SPECIFICATIONS AND CRITERIA FOR IDENTITY AND PURITY OF SOME FLAVOURING SUBSTANCES AND NON-NUTRITIVE SWEETENING AGENTS
SPECIFICATIONS AND CRITERIA FOR IDENTITY AND
PURITY OF SOME FLAVOURING SUBSTANCES
AND
NON-NUTRITIVE SWEETENING AGENTS

Issued jointly by FAO and WHO

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Expert Committee on Food Additives which met in Geneva, 21-28 August 1967/1

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**Test Solutions**

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INTRODUCTION

The specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents contained in this publication are the result of the deliberations of the Joint FAO/WHO Expert Committee on Food Additives in their eleventh meeting held at Geneva on 21-28 August 1967. These should therefore be considered together with the report of this meeting.  

While considering the specifications of these substances, the Committee had also agreed that:

1. Specific gravity should be given for 25°/25° as well as for 20°/20° where available.

2. Other additional criteria such as moisture limits and distillation range need not appear in the specifications, but may be useful in commercial transactions.

3. Assay: unless an upper limit is specified, there is an implied upper limit of 100-5%.

4. Toxic trace elements: Specific limits need not be set for arsenic, lead and other elements precipitable by hydrogen sulphide under acid conditions in the case of those flavouring substances that are distilled, because their absence is no proof of good manufacturing practice. The heavy metals list is included in the case of crystallised substances. It is recognised that the flavouring materials are used in concentrations expressible in parts per million (10^6) and these toxic elements may be present, if at all, in these materials in similar concentrations, giving net contents in the food in the range of parts per 10^{12} parts of food, amounts which would not increase significantly nor be detectable against the variable background load of these toxic trace elements naturally present in foods.

5. Other components: In general, on the basis of manufacturing processes, the other components making up the difference between the assay figure and 100% need not be mentioned if they are considered to be of the same general class as the material specified. For example, in the case of esters, the other components are likely to be starting acid and alcohol and the higher and lower homologous esters, acids and alcohols. In the case of aldehydes, the corresponding acids may be present as a result of oxidation. Further limits on impurities need to be specified only in the case of substances where in the light of present day technological practice this is deemed necessary for toxicological reasons.

6. The identification of the flavours substances would not be specific if based only on the criteria of identity and purity detailed in this publication. The identity can be determined more specifically using additional indicative methods, such as gas liquid chromatography, infra-red spectrometry and nuclear magnetic resonance. When using such techniques for comparing the substance with its standard sample, it needs to be realized that many flavouring substances are not completely stable.

Any new information and comments relating to the specifications of these products should be addressed to: Food Standards, Additives and Regulations Section, Nutrition Division, FAO, Rome, Italy.

For detailed monographs on toxicological evaluation of these substances a reference is invited to the publication: 
"Toxicological Evaluation of Some Flavouring Substances and Non-Nutritive Sweetening Agents." 1/

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1/ FAO Nutrition Meeting Report Series No. 44A; WHO/Food Add./68.33
TRANS-ANETHOLE
(trans-p-Propenylanisole)

Criteria

Refractive index, \( n^0_D \) 1.5570 - 1.5610

Specific gravity, 25\(^\circ\)/25\(^\circ\) 0.983 - 0.988

Solidification point Not below 20\(^\circ\)

cis isomer Not more than (1\%)*(by gas-liquid chromatography)

Aldehydes and Ketones (a)

No Phenolic colour reaction (b)

Solubility in ethanol 1 ml dissolves in 2 ml 96% ethanol

(a) Aldehydes and Ketones. Shake 10 ml with 50 ml of a saturated solution of sodium bisulfite in a glass-stoppered, graduated cylinder, and allow the mixture to stand for six hours. The volume of the sample does not diminish appreciably, and no crystalline deposit separates.

(b) Phenols. Shake 1 ml with 20 ml of water, and allow the liquids to separate. Filter the water layer through a filter paper previously moistened with water, and to 10 ml of the filtrate add 3 drops of ferric chloride T.S. No purplish colour is produced.

* Tentative
## BENZALDEHYDE
(Benzoic Aldehyde)

### Criteria

<table>
<thead>
<tr>
<th>Property</th>
<th>Requirement</th>
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<tr>
<td><strong>Assay</strong></td>
<td>Not less than 97% (b)</td>
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<td>C$_7$H$_6$O</td>
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<td><strong>Refractive index, $N^{20}_D$</strong></td>
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<td><strong>Specific gravity, 25°/25°</strong></td>
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<td><strong>Chlorinated compounds</strong></td>
<td>Limit test (a)</td>
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</table>

(a) Chlorinated compounds. Proceed as directed in the Annex.

(b) Assay. Weigh accurately about 1 gram, and proceed as directed in the Annex under Aldehydes, using 53.06 as the equivalence factor ($E$) in the calculation.
BENZYL ACETATE

(Acetate de benzyl)
Benzyl ethanoate

Criteria

Assay  Not less than 97% (a)

\[ C_{9}H_{10}O_{2} \]

Refractive index, \( n_{D}^{20} \)  1.5010 - 1.5040

Specific gravity, 25°/25° 1.052 - 1.056

20°/20° 1.055 - 1.059

Acid value  1 max

Chlorinated compounds  Limit test (b)

(a) Assay. Weigh accurately about 900 mg, and proceed as directed in the Annex under Ester Determination, using 75.10 as the equivalence factor (E) in the calculation. The results should be corrected for the acid value.

(b) Chlorinated compounds. Proceed as directed in the Annex.
BUTYL ACETATE
(Butyl ethanoate)

Criteria

Assay

Not less than 95% (a)

\( \text{C}_6\text{H}_{12}\text{O}_2 \)

Refractive index, \( N_D^{20} \)

1.3920 - 1.400

Specific gravity, \( 25^\circ/25^\circ \)

0.876 - 0.883

\( 20^\circ/20^\circ \)

0.880 - 0.887

Acid value

1 max

(a) Assay. Weigh accurately about 1 gram, and proceed as directed in the Annex under Ester Determination, using 58.08 as the equivalence factor (e) in the calculation.
ISOAMYL BUTYRATE*
(Butyrate d'isoamyl)

Criteria

Assay Not less than 98 - 101% C₉H₁₈O₂(a)

Refractive index, N°₀ ₁.₄₀₉₀ - 1.₄₁₄₀

Specific gravity, 25°/25° 0.₈₆₀ - 0.₈₆₄
20°/2₀° 0.₈₆₃ - 0.₈₆₈

Acid value 1 max

Solubility in ethanol 1 ml dissolves in
4 ml 70% ethanol

(a) Assay. Weigh accurately about 1 gram, and proceed as
directed in the Annex under Ester Determination, using
79.13 as the equivalence factor (e) in the calculation.

* There is the possibility of mixtures of iso- and normal amyl
butyrate.
d-CARVONE

(+ -Carvone; d-1-Methyl-4-isopropenyl-6-cyclohexen-2-one)

Criteria

Assay Not less than 95% (a)

Refractive index, \(N^D_{20}\) 1.4950 - 1.5010

Specific gravity, \(25^\circ/25^\circ\) 0.952 - 0.965
\(20^\circ/20^\circ\) 0.956 - 0.969

Angular rotation +56° to +60°

Solubility in ethanol 1 ml dissolves in 5 ml 60% ethanol

(a) Assay. Weigh accurately about 1 gram, and proceed as directed in the Annex under Aldehydes and Ketones, 4.1 Hydroxylamine Method, using 75.11 as the equivalence factor (E) in the calculation; or alternatively by 4.2, Neutral Sulphite Method.
1-CARVONE
(- -Carvone; 1-l-Methyl-4-isopropenyl-6-cyclohexen-2-one)

Criteria

Assay

Not less than 95% (a)

C_{10}H_{14}O

Refractive index, \( \frac{N^0}{D} \)

1.4950 - 1.5000

Specific gravity, 25°/25°

0.955 - 0.962

Angular rotation

-55° to -62°

Solubility in ethanol

1 ml dissolves in 2 ml 70% ethanol

(a) Assay. Weigh accurately about 1 gram, and proceed as directed in the Annex under Aldehydes and Ketones, 4.1 Hydroxylamine Method, using 75.11 as the equivalence factor (E) in the calculation.
CINNAMALDEHYDE
(Cinnamic Aldehyde; Cinnamal)
(Aldéhyde Cinnamique)

**Criteria**

**Assay**
Not less than 98% expressed as (b) 
\[ \text{C}_9\text{H}_8\text{O} \]

**Refractive index, \( n^D_{20} \)**

1.6190 - 1.6250

**Specific gravity, \( 25^\circ/25^\circ \)**

1.046 - 1.052

**Acid value**

10 max

**Chlorinated compounds**

Limit test (c)

**Hydrocarbons**

(a)

**Solubility in ethanol**

1 ml dissolves in 5 ml 60% ethanol

(a) **Hydrocarbons.** Measure 10 ml from a pipet into a 100 ml cassia flask and add 75 ml of a freshly prepared solution of sodium bisulfite (12 in 100) previously heated to a temperature of 85\(^\circ\). Shake the flask vigorously until solution is complete, then add sufficient sodium bisulfite solution to raise the meniscus within the graduated portion of the neck. No oil separates.

(b) **Assay.** Weigh accurately about 1.5 grams, and proceed as directed in the Annex under Aldehydes using 66.08 as the equivalence factor \((E)\) in the calculation.

(c) **Chlorinated compounds.** Proceed as directed in the Annex.
CITRAL

(3,7 Dimethyl-2,6-octadienal; a mixture of the geometric isomers neral and geranial)

Criteria

Assay

Not less than 95% expressed as (a)

\[ \text{C}_{10}\text{H}_{16} \]

Refractive index, \( \text{N}^{20}_\text{D} \)

1.4850 - 1.4910

Specific gravity, 25°/25° 0.883 - 0.891
20°/20° 0.886 - 0.894

Acid value

5 max.

Limits of Impurities

As 3 mg/kg max
Heavy metals (as Pb) 40 mg/kg max
Pb 10 mg/kg max
Solubility in ethanol 1 ml dissolves in
7 ml 70% ethanol

(a) Assay. Weigh accurately about 1 gram, and proceed as directed in the Annex under Aldehydes and Ketones, 4.1 Hydroxylamine Method, using 76.12 as the equivalence factor (E) in the calculation; or alternatively by 4.2, the Neutral Sulphite Method.
CITRONELLOL
(98% Total alcohols)

Criteria

Assay
Not less than 98% expressed as (a)
$\text{C}_{10}\text{H}_{20}\text{O}$
N$^{20}$
1.4530 - 1.4580

Specific gravity, 25$^\circ$/25$^\circ$
0.850 - 0.857

Angular rotation
-0$^\circ$ to +4$^\circ$

Ester value
2 max (b)

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

(a) Assay. Proceed as directed in the Annex under Total Alcohol. Weigh accurately about 1.2 grams of the acetylated alcohol for the saponification, and use 78.13 as the equivalence factor $(\text{E})$ in the calculation.

(b) Ester. Weigh accurately about 5 grams, and proceed as directed in the Annex under Ester Determination.
CITRONELLOL
(90% Total alcohols)

Criteria

Assay
Not less than 90% expressed as (a)
\[ C_{10}H_{20}O \]

Refractive index, \( n_D^{20} \) 1.4520 - 1.4620

Specific gravity, \( 25^\circ/25^\circ \) 0.848 - 0.860
\( 20^\circ/20^\circ \) 0.853 - 0.864

Angular rotation
-5\(^\circ\) to +5\(^\circ\)

Aldehydes
Not more than 2% (b) calculated as citronellal \( (C_{10}H_{18}O) \)

Ester Value
4 max (c)

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

(a) Assay. Proceed as directed in the Annex under Total Alcohol. Weigh accurately about 1.2 grams of the acetylated alcohol for the saponification, and use 78.13 as the equivalence factor (E) in the calculation.

(b) Aldehydes. Weigh accurately about 5 grams, and proceed as directed in the Annex under Aldehydes and Ketones, 4.1 Hydroxylamine Method, using 77.13 as the equivalence factor (E) in the calculation for citronellal \( (C_{10}H_{18}O) \).

(c) Ester. Weigh accurately about 5 grams, and proceed as directed in the Annex under Ester Determination, using 99.15 as the equivalence factor (E) in the calculation for citronellyl acetate \( (C_{12}H_{22}O_2) \).
DECANAL
(Aldehyde C-10; Capraldehyde, Decyl aldehyde)
(Aldéhyde caprique)

Criteria

Assay           Not less than 92% (a)
C_{10}H_{20}O

Refractive index, N^20_D  1.4270 - 1.4350

Specific gravity, 25°/25°  0.821 - 0.835

Acid value       10 max

(a) Assay. Weigh accurately about 1.5 grams and proceed as directed in the Annex under Aldehydes, using 78.14 as the equivalence factor (E) in the calculation.
DIACETYL
(2,3-Butanedione; Dimethyldiketone; Dimethylglyoxal) (Diacétyle)

Criteria

Assay Not less than 97% expressed as (a) 

\[ \text{C}_4\text{H}_6\text{O}_2 \]

Refractive index, \( n^{20}_D \) 1.3930 - 1.3970

Specific gravity \( 25^\circ/25^\circ \) 0.975 - 0.990

Solidification point -1.0\(^\circ\) to -4.0\(^\circ\)

(a) Assay. Weigh accurately about 1 gram, and proceed as directed in the Annex under Aldehydes and Ketones, 4.1 Hydroxylamine Method, using 21.52 as the equivalence factor (E) in the calculation.
**ETHYL ACETATE**

*(Acetate d'ethyle)*

**Criteria**

- **Assay**: Not less than 97% (a) expressed as $C_4H_8O_2$
- **Refractive index, $N_{20}^D$**: 1.3710 - 1.3760
- **Specific gravity, $25^\circ/25^\circ$**: 0.894 - 0.901
  - $20^\circ/20^\circ$: 0.897 - 0.906

(a) **Assay**: Transfer about 1.5 grams, accurately weighed in a tared, stoppered weighing bottle, to a suitable flask, add 50.0 ml of 0.5 N sodium hydroxide, and heat on a steam bath under a reflux condenser for 1 hour. Allow it to cool, add phenolphthalein T.S. and titrate the excess sodium hydroxide with 0.5 N hydrochloric acid. Perform a blank determination, and make any necessary correction. Each ml of 0.5 N sodium hydroxide is equivalent to 44.06 mg of $C_4H_8O_2$. 
ETHYL BUTYRATE
(Butyrate d'éthyle)

Criteria

Assay

Not less than 97% expressed as (a)
\( C_6H_{12}O_2 \)

Refractive index, \( N^\circ_{20D} \) 1.3900 - 1.3950

Specific gravity, \( 25^\circ/25^\circ \) 0.870 - 0.881
\( 20^\circ/20^\circ \) 0.874 - 0.884

Acid value

1 max

(a) Assay. Weigh accurately about 1 gram, and proceed as directed in the Annex under Ester Determination, using 58.08 as the equivalence factor (E) in the calculation.
ETHYL FORMATE

Criteria

Assay
Not less than 93% expressed as (a) C3H6O2

Refractive index, N20D
1.3580 - 1.3630

Specific gravity, 25°/25°
0.912 - 0.921

Acid value
2 max

(a) Assay. Weigh accurately about 500 mg, and proceed as directed in the Annex under Ester Determination, using 37.04 as the equivalence factor (E) in the calculation. Modify the procedure by allowing the mixture to stand at room temperature for 15 minutes, instead of heating on a steam bath for 1 hour.
ETHYL HEPTANOATE

(Ethyl Heptanoate, Ethyl Oenanthate)

(Oenanthate d'éthyle)

Criteria

Assay

Not less than 97% expressed as (a)

\( \text{C}_9\text{H}_{18}\text{O}_2 \)

Refractive index, \( \text{N}^{20}_D \)

1.4110 - 1.4160

Specific gravity, \( 25^\circ/25^\circ \)

0.864 - 0.872

\( 20^\circ/20^\circ \)

Acid value

1 max

(a) Assay. Weigh accurately about 1 gram, and proceed as directed in the Annex under Ester Determination, using 79.12 as the equivalence factor (E) in the calculation.
ETHYL LACTATE
(Ethyl 2-Hydroxypropionate)

Criteria

Assay

Not less than 98% expressed as \( \text{C}_6\text{H}_{10}\text{O}_3 \)

Refractive index, \( \eta^{20}_D \) 1.4090 - 1.4200

Specific gravity, \( 25^\circ/25^\circ \) 1.028 - 1.034
\( 20^\circ/20^\circ \) 1.031 - 1.037

Acid value 2 max

(a) Assay. Weigh accurately about 700 mg, and proceed as directed in the Annex under Ester Determination, using 59.07 as the equivalence factor \( (E) \) in the calculation.
ETHYL LAURATE
(Ethyl Dodecanoate)

Criteria

Assay  Not less than 98% expressed as (a)
\[ \text{C}_{14}\text{H}_{28}^0\text{O}_2 \]

Refractive index, \( n^\circ_D \) 1.4300 - 1.4340

Specific gravity, 25\(^\circ\)/25\(^\circ\) 0.858 - 0.862

Acid value  3.5 max

(a) Assay. Weigh accurately about 1.5 grams, and proceed as directed in the Annex under Ester Determination, using 114.2 as the equivalence factor (E) in the calculation.
ETHYL METHYLPHENYL GLYCIDATE
(Aldehyde C-16*)

Criteria

Assay
Not less than 98% expressed as (a)

\[ C_{12}H_{14}O_3 \]

Refractive index, \( \mu^D_{20} \) (1.5040 - 1.5100)**

Specific gravity, 25°/25° (1.086 - 1.092)**

Chlorinated compounds Limit test (b)

Acid value 3 max

---

(a) Assay. Weigh accurately about 1 gram, and proceed as directed in the Annex under Ester Determination, using 103.1 as the equivalence factor \((E)\) in the calculation.

(b) Chlorinated compounds. Proceed as directed in the Annex.

* Miscalled, but to be indexed under this name as well.

** Tentative
ETHYL NONANOATE
(Ethyl pelargonate)
(Félargonate d'ethyle)

Criteria

Assay

Not less than 98% expressed as (a)
$C_{11}H_{22}O_2$

Refractive index, $N^2_0/\rho$

1.4200 - 1.4260

Specific gravity, 25°/25°
0.863 - 0.867

Acid value

3 max

(a) Assay. Weigh accurately about 1.5 grams, and proceed as directed in the Annex under Ester Determination, using 93.15 as the equivalence factor (E) in the calculation.
ETHYL iso-VALERATE
(Ethyl 2-Methylbutyrate)
(Isovalérate d'ethyle)

Criteria

Assay

Not less than 98% and not more than 101% (a) expressed as C₇H₁₄O₂

Refractive index, N

1.3930 - 1.3990

Specific gravity, 25°/25°

0.862 - 0.866

20°/20°

0.866 - 0.871

Acid value

2 max

(a) Assay. Weigh accurately about 1.5 grams, and proceed as directed in the Annex under Ester Determination, using 65.10 as the equivalence factor (E) in the calculation.
**ETHYL VANILLIN**

(3-Ethoxy-4-hydroxybenzaldehyde)

(Aldhyde ethylprotocatachnique)

**Description**

Fine, white or slightly yellowish crystals, having a strong vanilla-like odour and taste; affected by light.

**Identification Tests**

A. Solubility:

- Water: Insoluble
- 60% Ethanol: 1 g in 10 ml.

B. Warm about 100 mg of the sample with 1 ml of 25 percent hydrochloric acid until complete solution is effected. Cool, add about 1 ml of hydrogen peroxide T.S., and allow the mixture to stand, with frequent shaking, until precipitation is complete (10 to 20 min.). Add an equal volume of benzene and shake thoroughly. The benzene layer is violet in colour (distinction from vanilla).

C. The sample is extracted completely from solution by shaking with a saturated solution of sodium bisulfite, from which it is precipitated by acids.

D. Add lead subacetate T.S. to a cold solution of the sample. A white precipitate which is sparingly soluble in hot water, but soluble in acetic acid, is produced.

**Limits of Impurities**

Heavy metals: Not more than 10 mg/kg (as Pb)
EUGENOL
(4- Allyl-2- methoxyphenol; 4- Allylguaiacol)

Criteria

Assay
Not less than 98% by volume, of phenols (a) as eugenol (C₁₀H₁₂O₂)

Refractive index, N²⁰¹
1.5380 - 1.5420

Specific gravity, 2⁰/2⁰
1.064 - 1.070
20⁰/20⁰
1.065 - 1.071

Hydrocarbons
(b)

Phenol
(c)

(a) Assay. Proceed as directed in the Annex under Phenols.

(b) Hydrocarbons. Dissolve 1 ml in 20 ml of 0.5 N sodium hydroxide in a stoppered 50 ml tube, add 18 ml of water, and mix. A clear mixture results immediately, but it may become turbid when exposed to air.

(c) Phenol. Shake 1 ml with 20 ml of water, filter, and add 1 drop of ferric chloride T.S. to 5 ml of the clear filtrate. The mixture exhibits a transient grayish green colour, but not a blue or violet colour.
GERANYL ACETATE
(2,6-Dimethyl-1,6-octadien-8-yl Acetate)

Criteria

Assay
Not less than 90% of total esters, (a) calculated as geranyl acetate
\( \text{C}_{12}\text{H}_{20}\text{O}_2 \)

Refractive index, \( N_{20}^D \)
1.4570 - 1.4640

Specific gravity, 25°/25°
0.896 - 0.914

Angular rotation
-2.0° to +3.0°

Solubility in ethanol
1 ml dissolves in 4 ml 80% ethanol

Acid value
1 max

(a) Assay. Weigh accurately about 1 gram, and proceed as directed in the Annex under Ester Determination, using 98.15 as the equivalence factor (E) in the calculation.
alpha-IONONE*

Criteria

**Assay**

Not less than 95% ketones expressed as (a) C\textsubscript{13}H\textsubscript{20}O

**Refractive index, \( N\textsubscript{D}^{20} \)**

1.4970 - 1.5025

**Specific gravity, \( 25^\circ/25^\circ \)**

0.927 - 0.933

20°/20° (0.930 - 0.934)**

---

(a) **Assay.** Weigh accurately about 1.3 grams, and proceed as directed in the Annex under Aldehydes and Ketones, 4.1 Hydroxylamine Method, using 96.15 as the equivalence factor (E) in the calculation.

* The product of commerce used in food under this name may contain varying proportions of isomeric ionones.

** Tentative
**Criteria**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Not less than 95% ketones expressed as (a) $C_{13}H_{20}O$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractive index, $N^{20}_D$</td>
<td>1.5070 - 1.5230</td>
</tr>
<tr>
<td>Specific gravity, $25^\circ/25^\circ$</td>
<td>0.935 - 0.947</td>
</tr>
</tbody>
</table>

(a) Assay. Weigh accurately about 1.3 grams, and proceed as directed in the Annex under Aldehydes and Ketones, 4.1 Hydroxylamine Method, using 96.15 as the equivalence factor (E) in the calculation.

* The product of commerce used in food under this name may contain varying proportions of isomeric ionones.
LINALOL, 95%
(Linalool; 3,7-Dimethyl-1,6-octadien-3-ol)

Criteria

Assay Not less than 95% expressed as (a) C_{10}H_{18}O

Refractive index, N
\[\text{D} = (1.4600 - 1.4640)*\]

Specific gravity, 25°/25° (0.858 - 0.867)*

Angular rotation (-2° to +2°)*

Ester value 1.5 max (b)

Solubility in ethanol 1 ml dissolves in 4 ml 60% ethanol to form a clear solution.

(a) Assay. Proceed as directed in the Annex under Linalool Determination, using about 1.2 grams of acetylated oil, accurately weighed.

(b) Esters. Weigh accurately about 10 grams, and proceed as directed in the Annex under Ester Determination.

* Tentative
LINALOL, 90%
(Linalool; 3,7-Dimethyl-1,6-octadien-3-ol)

Criteria

Assay
Not less than 90% expressed as (a) 
C_{10}H_{18}O

Refractive index, N^20_D
1.4604 - 1.4655

Specific gravity, 25°/25°
0.858 - 0.868

Ester value
4 max (b)

Solubility in ethanol
1 ml dissolves in 4 ml 60% ethanol to form a clear solution.

(a) Assay. Proceed as directed in the Annex under Linalool Determination using about 1.2 grams of acetylated oil, accurately weighed.

(b) Esters. Weigh accurately about 10 grams, and proceed as directed in the Annex under Ester Determination.
LINALYL ACETATE, 90%
(3,7-Dimethyl, 1,6-octadien-3-yl Acetate)

Criteria

Assay
Not less than 90% of esters, (a) calculated as linalyl acetate
($C_{12}H_{20}O_2$)

Refractive index, $N_{D}^{20}$
1.4490 - 1.4580

Specific gravity, $25^\circ/25^\circ$
0.895 - 0.914
$20^\circ/20^\circ$
0.905 - 0.917

Acid value
2 max

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

(a) Assay. Weigh accurately about 1 gram, and proceed as directed in the Annex under Ester Determination, using 98.15 as the equivalence factor (E) in the calculation. The volume of 0.5 N alcoholic potassium hydroxide consumed by the sample should be corrected for the Acid Value.
LINALYL ACETATE, 96%
(3,7-Dimethyl,1,6-octadien-3-yl Acetate)

Criteria

Assay                               Not less than 96% of esters, (a)
                                      calculated as linalyl acetate
                                      \( \text{C}_{12}\text{H}_{20}\text{O}_2 \)

Refractive index, \( n^\text{20}_D \) 1.4490 - 1.4530

Specific gravity, 25°/25° 0.895 - 0.908
                            20°/20° (0.899 - 0.906)*

Acid value                  1 max

(a) Assay. Weigh accurately about 1 gram, and proceed as directed in the Annex under Ester Determination, using 98.15 as the equivalence factor (e) in the calculation. The volume of 0.5 N alcoholic potassium hydroxide consumed by the sample should be corrected for the Acid Value.

* Tentative
MALTOL
(3-Hydroxy-2-methyl-4-pyrone)

Criteria

Assay

Not less than 98% (a)

C₆H₆O₃

Melting range

160° - 164°

(a) Assay: Standard Solution. Weigh accurately about 50 mg. of F.C.C. Maltol Reference Standard, dissolve it in sufficient 0.1 N hydrochloric acid to make 250.0 ml, and mix. Transfer 5.0 ml of this solution into a 100 ml volumetric flask, dilute to volume with 0.1 N hydrochloric acid, and mix.

Assay Solution. Weigh accurately about 50 mg. of the sample, dissolve it in sufficient 0.1 N hydrochloric acid to make 250.0 ml, and mix. Transfer 5.0 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 the wavelength of maximum absorption at about 274 mu, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg., of C₆H₆O₃ in the sample taken by the formula 50(Aₜ/ₘₜ), in which C is the concentration, in mcg. per ml, of F.C.C. Maltol Reference Standard in the Standard Solution, Aₜ is the absorbance of the Assay Solution, and Aₘ is the absorbance of the Standard Solution.
1-MENTHOL
(1-3-p-Menthanol)

Criteria

Melting range  
41° - 44°

Specific rotation, \((\alpha)_{D}^{25}\)
-40° to -52°
dl-MENTHOL
(d,1-3-p-Menthanol)

Criteria

Melting range  
32° - 38°

Solidification behaviour  
(a)

Specific rotation, (α)_{D}^{25°}  
-2° to +2°

Readily oxidizable substances  
(b)

(a) "Solidification behaviour of dl-menthol. Determine as directed in the Annex, using a sample previously dried in a desiccator over silica gel for 24 hours 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°."

(b) Readily oxidizable substances in dl-menthol. Transfer 500 mg. of dl-menthol into a clean, dry test tube, add 10 ml of potassium permanganate with water to 100 ml. Place the test tube in a beaker of water maintained between 45° and 50°. At 30-second intervals, quickly remove the test tube from the bath and shake. The colour of potassium permanganate is still apparent after 5 minutes.
MENTHYL ANTHRANILATE
(Methyl 2-Aminobenzoate)
(Anthranilate de Methylle)

Criteria

Assay

Not less than 98% expressed as (a) \( C_8H_7NO_2 \)

Refractive index, \( N^\circ_D \)

1.5800 - 1.5850

Specific gravity, 25°/25°

1.161 - 1.169

Solidification point

Not less than 22°

Acid value

1 max

(a) Assay. Weigh accurately about 1 gram, and proceed as directed in the Annex under Ester Determination, using 75.59 as the equivalence factor (E) in the calculation.
<table>
<thead>
<tr>
<th>Criterion</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assay</strong></td>
<td>Not less than 97% expressed as ( \text{C}<em>9\text{H}</em>{10}\text{O}_2 )</td>
</tr>
<tr>
<td><strong>Refractive index, ( N_{20}^D )</strong></td>
<td>1.5050 - 1.5090</td>
</tr>
<tr>
<td><strong>Specific gravity, 25°/25°</strong></td>
<td>1.061 - 0.067</td>
</tr>
<tr>
<td><strong>Acid value</strong></td>
<td>( 1 \max )</td>
</tr>
<tr>
<td><strong>Chlorinated compounds</strong></td>
<td>Limit test (b)</td>
</tr>
</tbody>
</table>

(a) **Assay.** Weigh accurately about 1 gram, and proceed as directed in the Annex under Ester Determination, using 75.09 as the equivalence factor \( (E) \) in the calculation.

(b) **Chlorinated compounds.** Proceed as directed in the Annex.
METHYL SALICYLATE
(Wintergreen oil, artificial)

Criteria

Assay  98% expressed as (a)

\[
\text{C}_8\text{H}_8\text{O}_3
\]

Refractive index, \( N^20_D \)  1.5340 - 1.5380

Specific gravity, 25°/25°  1.180 to 1.188

(a) Assay. Proceed as directed in the Annex under Ester Determination, using about 2 grams, accurately weighed. Use 50 ml of 0.5 N alcoholic potassium hydroxide and reflux on the steam bath for 2 hours. Each ml of 0.5 N potassium hydroxide consumed is equivalent to 76.08 mg of \( \text{C}_8\text{H}_8\text{O}_3 \).
### Criteria

**Assay**

Not less than 97% expressed as (a) $C_9H_{16}O_2$

**Refractive index, $N^{20}_D$**

1.4450 - 1.4500

**Specific gravity, $25^\circ/25^\circ$**

0.957 - 0.968

$20^\circ/20^\circ$ 0.965 - 0.970

**Acid value**

5 max

---

(a) Assay. Weigh accurately about 1 gram, and proceed as directed in the Annex under Ester Determination, 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.

* Miscalled Aldehyde C-18, but cross-indexed under this name.
NONANAL
(Pelargonic Aldehyde; Aldehyde C-9)
(Aldéhyde Pelargonique)

Criteria

Assay  Not less than 92% expressed as (a) $C_9H_{18}O$

Refractive index, $N^2_0D$  1.4220 - 1.4300

Specific gravity, $25^0/25^0$  0.820 - 0.830

Acid value  10 max

(a) Assay. Weigh accurately about 1.5 grams, and proceed as directed in the Annex under Aldehydes, using 71.12 as the equivalence factor ($E$) in the calculation.
OCTANAL
(Caprylic Aldehyde; Aldehyde C-8)
(Aldéhyde Caprylique)

**Criteria**

**Assay**
Not less than 92% expressed as (a)
\[ \text{C}_8\text{H}_{16}\text{O} \]

**Refractive index, \(N^\circ\)**
1.4170 - 1.4260

**Specific gravity, \(25^\circ/25^\circ\)**
0.817 - 0.830

**20^\circ/20^\circ**
0.820 - 0.837

**Acid value**
10 max

(a) **Assay.** Weigh accurately about 1.5 grams, and proceed as directed in the Annex under Aldehydes, using 64.11 as the equivalence factor (E) in the calculation.
PHENYLACETALDEHYDE
(α-Toluic Aldehyde)
(Aldéhyde α-toluique; Aldéhyde phénylacétique)

Criteria

<table>
<thead>
<tr>
<th>Assay</th>
<th>Not less than 90%* expressed as (a) C₈H₈O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractive index, N₀/₀²⁵</td>
<td>1.5240 - 1.5330</td>
</tr>
<tr>
<td>Specific gravity, 25°/25°</td>
<td>1.023 - 1.040</td>
</tr>
<tr>
<td>Chlorinated compounds</td>
<td>Limit test** (b)</td>
</tr>
<tr>
<td>Acid value</td>
<td>5 max</td>
</tr>
</tbody>
</table>

(a) Assay. Weigh accurately about 1 gram, and proceed as directed in the Annex under Aldehydes, using 60.08 as the equivalence factor (E) in the calculation.

(b) Chlorinated compounds: As in Annex.

* Caution: Because this compound is unstable, it is not stored and used in this form, but in solution, e.g. 50% in ethanol.

** Tentative
PIPERONAL
(Heliotropine; Piperonyl Aldehyde)

Criteria

Assay
Not less than 99% expressed as \( \text{(a)} \) \( \text{C}_8\text{H}_6\text{O}_3 \)

Solidification or melting point
Not less than 35°

Heavy metals (as Pb)
40 mg/kg max

(a) Assay. Weigh accurately about 1.5 grams, and proceed as directed in the Annex under Aldehydes, using 75.07 as the equivalence factor \((E)\) in the calculation.
**Y-UNDECALACTONE**

**Criteria**

**Assay**

Not less than 97% expressed as (a)
\[ \text{C}_{11}\text{H}_{20}\text{O}_2 \]

**Refractive index, \( N^D_{20} \)**

1.4500 - 1.4550

**Specific gravity, \( 25^\circ\text{o}/25^\circ\text{o} \)**

0.940 - 0.945

**20^\circ\text{o}/20^\circ\text{o}**

0.945 - 0.950

**Acid value**

5 max

(a) Assay. Weigh accurately about 1 gram, and proceed as directed in the Annex under Ester Determination, using 92.14 as the equivalence factor \( E \) in the calculation.

* Miscalled Aldehyde C-14, but to be cross-indexed under this name.
**VANILLIN**

(4-Hydroxy-3-methoxybenzaldehyde)

(Aldéhyde méthylproto catechique)

**Criteria**

**Assay**

97 - 103% expressed as \( \text{C}_8\text{H}_8\text{O}_3 \) (a)

**Melting range**

Between 80.8° and 83°

**Heavy metals**

Not more than 10 mg/kg

(a) **Assay. Standard Solution.** Transfer about 100 mg, accurately weighed of Vanillin Reference Standard into a 250 ml volumetric flask, add methanol to volume, and mix. Transfer 2.0 ml of this solution into a 100 ml volumetric flask, dilute to volume with methanol, and mix.

**Assay Solution.** Transfer about 100 mg., accurately weighed, of the sample into a 250 ml volumetric flask, add methanol to volume and mix. Transfer 2.0 ml of this solution into a 100 ml volumetric flask, dilute to volume with methanol, and mix.

**Procedure.** Determine the absorbance of each solution in a 1 cm. quartz cell at the wavelength of maximum absorption at about 308 mu, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg., of \( \text{C}_8\text{H}_8\text{O}_3 \) in the sample taken by the formula 12.5C \( \times \) \( \frac{A_u}{A_s} \), in which C is the concentration, in mcg. per ml, of Vanillin Reference Standard in the Standard Solution, \( A_u \) is the absorbance of the Assay Solution, and \( A_s \) is the absorbance of the Standard Solution.
CALCIUM CYCLAMATE
(Cyclamate de calcium)

Chemical Name
Calcium Cyclohexanesulfamate, Calcium Cyclohexylsulfamate

Empirical Formula
C_{12}H_{24}CaN_{2}O_{6}S_{2}\cdot H_{2}O

Molecular Weight
432.57

Definition
Calcium cyclamate contains not less than 98 percent and not more than the equivalent of 101 percent of C_{12}H_{24}CaN_{2}O_{6}S_{2} calculated on the dry basis, and conforms to the following specifications.

Description
White odourless crystals or crystalline powder.

Uses
Non-nutritive sweetener.

Identification Tests

A. Solubility:
Water: Soluble
Ethanol: Sparingly soluble

B. To 10 ml of a 1% solution add 1 ml of hydrochloric acid, mix, add 1 ml of barium chloride T.S. The solution remains clear, but upon the addition of 1 ml of a 10% sodium nitrite solution, a white precipitate is formed.

C. A 1 in 100 solution gives positive tests for calcium.
Calcium Cyclamate (cont'd)

Purity Tests

Loss on drying: Between 6 percent and 9 percent when dried at 140°C for 2 hours.

Heavy metals (as Pb): Not more than 10 mg/kg

Selenium: Not more than 30 mg/kg

Cyclohexylamine: Not more than 100 mg/kg. (Method to be established).

Assay

Dissolve about 400 mg of sample which has been accurately weighed in a mixture of 50 ml of water and 5 ml of hydrochloric acid, T.S. and titrate with 0.1 M sodium nitrite. Add the last ml of titrant dropwise until a blue colour is produced immediately when a glass rod dipped into the titrated solution is streaked on a piece of starch iodide test paper or alternatively the end point may be detected electrometrically. When the titration is complete, the end-point is reproducible after the mixture has been allowed to stand for 1 minute. Each ml of 0.1 M sodium nitrite is equivalent to 19.83 mg of \( \text{C}_\text{12}\text{H}_{24}\text{Ca H}_2\text{O}_6\text{S}_2 \), calculated on the dry basis.
CALCIUM SACCHARIN

Chemical Name
Calcium o-Benosulfimide, Calcium salt of
2,3-dihydro-3-oxobenzisulfarole

Empirical Formula
C_{14}H_{9}CaN_{2}O_{6}S_{2} \cdot 3 \frac{1}{2}H_{2}O

Molecular Weight
467.49

Definition
Calcium saccharin contains not less than
95 percent of C_{14}H_{9}CaN_{2}O_{6}S_{2} calculated on
anhydrous basis and conforms to the
following specifications.

Description
White crystals or a white crystalline
powder, which is odourless or has a
faint, aromatic odour and an intensely
sweet taste even in dilute solutions.

Uses
Non-nutritive sweetener.

Identification Tests

A. Solubility:
   Water: Freely soluble
   Ethanol: Soluble

B. Dissolve about 100 mg of the sample in 5 ml of 5 percent
   sodium hydroxide solution, evaporate to dryness and gently
   fuse the residue over a small flame until it no longer
   evolves ammonia. After the residue has cooled, dissolve
   it in 20 ml of water, neutralize the solution with diluted
   hydrochloric acid T.S. and filter. The addition of a drop
   of ferric chloride T.S. to the filtrate produces a violet
   colour.

C. Mix 20 mg of the sample with 40 mg of resorcinol, add
   10 drops of sulphuric acid and heat the mixture in a
   liquid bath at 200° for 3 minutes. After cooling, add
   10 ml of water and an excess of sodium hydroxide T.S.
   A fluorescent green liquid results.
D. A 1 in 10 solution gives positive test for calcium.

E. To 10 ml of a 1 in 10 solution add 1 ml of hydrochloric acid. A crystalline precipitate of saccharin is formed. Wash the precipitate well with cold water and dry at 105° for 2 hours. It melts between 226° and 230°.

Purity Tests

**Water:** Not more than 15 percent as determined by Karl Fischer Titrimeter Method.

**Heavy metals (as Pb):** Not more than 10 mg/kg

**Selenium:** Not more than 30 mg/kg.

Assay

Weigh accurately about 700 mg of the sample and transfer it quantitatively to a long-necked flask, having a capacity of 200 ml, and add 10 ml of a 30 percent w/v solution of sodium hydroxide in water; boil over a small flame for two minutes, avoiding loss by evaporation; cool, add 15 ml of hydrochloric acid, and boil again for fifty minutes under a reflux condenser. Cool, rinse the condenser with 50 ml of water, and pass a current of air through the flask to remove acid vapour. Connect with an ammonia distillation apparatus, add 20 ml of a 30 percent w/v solution of sodium hydroxide in water, and distil the ammonia into 40 ml of N/10 sulphuric acid; titrate the excess of acid with N/10 sodium hydroxide, using solution of methyl red as indicator. Each ml of N/10 sulphuric acid, neutralised by the ammonia in the distillate is equivalent to 20.22 mg of $C_{14}H_8CaN_2O_6S_2$. 
CYCLOHEXYLSULFAMIC ACID
(Acide cyclohexylsulfanique)

Synonyms
Cyclohexanesulfamic acid

Empirical Formula
\( \text{C}_6\text{H}_{13}\text{NO}_3\text{S} \)

Structural Formula

\[
\begin{array}{c}
\text{NH}_3\text{O}_3\text{H} \\
\text{N}
\end{array}
\]

Molecular Weight
179.24

Definition
Cyclohexylsulfamic acid contains not less than 98 percent and not more than the equivalent of 102 percent of \( \text{C}_6\text{H}_{13}\text{NO}_3\text{S} \), calculated on the anhydrous basis and conforms to the following specifications.

Description
A practically colourless, white crystalline powder with a sweet-sour taste.

Uses
Non-nutritive sweetener.

Identification Tests

A. Solubility:  
Water: Soluble
Ethanol: Soluble

B. Acidify a 2 percent solution with hydrochloric acid, add 1 ml of barium chloride T.S. and filter if any haze or precipitate forms. To the clear solution add 1 ml of a 10 percent solution of sodium nitrite. A white precipitate forms.
Cyclohexylsulfamic Acid (cont'd) JECFA/47/11/1967

Purity Tests

Loss on drying: Not more than 1 percent on drying at 105° for one hour.

Heavy metals (as Pb): Not more than 10 mg/kg.

Selenium: Not more than 30 mg/kg.

Cyclohexylamine: Not more than 100 mg/kg (method to be established).

Assay

Transfer about 350 mg, accurately weighed, into a 250 ml flask. Dissolve the sample in 50 ml of water, add phenolphthalein T.S., and titrate with 0.1 N sodium hydroxide. Each ml of 0.1 N sodium hydroxide is equivalent to 17.82 mg of C₆H₁₃NO₃S.
DULCIN (1)

Synonyms
Sucrol, Valzin

Chemical Name
4-orthoxyphenylurea

Empirical Formula
C₉H₁₂O₂N₂

Structural Formula

\[
\text{\text{OC}}_{\text{H}_2} \quad \text{\text{NH\cdot CONH}_2}
\]

Molecular Weight
180.21

Description
Colourless or white crystals, or a white crystalline powder, which is odourless and has a very sweet taste which is appreciable even after a 3000-fold dilution.

Uses
Non-nutritive sweetener.

Identification Tests

A. Solubility:
Water: Insoluble
Ethanol: Soluble

B. On heating a sample in a test-tube it decomposes with the evolution of ammonia (odour), and produces a white sublimate in the cool portion of the test-tube.

C. To 20 mg of the sample add 4 drops of liquified phenol and 4 drops of sulphuric acid, and heat to boil. Cool and carefully superimpose 3 ml of potassium hydroxide T.S. over this mixture. A blue colour appears at the plane of contact in 2 to 3 minutes.
**SACCHARIN**

(Saccharine)

**Chemical Name**

o-Benzosulfimide, 2,3-dihydro-3-oxobenzoisosulfonazole

**Empirical Formula**

C₇H₅NO₃S

**Structural Formula**

![Structural Formula of Saccharin]

**Molecular Weight**

183.19

**Definition**

Saccharin contains not less than 98 percent of C₇H₅NO₃S after drying and conforms to the following specifications.

**Description**

White crystals or a white, crystalline powder, odourless, or with a faint, aromatic odour.

**Uses**

Non-nutritive sweetener.

**Identification Tests**

A. **Solubility:**

Water: Slightly soluble, but soluble in basic solutions.

Ethanol: Sparingly soluble

B. **Dissolve about 100 mg in 5 ml of 5 percent sodium hydroxide solution, evaporate to dryness and gently fuse the residue over a small flame until it no longer evolves ammonia. After the residue has cooled, dissolve it in 20 ml of water, neutralize the solution with diluted hydrochloric acid T.S. and filter. The addition of a drop of ferric chloride T.S. to the filtrate produces a violet colour.
Saccharin (cont’d)  JECFA/49/11/1967

C. Mix 20 mg with 40 mg of resorcinol, add 10 drops of sulphuric acid, and heat the mixture in a liquid bath at 200° for 3 minutes. After cooling, add 10 ml of water and an excess of sodium hydroxide T.S. A fluorescent green liquid is produced.

D. Melting range: Between 226° and 230°.

Purity Tests

Loss on drying:  Not more than 1 percent.
Heavy metals (as Pb):  Not more than 20 mg/kg.
Selenium:  Not more than 30 mg/kg.

Assay

Transfer about 700 mg previously dried at 105° for 2 hours and accurately weighed, to a long-necked flask, having a capacity of 200 ml, and add 10 ml of a 30 percent w/v solution of sodium hydroxide in water; boil over a small flame for two minutes, avoiding loss by evaporation; cool, add 15 ml of hydrochloric acid, and boil again for fifty minutes under a reflux condenser. Cool, rinse the condenser with 50 ml of water, and pass a current of air through the flask to remove acid vapour. Connect with an ammonia distillation apparatus, add 20 ml of a 30 percent w/v solution of sodium hydroxide in water, and distil the ammonia into 40 ml of N/10 sulphuric acid; titrate the excess of acid with N/10 sodium hydroxide, using solution of methyl red as indicator. Each ml of N/10 sulphuric acid, neutralized by the ammonia in the distillate, is equivalent to 18.32 mg of C₇H₅NO₃S.
**Chemical Name**  
Sodium Cyclohexanesulfamate, Sodium Cyclohexylsulfamate

**Empirical Formula**  
C₆H₁₂NNaO₃S

**Structural Formula**  
\[ \text{NH-SO}_2-\text{ONa} \]

**Molecular Weight**  
201.22

**Definition**  
Sodium cyclamate contains not less than 98 percent and not more than the equivalent of 101 percent of C₆H₁₂NNaO₃S and conforms to the following specifications.

**Description**  
White, odourless crystals or crystalline powder which in dilute solution is about 30 times as sweet as sucrose.

**Uses**  
Non-nutritive sweetener.

**Identification Tests**

A. **Solubility:**  
- Water: Soluble
- Ethanol: Practically insoluble.

B. To 10 ml of a 1% solution add 1 ml of hydrochloric acid, mix, then add 1 ml of barium chloride T.S. The solution remains clear but upon the addition of 1 ml of sodium nitrite solution (1%) a white precipitate forms.

C. A 1% solution gives positive tests for sodium.
Sodium Cyclamate (cont'd)

Purity Tests

Loss on drying: Not more than 1 percent when dried at 105° for one hour.

Heavy metals (as Pb): Not more than 10 mg/kg.

Selenium: Not more than 30 mg/kg.

Cyclohexylamine: Not more than 100 mg/kg (method to be established).

Assay

Dissolve about 400 mg, previously dried at 105° for 1 hour and accurately weighed, in a mixture of 50 ml of water and 5 ml of hydrochloric acid, T.S. and titrate with 0.1 M sodium nitrite. Add the last ml of titrant dropwise until a blue colour is produced immediately when a glass rod dipped into the titrated solution is streaked on a piece of starch iodide test paper or alternatively, the end point may be detected electrometrically. When the titration is complete, the end point is reproducible after the mixture has been allowed to stand for 1 minute. Each ml of 0.1 M sodium nitrite is equivalent to 20.12 mg. of C₆H₁₂N-NaO₃S.
SODIUM SACCHARIN
(Sel de sodium de la saccharine)

Chemical Name: Sodium o-Benzoosulfinimide, Sodium salt of 2,3-diphydro-s-oxobenzisosulfazole

Empirical Formula: \( C_7H_4NNaO_3S \cdot 2H_2O \)

Molecular Weight: 241.20

Definition: Sodium saccharin contains not less than 98 percent and not more than the equivalent of 101 percent of \( C_7H_4NNaO_3S \) (205.17) after drying and conforms to the following specifications.

Description: White crystals or a white, crystalline, efflorescent powder which is odourless or has a faint, aromatic odour and an intensely sweet taste, even in very dilute solutions.

Uses: Non-nutritive sweetener.

Identification Tests

A. Solubility:
   Water: Freely soluble
   Ethanol: Sparingly soluble

B. Dissolve about 100 mg of the sample in 5 ml of 5% sodium hydroxide solution, evaporate to dryness and gently fuse the residue over a small flame until it no longer evolves ammonia. After the residue has cooled, dissolve it in 20 ml of water, neutralize the solution with diluted hydrochloric acid T.S. The addition of a drop of ferric chloride T.S. to the filtrate produces a violet colour.
C. Mix 20 mg of the sample with 40 mg of resorcinol, add 10 drops of sulphuric acid and heat the mixture in a liquid bath at 200° for 3 minutes. After cooling, add 10 ml of water and an excess of sodium hydroxide T.S. A fluorescent green liquid results.

D. Dissolve 0.5g of sample in 10 ml water; add 1 ml dilute hydrochloric acid T.S. and allow to stand 1 hour. A white crystalline precipitate is produced, which when washed and dried for 4 hours at 105° melts between 224° and 230°.

E. The residue obtained by igniting a 2-gram sample gives positive tests for sodium.

**Purity Tests**

**Water:** 12 to 16 percent by drying at 120°C for 4 hours.

**Heavy metals (as Pb):** Not more than 10 mg/kg.

**Selenium:** Not more than 30 mg/kg.

**Assay**

Weigh accurately about 700 mg of previously dried sample and transfer it quantitatively to a long-necked flask, having a capacity of 200 ml, and add 10 ml of a 30 percent w/v solution of sodium hydroxide in water; boil over a small flame for two minutes, avoiding loss by evaporation; cool, add 15 ml of hydrochloric acid, and boil again for fifty minutes under a reflux condenser. Cool, rinse the condenser with 50 ml of water, and pass a current of air through the flask to remove acid vapour. Connect with an ammonia distillation apparatus, add 20 ml of a 30 percent w/v solution of sodium hydroxide in water, and distil the ammonia into 40 ml of N/10 sulphuric acid; titrate the excess of acid with N/10 sodium hydroxide, using solution of methyl red as indicator. Each ml of N/10 sulphuric acid, neutralised by the ammonia in the distillate is equivalent to 20.52 mg of C7H4N3Na2O3S.
METHODS OF ANALYSIS

1. Acid Value

Dissolve about 10 grams of the sample, accurately weighed, in 50 ml of alcohol, previously neutralized to phenolphthalein with 0.1 N sodium hydroxide. Add 1 ml of phenolphthalein T.S. and titrate with 0.1 N sodium hydroxide until the solution remains faintly pink after shaking for 10 seconds, unless otherwise directed. Calculate the acid value (AV) by the formula:

\[
AV = \frac{5.61 \times S}{W}
\]

in which \( S \) = the number of ml of 0.1 N sodium hydroxide consumed in the titration of the sample and \( W \) = the weight of the sample in grams.

2. Total Alcohols

Transfer 10 grams of a solid sample, or 10 ml of a liquid sample, accurately weighed, into a 100 ml flask having a standard taper neck. Add 10 ml of acetic anhydride and 1 gram of anhydrous sodium acetate, mix these materials, attach a reflux condenser to the flask, and reflux the mixture for 1 hour. Cool and add 50 ml of water at a temperature between 50° and 60° through the condenser. Shake intermittently during a period of 15 minutes, cool to room temperature, transfer the mixture completely to a separator, allow the layers to separate, and then remove and reject the lower, aqueous layer. Wash the essential oil layer successively with 50 ml of a saturated sodium chloride solution, 50 ml of a 10 percent sodium carbonate solution, and 50 ml of saturated sodium chloride solution. If the oil is still acid to moistened litmus paper, wash it with additional portions of sodium chloride solution until it is free from acid. Drain off the oil, dry it with anhydrous sodium sulfate, then filter it.

Weigh the quantity of acetylated oil specified in the monograph into a tared 125 ml Erlenmeyer flask, add 10 ml of neutral alcohol, 10 drops of phenolphthalein T.S., and 0.1 N alcoholic potassium hydroxide, dropwise, until a pink end-point is obtained. If more than 0.35 ml is needed, reject the sample, and wash and test the remaining acetylated oil until its acid content is below this level. Prepare a blank using the same volume of alcohol and indicator, and add 1 drop of 0.1 N alkali to produce a pink end-point. Measure 25.0 ml of 0.5 N alcoholic potassium hydroxide into each of the flasks, reflux them simultaneously for one hour, cool, and titrate the contents of each flask with 0.5 N hydrochloric acid to the
disappearance of the pink colour. Calculate the percent of Total Alcohols by the formula:

\[ A = \frac{(b - S)(100e)}{W - 2l(b - S)} \]

in which \( b \) = the number of ml of 0.5 N hydrochloric acid consumed in the titration of the blank, \( S \) = the number of ml of 0.5 N hydrochloric acid consumed in the titration of the sample, \( e \) = the equivalence factor given in the monograph, and \( W \) = the weight of the sample of the acetylized oil in mg.

3. Determination of Aldehydes

Hydroxylamine Hydrochloride Solution. Dissolve 50 grams of hydroxylamine hydrochloride, preferably recrystallized before using, in 90 ml of water and dilute to 1000 ml with aldehyde-free alcohol. Adjust the solution to a pH of 3.4 with 0.5 N alcoholic potassium hydroxide.

Procedure. Transfer an accurately weighed quantity of sample specified in the monograph into a 125 ml Erlenmeyer flask. Add 30 ml of Hydroxylamine Hydrochloride Solution, mix thoroughly, and allow to stand at room temperature for 10 minutes, unless otherwise specified. Perform a blank determination simultaneously with the sample determination. Titrate the liberated hydrochloric acid with 0.5 N alcoholic potassium hydroxide to a greenish yellow end-point, using bromophenol blue T.S. as the indicator, or titrate to a pH of 3.4 using a suitable pH meter. Calculate the percent of aldehyde (A) by the formula:

\[ A = \frac{(S - b)(100W)}{W} \]

in which \( S \) = the number of ml of 0.5 N alcoholic potassium hydroxide consumed in the titration of the sample, \( b \) = the number of ml of 0.5 N alcoholic potassium hydroxide consumed in the titration of the blank, \( E \) = the equivalence factor given in the monograph, and \( W \) = the weight of the sample in mg.

4. Determination of Aldehydes and Ketones

4.1 Hydroxylamine Method

Hydroxylamine Solution. Dissolve 20 grams of hydroxylamine hydrochloride (reagent grade or preferably freshly recrystallized) in 40 ml of water and dilute to 400 ml with alcohol. Add, with stirring, 300 ml of 0.5 N alcoholic potassium hydroxide, and filter. Discard any solution which is two days or more old.
**Procedure.** Transfer an accurately weighed quantity of the sample specified in the individual monograph into a 250 ml glass-stoppered flask, add 75.0 ml of the Hydroxylamine Solution, and stopper the flask. If the sample is an aldehyde, allow the mixture to stand at room temperature for 1 hour, unless otherwise directed in the monograph. If the sample is a ketone, attach the flask to a suitable condenser, and reflux the mixture for 1 hour, unless otherwise directed in the monograph, and then cool to room temperature. Titrate the solution with 0.5 N hydrochloric acid to a greenish yellow end-point using bromophenol blue T.S. as indicator, or to a pH of 3.4 using a suitable pH meter. Perform a blank determination simultaneously with the sample determination. Calculate the percent of aldehyde or ketone by the formula:

\[
AK = \frac{(b - s)(100E)}{w}
\]

in which \(AK\) = percent of aldehyde or ketone, \(b\) = the number of ml of 0.5 N hydrochloric acid consumed in the titration of the blank, \(s\) = the number of ml of 0.5 N hydrochloric acid consumed in the titration of the sample, \(E\) = the equivalence factor given in the monograph, and \(w\) = the weight of the sample in mg.

**4.2 Neutral Sulphite Method**

Introduce 5 ml of substance into a 100 ml cassia flask fitted with a stopper. Add 50 ml of a freshly prepared 40 percent sodium sulphite solution and 4 drops of phenolphthalein T.S. Heat in a boiling water bath, agitating frequently, for at least 2 hours. Neutralize the liberated caustic soda from time to time by adding 50 percent (by volume) acetic acid solution, drop by drop, stoppering the flask to prevent loss of volatile material. Continue to heat until such time that any further addition of the sodium sulphite solution, plus 2 drops of phenolphthalein T.S. no longer causes pink colouration. Cool to room temperature. When the liquids have separated completely, add sufficient sodium sulphite solution to raise the lower level of the essential oil liberated into the graduated neck of the flask. The quantity of essential oil in the neck of the flask, at room temperature, is used to calculate the percentage of the aldehyde or ketone as follows:

\[
\% \text{ volume} = 100 - (a \times 20)
\]

\[
\% \text{ weight} = 100 - (a \times 20) \frac{D}{d}
\]

in which \(a\) = the number of ml of separated oil in the graduated neck of the flask, \(D\) = density of substance combined and \(d\) = density of the essential oil.
5. Determination of Esters

5.1 Ester Determination

Transfer an accurately weighed quantity of the sample specified in the monograph into a 125 ml Erlenmeyer flask containing a few boiling stones. Add to this flask, and, simultaneously, to a similar flask for a blank test, 25.0 ml of 0.5 N alcoholic potassium hydroxide. Connect each flask to a reflux condenser, and heat the mixtures on a steam bath for exactly 1 hour, unless otherwise directed in the monograph. Allow the mixtures to cool, add 10 drops of phenolphthalein T.S. to each flask, and titrate the excess alkali in each flask with 0.5 N hydrochloric acid. Calculate the percent of ester (E) in the sample by the formula:

\[
E = \frac{(b - S)(100e)}{W}
\]

in which \(b\) is the number of ml of 0.5 N hydrochloric acid consumed in the residual blank titration, \(S\) is the number of ml of 0.5 N hydrochloric acid consumed in the titration of the sample, \(e\) is the equivalence factor given in the monograph, and \(W\) is the weight of the sample in mg.

5.2 Ester Determination (High Boiling Solvent)

0.5 N Potassium Hydroxide Solution. Dissolve about 35 grams of potassium hydroxide in 75 ml of water, add 1000 ml of a suitable grade of monochloro ethyl ether of diethylene glycol, and mix.

Procedure. Transfer an accurately weighed quantity of the sample specified in the monograph into a 200 ml Erlenmeyer flask having a standard-taper joint, and add 2 glass beads and 25.0 ml of 0.5 N Potassium Hydroxide Solution, allowing exactly 1 minute for drainage from the buret or pipet. Attach an air condenser, reflux gently for 1 hour, and cool. Rinse down the condenser with water, using about 50 ml, then add phenolphthalein T.S. and titrate the excess alkali with 0.5 N sulphuric acid to the disappearance of the pink colour. Perform a blank determination, using 25.0 ml of the alkali and 50 ml of water. Calculate the percent of ester in the sample by the formula \((b - S)(100e)/W\), in which \(b\) is the number of ml of 0.5 N sulphuric acid consumed in the blank determination, \(S\) is the number of ml of 0.5 N sulphuric acid required in the titration of the sample, \(e\) is the equivalence factor given in the monograph, and \(W\) is the weight of the sample in mg.
Saponification Value. Proceed as directed for Ester Determination. Calculate the saponification value (SV) by the formula:

\[
SV = \frac{(b - S)(28.05)}{W}
\]

in which \(b\) and \(S\) are as defined under Ester Determination, and \(W = \) weight of the sample in grams.

Ester Value. If the sample contains no free fatty acids, the saponification value and the ester value are identical. If a determination of the Acid Value (AV) is specified in the monograph, calculate the ester value (EV) by the formula:

\[
EV = SV - AV.
\]

6. Determination of Linalool

Transfer a 10 ml sample, previously dried with sodium sulphate, 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 minutes, permit it to stand at room temperature for another 30 minutes, then immerse the flask in a water bath maintained at 40° ± 1° for 16 hours. Wash the acetylated oil with three 75 ml portions of ice water, followed by successive washes with 25 ml portions of 5 percent sulphuric 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 a 1 in 10 solution of sodium carbonate and then with successive portions of water until the washings are neutral to litmus. Finally dry the acetylated oil with anhydrous sodium sulphate and proceed as directed for Ester Determination under 5.1. Calculate the percent of linalool \((C_{10}H_{18}O)\) 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.

Note: When this method is applied to essential oils containing appreciable amounts of esters, perform an Ester Determination, 5.1, on a sample of the original oil and calculate the percent of total linalool by the formula:

\[
L = \frac{7.707(b - S)(1 - 0.062E)}{W - 0.021(b - S)}
\]
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.

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

7. *Determination of Phenols*

Pipet 10 ml of the oil into a 100 ml cassia flask, add 75 ml of potassium hydroxide T.S. and shake vigorously for 5 minutes to ensure complete extraction of the phenol by the alkali solution. Allow the mixture to stand for about 30 minutes, then add sufficient potassium hydroxide T.S. to raise the oily layer into the graduated portion of the flask, stopper the flask, and allow it to stand overnight. Read the volume of insoluble oil to 0.05 ml. Calculate the percent, by volume, of phenol by the formula:

\[
P = (10 - V) \times 10
\]

in which \( P \) = percent of phenols, by volume, and \( V \) = observed volume of insoluble oil in ml.

8. *Chlorinated Compounds*

Wind a 1.5 x 5 cm. strip of 20-mesh copper gauze around the edge of a copper wire. Heat the gauze in a non-luminous flame of a Bunsen burner until it glows without colouring the flame green. Permit the gauze to cool and re-ignite it several times until a good coat of oxide has formed. With a medicine dropper, apply 2 drops of the sample to the cooled gauze, ignite, and permit it to burn freely in the air. Again cool the gauze, add 2 more drops, and burn as before. Continue this process until a total of 6 drops has been added and ignited. Then hold the gauze in the outer edge of a Bunsen flame, adjusted to a height of 4 cm. Not even a transient green colour is imparted to the flame.

9. *Solubility in Alcohol*

Transfer a 1.0 ml sample into a calibrated 10 ml glass-stoppered cylinder graduated in 0.1 ml subdivisions, and add slowly, in small portions, alcohol of the concentration specified in the monograph. Maintain the temperature at 20° and shake the cylinder thoroughly after each addition of alcohol. When a clear solution is first obtained, record the number of ml of alcohol required. Continue the addition of the alcohol until a total of 10 ml has been added. If opalescence or cloudiness occurs during these subsequent additions of alcohol, record the number of ml of alcohol at which the phenomenon occurs.
10. **Solidification Point**

10.1 **Scope**

This method is designed to determine the solidification point of food grade chemicals having appreciable heats of fusion. It is applicable to chemicals having solidification points between \(-20^\circ\) and \(+150^\circ\).

10.2 **Definition**

**Solidification Point** is an empirical constant defined as the temperature at which the liquid phase of a substance is in approximate equilibrium with a relatively small portion of the solid phase. It is measured by noting the maximum temperature reached during a controlled cooling cycle after the appearance of a solid phase.

Solidification point is distinguished from freezing point in that the latter term applies to the temperature of equilibrium between the solid and liquid state of pure compounds.

Some chemical compounds have two temperatures at which there may be a temperature equilibrium between the solid and liquid state depending upon the crystal form of the solid which is present.

![Fig. 1 - Apparatus for Determining Solidification Point](image)

![Fig. 2 - Stirrer](image)
10.3 Apparatus

The apparatus illustrated in Figure 1 consists of the following components.

**Thermometer.** A thermometer having a range not exceeding 30°, graduated in 0.1° divisions, and calibrated for 76 mm immersion. A thermometer should be so chosen that the solidification point is not obscured by the cork stopper of the sample container.

**Sample Container.** Standard glass 25- x 150-mm test tube with lip, fitted with a two-hole cork stopper to hold the thermometer in place and to allow stirring with stirrer.

**Air Jacket.** A standard glass 38- x 200-mm test tube with lip, fitted with a cork or rubber stopper bored with a hole into which the sample container can easily be inserted up to the lip.

**Cooling Bath.** A 2-litre beaker or similar suitable container. Fill it with an appropriate cooling medium such as glycerin, mineral oil, water, water and ice, or alcohol and ice.

**Stirrer.** The stirrer (Figure 2) consists of a 1-mm diameter (Size 18), corrosion-resistant wire bent into a series of three loops about 25 mm apart. It should be made so that it will move freely in the space between the thermometer and the inner wall of the sample container. The shaft of the stirrer should be of a convenient length designed to pass loosely through a hole in the cork holding the thermometer. Stirring may be hand operated or mechanically activated at 20 to 30 strokes per minute.

**Assembly.** Assemble the apparatus in such a way that the cooling bath can be heated or cooled to control the desired temperature ranges. Clamp the air jacket so that it is held rigidly just below the lip and immerse it in the cooling bath to a depth of 160 mm.

10.4 Preparation of Sample

The solidification point is usually determined on chemicals as they are received. Some may be hygroscopic, however, and require special drying.

Products which are normally solid at room temperature must be carefully melted at a temperature about 10° above the expected solidification point. Care should be observed to avoid heating in such a way as to decompose or distill any portion of a sample.
10.5 Procedure

Adjust the temperature of the cooling bath to about 5° below the expected solidification point. Fit the thermometer and stirrer with a cork stopper so that the thermometer is centred and the bulb is about 20 mm from the bottom of the sample container. Transfer a sufficient amount of the sample, previously melted if necessary, into the sample container to fill it to a depth of about 90 mm when in the molten state. Place the thermometer and stirrer in the sample container and adjust the thermometer so that the immersion line will be at the surface of the liquid and the end of the bulb 20 ± 4 mm from the bottom of the sample container. When the temperature of the sample is about 5° above the expected solidification point, place the assembled sample tube in the air jacket.

Allow the sample to cool while stirring, at the rate of 20 to 30 strokes per minute, in such a manner that the stirrer does not touch the thermometer. Stir the sample continuously during the remainder of the test.

The temperature at first will gradually fall, then become constant as crystallisation starts and continues under equilibrium conditions, and finally will start to drop again. Some chemicals may supercool slightly below (0.5°) the solidification point; as crystallisation begins the temperature will rise and remain constant as equilibrium conditions are established. Other products may cool more than 0.5° and cause deviation from the normal pattern of temperature change. If the temperature rise exceeds 0.5° after the initial crystallisation begins, repeat the test and seed the melted compound with small crystals of the sample at 0.5° intervals as the temperature approaches the expected solidification point. Crystals for seeding may be obtained by freezing a small sample in a test tube directly in the cooling bath. It is preferable that seed of the stable phase be used from a previous determination.

Observe and record the temperature readings at regular intervals until the temperature rises from a minimum, due to supercooling, to a maximum and then finally drops. The maximum temperature reading is the solidification point. Readings 10 seconds apart should be taken in order to establish that the temperature is at the maximum level and continues until the drop in temperature is established.
TEST SOLUTIONS

Barium Chloride T.S.

Dissolve 12 grams of barium chloride, \( \text{BaCl}_2 \cdot 2\text{H}_2\text{O} \), in sufficient water to make 100 ml.

Bromophenol Blue T.S.

Dissolve 100 mg of bromophenol blue in 100 ml of dilute alcohol (1 in 2) and filter if necessary. For pH determinations, dissolve 100 mg in 3.0 ml of 0.05 N sodium hydroxide, and dilute with carbon dioxide-free water to 200 ml.

Ferric Chloride T.S.

Dissolve 9 grams of ferric chloride, \( \text{FeCl}_3 \cdot 6\text{H}_2\text{O} \), in sufficient water to make 100 ml.

Hydrochloric Acid T.S., Diluted

A solution containing 10 percent (w/v) of \( \text{HCl} \). Prepare by diluting 236 ml of hydrochloric acid (36 percent) with sufficient water to make 1000 ml.

Hydrogen Peroxide T.S.

A solution containing between 2.5 and 3.5 grams of \( \text{H}_2\text{O}_2 \) in each 100 ml. It may contain suitable preservatives, totalling not more than 0.05 percent.

Lead Subacetate T.S.

Triturate 14 grams of lead oxide, \( \text{PbO} \), to a smooth paste with 10 ml of water, and transfer the mixture to a bottle, using an additional 10 ml of water for rinsing. Dissolve 22 grams of lead acetate, \( \text{Pb(C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O} \), in 70 ml of water, and add the solution to the lead oxide mixture. Shake it vigorously for 5 minutes, then set it aside, shaking it frequently during 7 days. Finally filter, and add enough recently boiled water through the filter to make 100 ml.

Phenolphthalein T.S.

Dissolve 1 gram of phenolphthalein in 100 ml of alcohol.
Potassium Hydroxide T.S.

Dissolve 6.5 grams of potassium hydroxide, KOH, in water to make 100 ml.

Sodium Hydroxide T.S.

Dissolve 4.3 grams of sodium hydroxide, NaOH, in water to make 100 ml.