
<td>Treatment with sodium hypochlorite max 5.5% as chlorine</td>
<td>Carboxyl groups max 1.1% Residual SO₂ max 50 mg/kg</td>
</tr>
<tr>
<td>Monostarch phosphate (INS No. 1410)</td>
<td>Esterification with orthophosphoric acid or sodium or potassium ortho-phosphate, or sodium tripolyphosphate</td>
<td>Phosphate calculated as phosphorus max 0.5% for potato or wheat, and max 0.04% for other starches</td>
</tr>
<tr>
<td>Distarch phosphate (INS No. 1412)</td>
<td>Esterification with sodium trimetaphosphate or phosphorus oxychloride</td>
<td>Phosphate calculated as phosphorus max 0.14% for potato or wheat, and max 0.04% for other starches</td>
</tr>
<tr>
<td>Phosphated distarch phosphate (INS No. 1413)</td>
<td>Combination of treatments for Monostarch phosphate and Distarch phosphate</td>
<td>Phosphate calculated as phosphorus max 0.5% for potato or wheat, and max 0.4% for other starches</td>
</tr>
<tr>
<td>Acetylated distarch phosphate (INS No. 1414)</td>
<td>Esterification by sodium trimetaphosphate or phosphorus oxychloride combined with esterification by 10% max acetic anhydride or 7.5% max vinyl acetate</td>
<td>Acetyl groups max 2.5%. Phosphate calculated as phosphorus max 0.14% for potato or wheat, and max 0.04% for other starches Vinyl acetate max 0.1 mg/kg</td>
</tr>
<tr>
<td>Starch acetate (INS No. 1420)</td>
<td>Esterification with acetic anhydride or 7.5% max vinyl acetate</td>
<td>Acetyl groups max 2.5%</td>
</tr>
<tr>
<td>Acetylated distarch adipate (INS No. 1422)</td>
<td>Esterification with acetic anhydride and adipic anhydride max 0.12%</td>
<td>Acetyl groups max 2.5% Adipate groups max 0.135%</td>
</tr>
<tr>
<td>Material</td>
<td>Etherification Process</td>
<td>Specifications</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>-------------------------------------------------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>Hydroxypropyl starch (INS No. 1440)</td>
<td>Etherification with propylene oxide max 10.0%</td>
<td>Hydroxypropyl groups max 7.0%</td>
</tr>
<tr>
<td>(CAS No. 9049-76-7)</td>
<td></td>
<td>Propylene chlorohydrin max 1 mg/kg</td>
</tr>
<tr>
<td>Hydroxypropyl distarch phosphate (INS No. 1442)</td>
<td>Etherification by sodium trimetaphosphate or phosphorus oxychloride combined with etherification by 10% max propylene oxide</td>
<td>Hydroxypropyl groups max 7.0%</td>
</tr>
<tr>
<td>(CAS No. 53124-00-8)</td>
<td></td>
<td>Propylene chlorohydrin max 1 mg/kg</td>
</tr>
<tr>
<td>Starch sodium octenylsuccinate (INS No. 1450)</td>
<td>Esterification by octenylsuccinic anhydride max 3%</td>
<td>Degree of substitution with octenylsuccinyl groups max 0.02%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Residual octenylsuccinic acid max 0.3%</td>
</tr>
</tbody>
</table>

Residual phosphate calculated as phosphorus max 0.14% for potato and wheat, and max 0.04% for other starches.
Methods for additional purity specifications:

* pH

As specified in Column 3 of Table I

Mix 20 g of the sample with 80 ml of water, and agitate continuously at a moderate rate for 5 min. (In the case of pregelatinized starches, 3 g should be suspended in 97 ml of water). Determine the pH of the resulting suspension by the method outlined in General Methods.

Carboxyl groups

As specified in Column 3 of Table I.

Principle

The carboxyl containing starch is leached with mineral acid to convert carboxyl salts to the acid form. Cations and excess acid are removed by washing with water. The washed sample is gelatinized in water and titrated with standard alkali.

Note: Native phosphate groups present in potato starch increase the titre found in this method (See note 6)

Reagents

- Hydrochloric Acid Solution, 0.10 N: Standardization unnecessary
- Sodium Hydroxide Solution, 0.10 N: Standardized
- Phenolphthalein Indicator, 1%

Procedure

If necessary, grind sample completely through a laboratory cutting mill to 20 mesh or finer, taking precautions to prevent any significant change in moisture, and mix thoroughly.

Weigh accurately a sample containing not more than 0.25 milli-equivalent of carboxyl (Note 1), and transfer quantitatively to a 150-ml beaker. Add 25 ml of 0.1 N hydrochloric acid and stir occasionally over a period of 30 min. Vacuum filter the slurry through a medium porosity fritted-glass crucible or small funnel, using a fine stream of water from a wash bottle to aid quantitative transfer of the sample. Wash the sample with distilled water (300 ml usually sufficient) until the filtrate is free from chloride determined by silver nitrate test (Note 2).

Transfer the demineralized sample quantitatively to a 600 ml beaker with the aid of distilled water, and slurry the sample in 300 ml of distilled water. Heat sample dispersion in a steam bath or boiling water bath (Note 3), stirring continuously until the starch gelatinizes, and continue heating for 15 min to ensure complete gelatinization (Note 4).

Remove sample from bath and titrate while hot with standard 0.10 N sodium hydroxide solution to a phenolphthalein end-point. The end-point may be detected electrometrically at pH 8.3.

A blank determination is run on the original sample to correct for native acid substances (Note 5). Weigh the same quantity of starch as taken for carboxyl titration, and slurry in 10 ml of distilled water. Stir at about 5-min intervals for 30 min.

Vacuum filter the slurry quantitatively through a medium porosity fritted-glass crucible or small funnel, and wash sample with 200 ml of distilled water. Transfer, gelatinize, and titrate the sample with standard 0.10 N sodium hydroxide in the same manner as the demineralized sample.

Calculation

Carboxyl groups (%) = \( \frac{(\text{ml} \, 0.10 \, \text{N NaOH} - \text{Blank}) \times 0.0045 \times 100}{\text{Sample Wt. (g)}} \)

Notes and Precautions

1. Sample size should not exceed 5.0 g for a mildly oxidized nor less than 0.15 g for a highly oxidized commercial starch.
2. Add 1 ml of 1% aqueous silver nitrate solution to 5 ml of filtrate. Turbidity or precipitation occurs within 1 min. if chloride is present.
3. Heating on a hot plate or over a Bunsen burner is not recommended. Over-heating or scorched in amounts too small to be visible will cause sample decomposition and apparent high carboxyl results.
4. Thorough gelatinization facilitates rapid titration and accurate end-point detection.
5. A blank titration is run on a water washed sample to correct for acidic components which are not introduced by oxidation or derivatization. Free fatty acids complexed with amylose in common corn starch are the principal contributors to the blank titer.
6. A correction for phosphate content in potato starch (deduction) should be made after determining the phosphorus content of the sample being examined.

The deduction is calculated:

\[
\frac{2 \times 45.02 \times P}{30.97} = 2.907 \, P
\]

Where \( P \) = phosphorus content (%)

Manganese content

As specified in Column 3 of Table I.

Instrumentation

Determine the manganese content with the use of an atomic absorption spectrophotometer at 279.5 nm.

Preparation of solutions

- Standard solution: Prepare a solution containing 0.5 mg/kg of manganese.
- Sample solution: Transfer 10.000 g of the sample into a 200-ml Kohrausch volumetric flask, previously rinsed with 0.5 N hydrochloric acid, add 140 ml of 0.5 N hydrochloric acid, and shake vigorously for 15 min, preferably with
**Manganese content**

(continued)

a mechanical shaker. Dilute to volume with 0.5 N hydrochloric acid, and shake. Centrifuge approximately 100 ml of the mixture in a heavy-walled centrifuge tube at 2000 rpm for 5 min, and use the clear supernatant liquid in the following "procedure".

**Phosphorus content**

As specified in the Column 3 of Table I.

**Reagents**

- Ammonium Molybdate Solution (5%): Dissolve 50 g of ammonium molybdate tetrahydrate, (NH₄)₆Mo₇O₂₄•4H₂O, in 900 ml of warm water, cool to room temperature, dilute to 1000 ml with water, and mix.

- Ammonium Vanadate Solution (0.25%): Dissolve 2.5 of ammonium metavanadate, NH₄VO₃, in 600 ml of boiling water, cool to 60° to 70°, and add 20 ml of nitric acid. Cool to room temperature, dilute to 1000 ml with water, and mix.

- Zinc Acetate Solution (10%): Dissolve 120 g of zinc acetate dihydrate, Zn(C₂H₃O₂)₂•2H₂O, in 880 ml of water, and filter through Whatman No. 2V or equivalent filter paper before use.

- Nitric Acid Solution (29%): Add 300 ml of nitric acid (sp. gr 1.42) to 600 ml of water, and mix.

- Standard Phosphorus Solution: (100 µg P in 1 ml): Dissolve 438.7 mg of monobasic potassium phosphate, KH₂PO₄, in water in a 1000-ml volumetric flask, dilute to volume with water, and mix.

**Standard Curve**

Pipet 5.0, 10.0, and 15.0 ml of the Standard Phosphorus Solution into separate 100 ml volumetric flasks. To each of these flasks, and to a fourth blank flask, add in the order stated 10 ml of Nitric Acid Solution, 10 ml of Ammonium Vanadate Solution, and 10 ml of Ammonium Molybdate Solution, mixing thoroughly after each addition. Dilute to volume with water, mix, and allow to stand for 10 min. Determine the absorbance of each standard solution in a 1 cm cell at 460 nm, with a suitable spectrophotometer, using the blank to set the instrument at zero. Prepare a standard curve by plotting the absorbance of each solution versus its concentration, in mg P per 100 ml.
Phosphorus content (continued)

**Sample Pretreatment**
Place 20 to 25 g of the starch sample in a 250 ml beaker, add 200 ml of a 7 to 3 methanol-water mixture, disperse the sample, and agitate mechanically for 15 min. Recover the starch by vacuum filtration in a 150 ml medium-porosity fritted-glass or Buchner funnel, and wash the wet cake with 200 ml of the methanol-water mixture. Reslurry the wet cake in the solvent, and wash it a second time in the same manner. Dry the filter cake in an air oven at a temperature below 50°C, then grind the sample to 20-mesh or finer, and blend thoroughly. Determine the amount of dry substance by drying a 5 g portion in a vacuum oven, not exceeding 100 mm of Hg, at 120°C for 5 h. (NOTE: The treatment outlined above is satisfactory for starch products that are insoluble in cold water.

For pregelatinized starch and other water-soluble starches, prepare a 1% to 2% aqueous paste, place it in a cellophane tube, and dialyze against running distilled water for 30 to 40 h. Precipitate the starch by pouring the solution into 4 volumes of acetone per volume of paste, while stirring. Recover the starch by vacuum filtration in a medium-porosity fritted-glass or Buchner funnel, and wash the filter cake with absolute ethanol. Dry the filter cake, and determine the amount of dry substance as directed for water-insoluble starches).

**Sample preparation**
Transfer about 10 g of the Treated Sample, calculated on the dry-substance and accurately weighed, into a Vycor dish, and add 10 ml of Zinc Acetate Solution in a fine stream, distributing the solution uniformly in the sample. Carefully evaporate to dryness on a hot plate, then increase the heat, and carbonize the sample on the hot plate or over a gas flame. Ignite in a muffle furnace at 550°C until the ash is free from carbon (about 1 to 2 h), and cool. Wet the ash with 15 ml of water and wash slowly down the sides of the dish with 5 ml of Nitric Acid Solution. Heat to boiling, cool, and quantitatively transfer the mixture into a 200-ml volumetric flask, rinsing the dish with three 20-ml portions of water and adding the rinsings to the flask. Dilute to volume with water, and mix. Transfer an accurately measured aliquot (V, in ml) of this solution, containing not more than 1.5 mg of phosphorus, into a 100-ml volumetric flask and add 10 ml of Nitric Acid Solution, 10 ml of Ammonium Vanadate Solution, and 10 ml of Ammonium Molybdate Solution, mixing thoroughly after each addition. Dilute to volume with water, mix, and allow to stand for 10 min.

**Procedure**
Determine the absorbance of the Sample Preparation in a 1 cm cell at 460 nm, with a suitable spectrophotometer, using the blank to set the instrument at zero. From the Standard Curve, determine the mg of phosphorus in the aliquot taken, recording this value as a. Calculate the amount in mg/kg of Phosphorus (P) in the original sample by the formula:

\[(a \times 200 \times 1000) / (V \times W)\]

in which W is the weight of the sample taken, in g.
Acetyl groups content

As specified in Column 3 of Table 1.

Accurately weigh about 5 g of the sample and transfer into a 250 ml conical flask. Suspend in 50 ml of water, add a few drops of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide to a permanent pink end-point. Add 25.0 ml of 0.45 N sodium hydroxide, stopper the flask, and shake vigorously for 30 min., preferably with a mechanical shaker. (NOTE: the temperature should not exceed 30° or some starches may gelatinise). Remove the stopper, wash the stopper and sides of the flask with a few ml of water, and titrate the excess alkali with 0.2 N hydrochloric acid to the disappearance of the pink colour. Record the volume, in ml of 0.2 N hydrochloric acid required as S.

Perform a blank titration on 25.0 ml of 0.45 N sodium hydroxide, and record the volume, in ml, of 0.2 N hydrochloric acid required as B.

\[
\text{Acetyl groups (\%)} = \frac{(B - S) \times N \times 0.043 \times 100}{W}
\]

where \( N \) = the exact normality of the hydrochloric acid solution

\( W \) = the weight of the sample in grams.

Vinyl acetate content

Headspace Gas Chromatographic method*

Chromatographic system

Use a gas chromatograph with 2 m x 2 mm (i.d.) glass column containing Porapak Q, 80-100 mesh (or equivalent) fitted with a flame ionisation detector, under the following conditions:

- air flow: 200 ml/min
- hydrogen flow: 30 ml/min
- nitrogen flow: 20 ml/min
- injection port temperature: 200°
- column temperature: 150°
- detector temperature: 200°

Standard preparation

Accurately weigh 150 mg vinyl acetate (reagent grade) into a 100 ml volumetric flask. Dissolve and make up to volume with distilled water. Place 1 ml of this solution in a 10 ml volumetric flask and make up to volume with distilled water. Add 1 ml of this dilute solution to 30 g unmodified starch of the same botanical origin as the test substance in a 100 ml flask with a septum-liner. Seal the flask immediately with the septum-liner. This provides a standard starch preparation with a vinyl acetate content of 5 mg/kg.

Procedure

Weigh 30 g of the test substance into a 100 ml flask with a septum-liner. Seal the flask. Place the flask containing the test substance and the flask containing the standard.

Vinyl acetate content
(preparation in a constant temperature water bath at 70° for 30 min. Withdraw 2.0 ml from the headspace volume of the flask containing the standard preparation using a gas-tight syringe, inject directly into the injection port of the gas chromatograph and record the peak height of the chromatogram. Similarly inject 2.0 ml of the headspace volume from the flask containing the test substance into the chromatograph. Calculate the content of vinyl acetate in the test substance from a comparison of the peak heights of the two chromatograms.

Adipate group content
As specified in Column 3 of Table I.

Reagents and Solutions
- N,N-Bis-trimethylsilyl-trifluoroacetamide (BSTFA): Macherey-Nagel, D 5160 Duren, GFR or equivalent.
- Glutaric acid solution: Dissolve 1.00 g of glutaric acid (Merck or equivalent) in water and dilute to 1000 ml.
- Adipic acid solution: Dissolve 1.00 g of adipic acid (UCB, Brussels, Belgium or equivalent) in 900 ml of warm water, cool to room temperature, dilute to 1000 ml and mix.

Apparatus
Chromatograph
- Hewlett-Packard Model 7620A gas chromatograph or equivalent equipped with flame ionization detector and Model 3370A integrator.
- Column parameters: 2m stainless steel, 1.83 mm id, packed with 5% OV-17 on 80-100 mesh Chromosorb GAW-DMCS (Alltech Europe, Inc., B 9731 Eke, Belgium); precondition column 24 h at 350° with nitrogen carrier gas at 40 ml/min. Operating gas flow rates (ml/min): nitrogen carrier 30, hydrogen 40, air 400. Temperature: Injection 280°, detector 250°, column 140°. Retention times (min): glutaric acid 2.83, adipic acid 4.50.

Calibration
Weigh 1.0 g waxy corn starch into each of four 250 ml Erlenmeyer flasks. To each flask add 50 ml water and 1.0 ml of an aqueous solution containing 1.0 mg glutaric acid/ml. Add, to one flask, 0.25 ml of an aqueous solution containing 1.0 mg adipic acid per ml; to the other three, add 0.50 ml, 0.75 ml, and 1.0 ml respectively. Each flask then contains 1.0 mg glutaric acid and, respectively, 0.25, 0.50, 0.75 and 1.0 mg adipic acid. Agitate flasks manually to disperse the starch fully and add 50 ml 4N sodium hydroxide. Continue agitation another 5 min., place each flask in water bath at ambient temperature, and carefully add 20 ml 12N hydrochloric acid to each. When each flask is cool quantitatively transfer contents to 250 ml separatory funnel. Extract with 100 ml reagent grade ethyl acetate. Drain bottom aqueous layer into beaker and collect upper organic layer in 500 ml Erlenmeyer flask containing 20 g anhydrous sodium sulphate. Transfer aqueous portion back to separatory funnel and repeat ethyl acetate extraction twice more. Shake flasks periodically during 10 min and then filter contents through Whatman No. 1 paper into 1 L round-bottom
Adipate group content

(continued)

flasks. Rinse flasks and insoluble residues in filters twice with 50 ml of ethyl acetate. Under vacuum, (50 mm Hg) at temperature not exceeding 40°, evaporate total organic extraction and washings of each flask until completely dry.

The evaporation of ethyl acetate should be effected as quickly as possible because some hydrolysis takes place on standing. The products of hydrolysis cause a deterioration in the resolution of adipic acid in the chromatographic separation.

Successively add 2 ml pyridine and 1 ml N,N-bis-trimethylsilyl-trifluoroacetamide to the dry contents. Close round-bottom flasks with stopper and rinse internal surfaces thoroughly by swirling. Let flasks stand 1 h.; then transfer ca 2 ml from each to small glass vials and immediately seal. Inject 4 µl into gas chromatograph.

Calculations
Establish retention times for each acid and determine peak height for glutaric acid and for each level of adipic acid represented. A plot of peak height ratio of adipic acid to glutaric acid against amount of adipic acid is linear. This calibration curve may be used, but it is simpler to use a response factor (RF):

\[
RF = \frac{H_g \times W_s}{H_a}
\]

where \( H_a \) and \( H_g \) = peak heights of the standard adipic acid and glutaric acid, respectively;

\( W_s \) = weight of the standard adipic acid

RF should be verified weekly.

Total adipate content
Accurately weigh about 1.0 g of the sample into a 250 ml Erlenmeyer flask, and add 50 ml water and 1.0 ml of an aqueous solution containing 1.0 mg glutaric acid/ml. Proceed as in Calibration, beginning "Agitate flasks manually".

Free adipic acid content
Accurately weigh about 5.0 g of the sample into a 250 ml Erlenmeyer flask; add 100 ml water and 1.0 ml of the glutaric acid solution. Agitate for 1 h, filter through a 0.45 Millipore filter. Add 1 ml concentrated hydrochloric acid to the filtrate and transfer it quantitatively to a 250 ml separating funnel. Proceed as in Calibration, beginning "Extract with 100 ml..."

Calculation
For both preparations ("Total adipate content" and "Free adipic acid content") record peak heights for adipic acid and glutaric acid (internal standard). Calculate the amounts of total adipate and free adipic acid respectively contained in the sample as follows:
Adipate group content
(continued)

\[
A = \frac{H_x \times RF}{H_{ox} \times S \times 10}
\]

where:
A = content of total adipate or free adipic acid respectively (%)
H_x = peak height of adipic acid in the actual sample preparation
H_{ox} = peak height of glutaric acid in the actual sample preparation
RF = response factor for adipic acid
S = weight of sample in the actual preparation (g)

Adipate groups (%) =
Content of total adipate (%) - content of free adipic acid (%)

Hydroxypropyl group content

As specified in Column 3 of Table 1

- Ninhydrin reagent: A 3% solution of 1,2,3-triketo hydrindene crystals in 5% aqueous sodium bisulfite solution

Procedure
Accurately weigh 50 - 100 mg of the sample into a 100-ml volumetric flask and add 25 ml of 1 N sulfuric acid. Prepare a sample of unmodified starch of the same source (i.e., corn or potato) in the same manner. Place the flasks in a boiling water bath and heat until the samples are in solution. Cool and dilute the contents to 100 ml with water. If necessary, dilute the sample further to assure the presence of no more than 4 mg of hydroxypropyl group per 100 ml, and then dilute the blank starch in the same proportion. Pipet 1 ml of the solutions into 25-ml graduated test tubes with glass stoppers and, with the tubes immersed in cold water, add dropwise 8 ml of concentrated sulfuric acid to each. Mix well and place the tubes in a boiling water bath for exactly 3 min. Immediately transfer the tubes to an ice bath until the solution is chilled. Add 0.6 ml of ninhydrin reagent, carefully allowing the reagent to run down the walls of the test tubes. Immediately shake well, and place the tubes in a 25° water bath for 100 min. Adjust the volume in each tube to 25 ml with concentrated sulfuric acid and mix by inverting the tubes several times. (Do not shake). Immediately transfer portions of the solutions to 1-cm cells designed for a Beckman Model B spectrophotometer, and after exactly 5 min, measure the absorption (A) at 590nm, using the starch blank as the reference. Prepare a calibration curve with 1-ml aliquots of standard aqueous solutions, containing 10, 20, 30, 40 and 50 μg of propylene glycol per ml.

Calculations

Hydroxypropyl groups = \[ \frac{C \times 0.7763 \times 10 \times F}{W} \]

Where:
C = amount of propylene glycol in the sample solution read from the calibration curve (μg/ml)
F = dilution factor (if a further dilution has been necessary)
W = weight of sample (mg)
Propylene chlorohydrin

As specified in Column 3 of Table I.

Apparatus

Gas Chromatograph*

Use a Hewlett-Packard Model 5750 or equivalent. A dual-column instrument equipped with a flame-ionization detector is recommended. An integrator should be part of the recording system.

- Gas Chromatography column: Use a stainless steel column, 3 m x 3.2 mm (od), packed with 10% Carbowax 20 M on 80/100-mesh Gas Chrom 2, or equivalent. After packing and prior to use, condition the column overnight at 200°C, using a helium flow of 25 ml per min.


- Pressure Bottles: Use 200 ml pressure bottles, with a Neoprene washer, glass stopper, and attached wire clamp, available from Fisher Scientific Co., Pittsburg, PA, USA (Vitro 400, Catalog No. 3-100), or equivalent.

Reagents

- Diethyl ether: Use anhydrous, analytical reagent-grade diethyl ether, (NOTE: Some lots of diethyl ether contain foreign residues that interfere with the analysis and/or the interpretation of the chromatograms. If the ether quality is unknown or suspect, concentrate 50 ml to a volume of about 1 ml in the Concentrator, and then chromatograph a 2.0 ml portion using the conditions outlined under the Procedure. If the chromatogram is excessively noisy and contains signal peaks that overlap or interfere in the measurement of the peaks produced by the propylene chlorohydrin isomers, the ether should be redistilled.)

- Florisil: Use 60/100 mesh material, available from Floridin Co., 3 Penn Center, Pittsburg, PA. 15235, or an equivalent product available from Supelco, Bellefonte, PA. 16823.


- Standard preparation: Draw 25 µl of mixed propylene chlorohydrin isomers containing 75% of 1-chloro-2-propanol and 25% of 2-chloro-1-propanol) into a 50 µl syringe. Accurately weigh the syringe and discharge the contents into a 500 ml volumetric flask partially filled with water. Reweigh the syringe, and record the weight of the chlorohydrins taken. Dilute to the volume with water, and mix. This solution contains about 27.5 mg of mixed chlorohydrins, or about 55 µg per ml. Prepare this solution fresh on the day of use.

Propylene chlorohydrin
(continued)

Sample Preparation
Transfer a blended representative 50.0 g sample into a Pressure Bottle, and add 125 ml of 2 N sulfuric acid. Clamp the top in place, and swirl the contents until the sample is completely dispersed. Place the bottle in a boiling water bath, heat for 10 min, then swirl the bottle to mix the contents, and heat in the bath for an additional 15 min. Cool in air to room temperature, then neutralize the hydrolyzed sample to pH 7 with 25% sodium hydroxide solution, and filter through Whatman No. 1 paper, or equivalent, in a Buchner funnel, using suction. Wash the bottle and filter paper with 25 ml of water, and combine the washings with the filtrate. Add 30 g of anhydrous sodium sulfate, and stir with a magnetic stirring bar for 5 to 10 min, or until the sodium sulfate is completely dissolved. Transfer the solution into a 500-ml separator equipped with a Teflon plug, rinse the flask with 25 ml of water, and combine the washings with the sample solution. Extract with five 50 ml portions of Diethyl Ether, allowing at least 5 min in each extraction for adequate phase separation. Transfer the combined ether extracts in a Concentrator, place the graduated receiver of the concentrator in a water bath maintained at 50° to 55°C, and concentrate the extract to a volume of 4 ml.

(Note: Ether extracts of samples may contain foreign residues that interfere with the analysis and/or the interpretation of the chromatograms. These residues are believed to be degradation products arising during the hydrolysis treatment. Analytical problems created by their presence can be avoided through application of a clean-up treatment performed as follows: Concentrate the ether extract to about 8 ml, instead of 4 ml specified above. Add 10 g of Florisil, previously heated to 130°C for 16 h just before use, to a chromatographic tube of suitable size, then tap gently, and add 1 g of anhydrous sodium sulfate to the top of the column. Wet the column with 25 ml of Diethyl Ether, and quantitatively transfer the concentrated extract to the column with the aid of small portions of the ether. Elute with three 25-ml portions of the ether, collect all of the eluate, transfer it to a Concentrator, and concentrate to a volume of 4 ml. Cool the extract to room temperature, transfer it quantitatively to a 5.0-ml volumetric flask with the aid of small portions of Diethyl ether, dilute to volume with the ether, and mix.)

Control Preparations
Transfer 50.0 g portions of unmodified (underivatized) waxy corn starch into five separate Pressure Bottles, and add 125 ml of 2 N sulfuric acid to each bottle. Add 0.0, 0.5, 1.0, 2.0, and 5.0 ml of the Standard Preparation to the bottles, respectively, giving propylene chlorohydrin concentrations, on the starch basis, of 0, 0.5, 1.0, 2.0, and 5.0 mg/kg, respectively. Calculate the exact concentration in each bottle from the weight of Propylene Chlorohydrins used in making the Standard Preparation. Clamp the top in place, swirl until the contents of each bottle are completely dissolved, and proceed with the hydrolysis, neutralization, filtration, extraction, extract concentration, and final dilution as directed under Sample Preparation.
Propylene chlorohydrin
(continued)

Procedure
The operating conditions may be varied, depending upon the particular instrument used, but a suitable chromatogram is obtained with the Hewlett-Packard Model 5750 using a column oven temperature of 110°C, isothermal; injection port temperature of 210°C; detector temperature of 240°C; and hydrogen (30 ml per min), helium (25 ml per min), or air (350 ml per min) as the carrier gas. A 1.0 mV full-scale recorder is recommended; range, attenuation, and chart speed should be selected to optimize signal characteristics. Inject 2.0 µl aliquots of each of the concentrated extracts, prepared as directed under Control Preparation, allowing sufficient time between injections for signal peaks corresponding to the two chlorohydrin isomers to be recorded (and integrated) and for the column to be purged. Record and sum the signal areas (integrator outputs) from the two chlorohydrin isomers for each of the controls. Using identical operating conditions, inject a 2.0-µl aliquot of the concentrated extract prepared as directed under Sample Preparation, and record and sum the signal areas (integrator outputs) from the sample.

Calculation
Prepare a calibration plot on linear coordinate graph paper by plotting the summed signal areas for each of the controls against the calculated propylene chlorohydrin concentrations, in mg/kg, derived from the actual weight of chlorohydrin isomers used. Using the summed signal areas corresponding to the 1-chloro-2-propanol and 2-chloro-1-propanol from the sample, determine the concentration of mixed propylene chlorohydrins, in mg/kg, in the sample by reference to the calibration plot derived from the control samples. After gaining experience with the procedure and demonstrating that the calibration plot derived from the control samples is linear and reproducible, the number of controls can be reduced to one containing about 5 mg/kg of mixed propylene chlorohydrin isomers. The propylene chlorohydrin level in the sample can then be calculated as follows:

\[
\text{Propylene chlorohydrins, mg/kg} = \frac{(C \times a)}{A},
\]

in which C is the concentration, in mg/kg, of propylene chlorohydrins (sum of isomers) in the control; a is the sum of signal areas produced by the propylene chlorohydrin isomers in the sample; and A is the sum of the signal areas produced by the propylene chlorohydrin isomers in the control.

Degree of substitution of starch sodium octenyl succinate

Principle
The Degree of Substitution is determined by alkali consumed after acidification and thorough washing of the starch half ester.
Degree of substitution of starch sodium octenyl succinate (continued)

Procedure
Weigh out 5.0 g of sample in a 150 ml beaker. Wet out with a few ml of reagent grade isopropyl alcohol. Add, by pipette 25 ml of 2.5 N hydrochloric acid in isopropanol, allowing the acid to wash down any sample on the sides of the beaker. Stir for 30 min. on a magnetic stir plate. Add 100 ml of 90% isopropanol from a graduated cylinder. Stir for 10 min. Filter the sample through a Buchner funnel and wash the filter cake with 90% isopropanol until the filtrate is negative for chloride ions. Use 0.1 N AgNO₃ to check for chloride ions. Transfer the filter cake to a 600 ml beaker and rinse the Buchner funnel to wash any starch into the beaker. Bring to a 300 ml volume with distilled water. Place for 10 min. in a boiling water bath with stirring. Titrate while hot with 0.1 N NaOH to the phenolphthalein end-point.

Calculation: Calculate as follows:

\[
\text{Degree of Substitution (DS) = } \frac{0.162A}{1 - 0.210A}
\]

Where \( A = \) milliequivalents of sodium hydroxide required per g of starch octenyl succinate.

Residual octenyl succinic acid in starch sodium octenyl succinate

Extraction and Preparation of Sample Solution
Extract about 500 mg of starch with 15 ml of methanol overnight under constant shaking (weigh starch accurately). Filter the extraction mixture. Wash the starch on the filter with 7 ml of methanol. Repeat three times. Combine all filtrates (about 80% of the residuals are extracted by this procedure). Add 1 ml of 0.16 N methanolic KOH to the extracts. Dry the extracts with a flash evaporator at 30°. Dissolve the residue in 2 ml methanol. Take 0.5 ml of residue solution to the reaction vial. Add 0.5 ml derivative reagent (2.8 g of 2-p-dibromoacetophenone and 0.28 g 18-Crown-6 in 50 ml CH₃CN) to the reaction vial. Add 2 ml CH₃CN to the reaction vial. Cap the reaction vial and heat it at 80° for 30 min. Cool the reaction solution to room temperature (use within 24 h).

Liquid Chromatography Analysis*
- Column: Micro-Bondapack C₁₈ (Waters)
- Mobile Phase: Gradient elution of 70% methanol in water to 80% methanol in water in 5 min.
  Curve 6 (Waters 660 solvent programmer)
- Flow Rate: 1.5 ml/min.
- Detector: UV at 254 nm, attenuation 0.16 AUFS
- Injection volume: 5 µl

Residual octenyl succinic acid in starch sodium octenyl succinate (continued)

Preparation of Calibration Curve

Prepare a 0.5 M solution of sodium octenyl succinate (Solution A). With a syringe take 0.25 ml of Solution A and place into a 25-ml volumetric flask. Dilute to mark with methanol (Solution B). Prepare three calibration standards by taking 0.5, 1 and 2 ml of Solution B and placing into three 50-ml round bottom flasks. Add to each 1 ml of 0.16 N methanolic KOH. Dry each solution with a flash evaporator at 30°. Dissolve the residue in 2.0 ml of methanol (Solution C1, C2 and C3). Place 0.5 ml of the residue solution in the reaction vial. Add 0.5 ml derivative reagent (2.8 g of 2-p-dibromo-acetophenone and 0.28 g of 18-Crown-6 in 50 ml of CH3CN) to the reaction vial. Add 2 ml of CH3CH to the reaction vial. Cap the vial and heat to 80° for 30 min. Cool the reaction solution to room temperature (the derivative should be prepared as needed and used immediately). Inject 5 μl into the Liquid Chromatograph. The amount of residuals in each of the 5-μl injections are the following:

- for Solution C1: 0.2375 μg
- for Solution C2: 0.4750 μg
- for Solution C3: 0.9500 μg

Plot peak height from Liquid Chromatograph Chart versus μg of residuals per 5 ml of solution.

Calculations

Prepare a calibration curve according to the procedure. Using the peak height of the unknown sample from the Liquid Chromatograph Chart, determine the level of residuals (calculated as octenyl succinic acid) in the injected volume from the calibration curve.

\[
\text{% Residual in Starch} = \frac{300 \times \text{Value from Graph}}{\text{Weight of starch in mg}}
\]

Note: The formula is corrected to 100% recovery by dividing by 0.80, so that 240/0.80 = 300.
**MONO- AND DIGLYCERIDES**

SYNONYMS
Glyceryl monostearate, glyceryl monopalmitate, glyceryl monooleate, etc.; monostearin, monopalmitin, monoolein, etc.; GMS (for glyceryl monostearate)
INS No. 471, EEC No. E471

DEFINITION
Mono- and Diglycerides are a mixture of mono- and diglyceryl esters of long chain, saturated and unsaturated fatty acids that occur in food fats. They contain not less than 30% of α-monoglycerides and may also contain other isomeric monoglycerides, as well as di- and triglycerides, free glycerol, free fatty acids, soap and moisture. They are usually manufactured by the glycerolysis of edible fats and oils, but may also be prepared by esterification of fatty acids with glycerol, with or without molecular distillation of the product.

 Structural formula

\[
\begin{align*}
\alpha\text{-mono-} & : \quad CH_2OOCR \\
\beta\text{-mono-} & : \quad CH_2OH \\
\alpha\beta\text{-di-} & : \quad CH_2OOCR \quad CH_2OH \\
\alpha\alpha\text{-di-} & : \quad CH_2OH \quad CH_2OH \\
\end{align*}
\]

where -OCR represents the fatty acid moiety

Molecular weight
Glyceryl monostearate 358.6
Glyceryl distearate 625.0

These are two major components of commercial products

DESCRIPTION
White or cream coloured hard fats of waxy appearance, plastic products or viscous liquids

FUNCTIONAL USE
Emulsifier

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Insoluble in water. Soluble in ethanol, chloroform and benzene

** B. Infrared spectrum
Characteristic of a partial fatty acid ester of a polyol

** C. Positive test for fatty acids
Passes test

** D. Positive test for glycerol
Passes test

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

**PURITY TESTS**

* Water
  Not more than 2.0% (Karl Fischer method)

* Arsenic
  Not more than 3 mg/kg (Method II)

* Heavy metals
  Not more than 10 mg/kg
  Test 2 g of the sample as directed in the Limit Test (Method II)

* Acid value
  Not more than 6

* Free glycerol
  Not more than 7%

Soap
  Not more than 6%, calculated as a sodium oleate, C_{18}H_{33}O_{2}Na
  See description under TESTS

**TESTS**

**PURITY TEST**

Soap
Add 10.00 g of the sample to a mixture of 60 ml of acetone and 0.15 ml of bromophenol blue solution (0.5%), previously neutralized with 0.1 N hydrochloric acid or 0.1 N sodium hydroxide. Warm gently on a water bath until solution is complete, and titrate with 0.1 N hydrochloric acid until the blue colour is discharged. Allow to stand for 20 min, warm until any solidified matter has re-dissolved and, if the blue colour reappears, continue the titration. Each ml of 0.1 N hydrochloric acid is equivalent to 0.0304 g of C_{18}H_{33}O_{2}Na.

**METHODS OF ASSAY**

α-Monoglyceride content
Determine by the method given in the General Methods*
**MONOAMMONIUM L-GLUTAMATE**

**SYNONYMS**
Ammonium glutamate, INS No.624, EEC No.624

**DEFINITION**
- **Chemical names**: Monoammonium L-glutamate monohydrate,
- **C.A.S. number**: 7558-63-6
- **Chemical formula**: C₇H₇N₂O₅ • H₂O
- **Structural formula**: \( \text{HOOC-CH-CH}_2\text{-CH}_2\text{-COONH}_4 \cdot \text{H}_2\text{O} \)
- **Molecular weight**: 182.18
- **Assay**: Content not less than 99.0% of \( \text{C}_7\text{H}_7\text{N}_2\text{O}_5 \cdot \text{H}_2\text{O} \) on the dried basis

**DESCRIPTION**
White, practically odourless crystals or crystalline powder, having a characteristic taste

**FUNCTIONAL USE**
Flavor enhancer, salt substitute

**CHARACTERISTICS**

**IDENTIFICATION TESTS**
**A. Solubility**
Freely soluble in water.

**B. Positive test for glutamic acid**
Passes test
See description under TESTS

**C. Positive test for ammonium**
Passes test

**PURITY TESTS**

**A. Loss on drying**
Not more than 0.5% (50°, 4 h)

**pH**
6.0 - 7.0 (1 in 20 soln)

**Specific rotation**
\( \left[ \alpha \right]_D : +25.4 \text{ to } +26.4^\circ \)
Test a solution of 10 g of sample (dried basis) in 100 ml of 2N hydrochloric acid, using a 200-mm tube.

**Sulfated ash**
Not more than 0.1%.
Test 1 g of the sample as directed in the test for Ash (Sulfated Ash, Method I).

---

* These specifications were prepared at the 31st session of JECFA (1987) and published in FNP 38 (1988).

* **Arsenic**  
Not more than 3 mg/kg.  
Test 1 g of the sample as directed in the Arsenic Limit Test (Method II).

* **Lead**  
Not more than 10 mg/kg.  
Test 1 g of the sample as directed in the Lead Limit Test using 10 µg of lead ion (Pb) in the control.

* **Heavy metals**  
Not more than 20 mg/kg.  
Test a solution of 1 g of the sample in 25 ml of water as directed in the Heavy Metals Limit Test (Method II).

**Pyrrolidone carboxylic acid**  
Passes test  
Proceed as directed in the Purity Test for Pyrrolidone carboxylic acid under Monosodium L-Glutamate.

**TESTS**

**IDENTIFICATION TESTS**

B. Positive test for glutamic acid  
Proceed as directed under Chromatography (Thin-layer chromatography) in the General Methods* under following condition.

- Sample: 1 µl of 1 in 100 soln of the sample  
- Reference standard: 1 µl of a 1 in 100 soln of monosodium L-glutamate.  
- Developing solvent: a mixture of 2 volumes of n-butanol, 1 volume of glacial acetic acid and 1 volume of water.  
- Adsorbent: silica gel

Stop the development when the solvent front has advanced about 10 cm from the point of application. Dry the plate at 80° for 30 min. Spray ninhydrin TS on the plate, heat at 80° for 10 min and observe the plate under natural light. The Rf value of the sample and that of the reference standard are identical.

**METHOD OF ASSAY**

Dissolve about 200 mg of the sample, previously dried and weighed accurately, in 6 ml of formic acid, and add 100 ml of glacial acetic acid. Titrate with 0.1 N perchloric acid* determining the end-point potentiometrically. Run a blank determination in the same manner and correct for the blank.

Each ml of 0.1 N perchloric acid is equivalent to 9.106 mg of C₉H₆N₂O₄ · H₂O.

**MONOGLYCERIDE CITRATE**

**SYNONYM**

Citric acid ester of glyceryl monooleate

**DEFINITION**

Monoglyceride Citrate is a mixture of glyceryl monooleate and its citric acid monoester, manufactured by the reaction of glyceryl monooleate with citric acid under controlled conditions. It conforms to the following specifications.

**Structural formula**

\[
\begin{align*}
\text{CH}_2 \quad & \text{OR}_1 \\
\text{CH} \quad & \text{OR}_2 \\
\text{CH}_2 \quad & \text{OR}_3
\end{align*}
\]

where R₁ represents oleic acid moiety and R₂ and R₃ a citric acid moiety or hydrogen

**DESCRIPTION**

Soft, white to ivory coloured, waxy solid with a lard-like consistency and a bland odour and taste

**FUNCTIONAL USE**

Synergist and solubilizer for antioxidants and flavours

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

**A. Solubility**

Insoluble in water. Soluble in ethanol

**B. Positive test for fatty acid**

Passes test

**C. Positive test for citric acid**

Passes test

**D. Positive test for glycerol**

Passes test

**PURITY TESTS**

**Water content**

Not more than 0.2% (Karl Fisher Method)

**Sulfated ash**

Not more than 0.3%

1 g of the sample meets the requirements of the Limit Test for Sulfated Ash

**Arsenic**

Not more than 3 mg/kg (Method II)

**Heavy metals**

Not more than 10 mg/kg

Test 2 g of the sample as directed in the Limit Test

* These specifications were prepared at the 18th session of JECFA (1974) and published in FNP 4 (1978).

PURITY TESTS

- Acid value
  - Not less than 70 and not more than 100

- Saponification value
  - Not less than 260 and not more than 265

- Total citric acid
  - Not less than 14.0 and not more than 17.0%
  - See description under TESTS

TESTS

PURITY TESTS

Total citric acid

**Standard Solution**
Transfer about 35 mg of sodium citrate dihydrate, accurately weighed, into a 100-ml volumetric flask. Dissolve and dilute to volume with water, and mix. Calculate the concentration (C) in µg per ml of citric acid in the final solution by the formula:

\[
C = \frac{0.6533 \times W}{100}
\]

W is the weight, in mg, of the sodium citrate dihydrate taken, and 0.6533 is a factor converting sodium citrate dihydrate to citric acid.

**Sample Solution**
Transfer about 150 mg of the sample accurately weighed, into a saponification flask. Add 50 ml of 4% alcoholic potassium hydroxide solution, and reflux for 1 h. Acidify the reaction mixture with hydrochloric acid to a pH of 2.8 - 3.2, transfer to a 400-ml beaker, and evaporate to dryness on a steam bath. Quantitatively transfer the contents of the beaker into a separator, using no more than 50 ml of water. Extract with three 50-ml portions of petroleum ether (b.p. 30-60°) discarding the extracts. Transfer the water layer to a 100-ml volumetric flask, dilute to volume with water, and mix.

**Procedure**
Pipet 2.0 ml each of the **Standard Solution** and of the **Sample Solution** into separate 40-ml graduated centrifuge tubes. Add 2 ml of a 1 in 2 sulfuric acid solution and 11 ml of water to each tube. Boil for 3 min, cool, and add 5 ml of bromine TS to each tube. Dilute to the 20-ml mark, allow to stand for 10 min, and centrifuge. Transfer 4.0 ml of each solution into separate 19 × 110-mm test tubes. Add 1 ml of water, 0.5 ml of a 1 in 2 sulfuric acid solution, and 0.3 ml of 1 M potassium bromide, and shake. Add 0.3 ml of 1.5 N potassium permanganate, shake, and allow to stand for 2 min. Add 1 ml of a saturated solution of ferrous sulfate, shake, allow to stand for 2 min. Dilute to 10 ml with water. Add 10.0 ml of n-hexane (previously washed with sulfuric acid, followed by a water wash, and then dried over anhydrous sodium sulfate), shake vigorously for 2 min and centrifuge at a low speed for 1 min. Transfer 5.0 ml of the hexane extract into a 20 × 145-mm tube containing 10.0 ml of sodium sulfide solution (4 g of Na₂S·9 H₂O in 100 ml of water), and shake vigorously briefly (3 oscillations only). Centrifuge the mixture at low speed for 1 min.

Immediately determine the absorbance of each aqueous layer in a 1-cm cell at 450 nm with a suitable spectrophotometer, using a reagent blank in the reference cell. Calculate the quantity, in mg, of citric acid in the sample taken by the formula

$$0.1C \times \frac{A_s}{A_i}$$

$C$ is as defined under *Standard Solution*, $A_s$ is the absorbance of the final solution from the *Sample Solution*, and $A_i$ is that of the final solution from the *Standard Solution*. 
**MONOPOTASSIUM L-GLUTAMATE***

**SYNONYMS**

Potassium glutamate, MPG
INS No.622, EEC No.622

**DEFINITION**

- **Chemical name**: Monopotassium L-glutamate monohydrate,
- **C.A.S. number**: 19473-49-5
- **Chemical formula**: \( \text{C}_7\text{H}_7\text{KNO}_4 \cdot \text{H}_2\text{O} \)
- **Structural formula**: \[
\text{HOOC-CH-CH}_2\text{-CH}_2\text{-COOK} \cdot \text{H}_2\text{O} \\
\text{NH}_2
\]
- **Molecular weight**: 203.24
- **Assay**: Content not less than 99.0% of \( \text{C}_7\text{H}_7\text{KNO}_4 \cdot \text{H}_2\text{O} \) on the dried basis

**DESCRIPTION**

White, practically odourless crystals or crystalline powder, having a characteristic taste

**FUNCTIONAL USE**

Flavor enhancer, salt substitute

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

**A. Solubility**

Freely soluble in water. Slightly soluble in ethanol.

**B. Positive test for glutamic acid**

Passes test

Proceed as directed in the Positive test for glutamic acid under Monoammonium L-Glutamate.

**C. Positive test for potassium**

Passes test

**PURITY TESTS**

**Loss on drying**

Not more than 0.2% (80°, 5 h)

**pH**

6.7 - 7.3 (1 in 50 soln)

**Specific rotation**

\[ [\alpha]_{D}^o : +22.5 \text{ to } +24.0^o \]

Test a solution of 10 g of sample (dried basis) in 100 ml of 2N hydrochloric acid, using a 200-mm tube.

* These specifications were prepared at the 31st session of JECFA (1987) and published in FNP 38 (1988).

PURITY TESTS (continued)

* Chlorides

Not more than 0.2%.
Test 0.07 g of the sample as directed in the Chlorides Limit Test using 0.4 ml of 0.01 N hydrochloric acid in the control.

* Arsenic

Not more than 3 mg/kg.
Test 1 g of the sample as directed in the Arsenic Limit Test (Method II).

* Lead

Not more than 10 mg/kg.
Test 1 g of the sample as directed in the Lead Limit Test using 10 μg of lead ion (Pb) in the control.

* Heavy metals

Not more than 20 mg/kg.
Test a solution of 1 g of the sample in 25 ml of water as directed in the Heavy Metals Limit Test (Method II).

Pyrrolidone carboxylic acid
Passes test
Proceed as directed in the Purity Test for Pyrrolidone carboxylic acid under Monosodium L-Glutamate.

METHOD OF ASSAY

Dissolve about 200 mg of the sample, previously dried and weighed accurately, in 6 ml of formic acid, and add 100 ml of glacial acetic acid. Titrate with 0.1 N perchloric acid* determining the end-point potentiometrically. Run a blank determination in the same manner and correct for the blank.

Each ml of 0.1 N perchloric acid is equivalent to 10.162 mg of C₆H₅KNO₄·H₂O.

**MONOSODIUM L-GLUTAMATE**

SYNONYMS  
Sodium glutamate, MSG  
INS No.621, EEC No.621

**DEFINITION**

<table>
<thead>
<tr>
<th>Chemical names</th>
<th>Monosodium L-glutamate monohydrate, glutamic acid monosodium salt monohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A.S. number</td>
<td>142-47-2</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>C₅H₇NNaO₄ • H₂O</td>
</tr>
</tbody>
</table>
| Structural formula             | \[
|                               | \text{NaOOCCH₂CH₂} - \text{C} = \text{COOH} • H₂O \text{NH₂} \]             |
| Molecular weight               | 187.13                                                                        |
| Assay                          | Content not less than 99.0% of C₅H₇NNaO₄ • H₂O on the dried basis            |

**DESCRIPTION**

White, practically odourless crystals or crystalline powder, having a characteristic taste

**FUNCTIONAL USE**

Flavor enhancer

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

** A. Solubility**  

** B. Positive test for glutamic acid**  
Passes test  
See description under TESTS

** C. Positive test for sodium**  
Passes test

**PURITY TESTS**

** A. Loss on drying**  
Not more than 0.5% (98°, 5 h)

** pH**  
6.7 - 7.2 (1 in 20 soln)

---

* These specifications were prepared at the 31st session of JECFA (1987) and published in FNP 38 (1988).

**PURITY TESTS (continued)**

* **Specific rotation**

\[ \text{[\(\alpha\)]_D}^\circ : +24.8 \text{ to } +25.3^\circ \]

Test a solution of 10 g of sample (dried basis) in 100 ml of 2N hydrochloric acid, using a 200-mm tube.

* **Chlorides**

Not more than 0.2%.

Test 0.07 g of the sample as directed in the Chlorides Limit Test using 0.4 ml of 0.01 N hydrochloric acid in the control.

* **Arsenic**

Not more than 2 mg/kg.

Test 1 g of the sample as directed in the Arsenic Limit Test (Method II) using 2 ml of standard arsenic solution.

* **Lead**

Not more than 5 mg/kg.

Test 1 g of the sample as directed in the Lead Limit Test using 5 µg of lead ion (Pb) in the control.

* **Heavy metals**

Not more than 10 mg/kg.

Test a solution of 2 g of the sample in 25 ml of water as directed in the Heavy Metals Limit Test (Method I).

**Pyrrrolidone carboxylic acid**

Passes test

See description under TESTS

**TESTS**

**IDENTIFICATION TESTS**

B. Positive test for glutamic acid

Proceed as directed under Chromatography (Thin-layer chromatography)* in the General Methods* under following condition.

- **Sample:** 1 µl of 1 in 100 soln of the sample
- **Reference standard:** 1 µl of a 1 in 100 soln of monosodium L-glutamate
- **Developing solvent:** a mixture of 2 volumes of n-butanol, 1 volume of glacial acetic acid and 1 volume of water
- **Adsorbent:** silica gel

Stop the development when the solvent front has advanced about 10 cm from the point of application. Dry the plate at 80° for 30 min. Spray ninhydrin solution (1% ninhydrin in methanol + 3% acetic acid) on the plate, heat at 80° for 10 min, and observe the plate under natural light. The Re value of the sample and that of the reference standard are identical.

---

PURITY TESTS

Pyroolidone carboxylic acid

Proceed as directed under Chromatography (Thin-layer chromatography)* in the General Methods* under following condition.

Preparation of solutions

- Standard solution: Dissolve 500 mg of monosodium L-glutamate and 2.5 mg of pyroolidone carboxylic acid in water to make up to 100 ml.

- Sample solution: Dissolve 500 mg of the sample in water and make up to 100 ml.

- Potassium iodide-starch TS: Dissolve 0.5 g of starch in about 50 ml of water by heating, after cooling add 0.5 g of potassium iodide and water to make up to 100 ml.

Procedure

Use 2 μl of the Sample solution and 2 μl of the Standard solution, with a mixture of 2 volumes of n-butanol, 1 volume of glacial acetic acid and 1 volume of water as the developing solvent, and silica gel as the adsorbent. Stop the development when the solvent front has advanced about 10 cm from the point of the application, dry the plate for 30 min in air.

At the same time, prepare a similar chamber as that for developing, placing in the chamber a 50-ml beaker containing about 3 g of sodium hypochlorite; pour slowly 1 ml of hydrochloric acid into the beaker in order to generate chlorine gas, put on the lid and allow to stand for 30 sec to fill the chamber with the gas. Place the dried plate in this chamber, put on the lid and allow to stand for 20 min. Take out the plate, keep for 10 min in air and spray with ethanol. After drying, spray potassium iodide-starch TS and observe the plate under natural light immediately after the standard spot has appeared.

No spot corresponding to pyroolidone carboxylic acid standard is detected in the sample (sensitivity = 0.2%).

METHOD OF ASSAY

Dissolve about 200 mg of the sample, previously dried and weighed accurately, in 6 ml of formic acid, and add 100 ml of glacial acetic acid. Titrate with 0.1 N perchloric acid* determining the end-point potentiometrically. Run a blank determination in the same manner and correct for the blank.

Each ml of 0.1 N perchloric acid is equivalent to 9.356 mg of C\textsubscript{3}H\textsubscript{4}N\textsubscript{2}O \cdot H\textsubscript{2}O.

NISIN*

SYNONYM
INS No. 234

DEFINITION
Nisin consists of several closely related polypeptide antibiotics produced by strains of Streptococcus lactis, Lancefield group N of which major component is shown below.

Nisin concentrate contains not less than 900 units per mg. In a mixture of non-fat milk solids and a minimum sodium chloride content of 50%. The most potent preparation of nisin yet obtained is 40,000 units/mg. The Unit has been redefined by Tramer and Fowler, J.Sci.Fd.Agric., 15, 522 (1964) in terms of a standard preparation. This approximates to the activity unit described by Berridge (Biochem.J. 45, 436, 1949).

C.A.S. number
1414 - 45 -5

Chemical formula
C_{48}H_{60}N_{14}O_{20}S_{7}

Structural formula

Molecular weight
3354.12

DESCRIPTION
Nisin concentrate is a white, micronized, spray-dried powder.

STABILITY
Nisin concentrate is stable at ambient temperatures. Both purified nisin and nisin concentrate are stable to heating under acid conditions, and will withstand 121° for 30 min at pH 2.0 and 15 min at pH 3.0. Nisin is less stable at higher pH values; heating at 121° in buffer for 15 min. results in the following percentage decreases in activity

pH 4.0-29%, pH 5.0-69%, pH 6-86%, pH 7.0-99.7%

FUNCTIONAL USE
Antimicrobial preservative.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Passes test.
See description under TESTS

* These specifications were prepared at the 12th session of JECFA (1968) and published in NMRS 45A (1969).

IDENTIFICATION TESTS (continued)

B. Melting point
None. Chars on heating

C. Differentiation from other antibiotics
Passes test.
See description under TESTS

PURITY TESTS

* Loss on drying
Not more than 3.0% when dried to constant weight at 102-103°C.

* Arsenic
Not more than 1.0 mg/kg (Method II) using a 3 g sample

* Heavy metals
Not more than 2.0 mg/kg.

TESTS

IDENTIFICATION TESTS

B. Solubility
(a) Purified preparation of nisin containing 40,000 units/mg.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Units/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water, pH 2.5</td>
<td>4.8 x 10^6</td>
</tr>
<tr>
<td>Water, pH 5.0</td>
<td>1.6 x 10^7</td>
</tr>
<tr>
<td>Methanol, acidified with HCl to pH 2.8</td>
<td>3.2 x 10^6</td>
</tr>
</tbody>
</table>

Insoluble in non-polar solvents

(b) Nisin concentrate

Forms a cloudy suspension in water due to the presence of denatured protein, but the nisin component behaves similarly to the purified material.

C. Differentiation from other antibiotics

Differentiation of nisin and other common antibiotics in food extracts

The microbiological assay of nisin in foods is not specific and other antibiotics present in food could interfere. In preparation for nisin assay, samples of food are acidified and boiled in order to bring nisin into the aqueous phase. As a second stage the nisin-containing extracts are made alkaline and heated, causing rapid inactivation of nisin, in order to provide a suitable nisin-free diluent for the nisin standard. The fate of other antibiotics when subjected to heat under acid and alkaline conditions is an important factor when considering the possibility of interference during nisin assay.

a. Suspension of processed cheese to which various antibiotics had been added were adjusted to pH 2.0 by the addition of conc. HCl and boiled for 5 min. Antibiotic activity in the acidified suspensions was measured by diffusion assay against M. flavus before and after boiling. Table I gives the loss in activity, expressed as a percentage, attributable to boiling at pH 2.0.

Table I

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>% loss of activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nisin</td>
<td>0</td>
</tr>
<tr>
<td>Tylosin</td>
<td>0</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>0</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>68</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>32</td>
</tr>
<tr>
<td>Gramicidin</td>
<td>61</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>50</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>72</td>
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<td>Phenoxyethylpenicillin</td>
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<td>Streptomycin</td>
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<td>Erythromycin</td>
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<td>Phenoxybenzylpenicillin</td>
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<td>Phenylmethylpenicillin</td>
<td>97</td>
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<tr>
<td>Phenoxypropylpenicillin</td>
<td>74</td>
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</table>

Table II

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>% loss of activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nisin</td>
<td>100</td>
</tr>
<tr>
<td>Tylosin</td>
<td>100</td>
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<tr>
<td>Polymyxin B</td>
<td>100</td>
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<td>Tetracycline</td>
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<td>Cloxacillin</td>
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<tr>
<td>Gramicidin</td>
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<tr>
<td>Bacitracin</td>
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<td>Ampicillin</td>
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<tr>
<td>Benzylpenicillin</td>
<td>64</td>
</tr>
<tr>
<td>Neomycin</td>
<td>78</td>
</tr>
<tr>
<td>Novobiocin</td>
<td>100</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>99</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>100</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>99</td>
</tr>
<tr>
<td>Phenoxybenzylpenicillin</td>
<td>24</td>
</tr>
<tr>
<td>Phenoxymethylpenicillin</td>
<td>93</td>
</tr>
<tr>
<td>Phenoxyethylpenicillin</td>
<td>66</td>
</tr>
<tr>
<td>Phenoxypropylpenicillin</td>
<td>75</td>
</tr>
</tbody>
</table>

b. Cheese suspensions as in (a) were adjusted to pH 11.0 by the addition of 5 N NaOH and heated at 63° for 30 min. The suspensions were cooled and the pH adjusted back to 2.0 with conc. HCl. Antibiotic activity was measured before and after treatment. Percentage losses are listed in Table II.
Tylosin, polymyxin, gramicidin, bacitracin, novobiocin and erythromycin behaved in the same way as nisin. From the results obtained in a. and b. above it appears that two antibiotics in particular, namely tylosin and polymyxin B, might be confused with nisin when measuring antibiotic activity in foods.

It is a known that the *Streptococcus lactis* strains which produce nisin will grow in reasonably high concentrations of the antibiotic. The same strains may, however, be sensitive to a wide range of other antibiotics, thus providing a fairly simple means of distinction.

Sensitivity tests were set up in which sterile litmus milk containing aerial dilution of antibiotics was inoculated with 0.1% of an overnight milk culture of *Streptococcus lactis* NCIB 8586. After 18 h incubation at 30° the sensitivity of the organism to a particular antibiotic was taken as the lowest concentration in which no growth occurred (MIC). The results are given in Table III.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nisin</td>
<td>&gt; 5000 units/ml</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>&gt; 100 µg/ml</td>
</tr>
<tr>
<td>Gramicidin</td>
<td>&gt; 100 µg/ml</td>
</tr>
<tr>
<td>Benzylpenicillin</td>
<td>1.5 µg/ml</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>12 µg/ml</td>
</tr>
<tr>
<td>Tylosin</td>
<td>1.0 µg/ml</td>
</tr>
<tr>
<td>Benzylpenicillin</td>
<td>3 µg/ml</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1.5 µg/ml</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>0.75 µg/ml</td>
</tr>
<tr>
<td>Neomycin</td>
<td>2.5 µg/ml</td>
</tr>
<tr>
<td>Novobiocin</td>
<td>1.5 µg/ml</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.3 µg/ml</td>
</tr>
</tbody>
</table>

**METHOD OF ASSAY**

**Determination of sodium chloride content of nisin concentrate**

Weigh ca. 5 g material, transfer with 80% alcohol to 100 ml volumetric flask and add enough 80 percent alcohol to give volume of ca 50 ml. Shake well to suspend all insoluble material. Add 1 ml HNO₃ and with pipet add excess of 0.1 N AgNO₃ soln. Dilute to 100 ml with alcohol. Transfer mixture to centrifuge bottle and centrifuge 5 min. at ca 1800 rpm. Pipet 50 ml supernatant into 300 ml Erlenmeyer flask, add 2 ml saturated Fe(NH₄)₂(SO₄) solution and 2 ml HNO₃ and titrate to permanent light brown with 0.1N NH₄CNS. Divide ml 0.1N AgNO₃ used by 2 and subtract ml NH₄CNS solution used. Multiply difference by 0.005844 to obtain g NaCl present.
Assay of nisin concentrate

Preparation of test organism

Streptococcus cremoris, IP5 (NCDO 495) is subcultured daily in sterile separated milk by transferring (Based on the method of Friedman and Epstein, J. Gen. Microbiol. 5: 830, 1951) one loopful to a McCartney bottle of litmus milk and incubating at 30°. Inoculated milk for the assay is prepared by inoculating a suitable quantity of sterile separated milk with 2 percent of a 24 h culture, and placing it in a water-bath at 30° for 1-½ h. It is then used immediately.

Preparation of standard solution

The Standard Stock Solution is prepared by dissolving an accurately weighed quantity of standard nisin in 0.02N hydrochloric acid to give a solution containing 5 000 units/ml. The stock solution is diluted further with 0.02N hydrochloric acid immediately before use to give 50 units of nisin per ml.

Preparation of sample solution

The weight of sample taken for preparing the stock solution of the sample is such that corresponding tubes of the sample and standard series match, i.e. within close limits the sample and standard are of the same concentration with respect to nisin content. The sample stock solution is diluted in 0.02N hydrochloric acid to give an estimated concentration of 50 units of nisin per ml.

Preparation of resazurin solution

A 0.0125% solution of resazurin in distilled water is prepared immediately before use.

Assay procedure

Graded volume (0.60, 0.55, 0.50, 0.45, 0.41, 0.38, 0.34, 0.31, 0.28, 0.26 ml) of the 50 unit per ml sample and standard solutions are pipetted into rows of 10 dry 6-inches x 5/8-inch bacteriological test-tubes, and 4.6 ml of the inoculated milk is added to each by means of an automatic pipetting device. The addition of inoculated milk is made in turn across each row of tubes containing the same nominal concentration not along each row of ten tubes. The tubes are placed in a water-bath at 30° for 15 min, then cooled in an ice water bath while 1 ml resazurin solution is added to each. The addition is made with an automatic pipetting device, in the same order used for the addition of inoculated milk. The contents of the tubes are thoroughly mixed by shaking, and incubation at 30° is continued in a water-bath for a further 3-5 min.

The tubes are examined under 2 x 20 watt Osram "Natural" fluorescent light in a black matt-finish cabinet. The sample tube of the highest concentration which shows the first clear difference in colour (i.e. has changed from blue to mauve) is compared with tubes of the standard row to find the nearest in colour. Further matches are made at the next two lower concentrations of the sample and standard. Interpolation of matches may be made at half dilution steps. As the standard tubes contain known amounts of nisin, the concentration of nisin in the sample solution may be calculated. The three results are averaged.

SYNONYM

INS No. 941

DEFINITION

Chemical name: Nitrogen
C.A.S. number: 7727-37-9
Chemical formula: \( \text{N}_2 \)
Molecular weight: 28.0
Assay: Content not more than 99.0% v/v of \( \text{N}_2 \)

DESCRIPTION

Colourless, odourless gas or a liquid

FUNCTIONAL USE

Freezant, packing gas

CHARACTERISTICS

IDENTIFICATION TEST

A. Flame test: A flame is extinguished in an atmosphere of nitrogen

PURITY TESTS

- Oxygen: Not more than 10 \( \mu l/l \)
  
  See description under TESTS

- Hydrogen: Not more than 10 \( \mu l/l \)

- Argon, helium and neon: Not more than 5 \( \mu l/l \) as a total of all three

- Carbon dioxide and other carbon compounds: Not more than 10 \( \mu l/l \) (calculated as carbon dioxide)

- Moisture:
  
  In compressed gas: Not more than 30 \( \mu l/l \) at a cylinder pressure of 130 bar absolute at 15\(^\circ\) (as indicated by a dew point not higher than -16\(^\circ\)).

  In liquid nitrogen: Not more than 60 \( \mu l/l \) in the gas obtained by complete vapourisation.

* These specifications were prepared at the 26th session of JECFA (1982) and published in FNP 25 (1982).
TESTS

PURITY TESTS

**Gaseous components**

Gaseous components (oxygen, hydrogen, etc.) are determined by Gas chromatography* in accordance with the following requirements:

The analyzer must be capable of separating and determining the component with a sensitivity of 1 μL/L or 20% of the specified maximum amount of the component, whichever is greater. Appropriate impurity concentrating techniques may be used to attain the sensitivity. The analyzer is to be calibrated at appropriate intervals by the use of calibration gas standards which contain the applicable limiting characteristic gaseous components in nitrogen.

**METHOD OF ASSAY**

After determination of the total content of specified impurities, the balance consists of N₂ together with any traces of the inert gases argon, neon and helium that may be present.

NITROUS OXIDE*  

SYNONYMS  
Nitrogen oxide, dinitrogen monoxide;  
INS No. 942

DEFINITION  
Chemical name  
Dinitrogen monoxide  
C.A.S. number  
10024-97-2  
Chemical formula  
N₂O  
Formula weight  
44.01  
Assay  
Contains not less than 97% (v/v) of N₂O.

DESCRIPTION  
Colourless, odourless and tasteless gas.

FUNCTIONAL USE  
Propellant

CHARACTERISTICS  

IDENTIFICATION TESTS  
A. Solubility  
1 vol. dissolves in 1.5 vol. of water (at 20°, 760 mm Hg).

B. Flame test  
A glowing splinter of wood in contact with nitrous oxide bursts into flame.

C. Pyrogallol test  
Nitrous oxide is not absorbed by alkaline pyrogallol solution.

PURITY TESTS  

Carbon monoxide  
Not more than 10 µl/l  
See description under TESTS

Nitric oxide and nitrogen dioxide  
Not more than 5 µl/l  
See description under TESTS

Halogens and hydrogen sulfide  
Not more than 5 µl/l  
See description under TESTS

Arsine and phosphate  
Passes test  
See description under TESTS

* These specifications were prepared at the 29th session of JECFA (1985) and published in FNP 34 (1986).

PURITY TESTS

**NOTE:** For the following tests keep the cylinder of gas from which the sample is taken at room temperature for not less than 6 h before carrying out the tests. In all the tests the cylinder is kept in the vertical position with the outlet valve uppermost when delivery the gas. Pass the gas at a steady rate of 4 l/h unless otherwise stated and carry out the tests or calculate the results with reference to the gas at 20° and 760 mm Hg.

**Carbon monoxide**

**Principle**
Carry out the test on the first portion of gas issuing from the cylinder. Use 5.0 L of the nitrous oxide in the test and 5.0 l of carbon monoxide-free air as the control. The difference between the volumes of 0.002 N sodium thiosulfate used in the two titrations is not greater than 0.5 ml.

**Apparatus**
The apparatus consists of the following parts connected in series:
- U-tube containing anhydrous silica gel impregnated with chromium trioxide.
- Scrubber bottle (dreschel type) containing 100 ml of a 40% w/v solution of potassium hydroxide.
- U-tube containing pellets of potassium hydroxide.
- U-tube containing phosphorous pentoxide dispersed on previously granulated, fused pumice.
- Tube containing recrystallized iodic anhydride (I₂O₅) in granules, previously dried at 200° and kept at a temperature of 120°. The iodic anhydride is packed in the tube in 1 cm columns separated by 1 cm columns of glass wool to give an effective length of 5 cm.
- Flask containing 2.0 ml of potassium iodide TS and 3 drops of starch solution TS.

**Procedure**
Flush the apparatus with 5.0 L of carbon dioxide-free air and, if necessary, discharge the blue colour in the iodide solution by adding the smallest necessary quantity of freshly prepared 0.002 N sodium thiosulfate. Continue flushing until not more than 0.045 ml of 0.002 N sodium thiosulfate is required after passing 5.0 L of carbon dioxide-free air.

Pass the gas from the cylinder through the apparatus, using the volume and the rate of flow prescribed in the monograph. Flush the last traces of liberated iodine into the reaction flask by passing through the apparatus 1.0 l of carbon monoxide-free air. Titrate the liberated iodine with 0.002 N sodium thiosulfate.

Carry out a blank assay under the same conditions, using the carbon monoxide-free gas prescribed in the monograph. The difference between the volumes of 0.002 N sodium thiosulfate used in the two titrations is not greater than the limit prescribed in the monographs.
Nitric oxide and nitrogen dioxide

**Principle**
Carry out the test after the 5 l used in the test for carbon monoxide have been released from the cylinder. Pass the gas, at a rate of 15.0 l/h, through a solution containing 2.5% w/v of potassium permanganate and 1.2% v/v of sulfuric acid into an evacuated gas sampling tube of 1 l nominal capacity and fill to a pressure about 50 mm below that of the atmosphere, measured on a mercury manometer. Calculate the volume of gas at 20° and 760 mm Hg.

**Sulfanilic acid/naphthylenediamine TS**
- Solution I: Dissolve 2 g of sulfanilic acid TS in a mixture of 10 ml of glacial acetic acid and 180 ml of water.
- Solution II: Dissolve 0.2 g of naphthylenediamine dihydrochloride in 10 ml of a 50% v/v glacial acetic acid solution heating gently and dilute to 200 ml with water.

Mix 9 volumes of Solution I with 1 volume of Solution II.

**Procedure**
Introduce 20.0 ml of sulfanilic acid/naphthylenediamine TS into the sampling tube by dipping the end into a dish containing the mixture and carefully opening the tap. Shake the tube and allow to stand for 10 min with occasional shaking. Prepare the reference solution by adding 0.25 ml of 0.00308% w/v sodium nitrite solution to 20.0 ml of the sulfanilic acid/naphthylenediamine TS. Measure the absorbance of a 1 cm layer of the resulting solutions at 550 nm and correct the result to 1.0 l of the gas at 20° and 760 mm Hg. The extinction of 1.0 l of the test substance is not greater than that of the reference solution.

**Halogen and hydrogen sulfite**

**NOTE:** For the following test pass the gas into a closed, flat-bottomed glass cylinder fitted with (a) a delivery tube having an orifice of 1 mm internal diameter and reaching to 2 mm from the bottom of the cylinder and (b) an outlet tube. The delivery tube is immersed in the reagent to a depth of 12 to 14 cm.

Pass 10.0 l through 49 ml of water containing 1 ml of silver nitrate TS. The solution does not darken. After 5 min, any opalescence produced is not more intense than that obtained with a mixture of 1 ml of silver nitrate TS, 20 ml of chloride standard solution (5 mg of Cl per l), 0.15 ml of dilute nitric acid TS and sufficient water to make 50 ml of solution and left to stand for 5 min.

**Arsine and phosphine**

**Silver DDC/Quinolone TS**
Dissolve 50 mg of finely powdered silver nitrate in 100 ml of quinoline and add 0.2 g of silver diethyldithiocarbamate. The reagent should be freshly prepared.
Arsine and phosphine
(continued)

**METHOD OF ASSAY**

**Procedure**

Pass 10.0 l at a rate of 1.0 l/min through a glass tube packed with lead acetate TS on cotton wool and a gas distribution head of the domed, sintered type, of porosity 100, into a tube of about 2.5 cm internal diameter, containing 5 ml of Silver DDC/Quinoline TS, the domed head being almost in contact with the bottom of the tube.

The colour of the Silver DDC/Quinoline TS is not changed.

Use a gas burette (see Figure in the next page) of 100 ml capacity having as its upper end a two-way tap connected to two capillaries, one of which (tube A) is used to introduce the gas into the apparatus, the other (tube B) connected to the condenser (C) and the manometer (M). The lower part of the gas burette has a one-way tap connected to a mercury reservoir by a rubber tube. The gas burette is graduated from 0 to 5 ml in tenths of a ml in the upper part and from 99.5 to 100.5 ml in tenths of a ml in the lower part. The capillary tube (B) is connected to a vertical capillary arm to form a four-way junction. The descending arm of the junction is connected to a condenser (C) of about 60 ml capacity. The right arm of the junction is connected to a mercury manometer (M). A tap (D) on the upper vertical arm of the junction opens to the air.

Close the three taps and immerse the condenser in liquid nitrogen, keeping the level slightly above the upper part of the condenser. By manipulating the two-way tap and the mobile reservoir create a partial vacuum in the apparatus, choosing an arbitrary pressure Po between 50 and 60 mm Hg, accurately measured. This pressure must remain constant for 10 min to demonstrate that the apparatus is gas-tight.

Open the two-way tap to tube (A) and completely fill the burette and tube (A) with mercury. Close the two-way tap. Connect a rubber tube to the exit valve of the nitrous oxide cylinder through a suitable pressure relieving device and pass a current of nitrous oxide through the rubber tube for 1 min. Whilst the gas is still flowing, connect the rubber tube to the end of tube (A) and immediately open the two-way tap to tube (A). Allow 100 ml of nitrous oxide to enter the burette by lowering the mercury reservoir. Close the two-way taps.

Raise the mercury reservoir slightly above the tube (A) and lower the level of the liquid nitrogen to the middle of the condenser. Carefully open the tap of the burette to connect with the condenser and allow the mercury to rise in the burette until it reaches the tap. Close the tap. Raise the level of the liquid nitrogen so as to totally immerse the condenser. Read the pressure and wait until it remains steady for 2 min.
Place the mercury reservoir in its bottom position, open the tap of the burette to make connection with the condenser. Move the mercury reservoir until the manometer reading is the same as the initial pressure $P_0$. Close the tap of the burette and by means of the mercury reservoir bring the pressure of the gas in the burette to atmospheric pressure. The number of ml of gas represents the non-condensable volume in 100 ml of nitrous oxide. The volume of non-condensible gas must not exceed 3 ml. After each series of 10 determinations allow atmospheric air to enter by opening the tap (D), remove the liquid nitrogen from the condenser and allow the condenser to warm to room temperature.
### 2-NITROPROPANE*
(Tentative)**

**DEFINITION**

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>2-Nitropropane</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A.S. number</td>
<td>79-46-9</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>C₃H₇NO₃</td>
</tr>
<tr>
<td>Structural formula</td>
<td>[NO₂] CH₂ - CH - CH₃</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>89.09</td>
</tr>
<tr>
<td>Assay</td>
<td>Content not less than 94.0%</td>
</tr>
</tbody>
</table>

**DESCRIPTION**

Colourless liquid with a mild pleasant odour

**FUNCTIONAL USE**

Extraction solvent

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

- **A. Solubility**
  - Sparingly soluble in water
  - Soluble in many organic solvents

- **B. Specific gravity**
  - $d_2^0$: 0.984 - 0.988

- **C. Refractive index**
  - $n_0^0$: 1.392 - 1.396

**PURITY TESTS**

- **Distillation range**
  - Not less than 90% v/v distills between 119° and 122°

- **Colour**
  - Not more than colour standard No. 20

- **Water content**
  - Not more than 0.1% w/w

- **Arsenic**
  - Not more than 3 mg/kg (Method II)

- **Heavy metals**
  - Not more than 10 mg/kg
  - Test 2 g of the sample as directed in the Limit Test (Method II) using 20 μg of lead ion in the control (Solution A)

---

* These specifications were prepared at the 35th session of JECFA (1989) and published in FNP 49 (1990).

** Information required on the refractive index range applicable to the commercial product. Confirmation is also required on the adequacy of the method of assay.

PURITY TESTS (continued)

**Acidity**

Passes Tests
See description under TESTS

* Non-volatile residue

Not more than 2 mg/100 ml

TESTS

**Acidity**

Transfer 1.5 g of the sample into a 250-ml Erlenmeyer flask, add phenolphthalein TS and titrate with 0.02 N ethanolic potassium hydroxide to a pink end-point that persists for at least 15 sec. Not more than 1 ml is required.

METHOD OF ASSAY

Determine by Gas chromatography.* Use a suitable gas chromatograph with thermal conductivity detector. Set operating conditions as follows:

- Column: stainless steel, 1.8 m length and 3 mm diameter, packed with Porapak type Q (100/120 mesh)
- Carrier gas: helium, 30 ml/min
- Temperatures: injection port: 250°, column: 180°, detector: 250°

Inject 1.0 μl of sample into chromatograph; elute for 30 min.

2-Nitropropane is the major peak and elutes at about 9 min. 1-Nitropropane is the second largest peak and elutes at about 13 min. Measuring retention times relative to 2-Nitropropane (Rn), compute the total area of peaks eluting between 0.7 and 2.0 Rn. The area of the 2-Nitropropane peak is not less than 94.0% of the total area between 0.7-2.0 Rn.

** A. Refractive index

\( \rho^\circ : 1.4450 - 1.4500 \)

** B. Specific gravity

\( \rho^0 \): 0.965 - 0.970; \( \rho^2 \): 0.957 - 0.968

** Acid value

Not more than 5

**.method of assay**

Weigh accurately about 1 g, and proceed as directed under the method for Ester Determination in the General Methods**, using 78.11 as the equivalence factor (e) in the calculation. Correct the number of ml of 0.5 N alcoholic potassium hydroxide consumed in the saponification for the Acid value.

---

* These specifications were prepared at the 11th session of JECFA (1967) and published in NMRS 44B (1969).

NONANAL*

SYNONYMS Pelargonic aldehyde, aldehyde C-9, nonanoic aldehyde

DEFINITION

Chemical name Nonanal

C.A.S. number 124-19-6

Chemical formula C₉H₁₈O

Structural formula CH₃(CH₂)₇CHO

Molecular weight 142.24

Assay Content not less than 95% of C₉H₁₈O

DESCRIPTION Colourless liquid with a characteristic floral waxy odour

FUNCTIONAL USE Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility Practically insoluble in water and glycerol. Miscible with ethanol. Soluble in propane-1,2-diol, most fixed oils and mineral oils.

** B. Refractive index n₂⁰ : 1.422 - 1.426

** C. Specific gravity d₂⁰ : 0.820 - 0.829

PURITY TEST

** Acid value Not more than 5

METHOD OF ASSAY Determine by gas-liquid chromatography as directed in the Method of Assay for d-Carvone.

---

* These specifications were prepared at the 23rd session of JECFA (1979) and published in FNP 12 (1979) superseding the earlier specifications published in NMRS 44B (1969).

NORDIHYDROGUAIARETIC ACID

SYNONYM

NDGA

DEFINITION

Chemical names: 4,4'- (2 ,3-Dimethyltetramethylene)-dipyro-catechol; 1,4-dipyro-catechol-2,3-dimethyl-butane; nordihydroguaiaretic acid; 8,γ-dimethyl-α,δ-bis(3,4-dihydroxyphenyl) butane

C.A.S. number: 500-38-9

Chemical formula: C₁₈H₂₂O₄

Structural formula:

\[
\begin{align*}
\text{HO} & \quad \text{CH₃} & \quad \text{CH₃} \\
\text{HO} & \quad \text{-CH₂-CH - CH₂-CH₂-} & \quad \text{-OH} \\
\end{align*}
\]

Molecular weight: 302.36

Assay: Nordihydroguaiaretic acid contains not less than 95% and not more than the equivalent of 102% of C₁₈H₂₂O₄

DESCRIPTION

Nordihydroguaiaretic Acid is a white to greyish-white crystalline solid and may be prepared from the evergreen desert shrub, *Larrea divaricata*, (Fam. Zygophyllaceae)

FUNCTIONAL USE

Antioxidant

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility**

Freely soluble in ethanol and ether, and in propylene glycol at 116°

** B. Melting point**

About 184°

C. Colour reaction

Passes test

See description under TESTS

D. Colour reaction

Passes test

See description under TESTS

---

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

IDENTIFICATION TESTS (continued)

E. Colour reaction  
Passes test  
See description under TESTS

F. Colour reaction  
Passes test  
See description under TESTS

PURITY TESTS

* Arsenic  
Not more than 3 mg/kg (Method II)

* Lead  
Not more than 10 mg/kg  
Test 1 g of the sample using 10 μg lead ion (Pb) in the control as directed in the Limit Test

* Heavy metals  
Not more than 30 mg/kg  
Test 0.67 g of the sample as directed in the Limit Test

TESTS

IDENTIFICATION TESTS

C. Colour reaction  
Add 2 ml of ferric chloride reagent (0.2% solution of FeCl₃·6H₂O in absolute ethanol) and 2 ml of 0.2% of 2,2'-bipyridine in absolute ethanol to 5 ml of 0.5% solution of the sample in 50% ethanol. A deep cherry-red colour appears

D. Colour reaction  
To 5 ml of 1% solution of the sample in 75% ethanol, add 1 ml of strong ammonia TS. A yellow colour develops

E. Colour reaction  
To 10 ml of 0.5% solution of the sample in 50% ethanol, add 1.5 ml of 1% barium hydroxide (Ba(OH)₂·H₂O) in boiled water. A deep blue colour develops which is stable for approximately 1 h

F. Colour reaction  
To 10 ml of 10% sodium hydroxide, add 1 ml of 0.5% solution of the sample in 50% ethanol. A rose-red colour develops

METHOD OF ASSAY

Colorimetric method  
Weigh 1.00 g of the sample. Dilute with methanol so that the final concentration will be 1 mg of the sample per 100 ml of solution. Read the absorbance at 284 nm in a 1 cm quartz cell.

Calculation

% Nordihydroguaiaretic acid =

\[
\text{Absorbance} - 0.008 \times 100 \\
\frac{\text{Weight of sample} \times 0.21}{100}
\]

**OCTANAL**

**SYNONYMS**
Caprylic aldehyde, caprylaldehyde, aldehyde C-8, n-octanoic aldehyde

**DEFINITION**

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Octanal</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A.S. number</td>
<td>124-13-0</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>C₈H₁₆O</td>
</tr>
<tr>
<td>Structural formula</td>
<td>CH₃(CH₂)₆CHO</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>128.22</td>
</tr>
<tr>
<td>Assay</td>
<td>Content not less than 92% of C₈H₁₆O</td>
</tr>
</tbody>
</table>

**DESCRIPTION**
Colourless liquid with a sharp fatty and fruity odour

**FUNCTIONAL USE**
Flavouring agent

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

**A. Solubility**
Slightly soluble in water. Miscible with ethanol and with ether. Soluble in propane-1,2-diol, most fixed oils and mineral oils. Insoluble in glycerol.

**B. Refractive index**
\( n_\text{D} : 1.417 - 1.426 \)

**C. Specific gravity**
\( d_\text{D}^20 : 0.810 - 0.830 \)

**PURITY TEST**

**Acid value**
Not more than 10

**METHOD OF ASSAY**
Determine by gas-liquid chromatography as directed in the Method of Assay for \( \delta \)-Carvone.

---


OCTYL GALLATE*

SYNONYMS
INS No. 311, EEC No. E311

DEFINITION

Chemical names
Octyl gallate, octyl ester of gallic acid, n-octyl ester of 3,4,5-tri-hydroxybenzoic acid

C.A.S. number
1034-01-01

Chemical formula
C₁₅H₂₂O₅

Structural formula

![Structural formula image]

Molecular weight
282.34

Assay
Octyl Gallate contains not less than 98.5% of C₁₅H₂₂O₅ after drying at 90°C for 6 h.

DESCRIPTION
White to cream-white odourless solid which may have a slightly bitter taste.

FUNCTIONAL USE
Antioxidant

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Insoluble in water, freely soluble in ethanol, ether and propane-1,2-diol.

** B. Melting range
Between 99° and 102° after drying at 90° for 6 h

C. Gallic acid
Passes test.
Proceed as directed in the Identification Tests for Dodecyl Gallate.

D. TLC separation of
gallate esters
Passes test
See description under TESTS

* These specifications were prepared at the 30th session of JECFA (1986) and published in FNP 37 (1986).

IDENTIFICATION TESTS (continued)

E. Colour reaction

Dissolve 0.1 g of the sample in 5 ml of ethanol and add a drop of ferric chloride TS. A violet colour is produced.

PURITY TESTS

* Loss on drying

Not more than 0.5% (90°, 6 h.)
(Note: Ventilation during drying is advisable)

* Sulfated ash

Not more than 0.05%
Proceed as directed under the Limit Test for ash (Sulfated ash, Method I) using 2 g of the sample.

* Arsenic

Not more than 3 mg/kg (Method II)

* Lead

Not more than 10 mg/kg
Test 1 g of the sample using 10 μg lead ion (Pb) in the control as directed in the Limit Test.

* Heavy metals

Not more than 30 mg/kg
Test 0.67 g of the sample as directed in the Limit Test (Method II).

Chlorinated organic compound

Not more than 100 mg/kg as chlorine
See description under TESTS

Free acid

Not more than 0.5% as gallic acid
Proceed as directed in the specifications for Dodesyl Gallate.

TESTS

IDENTIFICATION TESTS

D. TLC separation of gallate esters

Use a thin layer plate prepared with silica gel G. Prepare a sample solution sample solution by dissolving 10 mg of sample in 10 ml ethyl alcohol. Prepare control solution A by dissolving 10 mg of Octyl gallate in 10 ml ethyl alcohol and control solution B by dissolving 10 mg of Propyl gallate and 10 mg of Octyl gallate in 10 ml ethyl alcohol.

Place 5 μl of each solution on the plate. Develop the chromatogram to about 15 cm from the starting point using a developing solvent containing: 20 volumes glacial acetic acid, 40 volumes petroleum ether and 40 volumes toluene. Dry the plate in air. Spray an Indicator solution, containing 20% w/v phosphomolybdic acid in ethanol, to the plate until a yellow coloration persists. Examine in daylight. After a few minutes there is a progressive change to blue coloration. After 5 to 10 min expose the plate to ammonia vapors until the background is white. Examine in daylight. The principle spot of the sample solution corresponds with that for Octyl gallate in the control solutions. Suitable resolution of Propyl and Octyl gallate spots is determined from control solution B.

PURITY TESTS

* Chlorinated organic compounds

Dissolve 1 g of the sample in 10 ml of 0.1 N sodium hydroxide. Acidify with nitric acid solution and filter off the precipitate. Mix the precipitate with 2 g of calcium carbonate, dry the mixture and then ignite. Take up the ignition residue in 20 ml of dilute nitric acid TS and filter. Mix the solution with 0.5 ml of 0.1 N silver nitrate. The turbidity should not be more than that obtained in 20 ml of dilute nitric acid TS by addition of 0.5 ml of 0.1 N silver nitrate and 0.3 ml of 0.01 N hydrochloric acid.

METHOD OF ASSAY

Weigh accurately about 0.2 g of the dried sample into a 400-ml beaker. Add 150 ml of water and heat to boiling. Then with constant and vigorous stirring add 50 ml of bismuth nitrate TS (II). Continue stirring for a few min. more until precipitation is complete, then allow the solution to cool to room temperature. Filter the yellow precipitate on a weighed sintered-glass crucible, wash first with cold 0.05 N nitric acid and then with ice-cold water, until free from acid. Dry at 110° to constant weight. Calculate the octyl gallate content by the formula:

\[
\% \text{ Octyl gallate} = \frac{\text{Weight of precipitate} \times 55.74}{\text{Weight of sample}}
\]
OXYSTEARIN*  
(Tentative)**

SYNONYM  
INS No. 387

DEFINITION  
Oxystearin is a mixture of glycerides of partially oxidized stearic and other fatty acids. The product is obtained by heating hydrogenated vegetable oil under controlled condition.

DESCRIPTION  
Oxystearin is a tan to light brown, fatty or wax-like substance

FUNCTIONAL USES  
Crystallization inhibitor in soils, sequestrant, defoaming agent

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility  
Insoluble in water. Soluble in ethanol.

B. Positive test for glycerol  
Heat 0.2 g of the sample with 0.5 g of potassium bisulfate until almost carbonized. A pungent odour of acrolein is evolved.

C. Positive test for partially oxidized fatty acids  
Passes test  
See description under TESTS

PURITY TESTS

*** Refractive index  
Between 1.465 and 1.467 at 48° by the following procedure. Melt the sample, filter through filter paper and determine the refractive index at 48°.

*** Arsenic  
Not more than 3 mg/kg  
A sample solution prepared as directed for organic compounds meets the requirements of the Limit Test for Arsenic (Method II)

*** Heavy metals  
Not more than 10 mg/kg  
Test 2.0 g of the sample as directed in Method II under the Limit Test for Heavy Metals using 20 μg of lead ion (Pb) in the control (Solution A).

* These specifications were prepared at the 20th session of JECFA (1976) and published in FNS 1B (1977).

** Suitable methods for epoxides needed.

**PURITY TESTS (continued)**

* Acid value  
Not more than 15  
Determine as directed in the General Methods under General Methods for Phosphates using about 8 g of the sample weighed to the nearest mg.

Epoxides  
Not more than 50 mg/kg**

* Hydroxyl value  
Between 30 and 45  
Determine as directed in General Methods using about 5 g of the sample weighed to the nearest mg.

* Iodine value  
Not more than 15  
Determine as directed in General Methods using about 1 g of the sample weighed to the nearest mg.

Non-urea-adduct forming methyl esters derived from oxystearin  
Not more than 40% w/w  
See description under TESTS

* Saponification value  
Between 225 and 240  
Determine as directed in General Methods using about 3 g of the sample weighed to the nearest mg.

**Unsaponifiable matter**  
Not more than 0.8%  
Determine as directed in Unsaponifiable Matter for SALTS OF FATTY ACIDS using about 5 g of the sample weighed to the nearest mg.

**TESTS**

**IDENTIFICATION TESTS**

C. Positive test for partially oxidized fatty acids  
Dissolve 0.1 g of the sample in 2 ml of ethanol by heating. Add 5 ml of dilute sulfuric acid TS, heat for 30 min in a water bath and cool. Oily drops or a white to yellowish white solid are formed. Separate the oily drops or solid from the solution. The fraction will dissolve with shaking in 3 ml of ether.

Non-urea-adduct forming methyl esters derived from oxystearin  
Reflux about 15 g of the sample, weighed to the nearest 10 mg, in a 500-ml round bottom flask on a steam bath for 30 min with 150 ml of methanol and 100 ml of sulfuric acid TS. Transfer the mixture into a 1 L separatory funnel, add 200 ml of water and extract with 300 ml of diethyl ether. Draw off the aqueous phase and extract with 150-ml portions of the ether until all colour is removed. Combine the ether extracts, wash twice with water, dry over anhydrous magnesium or sodium sulphate, evaporate the ether and dry in vacuum. Reflux the residue obtained in the 500-ml round bottom flask on a steam bath for 30 min with 250 ml of methanol and 0.5 g of sodium methoxide.

---


** Suitable method for epoxides needed.
Non-urea-adduct forming methyl esters derived from oxystearin (continued)

Cool slightly and add 5 ml of a 1:1 mixture of acetic acid and water to neutralize the sodium methoxide. Extract the ester fraction as described above, dry over anhydrous magnesium or sodium sulfate, evaporate the ether and dry in vacuum.

On the residue repeat the acid interesterification procedure as described in the first paragraph of this text. Weigh one part of the product obtained and add three parts of urea dissolved in 10 parts of acetone and 2 parts of methanol, stir constantly to prevent formation of lumps, let stand overnight at 1°C with constant stirring, and filter through a sintered glass funnel (medium porosity). Dilute the filtrate with water and extract with 150 ml of ether. Transfer the water layer into another separatory funnel and extract with 100-ml portions of ether until colourless. Combine the ether extracts (extract A). Slurry the adduct in the funnel with five 50-ml portions of cold ether and suck dry with vacuum after the addition of each 50 ml of solvent. Collect the washings and combine with ether extract A. Wash the combined ether extract twice with water. (Emulsions formed can be broken by addition of saturated aqueous sodium chloride). Evaporate the ether extract and dry in vacuum.

Repeat the urea treatment with the residue obtained as described. The weight of the final vacuum dried ether fraction represents the weight of methyl ester derived from 15 g of the sample that do not form urea adducts. Express the level of non-urea-adduct methyl esters as per cent by weight of the original sample weight.
PAPAIN

SYNONYM

INS No.1101(ii)

SOURCES

Commercial preparations of papain are a purified proteolytic substances derived from the fruit of Carica papaya (L) (Fam. Caricaceae).

ACTIVE PRINCIPLES

1. Papain (papaya peptidase I, cystein proteinase)
2. Chymopapain (cystein proteinase)

SYSTEMATIC NAMES AND NUMBERS

1. None - EC 3.4.22.2
2. None - EC 3.4.22.6

REACTIONS CATALYZED

These enzymes hydrolyze polypeptides, amides and esters, especially at linkages involving basic amino acids, or leucine or glycine, yielding peptides of lower molecular weight.

DESCRIPTION

The enzyme preparations occur as a white to light tan amorphous powder or liquids. They are soluble in water, the solutions being colourless to light yellow and somewhat opalescent. They are practically insoluble in alcohol, chroloform and ether.

FUNCTIONAL USES

Chillproofing of beer, tenderizing of meat, preparation of precooked cereals, and production of protein hydrolysates.

GENERAL SPECIFICATIONS

Must conform to the "General Specifications for Enzyme Preparations used in Food Processing".

CHARACTERISTICS

IDENTIFICATION TESTS

Papain activity The sample shows plant proteolytic activity

* These specifications were prepared at the 15th session of JECFA (1971) and published in NMRS 50B (1972).

** See Annex 1 at the end of this compendium.

PAPRIKA OLEORESIN*

SYNONYMS
Paprika extract, Oleoresin paprika
INS No. 160c, EEC No. E160c

DEFINITION
Paprika Oleoresin is obtained by solvent extraction of paprika, which consists of the ground fruit pods, with or without the seeds, of Capsicum annum L and contains the major flavouring and colouring principles of this spice. Only the following solvents may be used: trichloroethylene, acetone, propan-2-ol, methanol, ethanol, hexane. The solvent is subsequently removed. The major flavouring principle of paprika oleoresin is capsaicin. The major colouring principles of paprika oleoresin are capsanthin and capsorubin. A wide variety of other coloured compounds are known to be present.

Class
Carotenoid

C.A.S. number
68917-78-2

Chemical name
Major flavouring principle:
Capsaicin: (E)-N-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methyl-6-nonenamide

Major colouring principles:
Capsanthin: (3R,3'S,5'R)-3,3'-dihydroxy-β,β-carotene-6-one
Capsorubin: (3S,3'S,5R,5R')-3,3'-dihydroxy-x,x-carotene-6,6'-dione

Chemical formula
Capsaicin C₁₈H₂₇NO₅, Capsanthin C₄₀H₅₀O₃, Capsorubin C₄₀H₅₀O₄

Structural formula
Capsaicin

\[
\text{Capsanthin}
\]

\[
\text{Capsorubin}
\]

* These specifications were prepared at the 35th session of JECFA (1989) and published in FNP 49 (1990).
Molecular weight: Capsaicin: 305.40, Capsanthin: 584.85, Capsorubin: 600.85

Assay: Not less than 500 ASTA Color Value units

**DESCRIPTION**

Dark red viscous liquid

**FUNCTIONAL USE**

Food colour, flavouring agent

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

* A. Solubility
  - Practically insoluble in water
  - Partially soluble with oily separation in ethanol
  - Insoluble in glycerin

** B. Spectrophotometry
  - In hexane the maximum absorption is at about 470 nm.

C. Colour reaction
  - Passes test
  - To one drop of sample add 2-3 drops of chloroform and one drop of sulfuric acid. A deep blue colour is produced.

**PURITY TESTS**

* Arsenic
  - Not more than 3 mg/kg

* Lead
  - Not more than 10 mg/kg

* Heavy metals
  - Not more than 40 mg/kg

* Residual solvents
  - Dichloromethane: Not more than 30 mg/kg, singly or in combination
  - Trichloroethylene: Not more than 30 mg/kg
  - Acetone: Not more than 30 mg/kg
  - Propan-2-ol: Not more than 50 mg/kg
  - Methanol: Not more than 50 mg/kg
  - Ethanol: Not more than 50 mg/kg
  - Hexane: Not more than 25 mg/kg

**Capsaicin**

- Not more than 0.5%
- See description under TESTS


PURITY TESTS

Determination of Capsaicin

Weigh accurately about 5 g in a 300-ml ground joint flask. After addition of 100 ml of 70% methanol, shake for 30 minutes. Let the solution settle for 5 minutes and filter. Cover the funnel to avoid evaporation. The first 25 ml of the filtrate is discarded and the rest of the filtrate mixed well. Afterwards, solutions are prepared in 100-ml volumetric flasks in the following manner:

<table>
<thead>
<tr>
<th>flask 1</th>
<th>flask 2</th>
<th>flask 3</th>
<th>flask 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtrate solution</td>
<td>4.00 ml</td>
<td>4.00 ml</td>
<td>-</td>
</tr>
<tr>
<td>Distilled water</td>
<td>17.80 ml</td>
<td>16.80 ml</td>
<td>19.00 ml</td>
</tr>
<tr>
<td>1 N HCl</td>
<td>1.00 ml</td>
<td>-</td>
<td>1.00 ml</td>
</tr>
<tr>
<td>1 N NaOH</td>
<td>-</td>
<td>2.00 ml</td>
<td>-</td>
</tr>
</tbody>
</table>

Determined value $A_1$, $A_2$, $A_3$, $A_4$

The solutions are mixed well and the flasks filled to 100 ml with methanol. The absorbance values $A_1$, $A_4$ of the four solutions are measured at 248 nm and (Deuterium lamp, quartz cuvettes).

Calculation:

(a) at 248 nm

$$\frac{[(A_2 - A_1) - (A_4 - A_3)] \times [2500]}{314 \times \text{weight of sample (in gram)}} = \% \text{ Capsaicin}$$

(b) at 296 nm

$$\frac{[(A_2 - A_1) - (A_4 - A_3)] \times [2500]}{127 \times \text{weight of sample (in gram)}} = \% \text{ Capsaicin}$$

$$\frac{2500}{314 \text{ and } 127} = \text{dilution}$$

Replicate determinations of (a) and (b) must not differ more than 10%, otherwise the determination is to be repeated.
METHOD OF ASSAY

Determine the ASTA (American Spice Trade Association) Colour Value according to the following procedure:

Apparatus
Spectrophotometer, capable of accurately measuring absorbance at 460 nm, absorption cells, 1 cm, matched cells with stoppers, volumetric flasks, 100 ml, with ground glass stoppers, pipette, transfer-type, 10 ml, Whatman No. 40 filter paper or equivalent

Reagents
- Acetone, technical grade
- Cobaltous ammonium sulfate crystals
- Potassium dichromate, reagent grade

The cobaltous ammonium sulfate should be dried one week in a desiccator containing anhydrous calcium sulfate. No preliminary treatment is needed for the potassium dichromate.

Standard colour solution
0.3005 g/L potassium dichromate plus 34.96 g/L cobaltous sulfate crystals in 1.8 M sulfuric acid solution. The absorbance of this solution \((A_c)\) in a 1-cm cell at 460 nm should be about 0.600.

Procedure
Accurately weigh a sample of 50 to 80 mg in a 100-ml volumetric flask and dilute to the mark with acetone. Allow the extraction to proceed for at least 15 min with occasional shaking. With a 10-ml pipette, transfer 10.0 ml of the extract into another 100-ml volumetric flask, and dilute to the mark with acetone. Filter the diluted extract using Whatman No. 40 filter paper or equivalent; discard the first 10 or 15 ml of filtrate. Decant a portion of the filtrate into a cell and measure the absorbance at 460 nm using acetone as a blank. Determine the absorbance \((A_c)\) of the Standard colour solution at 460 nm.

Calculation

(a) Cell length and instrument correction factor = \(I_c\)

\[ I_c = \frac{0.600}{A_s} \]

(b) Extractable colour (ASTA colour value units) =

\[
\text{Absorbance of acetone extract at } 460 \text{ nm} \times 164 \times I_c
\]

sample weight in g
PARAFFIN WAX*
(Tentative)**

SYNONYMS  Petroleum wax, microcrystalline wax
          INS No. 905(c)

DEFINITION  Paraffin Wax is a refined mixture of solid hydrocarbons, mainly of paraffinic
            nature,** obtained from petroleum.

DESCRIPTION  Colourless or white, somewhat translucent, tasteless and odourless wax.

FUNCTIONAL USE  Chewing gum base, protective coating, defoaming agent.

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility  Insoluble in water. Very slightly soluble in ethanol
                   Sparingly soluble in ether and hexane.

*** B. Melting range  50° - 102°
                     See description under TESTS

*** C. Infrared spectrum  See appendix

PURITY TESTS

Residue on ignition  Not more than 0.1%
                    See description under TESTS

Colour  Passes test
        See description under TESTS

Sulfur  Information required**

*** Arsenic  Not more than 3 mg/kg (Method II)

*** Lead  Not more than 3 mg/kg

*** Heavy metals  Not more than 20 mg/kg
                 Test a 1 g sample as directed in Method II in the Limit Test.

---

* These specifications were prepared at the 33rd session of JECFA (1988) and published in FNP 38 (1988).

** Information required on actual composition of hydrocarbons, method(s) for identification of individual
   hydrocarbons, limit and method of analysis for sulfur and nature of sulfur compounds.

PURITY TESTS (continued)

Polycyclic aromatic hydrocarbons

The sample shall meet the following ultraviolet absorbance limits when subjected to the analytical procedure described in the TESTS.

\[
\begin{array}{cc}
\text{nm} & \text{max. absorbance per cm path length} \\
280 - 289 & 0.15 \\
290 - 299 & 0.12 \\
300 - 359 & 0.08 \\
360 - 400 & 0.02 \\
\end{array}
\]

TESTS

IDENTIFICATION TEST

B. Melting range

Melt a quantity of the substance slowly, while stirring, until it reaches a temperature of 90° to 92°. Remove the source of heat, and allow the molten substance to cool to a temperature of 8° to 10° above the expected melting point. Chill the bulb of an ASTM 14C or equal thermometer to 5°, wipe it dry and while it is still cold dip into the molten substance so that approximately the lower half of the bulb is submerged. Withdraw it immediately, and hold it vertically away from the heat until the wax surface dulls, then dip it for 5 min into a water bath having a temperature not higher than 16°.

Fix the thermometer securely in a test tube so that the lower point is 15 mm above the bottom of the test tube. Suspend the test tube in a water bath adjusted to about 16°, and raise the temperature of the bath at the rate of 2° per min to 30°, then change to a rate of 1° per min and note the temperature at which the first drop of melted substance leaves the thermometer. Repeat the determination twice on a freshly melted portion of the sample. If the variation of three determinations is less than 1°, take the average of the three as the melting point. If the variation of the three determinations is greater than 1°, make two additional determinations and take the average of the five.

PURITY TESTS

Residue on ignition

Accurately weight about 2 g of the sample in a tared porcelain or platinum dish and heat over a flame. The sample volatiles without emitting an acrid, odour. Ignite not exceeding a very dull redness until free from carbon. Cool on a desiccator and weigh.

Colour

Melt about 10 g of the sample on a steam bath, and pour 5 ml of the liquid into a clear-glass, 16- x 150-mm bacteriological test tube: the warm, melted liquid is not darker than a solution made by mixing 3.8 ml of ferric chloride TS and 1.2 ml of cobaltous chloride TS in a similar tube, the comparison of the two being made in reflected light against a white background, the tubes being held directly against the background at such an angle that there is no fluorescence.
Polycyclic aromatic hydrocarbons

General Instructions

Because of the sensitivity of the test, the possibility of errors arising from contamination is great. It is of the greatest importance that all glassware be scrupulously cleaned to remove all organic matter such as oil, grease, detergent residues, etc. Examine all glassware, including stoppers and stopcocks, under ultraviolet light to detect any residual fluorescent contamination. As a precautionary measure it is a recommended practice to rinse all glassware with purified isooctane immediately before use. No grease is to be used on stopcocks or joints. Great care to avoid contamination of wax samples in handling and to assure absence of any extraneous material arising from inadequate packaging is essential. Because some of the polynuclear hydrocarbons sought in this test are very susceptible to photo-oxidation, the entire procedure is to be carried out under subdued light.

Apparatus

- Separatory funnels: 250-ml, 500-ml, 1,000-ml, and preferably 2,000-ml capacity, equipped with tetrafluoroethylene polymer stopcocks.

- Reservoir: 500 ml capacity, equipped with a 24/40 standard taper male fitting at the bottom and a suitable balljoint at the top for connecting to the nitrogen supply. The male fitting should be equipped with glass hooks.

- Chromatographic tube: 180 mm in length, inside diameter to be 15.7 mm ± 0.1 mm, equipped with a coarse, fritted-glass disc, a tetrafluoroethylene polymer stopcock, and a female 24/40 standard tapered fitting at the opposite end. (Overall length of the column with the female joint is 235 mm). The female 24/40 standard tapered fitting at the opposite end.

- Disc: Tetrafluoroethylene polymer 2-inch diameter disc approximately 3/16-inch thick with a hole bored in the center to closely fit the stem of the chromatographic tube.

- Heating jacket: Conical, for 500-ml separatory funnel. (Used with variable transformer heat control).

- Suction flask: 250-ml or 500-ml filter flask.

- Condenser: 24/40 joints, fitted with a drying tube, length optional.

- Evaporation flask (optional): 250-ml or 500-ml capacity all-glass flask equipped with standard taper stopper having inlet and outlet tubes permit passage of nitrogen across the surface of the liquid to be evaporated.

- Vacuum distillation assembly: All glass (for purification of dimethyl sulfoxide); 2-L distillation flask with heating mantle; Vigreux vacuum-jacketed condenser (or equivalent) about 45 cm in length and distilling head with separable cold finger condenser. Use of tetrafluoroethylene polymer sleeves on the glass joints will prevent freezing. Do not use grease on stopcocks or joints.

* Information is requested on the performance of this method and on alternate methods.
Polycyclic aromatic hydrocarbons

Apparatus (continued)

- Spectrophotometric cells: Fused quartz cells, optical path length in the range of 5,000 ± 0.005 cm; also for checking spectrophotometer performance only, optical path length in the range 1,000 ± 0.005 cm. With distilled water in the cells, determine any absorbance differences.

- Spectrophotometer: Spectral range 250 nm-400 nm with spectral slit width of 2 nm or less, under instrument operating conditions for these absorbance measurements, the spectrophotometer shall, also meet the following performance requirements:
  
  Absorbance repeatability, ±0.01 at 0.4 absorbance.
  Absorbance accuracy, ±0.05 at 0.4 absorbance.
  Wavelength repeatability, ±0.2 nm.
  Wavelength accuracy, ±1.0 nm.

- Nitrogen cylinder: Water-pumped or equivalent purity nitrogen in cylinder equipped with regulator and valve to control flow at 5 p.s.i.g.

Reagents and materials

- Organic solvents: All solvents used throughout the procedure shall meet the specifications and tests described in this specification. The isooctane, benzene, acetone, and methyl alcohol designated in the list following this paragraph shall pass the following test:

  To the specified quantity of solvent in a 250-ml Erlenmeyer flask, add 1 ml of purified n-hexadecane and evaporate on the steam bath under a stream of nitrogen (a loose aluminium foil jacket around the flask will speed evaporation). Discontinue evaporation when not over 1 ml of residue remains. (to the residue from benzene add a 10 ml portion of purified isooctane, reevaporate, and repeat once to insure complete removal of benzene).

  Alternatively, the evaporation time can be reduced by using the optional evaporation flask. In this case the solvent and n-hexadecane are placed in the flask on the steam bath, the tube assembly is inserted, and a stream of nitrogen is fed through the inlet tube while the outlet tube is connection to a solvent trap and vacuum line in such a way as to prevent any flow-back of condensate into the flask.

  Dissolve the 1 ml of hexadecane residue in isooctane and make to 25 ml volume. Determine the absorbance in the 5 cm path length cells compared to isooctane as reference. The absorbance of the solution of the solvent residue (except for methyl alcohol) shall not exceed 0.01 per cm path length between 280 and 400 nm. For methyl alcohol this absorbance value shall be 0.00.
Reagents and materials (continued)

- Isooctane. (2,2,4-trimethylpentane): Use 180 ml for the test described in the preceding paragraph. Purify, if necessary, by passage through a column of activated silica gel (Grade 12, Davison Chemical Company, Baltimore, Maryland, or equivalent) about 90 cm in length and 5 cm to 8 cm in diameter.

- Benzene, reagent grade: Use 150 ml for the test. Purify, if necessary, by distillation or otherwise.

- Acetone, reagent grade: Use 200 ml for the test. Purify, if necessary, by distillation.

- Eluting mixtures:

1. 10% benzene in isooctane: Pipet 50 ml of benzene into a 500-ml glass-stoppered volumetric flask and adjust to volume with isooctane, with mixing.

2. 20% benzene in isooctane: Pipet 50 ml of benzene into a 250-ml glass-stoppered volumetric flask and adjust to volume with isooctane, with mixing.

3. Acetone-benzene-water mixture: Add 20 ml of water to 380 ml of acetone and 200 ml of benzene, and mix.

- n-Hexadecane, 99% olefin-free: Dilute 1.0 ml of n-hexadecane to 25 ml with isooctane and determine the absorbance in a 5-cm cell compared to isooctane as reference point between 280-400 nm. The absorbance per centimeter path length shall not exceed 0.00 in this range. Purify, if necessary, by percolation through activated silica gel or by distillation.

- Methyl alcohol, reagent grade: Use 10.0 ml of methyl alcohol. Purify, if necessary, by distillation.

- Dimethyl sulfoxide: Pure grade, clear, water-white, m.p. 18° minimum. Dilute 120 ml of dimethyl sulfoxide with 240 ml of distilled water in a 500-ml separatory funnel, mix and allow to cool for 5-10 min. Add 40 ml of isooctane to the solution and extract by shaking the funnel vigorously for 2 min. Draw off the lower aqueous layer into a second 500-ml separatory funnel and repeat the extraction with 40 ml of isooctane. Draw off and discard the aqueous layer. Wash each of the 40 ml extractives three times with 50 ml portions of distilled water. Shaking time for each wash is 1 min. Discard the aqueous layers. Filter the first extractive through anhydrous sodium sulfate prewashed with isooctane (see Sodium sulfate under "Reagents and Materials" for preparation of filter), into a 250-ml Erlenmeyer flask, or optionally into the evaporating flask. Wash the first separatory funnel with the second 40 ml isooctane extractive, and pass through the sodium sulfate into the flask. Then wash the second and first separatory funnels successively with a 10 ml portion of isooctane, and pass the solvent through the sodium sulfate into the flask. Add 1 ml of n-hexadecane and evaporate the isooctane on the steam bath under nitrogen. Discontinue evaporation when not over 1 ml of residue remains. To the residue, add a 10 ml portion of isooctane and reevaporate to 1 ml of hexadecane. Again, add 10 ml of isooctane to the residue and evaporate to 1 ml of hexadecane to insure complete removal of all volatile materials. Dissolve the 1 ml of hexadecane in isooctane and
Polycyclic aromatic hydrocarbons

- Dimethyl sulfoxide (continued)

  make to 25 ml volume. Determine the absorbance in 5 cm path length cells compared to isooctane as reference. The absorbance of the solution should not exceed 0.02 per cm path length in the 280-400 nm range. (Note - Difficulty in meeting this absorbance specification may be due to organic impurities in the distilled water. Repetition of the test omitting the dimethyl sulfoxide will disclose their presence. If necessary to meet the specification, purify the water by redistillation, passage through an ion-exchange resin, or otherwise).

  Purify, if necessary, by the following procedure: To 1,500 ml of dimethyl sulfoxide in a 2 l glass-stoppered flask, add 6.0 ml of phosphoric acid and 50 g of Norit A (decolorizing carbon, alkaline) or equivalent. Stopper the flask, and with the use of a magnetic stirrer (tetrafluoro-ethylene polymer coated bar) stir the solvent for 15 min. Filter the dimethyl sulfoxide through four thicknesses of fluted paper (18.5 cm, Schleicher & Schuell, No. 597, or equivalent). If the initial filtrate contains carbon fines, refilter through the same filter until a clear filtrate is obtained. Protect the sulfoxide from air and moisture during this operation by covering the solvent in the funnel and collection flask with a layer of isooctane. Transfer the filtrate to a 2-l separatory funnel and draw off the dimethyl sulfoxide into the 2-l distillation flask of the vacuum distillation assembly and distill at approximately 3 mm Hg pressure or less. Discard the first 200 ml fraction of the distillate and replace the distillate collection flask with a clean one. Continue the distillation until approximately 1 l of the sulfoxide has been collected.

  At completion of the distillation, the reagent should be stored in glass-stoppered bottles since it is very hygroscopic and will react with some metal containers in the presence of air.

  - Phosphoric acid, 85% reagent grade
  - Sodium borohydride, 98%

- Magnesium oxide (Sea Sorb 43, Food Machinery Company, Westvaco Division, distributed by chemical supply firms, or equivalent): Place 100 g of the magnesium oxide in a large beaker, add 700 ml of distilled water to make a thin slurry, and heat on a steam bath for 30 min with intermittent stirring. Stir well initially to insure that all the absorbent is completely wetted. Using a Buchner funnel and a filter paper (Schleicher & Schuell No. 597, or equivalent) of suitable diameter, filter with suction. Continue suction until water no longer drips from the funnel. Transfer the absorbent to a glass trough lined with aluminium foil (free from rolling oil). Break up the magnesia with a clean spatula and spread out the absorbent on the aluminium foil in a layer about 1-2 cm thick. Dry for 24 h at 160° ± 1°. Pulverize the magnesia with mortar and pestle. Sieve the pulverized absorbent between 60-180 mesh. Use the magnesia retained on the 180-mesh sieve.

- Celite 545: Johns-Manville Company, diatomaceous earth, or equivalent.
Polycyclic aromatic hydrocarbons

Reagents and materials (continued)

- Magnesium oxide-Celite 545 mixture (2+1) by weight: Place the magnesium oxide (60-180 mesh) and the Celite 545 in 2 to 1 proportions, respectively, by weight in a glass-stoppered flask large enough for adequate mixing. Shake vigorously for 10 min. Transfer the mixture to a glass trough lined with aluminum foil (free from rolling oil) and spread it out on a layer about 1 to 2 cm thick. Reheat the mixture at 160° ± 1° for 2 h, and store in a tightly closed flask.

- Sodium sulfate, anhydrous, reagent grade, preferably in granular form: For each bottle of sodium sulfate reagent used, establish as follows the necessary sodium sulfate prewash to provide such filters required in the method: Place approximately 35 g of anhydrous sodium sulfate in a 30 ml coarse, fritted-glass funnel or in a 65 ml filter funnel with glass wool plug; wash with successive 15 ml portions of the indicated solvent until a 15 ml portion of the wash shows 0.00 absorbance per cm path length between 280 nm and 400 nm when tested as prescribed under "Organic solvents." Usually three portions of wash solvent are sufficient.

Procedure

Before proceeding with the analysis of a sample, determine the absorbance in a 5 cm path cell between 250 nm and 400 nm for the reagent blank by carrying out the procedure, without a wax sample, at room temperature, recording the spectra after the extraction stage and after the complete procedure as prescribed. The absorbance per centimeter path length following the extraction stage should not exceed 0.040 in the wavelength range from 250 to 400 nm; the absorbance per cm path length following the complete procedure should not exceed 0.070 in the wavelength range from 250 to 299 nm, inclusive, nor 0.045 in the wavelength range from 300 nm to 400 nm. If in either spectrum the characteristic benzene peaks in the 250-260 nm region are present, remove the benzene by the procedure under "Organic solvents" and record absorbance again.

Place 300 ml of dimethyl sulfoxide in a 1-l separatory funnel and add 75 ml of phosphoric acid. Mix the contents of the funnel and allow to stand for 10 min. (The reaction between the sulfoxide and the acid is exothermic. Release pressure after mixing, then keep funnel stoppered). Add 150 ml of isooctane and shake to preequilibrate the solvents. Draw off the individual layers and store in glass-stoppered flasks.

Place a representative 1 kg sample of wax, or if this amount is not available, the entire sample, in a beaker of a capacity about three times the volume of the sample and heat with occasional stirring on a steam bath until the wax is completely melted and homogenous. Weigh four 25 ± 0.2 g portions of the melted wax in separate 100 ml beakers. Reserve three of the portions for later replicate analyses as necessary. Pour one weighed portion immediately after remelting (on the steam bath) into a 500 ml separatory funnel containing 100 ml of the preequilibrated sulfoxide-phosphoric acid mixture that has been heated in the heating jacket at a temperature just high enough to keep the wax melted. (Note: In preheating the sulfoxide-acid mixture, remove the stopper of the separatory funnel at intervals to release the pressure).
Polycyclic aromatic hydrocarbons

Procedure (continued)

Promptly complete the transfer of the sample to the funnel in the jacket with portions of the pre-equilibrated isooctane, warming the beaker, if necessary, and using a total volume of just 50 ml of the solvent. If the wax comes out of solution during these operations, let the stoppered funnel remain in the jacket until the wax redissolves. (Remove stopper from the funnel at intervals to release pressure).

When the wax is in solution, remove the funnel from the jacket and shake it vigorously for 2 min. Set up three 250 ml separatory funnels with each containing 30 ml of pre-equilibrated isooctane. After separation of the liquid phases, allow to cool until the main portion of the wax-isooctane solution begins to show a precipitate. Gently swirl the funnel when precipitation first occurs on the inside surface of the funnel to accelerate this process. Carefully draw off the lower layer, filter it slowly through a thin layer of glass wool fitted loosely in a filter funnel into the first 250 ml separatory funnel, and wash in tandem with the 30 ml portions of isooctane contained in the 250 ml separatory funnels. Shaking time for each wash is 1 min. Repeat the extraction operation with two additional portions of the sulfoxide-acid mixture, replacing the funnel in the jacket after each extraction to keep the wax in solution and washing each extractive in tandem through the same three portions of isooctane.

Collect the successive extractives (300 ml total) in a separatory funnel (preferably 2-liter), containing 480 ml of distilled water, mix, and allow to cool for a few minutes after the last extractive has been added. Add 80 ml of isooctane to the solution and extract by shaking the funnel vigorously for 2 min. Draw off the lower aqueous layer into a second separatory funnel (preferably 2-l) and repeat the extraction with 80 ml of isooctane. Draw off and discard the aqueous layer. Wash each of the 80 ml extractives three times with 100 ml portions of distilled water. Shaking time for each wash is 1 min. Discard the aqueous layers. Filter the first extractive through anhydrous sodium sulfate prewashed with isooctane (see Sodium Sulfate under “Reagents and Materials” for preparation of filter) into a 250-ml Erlenmeyer flask (or optionally into the evaporation flask). Wash the first separatory funnel with the second 80 ml isooctane extractive and pass through the sodium sulfate. Then wash the second and first separatory funnels successively with a 20 ml portion of isooctane and pass the solvent through the sodium sulfate into the flask. Add 1 ml of n-hexadecane and evaporate the isooctane on the steam bath under nitrogen. Discontinue evaporation when not over 1 ml of residue remains. To the residue, add a 10 ml portions of isooctane, re-evaporate to 1 ml of hexadecane, and repeat this operation once more.

Quantitatively transfer the residue with isooctane to a 25 ml volumetric flask, make to volume, and mix. Determine the absorbance of the solution in the 5 cm path length cells compared to isooctane as reference between 280 nm-400 nm (take care to lose none of the solution in filling the sample cell). Correct the absorbance values for any absorbance derived from reagents as determined by carrying out the procedure without a wax sample. If the corrected absorbance does not exceed the
Procedure (continued)

Quantitatively transfer the isooctane solution to a 125 ml flask equipped with 24/40 joint and evaporate the isooctane on the steam bath under a stream of nitrogen to a volume of 1 ml of hexadecane. Add 10 ml of methyl alcohol and approximately 0.3 g of sodium borohydride (Minimize exposure of the borohydride to the atmosphere. A measuring dipper may be used). Immediately fit a water-cooled condenser equipped with a 24/40 joint and with a drying tube into the flask, mix until the borohydride is dissolved, and allow to stand for 30 min at room temperature, with intermittent swirling. At the end of this period, disconnect the flask and evaporate the methyl alcohol on the steam bath under nitrogen until the sodium borohydride begins to come out of the solution. Then add 10 ml of isooctane and evaporate to a volume of about 2-3 ml. Again, add 10 ml of isooctane and concentrate to a volume of approximately 5 ml. Swirl the flask repeatedly to assure adequate washing of the sodium borohydride residues.

Fit the tetrafluoroethylene polymer disc on the upper part of the stem of the chromatographic tube, then place the tube with the disc on the suction flask and apply the vacuum (approximately 135 mm Hg pressure). Weigh out 14 g of the 2:1 magnesium oxide-Celite 545 mixture and pour the adsorbent mixture into the chromatographic tube in approximately 3 cm layers. After the addition of each layer, level off the top of the adsorbent with a flat glass rod or metal plunger by pressing down firmly until the adsorbent is well packed. Loosen the topmost few ml of each adsorbent layer with the end of a metal rod before the addition of the next layer. Continue packing in this manner until all the 14 g of the adsorbent is added to the tube. Level off the top of the adsorbent by pressing down firmly with a flat glass rod or metal plunger to make the depth of the adsorbent bed approximately 12.5 cm in depth. Turn off the vacuum and remove the suction flask. Fit the 500 ml reservoir onto the top of the chromatographic column and prewet the column by passing 100 ml of isooctane through the column. Adjust the nitrogen pressure so that the rate of descent of the isooctane coming off of the column is between 2-3 ml per min. Discontinue pressure just before the last of the isooctane reaches the level of the adsorbent. (Caution: Do not allow the liquid level to recede below the adsorbent level at any time). Remove the reservoir and decant the 5 ml isooctane concentrate solution onto the column and with slight pressure again allow the liquid level to recede to barely above the adsorbent level. Rapidly complete the transfer similarly with two 5 ml portions of isooctane, swirling the flask repeatedly each time to assure adequate washing of the residue. Just before the final 5 ml wash reaches the top of the adsorbent, add 100 ml of isooctane to the reservoir and continue the percolation at the 2-3 ml per minute rate. Just before the last of the isooctane reaches the adsorbent level, add 100 ml of 10% benzene in isooctane to the reservoir and continue the percolation at the aforementioned rate. Just before the solvent mixture reaches adsorbent level, add 25 ml of 20% benzene in isooctane to the
APPENDIX

Infrared spectrum: Paraffin Wax

The sample is melted and prepared for analyses on a cesium bromide plate.
**PATENT BLUE V**

**SYNONYMS**

CI Food Blue 5, Patent Blue 5;
INS No. 131, EEC No. E131

**DEFINITION**

Patent Blue V consists essentially of the calcium or sodium salt of 2-[(4-diethylaminophenyl)(4-diethylimino-2,5-cyclo-hexadien-1-yldiene)methyl]-4-hydroxy-1,5-benzenedisulfonate and subsidiary colouring matters together with water, sodium chloride and/or sodium sulfate and/or calcium chloride and/or calcium sulfate as the principal uncoloured components.

Patent Blue V may be converted to the corresponding aluminium lake in which case only the General Specifications for Aluminium Lakes of Colouring Matters shall apply.**

- **Class**: Triarylmethane
- **Code numbers**: CI (1975) No. 42051; CAS No. 3536-49-0
- **Chemical name**: Calcium or sodium salt of 2-[(4-diethylaminophenyl)(4-diethylimino-2,5-cyclohexadien-1-yldiene)methyl]-4-hydroxy-1,5-benzenedisulfonate; calcium or sodium salt of [4-α-(4-diethyl-aminophenyl)-5-hydroxy-2,4-disulfonatophenylmethylidene]2,5-cyclohexadien-1-yldene) diethylammonium hydroxide inner salt (alternative name).

**Chemical formula**

- **Calcium salt**: $\text{C}_{27}\text{H}_{31}\text{N}_{2}\text{O}_{6}\text{S}_{2}\frac{1}{2}\text{Ca}$
- **Sodium salt**: $\text{C}_{27}\text{H}_{31}\text{N}_{2}\text{O}_{6}\text{S}_{2}\text{Na}$

**Structural formula**

![Structural formula image]

where $X = \frac{1}{2}\text{Ca}$ for the calcium salt and $X = \text{Na}$ for the sodium salt

**Molecular weight**

- **Calcium salt**: 579.72
- **Sodium salt**: 582.67

* These specifications were prepared at the 31st session of JECFA (1987) and published in FNP 38 (1988).

** See Annex 2 at the end of this Compendium.
Assay

Content not less than 85% total colouring matters

DESCRIPTION

Blue powder or granules

FUNCTIONAL USE

Food colour

CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility
   Soluble in water. Slightly soluble in ethanol.

** B. Identification of colouring matters
   Passes test

PURITY TESTS

** Loss on drying at 135°C
   Not more than 15%

** Chloride and sulfate calculated as sodium salt

** Water-insoluble matter
   Not more than 0.5%

*** Arsenic
   Not more than 3 mg/kg

*** Lead
   Not more than 10 mg/kg

*** Mercury
   Not more than 1 mg/kg

*** Chromium
   Not more than 50 mg/kg

* Heavy metals
   Not more than 40 mg/kg
   Proceed as directed in the Heavy metals Limit Test.

Subsidiary colouring matters
   Not more than 2%
   See description under TESTS

Organic compounds other than colouring matters

3-Hydroxybenzaldehyde
3-Hydroxybenzoic acid
3-Hydroxy-4-sulfonato benzoic acid
N,N-Diethylaminobenzene sulfonic acids
   Total not more than 0.5%
   See description under TESTS

** Leuco base
   Not more than 4%


PURITY TESTS (continued)

* Unsulfonated primary aromatic amines
  Not more than 0.01% calculated as aniline

* Ether - extractable matters
  Not more than 0.2%

TESTS

PURITY TESTS

* Subsidiary colouring matters
  Proceed as directed under the method for the Determination of Subsidiary Colouring Matters in General Methods using the following conditions:
  
  Developing solvent: No. 2
  Height of ascent of solvent front: approximately 17 cm

* Organic compounds other than colouring matters
  Proceed as directed under HPLC using the following conditions:

  Instrument: High Performance Liquid Chromatograph fitted with a gradient elution accessory
  Detector: A UV HPLC detector recording absorbances at 254 nm
  Column: 250 x 4 mm (Kartusche). Li Chrosorb RP 18, 7 μm
  Solvent:
  (a) Acetate buffer pH 4.6: water (10% w/v) - the acetate buffer is prepared from 1 M sodium hydroxide, 1 M acetic acid and water (5:10:35)
  (b) Acetonitrile

  Gradient:

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<tr>
<td>40</td>
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METHOD OF ASSAY

Determination of total colouring matters by titration with titanous chloride *

Use the following conditions:

Weight of sample: 1.3-1.4 g
Buffer: 15 g sodium hydrogen tartrate
Weight (D) of colouring matters equivalent to 1.00 ml of 0.1 N TiCl3:
28.98 mg of the calcium salt, 29.13 mg of sodium salt.

PECTINS*

SYNONYMS
INS No. 440, EEC No. E440

DEFINITION
Pectin consists mainly of the partial methyl esters of polygalacturonic acid and their sodium, potassium, calcium and ammonium salts. It is obtained by aqueous extraction of appropriate edible plant material, usually citrus fruits or apples. No organic precipitants shall be used other than methanol, ethanol and isopropanol. In some types a portion of the methyl esters may have been converted to primary amides by treatment with ammonia under alkaline conditions. The commercial product is normally diluted with sugars for standardization purposes, and mixed with suitable food-grade buffer salts required for pH control and desirable setting characteristics. The article of commerce may be further specified as to pH value, jelly strength, viscosity, degree of esterification, and setting characteristics.

C.A.S. number 9000-69-5

DESCRIPTION
A white, yellowish, light greyish or light brownish powder

FUNCTIONAL USES
Gelling agent, thickening agent, stabilizer

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Soluble in water forming a colloidal, opalescent solution. Insoluble in ethanol

B. Gel formation
Passes test
See description under TESTS

C. Precipitate formation
Passes test
See description under TESTS

D. Gel formation
Passes test
See description under TESTS

E. Precipitate formation
Passes test
See description under TESTS

F. Positive test for amide group
Passes test
See description under TESTS

PURITY TESTS

** Loss on drying
Not more than 12% (105°C, 2 h)

Sulfur dioxide
Not more than 50 mg/kg
See description under TESTS

* These specifications were prepared at the 28th session of JECFA (1984) and published in FNP 31/2 (1984).

PURITY TESTS (continued)

* Acid-insoluble ash  Not more than 1%

* Nitrogen content  Not more than 2.5% after washing with acid and ethanol

* Arsenic  Not more than 3 mg/kg (Method II)

** Copper  Not more than 50 mg/kg

* Lead  Not more than 10 mg/kg

** Zinc  Not more than 25 mg/kg

Galacturonic acid  Not less than 65% calculated on the ash-free and dried bases.
See description under TESTS

Degree of amidation  Not more than 25% of total carboxyl groups of pectin.
See description under TESTS

TESTS

IDENTIFICATION TESTS

B. Gel formation  Heat 1 g of the sample with 9 ml of water on a steam bath until a solution is formed. Replace water lost by evaporation. A gel is formed upon cooling

C. Precipitate formation  To a 1-in-100 solution of the sample add an equal volume of ethanol. A translucent, gelatinous precipitate is formed

D. Gel formation  To 5 ml of a 1-in-100 solution of the sample add 1 ml of 2 N sodium hydroxide and allow to stand at room temperature for 15 min. A translucent to opaque gel or semi-gel is formed

E. Precipitate formation  Acidify the gel from the preceding test with dilute hydrochloric acid TS and shake well. A voluminous, colourless, gelatinous precipitate forms, which upon boiling becomes white and flocculent (pectic acid).

F. Positive test for amide group  Add 2 ml of hydrochloric acid and 50 ml of 60% ethanol to 0.5 g of the sample, and stir well for 20 min. Transfer to a fritted glass filter tube and wash with six 10 ml portions of the HCl-60% ethanol mixture. Dissolve in 100 ml distilled water; it may be necessary to add a few drops 0.1 N NaOH to achieve solution. Transfer 4 ml of this solution into a test tube (recommended dimensions 15.5 mm inner diameter and 146 mm length). Add 1 ml 5 N NaOH and mix. The mixture will form a gel. Fill a small glass tube (recommended dimensions 7.8 mm inner diameter and 89 mm length) with 2.5 ml boric acid indicator solution and let glide into the test tube. Close with parafilm and incubate overnight at 30°. In case of presence of amide groups the indicator changes its colour from red to green, due to release of ammonia.


** See Atomic absorption spectrophotometric method under General Instrumental Methods/Metallic Impurities (Part II) in General Methods.
PURITY TESTS

Sulfur dioxide

Suspend 100 g of the sample in 500 ml of methanol in a 1000-ml round-bottom flask, which is provided with a gas inlet tube reaching almost the bottom and connected to the neck with a reflux condenser. Prepare a glass joint connection from the condenser to an absorption flask or U-tube containing 10 ml of 3% H₂O₂ solution neutralized to methyl red TS. Connect the gas inlet tube with an oxygen-free source of CO₂ or N₂, and maintain a gas stream so as to cause steady bubbling. As soon as the apparatus is flushed free of air, pour 30 ml of HCl solution (10 ml conc. HCl + 20 ml H₂O) into the reflux condenser, and immediately connect the absorption flask or U-tube. Heat slowly until methanol starts refluxing, and reflux gently for 2 h. Disconnect the apparatus and titrate the H₂O₂ solution against methyl red TS with 0.01 N NaOH. Each ml of 0.01 N NaOH corresponds to 0.32 mg of SO₂.

Galacturonic acid

Degree of amidation

Degree of esterification

Weigh 5 g of the sample to the nearest 0.1 mg, and transfer to a suitable beaker. Stir for 10 min with a mixture of 5 ml of hydrochloric acid TS, and 100 ml of 60% ethanol. Transfer to a fritted-glass filter tube (30 to 60 ml capacity) and wash with six 15-ml portions of the HCl-60% ethanol mixture, followed by 60% ethanol until the filtrate is free of chlorides. Finally wash with 20 ml of ethanol, dry for 2.5 h in an oven at 105°, cool and weigh. Transfer exactly one-tenth of the total net weight of the dried sample (representing 0.5 g of the original unwashed sample) to a 250 ml conical flask and moisten the sample with 2 ml of ethanol TS. Add 100 ml of recently boiled and cooled distilled water, stopper and swirl occasionally until a complete solution is formed. Add 5 drops of phenolphthalein TS, titrate with 0.1 N sodium hydroxide TS and record the results as the initial titre (V₁).

Add exactly 20 ml of 0.5 N sodium hydroxide TS, stopper, shake vigorously and let stand for 15 min. Add exactly 20 ml of 0.5 N hydrochloric acid TS, and shake until the pink colour disappears. Titrate with 0.1 N sodium hydroxide TS to a faint pink colour which persists after vigorous shaking; record this value as the saponification titre (V₂).

Quantitatively transfer the contents of the conical flask into a 500-ml distillation flask fitted with a Kjeldahl trap and a water-cooled condenser, the delivery tube of which extends well beneath the surface of a mixture of 150 ml of carbon dioxide-free water and 20.0 ml of 0.1 N hydrochloric acid TS in a receiving flask. To the distillation flask add 20 ml of a 1-in-10 sodium hydroxide solution, seal the connections, and then begin heating carefully to avoid excessive foaming. Continue heating until 80-120 ml of distillate has been collected. Add a few drops of methyl red TS to the receiving flask, and titrate the excess acid with 0.1 N sodium hydroxide TS, recording the volume required, in ml, as S. Perform a blank determination on 20.0 ml of 0.1 N hydrochloric acid TS, and record the volume required, in ml, as B. Record the amide titre (B-S) as V₃.

Calculate degree of esterification (as % of total carboxyl groups) by the formula:

\[ 100 \times \frac{V₂}{V₁ + V₂ + V₃} \]
Galactouronic acid
Degree of amidation
Degree of esterification
(continued) Calculate degree of amidation (as % of total carboxyl groups) by the formula:

\[ 10 \times \frac{V_3}{V_1 + V_2 + V_3} \]

And calculate mg of galacturonic acid by the formula:

\[ 19.41 \times (V_1 + V_2 + V_3) \]

The mg of galacturonic acid obtained in this way is the content of one-tenth of the weight of the washed and dried sample. To calculate % galacturonic acid on a moisture-and-ash-free basis, multiply the number of mg obtained by 1000/x, x being the weight in mg of the washed and dried sample.

Methanol, ethanol and isopropanol

Principle
The alcohols are converted to the corresponding nitrite esters and determined by headspace gas chromatography.

Sample preparation
Dissolve 100 mg of sample in 10 ml of water using sodium chloride as a dispersing agent if necessary.

Internal standard solution
Prepare an aqueous solution containing 50 mg/l of n-propanol.

Standard alcohol solution
Prepare and aqueous solution containing 50 mg/l each of methanol, ethanol and isopropanol.

Procedure
Weigh 200 mg of urea into a 25-ml "dark vial" (Reacti-flasks, Pierce, Rockford 3, USA or equivalent). Purge with nitrogen for 5 min and then add 1 ml of saturated oxalic acid solution, close with a rubber stopper and swirl. Add 1 ml of sample solution, 1 ml of internal standard solution and simultaneously start a stop watch (T=0). Swirl the vial and recap with an open screw cap fitted with a silicone rubber septum. Swirl until T=30 sec. At T=45 sec inject through the septum 0.5 ml of an aqueous solution of sodium nitrite (250 g/l). Swirl until T=70 and at T=150 sec withdraw through the septum 1 ml of the headspace using a pressure lock syringe (Precision Sampling Corp., Baton Rouge, Louisiana, USA or equivalent).

Gas chromatography
Insert syringe needle in the injection port; pre-compress the sample, then open the syringe and inject the sample.
**Methanol, ethanol and isopropanol** (continued)

**GC conditions**

- **Column:** glass, 90 cm x 4 mm id
- **Column packing:** first 15 cm packed with chrompack (or equivalent) and the remainder with Propak R 120-150 mesh (or equivalent).
- **Column temperature:** 150° isothermal
- **Injection port temperature:** 250°
- **Carrier gas:** nitrogen
- **Flow rate:** 80 ml/min
- **Detector:** flame ionization

**Calculation**

Quantify the methanol, ethanol and isopropanol present in the sample by comparing the peak areas with the corresponding peaks obtained by chromatographing the headspace produced by substituting in the procedure 1 ml of Standard alcohol solution for 1 ml of Sample solution.
PECTINASE FROM *ASPERGILLUS NIGER, VAR.*, (Tentative)**

**SOURCES** Commercial enzyme preparations are produced by the controlled fermentation of *Aspergillus niger, var.* and isolated from the medium.

**ACTIVE PRINCIPLES**
1. Pectinesterase (pectin methylesterase)
2. Polygalacturonase
3. Pectin lyase (pectin depolymerase)

**SYSTEMATIC NAMES AND NUMBERS**

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<thead>
<tr>
<th>Systematic Name</th>
<th>EC Numbers</th>
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<td>Pectin pectylhydrolase</td>
<td>EC 3.1.1.11</td>
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<tr>
<td>Poly (1,4-α-D-galacturonide) glycanohydrolase</td>
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<tr>
<td>Poly (methoxygalacturonide) lyase</td>
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**REACTIONS CATALYZED**
1. Pectinesterase: demethylation of pectin
2. Polygalacturonase: hydrolysis of 1,4-α-galacturonide linkages in pectin.
3. Pectin lyase: eliminative cleavage of pectin to give oligosaccharides.

**SECONDARY ENZYME ACTIVITIES**

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<td>Beta-glucanase</td>
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<td>Xylanase</td>
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<td>Lactase</td>
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<tr>
<td>Cellulase</td>
<td>EC 3.2.1.4</td>
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**DESCRIPTION** The products are typically offered as off-white to tan amorphous powders, or as tan to dark brown liquids dispersed in food-grade diluents or carriers and may contain stabilizers and preservatives. They are soluble in water but practically insoluble in ethanol, chloroform and ether.

**FUNCTIONAL USES** Manufacture of fruit juice and wine.

**GENERAL SPECIFICATIONS** Must conform to the "General Specifications for Enzyme Preparations used in Food Processing***

*** These specifications were prepared at the 31st session of JECFA (1987) and published in FNP 38 (1988).

** Information required on major and minor enzymic activities, non-enzymic components; characterization of microbial strains used; a measure of specific or relative activity to more explicitly define product quality; a standard assay method for Pectinase activity.

*** See Annex 1 at the end of this compendium.
PENTAPOTASSIUM TRIPHOSPHATE*

SYNONYMS
Pentapotassium tripolyphosphate, potassium tripolyphosphate, potassium triphosphate;
INS No. 451(ii), EEC No. E450(b)

DEFINITION
Chemical names
Pentapotassium tripolyphosphate, pentapotassium tripolyphosphate
C.A.S. number
13845-36-8
Chemical formula
K₅O₁₀P₃
Formula weight
448.42
Assay
Content not less than 85% of K₅O₁₀P₃ on the dried basis, the remainder being principally other potassium phosphates.

DESCRIPTION
Hygroscopic white granules or powder

FUNCTIONAL USE
Texturizer

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Very soluble in water

** B. pH
9.2 - 10.1 (1 in 100 Soln)

** C. Positive test for potassium
To 1 ml of 1 in 100 solution of the sample add a few drops of phosphate silver nitrate TS. A white precipitate is formed which is soluble in dilute nitric acid TS.

** D. Positive test for potassium
Passes test

PURITY TESTS

** Loss on ignition
Not more than 0.4% after drying (105°, 4 h), followed by ignition at 550° for 30 min.

** Water insoluble matter
Not more than 2%

* These specifications were prepared at the 29th session of JECFA (1985) and published in FNP 34 (1986).

PURITY TESTS (continued)

* P₂O₅ content

Not less than 46.5% and not more than 48.0%
Proceed as directed in the Phosphate Determination as P₂O₅ in the General Methods using about 1.5 g of the sample previously dried at 105° for 4 h and accurately weighed.

Fluoride

Not more than 10 mg/kg
See description under TESTS

* Arsenic

Not more than 3 mg/kg
Test a solution of 1 g of the sample in 35 ml of water as directed in the Limit Test (Method II).

* Heavy metals

Not more than 10 mg/kg
Test a solution of 2 g of the sample in 25 ml of water as directed in the Limit Test (Method I).

TESTS

PURITY TESTS

* Fluoride

Place 5 g of the sample, 25 ml of water, 50 ml of perchloric acid, 5 drops of silver nitrate solution (1 in 2), and a few glass beads in a 250-ml distilling flask connected with a condenser and carrying a thermometer and a capillary tube, both of which must extend into the liquid. Connect a small dropping funnel, filled with water, or a steam generator to the capillary tube. Support the flask on an asbestos mat with a hole which exposes about one-third of the flask to the flame. Distil into a 250-ml flask until the temperature reaches 135°. Add water from the funnel or introduce steam through the capillary to maintain the temperature between 135° and 140°. Continue the distillation until 225-240 ml has been collected, then dilute to 250 ml with water, and mix. Place a 50-ml aliquot of this solution in a 100-ml Nessler tube. In another similar Nessler tube place 50 ml of water as a control. Add to each tube 0.1 ml of a filtered solution of sodium alizarin-sulphonate (1 in 1000) and 1 ml of freshly prepared hydroxylamine hydrochloride solution (1 in 4000), and mix well. Add, dropwise, and with stirring, 0.05 N sodium hydroxide to the tube containing the distillate until its colour just matches that of the control, which is faintly pink. Then add to each tube exactly 1 ml of 0.01 N hydrochloric acid, and mix well. From a buret, graduated in 0.05 ml, add slowly to the tube containing the distillate enough thorium nitrate solution (1 in 4000) so that, after mixing, the colour of the liquid just changes to a faint pink. Note the volume of the solution added, add exactly the same volume to the control, and mix. Now add to the control sodium fluoride TS (10 µg, F per ml) from a buret to make the colours of the two tubes match after dilution to the same volume. Mix well, and allow all air bubbles to escape before making the final drops of sodium fluoride TS to the control. A distant change in colour should take place. Note the volume of sodium fluoride added. The volume of sodium fluoride TS required for the control solution should not exceed 10 ml.

METHOD OF ASSAY

Reagents and Solutions
- **Potassium Acetate Buffer (pH 5.0):** Dissolve 78.5 g of potassium acetate in 1000 ml of water, and adjust the pH of the solution to 5.0 with acetic acid. Add a few mg of mercuric iodide to inhibit mould growth.

- **0.3 M Potassium Chloride:** Dissolve 22.35 g of potassium chloride in water, add 5 ml of Potassium Acetate Buffer, dilute with water to 1000 ml, and mix. Add a few mg of mercuric iodide.

- **0.6 M Potassium Chloride:** Dissolve 44.7 g of potassium chloride in water, add 5 ml of Potassium Acetate Buffer, dilute with water to 1000 ml, and mix. Add a few mg of mercuric iodide.

- **1 M Potassium Chloride:** Dissolve 74.5 g of potassium chloride in water, add 5 ml of Potassium Acetate Buffer, dilute to 1000 ml with water, and mix. Add a few mg of mercuric iodide.

Chromatographic Column
Use a standard chromatographic column 20 to 40 cm in length, 20 to 28 cm in inside diameter, with a sealed-in, coarse porosity fritted disk. If a stopcock is not provided, attach a stopcock having a 3 to 4 mm diameter bore to the outlet of the column with a short length of flexible vinyl tubing.

Procedure
Close the column stopcock, fill the space between the fritted disk and the stopcock with water, and connect a vacuum line to the stopcock. Prepare a 1:1 water slurry of Dowex F x 8, or equivalent, chloride form, 100-200 or 200-400 mesh, styrene/divinylbenzene ion exchange resin, and decant off any fine particles and any foam. Do this two or three times or until no more finely suspended material or foaming is observed. Fill the column with the slurry, and open the stopcock to allow the vacuum to pack the resin bed until the water level is slightly above the top of the resin, then immediately close the stopcock. Do not allow the liquid level to fall below the resin level at any time. Repeat this procedure until the packed resin column is 15 cm above the fritted disk. Place one circle of tightly fitting fiber filter paper on top of the resin bed, then place a perforated polyethylene disk on top of the paper. Alternatively, a loosely packed plug of glass wool may be placed on top of the bed. Close the top of the column with a rubber stopper in which a 7.6 cm length of capillary tubing (1.5 mm i.d., 7 mm o.d.) has been inserted through the centre, so that about 12 mm of the tubing extends through the bottom of the stopper. Connect the top of the capillary tubing to the stem of a 500 ml separator with flexible vinyl tubing, and clamp the separator to a ring stand above the column. Wash the column by adding 100 ml of water to the separator with all stopcocks closed. First open the separator stopcock, then open the column stopcock. The rate of flow should be about 5 ml per min. When the separator is empty, close the stopcock on the column then close the separator stopcock.
Transfer about 500 mg of the sample previously dried at 105° for 4 h and accurately weighed, into a 250 ml volumetric flask, dissolve and dilute to volume with water, and mix. Transfer 10 ml of this solution into the separator, open both stopcocks and allow the solution to drain into the column, rinsing the separator with 20 ml of water. Discard the eluate. Add 370 ml of 0.3 M Potassium Chloride to the separator, and allow this solution to pass through the column, discarding the eluate. Add 250 ml of 0.6 M Potassium Chloride to the column, allow the solution to pass through the column, and receive the eluate in a 400 ml beaker. (To ensure a clean column for the next run, pass 100 ml of 1 M Potassium Chloride through the column, followed by 100 ml of water. Discard all washings.) To the beaker add 15 ml of nitric acid, mix, and boil for 15 to 20 min. Add methyl orange TS, and neutralize the solution with stronger ammonia TS. Add 1 g of ammonium nitrate crystals, stir to dissolve, and cool. Add 15 ml of ammonium molybdate TS, with stirring, and stir vigorously for 3 min or allow to stand with occasional stirring for 10 to 15 min. Filter the contents of the beaker with suction through a 6-7 mm paper pulp filter pad supported in a 25 mm porcelain disk. The filter pad should be covered with a suspension of infusorial earth. After the contents of the beaker have been transferred to the filter, wash the beaker with five 10 ml portions of a 1 in 100 solution of sodium or potassium nitrate, passing the washings through the filter, then, wash the filter with five 5-ml portions of the wash solution. Return the filter pad and the precipitate to the beaker, wash the funnel throughly with water into the beaker, and dilute to about 150 ml. Add 0.1 N sodium hydroxide from a buret until the yellow precipitate is dissolved, then add 5 to 8 ml in excess. Add phenolphthalein TS, and titrate the excess alkali with 0.1 N, nitric acid. Finally, titrate with 0.1 N sodium hydroxide to the first appearance of the pink colour. The difference between the total volume of 0.1 N sodium hydroxide added and the volume of nitric acid required represents the volume, V, in ml, of 0.1 N sodium hydroxide consumed by the phosphomolybdate complex. Calculate the quantity, in mg, of K$_2$O$_{10}$P$_3$ in the sample taken by the formula 0.650 x 25V.
PENTASODIUM TRIPHOSPHATE*
(Tentative)**

SYNONYMS
Pentasodium tripolyphosphate, sodium triphosphate; INS No. 451(i), EEC No. E450(b)

DEFINITION

Chemical names
Pentasodium triphosphate, pentasodium tripolyphosphate

C.A.S. number
7758-29-4

Chemical formula
$\text{Na}_5\text{O}_4\text{P}_3$

Structural formula

\[
\begin{array}{cccc}
\text{O} & \text{O} & \text{O} \\
\text{Na} & \text{P} & \text{O} & \text{P} & \text{O} & \text{P} & \text{ONa} \\
\text{ONa} & \text{ONa} & \text{ONa}
\end{array}
\]

Formula weight
367.86

Assay
Content not less than 85.0% of $\text{Na}_5\text{O}_4\text{P}_3$, and not less than 56.0% and not more than 58.0% of $\text{P}_2\text{O}_5$. 

DESCRIPTION
White, slightly hygroscopic granules or powder

FUNCTIONAL USE
Texturizer

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility
Freely soluble in water. Insoluble in ethanol.

*** B. pH
9.1 - 10.1 (1 in 100 Soln)

*** C. Positive test for phosphate
To 5 ml of 1 in 100 solution of the sample add 1 ml of concentrated nitric acid and 5 ml of ammonium molybdate TS and warm. A bright canary-yellow precipitate is formed.

*** D. Positive test for sodium
To 5 ml of a 1 in 20 solution of the sample add 1 ml of acetic acid TS and 1 ml of uranyl zinc TS. A yellow crystalline precipitate is formed within a few minutes.

* These specifications were prepared at the 20th session of JECFA (1976) and published in FNS 1B (1977).

** Method for contents of $\text{P}_2\text{O}_5$ to be provided

PURITY TESTS

* Loss on drying
  Not more than 0.7% (105°, 1 h)

* Water insoluble matter
  Not more than 0.1%

Higher polyphosphates
  Not detectable
  See description under TESTS

* Fluoride
  Not more than 50 mg/kg (Method I or III)

* Arsenic
  Not more than 3 mg/kg
  Test a solution of 1 g of the sample in 35 ml of water as directed in the Limit Test (Method II).

* Lead
  Not more than 10 mg/kg
  Dissolve 1 g of the sample in 20 ml of water and neutralize to phenolphthalein TS. this solution meets the requirements of the Limit Test for Lead.

* Heavy metals
  Not more than 30 mg/kg
  Test a solution of 0.67 g of the sample in 25 ml of water as directed in the Limit Test (Method I).

TESTS

PURITY TESTS

Higher polyphosphates

Reagents and Solutions
- Chromatographic solvent: Mix 75 ml of isopropanol, 10 ml of water, 20 ml of 20% trichloroacetic acid and 0.3 ml of 20% ammonia. Make fresh every week.
- Chromatographic spray: Dissolve 1 g of ammonium molybdate in 85 ml of water, 10 ml of N hydrochloric acid and 5 ml of 60% perchloric acid.
- Sample solution: Dissolve 1 g of the sample in 50 ml of water.
- Reference solution: Dissolve 1 g of a standard sample of pentasodium triphosphate in 50 ml of water.

Procedure
Place 0.01 ml of the sample solution and 0.01 ml of reference solution on the starting line of the chromatographic paper and allow to dry in a stream of warm air. Use ascending chromatography at 18°-20° until the solvent has ascended about 25 cm from the starting line (12 - 15 h). Dry at 60° in an oven and spray with the chromatographic spray. Place the paper under an ultraviolet lamp and irradiate until the phosphates are visible as blue spots (about 2 min). Three spots (one from the monophosphate \( R_t = 0.69 \), a second from the diphosphate \( R_t = 0.44 \) and the third from the triphosphate \( R_t = 0.29 \) ) are observed, and no other spot is observed.

METHOD OF ASSAY

Reagents and Solutions
- **Potassium Acetate Buffer (pH 5.0):** Dissolve 78.5 g of potassium acetate in 1000 ml of water, and adjust the pH of the solution to 5.0 with acetic acid. Add a few mg of mercuric iodide to inhibit mould growth.

- **0.3 M Potassium Chloride:** Dissolve 22.35 g of potassium chloride in water, add 5 ml of Potassium Acetate Buffer, dilute with water to 1000 ml, and mix. Add a few mg of mercuric iodide.

- **0.6 M Potassium Chloride:** Dissolve 44.7 g of potassium chloride in water, add 5 ml of Potassium Acetate Buffer, dilute with water to 1000 ml, and mix. Add a few mg of mercuric iodide.

- **1 M Potassium Chloride:** Dissolve 74.5 g of potassium chloride in water, add 5 ml of Potassium Acetate Buffer, dilute to 1000 ml with water, and mix. Add a few mg of mercuric iodide.

Chromatographic Column
Use a standard chromatographic column ~0 to 40 cm in length, 20 to 28 cm in inside diameter, with a sealed-in, coarse porosity fritted disk. If a stopcock is not provided, attach a stopcock having a 3 to 4 mm diameter bore to the outlet of the column with a short length of flexible vinyl tubing.

Procedure
Close the column stopcock, fill the space between the fritted disk and the stopcock with water, and connect a vacuum line to the stopcock. Prepare a 1:1 water slurry of Dowex F x 8, or equivalent, chloride form, 100-200 or 200-400 mesh, styrenedivinylbenzene ion exchange resin, and decant off any fine particles and any foam. Do this two or three times or until no more finely suspended material or foaming is observed. Fill the column with the slurry, and open the stopcock to allow the vacuum to pack the resin bed until the water level is slightly above the top of the resin, then immediately close the stopcock. Do not allow the liquid level to fall below the resin level at any time. Repeat this procedure until the packed resin column is 15 cm above the fritted disk. Place one circle of tightly fitting fiber filter paper on top of the resin bed, then place a perforated polyethylene disk on top of the paper. Alternatively, a loosely packed plug of glass wool may be placed on top of the bed. Close the top of the column with a rubber stopper in which a 7.6 cm length of capillary tubing (1.5 mm i.d., 7 mm o.d.) has been inserted through the centre, so that about 12 mm of the tubing extends through the bottom of the stopper. Connect the top of the capillary tubing to the stem of a 500 ml separator with flexible vinyl tubing, and clamp the separator to a ring stand above the column. Wash the column by adding 100 ml of water to the separator with all stopcocks closed. First open the separator stopcock, then open the column stopcock. The rate of flow should be about 5 ml per min. When the separator is empty, close the stopcock on the column then close the separator stopcock.
METHOD OF ASSAY (continued) Transfer about 500 mg of the sample (a), accurately weighed, into a 250 ml volumetric flask, dissolve and dilute to volume with water, and mix. Transfer 10 ml of this solution into the separator, open both stopcocks and allow the solution to drain into the column, rinsing the separator with 20 ml of water. Discard the eluate. Add 370 ml of 0.3 M Potassium Chloride to the separator, and allow this solution to pass through the column, discarding the eluate. Add 250 ml of 0.6 M Potassium Chloride to the column, allow the solution to pass through the column, and receive the eluate in a 400-ml beaker. (To ensure a clean column for the next run, pass 100 ml of 1 M Potassium Chloride through the column, followed by 100 ml of water. Discard all washings.) To the beaker add 15 ml of nitric acid, mix, and boil for 15 to 20 min. Add methyl orange TS, and neutralize the solution with stronger ammonia TS. Add 1 g of ammonium nitrate crystals, stir to dissolve, and cool. Add 15 ml of ammonium molybdate TS, with stirring, and stir vigorously for 3 min or allow to stand with occasional stirring for 10 to 15 min. Filter the contents of the beaker with suction through a 6-7 mm paper pulp filter pad supported in a 25 mm porcelain disk. The filter pad should be covered with a suspension of infusorial earth. After the contents of the beaker have been transferred to the filter, wash the beaker with five 10 ml portions of a 1 in 100 solution of sodium or potassium nitrate, passing the washings through the filter, then, wash the filter with five 5-ml portions of the wash solution. Return the filter pad and the precipitate to the beaker, wash the funnel thoroughly with water into the beaker, and dilute to about 150 ml. Add 0.1 N sodium hydroxide from a buret until the yellow precipitate is dissolved, then add 5 to 8 ml in excess. Add phenolphthalein TS, and titrate the excess alkali with 0.1 N nitric acid. Finally, titrate with 0.1 N sodium hydroxide to the first appearance of the pink colour. The difference between the total volume of 0.1 N sodium hydroxide added and the volume of nitric acid required represents the volume, V, in ml, of 0.1 N sodium hydroxide consumed by the phosphomolybdate complex.

Calculation

Calculate the Na$_3$O$_{10}$P$_3$ content in the sample by the formula:

\[
\% \text{ Na}_3\text{O}_{10}\text{P}_3 = \frac{0.533 \times 25 \times V}{a} \times 100
\]
PEPSIN FROM HOG STOMACH*

SYNONYM
Pepsin

SOURCES
Commercial preparations of Pepsin contain proteolytic enzymes obtained from the glandular layer of hog stomach.

ACTIVE PRINCIPLE
Pepsin (acid proteinase)
Composed of Pepsin A (major component), Pepsin B and Pepsin C

SYSTEMATIC NAMES AND NUMBERS
None - EC 3.4.23.1
None - EC 3.4.23.2
None - EC 3.4.23.3

REACTIONS CATALYZED
The enzyme preparations hydrolyze polypeptides, including those with linkages adjacent to aromatic or L-leucine residues, yielding peptides of lower molecular weight. Pepsin B and Pepsin C, which are minor components, have more restricted specificity.

DESCRIPTION
The enzyme preparations occur as white to light tan water soluble powders, amber pastes or clear amber to brown aqueous liquids.

FUNCTIONAL USES
Preparation of fish meal and other protein hydrolysates, and the clotting of milk in cheese making in combination with one of the rennet.

GENERAL SPECIFICATIONS
Must conform to the "General Specifications for Enzyme Preparations used in Food Processing"**

CHARACTERISTICS
IDENTIFICATION TESTS
Pepsin activity
The sample shows proteolytic activity
See description under TESTS (next pages)

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* This specification was prepared at the 15th session of JECFA (1971) and published in NMRS 50B (1972).

** See Annex 1 at the end of this compendium.
DETERMINATION OF PROTEOLYTIC ACTIVITY IN PEPsin* 

**Principle of Method**
Coagulated egg albumin is digested with a pepsin solution and the residue of the undissolved albumen is compared with that from a test with a reference standard.

**Definition of Activity**
The activity of pepsin is expressed as the multiple of its own weight with that of coagulated egg albumen which it can digest. The Food Chemicals Codex defines, that one pepsin unit digests 3,000 times its weight of coagulated egg albumen under the condition of the assay.

**Procedure of Assay**
Mix 35 ml of 1.0N hydrochloric acid with 385 ml of water. Dissolve 100 mg of pepsin, accurately weighed, in 150 ml of this solution. Prepare a similar solution of N.F. Pepsin Reference Standard, accurately weighed. Boil one or more hen eggs for 15 min to provide coagulated albumen for the assay. Cool them rapidly to room temperature by immersion in cold water; remove the shell and pellicle and all of the yolk and at once rub the albumen through a clean, dry No. 40 sieve rejecting the first portion that passes through the sieve. Place 10 g of the succeeding well mixed portion in each of 3 wide-mouth bottles of about 100 ml capacity. Immediately add 35 ml of the dilute acid at one time or in portions and, by suitable means, thoroughly disintegrate the particles of albumen.

Place the bottles in a water bath at 52°. After the contents of the bottles have reached that temperature, add 5.0 ml of the acidified solution of pepsin to one bottle, 4.30 ml of the same solution and 0.7 ml of the dilute acid to another bottle, and 5.0 ml of the Reference Standard solution to the third bottle. At once stopper the bottles securely, invert them 3 times, and maintain them at 52° for 2 h and 30 min, agitating the contents equally every 10 min by inverting the bottles once.

Remove the bottles from the bath, pour the contents into 100 ml conically shaped measuring vessels, having diameters not exceeding 1 cm at the bottom and complying in other respects with the water and sediment tube ASTM Standard Methods D 96-68, graduated from 0 to 0.5 ml in 0.05 ml graduations; from 0.5 to 2 ml in 0.1 ml graduations; from 2 to 3 in 0.2 ml graduations; from 3 to 5 ml in 0.5 ml graduations; from 5 to 10 ml in 1 ml graduations; from 10 to 25 ml in 5 ml graduations; and with graduation marks at 50, 75, and 100 ml points.

Transfer the undigested egg albumen which adheres to the sides of the bottles to the respective measuring vessels with the aid of small portions of water until 50 ml has been used for each. Mix the contents of each measuring vessel, and allow them to stand for 30 min. The volume of the undissolved albumen in the measuring vessel corresponding to 5.0 ml of the solution of pepsin being assayed does not exceed the volume of the undissolved albumen in the measuring vessel corresponding to 5.0 ml of the Reference Standard solution, and the volume of the undissolved albumen in the measuring vessel corresponding to 4.30 ml of the solution of pepsin being assayed is not less than the volume of the undissolved albumen in the measuring vessel corresponding to 5.0 ml of the Reference Standard solution.

Note: Other measuring vessels than the type described in this monograph may be used if they are of such design and graduation as to measure the residue accurately.
PETROLEUM JELLY*
(Tentative)**

SYNONYMS
Vaseline, Petrolatum;
INS No. 905b

DEFINITION
Petroleum Jelly is a purified mixture of semi-solid hydrocarbons, mainly of paraffinic nature,** obtained from petroleum. It may contain antioxidants approved for food use.

C.A.S. number
8009-03-8

DESCRIPTION
White to yellowish or light amber semisolid substance. It is transparent in thin layers and have not more than a slight fluorescence.

FUNCTIONAL USE
Lubricant, release agent, protective coating, anti-foaming agent.

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility
Insoluble in water. Very soluble in carbon disulfide
Soluble in ether and hexane.

B. Melting range
Between 38° and 60°
See description under TESTS

PURITY TESTS

Residue on ignition
Not more than 0.1%
See description under TESTS

Colour
Passes test
See description under TESTS

Acidity or alkalinity
Passes test
See description under TESTS

Sulfur
Information required

Arsenic
Information required

Lead
Information required

Heavy metals
Information required

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* These specifications were prepared at the 33rd session of JECFA (1988) and published in FNP 38 (1988).

** Information required on actual composition of hydrocarbons, method(s) for identification of individual hydrocarbons, limits and methods of analysis for sulfur, arsenic, lead and heavy metals and nature of sulfur compounds.

PURITY TESTS (continued)

**Organic acids**

Passes tests
See description under TESTS

**Fixed oils, fats and resins**

Passes test
See description under TESTS

**Polycyclic aromatic hydrocarbons**

The sample shall meet the following ultraviolet absorbance when subjected to the analytical procedure as directed in the specifications for Paraffin Wax.

<table>
<thead>
<tr>
<th>nm</th>
<th>max. absorbance per cm path length</th>
</tr>
</thead>
<tbody>
<tr>
<td>280-289</td>
<td>0.25</td>
</tr>
<tr>
<td>290-299</td>
<td>0.20</td>
</tr>
<tr>
<td>300-359</td>
<td>0.14</td>
</tr>
<tr>
<td>360-400</td>
<td>0.04</td>
</tr>
</tbody>
</table>

TESTS

**IDENTIFICATION TESTS**

* B. Melting range

Carefully melt the material to be tested at as low temperature as possible, and draw it into a capillary tube that is left open at both ends to a depth of about 10 mm. Cool the charged tube at 10° or lower, for 24 h or in contact with ice for at least 2 h. Then attach the tube to the thermometer by means of a rubber band, adjust it in a water bath so that the upper edge of the material is 10 mm below the water level and heat with constant stirring at a rate of rise of approximately 3° per min. until a temperature 5° below the melting point is attained, then carefully regulate the rise of temperature to 0.5° to 1.0° per min. The temperature at which the material is observed to rise in the capillary tube is the melting temperature.

**PURITY TESTS**

* Residue on ignition

Accurately weigh about 4 g of the sample in a tared porcelain or platinum dish and heat over a flame. The sample volatiles without emitting any acid odour. Ignite not to exceed very dull redness, until free from carbon. Cool in a desiccator and weigh.

**Colour**

Melt about 10 g of the sample on a steam bath, and pour about 5 ml of the liquid into a 150 x 16-mm clear-glass bacteriological test tube, keeping the sample melted. The petrolatum is not darker than a solution made by mixing 3.8 ml of ferric chloride TS and 1.2 ml of cobaltous chloride TS in a similar tube, the comparison of the two being made in reflected light against a white background, holding the sample tube directly against the background at such an angle that there is no fluorescence.

Acidity or alkalinity

Introduce 35 g of the sample into a 250 ml separator, add 100 ml of boiling water, and shake vigorously for 5 min. After the petrolatum and water have separated, draw off the water into a casserole, wash the sample in the separator with two 50-ml portions of boiling water, and add the washings to the casserole. To the accumulated 200 ml of water add 1 drop of phenolphthalein TS and boil. The solution does not acquire a pink colour. If the addition of phenolphthalein produces no pink colour, add 0.1 ml of methyl orange TS. No red pink colour is produced.

Organic acids

Weigh 20 g of the sample, add 50 ml of neutralized ethanol, and 50 ml of water, agitate thoroughly, and heat to boiling. Add 1 ml phenolphthalein TS, and titrate rapidly with 0.1 M sodium hydroxide, with vigorous agitation to a sharp pink end-point, noting the colour change in the alcohol - water layer. Not more than 0.4 ml is required.

Fixed oils, fats and rosin

Digest 10 g of the sample at 100° with 10 g of sodium hydroxide and 50 ml of water for 30 min. Separate the water layer, and add to it an excess of diluted sulfuric acid TS. No oily or solid matter separates.
Phenylacetaldehyde*  

**SYNONYM**  
α-Toluic aldehyde

**DEFINITION**

- **Chemical name**: Phenylacetaldehyde  
- **C.A.S. number**: 122-78-1  
- **Chemical formula**: C₈H₈O  
- **Structural formula**

![structural_formula](image)

- **Molecular weight**: 120.14  
- **Assay**: Content not less than 90% of C₈H₈O

**DESCRIPTION**

Colourless to slightly yellow, oily liquid having harsh, green odour reminiscent of hyacinth.

**Caution**: Because this compound is unstable, it is not stored and used in the isolated form, but in solution, e.g. 50% in ethanol.

**FUNCTIONAL USE**

Flavouring agent

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

- **A. Refractive index**: n₀° : 1.5240 - 1.5330
- **B. Specific gravity**: d₅₀° : 1.023 - 1.040

**PURITY TESTS**

- **Chlorinated compounds**: Passes test.
- **Acid value**: Not more than 5.

**METHOD OF ASSAY**

Weigh accurately about 1 g, and proceed as directed for Aldehyde Determination in the General Methods**, using 60.08 as the equivalence factor (e) in the calculation.

---

* These specifications were prepared at the 11th session of JECFA (1961) and published in NMRS 44B (1969).

**o-PHENYLPHENOL**

SYNONYMS
Orthoxenol;
INS No. 231, EEC No. E231

DEFINITION

<table>
<thead>
<tr>
<th>Chemical names</th>
<th>(1,1'-Biphenyl)-2-ol, 2-hydroxydiphenyl, o-hydroxydiphenyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A.S. number</td>
<td>90-43-7</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>$\text{C}<em>{12}\text{H}</em>{10}\text{O}$</td>
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<tr>
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<td><img src="image" alt="Structural formula" /></td>
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<tr>
<td>Molecular weight</td>
<td>170.20</td>
</tr>
<tr>
<td>Assay</td>
<td>Contains not less than 98% of $\text{C}<em>{12}\text{H}</em>{10}\text{O}$</td>
</tr>
</tbody>
</table>

DESCRIPTION
White, slightly yellow or pink flaky crystals or solid, having a mild characteristic odour.

FUNCTIONAL USES
For the post-harvest treatment of fruits and vegetables to protect against microbial damage.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Practically insoluble in water, very soluble in ethanol.

** B. Melting point
About 57°.

C. Positive test for phenolate
An ethanolic solution (1 g in 10 ml) produces a green colour upon addition of 10% ferric chloride solution.

PURITY TESTS

** Total ash
Not more than 0.05%.

** Arsenic
Not more than 3 mg/kg (Method II).

** Lead
Not more than 10 mg/kg.

---

* These specifications were prepared at the 8th session of JECFA (1964) and published in NMRS (1965).

METHOD OF ASSAY

Redox titration

Weigh 2.000 g of o-phenylphenol, dissolve in 10 ml of 10% sodium hydroxide solution by warming and dilute to 500.0 ml. Pipette 25.0 ml into a 250-ml iodine flask and add 30.0 ml of 0.1 N bromide-bromate TS and 50 ml of anhydrous methanol. Place the stopper in the flask and add 10 ml of dilute (1 to 1) hydrochloric acid to the well. Raise the stopper slightly to allow the acid to flow down the sides inside the flask, but retain a small amount of the acid in the well to act as a seal. Mix the contents by swirling and allow it to react for exactly 30 seconds at 25° ± 5°. Immediately add 10 ml of 20% potassium iodide solution to the well and allow it to drain into the assay flask as for the acid. Mix well, allow the solution to stand for 5 min, shaking occasionally. Wash the stopper and the sides of the flask with water. Titrate the liberated iodine with 0.1 N sodium thiosulfate adding starch TS as the endpoint is approached. Each ml of 0.1 N bromide-bromate TS consumed is equivalent to 4.255 mg of C₁₂H₁₀O₆.
PHOSPHORIC ACID*

SYNONYMS
Orthophosphoric acid;
INS No. 338, EEC No. E338

DEFINITION
Chemical names
Phosphoric acid, orthophosphoric acid
C.A.S. number
7664-38-20
Chemical formula
H₃PO₄
Molecular weight
98.00
Assay
Phosphoric Acid contains not less than 75% of H₃PO₄, and not less than the minimum or within the range of percent claimed by the vendor.

DESCRIPTION
Phosphoric acid is a clear, colourless, odourless, viscous liquid.

FUNCTIONAL USES
Acidulant, sequestrant, synergist for antioxidants

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Miscible with water and with ethanol

** B. Positive test for acid
Strongly acid, even at high dilution

C. Positive test for phosphate
Passes test
See description under TESTS

PURITY TESTS

Nitrates
Not more than 5 mg/kg
See description under TESTS

Volatile acids
Not more than 10 mg/kg as acetic acid
See description under TESTS

** Chlorides
Not more than 200 mg/kg as chlorine.
1.78 g of the sample meets the requirements of the Limit Test for Chlorides.

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

PURITY TESTS

* Sulfates
Not more than 0.15% as sulfate.
1.25 g of the sample meets the requirements of the Limit Test for Sulfate.

* Fluoride
Not more than 10 mg/kg

* Arsenic
Not more than 2 mg/kg
See description under TESTS

* Lead
Not more than 5 mg/kg

Heavy metals
Passes test
See description under TESTS

TESTS

IDENTIFICATION TESTS

* Positive test for phosphate
Neutralize a few ml of phosphoric acid and add dilute nitric acid TS. Then add an equal volume of ammonium molybdate TS and warm. A bright canary-yellow precipitate is obtained which is soluble in dilute ammonia TS.

PURITY TESTS

Nitrate
Dilute 3.48 g of the sample to 10 ml with water and add 5 mg of sodium chloride, 0.1 ml of indigo carmine TS, and 10 ml of sulfuric acid. The blue colour shall not disappear entirely within 5 min.

Volatile acids
Dilute 60.05 g of the sample with 75 ml of freshly boiled and cooled water in a distilling flask with a spray trap, and distil 50 ml. To the distillate add phenolphthalein TS and titrate with 0.1 N sodium hydroxide. Not more than 0.1 ml of 0.1 N sodium hydroxide should be required for neutralization.

Arsenic
A solution of 1.5 g of the sample in 35 ml of water meets the requirements of the Limit Test for Arsenic (Method II) using as control a mixture of 3 ml Standard Arsenic Solution (3 µg As) and 1.5 g reagent grade Phosphoric Acid.

* Heavy metals
Dilute 2.4 ml of the sample with 10 ml of water, add 12 ml of 20% ammonia and dilute to 40 ml. To 30 ml of this solution add 10 ml of hydrogen sulfide TS. Any brown colour produced should not be darker than that produced by the addition of 10 ml of hydrogen sulfide TS to 30 ml of a control containing the remaining 10 ml of the solution and 0.02 mg of added Pb (prepared from lead nitrate TS, containing 0.01 mg Pb/ml).

METHOD OF ASSAY

Titrimetric method
Weigh 1.00 g of the sample into a glass-stoppered flask, dilute with about 100 ml of water, add 0.5 ml of thymolphthalein TS, and titrate with N sodium hydroxide. Each ml of N sodium hydroxide is equivalent to 0.049 g of H₃PO₄.

PIMARICIN

SYNONYMS
INS No. 235, EEC No. E235

DEFINITION
Pimaricin is a fungicidal antibiotic of the polyene macrocycle group, and is produced by Streptomyces natalensis.

Chemical name
22-(3-Amino-3,6-dideoxy-β-D-mannopyranosyloxy)-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo[22.3.1.05.7]octacosa-8,14,16,18,20-pentaene-25-carboxylic acid

C.A.S. number
7681-93-8

Chemical formula
C_{33}H_{47}N_{13}O_{13}

Structural formula

Molecular weight
665.74

Assay
Contains not less than 95.0% of C_{33}H_{47}N_{13}O_{13}, calculated on the dried basis.

DESCRIPTION
White to creamy-white, almost tasteless and almost odourless, crystalline powder.

FUNCTIONAL USE
Fungicidal preservative.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Practically insoluble in water and in fatty and mineral oils. Slightly soluble in methanol. Soluble in glacial acetic acid and dimethyl-formamide.

* These specifications were prepared at the 20th session of JECFA (1976) and published in FNS 1B (1977).

IDENTIFICATION TESTS (continued)

B. Colour reactions
On adding a few crystals of pimaricin, on a spot plate, to a drop of
- concentrated hydrochloric acid, a blue colour develops;
- concentrated phosphoric acid, a green colour develops, which changes into pale-
red after a few minutes.

C. Spectrophotometry:
A 0.0005% w/v solution in 1% methanolic acetic acid solution has absorption
maxima at about 290, 303 and 318 nm, a shoulder at about 280 nm and exhibits
minima at about 250, 295.5 and 311 nm. See appendix A at the end of these
specifications.

D. Infrared spectrum:
The infrared spectrum exhibits maxima which are only at the same wavelengths
as, and have similar relative intensities to, those in the reference spectrum. See
appendix B at the end of these specifications.

PURITY TESTS

* Loss on drying
Not more than 8.0%
Test 2 g by drying to constant weight at 60°, over phosphorus pentoxide at a
pressure not exceeding 5 mm Hg (6.5 m Barr).

* Specific rotation
[α]D: + 250° to + 295° (a 1% w/v solution in glacial acetic
acid, at 20° and calculated with reference to the dried material).

* pH
5.5 - 7.5 (1.0% w/v solution in a previously neutralized mixture of 20 parts
dimethylformamide and 80 parts of water).

* Sulfated ash
Not more than 0.5%
Proceed as directed under the test for Ash (Sulfated ash, Method I) using 2 g of
the sample.

* Heavy metals
Not more than 30 mg/kg
Test 0.67 g of the sample as directed in Method II under the Limit Test for
Heavy Metals using 20 µg of lead ion (Pb) in the control (Solution A).

METHOD OF ASSAY

Principle
A parallel line assay is carried out on agar plates, inoculated with a
microorganism which is sensitive to piramicin.
The precision of the assay is such that the fiducial limits of error of the estimated
content (P = 0.95) are not less than 95% and not more than 105% of the
estimated content. The upper fiducial limit of error of the estimated content is not
less than 95% calculated with reference to dried substance.

Materials and equipment
- Glass plates: plan-parallel, 16 x 16 cm.
- Petri dishes: diameter 9 cm, flat bottom.
- Agar pipettes: wide tip; 20 ml graduated in 2.5 ml.
- Wide tip; 60 ml graduated in 10 ml.
- Pipettes: 5 and 25 ml, 50 µl.

METHOD OF ASSAY

Materials and equipment (continued)

- Glassware: all glassware used should be brown.
  (Note: Dilute solutions of pimaricin are very sensitive to light).
- Waterbath: 45°
- Incubator: 30°
- Ruler: white, graduated in mm.

Test-organism

Saccharomyces cerevisiae: ATCC 9763)
Maintain the yeast on malt-agar slants.
Transfer the test-organism every four weeks; incubate for 24 h at 25-30° after transfer, then store at 5-15°. For preparation of the inoculum transfer a loopful to a 100 ml flask containing 30 ml liquid Malt extract and incubate for approximately 48 h at 26° (stationary). Keep the inoculum at 6° for no longer than 24 h.

Media and buffers

- Whiffen agar medium
  Yeast extract (Difco) 2.5 g
  Potassium dihydrogen phosphate 6.8 g
  Sodium hydroxide 0.6 g
  Agar 15.0 g
  Dissolve the ingredients in sufficient distilled water to make 1000 ml by heating for 30 min at 100-110°. Filter the solution hot, adjust the pH to 6.6 with N sodium hydroxide and fill into suitable flasks. Sterilize for 30 min at 110°. Check the pH; it should be 6.5.

- Liquid Malt extract
  Dilute Malt extract with distilled water to 12° Balling (equivalent to 125 g Difco Malt extract per L).
  Sterilize for 1 h at 110°, filter and sterilize for 20 min at 120°. Filter again, fill 100 ml flasks with 30 ml each and sterilize for 30 min at 110°.

- Malt agar medium (for maintenance of the test organism).
  Dilute Malt extract with distilled water to 10° Balling (equivalent to 100 g Difco Malt extract per L) and add 20 g agar per L. Dissolve the agar by heating at 100-110° for 30 min, filter and sterilize for 30 min at 110°. After cooling the final pH is 5.4.

- Glucose-cane sugar molasses solution
  Sterilize 50 ml of a 50% w/v solution of glucose (dextrose) in distilled water by Seitz filtration. Add aseptically, 5 ml of a 50% w/v solution of cane sugar molasses in distilled water. Sterilize the cane sugar molasses solution before use by heating for 30 min at 110°.

- Sterile 0.05 M phosphate buffer pH 6.8
  Dissolve 4.35 g dipotassium hydrogen phosphate and 3.4 g potassium dihydrogen phosphate in distilled water, make up to 1 L with same and adjust the pH of the solution to 6.8 with N sodium hydroxide. Sterilize for 30 min at 100°. Check the pH again.
METHOD OF ASSAY

Procedure

- **Preparation of the test plates**
  Add to 300 ml molten Whiffen agar medium cooled to 45°, 6 ml sterile glucose-cane sugar molasses solution and sufficient inoculum to reach a final concentration of approximately 100,000 organisms per ml agar medium. Shake well and pour with an agar pipette 60 ml of inoculated agar medium on the square plan-parallel glass plates or 20 ml into a Petri dish. After solidification of the agar punch 12 holes, 5 mm diameter, in the agar on the glass plates in the pattern shown or 6 holes, 5 mm diameter, in the agar in the Petri dishes at regular distances in a circle with a diameter of 5.7 cm.

- **Reference standard solutions**
  Weigh accurately approximately 50 mg Pimaricin reference standard and dissolve in 100 ml methanol in a brown 200-ml volumetric flask. Make up to volume with sterile 0.05 M phosphate buffer pH 6.8 and mix. The stock solution must contain exactly 250 μg Pimaricin/ml. Dilute the stock solution 5, 10 and 20 times with a 1:1 mixture of methanol and sterile 0.05 M phosphate buffer/pH 6.8 to concentrations of exactly 50, 25 and 12.5 μg Pimaricin/ml.

- **Sample solutions**
  Weigh accurately approximately 55 mg sample, dissolve in 100 ml methanol in a brown 200 ml volumetric flask, make up to volume with sterile 0.05 M phosphate buffer pH 6.8 and mix. This solution contains approximately 250 μg Pimaricin/ml. Dilute this solution 5, 10 and 20 times with a 1:1 mixture of methanol and sterile 0.05 M phosphate buffer pH 6.8 to concentrations of approximately 50, 25 and 12.5 μg Pimaricin/ml.

- **Depositing sample solutions and reference standard solutions**
  Deposit with the 50 μl pipette the three sample solutions and reference standard solutions according to the pattern shown in duplo in the holes on the plates or singly on the Petri dishes. Use per sample at least 4 Petri dishes or 2 glass plates. Use for routine assays 2 weighings per batch.

- **Incubation**
  Incubate the plates for 18 h at 30°.

- **Determination of the zones of inhibition**
  Following incubation measure the diameters of the zones of inhibition in two perpendicular directions to the nearest 0.5 mm with a ruler. Calculate the values of the sample concentrations on each plate according to the "parallel line assay" principle.

Storage:
Store in a well-closed container, protected from light and at a temperature not exceeding 15°.

* Oberzill - Mikrobiologische Analytik - Verlag Hans Karl, Nurnberg.
Petri dish pattern

\[ S_i, R_2, R_4, S_4, S_2, R_1 \]

Glass plate pattern

Plate 1

\[ S_i, R_1, R_4, R_3, S_4, S_1, S_1, S_2, R_2, R_3, R_4, S_2 \]

Note: the plates are not drawn to scale.

Plate 2

\[ S_i, R_2, R_4, R_3, S_2, S_2, S_1, S_4, R_1, R_4, R_4, S_4 \]

\[ R = \text{Reference standard solution} \]
\[ R_1 = 12.5 \, \mu g/ml \]
\[ R_2 = 25 \, \mu g/ml \]
\[ R_4 = 50 \, \mu g/ml \]

\[ S = \text{Sample solution} \]
\[ S_1 = \text{Approx. } 12.5 \, \mu g/ml \]
\[ S_2 = 2 \times S_1 \]
\[ S_4 = 4 \times S_1 \]
APPENDIX A.

Absorption spectrum of pimaricin

Concentration: 5 µg/ml in methanol/glacial acetic acid mixt.
Scan speed: 0.2 nm/sec
Band width: 2 - 3 nm
Spectrophotometer: Beckman Acta C III
APPENDIX B

Pimaricin - Reference Infrared Spectrum (1.3 mg solid in 300 mg potassium bromide).
**PIPERONAL*\**

**SYNONYMS**
Heliotropine, piperonyl aldehyde

**DEFINITION**

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Piperonal</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A.S. number</td>
<td>120-57-0</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>C₈H₆O₃</td>
</tr>
<tr>
<td>Structural formula</td>
<td><img src="image" alt="Structural formula" /></td>
</tr>
</tbody>
</table>

Molecular weight 150.14
Assay Content not less than 99% of C₈H₆O₃

**DESCRIPTION**
White, lustrous crystals having sweet, flowery odour reminiscent of heliotrope.

**FUNCTIONAL USE**
Flavouring agent

**CHARACTERISTICS**

**IDENTIFICATION TEST**

** A. Solidification point**
Not less than 35°

**PURITY TEST**

**Heavy metals**
Not more than 40 mg/kg

**METHOD OF ASSAY**
Weigh accurately about 1.5 g, and proceed as directed for Aldehyde Determination in the General Methods***, using 75.07 as the equivalence factor (e) in the calculation.

---

* These specifications were prepared at the 11th session of JECFA (1967) and published in NMRS 44B (1969).

POLYDEXTROSES*

SYNONYMS
Modified polydextroses; INS No. 1200

DEFINITION
Randomly bonded glucose polymers with some sorbitol end-groups, and with citric acid residues attached to the polymers by mono or diester bonds. They are obtained by melting and condensation of the ingredients and consist of approximately 90 parts d-glucose, 10 parts sorbitol and 1 part citric acid. The 1,6-glucosidic linkage predominates in the polymers but other linkages are present. The products contain small quantities of free glucose, sorbitol, levoglucosan and citric acid and may be neutralized with potassium hydroxide and/or decolourized. Polydextrose-N is neutralized Polydextrose.

C.A.S. number
68424-04-4

Assay
Content not less than 90% of polymer on the ash-free and water-free bases

DESCRIPTION
An off-white to light tan coloured solid

FUNCTIONAL USES
Bulking agent, formulation aid, humectant, stabilizer, texturizer, thickening agent.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Very soluble in water

B. Positive test for sugar
To 1 drop of 1 in 10 solution of the sample, add 4 drops of 5% phenol solution, then rapidly add 15 drops of sulfuric acid TS. A deep yellow to orange colour is produced.

C. Solubility in acetone
With vigorous swirling add 1 ml of acetone to 1 ml of a 1 in 10 solution of the sample. The solution remains clear.

D. Solubility in acetone
With vigorous swirling add 2 ml of acetone to the solution from B. A heavy, milky turbidity develops immediately.

E. Positive test for reducing sugar
To 1 ml of a 1 in 50 solution of the sample, add 4 ml of alkaline cupric citrate TS. Boil vigorously 2-4 min. Remove from heat and let precipitate (if any) settle. The supernatant is blue or blue-green.

* These specifications were prepared at the 25th session of JECFA (1981) and published in FNP 19 (1981).

Confirmation of the name may be found in United States Adopted Names (USAN) and USP Dictionary of Drug Names, USAN 1981; 1961-1980 Cumulative List. The product of commerce is designated as Polydextrose and Polydextrose-N (Type N).

PURITY TESTS

* pH
2.5 - 3.5 (for Polydextrose) (1 in 10 soln)
5.0 - 6.0 (for Polydextrose-N) 1 in 10 soln

* Water content
Not more than 4% (Karl Fischer Method)

* Sulfated ash
Not more than 0.3% (for Polydextrose)
Not more than 3.0% (for Polydextrose-N)

* Arsenic
Not more than 1 mg/kg (3 g sample, Method II)

* Heavy metals
Not more than 5 mg/kg (4 g sample, Method II)
Test 4 g of the sample as directed in the Limit Test (Method II)

* 1,6-Anhydro-D-glucose
Not more than 4% on the ash-free and the dried bases
See description under TESTS

Glucose
Not more than 4% on the ash-free and the dried bases
See description under TESTS

Sorbitol
Not more than 2% on the ash-free and the dried bases
See description under TESTS

High Molecular Weight Limit
Negative to test for polymer of molecular weight greater than 22,000
See description under TESTS

5-Hydroxymethylfurfural
Not more than 0.1% in Polydextrose
Not more than 0.05% in Polydextrose-N
See description under TESTS

TESTS

PURITY TESTS

1,6-Anhydro-D-glucose, glucose and sorbitol

Gas chromatography
- Octadecane solution: Weigh 50 mg of n-octadecane into a 100-ml volumetric flask and make up to volume with pyridine
- Monomer standard solution: Weigh accurately 50 mg reagent grade α-D-glucose, 40 mg anhydrous D-sorbitol (min. 97% purity), and 35 mg of reagent grade levoglucosan into a 100-ml volumetric flask and make up to volume with pyridine.

Silylation of monomer standard solution
Transfer 1.0 ml of Monomer Standard Solution to a screw-cap vial and add 1 ml of Octadecane Solution and 0.5 ml of N-trimethylsilylimidazole. Cap vial and immerse in an ultrasonic bath at 50° for 10 min.

Gas Chromatograph conditions
Glass column, 2.44 m by 2 mm i.d. packed with 3% OV-1 on Gas Chrom Q 100/120 mesh. Flame ionization detector. Temperatures: column 175°; injection port 210°; detector 230°. Retention times (min): levoglucosan, pyranose form 3.7; levoglucosan, furanose form (not present in standard) 4.3; n-octadecane 5.1; α-D-glucose 8.7; D-sorbitol 11.3; β-D-glucose 13.3.

1,6-Anhydro-\(\alpha\)-glucose, glucose and sorbitol (continued)

**Procedure**

Accurately weigh 20 mg of the sample into a screw-cap vial and add 1 ml of Octadecane Solution, 1 ml of pyridine, and 0.5 ml of N-trimethylsilylimidazole. Cap vial and immerse in an ultrasonic bath at 50° for 10 min. Prior to sample analysis, inject 3 \(\mu\)l of the silylated Monomer Standard Solution into the gas chromatograph. Repeat two times, then inject 3 \(\mu\)l of the sample solution. Calculate the percentage of each monomer by the formula:

\[
\frac{(R \times W)}{(R_s \times W)}
\]

in which \(W\) is the weight of the sample in mg, adjusted for ash and moisture; \(W_s\) is the weight in mg of the monomer in the Monomer Standard Solution; \(R\) is the ratio of the area of the monomer peak to the area of the octadecane peak in the sample injection; \(R_s\) is the mean ratio of the area of the monomer peak to the area of the octadecane peak in the standard injections. In the case of glucose, the peak areas for the \(\alpha\) and \(\beta\) epimers and in the case of levoglucosan the peak areas for the pyranose form and furanose form are combined.

**High molecular weight limit**

**Column Chromatography**

**Apparatus**

Chromatographic separations are conducted in a 25 mm by 250 mm glass chromatographic column equipped at the upper end with a removable 1-litre solvent reservoir, and at the lower end with a low-dead-volume fitting accommodating 1 mm i.d. tubing. Eluent is supplied to the column by gravity and pumped from the bottom of the column with a precision, low-dead-volume, reciprocating piston capable of providing flow rates between 1 ml/min and 2 ml/min reproducible to 1%. Flow pulsations are damped by installing a blanked-off 0.32 cm by 91.40 cm stainless steel tube on the pump outlet line. Connections to and from the pump are made with 1 mm i.d. silicone, Teflon, or stainless steel tubing. Eluent is conducted from the pump to a low-dead-volume recording differential refractometer having a sensitivity of at least \(4 \times 10^3\) refractive index units full scale. Noise attributable to the detector and electronics should be less than 0.5% of full scale. The detector cell is thermostated to a constant (±0.1°) temperature of about 30°.

**Buffer**

0.05 N ammonium carbonate containing 0.02% sodium azide in degassed distilled water (pH 8.8)

**Column preparation**

Swell 15 g of Sephadex G-50, or equivalent, in 300 ml of water for 3 h. Pack the chromatographic column with the gel suspension to a height of 230 mm. Equilibrate by eluting with Buffer for 24 h at 1 ml/min. Adjust height of gel to 220 mm and place a circle of filter paper on top of the gel.
High molecular weight limit (continued)

Column standardization
Adjust the refractometer so that elution of standards as described below causes a peak recorder deflection of about 50% of full scale. Weigh accurately 20 mg of Blue Dextran 2000 or equivalent and 20 mg of sodium chloride into a 10 ml volumetric flask and make up to volume with Buffer. Set the pump to give a flow rate of 2.3–2.4 ml/min. With the eluent reservoir disconnected, elute until the liquid level falls just below the top of the gel. Add a 1 ml aliquot of standard solution to the column and start the pump and recorder. After the standard solution flows into the column, add a 1 ml aliquot of Buffer two times, connect the eluent reservoir and record the monitoring curve. Mark on the curve the position $V_0$ where Blue Dextran can first be detected, $S_P$ where the peak recorder deflection for sodium chloride occurs and $M$, a point 23.0% of the distance from $V_0$ to $S_P$, corresponding to molecular weight 22,000.

Procedure
Weigh accurately 500 mg of sample into a 50-ml volumetric flask and make up to volume with Buffer. Elute a 1 ml aliquot of the sample solution using the same conditions and procedure as for Column Standardization and record the monitoring curve. Peak recorder deflection should be 50-80% of full scale. Mark the positions $V_0$ and $M$ on the curve to correspond to the standards curve. No significant response between $V_0$ and $M$ (ratio of Signal to height of main peak less than 0.02) indicates negative to test for polymer of molecular weight greater than 22,000.

5-Hydroxymethylfurfural (HMF)

Principle
HMF solutions absorb light in the ultraviolet region. The maximum absorption occurs at 283 nm and the molar extinction coefficient is 16,830 at that wavelength. The HMF concentration in polydextrase solutions is determined from the optical density at 283 nm and the application of the Beer-Lambert law.

Apparatus
- Standard laboratory equipment
- Ultraviolet spectrophotometer
- Spectrophotometer cells (quartz) 1.00 cm path length

Procedure
Accurately weigh 1.00 ± 0.01 g of the sample into a 100-ml volumetric flask and make up to volume with distilled water (for polydextrase-N 70% solution use 1.43 ± 0.01 g sample). Read the optical density of this solution against a water blank at 283 nm in a 1.00 cm quartz cell in the spectrophotometer according to the directions supplied with the instrument. Under these conditions, the % HMF in the original sample is $0.0749 \times$ optical density, on the dried basis.
5-Hydroxymethylfurfural (HMF)
(continued)

Calculation

\[ C = \frac{(100) \times (M) \times (D)}{(10) \times (L) \times (E)} \]

where C is % HMF in the original polydextrose sample; M is HMF molecular weight; D is optical density of the solution; L is the path length of the spectrophotometer cell and E is the molar extinction coefficient for HMF. The numbers 100 and 10 are factors to convert solution concentration in mg/l to % HMF in the original sample, on the dried basis.

METHOD OF ASSAY

Phenol Solution
Add 20 ml of water to 80 g of phenol

Glucose Standard Solutions
Weigh accurately 100 mg of α-D-glucose (minimum 97% purity) into a 500-ml volumetric flask and make up to volume with distilled water. Dilute five aliquots of the solution with distilled water to obtain the following concentrations of standard: 50, 40, 20, 10 and 5 μg/ml.

Standard Curve
On a daily basis, pipet 2.0 ml of each of the Glucose Standard Solutions into 4 dram acetone-free screw-cap vials. Add 0.12 ml of the phenol solution and mix gently. Uncap vials and add rapidly 5 ml of sulfuric acid TS. Immediately recap the vials and shake vigorously.

CAUTION: Rubber gloves and safety shield should be used in the sulfuric acid addition step.

Let the vials stand at room temperature for 45 min. Determine absorbances at 490 nm in a suitable spectrophotometer, using a Phenol Solution-sulfuric acid mixture as a blank in the reference cell. Assays should be run in triplicate. Plot mean absorbances versus concentrations in μg/ml.

Procedure
Weigh accurately about 250 mg of the sample into a 250-ml volumetric flask and make up to volume with distilled water. Transfer a 10 ml aliquot to a 250-ml volumetric flask and make up to volume with distilled water. Proceed as in Standard Curve. Calculate the percentage of polymer by the formula:

\[ \text{Polymer (\%)} = 1.07 \times \left( \frac{100 \times A}{S \times C} - P_1 - 1.11 P_2 \right) \]

in which A is the absorbance; S is the slope of absorbance versus glucose concentration in μg/ml obtained from the Standard Curve; C is the concentration of the sample solution in μg/ml (adjusted for ash and moisture); and P_1 and P_2 are the percentages of glucose and levoglucosan determined by the tests for monomers (see Purity tests for 1,6-Anhydro-α-glucose, glucose and sorbitol as described above).
POLYDIMETHYLSILOXANE*

SYNONYMS

Poly(dimethylsiloxane), dimethylpolysiloxane, dimethylsilicone fluid, dimethylsilicone oil; INS No. 900, EEC No. 900

DEFINITION

Polydimethylsiloxane consists of fully methylated linear siloxane polymers containing repeating units of the formula \((\text{CH}_3)_2\text{SiO}\), with trimethylsiloxy end-blocking units of the formula \((\text{CH}_3)_3\text{SiO}\). Polydimethylsiloxane in the article of commerce used as an antifoaming agent can be further specified as to total silicon.

Chemical name

Simethicone (CAS name)

C.A.S. number

8050-81-5

Structural formula

\[
\begin{align*}
\text{CH}_3 & \text{Si} & \text{O} & \text{Si} & \text{O} & \text{Si} & \text{O} & \text{CH}_3 \\
\text{CH}_3 & & & & & & & \\
\text{CH}_3 & & & & & & \text{Si} & \text{O} & \text{Si} & \text{O} & \text{Si} & \text{O} & \text{CH}_3 \\
\end{align*}
\]

The average value for \(n\) is 90 to 410.

Molecular weight

6,800 to 30,000 (average and approximate)

Assay

Silicon content not less than 37.3% and not more than 38.5% of the total.

DESCRIPTION

A clear, colourless, viscous liquid. Polydimethylsiloxane is frequently used in commerce as such, as a liquid containing 4-5% silica gel, and as an aqueous emulsion formulation containing, in addition to silica gel, emulsifiers and preservatives. The pure substance described here can be isolated by centrifuging from the silica gel-containing liquid at about 20,000 rpm.

FUNCTIONAL USES

Antifoaming agent, anticaking agent

* These specifications were prepared at the 37th session of JECFA (1990) superseding the earlier specifications published in FNP 34 (1986).
CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility
Insoluble in water and in ethanol
Soluble in carbon tetrachloride, benzene, chloroform, diethyl ether, toluene and other organic solvents.

* B. Specific gravity
\( \rho : 0.964 - 0.977 \)

C. Refractive index
\( n_0^\infty : 1.400 - 1.405 \)

D. Infrared spectrum
Passes test
See description under TESTS

PURITY TESTS

Viscosity
100 - 1500 centistokes at 25°
See description under TESTS

* Loss on drying
Not more than 0.5% (150°, 4 h)

* Arsenic
Not more than 3 mg/kg (Method II)

* Heavy metals
Not more than 10 mg/kg
See description under TESTS

TESTS

IDENTIFICATION TESTS

D. Infrared spectrum

**Apparatus**
Double-beam infrared Spectrometer. Two potassium bromide cells, 0.1 mm path length.

**Reagents**
Carbon tetrachloride
Carbon disulfide

**Procedure**
Prepare two solutions containing: (1) 10% of sample in carbon tetrachloride and (2) 2% of sample in carbon disulfide. Obtain the infrared spectrum for the sample using the carbon disulfide solution (2) from 1300 to 650 cm\(^{-1}\). The solvent in the reference cell is also changed at the appropriate wave numbers to correspond to the sample solution.

The major absorbance peaks of the sample correspond with those of the infrared spectrum in the Appendix.

PURITY TESTS

Viscosity

Apparatus

The Ubbelohde suspended level viscometer, shown in the accompanying diagram, is preferred for the determination of the viscosity.

Figure: Ubbelohde Viscometer for Polydimethylsiloxane (all dimensions are in millimeters)

For use in the range of 100 to 1,500 centistokes, a No. 3 size viscometer, having a capillary diameter of 2.00 ± 0.04 mm, is required. The viscometer should be fitted with holders which satisfy the dimensional positions of the separate tubes as shown in the diagram, and which hold the viscometer vertical. Filling lines in bulb A indicate the minimum and maximum volumes of liquid to be used for convenient operation. The volume of bulb B is approximately 5 ml.

Calibration of the Viscometer

Determine the viscosity constant C for each viscometer by using an oil of known viscosity. Charge the viscometer by tilting the instrument about 30 degrees from the vertical, with bulb A below the capillary, and then introduce enough of the sample into tube 1 to bring the level up to the lower filling line.
Calibration of the Viscometer (continued)
The level should not be above the upper filling line when the viscometer is returned to the vertical position and the sample has drained from tube 1. Charge the viscometer in such a manner that the U-tube at the bottom fills completely without trapping air.

After the viscometer has been in a constant-temperature bath long enough for the sample to reach temperature equilibrium, place a finger over tube 3 and apply suction to tube 2 until the liquid reaches the center of bulb C. Remove suction from tube 2, then remove the finger from tube 3 and place it over tube 2 until the sample drops away from the lower end of the capillary. Remove the finger from tube 2, and measure the time, to the nearest 0.1 sec. required for the meniscus to pass from the first time mark \( T_1 \) to the second \( T_2 \). In order to obtain accurate results within a reasonable time, the apparatus should be adjusted to give an elapsed time of from 80 to 100 sec.

Calculate the viscometer constant \( C \) by the equation, \( C = \frac{cs}{t_1} \), in which \( cs \) is the viscosity, in centistokes, and \( t_1 \) is the efflux time, in sec. for the standard liquid.

Test 2 g of the sample as directed in the Limit Test (Method II).

**METHOD OF ASSAY**

**Principle**

Silicon in the sample is converted to a soluble form by fusion with sodium peroxide. Soluble silicon is measured in the percent range as total silicon by atomic absorption spectrometry.

**Apparatus**

- Apparatus for fusion: a Parr-type fusion cup and nickel beaker, or equivalent (avoid use of glassware during fusion and solubilization).
- Instrument: atomic absorption spectrometer with silicon hollow cathode lamp; nitrous oxide - acetylene burner, or equivalent.

**Reagents**

- Sodium peroxide, glacial acetic acid, silica (of known purity for use as standard).

**Procedure**

1. **Solubilization**

   NOTE: Normal safe laboratory practices for Parr-type bomb fusion should be followed.

   Equivalent fusions must be performed on sample(s), reagent blank(s) and silica standards for each series of samples. For each sample weigh a portion (W) not to exceed 0.3 g into a Parr-type fusion cup (use gelatin capsules for liquid samples). Add 15.0 ± 0.5 g sodium peroxide.

   Assemble the fusion apparatus and place it in a protective ignition rack. Fill the cavity above the cap with water and keep it full during ignition to prevent the gasket from melting. Heat the bottom of the cup with a blast lamp until the cup is cherry red about 100 mm up from the bottom within 90 sec. Quench the apparatus.

---

* See General Methods (Guide to JECFA Specifications) FNP 5/Rev. 2 (1991)
METHOD OF ASSAY
(continued)

in ice water and disassemble the apparatus. Place the cup in a 500 ml nickel
beaker containing 150 to 200 ml of distilled water. Rinse any material adhering
to the inside of the assembly cup into the beaker with distilled water. Cover the
beaker with a nickel lid. When the solution is complete and cooled, remove the
cup from the beaker and rinse with distilled water into the beaker. Add 55.0 ml
of reagent grade glacial acetic acid to the beaker. Cool the solution to room
temperature and transfer to a 500 ml volumetric flask. Dilute to volume with
distilled water. The solution should contain about 100 μg silicon/ml for a sample
weight of about 0.13 g. This method performs best if the silicon concentration
of the final analysis solution is 1 to 200 μg/ml. Prepare a series of standards,
using the same fusion technique, which bracket the sample.

Adjust the spectrophotometer according to operating instructions to obtain
optimum analysis conditions for: maximum lamp output when monochromator is
at 251.6 nm, fuel and oxidant flow rate to burner (or equivalent procedures for
other vaporizing techniques). Adjust zero absorbance while aspirating the solvent
blank used to dilute the samples. Measure the absorbance of sample(s), reagent
blank and standards. Estimate the concentration (C) of silicon in the sample
solution from the standards, correcting for the reagent blank. Calculate percent
total silicon in the sample by the formula

% silicon = 0.05 (C/W) in which C is the silicon concentration of the sample
solution, in μg per ml, and W is the weight of sample taken in g.

APPENDIX

* Infrared spectrum: Polydimethylsiloxane

* Infrared spectrum through the courtesy of the International Organization of the Flavour Industry (IOFI), Geneva,
Switzerland, and of the SADTLER RESEARCH LABORATORIES Inc., Philadelphia.
POLYETHYLENE GLYCOLS*

SYNONYMS
PEG, Macrogol; INS No. 1521

DEFINITION
Polyethylene glycols are addition polymers of ethylene oxide and water usually designated by a number roughly corresponding to the molecular weight.

Chemical name
α-Hydro-ω-hydroxypoly (oxy-1,2-ethanediol)

C.A.S. number
25322-68-3

Chemical formula
(C₂H₄O)ₓ·H₂O

Structural formula
HOCH₂ - (CH₂ - O - CH₂)ₓ - CH₂OH

Molecular weight
200-9500

DESCRIPTION
PEG's below 700 molecular weight occur as clear to slightly hazy, colourless, slightly hygroscopic liquids with a slight characteristic odour. PEG's between 700-900 are semi-solid. PEG's over 1000 molecular weight are creamy white waxy solids, flakes, or free-flowing powders.

FUNCTIONAL USES
Carrier solvent, excipient

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Polyethylene glycols having a molecular weight of 1000 or above are freely soluble in water. The polyethylene glycols are soluble in many organic solvents, including aliphatic ketones and alcohols, chloroform, glycol ethers, esters, and aromatic hydrocarbons; they are insoluble in ether and in most aliphatic hydrocarbons. As their molecular weight increases, water solubility and solubility in organic solvents decrease.

B. Molecular weight
PEG's having molecular weight below 1000: not less than 95.0% and not more than 105.0% of the declared value.

PEG's having molecular weight between 1000 and 7000: not less than 90.0% and not more than 110.0% of the declared value.

PEG's having molecular weight above 7000: not less than 87.5% and not more than 112.5% of the declared value.

See description under TESTS

* These specifications were prepared at the 31st session of JECFA (1987) and published in FNP 38 (1988).

IDENTIFICATION TESTS (continued)

C. Viscosity at 100° (± 0.3°), Centistokes (See description under TESTS)

<table>
<thead>
<tr>
<th>Average Mol weight</th>
<th>Viscosity Range (centistokes)</th>
<th>Average Mol weight</th>
<th>Viscosity Range (centistokes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>4.1 - 4.8</td>
<td>2400</td>
<td>49 - 65</td>
</tr>
<tr>
<td>300</td>
<td>5.4 - 6.4</td>
<td>2500</td>
<td>51 - 70</td>
</tr>
<tr>
<td>400</td>
<td>6.8 - 8.0</td>
<td>2600</td>
<td>54 - 74</td>
</tr>
<tr>
<td>500</td>
<td>8.3 - 9.6</td>
<td>2700</td>
<td>57 - 78</td>
</tr>
<tr>
<td>600</td>
<td>9.9 - 11.3</td>
<td>2800</td>
<td>60 - 83</td>
</tr>
<tr>
<td>700</td>
<td>11.5 - 13.0</td>
<td>2900</td>
<td>64 - 88</td>
</tr>
<tr>
<td>800</td>
<td>12.5 - 14.5</td>
<td>3000</td>
<td>67 - 93</td>
</tr>
<tr>
<td>900</td>
<td>15.0 - 17.0</td>
<td>3250</td>
<td>73 - 105</td>
</tr>
<tr>
<td>1000</td>
<td>16.0 - 19.0</td>
<td>3350</td>
<td>76 - 110</td>
</tr>
<tr>
<td>1100</td>
<td>18.0 - 22.0</td>
<td>3500</td>
<td>87 - 123</td>
</tr>
<tr>
<td>1200</td>
<td>20.0 - 24.5</td>
<td>3750</td>
<td>99 - 140</td>
</tr>
<tr>
<td>1300</td>
<td>22.0 - 27.0</td>
<td>4000</td>
<td>110 - 158</td>
</tr>
<tr>
<td>1400</td>
<td>24.0 - 30.0</td>
<td>4250</td>
<td>123 - 177</td>
</tr>
<tr>
<td>1450</td>
<td>25.0 - 32.0</td>
<td>4500</td>
<td>140 - 200</td>
</tr>
<tr>
<td>1500</td>
<td>26.0 - 33.0</td>
<td>4750</td>
<td>150 - 228</td>
</tr>
<tr>
<td>1600</td>
<td>28.0 - 36.0</td>
<td>5000</td>
<td>170 - 250</td>
</tr>
<tr>
<td>1700</td>
<td>31.0 - 39.0</td>
<td>5500</td>
<td>206 - 315</td>
</tr>
<tr>
<td>1800</td>
<td>33.0 - 42.0</td>
<td>6000</td>
<td>250 - 390</td>
</tr>
<tr>
<td>1900</td>
<td>35.0 - 45.0</td>
<td>6500</td>
<td>295 - 480</td>
</tr>
<tr>
<td>2000</td>
<td>38.0 - 49.0</td>
<td>7000</td>
<td>350 - 590</td>
</tr>
<tr>
<td>2100</td>
<td>40.0 - 53.0</td>
<td>7500</td>
<td>405 - 735</td>
</tr>
<tr>
<td>2200</td>
<td>43.0 - 56.0</td>
<td>8000</td>
<td>470 - 900</td>
</tr>
<tr>
<td>2300</td>
<td>46.0 - 60.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For PEG's not listed in the table, calculate the limits by interpolation.

See description under TESTS

PURITY TESTS

* **pH**
  4.5 - 7.5 (1 in 20 soln)

* **Sulfated ash**
  Not more than 0.1% w/w
  Test 5 g of the sample as directed in the General Methods

* **Arsenic**
  Not more than 3 mg/kg (Method II)

* **Heavy metals**
  Not more than 10 mg/kg
  Test 2 g of the sample as directed in the General Methods

**Acidity**
Not more than 0.05% w/w (as acetic acid).
See description under TESTS

**1,4-Dioxane**
Not more than 10 mg/kg.
See description under TESTS

**Ethylene oxide**
Not more than 0.02%
See description under TESTS

**Ethylene glycol and diethylene glycol**
Total not more than 0.25% w/w individually or in combination.
See description under TESTS

IDENTIFICATION TESTS

B. Molecular weight

**Chemical method**

**Phthalic anhydride solution**
Place 49 g of phthalic anhydride in an amber bottle and dissolve it in 300 ml of pyridine that has been freshly distilled over phthalic anhydride. Shake the bottle vigorously until solution is effected, and allow to stand overnight before using.

**Sample preparation for liquid polyethylene glycols**
Carefully introduce 25 ml of the Phthalic anhydride solution into a clean, dry, heat-resistant pressure bottle. To the bottle add an accurately weighed amount of the sample equivalent to its expected molecular weight divided by 160. (Thus, a sample of about 1.3 g would be taken for PEG 200, or about 3.8 g for PEG 600). Stopper the bottle, and wrap it securely in a fabric bag.

**Sample preparation for solid polyethylene glycols**
Carefully introduce 25 ml of the Phthalic anhydride solution into a clean, dry, heat-resistant pressure bottle. To the bottle add an accurately weighed amount of the sample, previously melted, equivalent to its expected molecular weight divided by 160; because of limited solubility, however, do not use more than 25 g of any sample. Add 25 ml of pyridine, freshly distilled over phthalic anhydride, swirl to effect solution, stopper the bottle, and wrap it securely in a fabric bag.

**Procedure**
Immerse the sample bottle in a water bath, maintained between 96° and 100°, to the same depth as that of the mixture in the bottle. Heat it in the water bath for 30 to 60 min., then remove the bottle from the bath and allow it to cool to room temperature. Uncap the bottle carefully to release any pressure, remove the bottle from the fabric bag, add 5 drops of a 1 in 100 solution of phenolphthalein in pyridine, and titrate with 0.5 N sodium hydroxide to the first pink colour that persists for 15 sec, recording the volume, in ml of 0.5 N sodium hydroxide required as S. Perform a blank determination on 25 ml of the Phthalic anhydride solution plus any additional pyridine added to the sample bottle, and record the volume, in ml of 0.5 N sodium hydroxide required as B. Calculate the molecular weight of the sample by the formula:

\[
\text{Molecular weight} = \frac{2000W}{(B-S)N}
\]

where

- \( W \) = weight of the sample in g
- \( B \) = volume of 0.5 N NaOH consumed by the blank, in ml
- \( S \) = volume of 0.5 N NaOH consumed by the sample, in ml
- \( N \) = exact normality of NaOH
B. Molecular weight  

Alternative Tentative Method:  

**Size exclusion chromatography (Gel permeation chromatography)**

1. Polyethylene glycols having nominal molecular weight below 1000

**Apparatus**

Use a suitable HPLC chromatograph equipped with a differential refractometer fitted with a 0.60 m x 7.7 mm (inside diameter) column packed with PL gel 10 μm 50 Å with tetrahydrofuran as the mobile phase.

**Operating Conditions**

The operating parameters may vary depending upon the particular instrument used but a suitable chromatogram may be obtained using the following conditions:

- Mobile phase flow rate: 1 ml/min
- Pressure: 35 bars
- Injected volume: 20 μl of a 1% (w/v) solution
- Temperature of detection: 25° ± 0.1°

The procedure allows the identification of PEG by comparison with a standard and to examine mixtures of PEG.

2. Polyethylene glycols having a nominal molecular weight of 1000 and higher

The determination is carried out with the same procedure but with a mobile phase: Tetrahydrofuran/n-heptane (80/20).

The elution volumes of PEG are approximately as follows depending on the particular instrument and operating conditions.

<table>
<thead>
<tr>
<th>Molecular Mass (g/mol)</th>
<th>35 000</th>
<th>10 000</th>
<th>6 000</th>
<th>4 000</th>
<th>2 000</th>
<th>1 000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elution Volume ml</td>
<td>21.2</td>
<td>22.8</td>
<td>24.2</td>
<td>25.1</td>
<td>26.8</td>
<td>28.4</td>
</tr>
</tbody>
</table>

C. Viscosity

**Method for Determination of Viscosity**

**Apparatus**

The Ubbelohde suspended level viscometer, shown on the following page is efficient for the determination of viscosity in the case of polyethylene glycols. This apparatus is preferred for the determination of viscosity.

For use in the range of 300 to 600 centistrokes, a number 3 size viscometer, having a capillary diameter of 2.00 ± 0.04 mm, is required. The viscometer should be fitted with holders which satisfy the dimensional positions of the separate tubes as shown in the diagram, and which hold the viscometer vertical. Filling lines in bulb A indicate the minimum and maximum volumes of liquid to be used for convenient operation. The volume of bulb B is approximately 5 ml.
C. Viscosity

Method for Determination of Viscosity (continued)

Calibration of the Viscometer

Determine the viscosity constant (C) for each viscometer by using an oil of known viscosity. Charge the viscometer by tilting the instrument about 30 degrees from the vertical, with bulb A below the capillary, and then introduce enough of the sample into tube 1 to bring the level up to the lower filling line. The level should not be above the upper filling line when the viscometer is returned to the vertical position and the sample has drained from tube 1. Charge the viscometer in such a manner that the U-tube at the bottom fills completely without trapping air. After the viscometer has been in a constant temperature bath long enough for the sample to reach temperature equilibrium, place a finger over tube 3 and apply suction to tube 2 until the liquid reaches the center of bulb C. Remove suction from tube 2, then remove the finger from tube 3 and place it over tube 2 until the sample drops away from the lower end of the capillary. Remove the finger from tube 2, and measure the time, to the nearest 0.1 sec., required for the meniscus to pass from the first timing mark (T₁) to the second (T₂). In order to obtain accurate results within a reasonable time, the apparatus should be adjusted to give an elapsed time of from 80 to 100 sec.

Calculate the viscometer constant C by the equation \( C = \frac{cSt}{t₁} \), in which cSt is the viscosity, in centistokes, and t₁ is the efflux time, in sec, for the standard liquid.

Determine the viscosity of the sample, maintaining the constant temperature bath at 100° ± 0.3° and using a capillary viscometer having a flow time of at least 200 sec for the PEG being tested. The viscosity must be within the limits specified in the table, or interpolated from the table.

![Figure. Ubbelohde Viscometer](image)

(All dimensions are in mm)
PURITY TESTS

**Acidity**
Transfer 6 g of the sample into a 250-ml Erlenmeyer flask, add phenolphthalein TS and 50 ml neutral ethanol and titrate with 0.1 N ethanolic potassium hydroxide to a pink end-point that persists for at least 15 sec. Not more than 0.5 ml is required.

* **1,4-Dioxane**
  Proceed as directed in the General Methods using Gas chromatography. See also Headspace gas chromatography method described in the next page (alternative tentative method).

**Ethylene oxide**

**Titration method**
Mix one part of redistilled morpholine with nine parts of anhydrous methanol.

**Mixed indicator**
Weigh 0.050 g of 4,4'-bis-(amino-l-naphthylazo-2,2'-stilbenedisulfonic acid) and 0.010 g of brilliant yellow into a 60-ml vial. Pipet 1.5 ml of 0.1 N sodium hydroxide into the vial, and mix. Add 3.5 ml of water, and mix. Transfer the mixture to a storage bottle with the aid of 45 ml of methanol as a rinse, and mix.

**Standard methanolic hydrochloric acid**
Mix 8.5 ml of hydrochloric acid and 1000 ml of anhydrous methanol, and standardize by titrating 9.00 ml with 0.1 N sodium hydroxide TS to a phenolphthalein end-point. Restandardize if this solution is used more than 48 h after standardization.

**Procedure**
Place 50 ml of anhydrous methanol into a 250-ml conical flask. Add 4 to 6 drops of Mixed indicator, and titrate with Standard methanolic hydrochloric acid to a clear blue colour.

Transfer to the flask about 25 g of the sample, accurately weighed, to provide the specimen blank, swirl to effect complete solution. Titrate with Standard methanolic hydrochloric acid to a clear blue colour, approaching the end-point carefully using small increments of titrant. Place 50 ml of Morpholine solution into a heat-resistant pressure bottle, and place an equal amount into a similar bottle to provide the reagent blank. To the first bottle add about 25 g of the sample, accurately weighed, and swirl to effect complete solution. Wrap the bottles securely in a cloth bag, and place them close together in a water bath maintained at 98 ± 2° for 30 min., keeping the water level in the bath just above the liquid level in the bottles. Remove the bottles from the bath, and allow them to cool in air to room temperature. When the bottles have cooled, loosen the wrappers, uncap to release any pressure, and remove the wrappers. Slowly add 20 ml of acetic anhydride to each bottle, and swirl to effect complete solution. Allow to stand at room temperature for 15 min. If the bottles are still warm, cool them to room temperature. To each bottle add 4 to 6 drops of Mixed indicator and titrate with Standard methanolic hydrochloric acid to a clear blue colour, adding very small increments when approaching the end-point.

Calculate the percentage of ethylene oxide by the formula:

$$4.41N(A - B)/W_1 - C/W_2$$

in which $N$ is the normality of the Standard methanolic hydrochloric acid, $A$, $B$, and $C$ are the volumes, in ml, required in the titration of the specimen, the reagent blank, and the specimen blank, respectively and $W_1$ and $W_2$ are the weights, in g, of the sample taken for the reaction and the specimen blank, respectively. The limit is 0.02% w/w.

* **Headspace gas chromatography**

Alternative tentative method for 1,4-Dioxane and Ethylene oxide

**Principle**

After addition of water to the sample, ethylene oxide and 1,4-dioxane are analysed by headspace gas chromatography.

**Standard Solutions**

- **1,4-Dioxane Standard Stock Solution**

  Standard solutions of 1,4-dioxane in water are prepared by weighing out about 1.00 g 1,4-dioxane/100 ml distilled water (stock solution) with successive dilutions. Two standard solutions of about 20 and 100 µg 1,4-dioxane/ml water are used.

- **Ethylene Oxide (EO) Standard Stock Solution**

  In a 25 ml multidose injection vial, introduce 25 ml of distilled water. Close the vial with septum and cap with a gas tight syringe. Introduce slowly into the liquid 20 ml of ethylene oxide gas (about 40 mg). Determine the exact amount of ethylene oxide added by weighing the vial before and after the introduction of the gas (stock solution).

  Prepare two working standard solutions by dilution with about 2 and 10 µg ethylene oxide per ml of water by successive dilutions.

**Apparatus**

Use a suitable gas chromatograph equipped with a flame-ionization detector (FID) containing a 30 m fused silica capillary column coated with dimethylpolysiloxane, internal diameter 0.25 mm, film thickness 1.0 µm.

**Operating Conditions**

The operating conditions may vary depending upon the particular instrument used, but the suitable chromatogram can be obtained using the following conditions:

**Headspace Sampler Setting**

- Temperature equilibration time: 45 min
- Temperature: 70°
- Transfer line temperature: 150°
- Pressurization time: 30 sec
- Injection time: 6 sec
- Analysis time: 45 min

Headspace gas Chromatography (continued)

GC - Conditions
- Temperature: Column, 50° (5 min isothermal)
  50° to 180° at 5%/min.
- Detector (FID), 250°
- Carrier gas: Helium, 1 ml/min
- Carrier pressure: 0.7 bar
- Split ratio: 40 : 1
- Hydrogen and air: for FID

Sample Preparations
Transfer about 1 g of the sample accurately weighed (± 0.1 mg) in a headspace vial and add 1 ml of distilled water. Seal the vial and insert it into the headspace analyser for equilibration 45 min at 70°.

Prepare in the same conditions 2 vials with each 1 g sample accurately weighed (± 0.1 mg) and 1 ml of standard stock solutions of 1,4-dioxane and EO.

Standard Solutions for Work
Two standard solutions A and B (for spiking) are prepared as follows:

A. 1 ml EO stock solution with ca. 200 mg EO/100 ml + 2 ml 1,4-dioxane stock solution with ca. 1000 mg dioxane/100 ml + water make up to 100 ml. This solution will be diluted 1 : 10 with water to yield a concentration of about 2 µg EO/ml and 20 µg 1,4-dioxane/ml.

B. 1 ml EO stock solution with ca. 500 mg EO/100 ml + 5 ml 1,4 dioxane stock solution with ca. 1000 mg dioxane/100 ml + water make up to 100 ml. This solution will be diluted 2 : 10 with water to yield a concentration of about 10 µg EO/ml and 100 µg 1,4-dioxane/ml.

Calculation
The concentration of compound i can be calculated by the following formula:

\[
\mu g \text{ compound } i/g = \frac{W_i \times A_i}{A_i}
\]

Where:

\[
\mu g \text{ compound } i/g = \text{ Mass portion of 1,4-dioxane or EO in the sample } \frac{[\mu g/g]}{W_i}
\]
\[
W_i = \text{ Mass of spiked compound } i \text{ normalized to } 1 \text{ g sample } \frac{[\mu g/g]}{A_i}
\]
\[
A_i = \text{ Peak area of compound } i \text{ in the sample, normalized to } 1 \text{ g sample } \frac{}{A_i}
\]
\[
A_i = \text{ Peak area of compound } i \text{ in the spiked sample, normalized to } 1 \text{ g sample } \frac{}{A_i}
\]
Ethylene glycol and diethylene glycol

Polyethylene glycols having molecular weights below 450

Apparatus
Use a suitable gas chromatograph equipped with a hydrogen flame ionization detector, containing a 1.5 m x 3 mm (inside diameter) stainless steel column packed with sorbitol 12%, by weight, on 60/80 mesh non-acid-washed diatomaceous earth (Chromosorb W, or equivalent).

Operating conditions
The operating parameters may vary, depending upon the particular instrument used, but a suitable chromatogram may be obtained using the following conditions: Column temperature: 165°; Inlet temperature: 260°; Carrier gas: nitrogen (or suitable inert gas); flowing at a rate of 70 ml per min; Recorder: -0.5 to 1.05 mv, full span, 1 sec. full response time; Hydrogen and air flow to burner, optimize to give maximum sensitivity.

Standard solutions
Prepare chromatographic standards by dissolving accurately weighed amounts of commercial ethylene glycol and diethylene glycol, previously purified by distillation if necessary, in water. Suitable concentrations range from 1 to 6 mg of each glycol per ml.

Sample preparation
Transfer about 4 g of the sample, accurately weighed, into a 10-ml volumetric flask, dilute to volume with water and mix.

Procedure
Inject a 2 μl portion of each of the Standard solutions into the chromatograph, and obtain the chromatogram for each solution. Under the stated conditions, the elution time is approximately 2 min for ethylene glycol and 6.5 min for diethylene glycol. Measure the peak heights, and record the values as follows:

\[ A = \text{height, in mm, of the ethylene glycol peak;} \]
\[ B = \text{weight, in mg, of ethylene glycol per ml of the Standard solution;} \]
\[ C = \text{height, in mm, of the diethylene glycol peak; and} \]
\[ D = \text{weight, in mg, of diethylene glycol per ml of the Standard solution} \]

Similarly, inject a 2 μl portion of the Sample preparation into the chromatograph, and obtain the chromatogram, recording the height of the ethylene glycol peak as E and that of the diethylene glycol peak as F.

Calculation
Calculate the percent of ethylene glycol in the sample by the formula: \((E \times B) / A \times \text{sample weight in g.}\)

Calculate the percent of diethylene glycol in the sample by the formula: \((F \times D) / C \times \text{sample weight in g.}\)
Polyethylene glycols having molecular weights of 450 or higher

**Sample preparation**
Dissolve 50 g of the sample in 75 ml of diphenyl ether in a 250-ml distillation flask. Slowly distil at a pressure of 1-2 mm of mercury into a receiver graduated to 100 ml in 1-ml subdivisions, until 25 ml of distillate has been collected. Add 25 ml of water to the distillate, shake vigorously, and allow the layers to separate. Cool the container in an ice bath to solidify the diphenyl ether and to facilitate its removal. Filter the water layer through filter paper into a 50-ml glass-stoppered graduated cylinder, and to the filtrate add an equal volume of freshly distilled acetonitrile.

**Standard preparation**
Transfer 50 mg of diethylene glycol to a 25-ml volumetric flask, dilute to volume with a 1:1 mixture of freshly distilled acetonitrile and water, and mix.

**Procedure**
Transfer 10 ml of each of the Sample preparation and of the Standard preparation into separate 50-ml flasks each containing 15 ml of ceric ammonium nitrate TS, and mix. Within 2 to 5 min., determine the absorbance of each solution in a 1-cm cell at 450 nm, with a suitable spectrophotometer, using a blank, consisting of 15 ml of ceric ammonium nitrate TS and 10 ml of a 1:1 mixture of acetonitrile and water. The absorbance of the solution from the Sample preparation does not exceed that from the Standard preparation.

**Alternative tentative method for the determination of mono and diethylene glycol**

**Test solution**
In a 100 ml volumetric flask weigh 5.0 g of the substance to be examined, dissolve in acetone and dilute to 100.0 ml with acetone.

**Reference solution**
In a 100 ml volumetric flask weigh 100 mg of monoethylene glycol and 500 mg of diethylene glycol. Dilute to 100.0 ml with acetone. Dilute 1.0 ml of this solution to 10.0 ml with acetone.

**Procedure**
Gas chromatographic procedure may be carried out using:

- Glass column 1.8 m and 2 mm internal diameter, packed with diatomaceous earth for gas chromatography, washed and silanised (Chromosorb G.AW.DMCS 100-125 mesh is suitable), impregnated with 4% (m/m) of polyethylene glycol 20.000 (Carbowax 20 M is suitable).
Alternative tentative method for the determination of mono- and diethylene glycol

Procedure (continued)

- Nitrogen as carrier gas with a flow rate of 30 ml/min,
- Flame ionization detector

If necessary, preconditioning of the column may be carried out by heating at 200° for about 15 h.

Adjust the initial temperature to obtain a retention time of 14 min to 16 min for diethylene glycol. Lower the temperature of the column to 140°. Inject the solutions and raise the temperature of the column to 170°, at a rate of 2° per minute. Maintain the temperature of the injection port at 250° and that of the detector at 250°. Inject 2.0 μl of the test solution and of the reference solution. Verify after five injections the repeatability of the response.

Calculation

Measure the peak areas of the mono and diethylene glycol in the test and reference solutions. Calculate the concentration of the mono and diethylene glycol in the test solution from the peak areas.
POLYGLYCEROL ESTERS OF FATTY ACIDS*

SYNONYMS
Polyglycerol fatty acid esters, glycerin fatty acid esters
INS No. 475, EEC No. E475

DEFINITION
Polyglycerol Esters of Fatty Acids are mixed partial esters formed by reacting polymerized glycerols with edible fats, oils, or fatty acids. Minor amounts of mono-, di-, and triglycerides, free glycerol and polyglycerols, free fatty acids, and sodium salts of fatty acids may be present. The polyglycerols vary in degree of polymerization, which is specified by a number (such as tri-) that is related to the average number of glycerol residues per polyglycerol molecule. A specified polyglycerol consists of a distribution of molecular species characteristic of its nominal degree of polymerization. By varying the proportions as well as the nature of the fats or fatty acids to be reacted with the polyglycerols, a large and diverse class of products may be obtained.

The article of commerce may be further specified as to saponification value, solidification point of the free fatty acids, iodine value, hydroxyl value and ash content.

Structural formula

OR₂

R₁O—(CH₂—CH—CH₃)ₙ—R₃

where the average value of n is about 3 and R₁, R₂ and R₃ each may be a fatty acid moiety or hydrogen

DESCRIPTION
Light yellow to amber, oily to very viscous liquids; light tan to medium brown, plastic or soft solids; and light tan to brown, hard, waxy solids

FUNCTIONAL USE
Emulsifier

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
The esters range from very hydrophilic to very lipophilic, but as a class tend to be dispersible in water and soluble in organic solvents and oils

** B. Positive test for fatty acids
Passes test

C. Positive test for glycerol and polyglycerols
Passes test
See description under TESTS

* These specifications were prepared at the 35th session of JECFA (1989) superseding the earlier specifications published in FNP 28 (1983).

PURITY TESTS

* Arsenic

Not more than 3 mg/kg (Method II)

* Heavy metals

Not more than 10 mg/kg
Test 2 g of the sample as directed in the Heavy Metals Limit Test (Method II)

* Acids

Acids other than fatty acids shall not be detectable

* Polyglycerols

The polyglycerol moiety shall be composed of not less than 70% of di-, tri- and tetragelycerols and shall contain not more than 10% of polyglycerols equal to or higher than heptaglycerol.

TESTS

IDENTIFICATION TESTS

* C. Positive test for glycerol and polyglycerols

Spot 5 to 20 μl of the aqueous layer obtained in A under Identification tests for functional groups* alongside control spots of glycerol on paper such as Whatman No. 3 and develop using descending chromatography for 36 h with isopropanol: water (90:10). The glycerol spot moves 40 cm and the polyglycerols are revealed in succession below that for glycerol when the paper is sprayed with either permanganate in acetone or ammoniacal silver nitrate.

POLYGLYCEROL ESTERS OF INTERESTERIFIED RICINOLEIC ACID

SYNONYMS
Glyceran esters of condensed castor oil fatty acids; poly-glycerol esters of polycondensed fatty acids from castor oil
INS No. 476

DEFINITION
Polyglycerol Esters of Intesterified Ricinoleic Acid are prepared by the esterification of polyglycerol with condensed castor oil fatty acids

The article of commerce may be specified further as to saponification value, solidification point of the free fatty acids, iodine value, acid value, hydroxyl value, ash content and Refractive index.

Structural formula
The major components have the general structure:

\[
\text{OR}_2
\]

\[
\text{R}_1\text{O}-(\text{CH}_2-\text{CH}-\text{CH}_2\text{O})_n-\text{R}_3
\]

where the average value of n is about 3 and R₁, R₂, and R₃ each may be hydrogen or a linear condensation polymer of ricinoleic acid with itself thus:

\[
(\text{CH}_3)_2\text{CH}_3
\]

\[
\text{H}-(\text{O}--\text{CH}-\text{CH}_2-\text{CH}==\text{CH}-(\text{CH}_3\text{C})_n-\text{OH}
\]

where the average value of m is between 5 and 8

DESCRIPTION
Polyglycerol esters of intesterified ricinoleic acid are highly viscous liquids and conform to the following specifications

FUNCTIONAL USE
Emulsifier

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Insoluble in water and in ethanol; soluble in ether, hydrocarbons and halogenated hydrocarbons

** B. Positive test for fatty acids
Passes test

** C. Positive test for ricinoleic acid
The fatty acids liberated in A under Identification test for functional groups** should have a hydroxyl value corresponding to that for castor oil fatty acids (about 150 to 170)

D. Positive test for glycerol and poly-glycerol
Passes test
See description under TESTS

** These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

PURITY TESTS

* Arsenic Not more than 3 mg/kg (Method II)

* Heavy metals Not more than 10 mg/kg

Test 2 g of the sample as directed in Method II under the Limit test for Heavy Metals

* Polyglycerols

The polyglycerol moiety shall be composed of not less than 75% of di-, tri- and tetracylglycerols and shall contain not more than 10% of polyglycerols equal to or higher than heptaglycerol.

IDENTIFICATION TESTS

* C. Positive test for glycerol and polyglycerols

Spot 5 to 20 μl of the aqueous layer obtained in A under Identification test for functional groups* alongside control spots of glycerol on paper such as Whatman No. 3 and develop using descending chromatography for 36 h with isopropanol : water, 90:10. The glycerol spot moves 40 cm and the polyglycerols are revealed in succession below that for glycerol when the paper is sprayed with either permanganate in acetone or ammoniacal silver nitrate.

POLYOXYETHYLENE (20) SORBITAN MONOLAURATE*

SYNONYMS
Polysorbate 20
INS No. 432, EEC No. E432

DEFINITION
Polyoxyethylene (20) Sorbitan Monolaurate consists of a mixture of the partial esters of sorbitol and its mono- and dianhydrides (which have an acid value below 7 and a water content below 0.2%) with edible commercial lauric acid and condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides.

Structural formula and Approximate composition
A nominal formula for polyoxylethylene (20) sorbitan monolaurate is as follows:

```
CH₂
HCO(C₂H₄O)₉H
H(OC₂H₄)OCH
CH
HCO(C₂H₄O)₂H
CH₂O(C₂H₄O)₉OCR
```

where \( w + x + y + z \approx 20 \) and \( RCO- \) is the fatty acid moiety

Assay
Not less than 70.0 and not more than 74.0% of oxyethylene groups, equivalent to not less than 97.3 and not more than 103.0% of polyoxyethylene (20) sorbitan monolaurate calculated on the anhydrous basis.

DESCRIPTION
Polyoxyethylene (20) sorbitan monolaurate is a lemon to amber coloured oily liquid at 25°C, with a faint characteristic odour, and a warm, somewhat bitter taste

FUNCTIONAL USES
Emulsifier, dispersing agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Soluble in water, ethanol, methanol, ethyl acetate and dioxane. Insoluble in mineral oil and petroleum ether

B. Infrared absorption spectrum
Characteristic of a partial fatty acid ester of a polyoxylethylated polyol

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

IDENTIFICATION TESTS (continued)

C. Colour reaction

Passes test
See description under TESTS

D. Positive test for fatty acids

Passes test
See description under TESTS

* E. Saponification

100 g of the sample shall yield upon alkaline saponification approximately 16 g of fatty acids and 81 g of polyol

PURITY TESTS

* Water

Not more than 3% (Karl Fischer Method)

* Sulfated ash

Not more than 0.25%
5 g of the sample meets the requirements of the Limit Test for Sulfated Ash

* Arsenic

Not more than 3 mg/kg (Method II)

* Heavy metals

Not more than 10 mg/kg
Test 2 g of the sample as directed in Method II under the Limit test for Heavy Metals

* Acid value

Not more than 2

* Saponification value

Not less than 40 and not more than 50

* Hydroxyl value

Not less than 96 and not more than 108

TESTS

IDENTIFICATION TESTS

C. Colour reaction

To 5 ml of a 5% (w/v) aqueous solution of the sample add 10 ml of ammonium cobaltithiocyanate solution and 5 ml of chloroform, shake well and allow to separate; a blue colour is produced in the chloroform layer. (Ammonium cobaltithiocyanate solution: 37.5 g of cobalt nitrate and 150 g of ammonium thiocyanate made up to 100 ml with water - freshly prepared).

D. Positive test for fatty acids

Test for fatty acids: To 5 ml of a solution of polyoxyethylene (20) sorbitan (20) sorbitan monolaurate (1 in 20) add 5 ml sodium hydroxide TS. Boil for a few min, cool, and acidify with dilute hydrochloric acid. The solution is strongly opalescent, owing to the fatty acids liberated.

METHOD OF ASSAY

Determine the content of Oxyethylene groups as directed in the General Methods*

POLYOXYETHYLENE (20) SORBITAN MONOOLEATE

SYNONYMS
Polysorbate 80
INS No. 433, EEC No. E433

DEFINITION
Polyoxyethylene (20) Sorbitan Monoooleate consists of a mixture of the partial
esters of sorbitol and its mono- and dianhydrides (which have an acid value below
7.5 and a water content below 0.2%) with edible commercial oleic acid and
condensed with approximately 20 moles of ethylene oxide per mole of sorbitol
and its anhydrides.

C.A.S. number
9005-65-6

Structural formula
A nominal formula for polyoxyethylene (20) sorbitan monoooleate is
approximately as follows:

\[
\begin{align*}
\text{CH}_2 & - \text{HCO} (\text{C}_2 \text{H}_4 \text{O})_w \text{H} \\
\text{HCO} (\text{C}_2 \text{H}_4 \text{O})_x & \text{OCH} \quad \text{O} \\
\text{H} (\text{OC}_2 \text{H}_4)_y & \text{OCH} \\
\text{HCO} (\text{C}_2 \text{H}_4 \text{O})_z & \text{H} \\
\text{CH}_2 \text{O} (\text{C}_2 \text{H}_4 \text{O})_w \text{OCR}
\end{align*}
\]

where \( w + x + y + z \approx \text{approx. 20} \) and \( RCO- \) is the fatty acid moiety

Assay
Not less than 65.0 and not more than 69.5% of oxyethylene groups, equivalent
to not less than 96.5 and not more than 103.5% of polyoxyethylene (20) sorbitan
monoooleate, calculated on the anhydrous basis.

DESCRIPTION
Polyoxyethylene (20) Sorbitan Monoooleate is a lemon to amber coloured oily
liquid at 25°C, with a faint characteristic odour, and a warm, somewhat bitter taste.

FUNCTIONAL USES
Emulsifier, dispersing agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Soluble in water, ethanol, methanol, ethyl acetate and toluene. Insoluble in
mineral oil and petroleum ether

B. Infrared absorption
spectrum
Characteristic of a partial fatty acid ester of a polyoxyethylated polyol

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

IDENTIFICATION TESTS (continued)

C. Colour reaction
   Passes test
   Proceed as directed in the TESTS for Polyoxyethylene (20) Sorbitan Monolaurate.

D. Positive test for fatty acids
   Passes test
   Proceed as directed in the TESTS for Polyoxyethylene (20) Sorbitan Monolaurate

E. Positive test for unsaturation
   To a solution of the sample (1 in 20) add bromine TS dropwise. The bromine is decolourized

F. Gelatinization
   A mixture of 60 parts by volume of the sample and 40 parts of water yields a gelatinous mass at or below room temperature

G. Saponification
   100 g of the sample yields upon alkaline saponification approximately 23 g of fatty acids and 75 g of polyols

PURITY TESTS

* Water
   Not more than 3% (Karl Fischer Method)

* Sulfated ash
   Not more than 0.25%
   5 g of the sample meets the requirements of the Limit Test for Sulfated Ash

* Arsenic
   Not more than 3 mg/kg (Method II)

* Heavy metals
   Not more than 10 mg/kg
   Test 2 g of the sample as directed in Method II under the Limit Test for Heavy Metals (Solution A)

* Acid value
   Not more than 2

* Saponification value
   Not less than 45 and not more than 55

* Hydroxyl value
   Not less than 65 and not more than 80

METHOD OF ASSAY

Determine the content of Oxyethylene groups as directed in the General Methods*

POLYOXYETHYLENE (20) SORBITAN MONopalmitate

SYNONYMS

Polysorbate 40  
INS No. 434, EEC No. E434

DEFINITION

Polyoxyethylene (20) Sorbitan Monopalmitate consists of a mixture of the partial esters of sorbitol and its mono- and di-anhydrides (which have an acid value below 7.5 and a water content below 0.2%) with edible commercial palmitic acid condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides.

C.A.S. number

9005-66-7

Structural formula

A nominal formula for polyoxyethylene (20) sorbitan monopalmitate is approximately as follows:

\[
\begin{align*}
&\text{CH}_2 \\
&D\text{HCO}((\text{C}_2\text{H}_4\text{O})_w\text{H} \\
&D\text{H(O}_2\text{C}_2\text{H}_4_{x+y+z}\text{OCH}_2\text{O} \\
&D\text{HCO}((\text{C}_2\text{H}_4\text{O})_y\text{H} \\
&\text{CH}_2\text{O(C}_2\text{H}_4\text{O})_z\text{OCR}
\end{align*}
\]

where \( w + x + y + z \approx 20 \) and \( RCO- \) is the fatty acid moiety

Assay

Not less than 66.0 and not more than 70.5\% of oxyethylene groups, equivalent to not less than 97.0 and not more than 103.0\% of polyoxyethylene (20) sorbitan monopalmitate calculated on the anhydrous basis.

DESCRIPTION

Polyoxyethylene (20) Sorbitan Monopalmitate is a lemon to orange coloured, oily liquid or semi-gel at 25\°C, with a faint characteristic odour, and a warm, somewhat bitter taste

FUNCTIONAL USES

Emulsifier, dispersing agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility

Soluble in water, ethanol, methanol, ethyl acetate and acetone. Insoluble in mineral oil

B. Infrared absorption spectrum

Characteristic of a partial fatty acid ester of a polyoxyethylated polyol

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

IDENTIFICATION TESTS (continued)

C. Colour reaction

Passes test
Proceed as directed in the TESTS for Polyoxyethylene (20) Sorbitan Monolaurate.

D. Positive test for fatty acids

Passes test
Proceed as directed in the TESTS for Polyoxyethylene (20) Sorbitan Monolaurate.

E. Gelatinization

A mixture of 60 parts by volume of polyoxyethylene (20) sorbitan monopalmitate and 40 parts of water yields a gelatinous mass at or below room temperature.

* F. Saponification

100 g of the sample yield upon alkaline saponification approximately 20 g of fatty acids and 78 g of polyols.

PURITY TESTS

* Water

Not more than 3% (Karl Fischer Method)

* Sulfated ash

Not more than 0.25%
5 g of the sample meets the requirements of the Limit Test for Sulfated Ash

* Arsenic

Not more than 3 mg/kg (Method II)

* Heavy metals

Not more than 10 mg/kg
Test 2 g of the sample as directed in Method II under the Limit Test for Heavy Metals

* Acid value

Not more than 2

* Saponification value

Not less than 41 and not more than 52

* Hydroxyl value

Not less than 90 and not more than 107

METHOD OF ASSAY

Determine the content of Oxyethylene groups as directed in the General Methods*

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POLYOXYETHYLENE (20) SORBITAN MONOSTEARATE*

SYNONYMS
Polysorbate 60
INS No. 435, EEC No. E435

DEFINITION
Polyoxyethylene (20) Sorbitan Monostearate consists of a mixture of the partial esters of sorbitol and its mono- and di-anhydrides (which have an acid value below 10 and a water content below 0.2%) with the edible commercial stearic acid and condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides.

C.A.S. number 9005-07-6
Structural formula A nominal formula for polyoxyethylene (20) sorbitan monostearate is approximately as follows:

\[
\text{CH}_2 \quad \text{HCO(C}_2\text{H}_4\text{O})_y\text{H} \\
\text{H(O(C}_2\text{H}_4\text{O})_x\text{OCH}_2\text{O(C}_2\text{H}_4\text{O})_z\text{OCR}}
\]

where \( w + x + y + z \approx 20 \), and RCO- is the fatty acid moiety

Assay
Content not less than 65.0 and not more than 69.5% of oxyethylene groups, equivalent to not less than 97.0 and not more than 103.0% of polyoxyethylene (20) sorbitan monostearate, on the dried basis.

DESCRIPTION
A lemon to orange coloured oily liquid or semi-gel at 25°C, with a faint characteristic odour, and a waxy somewhat bitter taste

FUNCTIONAL USES
Emulsifier, dispersing agent

* These specifications were prepared at the 25th session of JECFA (1981) and published in FNP 19 (1981).

CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility Soluble in water, ethyl acetate, aniline and toluene. Insoluble in mineral oil and vegetable oils

B. Infrared absorption spectrum Characteristic of a partial fatty acid ester of a polyoxyethylated polyol

C. Colour reaction Passes test Proceed as directed in the TESTS for Polyoxyethylene (20) Sorbitan Monolaurate.

D. Positive test for fatty acids Passes test Proceed as directed in the TESTS for Polyoxyethylene (20) Sorbitan Monolaurate.

E. Gelatinization A mixture of 60 parts by volume of the sample and 40 parts of water yields a gelatinous mass at or below room temperature

* F. Saponification 100 g of the sample yield upon alkaline saponification approximately 25 g of fatty acids and 77 g of polyols

PURITY TESTS

* Water Not more than 3% (Karl Fischer Method)

* Sulfated ash Not more than 0.25% Proceed as directed under the test for Ash (Sulfated ash, Method I), using 2 g of the sample

* Arsenic Not more than 3 mg/kg (Method II)

* Heavy metals Not more than 10 mg/kg Test 2 g of the sample as directed in Method II under the Limit Test for Heavy Metals

* Acid value Not more than 2

* 1,4-Dioxane Not more than 10 mg/kg

* Saponification value Not less than 41 and not more than 52

* Hydroxyl value Not less than 90 and not more than 107

METHOD OF ASSAY Determine the content of Oxyethylene groups as directed in the General Method*
POLYOXYETHYLENE (20) SORBITAN TRISTEARATE*

SYNONYMS
Polysorbate 65
INS No. 436, EEC No. E436

DEFINITION
Polyoxyethylene (20) Sorbitan Tristearate consists of a mixture of the partial esters of sorbitol and its mono- and dianhydrides (which have an acid value below 15 and a water content below 0.2%) with edible commercial stearic acid and condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides.

Structural formula
A nominal formula for polyoxyethylene (20) sorbitan tristearate is approximately as follows:

\[
\begin{align*}
\text{HCO(C}_2\text{H}_4\text{O})_w\text{H} \\
\text{H(O}_2\text{C}_2\text{H}_4\text{O})_x\text{OCH}_2\text{O} \\
\text{HCO(C}_2\text{H}_4\text{O})_y\text{H} \\
\text{CH}_2\text{O(C}_2\text{H}_4\text{O})_z\text{OCR}
\end{align*}
\]

where \( w + x + y + z \approx 20 \) and \( RCO^- \) is the fatty acid moiety.

Assay
Content not less than 46.0 and not more than 50.0% of oxyethylene groups, equivalent to not less than 96.0 and not more than 104.0% of polyoxyethylene (20) sorbitan tristearate on the dried basis.

DESCRIPTION
A tan coloured, waxy solid at 25°C, with a faint characteristic odour and a waxy, somewhat bitter taste.

FUNCTIONAL USES
Emulsifier, dispersing agent

* These specifications were prepared at the 25th session of JECFA (1981) and published in FNP 19 (1981).

CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility
  Dispersible in water. Soluble in mineral oil, vegetable oils, petroleum ether, acetone, ether, dioxane, ethanol and methanol

* B. Congealing range
  29° - 33°

C. Infrared absorption spectrum
  Characteristic of a partial fatty acid ester of a polyoxyethylated polyol

D. Colour reaction
  Passes test
  Proceed as directed in the TESTS for Polyoxyethylene (20) Sorbitan Monolaurate

E. Positive test for fatty acids
  Passes test
  Proceed as directed in the TESTS for Polyoxyethylene (20) Sorbitan Monolaurate

* F. Saponification
  100 g of the sample yield upon alkaline saponification approximately 43 g of fatty acids and 56 g of polyols

PURITY TESTS

* Water
  Not more than 3% (Karl Fischer Method)

* Sulfated ash
  Not more than 0.25%
  Proceed as directed under the test for Ash (Sulfated ash, Method I), using 2 g of the sample

* Arsenic
  Not more than 3 mg/kg (Method II)

* Heavy metals
  Not more than 10 mg/kg
  Test 2 g of the sample as directed in Method II under the Limit test for Heavy Metals

* Acid value
  Not more than 2

* 1,4-Dioxane
  Not more than 10 mg/kg

* Saponification value
  Not less than 88 and not more than 98

* Hydroxyl value
  Not less than 40 and not more than 60

METHOD OF ASSAY

Determine the content of Oxyethylene groups as directed in the General Methods*

POLYOXYETHYLENE (8) STEARATE*

SYNONYMS

Polyoxyl (8) stearate
INS No. 430, EEC No. 430

DEFINITION

Polyoxyethylene (8) Stearate consists of a mixture of the mono- and diesters of edible commercial stearic acid and mixed polyoxyethylene diols (having an average polymer length of about 7.5 oxyethylene units) together with free polyol.

C.A.S. number

9004-99-3

Structural formula and Approximate composition

Polyoxyethylene (8) Stearate may be represented by the formulae:

\[ \text{HO(CH}_2\text{CH}_2\text{O)}_n\text{H} \quad \text{RCOO(CH}_2\text{CH}_2\text{O)}_n\text{H} \quad \text{RCOO(CH}_2\text{CH}_2\text{O)}_n\text{OCR} \]

free polyol monoester diester

where \( \text{RCO}^- \) is the fatty acid moiety, and \( n \) has an average value of approximately 7.5. The distribution of polymers is approximately in accordance with the Poisson expression.

Assay

Not less than 53.0 and not more than 57.0% of oxyethylene groups equivalent to not less than 96.0 and not more than 103.0% of polyoxyethylene (8) stearate calculated on the anhydrous basis.

DESCRIPTION

Polyoxyethylene (8) Stearate is a cream-coloured, soft, waxy or pasty solid at 25°, with a faint fatty odour and a slightly bitter, fatty taste

FUNCTIONAL USES

Emulsifier

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility

Soluble in ethanol, methanol, acetone, ether, ethyl acetate and dioxane. Dispersible in warm water. Soluble with haze in mineral oil

** B. Congealing range

21° - 29°

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

IDENTIFICATION TESTS (continued)

- C. Infrared absorption spectrum
  Characteristic of a partial fatty acid ester of a polyoxyethylated polyol

- D. Colour reaction
  Passes test
  Proceed as directed in the TESTS for Polyoxyethylene (20) Sorbitan Monolaurate.

- E. Saponification
  100 g of the sample shall yield upon alkaline saponification approximately 44 g of fatty acids and 59 g of polyols

PURITY TESTS

- Water
  Not more than 3% (Karl Fischer Method)

- Arsenic
  Not more than 3 mg/kg (Method II)

- Heavy metals
  Not more than 10 mg/kg
  Test 2 g of the sample as directed in Method II under the Limit test for Heavy Metals

- Acid value
  Not more than 2

- Saponification value
  Not less than 87 and not more than 97

- Hydroxyl value
  Not less than 85 and not more than 100

METHOD OF ASSAY

Determine the content of Oxyethylene groups as directed in the General Methods*
POLYOXYETHYLENE (40) STEARATE*  

SYNONYMS  
Polyoxyl (40) stearate, polyoxyethylene (40) monostearate  
INS No. 431, EEC No. 431  

DEFINITION  
Polyoxyethylene (40) Stearate consists of a mixture of the mono- and diesters of edible commercial stearic acid and mixed polyoxyethylene diols (having an average polymer length of about 40 oxyethylene units) together with free polyol.  

Structural formula and Approximate composition  
Polyoxyethylene (40) Stearate may be represented by the formulae:  
\[ \text{HO} \left( \text{CH}_2 \text{CH}_2 \text{O} \right)_n \text{H} \quad \text{RCOO} \left( \text{CH}_2 \text{CH}_2 \text{O} \right)_m \text{H} \quad \text{RCOO} \left( \text{CH}_2 \text{CH}_2 \text{O} \right)_n \text{OCR} \]  
free polyol monoester diester  

where RCO- is a fatty acid moiety, and "n" has an average value of approximately 40. The distribution of polymers is approximately in accordance with the Poisson expression.  

Assay  
Not less than 84.0 and not more than 88.0% of oxyethylene groups equivalent to not less than 97.5 and not more than 102.5% of polyoxyethylene (40) stearate calculated on the anhydrous basis.  

DESCRIPTION  
Polyoxyethylene (40) Stearate is cream-coloured and exists as flakes or as a waxy solid at 25° with a faint odour and a waxy, somewhat bitter taste.  

FUNCTIONAL USES  
Emulsifier  

CHARACTERISTICS  
IDENTIFICATION TESTS  
** A. Solubility  
Soluble in water, ethanol, methanol and ethylacetate. Insoluble in mineral oil  
** B. Congealing range  
39° - 44°  

---  
* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).  
IDENTIFICATION TESTS (continued)

* C. Infrared absorption spectrum

Characteristic of a partial fatty acid ester of a polyoxyethylated polyol.

* D. Colour reaction

Passes test
Proceed as directed in the TESTS for Polyoxymethylene (20) Sorbitan Monolaurate.

* E. Saponification

100 g of the sample shall yield upon alkaline saponification approximately 13-14 g of fatty acids and 85-87 g of polyols.

PURITY TESTS

* Water

Not more than 3% (Karl Fischer Method)

* Arsenic

Not more than 3 mg/kg (Method II)

* Heavy metals

Not more than 10 mg/kg
Test 2 g of the sample as directed in Method II under the Limit test for Heavy Metals.

* Acid value

Not more than 1

* Saponification value

Not less than 25 and not more than 35

* Hydroxyl value

Not less than 27 and not more than 40

METHOD OF ASSAY

Determine the content of Oxyethylene groups as directed in the General Methods*. 

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POLYVINYLPYRROLIDONE

SYNONYMS
Povidone, PVP; INS No. 1201

DEFINITION
Chemical names Polyvinylpyrrolidone, poly-[1-(2-oxo-1-pyrrolidinyl)-ethylene]
C.A.S. number 9003-39-8
Chemical formula \((\text{C}_n\text{H}_{4n}\text{NO})_n\)
Structural formula

\[
\begin{align*}
\text{H}_2\text{C} & \text{-N} \text{-C=O} \\
\text{H}_2\text{C} & \text{-CH-CH}_2 \text{-} \\
& \text{n}
\end{align*}
\]

Molecular weight
Lower molecular weight range product: about 40 000
Higher molecular weight range product: about 360 000
Assay
Content not less than 12.2% and not more than 13.0% of Nitrogen (N) on the anhydrous basis.

DESCRIPTION
White to tan powder.
Commercial products for food use are supplied in two molecular weight forms. The molecular weight value is an average molecular weight for the two forms.

FUNCTIONAL USES
Clarifying agent, stabilizer, bodying agent, tableting adjunct, dispersing agent.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Soluble in water, in ethanol and in chloroform.
Insoluble in ether.

** B. pH
3.0 - 7.0 (5% soln)

* These specifications were prepared at the 30th session of JECFA (1986) and published in FNP 37 (1986).

IDENTIFICATION TESTS (continued)

C. Precipitation with dichromate
   Passes test
   See description under TESTS

D. Precipitation test
   Passes test
   See description under TESTS

E. Precipitation test
   Passes test
   See description under TESTS

PURITY TESTS

Relative viscosity
   Between 1.188 and 1.325 for lower molecular weight product, and between 3.225 and 5.662 for higher molecular weight product. See description under TESTS.

* Water
   Not more than 5% (Karl Fischer Method)

* Total ash
   Not more than 0.02%
   Proceed as directed under the tests for Ash (Total) using 10 g of the sample.

* Arsenic
   Not more than 3 mg/kg (Method II)

* Heavy metals
   Not more than 10 mg/kg
   Test 2 g of the sample as directed in the Limit Test (Method II).

Aldehyde
   Not more than 0.2% (as acetaldehyde)
   See description under TESTS

Monomer content
   Not more than 1% (as vinylpyrrolidone)
   See description under Tests

Hydrazine
   Not more than 1 mg/kg
   See description under TESTS

TESTS

IDENTIFICATION TESTS

C. Precipitation with dichromate
   To 5 ml of a 1 in 50 solution of the sample add 5 ml of dilute hydrochloric acid TS, 5 ml of water and 2 ml of 1 in 10 solution of potassium dichromate. A yellow precipitate forms.

D. Precipitation test
   Add 5 ml of a 1 in 50 solution of the sample to 75 mg of cobalt nitrate and 0.3 g of ammonium thiocyanate dissolved in 2 ml of water, mix and acidify with dilute hydrochloric acid TS. A pale blue precipitate forms.

IDENTIFICATION TESTS (continued)

E. Precipitation test

To 5 ml of a 1 in 50 solution of the sample add 1 ml of 25% hydrochloric acid and 5 ml of 5% barium chloride solution and 1 ml of 5% phosphomolybdotungstic acid solution. A voluminous white precipitate is formed which becomes gradually blue on standing in daylight. (Note: The blue colouration on exposure to light distinguishes polyvinylpyrrolidone from polyethylene oxide adducts which give similar precipitates with the same reagents but which retain their white colour in light).

PURITY TESTS

Relative viscosity

Transfer an accurately weighed portion of the sample, equivalent to 1 g of anhydrous polyvinylpyrrolidone, into a 250 ml conical flask, and calculate the amount of water to be added to make a 1% solution. Allow the mixture to stand at room temperature, with occasional swirling, until solution is complete, and then allow to stand for 1 h. longer. Filter through a dry sintered-glass filter funnel of coarse porosity, then pipet 10 ml of the filtrate into a Cannon-Fenske viscometer, or equivalent, and place the viscometer in a water bath maintained at 25° ± 0.05°. After allowing the sample solution and pipet to warm in the water bath for 10 min., draw the solution by means of very gentle suction up through the capillary until the meniscus is formed from 3 to 4 mm above the upper etched mark. Release the vacuum, and, when the meniscus reaches the upper etched mark, begin timing the flow through the capillary. Record the exact time when the meniscus reaches the lower etched mark, and calculate the flow time to the nearest 0.1 sec. Repeat this operation until at least three readings are obtained. The readings must agree within 0.3 sec.; if not, repeat the determination with additional 10 ml portions of the sample solution after recleaning the viscosity pipet with sulfuric acid-dichromate cleaning solution.

Calculate the average flow time for the sample solution, and then obtain the average flow time in similar manner for 10 ml of filtered water for the same viscosity pipet. Calculate the relative viscosity of the sample by dividing the average flow time for the sample solution by that of the water sample.

Aldehyde

Transfer about 10 g of the sample, accurately weighed and dissolved in 300 ml of water, into a 1000 ml round-bottom flask containing 80 ml of 25% sulfuric acid, reflux for about 45 min. under a water-cooled condenser, and then distil about 100 ml into a receiver containing 20 ml of 1 N hydroxylamine hydrochloride previously adjusted to pH 3.1. Titrate the contents of the receiver with 0.1 N sodium hydroxide to a pH of 3.1, using a pH meter. Perform a blank determination and make any necessary correction.

Each ml of 0.1 N sodium hydroxide is equivalent to 4.405 mg of C₆H₈O.
**Monomer content**  
Dissolve about 4 g of the sample, accurately weighed, in 30 ml of water in a 125 ml round-bottom flask, add 0.5 g of sodium acetate, mix and begin titrating with 0.1 N iodine. When the iodine colour no longer fades, add additional 3.0 ml of the titrant, and allow the solution to stand for 5 to 10 min. Add starch TS, and titrate the excess iodine with 0.1 N sodium thiosulfate. Perform a blank determination, using the same volume of 0.1 N iodine, accurately measured, as was used for the sample.

Each ml of 0.1 N iodine is equivalent to 5.56 mg of vinylpyrrolidone.

**Hydrazine**
Transfer 2.5 g of the sample into a 50-ml centrifuge tube, add 25 ml of a 1 in 20 solution of salicylaldehyde in methanol, swirl, and heat in a water bath at 60° for 15 min. Allow to cool, add 2.0 ml of toluene, insert a stopper in the tube, shake vigorously for 2 min, and centrifuge. On a suitable thin-layer chromatographic plate, coated with a 0.25-mm layer of dimethylsilanized chromatographic silica gel mixture, apply 10 µl of the clear upper toluene layer in the centrifuge tube and 10 µl of a Standard solution of salicylaldazine in toluene containing 9.38 µg per ml. Allow the spots to dry, and develop the chromatogram in a solvent system of methanol and water (2:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under ultraviolet light at a wavelength of 365 nm: salicylaldazine appears as a fluorescent spot having an Rf value of about 0.3, and the fluorescence of any salicylaldazine spot from the test specimen is not more intense than that produced by the spot obtained from the Standard solution (1 ppm of hydrazine).

**Preparation of Salicylaldazine Standard**
Dissolve 300 mg of hydrazine sulfate in 5 ml of a freshly prepared 20% (v/v) solution of salicylaldehyde in isopropyl alcohol, mix, and allow to stand until a yellow precipitate forms. Extract the mixture with two 15-ml portions of methylene chloride. Combine the methylene chloride extracts, and dry over anhydrous sodium sulfate. Decant the methylene chloride solution, and evaporate it to dryness. Recrystallize the residue of salicylaldazine from a mixture of warm toluene and methanol (60:40) with cooling; filter and dry the crystals in vacuum. The crystals have a melting range of 213° to 214°.

**METHOD OF ASSAY**
Determine as directed under "Nitrogen Determination" in General Methods*, using about 1 g of the sample, accurately weighed.

SYNONYMS
Cl Food Red 7, Cochineal Red A, New Coccine; INS No. 124, EEC No. E124

DEFINITION
Ponceau 4R consists essentially of trisodium d-2-hydroxy-1-(4-sulfonato-1-naphthylazo)-6,8-naphthalenedisulfonate, and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

Ponceau 4R may be converted to the corresponding aluminium lake in which case only the General Specifications for Aluminium Lakes of Colouring Matters shall apply.**

Class
Monoazo

Code numbers
CI (1975) No. 16255
CAS No. 2611-82-7

Chemical name
Trisodium-2-hydroxy-1-(4-sulfonato-1-naphthylazo)-6,8-naphthalenedisulfonate.

Chemical formula
$C_{26}H_{16}N_{12}Na_{6}O_{26}S_6 \cdot 3/2H_2O$

Structural formula

\[
\text{Structural formula image}
\]

Molecular weight
631.51

Assay
Content not less than 85% total colouring matters

DESCRIPTION
Reddish powder or granules

FUNCTIONAL USE
Food colour

* These specifications were prepared at the 28th session of JECFA (1984) and published in FNP 31/1 (1984).

** See Annex 2 at the end of this Compendium.
CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility
   Soluble in water, Sparingly soluble in ethanol

** B. Identification of colouring matters
   Passes test

PURITY TESTS

** Loss on drying at 135°
   } Not more than 20%

** Chloride and sulfate calculated as sodium salts

** Water insoluble matter
   Not more than 0.2%

*** Arsenic
   Not more than 3 mg/kg

*** Lead
   Not more than 10 mg/kg

* Heavy metals
   Not more than 40 mg/kg
   Proceed as directed in the Limit Test for Heavy Metals

Subsidiary colouring matters Not more than 1%
   See description under TESTS

Organic compounds other than colouring matters

4-Amino-1-naphthalenesulfonic acid
7-Hydroxy-1,3-naphthalenedisulfonic acid
3-Hydroxy-2,7-naphthalenesulfonic acid } Total not more than 0.5%
6-Hydroxy-2-naphthalenesulfonic acid
7-Hydroxy-1,3,6-naphthalenetrisulfonic acid

** Unsulfonated primary aromatic amines Not more than 0.01% calculated as aniline

** Ether extractable matter Not more than 0.2%


TESTS

PURITY TESTS

* Subsidiary colouring matters

Use the following conditions:
- Developing solvent: No. 3
- Height of ascent of solvent front: 17 cm, then
- 1 h further development

* Organic compounds other than colouring matters

Use HPLC under the following conditions:
- HPLC elution gradient: 2 to 100% at 4% per min (linear)

METHOD OF ASSAY

Determination of Total Colouring Matters by Titration with Titanous Chloride*

Use the following:
- Weight of sample: 0.7-0.8 g
- Buffer: 10 g sodium citrate
- Weight (D) of colouring matters equivalent to 1.00 ml of 0.1 N TiCl₃: 15.78 mg

POTASSIUM ACETATE*

SYNONYMS
INS No.261, EEC No.E261

DEFINITION
Chemical name: Potassium acetate
C.A.S. number: 127-08-2
Chemical formula: C₂H₃KO₂
Structural formula: CH₃-COOK
Molecular weight: 98.14
Assay: After drying at 150°C for 2 h, contains not less than 99.0% of C₂H₃KO₂

DESCRIPTION
Colourless, deliquescent crystals or a white, crystalline powder, odourless or with a faint acetic odour, having a saline taste.

FUNCTIONAL USES
Buffer, antimicrobial preservative

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Very soluble in water. Freely soluble in ethanol.

** B. pH
7.5 - 9.0 (1 in 20 soln)

** C. Positive test for potassium
Passes test

** D. Positive test for acetate
Passes test

PURITY TESTS

** Loss on drying
Not more than 8.0% (150°C, 2 h)

Alkalinity
Passes test
See description under TESTS

Sodium compounds
Passes test
See description under TESTS

* These specifications were prepared at the 18th session of JECFA (1974) and published in NMRS 54B (1975).

** See General Methods (Guide to JECFA Specifications), FNP 5/Rev.2 (1991)
PURITY TESTS (continued)

* **Arsenic**
  Not more than 3 mg/kg
  Proceed as directed in the specifications for DL-Malic Acid

* **Lead**
  Not more than 10 mg/kg
  Proceed as directed in the specifications for DL-Malic Acid

* **Heavy metals**
  Not more than 20 mg/kg
  Proceed as directed in the specifications for DL-Malic Acid

**TESTS**

**PURITY TESTS**

**Alkalinity**
Dissolve 1 g of the sample in 20 ml of freshly boiled and cooled water, and add 3 drops of phenolphthalein TS. If a pink colour is produced, not more than 0.5 ml of 0.1 N hydrochloric acid should be required to discharge it.

**Sodium compounds**
To 3 ml of a 1 in 20 solution add 1.5 ml of water, 1.0 ml of 95% ethanol, and 3.0 ml of potassium antimonate solution, and allow to stand. No white crystalline precipitate or sediments should be visible to the naked eye within 15 min.

**METHOD OF ASSAY**
Dissolve about 200 mg of previously dried sample, accurately weighed, in 25 ml of glacial acetic acid. Add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid in glacial acetic acid. Perform a blank determination, and make any necessary correction. Each ml of 0.1 N perchloric acid is equivalent to 9.814 mg of C\textsubscript{2}H\textsubscript{3}K\textsubscript{2}O\textsubscript{2}.

POTASSIUM ALGINATE*

SYNONYMS

INS No. 402, EEC No. E402

DEFINITION

Potassium Alginate, the potassium salt of alginic acid, is a hydrophilic, colloidal substance. The alginic acid is a linear, high-polymer consisting mainly of β-(1→4) linked β-mannuronic acid in the pyranose ring form, with part of the mannuronic acid replaced by L-guluronic acid.

C.A.S. number

9005-36-1

Chemical formula

(C₆H₇KO₉)ₙ

Structural formula

![Structural formula of Potassium Alginate](attachment:structural_formula.png)

Units of the salt of mannuronic acid

Molecular weight

Structural unit: 214.22 (theoretical)
(238.00 actual average)

Macromolecule: 32 000-250 000

Assay

Potassium Alginate yields, on the dried basis, not less than 16.5% and not more than 19.5% of carbon dioxide (CO₂), equivalent to not less than 89.25% and not more than 105.50% of potassium alginate (C₆H₇KO₉)ₙ.

DESCRIPTION

Potassium Alginate occurs in filamentous, grainy, granular and powdered forms. It is colourless or slightly yellow and may have a slight characteristic smell and taste.

FUNCTIONAL USES

Thickening agent and stabilizer

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility

Slowly soluble forming a viscous solution in water; insoluble in ethanol, ether and chloroform

** B. Specific rotation

Clarify an 0.5% solution of the sample in sodium hydroxide TS with kieselguhr and determine the rotation in a 2-dm tube. The specific rotation is not less than -0.8° at 20°

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

IDENTIFICATION TESTS (continued)

C. pH 6.0 - 8.0 (1 in 100 soln)

D. Precipitate formation with calcium chloride solution
   Passes test
   Proceed as directed in the same test under Alginic Acid

E. No precipitate formation with ammonium sulfate solution
   Passes test
   Proceed as directed in the same test under Alginic Acid

F. Colour reaction
   Moisten 1-5 mg of the sample with water and add 1 ml of acid ferric sulfate TS. Within 5 min, a cherry-red colour develops that finally becomes deep purple

G. Positive test for potassium
   Dissolve the sulfated ash of the sample in dilute acetic acid TS and filter. Add 1 volume of saturated sodium hydrogen tartrate solution and 1 volume of ethanol and shake. A white crystalline precipitation is formed

PURITY TESTS

* Loss on drying
   Not more than 15% (105°, 4 h)

Phosphate
   Not detectable
   Proceed as directed in the Purity Test for Phosphate under Sodium Alginate

Water insolubles
   Not more than 1% on the dried basis
   Proceed as directed in the Purity Test for Water Insolubles under Sodium Alginate

* Total ash
   Not less than 23% and not more than 32% on the dried basis.
   Proceed as directed in the Total Ash test under Alginic Acid.

* Sodium
   Not detectable by the following test
   Dissolve the ash of the sample in dilute acetic acid TS and filter. Add to the filtrate uranyl zinc acetate TS. No precipitate should be formed

* Arsenic
   Not more than 3 mg/kg
   A sample solution prepared as directed for organic compounds will meet the requirements of the Limit Test for Arsenic

* Lead
   Not more than 10 mg/kg
   A sample solution prepared as directed for organic compounds will meet the requirements of the Limit Test for Lead

PURITY TESTS (continued)

**Heavy metals**

Not more than 40 mg/kg

Test 0.5 g of the sample as directed in Method II under The Limit Test for Heavy metals using 20 μg of lead ion (Pb) in the control (Solution A)

**METHOD OF ASSAY**

*Decarboxylation method*

Proceed as directed under Carbon Dioxide Determination by Decarboxylation in the General Methods.* Each ml of 0.25 N sodium hydroxide consumed is equivalent to 5.5 mg of carbon dioxide (CO₂) or 29.75 mg of potassium alginate (equivalent weight 238.00).

*Gravimetric method*

Dissolve 0.500 g of the sample in 10 ml of sodium hydroxide TS, add 90 ml of water and filter if necessary. Add 15 ml of 4 N hydrochloric acid and 100 ml of 90% ethanol. Allow this mixture to stand for 2 h, decant the supernatant liquid as far as possible, and centrifuge. Decant the liquid and replace it by 90% v/v ethanol. Mix well, centrifuge and decant again. This washing is repeated until the hydrochloric acid is removed. Then transfer the precipitate by means of 90% ethanol to a fine glass filter, wash with dry acetone, place the filter in an vacuum desiccator, and dry to constant weight at 100°.

Calculate the percent purity of the sample by the formula:

\[
\% \text{ Purity} = \frac{200 \times F \times W}{\% \text{ dry substance in sample}} \times 100
\]

in which F is a conversion factor specified as 1.216 and W is the weight (in grams of the dried precipitate)

POTASSIUM BENZOATE*

SYNONYMS

INS No. 212, EEC No. E212

DEFINITION

Chemical names

Potassium benzoate, potassium salt of benzenecarboxylic acid, potassium salt of phenylcarboxylic acid.

C.A.S. number

582-25-2

Chemical formula

C₇H₅KO₂·3H₂O

Structural formula

\[
\text{COOK}
\]

Molecular weight

214.27

Assay

Contains not less than 99% C₇H₅KO₂ after drying at 105° to constant weight.

DESCRIPTION

A white crystalline powder

FUNCTIONAL USE

Antimicrobial preservative

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility

Freely soluble in water, soluble in ethanol.

** B. Melting range

for benzoic acid

121.5 - 123.5°

Produced as directed in the specification for Sodium Benzoate.

** C. Positive test

for benzoate

Passes tests

To a 10% solution of the sample add ferric chloride TS. A buff coloured precipitate is produced.

D. Positive test for potassium

Passes tests

See description under TESTS

PURITY TESTS

Acidity and alkalinity

Passes tests

Proceed as directed in the specifications for Sodium Benzoate.

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

PURITY TESTS (continued)

* Arsenic
Not more than 3 mg/kg. A sample solution prepared as directed for organic compounds meets the requirements of the Limit Test for Arsenic (Method II).

Heavy metals
Not more than 10 mg/kg.
Proceed as directed in the specifications for Sodium Benzoate.

Readily carbonizable substances
Dissolve 0.5 g of the sample, weighed to the nearest mg, in 5 ml of sulfuric acid TS. The colour produced should not be darker than a light pink ("Matching Fluid Q")

Readily oxidizable substances
Passes tests.
Proceed as directed in the specifications for Benzoic Acid.

Chlorinated organic compounds
Not more than 0.07% (as Cl)
Proceed as directed in the specifications for Benzoic Acid.

TESTS

IDENTIFICATION TESTS

* D. Positive test for potassium
Acidify a 10% solution of the sample with dilute hydrochloric acid TS and filter. Make the filtrate neutral with sodium hydroxide TS and add 1 volume of saturated sodium hydrogen tartrate solution and 1 volume of ethanol and shake. A white crystalline precipitate is formed.

METHOD OF ASSAY

Neutralization titration

Weigh to the nearest 0.1 mg, 2.5 to 3 g of the sample, previously dried at 105°C to constant weight, and dissolve in 50 ml of water. Neutralize the solution, if necessary, with 0.1 N hydrochloric acid, using phenolphthalein TS as indicator. Add 50 ml of ether and a few drops of bromophenol blue TS and titrate with 0.5 N hydrochloric acid, shaking constantly, until the colour of the indicator begins to change. Separate the lower layer, wash the ethereal layer with 10 ml of water, and add the washing and an additional 20 ml of ether to the separated aqueous layer. Complete the titration with the 0.5 N hydrochloric acid, shaking constantly. Each ml of 0.5 N hydrochloric acid is equivalent to 80.11 mg of C₇H₇KO₂.

POTASSIUM BROMATE*

SYNONYMS

INS No.924a, EEC No.924

DEFINITION

Chemical name: Potassium bromate
C.A.S. number: 7758-01-2
Chemical formula: KBrO₃

Assay: Content not less than 99.0% and not more than 101.0% of KBrO₃ on the dried basis

DESCRIPTION

White, odourless crystals or a granular powder

FUNCTIONAL USES

Flour treatment agent, oxidizing agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Soluble in water. Slightly soluble in ethanol.

** B. Positive test for potassium
A 1 in 20 solution of the sample imparts a violet colour to a non-luminous flame

** C. Positive test for bromate
To a 1 in 20 solution of the sample add sulfurous acid dropwise. A yellow colour is produced which disappears upon the addition of an excess of sulfurous acid

PURITY TESTS

** Loss on drying
Not more than 0.5%. Dry over a suitable desiccant to constant weight

Free acid or alkali
Passes test
See description under TESTS

Arsenic
Not more than 3 mg/kg
See description under TESTS

Heavy metals
Not more than 10 mg/kg
See description under TESTS

* These specifications were prepared at the 31st session of JECFA (1987) and published in FNP 38 (1988).

TESTS

PURITY TESTS

Free acid or alkali
Dissolve by warming 5 g of the sample in 60 ml of freshly boiled and cooled water, cool and add 3 drops of phenolphthalein; observe colour:

(a) if the solution is pink, add 0.4 ml of 0.01 N hydrochloric acid. The pink colour disappears.
(b) if the solution is colourless, add 1.2 ml of 0.01 N sodium hydroxide. The solution turns pink.

* Arsenic
Dissolve 1 g of the sample in a mixture of 5 ml hydrochloric acid and 5 ml of water, and evaporate the solution until crystals appears. Cool, dissolve the residue in water, and dilute to 35 ml. This solution meets the requirements of the Limit Test for Arsenic (Method II).

* Heavy metals
Dissolve 2 g of the sample in 10 ml of water, add 10 ml of hydrochloric acid, and evaporate to dryness on a steam bath. Dissolve the residue in 10 ml of hydrochloric acid, again evaporate to dryness, and then dissolve the residue in 25 ml of water. Test this solution as directed in the Heavy Metals Limit Test (Method I).

METHOD OF ASSAY

Redox titration
Dissolve about 100 mg of the sample, previously dried over a suitable desiccant to constant weight and accurately weighed, in 50 ml of water contained in a 250-ml glass-stoppered conical flask. Add 3 g of potassium iodide, followed by 3 ml of concentrated hydrochloric acid. Allow the mixture to stand for 5 min., add 100 ml of cold water, and titrate the liberated iodine with 0.1 N sodium thiosulfate, adding starch as the end-point is approached. Perform a blank determination. Each ml of 0.1 N sodium thiosulfate is equivalent to 2.783 mg of KBrO₃.

POTASSIUM CARBONATE

SYNONYMS  INS No.501(i),  EEC No.501

DEFINITION

Chemical names  Potassium carbonate, potassium salt of carbonic acid
C.A.S. number  584-08-7
Chemical formula  Anhydrous K₂CO₃;  Hydrated: K₂CO₃·½H₂O
Formula weight  138.21 (anhydrous)
Assay  After drying at 180° for 4 h contains not less than 99.0% of K₂CO₃

DESCRIPTION

White, odourless, very deliquescent powder, having an alkaline taste. The hydrated form occurs as small, white, translucent crystals or granules.

FUNCTIONAL USE

Alkali

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Very soluble in water. Insoluble in ethanol.

** B. Positive test for potassium
To a 1 in 100 solution of the sample add 1 volume of saturated sodium hydrogen tartrate solution and 1 volume of ethanol, and mix. A white crystalline precipitate is formed.

** C. Positive test for carbonate
A 1 in 20 solution of the sample effervescences upon the addition of acetic acid.

PURITY TESTS

** Loss on drying
Anhydrous: not more than 5%.
Hydrated form: Between 10% and 18%.
Heat the sample at 180° for 4 h.

Arsenic
Not more than 3 mg/kg
See description under TESTS

Lead
Not more than 10 mg/kg
See description under TESTS

Heavy metals
Not more than 20 mg/kg
See description under TESTS

** These specifications were prepared at the 19th session of JECFA (1975) and published in NMRS 55B (1976).

TESTS

PURITY TESTS

* **Arsenic**

Dissolve 1 g of the sample in a mixture of 5 ml of dilute hydrochloric acid TS and 30 ml of water. This solution meets the requirements of the Limit Test for Arsenic (Method II).

* **Lead**

Dissolve 1 g of the sample cautiously in 5 ml of dilute hydrochloric acid TS, neutralize to phenolphthalein TS, and dilute to about 20 ml with water. This solution meets the requirements of the Limit Test for Lead.

* **Heavy metals**

To 1 g of the sample add 2 ml of water and 6 ml of dilute hydrochloric acid TS, boil for 1 min, cool, and dilute to 25 ml with water. This solution meets the requirements of the Limit Test for Heavy Metals (Method I) using 20 µg of lead ion (Pb) in the control (Solution A).

METHOD OF ASSAY

Weigh accurately about 1 g of the sample previously dried at 180° for 4 h. Dissolve carefully in 50 ml of 1 N sulfuric acid, add methyl orange TS and titrate the excess acid with 1 N sodium hydroxide. Each ml of 1 N sulfuric acid is equivalent to 69.11 mg of K₂CO₃.

**POTASSIUM CHLORIDE**

SYNONYMS
Sylvine, sylvite;
INS No. 508, EEC No. 508

DEFINITION

**Chemical name**
Potassium chloride

**C.A.S. number**
7447-40-7

**Chemical formula**
KCl

**Formula weight**
74.56

**Assay**
Content not less than 99.0% on the dried basis

DESCRIPTION
Colourless, elongated, prismatic, or cubital crystals, or white granular powder. Odourless with a saline taste.

FUNCTIONAL USE
Seasoning agent, gelling agent, yeast food.

CHARACTERISTICS

IDENTIFICATION TESTS

**A.** Solubility
Freely soluble in water. Insoluble in ethanol.

**B.** Positive test for potassium
Passes test

**C.** Positive test for chloride
Passes test

PURITY TESTS

**Loss on drying**
Not more than 1% (105°, 2 h)

**Acidity or alkalinity**
Passes test
See description under TESTS

**Iodide or bromide**
Passes test
See description under TESTS

**Sodium**
Passes test
See description under TESTS

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* These specifications were prepared at the 23rd session of JECFA (1979) and published in FNP 12 (1979).

PURITY TESTS (continued)

* Arsenic

Not more than 3 mg/kg
Test a solution of 1 g of the sample in 35 ml of water as directed in the Limit Test (Method II)

* Heavy metals

Not more than 10 mg/kg
Test a solution of 2 g of the sample in 25 ml of water as directed in the Limit Test

TESTS

PURITY TESTS

Acidity or alkalinity

To a solution of 5 g of the sample in 50 ml of recently boiled and cooled water, add 3 drops of phenolphthalein TS. No pink colour is produced. Then add 0.3 ml of 0.02 N sodium hydroxide. A pink colour is produced.

Iodide or bromide

Dissolve 2 g of the sample in 6 ml of water, add 1 ml of chloroform, and then add, dropwise and with constant agitation, 5 ml of a mixture of equal parts of chlorine TS and water. The chloroform is free from even a transient violet or permanent orange colour.

Sodium

A solution of 1 g of the sample in 20 ml of water, tested on a platinum wire does not impart a pronounced yellow colour to a non-luminous flame.

METHOD OF ASSAY

Dry about 250 mg of the sample at 105° for 2 h, weigh accurately, and dissolve in 50 ml of water in a glass-stoppered flask. Add, while agitating, 50 ml of 0.1 N silver nitrate, 3 ml of nitric acid, and 5 ml of nitrobenzene, shake vigorously, add 2 ml of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate. Each ml of 0.1 N silver nitrate is equivalent to 7.456 mg of KCl.

POTASSIUM DIHYDROGEN CITRATE*

SYNONYMS
Monopotassium citrate, potassium citrate monobasic;
INS No. 332(i), EEC No. E332

DEFINITION

Chemical names
Potassium dihydrogen citrate, monopotassium salt of 2-hydroxypropan-1,2,3-
tricarboxylic acid

C.A.S. number
866-83-1

Chemical formula
C$_6$H$_7$KO$_7$

Structural formula
\[
\begin{align*}
\text{CH}_2\text{COOH} \\
\text{HO} \cdot \text{C} \cdot \text{COOK} \\
\text{CH}_2\text{COOH}
\end{align*}
\]

Molecular weight
230.21

Assay
Contains not less than 99.0% and not more than the equivalent of 101.0% on
the dried basis

DESCRIPTION
Odourless, transparent crystals or white powder, with a slight acidic taste

FUNCTIONAL USES
Buffering agent, sequestrant, yeast food

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Freely soluble in water
Very slightly soluble in ethanol

** B. pH
3.5 - 3.9 (1 in 10 soln)

** C. Positive test for citrate
Passes test

** D. Positive test for potassium
Passes test

* These specifications were prepared at the 31st session of JECFA (1987) and published in FNP 38 (1988).

<table>
<thead>
<tr>
<th>PURITY TESTS</th>
<th>METHOD OF ASSAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Loss on drying</td>
<td>Weigh accurately about 180 mg of the sample, previously dried at 105° for 4 h, dissolve it in 25 ml of water and titrate with 0.1 N sodium hydroxide (potentiometric end-point determination). Each ml of 0.1 N sodium hydroxide is equivalent to 11.511 mg of C₆H₇KO₇.</td>
</tr>
<tr>
<td>* Oxalate</td>
<td></td>
</tr>
<tr>
<td>* Arsenic</td>
<td></td>
</tr>
<tr>
<td>* Heavy metals</td>
<td></td>
</tr>
</tbody>
</table>
POTASSIUM DIHYDROGEN PHOSPHATE*

SYNONYMS
Monobasic potassium phosphate, monopotassium monophosphate potassium acid phosphate, potassium biphosphate; INS No. 340(i), EEC No. E340

DEFINITION
Chemical names
Potassium dihydrogen phosphate, monopotassium dihydrogenorthophosphate, monopotassium dihydrogen monophosphate

C.A.S. number
7778-77-0

Chemical formula
KH₂PO₄

Formula weight
136.09

Assay
After drying at 105° for 4 h, contains not less than 98.0% of KH₂PO₄

DESCRIPTION
Odourless, colourless crystals or white granular or crystalline powder

FUNCTIONAL USES
Buffer, neutralizing agent, sequestrant, yeast food.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Freely soluble in water. Insoluble in ethanol.

** B. pH
4.2 - 4.7 (1 in 100 soln)

** C. Positive test for potassium
To a 1 in 100 solution of the sample add 1 volume of saturated sodium hydrogen tartrate solution and 1 volume of ethanol and shake. A white crystalline precipitate is formed.

** D. Positive test for phosphate
To 5 ml of a 1 in 100 solution of the sample add 1 ml of concentrated nitric acid and 5 ml of ammonium molybdate TS and warm. A bright canary-yellow precipitate is obtained.

E. Positive test for orthophosphate
To 5 ml of a 1 in 100 solution of the sample add silver nitrate TS. A yellow precipitate is formed.

* These specifications were prepared at the 19th session of JECFA (1975) and published in NMRS 55B (1976).

PURITY TESTS

* Loss on drying

Not more than 2% (105°, 4 h)

* Water insoluble substances

Fluoride

Not more than 10 mg/kg
Proceed as directed in the Limit Test for Fluoride under DIPOTASSIUM HYDROGEN PHOSPHATE

* Arsenic

Not more than 3 mg/kg
A solution of 1 g of the sample in 35 ml of water meets the requirements of the Limit Test for Arsenic (Method II).

* Lead

Not more than 10 mg/kg
Dissolve 1 g of the sample in 20 ml of water, and neutralize to phenolphthalein TS. This solution meets the requirements of the Limit Test for Lead.

* Heavy metals

Not more than 30 mg/kg
A solution of 0.67 g of the sample in 25 ml of water meets the requirements of the Limit Test for Heavy Metals (Method I) using 20 µg of lead ion (Pb) in the control (Solution A).

METHOD OF ASSAY

Transfer about 5 g of the sample, previously dried at 105° for 4 h and accurately weighed, into a 250-ml beaker. Add 100 ml of water and 5 ml of 1 N hydrochloric acid, and stir until the sample is completely dissolved. Place the electrodes of a suitable pH meter in the solution, and slowly titrate the excess acid, stirring constantly, with 1 N sodium hydroxide to the inflection point occurring at about pH 4. Record the buret reading, and calculate the volume (A), if any, of 1 N hydrochloric acid consumed by the sample. Continue the titration with 1 N sodium hydroxide until the inflection point occurring at about pH 8.8 is reached, record the buret reading, and calculate the volume (B) of 1 N sodium hydroxide required in the titration between the two inflection points (pH 4 and pH 8.8). Each ml of the volume (B) - (A) of 1 N sodium hydroxide is equivalent to 136.1 mg of KH₂PO₄.

**POTASSIUM GLUCONATE**

**SYNONYMS**
Potassium d-gluconate, d-gluconic acid potassium salt
INS No. 577, EEC No. E577

**DEFINITION**

<table>
<thead>
<tr>
<th>Chemical names</th>
<th>Potassium d-gluconate, d-gluconic acid potassium salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A.S. number</td>
<td>299-27-4</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>C_{6}H_{11}KO_{7}</td>
</tr>
</tbody>
</table>

**Structural formula**

```
COOK
| H—C—OH
| OH—C—H
| H—C—OH
| H—C—OH
| CH_{2}OH
```

**Molecular weight**

234.25

**Assay**

Content not less than 97.0% and not more than the equivalent of 103.0% on the dried basis

**DESCRIPTION**

Odourless, free flowing white to yellowish white, crystalline powder or granules

**FUNCTIONAL USES**

Yeast food

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

**A. pH**

7.2 - 8.5 (1 in 10 soln)

**B. Positive test for potassium**

Passes test

**C. Derivation to phenylhydrazide of gluconic acid**

Passes test

Proceed as directed in the specifications for Sodium Gluconate

---

* These specifications were prepared at the 23rd session of JECFA (1979) and published in FNP 12 (1979).

PURITY TESTS

* Loss on drying
  Not more than 3% (105°, 4 h, under vacuum)

* Arsenic
  Not more than 3 mg/kg (Method II)

* Lead
  Not more than 10 mg/kg

* Heavy metals
  Not more than 20 mg/kg
  Test a solution of 1 g of the sample in 25 ml of water as directed in the Limit Test (Method II)

* Reducing substances
  Not more than 0.5% calculated as D-glucose (Method I)

METHOD OF ASSAY

Flame spectrochemical analysis

Solution
- Sodium stock solution: Transfer 7.306 g of sodium chloride, previously dried at 125° for 30 min, and accurately weighed, to a 500-ml volumetric flask, add water to volume and mix. This solution contains 250 meq (equivalent to 5.76 g) of Na per 1000 ml.

- Potassium stock solution: Transfer 745.5 mg of potassium chloride, previously dried at 125° for 30 min, and accurately weighed, to a 1000-ml volumetric flask, add water to volume, and mix. This solution contains 10 meq (equivalent to 391 mg) of K per 1000 ml.

- Surfactant solution: Transfer 5 g of suitable nonionic surfactant to a 250-ml beaker, add 200 ml of water, and stir to dissolve. Quantitatively transfer this solution to a 500-ml volumetric flask, dilute to volume with water, and mix. (Note: To prevent foaming when using this solution, gently run the solution down the sides of the vessel, and use gentle action when mixing.)

- Diluted sodium solution: Transfer 50 ml of Sodium stock solution and 10 ml of Surfactant solution to a 100-ml volumetric flask, dilute to volume with water, and mix gently to prevent foaming.

Preparation
- Standard preparation: Transfer 5 ml of Potassium stock solution, 50 ml of Sodium stock solution, and 10 ml of Surfactant solution to a 500-ml volumetric flask, dilute to volume with water, and mix gently to prevent foaming. Each ml of this solution contains 3.910 μg of K.

- Assay preparation: Transfer about 120 mg of the sample, previously dried at 105° for 4 h under vacuum and accurately weighed, to a 100-ml volumetric flask, add water to volume, and mix. Filter a portion of the solution through Whatman No. 2V, or equivalent, filter paper, and transfer 2 ml of the filtrate to a 100-ml volumetric flask. To the flask add 50 ml of water, then add 20 ml of Diluted sodium solution, dilute to volume with water, and mix gently to prevent foaming.

CONCOMITANTLY, determine the emittance of the Standard preparation and of the Assay preparation, at 766 and 767 nm, with a suitable flame photometer, setting the instrument to zero emittance with a solution prepared by mixing 50.0 ml of Sodium stock solution and 10 ml of Surfactant solution and diluting to 500 ml with water and adjusting the instrument so that the Standard preparation gives a reading near mid-scale. Record the emittance of the Standard preparation as Ts and that of the Assay preparation as Tu. Calculate the weight, in mg, of C_{6}H_{11}KO_{3} in the sample taken by the formula:

\[
\frac{234.25 \times 5 \times 3.91 \times Tu}{39.10 \times Ts}
\]

in which 234.25 is the molecular weight of potassium gluconate, and 39.10 is the atomic weight of potassium.
POTASSIUM HYDROGEN CARBONATE*

SYNONYMS  
Potassium bicarbonate;  
INS No. 501(ii), EEC No. 501

DEFINITION

<table>
<thead>
<tr>
<th>Chemical names</th>
<th>Potassium hydrogen carbonate, potassium acid carbonate</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A.S. number</td>
<td>298-14-6</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>KHCO₃</td>
</tr>
<tr>
<td>Formula weight</td>
<td>100.11</td>
</tr>
<tr>
<td>Assay</td>
<td>Potassium Hydrogen Carbonate contains not less than 99.0% and not more than the equivalent of 101% of KHCO₃, calculated on the dried bases</td>
</tr>
</tbody>
</table>

DESCRIPTION

Odourless, colourless crystals or white powder or granules.

FUNCTIONAL USES

Alkali, leavening agent, buffer.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility  
Freely soluble in water. Insoluble in ethanol.

** B. Positive test for potassium  
To a 1 in 100 solution of the sample add 1 volume of saturated sodium hydrogen tartrate solution and 1 volume of ethanol and shake. A white crystalline precipitate is formed.

** D. Positive test for carbonate  
A 1 in 20 solution of the sample effervesces upon the addition of acetic acid TS

PURITY TESTS

** Loss on drying  
Not more than 0.25% (over silica gel, 4 h)

Normal carbonate  
Passes test  
See description under TESTS

** Arsenic  
Not more than 3 mg/kg  
Dissolve 1 g of the sample in 4 ml of dilute hydrochloric acid TS, and dilute to 35 ml with water. This solution meets the requirements of the Limit Test for Arsenic (Method II).

* These specifications were prepared at the 19th session of JECFA (1975) and published in NMRS 55B (1976).

TESTS

PURITY TESTS

Normal carbonate

Dissolve 1 g of the sample without agitation in 20 ml of water at a temperature not above 5°C. Add 2 ml of 0.1 N hydrochloric acid and 2 drops of phenolphthalein TS and observe immediately. The colour of the solution is not deeper than a faint pink.

* Heavy metals

Dissolve 2 g of the sample in 5 ml of water and 8 ml of dilute hydrochloric acid TS, boil gently for 1 minute, and dilute to 25 ml with water. This solution meets the requirements of the Limit Test for Heavy Metals (Method I) using 20 μg of lead ion (Pb) in the control (Solution A).

METHOD OF ASSAY

Dissolve about 4 g of the sample, accurately weighed, in 25 ml of water, add methylorange TS and titrate with 1 N sulfuric acid. Each ml of 1 N sulfuric acid is equivalent to 100.1 mg of KHCO₃.

POTASSIUM HYDROXIDE*

SYNONYMS
Caustic potash, Potassium hydrate; INS No.525, EEC No.525

DEFINITION
Chemical name
Potassium hydroxide
C.A.S. number
1310-58-3
Chemical formula
KOH
Molecular weight
56.11
Assay
Potassium Hydroxide contains not less than 85.0% of total alkali calculated as KOH

DESCRIPTION
White or nearly white pellets, flakes, sticks, fused masses or other forms

FUNCTIONAL USE
Alkali

CHARACTERISTICS

IDENTIFICATION TESTS
** A. Solubility
Very soluble in water. Freely soluble in ethanol.

B. Positive test for alkali
A 1 in 100 solution of the sample is strongly alkaline.

** C. Positive test for potassium
To a 1 in 100 solution of the sample add 1 volume of saturated sodium hydrogen tartrate solution and 1 volume of ethanol and shake. A white crystalline precipitate is formed.

PURITY TESTS

Water insoluble substances
A 1 in 20 solution of the sample is complete, clear, and colourless.

Carbonate
Not more than 3.5% (as K₂CO₃)
Each ml of 1 N sulfuric acid required between the phenolphthalein and methyl orange endpoints in the "METHOD OF ASSAY" is equivalent to 138.2 mg of K₂CO₃.

Arsenic
Not more than 3 mg/kg
See description under TESTS

* These specifications were prepared at the 19th session of JECFA (1975) and published in NMRS 55B (1976).

PURITY TESTS (continued)

**Lead**
Not more than 10 mg/kg
See description under TESTS

**Heavy metals**
Not more than 30 mg/kg
See description under TESTS

**Mercury**
Not more than 1 mg/kg
See description under TESTS

**TESTS**

* **Arsenic**
Dissolve 1 g of the sample in about 10 ml of water, cautiously neutralize to litmus paper with dilute sulfuric acid TS and dilute to 35 ml with water. This solution meets the requirements of the Limit Test for Arsenic (Method II).

* **Lead**
Dissolve 1 g of the sample in 5 ml of water, and neutralize to phenolphthalein TS with dilute hydrochloric acid TS and cool. This solution meets the requirements of the Limit Test for Lead.

* **Heavy metals**
Dissolve 0.67 g of the sample in a mixture of 5 ml of water and 5 ml of dilute hydrochloric acid TS. Heat to boiling, cool, dilute to 25 ml with water and filter. This solution meets the requirements of the Limit Test for Heavy Metals (Method I) using 20 Jg of lead ion (Pb) in the control (Solution A).

* **Mercury**
Transfer 2 g of the sample into a 50-ml beaker, dissolve in 10 ml of water, add 2 drops of phenolphthalein TS, and slowly neutralize, with constant stirring, with dilute hydrochloric acid solution (1 in 2). Add 1 ml of dilute sulfuric acid solution (1 in 5) and 1 ml of potassium permanganate solution (1 in 25), cover the beaker with a watch glass, boil for a few seconds and cool. This solution meets the requirements of the Limit Test for Mercury.

**METHOD OF ASSAY**
Dissolve about 1.5 g of the sample, accurately weighed, in 40 ml of recently boiled and cooled water, cool to 15°C, add phenolphthalein TS and titrate with 1 N sulfuric acid. At the discharge of the pink colour, record the volume of acid required, then add methyl orange TS and continue to titrate to a persistent pink colour. Record the total volume of acid required for the titration. Each ml of 1 N sulfuric acid is equivalent to 56.11 mg of total alkali, calculated as KOH.

POTASSIUM IODATE*

SYNONYM
INS No.917

DEFINITION
Chemical name
Potassium iodate
C.A.S. number
7758-05-6
Chemical formula
KIO₃
Formula weight
214.02
Assay
Content not less than 99.0% and not more than 101.0% of KIO₃ on the dried basis

DESCRIPTION
White, odourless crystalline powder

FUNCTIONAL USES
Flour treatment agent, oxidizing agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Soluble in water. Insoluble in ethanol.

** B. Positive test for potassium
A 1 in 20 solution of the sample imparts a violet colour to a non-luminous flame

** C. Positive test for iodate
To a 1 in 20 solution of the sample add one drop of starch TS and a few drops of 20% hypophosphorous acid. A transient blue colour appears

PURITY TESTS

** Loss on drying
Not more than 0.5% (150°C, 3 h)

Free acid or alkali
Passes test
See description under TESTS

Arsenic
Not more than 3 mg/kg
See description under TESTS

Heavy metals
Not more than 10 mg/kg
See description under TESTS

* These specifications were prepared at the 33rd session of JECFA (1988) and published in FNP 38 (1988).

TESTS

PURITY TESTS

Free acid or alkali
Dissolve by warming 5 g of the sample in 40 ml of freshly boiled and cooled water, cool and add 3 drops of phenolphthalein TS; observe colour:

(a) if the solution is pink, add 0.4 ml of 0.01 N hydrochloric acid. The pink colour disappears.
(b) if the solution is colourless, add 1.2 ml of 0.01 N sodium hydroxide. The solution turns pink.

Arsenic
Dissolve 1 g of the sample in a mixture of 5 ml hydrochloric acid and 5 ml of water, and evaporate the solution until crystals appears. Cool, dissolve the residue in water, and dilute to 35 ml. This solution meets the requirements of the Limit Test for Arsenic (Method II).

Heavy metals
Dissolve 2 g of the sample in 10 ml of water, add 10 ml of hydrochloric acid, and evaporate to dryness on a steam bath. Dissolve the residue in 10 ml of hydrochloric acid, again evaporate to dryness, and then dissolve the residue in 25 ml of water. Test this solution as directed in the Heavy Metals Limit Test (Method I).

METHOD OF ASSAY

Redox titration
Weigh accurately about 100 mg of the sample, previously dried at 105° for 3 h, and dissolve in 50 ml of water contained in a 250 ml glass-stoppered conical flask. Add 3 g of potassium iodide, followed by 3 ml of dilute hydrochloric acid (3 in 10), and stopper the flask. Allow the mixture to stand for 5 min., add 100 ml of cold water, and titrate the liberated iodine with 0.1 N sodium thiosulfate, adding starch TS as the end-point is approached. Perform a blank determination. Each ml of 0.1 N sodium thiosulfate is equivalent to 3.567 mg of KIO₃.

### SYNONYMS
INS No.326, EEC No. E326

### DEFINITION

<table>
<thead>
<tr>
<th>Chemical names</th>
<th>Potassium lactate, potassium 2-hydroxypropanoate</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A.S. number</td>
<td>996-31-6</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>( \text{C}_2\text{H}_5\text{KO}_3 )</td>
</tr>
<tr>
<td>Structural formula</td>
<td>( \text{CH}_3 - \text{CH} - \text{COOK} )</td>
</tr>
<tr>
<td></td>
<td>( \mid )</td>
</tr>
<tr>
<td></td>
<td>( \text{OH} )</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>128.17 (anhydrous)</td>
</tr>
<tr>
<td>Assay</td>
<td>Potassium lactate solution contains not less than 95% and not more than 110% of the labelled amount ( \text{C}_2\text{H}_5\text{KO}_3 ) and conform to the following specifications. The specification is based on a 60% w/w solution of ( \text{C}_2\text{H}_5\text{KO}_3 ) in water.</td>
</tr>
</tbody>
</table>

### DESCRIPTION
Slightly viscous, almost odourless clear liquid. Odourless, or with a slight, characteristic odour

### FUNCTIONAL USES
Antioxidant, synergist

### CHARACTERISTICS

### IDENTIFICATION TESTS

| A. Ignition | Ignite potassium lactate solution to an ash. The ash is alkaline, and an effervescence occurs when acid is added |
| B. Colour reaction | Overlay 2 ml of potassium lactate solution on 5 ml of a 1 in 100 solution of catechol in sulfuric acid. A deep red colour is produced at the zone of contact |
| ** C. Positive test for potassium | To 2 ml of potassium lactate solution add 5 ml of dilute sulfuric acid and 2 ml of potassium permanganate TS, and heat. The odour of acetaldehyde is evolved |

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* These specifications were prepared at the 18th meeting of JECFA (1974) and published in NMRS 54B (1975).

PURITY TESTS (calculated on the 60% solution basis)

* **Arsenic**

Not more than 3 mg/kg (Method II)

* **Lead**

Not more than 10 mg/kg
Test 1 g of the sample using 10 μg lead ion (Pb) in the control as directed in the Limit Test

* **Heavy metals**

Not more than 20 mg/kg
Test 1 g of the sample as directed in the Limit Test

* **Acidity**

Dissolve 1 g of potassium lactate solution in 20 ml of water, add 3 drops of phenolphthalein TS and titrate with 0.1 N sodium hydroxide. Not more than 0.2 ml should be required

* **Reducing substances**

Potassium lactate solution shall not cause any reduction of Fehling’s solution

METHOD OF ASSAY

Weigh accurately about 0.6 g of potassium lactate solution into a small beaker, and evaporate to dryness. Add to the residue 60 ml of a 1 in 5 mixture of acetic anhydride in glacial acetic acid, and stir until the residue is completely dissolved. Add crystal violet TS, and titrate with 0.1 N perchloric acid to a blue end-point. Perform a blank determination, and make any necessary correction. Each ml of 0.1 N perchloric acid is equivalent to 12.82 mg of C₃H₅KOO₃.

POTASSIUM METABISULFITE*

SYNONYMS
INS No. 224, EEC No. E224

DEFINITION

Chemical names
Potassium disulfite, potassium pentaoxodisulfate

C.A.S. number
16731-55-8

Chemical formula
K₂S₂O₅

Formula weight
222.33

Assay
Contains not less than 90% of K₂S₂O₅.

DESCRIPTION
Colourless free-flowing crystals, crystalline powder, or granules, usually having and odour of sulfur dioxide.

FUNCTIONAL USES
Antimicrobial preservative, antibrowning agent.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Soluble in water, insoluble in ethanol.

B. Positive test for potassium
To 1 volume of a 1% solution of the sample add 1 volume of saturated sodium hydrogen tartrate solution and 1 volume of ethanol and shake. A white crystalline precipitate is formed.

C. Positive test for sulfite
Passes test
See description under TESTS

PURITY TESTS

Water insolubles
20 g of the sample, dissolved in 200 ml of water, should give a clear solution with only a trace of suspended matter.

Thiosulfate
Not more than 0.1%.
A 10% solution of the sample should remain clear on acidification with sulfuric or hydrochloric acid.

Iron
Not more than 5 mg/kg
See description under TESTS.

** Selenium
Not more than 30 mg/kg
Determine as directed in Method I under the Limit Test for Selenium using 200 mg of the sample and 100 mg of magnesium oxide for sample preparation.

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

PURITY TESTS (continued)

* **Arsenic**

Not more than 3 mg/kg
See description under TESTS.

* **Heavy Metals**

Not more than 10 mg/kg
See description under TESTS

TESTS

IDENTIFICATIONS TESTS

C. Positive test for sulfite

Add to the sample dilute hydrochloric acid. The sulfur dioxide evolved may be recognized by: its odour; the blackening of filter paper moistened with mercurous nitrate TS; and the development of a blue colour on filter paper treated with potassium iodate and starch TS.

PURITY TESTS

**Iron**

To 0.5 g of the sample, weighed to the nearest mg, add 2 ml of hydrochloric acid, and evaporate to dryness on a steam bath. Dissolve the residue in 2 ml of hydrochloric acid and 20 ml of water, and add a few drops of bromine TS. Boil the solution to remove the bromine, cool, dilute with water to 25 ml, and then add 50 mg of ammonia persulfate and 5 ml of ammonium thiocyanate TS. Any red colour produced should not exceed that of a control solution made the same way as the test solution, but instead of the sample containing 0.25 ml of Iron Standard Solution (2.5 μg Fe).

* **Arsenic**

Dissolve 1 g of the sample, weighed to the nearest mg, in 10 ml of water in a 150-ml beaker. Cautiously add 10 ml of nitric acid and 5 ml of sulfuric acid, and evaporate on a steam bath to a volume of about 5 ml. Place the beaker on a hot plate, and heat just to dense fumes of sulfur trioxide. Cool, cautiously wash down the side of the beaker with about 10 ml of water, and again heat to dense fumes. Cool repeat the washing and fuming procedure, then cool and dilute to 35 ml with water. This solution meets the requirements of the Limit Test for Arsenic (Method II), omitting the addition of 20 ml of dilute sulfuric acid.

* **Heavy metals**

Dissolve 2 g of the sample, weighed to the nearest g, in 10 ml of water. Add 5 ml of hydrochloric acid, evaporate to dryness on a steam bath and dissolve the residue in 25 ml of water. This solution meets the requirements of the Limit Test for Heavy Metals (Method I) using 20 μg of lead ion (Pb) in the control (Solution A).

METHOD OF ASSAY

Redox titration

Weigh 250 mg of the sample, add to 50.0 ml of 0.1 N iodine in a glass stoppered flask, and stopper the flask. Allow to stand for 5 min, add 1 ml of dilute hydrochloric acid TS and titrate the excess iodine with 0.1 N sodium thiosulfate, using starch TS as the indicator. Each ml of 0.1 N iodine is equivalent to 5.558 mg of K₂S₂O₃ or 3.203 mg of SO₂.

POTASSIUM NITRATE*

SYNONYM

INS No. 252, EEC No. E252

DEFINITION

Chemical name
Potassium nitrate

C.A.S. number
7757-79-1

Chemical formula
KNO₃

Formula weight
101.11

Assay
Content not less than 99% of KNO₃ on the dried basis

DESCRIPTION

Colourless, odourless, transparent prisms, or white granular or crystalline powder, having a cooling, saline, pungent taste.

FUNCTIONAL USES

Antimicrobial preservative, colour fixative.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Soluble in water, slightly soluble in ethanol and in glycerol.

** B. Positive test for potassium
Passes test

** C. Positive test for nitrate
Passes test

PURITY TESTS

** Loss on drying
Not more than 1% (105°C, 4 h)

** pH
4.5-8.5 (1 in 20 soln)

Nitrite
Not more than 20 mg/kg
See description under TESTS

Arsenic
Not more than 3 mg/kg
See description under TESTS

* These specifications were prepared at the 27th session of JECFA (1983) and published in FNP 28 (1983).

**PURITY TESTS** (continued)

* **Lead**
  Not more than 10 mg/kg
  Test a solution of 1 g of the sample in 10 ml of water as directed in the Limit Test using 10 μg of lead ion in the control.

* **Heavy metals**
  Not more than 20 mg/kg
  Test a solution of 2 g of the sample in 25 ml of water as directed in the Limit Test for Heavy Metals (Method I), using 20 μg of lead ion and 1 g of the sample in the control.

**TESTS**

**PURITY TESTS**

* **Arsenic**
  Dissolve 1 g of the sample in 3 ml of water, add 2 ml of sulfuric acid, and evaporate to strong fumes of sulfur trioxide. Cool, wash down the side of container with water, and heat again to strong fuming. Repeat the washing and fuming three more times, then cool and dilute to 35 ml with water. Test this solution as directed in the Limit Test (Method II).

**Determination of nitrite**

**Principle**
Excess of 0.1 N ferrous ammonium sulfate is added to a mixture of a solution of the sample and a measured amount of 0.1 N potassium permanganate, and the excess is back titrated with 0.1 N potassium permanganate.

**Apparatus**
- P Glass filter crucible, 1 G 4
- P Volumetric flasks, 250 ml, 1000 ml
- P Mortar
- P Burettes, pipettes

**Reagents**
- Sulfuric acid, conc., sp.gr. 1.84
- Potassium permanganate, 0.1 N: 3.3 g of potassium permanganate (KMnO₄) are dissolved in an Erlenmeyer flask in 1050 ml of water. The solution is heated to the boiling for 30 min. After cooling, the neck of the flask is covered with a beaker, and the solution is put to stand in a dark place for a few days. A glass filter crucible 1G4 and a 1-L brown bottle with glass stopper are cleaned with a chromium-sulfuric acid mixture, and rinsed acid-free with water. The potassium permanganate solution is filtered through the glass filter crucible. The first about 25 ml passed through the filter are rejected; the rest is collected in the brown bottle. After use the glass filter crucible is most easily cleaned with dilute sulfuric acid admixed a little hydrogen peroxide.

Determination of the normality of the potassium permanganate solution:

Sodium oxalate, "Sorensen" (Na$_2$C$_2$O$_4$) is dried at 105° to constant weight. About 0.25 g of the dried sodium oxalate is weighed with an accuracy of 0.1 mg and transferred to a mixture of 10 ml of sulfuric acid and 250 ml of water, which has previously been kept boiling for 10 min. and then cooled to room temperature. About 30 ml of potassium permanganate solution (about 75% of the amount required for the titration) are added to the sodium oxalate solution from a burette at the rate of 25-30 ml per min. After standing till the pink colour has disappeared, the solution has heated to about 60°, and the titration is continued until a faint pink colour remains constant for 30 sec. The last few ml are added dropwise, each drop being allowed to decolorize before adding the next drop. Then the exact quantity of potassium permanganate solution required to produce the same pink colour in the same quantity of boiled sulfuric acid solution (see above) is determined; this blank value, usually amounting to 0.03-0.05 ml, is deducted from the value used in titrating the sodium oxalate.

$$\text{Normality} x = \frac{\text{g of sodium oxalate} \times 1000}{67 \times \text{ml of potassium permanganate}}$$

The normality of the solution should be frequently controlled.

- Ferrous ammonium sulfate, 0.1 N: 40 g of ferrous ammonium sulfate (FeSO$_4$ · (NH$_4$)$_2$SO$_4$ · 6H$_2$O) are dissolved in a cooled mixture of 30 ml of concentrated sulfuric acid and 300 ml of water; the solution is diluted to one litre with water, and mixed. 4.00 ml of the solution are measured with a pipette; 25 ml of water and 3 ml of phosphoric acid are added, when the solution is titrated with 0.1 N potassium permanganate to constant pink colour.

$$\text{Normality} y = \frac{\text{number of ml KMnO}_4 \times X}{40.00}$$

where X = the normality of the potassium permanganate solution.

- Phosphoric acid, 85%

- Zimmermann-Reinhardt solution: 70 g of manganous sulfate (MnSO$_4$ · 4H$_2$O) are dissolved in 500 ml of water, 125 ml of phosphoric acid are added with stirring, and diluted to one litre with water.

Procedure

Sample solution

"Nitrite salt" (about 0.5% NaNO$_2$ or KNO$_2$).

The sample is finely pulverized in a mortar. 50.00 g of the pulverized and thoroughly mixed sample are transferred to a 250 ml volumetric flask, which is made up to the mark with water and mixed. For each titration 50 ml of the solution corresponding to 10.00 g of the sample are pipetted.
Determination of nitrite (continued)

"Nitrite salpetre" (about 5% NaNO₂ or KNO₂).
The sample is finely pulverized in a mortar. 25.00 g of the pulverized and thoroughly mixed sample are transferred to a 250 ml volumetric flask, which is made up to the mark with water, and mixed. For each titration 25 ml of the solution corresponding to 2.500 g of the sample are pipetted.

Titration
5 ml of sulfuric acid are added to 300 ml of water and then immediately the amount of 0.1 N potassium permanganate required to produce a pink colour which keeps constant for 2 min. This volume of potassium permanganate is not noted. Then, from a pipette 25 ml of 0.1 N potassium permanganate in titrating "nitrite salt" and 50 ml of 0.1 N potassium permanganate in titrating "nitrite salpetre" are added. The solution is mixed, and the above mentioned amount of sample solution is added slowly from a pipette with stirring (for "nitrite salt" 50 ml - for "nitrite salpetre" 25 ml), the point of pipette being held below the surface of the liquid. After standing for 5 min, 25 ml of Zimmermann-Reinhardt solution and excess of 0.1 N ferrous ammonium sulfate are added, which causes the liquid to decolorize. The excess of ferrous ammonium sulfate is titrated back with 0.1 N potassium permanganate.

Calculation
1 ml of 1.000 N KMnO₄ corresponds to 0.03451 g of NaNO₂ and 0.04255 g of KNO₂, respectively.

\[
\begin{align*}
\% \text{NaNO}_2 &= \frac{[(a + c) \cdot x + b \cdot y] \cdot 0.0345 \cdot 100}{d} \\
\% \text{KNO}_2 &= \frac{[(a + c) \cdot x + b \cdot y] \cdot 0.04255 \cdot 100}{d}
\end{align*}
\]

where,

- \(a\) = number of ml of 0.1 N potassium permanganate added.
- \(b\) = number of ml of 0.1 N ferrous ammonium sulfate added.
- \(c\) = number of ml of potassium permanganate used in back titrating the excess of ferrous ammonium sulfate.
- \(d\) = number of g of sample taken out for the titration (for "nitrite salt" 10.00 g, for "nitrite salpetre" 2.500 g).
- \(x\) = normality of potassium permanganate
- \(y\) = normality of ferrous ammonium sulfate
METHOD OF ASSAY

Weigh accurately about 0.4 g of the sample previously dried at 105° for 4 h. and dissolve in about 300 ml of water in a 500 ml round flask. Add 3 g of a powder of Devarda's alloy and 15 ml of sodium hydroxide solution (2 in 5), and connect with a spray-preventing device and condenser to the flask. Transfer 50 ml of 0.1 N sulfuric acid in a receptacle, allow to stand for 2 h., distil to obtain 250 ml of the distillate, and titrate the excess sulfuric acid with 0.1 N sodium hydroxide, using 3 drops of methyl red/Methylene blue TS as the indicator. Perform a blank test in the same manner as the sample to make any necessary correction.

Each ml of 0.1 N sulfuric acid is equivalent to 10.11 mg of KNO₃.
POTASSIUM NITRITE*  
(Tentative)

SYNONYMS  INS No. 249, EEC No. E249

DEFINITION
Chemical name Potassium nitrite  
C.A.S. number 7758-09-0  
Chemical formula KNO₂  
Formula weight 85.11  
Assay After drying over silica gel for 4 h, contains not less than 90% of KNO₂

DESCRIPTION  Potassium Nitrite occurs as small, white or slightly yellow, deliquescent granules or rods

FUNCTIONAL USE  Colour fixative

CHARACTERISTICS

IDENTIFICATION TESTS
** A. Solubility  Freely soluble in water. Sparingly soluble in ethanol.
** B. Positive test for potassium  Passes test  
** C. Positive test for nitrite  Passes test

PURITY TESTS
** Loss on drying  Not more than 3% after drying over silica gel for 4 h
** pH  6.0 - 9.0 (1 in 20 soln)
** Arsenic  Not more than 3 mg/kg  
See description under TESTS
** Lead  Not more than 10 mg/kg  
See description under TESTS
** Heavy metals  Not more than 20 mg/kg  
See description under TESTS

* These specifications were prepared at the 20th session of JECFA (1976) and published in FNP 4 (1978).
TESTS

PURITY TESTS

* Arsenic
Dissolve 1 g of the sample, weighed to the nearest mg, in 10 ml of dilute sulfuric acid TS. Boil gently for 1 min, cool, and dilute to 35 ml with water. This solution meets the requirements of the Limit Test for Arsenic (Method II).

* Lead
A solution of 1 g of the sample, weighed to the nearest mg, in 10 ml of water, meets the requirements of the Limit Test for Lead, using 10 µg of lead ion (Pb) in the control.

* Heavy metals
Dissolve 1 g of the sample, weighed to the nearest mg, in 15 ml of dilute hydrochloric acid TS, and evaporate to dryness on a steam bath. To the residue add 2 ml of hydrochloric acid, again evaporate to dryness, and dissolve the residue in 25 ml of water. This solution meets the requirements of the Limit Test for Heavy Metals (Method I), using 20 µg of lead ion (Pb) in the control (Solution A).

METHOD OF ASSAY

Weigh, to the nearest mg, 1 g of the sample, previously dried over silica gel for 4 h, dissolve in water and make to 100.0 ml. Pipette 10.0 ml of this solution into a mixture of 50.0 ml of 0.1 N potassium permanganate, 100 ml of water and 5 ml of sulfuric acid, keeping the tip of the pipette well below the surface of the liquid. Warm the solution to 40°, allow it to stand for 5 min and add 25.0 ml of 0.1 N oxalic acid. Heat the mixture to about 80° and titrate with 0.1 N potassium permanganate.

\[
\% \text{ KNO}_2 = \frac{(25 + X)}{W} \times 4.256
\]

in which:
- \( X \) = ml of 0.1 N potassium permanganate used for titration
- \( W \) = weight (in grams) of the sample.

POTASSIUM PERSULFATE*  
(Tentative)

SYNONYM
INS No. 922

DEFINITION

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Potassium persulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A.S. number</td>
<td>7727-21-1</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>K₂S₂O₈</td>
</tr>
<tr>
<td>(empirical)</td>
<td></td>
</tr>
<tr>
<td>Formula weight</td>
<td>270.32</td>
</tr>
<tr>
<td>Assay</td>
<td>The product contains not less than 95% of K₂S₂O₈</td>
</tr>
</tbody>
</table>

DESCRIPTION
Potassium persulfate occurs as colourless or white, odourless crystals

Caution: Powerful oxidizing substance

FUNCTIONAL USE
Strengthening agent for flour

* These specifications were prepared at the 9th session of JECFA (1965) and published in NMRS 40ABC (1969).
POTASSIUM POLYPHOSPHATES*

SYNONYMS

Potassium metaphosphate; INS No 452 (ii), EEC No. E450c

DEFINITION

Potassium polyphosphate is a heterogeneous mixture of potassium salts of linear condensed polyphosphoric acids of general formula $H_{n+2}P_2O_{3n+1}$, where "n" is not less than 2

Chemical names

Potassium metaphosphate, potassium polymetaphosphate, potassium polyphosphate

C.A.S. number

7790-53-6

Assay

Content not less than 53.5% and not more than 61.5% of $P_2O_5$ on the ignited basis

DESCRIPTION

Odourless, colourless or white glassy masses, fragments, crystals or powder

FUNCTIONAL USES

Emulsifier, moisture-retaining agent, sequestrant, texturizer

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility

1 g dissolves in 100 ml of a 1 in 25 soln of sodium acetate

B. Gelatinous mass formation

Finely powder about 1 g of the sample, and add it slowly to 100 ml of a 1 in 50 solution of sodium chloride while stirring vigorously. A gelatinous mass is formed.

** C. Positive test for phosphate and potassium

Mix 0.5 g of the sample with 10 ml of nitric acid and 50 ml of water, boil for about 30 min, and cool. The resulting solution is used for the positive test for phosphate and potassium.

PURITY TESTS

** Loss on ignition

Not more than 2 % after drying (105°C, 4 h) followed by ignition at 550°C for 30 min

** Cyclic phosphate

Not more than 8.0%

Fluoride

Not more than 10 mg/kg

See description under TESTS

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* These specifications were prepared at the 26th session of JECFA (1982) and published in FNP 25 (1982).

**PURITY TESTS (continued)**

* Arsenic

Not more than 3 mg/kg
Dissolve 1 g of the sample in 15 ml of dilute hydrochloric acid TS, add 20 ml of water. Test this solution as directed in the Limit Test (Method II).

* Lead

Not more than 10 mg/kg
Dissolve 0.5 g of the sample in 10 ml of dilute hydrochloric acid TS, add 10 ml of water and neutralize to phenolphthalein TS by the addition of strong ammonia TS. Test this solution using 5 mg of lead ion (Pb) in the control as directed in the Limit Test.

* Heavy metals

Not more than 20 mg/kg
Warm 1 g of the sample with 10 ml of dilute hydrochloric acid TS until no more dissolves, dilute with water to 25 ml, and filter. Test this solution as directed in the Limit Test (Method I).

**TESTS**

**PURITY TESTS**

* Fluoride

**Thorium Nitrate Colorimetric Method**

Place 5 g of the sample, 25 ml of water, 50 ml of perchloric acid, 5 drops of silver nitrate solution (1 in 2), and a few glass beads in a 250-ml distilling flask connected with a condenser and carrying a thermometer and capillary tube, both of which must extend into the liquid. Connect a small dropping funnel, filled with water, or a steam generator to the capillary tube. Support the flask on an asbestos mat with a hole which exposes about one-third of the flask to the flame. Distil into a 250-ml flask until the temperature reaches 135°.

Add water from the funnel or introduce steam through the capillary to maintain the temperature between 135° and 140°. Continue the distillation until 225-240 ml has been collected, then dilute to 250 ml with water, and mix.

Place a 50-ml aliquot of this solution in a 100-ml Neustor tube. In another similar Neustor tube place 50 ml of water as a control. Add to each tube 0.1 ml of filtered solution of sodium alizarinsulfonate (1 in 1,000) and 1 ml of freshly prepared hydroxylamine hydrochloride solution (1 in 4,000), and mix well. Add, dropwise, and with stirring, 0.05 N sodium hydroxide to the tube containing the distillate until its colour just matches that of the control, which is faintly pink. Then add to each tube exactly 1 ml of 0.1 N hydrochloric acid, and mix well. From a buret, graduated in 0.05-ml, add slowly to the tube containing the distillate enough thorium nitrate solution (1 in 4,000) so that, after mixing, the colour of the liquid just changes to a faint pink. Note the

---

volume of the solution added, add exactly the same volume to the control, and mix. Now add to the control sodium fluoride TS (10 \( \mu \text{g} \) F per ml) from a buret to make the colours of the two tubes match after dilution to the same volume. Mix well, and allow all air bubbles to escape before making the final colour comparison. Check the end-point by adding 1 or 2 drops of sodium fluoride TS to the control. A distinct change in colour should take place. Note the volume of sodium fluoride added. The volume of sodium fluoride TS required for the control solution should not exceed 1.0 ml.

Mix about 300 mg of the sample, accurately weighed, with 15 ml of nitric acid and 30 ml of water, boil for 30 min, and dilute with water to about 100 ml. Heat at 60°, add an excess of ammonium molybdate TS, and heat at 50° for 30 min. Filter, and wash the precipitate with dilute nitric acid (1 in 36 soln), followed by potassium nitrate solution (1 in 100 soln) until the filtrate is no longer acid to litmus. Dissolve the precipitate in 50 ml of 1 N sodium hydroxide, add phenolphthalein TS, and titrate the excess sodium hydroxide with 1 N sulfuric acid. Each ml of 1 N sodium hydroxide is equivalent to 3.086 mg of P\(_2\)O\(_5\).

POTASSIUM SACCHARIN*

SYNONYM
INS No. 954

DEFINITION

Chemical names
Potassium salt of 1,2-benzisothiazole-3(2H)-one-1,1-dioxide monohydrate, 3-oxo-2,3-dihydrobenzo[d]isothiazol-1,1-dioxide monohydrate, 2,3-dihydro-3-oxobenzisulfonazole monohydrate; potassium o-benzosulfinamide.

C.A.S. number
10332-51-1

Chemical formula
C₇H₆KNO₃S · H₂O

Structural formula

![Structural formula of potassium saccharin](image)

Molecular weight
239.77

Assay
Content not less than 99% and not more than 101% of C₇H₆KNO₃S on the dried basis

DESCRIPTION
White crystals or a white, crystalline powder, odourless or with a faint, aromatic odour having a sweet taste even in very dilute solutions. About 500 times as sweet as sucrose in dilute solutions.

FUNCTIONAL USE
Sweetening agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Freely soluble in water. Sparingly soluble in ethanol

B. Melting range
Passes test
Proceed as directed in the specifications for Sodium Saccharin.

C. Derivation to salicylic acid
Passes test
Proceed as directed in the specifications for Saccharin.

* These specifications were prepared at the 28th session of JECFA (1984) and published in FNP 31/2 (1984).

IDENTIFICATION TESTS (continued)

D. Derivation to fluorescent substance

E. Positive test for Potassium

* E. Positive test for Potassium

PURITY TESTS

* Loss on drying

Acidity and alkalinity

* Arsenic

* Selenium

* Heavy metals

Benzoic and salicylic acid

* Readily carbonizable substances

Toluenesulfonamides

TESTS

PURITY TESTS

Benzoin and salicylic acid

METHOD OF ASSAY

Dissolve about 0.3 g of previously dried sample, accurately weighed, in 20 ml of glacial acetic acid. Add 2 drops of crystal violet-glacial acetic acid TS as indicator, and titrate with 0.1 N perchloric acid. End-point is where violet colour of the solution changes to green, via blue. Perform a blank determination, and make any necessary correction. Each ml of 0.1 N perchloric acid is equivalent to 22.18 mg of C_7H_4KNO_3S.

POTASSIUM SODIUM L(+)-TARTRATE*

SYNONYMS
Rochelle salt, Seignette salt, Potassium sodium dextro-tartrate; INS No. 337, EEC No. E337

DEFINITION
Chemical names
Potassium sodium L-tartrate, potassium sodium (+)-tartrate
Potassium sodium (+)-2,3-dihydroxybutanedioic acid

C.A.S. number
304-59-6

Chemical formula
C₆H₇KNaO₄·4H₂O

Structural formula

\[
\text{COOK}
\]

\[
\text{H} - \text{C} - \text{OH}
\]

\[
\text{HO} - \text{C} - \text{H}
\]

\[
\text{COONa}
\]

Molecular weight
282.23

Assay
After drying at 150° for 3 h, it contains not less than 99% of C₆H₇O₄KNa

DESCRIPTION
Potassium Sodium L(+)-Tartrate occurs as colourless crystals, or as a white, crystalline powder, having a cooling saline taste

FUNCTIONAL USES
Sequestrant, stabilizer in cheese products, minced meat, and sausage casings

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
One gram is soluble in 1 ml of water. Insoluble to ethanol.

** B. Positive test for tartrate
On ignition, potassium sodium tartrate emits the odour of burning sugar and leaves a residue which is alkaline to litmus and effervesces with acids.

** C. Positive test for tartrate
Heat a few mg of potassium sodium tartrate on a steam bath with 2 ml of sulfuric acid containing 0.5% pyrogallol. An intense violet colour is produced.

* These specifications were prepared at the 7th session of JECFA (1963) and published in NMRS 35 (1964).

IDENTIFICATION TESTS (continued)

D. Positive test for sodium

A solution of potassium sodium tartrate, acidified with dilute acetic acid TS, filtered if necessary, and treated with uranyl zinc acetate TS, yields a yellow crystalline precipitate within a few minutes.

E. Positive test for potassium

A solution of potassium sodium tartrate acidified with dilute hydrochloric acid TS gives with platinic chloride TS a yellow crystalline precipitate which on ignition leaves a residue of potassium chloride and platinum.

PURITY TESTS

* Loss on drying

Not more than 26.0% and not less than 21.0% after drying at 150° for 3 h

* pH

6.5 - 7.5 (1 in 10 soln)

* Arsenic

Not more than 3 mg/kg (Method II)

* Lead

Not more than 5 mg/kg

* Heavy metals

Not more than 20 mg/kg (Method II)

* Oxalate

Add 3 drops of dilute acetic acid TS and 2 ml of calcium chloride TS to 10 ml of a 10% solution of potassium sodium tartrate. No turbidity is produced within 1 h.

METHOD OF ASSAY

Potassium and sodium analysis on ash

Weigh 1.500 g of potassium sodium tartrate, previously dried at 150° for 3 h, into a tared porcelain crucible and ignite. Heat gently at first, until the salt is thoroughly carbonized, protecting the carbonized salt from contact with the flame at all times. The final temperature must not be above that of a dull red heat. Cool the crucible, place in a glass beaker, and break up the carbonized mass with a glass rod. Without removing the glass rod or the crucible, add 50 ml of water, 50 ml of 0.5 N sulfuric acid, cover the beaker, and boil the solution for 30 min. Filter, and wash with hot water until the last washing is neutral to litmus. Cool the combined filtrate and washings, add methyl orange TS, and titrate the excess acid with 0.5 N sodium hydroxide. Each ml of 0.5 N sulfuric acid is equivalent to 0.05254 g of C₂H₄KNaO₆.

**POTASSIUM SORBATE**

**SYNONYMS**

INS No. 202, EEC No. E202

**DEFINITION**

Chemical name

Potassium sorbate, potassium (E,E)-2,4-hexadienoate, potassium salt of trans, trans 2,4-hexadienoic acid.

C.A.S. number

24634-61-5

Chemical formula

C_6H_6KO_2

Structural formula

H
H
H

H
H

COOK

**DESCRIPTION**

White or yellowish-white crystals or crystalline powder.

**FUNCTIONAL USES**

Antimicrobial preservative, fungistatic agent.

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

**A. Solubility**

Freely soluble in water, soluble in ethanol.

**B. Positive test for potassium**

Passes test See description under TESTS

**C. Positive test for sorbate**

Melting range of sorbic acid derived from the sample is between 130° to 135°. See description under TESTS.

**D. Positive test for double bond**

To 2 ml of a 1 in 10 solution of the sample add a few drops of bromine TS. The colour of the bromine disappears.

**PURITY TESTS**

**Loss on drying**

Not more than 1% after drying at 105° for 3 h.

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* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

PURITY TESTS (continued)

**Acidity or alkalinity**
Not more than about 1% (as sorbic acid or \( K_2CO_3 \))
See description under TESTS

* **Arsenic**
Not more than 3 mg/kg
A sample solution prepared as directed for organic compounds meets the requirements of the Limit Test for Arsenic (Method II).

* **Heavy metals**
Not more than 10 mg/kg
Test 2 g of the sample as directed in Method II under the Limit Test for Heavy Metals using 20 \( \mu g \) of lead ion (Pb) in the control (Solution A).

**Aldehydes**
Not more than 0.1% (as formaldehyde)
See description under TESTS.

TESTS

IDENTIFICATION TESTS

* **B. Positive test for potassium**
To 1 volume of a 1% solution of the sample add 1 volume of saturated sodium hydrogen tartrate solution and 1 volume of ethanol and shake. A white crystalline precipitate is formed.

* **C. Positive test for sorbate**
Acidify a solution of the sample with dilute hydrochloric acid TS. Collect the precipitated sorbic acid on a filter paper, wash free of chloride with water and dry under vacuum over sulfuric acid. Determine the melting range as directed in the General Methods.

PURITY TESTS

**Acidity or alkalinity**
Dissolve 1.1 g of the sample in 20 ml of water and add 3 drops of phenolphthalein TS. If the solution is colourless, titrate with 0.1 N sodium hydroxide to a pink colour that persists for 15 sec. Not more than 1.1 ml should be required. If the solution is pink in colour titrate with 0.1 N hydrochloric acid. Not more than 0.8 ml should be required to discharge the pink colour.

**Aldehydes**
To 5 ml of a 0.3% solution of the sample add 2.5 ml of Schiff's reagent TS and allow to stand for 10 - 15 min. Compare the colour with that produced by 5 ml of a control solution containing 15 \( \mu g \) of formaldehyde and the same amount of Schiff's reagent and tested under the same conditions as the test solution. The colour of the test solution should not be more intense than that of the control solution.

METHOD OF ASSAY

**Non-aqueous titration**
Weigh, to the nearest 0.1 mg, 0.25 g of the sample, previously dried at 105° for 3 h. Dissolve in 36 ml of glacial acetic acid and 4 ml acetic anhydride in a 250-ml glass-stoppered flask, warming to effect solution. Cool to room temperature, add 2 drops of crystal violet TS and titrate with 0.1 N perchloric acid in glacial acetic acid to a blue-green end point which persists for at least 30 sec. Perform a blank determination and make any necessary correction. Each ml of 0.1 N perchloric acid is equivalent to 15.02 mg of \( C_4H_4KO_2 \).

POTASSIUM SULFATE*

SYNONYMS

INS No. 515, EEC No. 515

DEFINITION

Chemical name
Potassium sulfate

C.A.S. number
7778-80-5

Chemical formula
K₂O₆S

Structural formula
K₂SO₄

Formula weight
174.25

Assay
Content not less than 99.0% of K₂SO₄.

DESCRIPTION

Colourless or white crystals or crystalline powder having a bitter, saline taste.

FUNCTIONAL USES

Salt substitute

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Freely soluble in water, insoluble in ethanol.

** B. pH
5.5 - 8.5 (1 in 20 soln)

** C. Positive test for potassium
Passes test

** D. Positive test for sulfate
Passes test

PURITY TESTS

** Selenium
Not more than 30 mg/kg
Test 0.2 g of the sample as directed in the Limit Test (Method II).

** Arsenic
Not more than 3 mg/kg
Test a solution of 1 g of the sample in 35 ml of water as directed in the Limit Test (Method II).

* These specifications were prepared at the 29th session of JECFA (1985) and published in FNP 34 (1986).

PURITY TESTS (continued)

* **Heavy metals**

Not more than 10 mg/kg

Test a solution of 3 g of the sample in 25 ml of water as directed in the Limit Test (Method I), using 2 ml of Standard lead solution (20 μg of Pb) and 1 g of the sample in the control (Solution A).

**METHOD OF ASSAY**

Weigh accurately about 0.5 g of the sample, dissolve in 200 ml of water, add 1 ml of hydrochloric acid, and heat to boiling. Gradually add, in small portions and while stirring constantly, an excess of hot barium chloride TS (about 8 or 9 ml), and heat the mixture on a steam bath for 1 h. Collect the precipitate on a filter, wash until free from chloride, dry, ignite, and weigh. The weight of the barium sulfate so obtained, multiplied by 0.7466, indicates its equivalent of K₂SO₄.

POTASSIUM SULFITE*

SYNONYM
INS No. 225

DEFINITION

Chemical name
Potassium sulfite

C.A.S. number
10117-38-1

Chemical formula
K₂O₇S

Formula weight
158.25

Assay
Not less than 90.0% of K₂SO₅

DESCRIPTION
A white, odourless, granular powder

FUNCTIONAL USES
Preservative, antioxidant

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Freely soluble in water. Slightly soluble in ethanol

** B. Positive test for potassium
Passes test

** C. Positive test for sulfite
Passes test

PURITY TESTS

Alkalinity
0.25 - 0.45% as K₂CO₃

** Selenium
Not more than 30 mg/kg
Test 0.2 g of the sample as directed in the Limit Test (Method I), using 0.1 g of magnesium oxide.

Arsenic
Not more than 3 mg/kg
See description under TESTS

Heavy metals
Not more than 10 mg/kg
See description under TESTS

* These specifications were prepared at the 29th session of JECFA (1985) and published in FNP 34 (1986).

TESTS

PURITY TESTS

Alkalinity
Dissolve 1 g of the sample in 20 ml of water, add 25 ml of 3% hydrogen peroxide, previously neutralized to methyl red TS, mix thoroughly, cool to room temperature, and titrate with 0.02 N hydrochloric acid. Perform a blank determination using 25 ml of neutralized hydrogen peroxide solution. Each ml of 0.02 N hydrochloric acid is equivalent to 1.38 mg of K₂CO₃.

* Arsenic
Dissolve 1 g of the sample in 10 ml of water in a 150 ml beaker, cautiously add 10 ml of nitric acid and 5 ml of sulfuric acid, and evaporate on a steam bath to a volume of about 5 ml. Place the beaker on a hot plate, and heat just to dense fumes of sulfur trioxide. Cool, cautiously wash down the side of the beaker with about 10 ml of water, and again heat to dense fumes. Cool, repeat the washing and fuming procedure, and cool again. Test this solution as directed in the Limit Test (Method II), omitting the addition of 20 ml of dilute sulfuric acid (1 in 5).

* Heavy metals
Dissolve 2 g of the sample in 10 ml of water, add 4 ml of hydrochloric acid, and evaporate to dryness on a steam bath. To the residue add 5 ml of hot water and 1 ml of hydrochloric acid, and again evaporate to dryness. Dissolve the residue in water and dilute to 25 ml. Test this solution as directed in the Limit Test (Method I).

METHOD OF ASSAY

Redox titration
Weigh accurately about 0.75 g of the sample, dissolve in a mixture of 100 ml of 0.1 N iodine and 5 ml of dilute hydrochloric acid TS, and titrate the excess iodine with 0.1 N sodium thiosulfate, adding starch TS as the indicator. Each ml of 0.1 N iodine is equivalent to 7.912 mg of K₂SO₃.