WHO Expert Committee on Specifications for Pharmaceutical Preparations

Twenty-sixth Report

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WHO EXPERT COMMITTEE
ON SPECIFICATIONS
FOR PHARMACEUTICAL PREPARATIONS

Twenty-sixth Report

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## CONTENTS

1. Analytical criteria for drug quality assessment ........................................... 5
2. Revision of the International Pharmacopoeia .................................................. 6
3. International chemical reference substances ............................................... 7
4. Basic tests for drugs ...................................................................................... 8
5. Plastic containers for pharmaceuticals ............................................................ 9
6. Quality assurance in pharmaceutical supply systems ...................................... 11

Annex 1. Analytical criteria for drug quality assessment .................................. 13
Annex 2. Basic tests for drugs ........................................................................... 21
Annex 3. Requirements for plastic containers for pharmaceutical preparations 25
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FOR PHARMACEUTICAL PREPARATIONS

Geneva, 25-30 April 1977

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WHO EXPERT COMMITTEE ON
SPECIFICATIONS FOR PHARMACEUTICAL
PREPARATIONS

Twenty-sixth Report

The WHO Expert Committee on Specifications for Pharmaceutical Preparations met in Geneva from 25 to 30 April 1977. The meeting was opened on behalf of the Director-General by Dr Ch’en Wen-chieh, Assistant Director-General. He recalled that in its twenty-fifth report the Expert Committee had proposed revised texts of "Good practices in the manufacture and quality control of drugs" and "Certification scheme on the quality of pharmaceutical products moving in international commerce". He informed the Committee that the Twenty-eighth World Health Assembly, in resolution WHA28.65, had adopted those texts and had recommended Member States to apply the revised requirements. WHO had so far received 14 positive replies from countries agreeing to apply the certification scheme. Cyprus, Egypt, France, Italy, New Zealand, Poland, Portugal, and the Syrian Arab Republic had given positive replies without reservations or comments. Australia, Japan, Norway, Sweden, the United Kingdom and the USA had given positive replies with reservations or comments.

1. ANALYTICAL CRITERIA FOR DRUG QUALITY ASSESSMENT

Specifications are an important element in a drug quality assurance system, forming a basis for the laboratory examination of drugs. The selection of standards and associated methods of analysis depends partly on the intended area of application of the specification.

There are two principal considerations—one concerns the types of criteria for judging drug quality (e.g., standards and tests for identity

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and purity, standards and assays of strength, and standards and tests of performance for dosage forms) and the other concerns the use of criteria in quality specifications (e.g., pharmacopoeial monographs, manufacturer's batch release specifications, government regulations).

In the belief that an examination of various aspects of the analytical criteria used for drug quality assessment would be useful to all involved in drug regulatory activities, the Committee carried out a review of these criteria, which is published in Annex 1.

2. REVISION OF THE INTERNATIONAL PHARMACOPOEIA

In resolution WHA28.66, the Twenty-eighth World Health Assembly requested the Director-General "to continue to develop activities related to the establishment and revision of international standards, requirements and guidelines for prophylactic and therapeutic substances in consultation, as appropriate, with relevant governmental and non-governmental organizations in official relations with WHO ".

It was recommended in the twenty-fifth report of the Expert Committee that the Secretariat, working with members of the Expert Advisory Panel and with other specialists, should establish specifications for raw materials (comprising active and inactive ingredients) in pharmaceutical products and general methods and tests necessary to support such specifications.

Following these recommendations, work was started on the revision of the general methods of analysis contained in the International Pharmacopoeia. Drafts of a number of general methods of analysis to be used in the third edition of the International Pharmacopoeia were produced with the help of specialists and circulated to all members of the Expert Advisory Panel and to national and regional pharmacopoeia commissions. On the basis of the comments received revised drafts of those methods were prepared. Work was also started on the revision of specifications for the third edition of the International Pharmacopoeia.

The Committee welcomed the progress that had been made and requested the Secretariat to continue the process of revision of the International Pharmacopoeia, giving priority to those substances that are most widely used in general health care.

3. INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES

3.1 Reports from the WHO Centre

Reports from the WHO Collaborating Centre for Chemical Reference Substances were received by the Committee.¹

3.1.1 Establishment of new reference substances

The Committee noted that in accordance with the authorization given in its previous reports the following new International Chemical Reference Substances had been established:

- Chloramphenicol Palmitate
- Chloramphenicol Palmitate (polymorph A)
- Dicloxacillin Sodium
- Fluphenazine Hydrochloride
- Phenoxymethylpenicillin Potassium
- Tolnaftate

3.1.2 Replacement of current reference substances

The Committee also noted that replacement batches of the following International Chemical Reference Substances had been introduced:

- Carbenicillin Sodium
- Ergotamine Tartrate
- Oxacillin Sodium
- Riboflavin
- Vitamin A Acetate

3.1.3 Future work

The Committee was informed that work had been initiated to replace the following International Chemical Reference Substances since stocks of them were nearing depletion:

- Digitoxin
- Ergometrine Maleate
- Digoxin
- Folic Acid

It was noted that the Centre had, in consultation with the Secretariat, prepared a tentative list of new International Chemical Reference Substances to be established in the next few years. This list had been sent to a number of other organizations known to be concerned with the establishment of reference materials, together with an invitation to collaborate on substances of mutual interest.

The Committee was also informed that the WHO Expert Committee on Biological Standardization had asked the Secretariat to investigate the possibilities of providing chemical reference substances for some antitumour antibiotics. It was recommended that the need for chemical reference substances for these products should be further explored by the Secretariat before any work to provide them is undertaken by the Centre.

It was noted that International Chemical Reference Substances are being increasingly used, particularly in several developing countries where official drug quality control systems are now being built up. It was also noted that the use of International Chemical Reference Substances is now prescribed in various national and regional pharmacopoeias. The Committee considered this to be a commendable development since it minimizes the proliferation of differing reference substances of the same material. It was, however, urged that, before reference to the use of an International Chemical Reference Substance is made in published national or regional quality control specifications, the WHO Collaborating Centre for Chemical Reference Substances should be informed so that adequate stocks of the substances concerned can be maintained to meet the resulting increased demand for them. In addition it would be helpful if users of International Chemical Reference Substances would make careful estimates of their requirements from time to time and inform the Centre accordingly.

In discussing the possibility of fostering increased international cooperation with regard to chemical reference substances, the Committee requested the Secretariat to investigate the feasibility of producing and maintaining an up-to-date computerized list of the reference substances available from various authorities, including information about the purposes for which such substances are offered and the names and addresses of the issuing authorities.

The Committee expressed its satisfaction with the work carried out so far by the Centre.

4. BASIC TESTS FOR DRUGS

Procedures for the quality control of drugs that are usually included in pharmacopoeial monographs often demand resources and equipment that may be found only in large and well equipped laboratories.

Where such laboratories do not exist, a full examination for the quality of a drug based on the use of adequate specifications is not
feasible, and the Committee felt that it might be useful to evolve simplified tests for establishing the identity of a drug and for ascertaining the absence of gross degradation. Such basic tests are discussed in Annex 2.

The Committee considered that the use of basic tests should be strictly limited to situations in which full testing according to established quality specifications is not possible. By their nature, simplified tests cannot be as reliable as full drug quality specifications, and an adverse result of such a simplified test should serve only as a warning of potential unsuitability of a drug; no final conclusions should be drawn until a full analytical examination has been carried out.

The Committee recommended that the Secretariat should proceed with the elaboration of basic tests with the help of specialists, especially for drugs widely used in general health care. At the initial stage, the proposals should cover pharmaceutical substances (starting materials) both in respect of tests for identity and for absence of gross degradation. At a later stage these basic tests should be adapted to allow examination of dosage forms subjected to extreme climatic conditions.

The validity of the proposed tests should be established by application in the field in developing countries with extreme climatic conditions. Reports on such applications should be evaluated before final recommendations are made. Consideration could then be given to the publication of the tests, together with the respective monographs of the International Pharmacopoeia.

5. PLASTIC CONTAINERS FOR PHARMACEUTICALS

In its twenty-fifth report the Expert Committee published provisional requirements for plastic containers¹ in order to encourage further work. Comments and suggestions concerning these requirements were received from numerous experts and organizations and were subsequently reviewed by consultants.

In the light of this review the Committee revised the requirements. The advisory information in the introductory part of the requirements was considerably expanded in order to increase its value to pharmaceutical manufacturers and regulatory authorities. Some test procedures were also modified. The revised text is contained in Annex 3. Although the requirements concern plastic containers it is recognized that in certain situations there is a need to examine the plastic material prior to fabrication of the container.

The Committee drew attention to the existence of other criteria that might be of value in examining plastic containers. These include a limit test for primary aromatic amines, measurement of the ultraviolet light absorption of a suitable extract, and the use of tissue culture tests for toxicity. It was recommended that these tests be considered during any future revision of the requirements.

The requirements given in Annex 3 indicate the existence of published methods for the detection and measurement of traces of vinyl chloride monomer in poly(vinyl chloride). However, it was considered premature to recommend a method and a limit until further experience in the applicability of these methods is obtained.

As plastic giving sets are being used for administration of infusions the Committee recommended that any plastic component in such sets should meet equivalent requirements to those for plastic containers for infusions.

In selecting plastic containers for use in extreme climatic conditions it is necessary to ascertain that physical damage will not be induced by the temperatures encountered.

The requirements given in Annex 3 draw attention to the necessity of performing stability tests on the pharmaceutical preparation in the container selected for use. They also refer to the permeability of plastic material by water, which may result in a change in concentration of the contents. Such permeability is a particular problem in hot and dry climatic conditions, and special precautions may have to be taken, such as the placing of an external wrapper around the container to minimize solvent losses.

The Committee considered the advisability of providing lists of plastic polymers and additives that are approved by national authorities for use in containers for pharmaceuticals. It was recognized that delays in introducing new additives to the approved lists might hinder development and also that information derived from the list might be misused; nevertheless it was considered that the probable value of such lists would outweigh these disadvantages. The entries in the lists should relate to different types of dosage form and should be accompanied by adequate explanation to minimize misuse of the information. In this connexion, the recent elaboration by some authorities of monographs for certain plastic materials intended for the manufacture of containers was noted.

The Committee recognized that additional assurances of quality would result from the international acceptance of a code of good manufacturing practices in the production of plastics and the fabrication
of plastic containers. The development of such a code should be encouraged.

A related use of plastics for fabrication of medical devices, such as contact lenses, catheters, and prostheses was noted, and a need for appropriate quality requirements was recognized. Such requirements are already described in the Pharmacopoeia of the German Democratic Republic.

The observer for UNIDO informed the Committee of the interest of his organization in the overall industrialization of developing countries, particular emphasis being given to the petrochemical and pharmaceutical industries. His organization was thus particularly interested in the application of plastic materials to pharmaceutical packaging. He drew attention to the value of cooperation between WHO and UNIDO in this regard.

6. QUALITY ASSURANCE IN PHARMACEUTICAL SUPPLY SYSTEMS

The Twenty-eighth World Health Assembly, in resolution WHA 28.66, requested the Director-General “to develop means by which the Organization can be of greater direct assistance to Member States in the implementation of national programmes in research, regulatory control, management and monitoring of drugs and, in so doing, also in the formulation of national drug policies”.

The Committee was of the opinion that a comprehensive review of quality assurance in pharmaceutical supply systems might be of value for national programmes in the regulatory control of drugs and suggested that a suitable document be prepared for discussion by the Committee. The document should refer to international problems in the field of drug quality assurance and should include a consideration of related major WHO programmes.

Since a lack of resources in certain areas might prevent the application of a comprehensive system of drug quality assurance, the Committee considered that the document should also recommend the courses of action that might be taken to assure the quality of drugs being supplied and distributed under such conditions. It was also suggested that the information contained in Annex 1 be taken into consideration in the preparation of the document.

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Annex 1

ANALYTICAL CRITERIA FOR DRUG QUALITY
ASSESSMENT

1. Quality assessment as an element of drug regulatory activities

The production of drugs with assured quality and the maintenance of that quality while the drugs pass along commercial channels are matters that concern both manufacturers and consumers. The primary responsibility for systematic surveillance over the integrity of drugs and starting materials is vested in government authorities. The regulatory functions required for such surveillance include: (1) inspection of manufacturing and quality control activities to ensure that drugs of demonstrated safety and efficacy are fabricated by means of good manufacturing practices in accordance with approved specifications; (2) examination of starting materials, containers, and closures to determine whether they are sound; (3) examination of drugs both before and after distribution to ascertain whether their integrity has been maintained; and (4) examination of imported drugs both at their point of entry and thereafter. All these essential regulatory activities involve the sampling and quality assessment of drugs in commerce to determine whether they meet the standards of excellence established for judging their acceptability. For information about drugs at the stage of clinical evaluation see "Pharmaceutical aspects of drug evaluation for registration".  

2. Types of criteria for judging drug quality

Judgements concerning drug quality may be based on both objective and subjective criteria. Criteria such as the general appearance of a drug and its colour, odour, and taste are relevant to its integrity and quality but may not be amenable to analytical measurement and are

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1 For the purposes of this document the definitions of the terms drug, manufacturing, starting materials, batch, and quality control are identical with those given in "Good practices in the manufacture and quality control of drugs" (WHO Technical Report Series, No. 567, 1975, p. 17, Annex 1.) A drug is defined as any substance or mixture of substances that is manufactured, sold, offered for sale, or represented for use in (1) the treatment, mitigation, prevention, or diagnosis of disease, an abnormal physical state, or the symptoms thereof in man or animal; or (2) the restoration, correction, or modification of organic functions in man or animal.

are therefore subjective in nature. An objective standard, by contrast, is a criterion for which a defined range of values can be stated or that may be expressed as a response under specified conditions of examination. Every standard is associated with a complementary test or assay. The standards and associated tests and assays that are most useful in quality assessments are those relating to the identity, purity, strength, and performance of drug products. Depending on the nature of the material, these may be based on chemical, physical, or biological responses.

2.1 Standards and tests of identity

Standards and tests of identity for starting materials, whether active or inactive ingredients, are designed to demonstrate that the specimen examined consists of the substance it purports to be. Standards and tests of identity for drugs are designed to demonstrate that the specimen examined contains the active ingredient (or ingredients) it purports to contain. Tests of identity are most frequently based on qualitative chemical procedures or physical observations, but microbiological or biological methods also may be employed.

Tests of identity should yield unambiguous results. Specificity, the hallmark of reliable tests of identity, is not always assured or easily attained. Accuracy is not usually a requirement for these tests. Typical tests of identity include the use of: melting point characteristics; ultraviolet, infrared and other types of spectrophotometry; chromatography; specific colour reactions; and degradative reactions. Subjective criteria are often taken into consideration for identification purposes.

2.2 Standards and tests of purity

Standards of purity set limits on the concentrations of impurities permitted in drugs and in starting materials. Modern analytical techniques allow the detection and estimation of exceedingly small quantities of impurities. Standards are set for those impurities that are potentially harmful and for those whose presence may indicate a deviation from good manufacturing practice. The stringency of the tolerance depends on the nature of the impurity.

The test methods associated with standards of purity are semi-quantitative or quantitative procedures designed to detect and estimate the impurities. Where the nature of the impurity is known, it is often possible to express the standard as a numerical limit. Where the nature of the impurity is unknown, the tolerance is frequently expressed as
a response under specified conditions of examination. The specificity needed for tests of purity varies with the nature of the impurity. The heavy metals test, for example, is based on a nonspecific reaction; it is designed to detect and estimate all the ions that yield a discernible sulfide precipitate in aqueous acid. By contrast, a test to determine lead in a drug or starting material requires a specific method that permits the quantitation of that element in the presence of other metallic impurities.

The sensitivity, accuracy, and precision of the purity test must be suited to the requirements of the tolerance implicit in the standard. Purity tests may range from the simple (e.g., loss on drying) to the complex (e.g., the combination of gas liquid chromatography and mass spectrometry to estimate the concentration of a toxic impurity—though such complex tests are rare). Biological procedures such as sterility tests and pyrogen tests may be employed, as well as chemical and physical procedures.

2.3 Standards and assays of strength

The standard of strength is expressed quantitatively as the permitted range of concentration of the active ingredient (or ingredients) in the drug or starting material, as determined in the required assay.

Quantitative analytical procedures that are employed in assays include such diverse methods as spectrophotometry, titrimetry, gravimetry, and methods based on electrical, thermal, and biological responses. Accuracy, precision, and specificity are the principal considerations in devising assays. Ideally, the assay should permit the specific determination of the intact active substance, but because closely related substances (and other sources of interference) may be present it is usually necessary to apply separative processes such as extraction, volatilization, precipitation, or chromatography to assure the specificity of the determination. These separative processes may introduce significant analytical errors. Therefore it is often preferable in practice to employ an accurate and precise assay that is not specific and to rely both on associated purity tests to demonstrate that significant quantities of interfering impurities are absent and on unequivocal identification of the substance being assayed.

The range of permissible strength stated in the standard must take into account all the anticipated errors due to the effects of interfering substances and the inaccuracy and imprecision of the assay. An assayed strength that is then outside the permitted range must be deemed a
contravention of the standard, regardless of any errors inherent in the method.

2.4 Standards and tests of performance for dosage forms

Standards and tests of performance are designed to provide some warranty that the dosage form will function as it is intended to do. An example of a performance test whose execution directly parallels the conditions of intended administration is the measurement of the “unit spray content” of aerosol products. In this performance test, the average quantity of the active ingredient delivered by a single actuation of a metered-dose aerosol is compared with the intended dose declared on the label.

In vitro disintegration tests, such as those for enteric-coated tablets, are performance tests that are only indirectly related to the in vivo action of the drug. Enteric-coated tablets are oral dosage forms that are designed to pass through the stomach intact, and to release the active ingredient in the intestines. In the disintegration test, the product is treated with dilute acid solution for a period of time, and is then treated with a slightly alkaline solution whose pH is similar to that of the intestinal fluids. The tablets should remain unaffected by the acid solution, and should disintegrate within a specified time interval when agitated with the alkaline solution. The operational conditions of the disintegration test are obviously designed to simulate the conditions of the physiological milieu to which the enteric-coated tablets are exposed, but it is difficult to demonstrate a high degree of correlation between the results observed in vitro and the effects of oral administration. Nevertheless, this performance test has been found useful for quality assessments and as an indicator of good manufacturing practice.

Similarly dissolution requirements for tablets and other oral dosage forms have been used as criteria in quality assessment. The dissolution rate of some active ingredients has been shown to correlate with one or more of the following: rate of absorption of the substance from the gastrointestinal tract into the bloodstream; extent of absorption of the substance into the circulatory system; rate of excretion of the substance (or a metabolite) in the urine; amount of drug (or a metabolite) excreted in the urine. For drugs where such correlation exists, the dissolution test may be considered an indirect performance test for bio-availability. In the absence of such a correlation, bioavailability may be measured by means of other, more direct performance tests, although a dissolution requirement may still provide useful evidence of uniformity between batches.
3. Quality specifications

Quality specifications comprise a set of selected standards associated with methods of analysis that may be used to assess the integrity of drugs and starting materials. Such specifications must not be so exacting that they exclude effective drugs that have been prepared in accordance with acceptable manufacturing procedures, but they must be searching enough to differentiate clearly between properly constituted entities and inferior entities. Certain major principles govern the selection of standards that are included in specifications. Firstly, they should incorporate the characteristic properties observed in specimens whose efficacy and safety have been demonstrated. Secondly, they must recognize and limit the potentially dangerous impurities, including degradation products, that might be present at unacceptable levels. Thirdly, they must rely on discriminating analytical methods that permit the detection and measurement of important constituents of the drug.

The selection of appropriate specifications depends on their intended uses. Specifications appropriate to pharmacopoeias are intended to be used in examining drugs within their entire shelf-life and may be applied as legal regulatory instruments. However, another set of related specifications might be deemed more appropriate for other purposes, such as determining a manufacturer's decision whether to release or to reject batches of production material.

3.1 Drug quality criteria in pharmacopoeial monographs

All drugs and starting materials introduced into commerce within a jurisdiction must comply with the laws, the regulations, and the official pharmacopoeia recognized in that jurisdiction. To serve as satisfactory instruments for the regulation of drugs in commerce, the standards in pharmacopoeial monographs must be stated unambiguously in terms that permit objective evaluation of product quality.

Ideally, pharmacopoeial specifications are universally applicable to all commercial products that purport to be the entity defined in the official monograph. The analytical methods in the pharmacopoeia must yield reliable, consistent results when employed by any competent analyst.

A pharmacopoeial monograph should include all provisions that are necessary and sufficient to permit a definitive judgement on the quality of the drug it defines. A specimen of the drug that fails to comply with
any monograph requirement is not of pharmacopoeial quality, even though it conforms to all the other requirements in the monograph.

The results obtained by applying the pharmacopoeial assay and tests to a discrete specimen of a drug are valid for judging the quality of that specimen. To draw valid inferences regarding the presumed quality of unexamined units in a batch from which the examined specimen was taken, it is necessary to define the relationship of the specimen to the batch by means of a sound sampling plan. The development and application of such sampling plans are in the domain of production quality assurance and governmental control functions; they are usually not within the regulatory purview of the pharmacopoeia.

The legal status of pharmacopoeial drug quality criteria as enforceable obligatory standards for articles in commerce derives from the law or ordinance conferring official recognition on the pharmacopoeia. The applicability and binding force of these pharmacopoeial criteria in private commercial transactions depend upon the tacit or explicit agreement between purchaser and vendor that these published standards shall determine the quality of the commodities involved in their dealings.

3.2 Drug quality criteria in manufacturers' quality control systems

Manufacturers' quality control systems are designed to ensure that all batches of drugs released for distribution in commerce meet their established specifications. The drug quality criteria that comprise the batch release specifications must be sufficiently stringent to provide assurance that any specimen drawn from the batch conforms to all official requirements at the time of manufacture and will continue to do so while the drug is an article of commerce. Thus, although release specifications must be based on and must be compatible with pharmacopoeial specifications, they can differ in several respects. In general, manufacturers' batch release specifications for a drug are more exacting than the corresponding pharmacopoeial requirements.

Manufacturers' batch release specifications may vary from manufacturer to manufacturer and sometimes even among several production centres of the same manufacturing enterprise. Such specifications not only relate to pharmacopoeial requirements for the product but are influenced by many additional factors, some of which may be unique. For example, the batch release specifications of a production centre are based on the formulae and procedures employed in that centre's manufacturing process (including those for intermediate products) and on the attributes of high-quality specimens consistently fabricated at that
centre. Among other data considered in setting batch release specifications are: the results of studies on the stability of the finished product in its container under various conditions of storage; the expiration date assigned to the product; statistically valid sampling plans that take into consideration the size of the batch; the number of specimens drawn from the batch for quality assessments; and the mode of selecting the specimens for examination during intermediate stages of production, as well as after final packaging.

The manufacturer's analytical methods, too, can differ from the tests and assays set forth in pharmacopoeial monographs. The manufacturer's laboratory may be able to devise analytical methods more appropriate and more convenient to production within the factory since it knows the identity and expected behaviour of every ingredient in the drug and of its precursors and intermediates. Alternative methods so chosen are preferable if the information they yield regarding the integrity of the batch is more cogent than the information furnished by officially recognized methods.

Moreover, the character of the conclusions afforded by the manufacturer's quality control system differs from the character of judgements based on pharmacopoeial criteria. Pharmacopoeial requirements permit a definitive judgement, affirmative or negative, regarding the quality of a drug specimen in commerce. The manufacturer's quality control system is designed to evolve a prediction, with some calculated degree of confidence, whether the untested population in a batch will comply with all official requirements over a specified period of time when exposed to stipulated conditions of storage.

3.3 Drug quality criteria in governmental regulatory activities

For drugs and starting materials that are defined in pharmacopoeial monographs, the criteria that determine the legally acceptable quality characteristics are the specifications set forth in the monograph. Where the pharmacopoeia does not provide a monograph for a drug or starting material, the regulatory government body may promulgate appropriate quality criteria. Regulatory agencies and industry alike may employ alternative analytical methods if they deem them more suitable in checking the quality of products. However, in the event of a dispute whether a drug or starting material complies with the pharmacopoeial requirements, only the results obtained by the procedures given in the pharmacopoeia are conclusive.

Although the primary objective of the pharmacopoeia is to ensure the integrity of the drugs and starting materials it defines, it is
manifestly impossible to include in each monograph a test for every impurity that might be present, including microbial contaminants. Any reliable and validated test results that demonstrate the presence of impurities at levels that are dangerous or otherwise objectionable may be cited by the regulatory agency to impugn the quality of a product, even though the pharmacopoeia provides no standards regarding those impurities.

Analytical results obtained by examining individual specimens according to pharmacopoeial tests and assays may be used to judge whether the quality of these specimens is acceptable. To characterize the quality of the larger population from which the specimens have been drawn, the regulatory agency must develop an appropriate sampling plan and must consider the distribution of the analytical values actually obtained.
Annex 2

BASIC TESTS FOR DRUGS

General

In many countries, including those that satisfy their drug needs by importation, labelling and visual characteristics serve as the sole indication of drug identity throughout the drug distribution chain. Simplified analytical tests might be used in such circumstances to ensure the identity of drugs.

In several European countries simple tests have already been introduced for use at peripheral levels of distribution (wholesale drugstores, pharmacies) to confirm the identity of pharmaceutical substances (so preventing mix-ups) and sometimes to ascertain the absence of important impurities. Such systems have been fostered by several pharmaceutical associations.

Besides the question of identification of pharmaceutical substances, another problem of great importance especially in tropical countries is the degradation of pharmaceuticals during storage and transportation. The expiry date, which sometimes serves as an adequate safeguard in temperate countries, may be misleading in tropical areas even when adequate containers are provided. Simplified analytical techniques to ensure the absence of gross degradation should be developed. Such tests should be introduced first of all for well established drugs used in general health care.

Basic tests need not be carried out by fully qualified pharmacists or chemists, but they should be performed by persons who have some understanding of analytical chemistry such as that acquired in pharmacy assistant schools or obtained in special courses.

The application of basic tests should be strictly limited to situations in which full testing according to established quality specifications is not possible. By their nature, simplified tests cannot be completely reliable, and an adverse result should serve only as a warning of potential unsuitability of a drug. No final conclusion should be drawn until a full analytical examination has been carried out.

In developing simplified methods, research workers should initially concentrate on tests for identity and stability of starting materials. A pilot project should be undertaken to demonstrate the viability of the techniques in a few interested countries prior to wider implementation. The application to dosage forms should be undertaken at a later stage.
In every case the results obtained in the stability studies on the starting materials will have a bearing on the tests for dosage forms.

Technical approaches

The procedures used for basic tests should be as simple as possible and the techniques selected should be capable of being carried out even under adverse conditions. To achieve a reasonable degree of assurance in respect of drug identity, several tests should usually be performed, possibly based on different functions of the tested molecule. An excessive number of tests should be avoided and a compromise solution sought that will give the desired degree of certainty.

The number of reagents and appliances should be kept to a minimum, and attempts should be made to avoid the use of reagents that are expensive or difficult to obtain. The reagents should be stable (unaffected by storage at elevated temperatures), and every effort should be made to avoid using reagents with corrosive properties. The possible use of some pharmaceutical starting materials as reagents has also been suggested since these materials are normally available where the basic tests will be carried out.

The use of visual or similar characteristics for initial assessment is self-evident, and all such attributes as colour and characteristic odour (or their absence) should be utilized as far as possible. Taste should be used only in special cases.

To appraise each drug a number of basic tests would have to be performed. A negative result, even in a single test, would be sufficient to declare the substance unsuitable for use until a thorough test could be carried out in a properly equipped analytical laboratory.

Among the techniques that might be used to carry out basic tests are test-tube techniques, the determination of melting temperatures, simple chromatographic procedures, and the use of impregnated reagent papers.

(1) Test-tube techniques

Classical chemical techniques such as colour reactions, the formation of precipitates with specific reagents, the evolution of gas and its identification, and the behaviour of substances on heating are widely used for identification purposes by the basic test system described. In some instances these reactions lack specificity, the reason being that many drugs have similar functional groups that are indistinguishable by
simple chemical reactions. On the other hand, the tests are usually simple to perform and no great skill is required.

The appearance of a concentrated solution of a substance in selected solvents can serve as a useful procedure for determining degradation and the presence of some impurities.

(2) Melting temperature technique

The determination of melting temperature in a capillary tube in an apparatus heated even by a small alcohol burner is considered suitable as a basic test. The use of a Koehler hot bar or an electrically heated Maquenne block is likely to be more convenient. Where considerable differences exist in melting temperature by the use of different methods, separate ranges of melting temperatures should be given for each method.

The use of eutectic melting temperature determination with a limited number (about five) of standard substances greatly increases the degree of assurance of the identification.

It is necessary to define the terms used in this technique, especially for eutectic melting temperature determination, and to calibrate the apparatus with substances of known melting temperatures.

Because of difficulties that may be associated with methods involving special apparatus, possibly requiring unusual skills, the use of such methods should be kept to a minimum.

(3) Simple chromatographic procedures

Simple chromatographic techniques, especially thin-layer and paper chromatography, are highly suitable for checking the presence of degradation products. They usually give a high degree of identity assurance if used together with an authentic reference substance.

Several issues have to be resolved, however, before suitable chromatographic procedures can be adopted as basic tests. Apart from the provision of a suitable reference substance, systems should be selected that use high-boiling solvents and thus permit the development of chromatograms at temperatures of about 30–35°C. Other issues to be resolved include the detection of spots by ultraviolet lamps or by reagents and the availability of solvents of sufficient purity. The use of only a limited number of detection reagents should be envisaged, and the provision of commercially prepared chromatographic plates may obviate the need for equipment for coating plates.

(4) Impregnated reagent papers

An extension of the test-tube technique based on colour reactions may be obtained by using specially prepared reagent papers, which
may be either dipped into the solution to be tested or spotted with that solution. The procedures for the preparation of the reagent papers should be simple so that they could be prepared locally. The advantage of this approach lies in the decrease of the number of places where the full set of reagents for basic tests has to be made available.

At the moment there are several problems hindering the extensive use of reagent papers. Only a limited number of such papers are available commercially, and very little work has been done on the degradation of such papers during storage.
Annex 3

REQUIREMENTS FOR PLASTIC CONTAINERS
FOR PHARMACEUTICAL PREPARATIONS

1. Introduction .............................................. 26
   1.1 General .............................................. 26
   1.2 Plastic containers for parenteral and aqueous ophthalmic preparations 27
   1.3 Plastic containers for other pharmaceutical dosage forms .............. 29

2. Recommended procedures for containers for infusions and injections .... 30
   2.1 General .............................................. 30
   2.2 Physicochemical tests ................................ 31
       2.2.1 Aqueous extractives obtained at 70°C ....................... 31
       2.2.2 Aqueous extractives obtained at 120°C ...................... 33
       2.2.3 Additional test for infusions autoclaved in a container ........ 34
       2.2.4 Additional test for tin .................................. 35
       2.2.5 Additional test for barium .............................. 35
   2.3 Biological test in vitro .............................. 36
       2.3.1 Aqueous extractives obtained at 120°C ...................... 36
   2.4 Biological tests in vivo .............................. 37
       2.4.1 General .............................................. 37
       2.4.2 Extractives obtained with standard extraction media ............ 37
       2.4.3 Additional test for infusions autoclaved in a container ........ 39

3. Recommended procedures for containers for aqueous ophthalmic preparations 41
   3.1 General .............................................. 41
   3.2 Chemical tests ....................................... 41
       3.2.1 Aqueous extractives obtained at 70°C ....................... 41
       3.2.2 Aqueous extractives obtained at 120°C ...................... 42
   3.3 Biological test ....................................... 42
       3.3.1 General .............................................. 42
       3.3.2 Extractives obtained with standard extraction media ............ 43

Appendix. List of reagents, test solutions, and volumetric solutions .... 43
1. INTRODUCTION

1.1 General

According to the general requirements of the International Pharmacopoeia, the container of a substance and its closure must not interact physically or chemically with the substance in such a way as to alter the strength, quality, purity or physical characteristics of the substance. If interaction is unavoidable, the alteration must not be so great as to bring the substance below the pharmacopoeial requirements. These requirements are applicable to containers of any type of material.

Containers for pharmaceutical use made of plastic materials are produced from a wide range of largely organic solid polymers based on synthetic resins (polyolefins, polyamides, polyesters, polystyrenes, halogenated vinyls, and various copolymers) or of modified polymers of natural origin (cellulose esters). They possess appreciable mechanical strength and are capable of being cast, moulded, or polymerized into shape. These characteristics have led to the development of a large variety of rigid bottles, flexible forms (bags) for blood and infusions, as well as films and laminates for enclosing many different solid dosage forms.

The mechanical, physicochemical, and biological properties of formed plastic containers depend on the chemical structure, molecular weight, and class of the polymer and on the type and concentration of additives. Some plastics may contain as impurities monomers, catalytic materials, and other residues of ingredients that are used in processing. The concentration of such impurities generally represents only a small fraction of the total polymer weight. Plastics may also contain deliberately added substances such as antioxidants, impact modifiers, lubricants, plasticizers, stabilizers, and mould release agents, which are frequently highly reactive and potentially incompatible with various ingredients in the pharmaceutical dosage forms. Some of these additives may be capable of migrating into the preparation.

The selection of a plastic material, a combination of plastic materials, or a combination of plastic and other materials for the container for packaging a pharmaceutical preparation will depend on the nature of the dosage form for which it is intended. The kind of tests that will be needed will depend on the nature of the plastic and the characteristics of the pharmaceutical preparation. The plastic might, for example, be an entirely new material, or it might be one that is well known but produced by another manufacturer. The pharmaceutical preparation
may be a newly developed product or a stock product that is to be
differently packed. In addition, there is a need for routine tests that
can be carried out on deliveries of the chosen container before release
for use. The testing will confirm that the processing of the plastic has
been carried out satisfactorily and will establish the protective qualities
of the container and the compatibility, toxicological safety, and stability
of the pharmaceutical preparation.

In order to maintain consistency in the quality of plastic containers
it is essential that an agreement be reached between the pharmaceutical
manufacturer, the supplier of plastic materials, and the container
manufacturer regarding the detailed composition of the plastic, the
method of processing it, and the quality specifications for ingredients
and plastic product. Any change in composition or processing of the
plastic containers or components should be reviewed in advance with
the pharmaceutical manufacturer.

The purpose of this document is to offer guidance to pharmaceutical
manufacturers on the selection of plastic containers and on the tests
that may be undertaken as part of a system of good pharmaceutical
manufacturing practice. It is not the intention of the document to
supply a series of specifications for regulatory purposes. The pharma-
aceutical manufacturer will need to perform stability tests on the prepa-
ration in the container selected for use and determine whether any
degradation of the medicament occurs and, if it does, its degree and
type. Any loss of antimicrobial agent, if included, should be measured
and the life to expiry of the product determined under appropriate
conditions. Those tests that may be used routinely will form the basis
of a release specification for the containers.

When an established pharmaceutical product is to be manufactured
in a new plant, particularly in a new geographical area, then even
though the same plastic containers are used it may be necessary to apply
not only the routine quality control tests recommended in the present
document but also some of the specific tests used in the original develop-
ment of the product, these tests being made after the product has been
stored in the container.

1.2 Plastic containers for parenteral and aqueous ophthalmic
preparations

Since all parenteral and aqueous ophthalmic pharmaceutical prepa-
trations are required to be sterile, consideration must be given to the
use of suitable methods of cleaning and sterilizing plastic containers
for such products. Detergents and other substances used for washing, if not completely removed, may be absorbed into the plastic and subsequently released into the preparation; they may also damage the plastic. The method of sterilization that is selected must not significantly alter the properties of the plastic container, leave undesirable residues, or form objectionable new impurities. In sufficient concentrations, such residues or impurities may be hazardous. Steam, ethylene oxide, or penetrating radiation have been employed successfully for sterilizing empty plastic containers. Steam is frequently used for sterilizing blood and infusion bags, either empty or filled with intravenous infusion solution. When plastic containers have been sterilized by exposure to ethylene oxide, the removal of residues of the gas and its reaction products should be demonstrated by analytical assessment. Suitable methods of determining such residues have been published.

When considering containers for large-volume infusion solutions, specific properties such as flexibility, collapsibility, clarity, and temperature resistance are important and should be controlled. Attention must also be given to the permeability of the plastic to water and solvent vapours and other gases to ensure that solutions become neither unduly concentrated on storage nor contaminated with extraneous materials. The design of the container and its closure should be appropriately selected and it should be established experimentally that the product in the chosen container is adequately protected from microbial contamination during storage. Requirements for parenteral pharmaceutical preparations, such as the requirements for sterility, particulate matter, and freedom from pyrogens, should be borne in mind when selecting a plastic material and container.

Plastic materials similar to those used for containers for large-volume intravenous infusion solutions are used for making containers for blood and blood substitutes. For these applications, additional tests are required to ensure that the properties and characteristics of blood and its substitutes are maintained. Such tests are not included in this document.

Plastic containers for parenteral pharmaceutical preparations should be, and should remain, sufficiently transparent that the appearance and the clarity of the preparations can be examined at any time. The container should not impart an objectionable odour to the contents nor release solid particles.

The test procedures recommended in this document for application to plastic containers for parenteral and aqueous ophthalmic preparations include physicochemical and biological tests, and are mainly based on
extraction procedures. Some of these tests will be of value in the examination of new plastic materials or of plastic containers in which changed ingredients have been introduced, but very much more searching and prolonged biological tests will be needed to establish toxicological safety in such instances. In addition chromatographic or spectrophotometric techniques are highly desirable in order to establish the nature and quantities of materials leached from the plastic into the contents of the container.

At present, four main types of plastics are used for containers for parenteral products and aqueous ophthalmic preparations: low-density polyethylene, high-density polyethylene, polypropylene, and poly(vinyl chloride). The different compositions and possible impurities in these types of plastic may require different testing methods, and these are recommended where appropriate. The identity of the type of plastic under examination may be confirmed by infrared spectroscopy (preferably) or by such measurements as melt-flow index, softening-point, hardness, density, or refractive index.

The biological tests recommended in the document are designed mainly to detect nonspecific toxicity of the plastic material arising from such sources as errors or cross-contamination during manufacture of the plastic or fabrication of the container. Various extraction fluids are specified for the biological tests, and it is recommended that the fluids nearest in properties and composition to the intended products should be employed. Although some pharmaceutical manufacturers may wish to undertake the tests on all deliveries of containers, it must be recognized that some of the biological tests (particularly that for absence of cardiovascular toxicity in the cat) may be too costly and difficult to perform routinely. However an adequate knowledge of the composition and analytical characteristics of the plastic may render the performance of biological tests inessential for routine control purposes.

Certain additional chemical tests, such as the measurement of light absorption in the ultraviolet region, may be useful for control purposes but are not described in this document. Attention is also drawn to published methods for the determination of low levels of vinyl chloride monomer in poly(vinyl chloride) by gas-liquid chromatography.

1.3 Plastic containers for other pharmaceutical dosage forms

Plastics are also used as containers (or components of containers) for dosage forms other than parenteral and ophthalmic pharmaceutical products. These include preparations for direct application to the skin
(ointments, creams, lotions, gels, sprays), nasal drops or sprays, ear drops, enemas, suppositories, and inhalation aerosols. For these applications it must be ascertained that nothing will be extracted from the plastic by the preparation that will give rise to adverse effects during use—e.g., irritation or sensitization of the skin, irritation of mucous membranes (nasal, bronchial, intestinal, and rectal), or unacceptable taste and/or odour on inhalation. For example, materials known to produce skin sensitization (compounds of nickel and chromium) or surface anaesthesia should not be included unless evidence is available that they cannot migrate into the product.

Ultimately, the acceptability of a plastic container for a particular dosage form can be assessed only by carrying out animal toxicity or irritation tests on the preparation after it has been stored in the container for an appropriate period of time. The storage tests should also be designed to ascertain whether loss of active ingredient, preservative, or other essential components of the product, where it occurs, is attributable to the plastic.

2. RECOMMENDED PROCEDURES FOR CONTAINERS
FOR INFUSIONS AND INJECTIONS

2.1 General

To carry out the examination (which should include all specified tests) a quantity of plastic material of the required surface area and, where appropriate, a further quantity of the material of the required weight should be available. Normally plastic material used for the test should be taken from parts of containers not covered with print or glued-on labels. Any adherent dust should be removed with a soft brush or a jet of clean dry air. A dry or wet cloth, organic solvents, or cleaning solutions of surfactants should not be used for this purpose.

In the process of extraction the important factors are the contact of the extracting medium with the available surface area of the plastic, the time and temperature during extraction, and proper cooling, agitation, and decanting. The cleaned pieces of plastic should always be added individually to the extracting medium.

The extraction conditions should not in any instance cause physical changes (such as fusion or melting of the plastic pieces) that would result in a decrease in the available surface area. A slight adherence of the pieces can be tolerated. Extraction procedures described in sections
2.2.1, 2.2.2, 2.2.3, and 2.3.1 below may be employed only with plastic materials of sufficient heat resistance, otherwise a lower extraction temperature should be selected.

The tests described are designed for application to plastics in the condition in which they are used. If a plastic is to be exposed to any cleansing or sterilization process before use, the tests must be conducted on material prepared from a specimen preconditioned by the same process.

The suitability of plastic used for a specific product for infusion or injection may be affected by factors such as plastic composition, processing and cleaning procedures, contacting media, ink, adhesives, absorption and adsorption of preservatives, and conditions of storage. Evaluation of such factors should be made by appropriate additional specific tests to determine the suitability of a plastic for its intended use.

2.2 Physicochemical tests

Tests 2.2.1 to 2.2.3, designed to determine the physical and chemical properties of plastic materials intended for use in containers and relevant accessories for parenteral preparations, are based on the extraction of the plastic material. Tests 2.2.4 and 2.2.5, which are intended for poly(vinyl chloride) plastic materials only, are based on wet combustion of the material. The chemicals used in these tests are described in the Appendix, and the following abbreviations are used: R = reagent; TS = test solution; VS = volumetric solution; AsTS = solution used for arsenic test; PbTS = solution used for heavy metals test.

2.2.1 Aqueous extractives obtained at 70°C

It is recommended that tests for nonvolatile residue, for heavy metals, and for buffering capacity be carried out on plastic containers made of high-density polyethylene, polypropylene, and poly(vinyl chloride) and that tests for nonvolatile residue and for buffering capacity only be carried out on containers made of low-density polyethylene.

2.2.1.1 Procedure for extraction

Extracting medium. Water for injection R.

Oven. Use an oven, preferably a forced-circulation model, that will maintain an operating temperature of 70 ± 1°C.

Extraction vessels. Use suitable vessels such as flasks or large test-tubes of borosilicate glass. If vessels closed with screw caps having rubber liners are used, the
exposed surface of the rubber liner should be completely protected with an inert solid disc 0.05-0.075 mm in thickness. A suitable disc may be fabricated from a polytetrafluoroethylene resin.

Cleaning of equipment. Clean all glassware thoroughly with chromic acid TS or, if necessary, with hot nitric acid (1000 g/l) TS, followed by prolonged rinsing with distilled or demineralized water. Clean cutting devices by an appropriated method (for example, successive cleaning with acetone R and dichloromethane R) prior to use in subdividing a specimen. Clean all other equipment by thorough scrubbing with a suitable detergent and prolonged rinsing with distilled or demineralized water.

Preparation of test material. Calculate in advance the total volume of extract needed to carry out all the required tests and the appropriate amount of plastic to be extracted. For each 20 ml of water for injection R necessary for the subsequent extraction, use homogeneous plastic material (as described in section 2.1) 120 cm² in total surface area when the thickness is 500 μm or less or 60 cm² in area when the thickness is greater than 500 μm. Divide the material into pieces of about 3 cm³ total surface, preferably in the form of strips approximately 3 mm in width and 5 cm in length. Transfer the material to a suitable glass-stoppered vessel made of borosilicate glass, and add at least 150 ml of water for injection R. Agitate for about 30 seconds, drain off the water, and repeat with a second washing.

Extraction. Transfer the prepared material to a suitable extraction vessel, and add the required amount of water for injection R. Cover the vessel in an appropriate manner (e.g., with an inverted beaker or a screwed-on cap). Extract by heating in an oven at 70°C for 24 hours, allowing adequate time for the liquid in the vessel to reach the extraction temperature. Cool the contents to room temperature (e.g., under running water) but not below 22°C. Remove with a pipette the portion of the extract necessary for the test for buffering capacity (section 2.2.1.4), and decant the remaining extract immediately into a suitable container, and tightly stopper it.

Blank. Use water for injection R and treat it as described above.

2.2.1.2 Test for non-volatile residue

Transfer 50.0 ml of the extract (section 2.2.1.1) in portions to a tared crucible, preferably a fused silica crucible that has been acid-cleaned, and evaporate the volatile substances in a bath of boiling water. Proceed similarly with 50.0 ml of the blank. If any oily residue is expected, inspect the crucible repeatedly during the evaporation and drying period, and reduce the amount of heat if the oil tends to creep along the walls of the crucible. Dry at 105°C for one hour, cool, and weigh. The difference between the quantity obtained from the extract and that obtained from the blank should not exceed 10 mg.

2.2.1.3 Test for heavy metals

The test for heavy metals described below may be carried out by other methods provided the results obtained are of equivalent accuracy. Atomic absorption spectrophotometry is particularly useful for the separate determination of individual metals.

Comparison tubes. Carry out the test in matched flat-bottomed comparison tubes of transparent glass with a capacity of about 70 ml and an internal diameter of about 23 mm and bearing a 25-ml mark. Nessler cylinders complying with the above dimensions are suitable. The expression "matched tubes" means tubes that are matched as closely as possible in internal diameter and in all other respects.
Procedure. Transfer 20.0 ml of the extract (section 2.2.1.1) to a comparison tube, add 2 ml of acetic acid (60 g/l) PbTS, then dilute to 25 ml with water. If the extract is turbid, clarify it by centrifuging or filtering. Into a second comparison tube transfer 20.0 ml of water for injection R, 2 ml of acetic acid (60 g/l) PbTS, and 2.0 ml of lead, dilute, PbTS (representing the lead equivalent of a heavy metals limit of 1 μg/ml of the extract), then dilute to 25 ml with water. To each comparison tube add 10 ml of hydrogen sulfide TS, mix, allow to stand for 10 minutes, and compare the colours by viewing down the vertical axis of the tube in diffused light against a white background. Any colour of the test solution must not be darker than the brown colour of the blank containing the lead standard.

2.2.1.4 Test for buffering capacity

Pipette a 20.0 ml portion of the extract (section 2.2.1.1) into a suitable vessel prior to decanting the extract from the extraction vessel. Titrate potentiometrically to a pH of 7.0, using either hydrochloric acid (0.01 mol/l) VS or sodium hydroxide (0.01 mol/l) VS, as required. Treat a 20.0 ml portion of water for injection R similarly: if the same titrant is required for both extract and blank, the difference between the two volumes must not be greater than 10 ml; and if acid is required for either the extract or the blank and alkali for the other, the total of the two volumes required must not be greater than 10 ml.

2.2.2 Aqueous extractives obtained at 120°C

2.2.2.1 Procedure for extraction

Extracting medium. Water for injection R.

Autoclave. Use a suitable steam autoclave capable of maintaining a temperature of 120 ± 1°C.

Extraction vessels. Use suitable vessels such as flasks or large test-tubes as described in section 2.2.1.1.

Cleaning of equipment. Use the procedure described in section 2.2.1.1.

Preparation of test material. Use homogeneous plastic material (as described in section 2.1) 300 cm² in total surface area. Divide the material into pieces of about 3 cm² total surface, preferably in the form of strips approximately 3 mm in width and 5 cm in length. Transfer the material to a suitable glass-stoppered vessel made of borosilicate glass, and add at least 25 ml of water for injection R. Agitate for about 30 seconds, drain off the water, and repeat with a second washing.

Extraction. Transfer the prepared material to a suitable extraction vessel and add 50 ml of water for injection R. Cover the vessel in an appropriate manner (e.g., with an inverted beaker or a screwed-on cap). Extract by heating in an autoclave at 120°C for 30 minutes, allowing adequate time for the liquid in the vessel to reach the extraction temperature. Immediately after the autoclave has cooled down to under 100°C, cool the contents to room temperature (e.g., under running water) but not below 22°C. Decant the extract immediately into a suitable container and tightly stopper it.

Blank. Use water for injection R and treat as described above.

2.2.2.2 Test for reducing substances

It is recommended that the test for reducing substances be carried out on plastic containers made of polyolefins (low-density and high-density polyethylene and polypropylene) and of poly(vinyl chloride).
Procedure. Transfer 10.0 ml of the extract (section 2.2.2.1) to a suitable flask, add 20.0 ml of potassium permanganate (0.002 mol/l) VS and 1 ml of sulfuric acid (190 g/l) TS. Heat to boiling for exactly 3 minutes and cool immediately. Add 0.1 g of potassium iodide R and titrate the liberated iodine with sodium thiosulphate (0.01 mol/l) VS using five drops of starch TS as indicator. Proceed similarly with 10.0 ml of the blank. The consumption of potassium permanganate (0.002 mol/l) VS for the test solution should not exceed 1.0 ml after deduction of the volume of potassium permanganate (0.002 mol/l) VS observed for the blank.

2.2.2.3 Test for turbidity of solution

It is recommended that the test for turbidity of solution be carried out on plastic containers made of polyolefins (low-density and high-density polyethylene and polypropylene) and of poly(vinyl chloride). 

Comparison tubes. Carry out the test in matched flat-bottomed comparison tubes of transparent glass with a capacity of about 70 ml and an internal diameter of about 23 mm. Nessler cylinders complying with the above dimensions are suitable. The expression “matched tubes” means tubes that are matched as closely as possible in internal diameter and in all other respects.

Procedure. Transfer 1.0 ml of the extract (section 2.2.2.1) to a comparison tube. To a second tube add 1.0 ml of barium chloride, ethanolic, TS. Add to both tubes, while shaking them, 1 ml of sulfuric acid (1 mol/l) VS. After 5 minutes dilute both tubes with 50 ml of water. The tubes are compared within 20 minutes. The turbidity produced with the extract must not be greater than the standard turbidity when viewed down the vertical axis of the tube in diffused light against a black background.

2.2.3 Additional test for infusions autoclaved in a container

This test is carried out on aqueous infusions that in the process of manufacture are placed in plastic containers of at least 50 ml capacity and autoclaved in them. The temperature at which the test is carried out and the period of heating should be the same as those actually used in the process of sterilization.

2.2.3.1 Procedure for extraction

Test liquid. Pharmaceutical preparation for which the container is used.

Autoclave. Use a suitable steam autoclave capable of maintaining the required temperature ±1°C.

Cleaning of equipment. Use the procedure described in section 2.2.1.1.

Extraction. Place 1000 ml of the test liquid into one or more of the plastic containers and heat it in an autoclave.

Blank. Place 1000 ml of a solution of the same composition as the test liquid in a suitable glass container and treat it identically.

2.2.3.2 Test for ether-soluble extractives

Add 10 ml of sulfuric acid (1760 g/l) TS to the test liquid (section 2.2.3.1), mix, and extract with successive quantities of 200 ml, 200 ml, and 100 ml of ether R. Filter
the combined extracts through cotton wool, washing the filter with 20 ml of ether R. Evaporate the ether in a water-bath and dry the residue under reduced pressure. Transfer to a vacuum desiccator, allow to cool, and weigh. Proceed similarly with the blank. The difference between the weights of the residues must not be more than 30 mg.

2.2.4 Additional test for tin

The test for tin is carried out on plastic containers made of poly(vinyl chloride). Alternative methods may be chosen, as convenient, provided the results obtained are as accurate as those obtained in the test described below. The atomic absorption spectrophotometric method is particularly useful for the purpose.

2.2.4.1 Cleaning of equipment

Use the procedure described in section 2.2.1.1.

2.2.4.2 Preparation of test solution

Take about 3 g of homogeneous plastic material (as described in section 2.1) and divide it into small pieces. Wash the pieces thoroughly with water and dry them.

Transfer 2.5 g of the material to a long-necked round-bottomed flask, add 20 ml of sulfuric acid (1760 g/l) PbTS and char for 5–10 minutes. Add hydrogen peroxide (500 g/l) TS dropwise to the hot solution until it becomes colourless, heating until fumes of sulfur trioxide evolve between each addition. Cool, transfer to a suitable dish (e.g., platinum or fused silica) with the aid of 10 ml of water, and evaporate to dryness, preferably under a silica infrared heater. Dissolve the residue in 10 ml of hydrochloric acid (1 mol/l) VS, filter (if necessary), and add sufficient water to produce 25 ml.

2.2.4.3 Procedure

To 10 ml of the test solution (section 2.2.4.2) add 5 ml of sulfuric acid (360 g/l) TS, 1 ml of sodium laurylsulfate (10 g/l) TS, and 1 ml of zinc dithiol TS. Heat in a water-bath for exactly 1 minute, cool, and allow to stand for 30 minutes. Any red colour in the solution should not be more intense than the red colour obtained by repeating the operation using 10 ml of tin, dilute, TS.

2.2.5 Additional test for barium

The test for barium is intended to be carried out on plastic containers made of poly(vinyl chloride). Alternative methods may be chosen, as convenient, provided the results obtained are as accurate as those obtained in the test described below. The atomic absorption spectrophotometric method is particularly useful for the purpose.

2.2.5.1 Cleaning of equipment

Use the procedure described in section 2.2.1.1.
2.2.5.2 Procedure

Take about 2.5 g of homogeneous plastic material (as described in section 2.1) and divide it into small pieces. Wash the pieces thoroughly with water and dry them.

Transfer 2.0 g of the material to a suitable dish (platinum or fused silica), moisten with hydrochloric acid (250 g/l) TS and ignite. Dissolve the residue in 10 ml of hydrochloric acid (1 mol/l) VS, filter, and add 1 ml of sulfuric acid (190 g/l) TS to the filtrate. Any turbidity obtained should not be greater than that produced on adding 1 ml of sulfuric acid (190 g/l) TS to 10 ml of barium, dilute, TS and 10 ml of hydrochloric acid (1 mol/l) VS.

2.3 Biological test in vitro

The following procedure is designed to test the suitability of plastic materials for use in containers and relevant accessories for parenteral preparations and is based on the extraction of the plastic.

2.3.1 Aqueous extractives obtained at 120°C

2.3.1.1 Procedure for extraction

Use an autoclave and extraction vessels, clean the equipment, and prepare the test material, the extract, and the blank as described in section 2.2.2.1.

2.3.1.2 Test for haemolytic effect

Dilute sodium phosphate/sodium chloride buffer TS as follows:

<table>
<thead>
<tr>
<th>Designation of intermediate solution</th>
<th>Sodium phosphate/sodium chloride buffer TS (ml)</th>
<th>Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a₀</td>
<td>30.0</td>
<td>10.0</td>
</tr>
<tr>
<td>b₀</td>
<td>30.0</td>
<td>20.0</td>
</tr>
<tr>
<td>c₀</td>
<td>15.0</td>
<td>85.0</td>
</tr>
</tbody>
</table>

Dilate further the intermediate solutions a₀ and b₀ as follows:

<table>
<thead>
<tr>
<th>Diluted solution</th>
<th>Intermediate solution</th>
<th>Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a₁</td>
<td>3.0 ml a₀</td>
<td>12.0</td>
</tr>
<tr>
<td>b₁</td>
<td>4.0 ml b₀</td>
<td>11.0</td>
</tr>
<tr>
<td>c₁</td>
<td>4.75 ml b₀</td>
<td>10.25</td>
</tr>
</tbody>
</table>

To each of three centrifuge tubes (Nos. 1, 2, and 3) add 1.40 ml of the extract (section 2.2.2), and to a fourth centrifuge tube (No. 4) add 1.40 ml of the blank. To tube No. 1 add 0.10 ml of a₀, to tube No. 2 and tube No. 4 add 0.10 ml of b₀, and to tube No. 3 add 0.10 ml of c₀. (Note: The osmotic effects of the solutions correspond to those of sodium chloride solutions of concentration 5 g/l, 4 g/l, and 1 g/l respectively for tube No. 1, tubes No. 2 and No. 4, and tube No. 3). To each tube add 0.02 ml of freshly prepared, well mixed, heparinized human blood TS and place the tubes in a water-bath at 30 ± 1°C for exactly 40 minutes. Immediately to tube No. 1 add 1.50 ml of a₁, to tube No. 2 and tube No. 4 add 1.50 ml of b₁, and
to tube No. 3 add 1.50 ml of c1. Centrifuge the tubes for 5 minutes, preferably in a horizontal centrifuge.

Measure the absorbance at the maximum of about 540 nm, using sodium phosphate/sodium chloride buffer TS as a blank.

Calculate the degree of haemolysis (hₙ) from the formula:

\[ hₙ = \frac{Aₙ}{A₁₀₀} \times 100 \]

where \( A₁₀₀ \) is the absorbance of the solution in tube No. 3, \( n \) is the tube number, and \( Aₙ \) is the absorbance of the solutions in tubes Nos. 1, 2, and 4 respectively.

The degree of haemolysis (hₙ) in tube No. 1 should not exceed 10, and the difference between the degrees of haemolysis in tubes No. 2 and No. 4 should not exceed 10.

The degree of haemolysis (hₙ) for tube No. 4 should be between 60 and 75.

### 2.4 Biological tests in vivo

#### 2.4.1 General

The following biological procedures are designed to test the suitability of plastic materials for use in containers and accessories for parenteral preparations. The procedures test the reaction of living animal tissue and of normal animals to extracts prepared from plastic materials. General observations on the selection of material for testing and on extraction procedures are given in section 2.1. The temperature and duration of extraction should depend on the heat resistance of the plastic as indicated below in section 2.4.2.2.

Aseptic handling and storage of the extracts following extraction are important. It is essential to exercise care in the preparation of the materials to be injected in order to prevent contamination with microorganisms and other foreign matter.

#### 2.4.2 Extractives obtained with standard extraction media

The tests for acute systemic toxicity (section 2.4.2.3) and for intracutaneous activity (section 2.4.2.4) are designed to determine the biological response of animals to plastics after single-dose injection of extracts prepared from a test material.

##### 2.4.2.1 Test animals

**Mice.** Use healthy and hitherto unused albino mice weighing between 17 g and 23 g. For each test group use only mice of the same strain. Offer ad libitum water and food commonly used for laboratory animals and known with respect to composition.
Rabbits. Select healthy, hitherto unused, thin-skinned albino rabbits whose fur can be clipped closely and whose skin is free from mechanical irritation or trauma. In handling the animals, avoid touching the injection sites during observation periods.

Cats. Use adult, healthy, male or female cats.

2.4.2.2 Procedure for extraction

Extracting media. Sodium chloride injection TS; sodium chloride injection with ethanol TS; macrogol 400 R; vegetable oil R.

Autoclave. Use an autoclave as described in section 2.2.2.1.

Oven. Use an oven, preferably a forced-circulation model, that will maintain operating temperatures of 50 ± 1°C and 70 ± 1°C.

Extraction vessels. Use suitable vessels such as flasks or test-tubes as described in section 2.2.1.1. If culture tubes are used for autoclave extractions with vegetable oil R, seal the screw caps adequately with pressure-sensitive tape.

Cleaning of equipment. Clean all glassware thoroughly with chromic acid TS or, if necessary, with hot nitric acid (1000 g/l) TS, followed by prolonged rinsing with distilled or demineralized water. Clean cutting devices by an appropriate method (for example, successive cleaning with acetone R and dichloromethane R) prior to use in subdividing a specimen. Clean all other equipment by thorough scrubbing with a suitable detergent and prolonged rinsing with distilled or demineralized water. Containers and devices used for extraction, transfer, and administration of test material should be rendered sterile and dry by a suitable process. (Note: if ethylene oxide is used as the sterilizing agent, allow adequate time for complete degassing).

Preparation of test material. For each 20 ml of the extracting medium use homogeneous plastic material (as described in section 2.1) 120 cm² in total surface area when the thickness is 500 μm or less or 60 cm² in area when the thickness is greater than 500 μm. Divide the material, preferably in the form of strips approximately 3 mm in width and 5 cm in length. Transfer the material to a suitable glass-stoppered vessel made of borosilicate glass, and add at least 70 ml of water for injection R. Agitate for about 30 seconds, drain off the water, and repeat with a second washing. Dry the pieces prepared for the extraction with vegetable oil R in an oven at a temperature not exceeding 50°C.

Extraction. Prepare two separate extracts with each extracting medium. Transfer the prepared material to the extraction vessels and add to each vessel 20 ml of the appropriate extracting medium. Depending on the heat resistance of the plastic, extract by heating in an autoclave at 120°C for 1 hour or in an oven at 70°C for 24 hours or at 50°C for 72 hours, allowing adequate time for the liquid in the vessel to reach the extraction temperature. Cool the contents to room temperature (e.g., under running water) but not below 22°C. Shake vigorously and decant each extract, using aseptic precautions, into a dry sterile vessel. Keep the extracts at a temperature between 22°C and 30°C but do not use them for tests after 24 hours.

Blank. Use the same quantity of the same extracting medium as for the extraction of the material and treat it as described above.

2.4.2.3 Test for acute systemic toxicity (in mice)

Inject each extract (section 2.4.2.2) and the corresponding blank into groups of 5 mice, each injection being in the amount and by the route given in the following table.
Take care to agitate each extract vigorously prior to withdrawal of each injection dose in order to ensure an even distribution of the extracted matter.

<table>
<thead>
<tr>
<th>Extract or blank</th>
<th>Dose (per kg)</th>
<th>Route of injection</th>
<th>Rate of injection (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride injection TS</td>
<td>50 ml</td>
<td>intravenous</td>
<td>2</td>
</tr>
<tr>
<td>Sodium chloride injection with ethanol TS</td>
<td>50 ml</td>
<td>intravenous</td>
<td>2</td>
</tr>
<tr>
<td>Vegetable oil R</td>
<td>50 ml</td>
<td>intraperitoneal</td>
<td>--</td>
</tr>
</tbody>
</table>

Observe the animals immediately after injection, again 4 hours after injection, and again 24, 48, and 72 hours after injection. During the observation period none of the animals treated with the extract should show a significantly greater reaction than that shown by the animals treated with the blank. If any of the animals treated with the extract show signs of toxicity, and if not more than one animal shows gross symptoms of toxicity or dies, the test must be repeated using groups of 10 mice each. On the repeated test, the requirements are met if none of the animals treated with the extract show an appreciably greater reaction than that observed in the animals treated with the blank.

Larger groups of mice may be used in the test.

2.4.2.4 Test for intracutaneous activity (in rabbits)

The test for intracutaneous activity described below may be alternatively performed by the patch technique.

On the day of the test, closely clip the fur on the backs of 2 rabbits on both sides of the spinal column over a sufficiently large test area. Avoid mechanical irritation and trauma. Remove loose hair by means of vacuum. If necessary, swab the skin lightly with ethanol (500 g/l) TS, and dry the skin prior to injection.

Inject intracutaneously 0.2 ml of each extract (section 2.4.2.2) at 10 sites on one side of each of the 2 rabbits. Similarly at 5 other sites on each rabbit, inject 0.2 ml of the corresponding blank. The extract prepared with macrogol 400 R and the corresponding blank should be diluted prior to injection with 8.3 volumes of sodium chloride injection TS to obtain a concentration of 120 mg of macrogol per ml. Examine the injected sites 24, 48, and 72 hours after the injection for gross evidence of tissue reaction such as erythema, oedema, and necrosis. To facilitate the examination, swab the skin lightly with ethanol (500 g/l) TS and clip the fur if necessary. Rate the observations on a numerical scale that permits the striking of averages for both the extract and the blank. The average for the extract should not be significantly greater than that for the blank. If the result is doubtful, repeat the test using fresh extracts in 3 more rabbits. The requirements are met if on the repeated test the average for the extract is not appreciably greater than that for the blank.

2.4.3 Additional test for infusions autoclaved in a container

This test is carried out on aqueous infusions that in the process of manufacture are placed in plastic containers of at least 50 ml capacity and autoclaved in them. The temperature at which the test is carried
out and the period of heating should be the same as those actually used in the process of sterilization.

2.4.3.1 Procedure for extraction

Follow the procedure described in section 2.2.3.1. If the liquid preparation is hypertonic or contains excessive amounts of potassium, dilute it as follows:

Glucose injection—use water for injection R to produce a concentration of 100 g/l immediately before infusion.

Fructose injection—proceed as with glucose injection.

Sodium chloride and glucose injection—use water for injection R to produce a concentration of 50 g/l of glucose immediately before infusion.

Potassium chloride (3 g/l) and glucose (50 g/l) injection—add an equal volume of sodium chloride injection TS immediately before infusion.

Potassium chloride (3 g/l) and sodium chloride (9 g/l) injection—proceed as with potassium chloride (3 g/l) and glucose (50 g/l) injection.

2.4.3.2 Test for cardiovascular toxicity (in the cat)

Anaesthetize a cat by the intravenous or intraperitoneal administration of chloralose R using 70 mg per kg of body weight. Insert into a femoral artery suitable tubing connected to a device capable of giving a permanent record of the blood pressure. Insert into a femoral vein tubing of a similar kind attached to a suitable pump for infusion of the test liquid (section 2.4.3.1). The liquid is infused for 15 minutes at a rate of 0.5 ml per kg of body weight per minute. Isolate the common carotid arteries, cannulate the trachea, connect to a respiration recorder, and record the respiration continuously.

Before infusing the test liquid, record two carotid reflexes with an interval of 7 minutes. Elicit the reflexes by occluding the common carotid arteries for 30 seconds. Record an electrocardiograph trace between the carotid reflexes using electrodes on the right foreleg and left hindleg. The animal should be discarded if the second response is not within 20% of the first or if the smaller of the two does not correspond to an increase in pressure of at least 3 kPa (20 mm Hg). Infusion of the test liquid should begin 2 minutes after recording the second carotid effect.

Five and 12 minutes after beginning the infusion, record two more carotid reflexes and record an electrocardiograph trace between them. Four minutes after completing the infusion, record a further reflex. The test liquid complies with the test if the height of each of the reflexes recorded during and after infusion of the test liquid is within 20% of the mean of the heights of the reflexes recorded before infusion and if the blood pressure, electrocardiogram, and both rate and depth of respiration do not differ appreciably from the recordings obtained before infusion.

If the test liquid does not comply, repeat the whole test in another animal using a blank solution. The height of each of the reflexes recorded during and after infusion of the blank solution should be within 20% of the mean of the heights of the reflexes recorded before infusion, and the blood pressure, electrocardiogram, and both rate and depth of respiration should not differ from the recordings obtained before infusion. The test liquid is regarded as complying on retesting if it produces no responses that are not produced by the blank solution.
3. RECOMMENDED PROCEDURES FOR CONTAINERS
   FOR AQUEOUS OPHTHALMIC PREPARATIONS

3.1 General

To carry out the examination (which should include all specified tests) a quantity of plastic material of the required surface area should be available. Normally plastic material used for the test should be taken from parts of containers not covered with print or glued-on labels. Any adherent dust should be removed with a soft brush or a jet of clean dry air. A dry or wet cloth, organic solvents, or cleaning solutions of surfactants should not be used for this purpose.

In the process of extraction the important factors are the contact of the extracting medium with the available surface area of the plastic, the time and temperature during extraction, and proper cooling, agitation, and decanting. The cleaned pieces of plastic should always be added individually to the extracting medium.

The extraction conditions should not in any instance cause physical changes (such as fusion or melting of the plastic pieces) that would result in a decrease in the available surface area. A slight adherence of the pieces can be tolerated. The temperature and duration of extraction should depend on the heat resistance of the plastic. Extraction procedures described in sections 3.2.1 and 3.2.2 below may be employed only with plastic materials of sufficient heat resistance, otherwise a lower extraction temperature should be selected.

The tests described are designed for application to plastics in the condition in which they are used. If a plastic is to be exposed to any cleansing or sterilization process before use, the tests must be conducted on material prepared from a specimen preconditioned by the same process.

3.2 Chemical tests

The following tests, designed to determine the chemical properties of plastic materials intended for use in containers for aqueous ophthalmic preparations, are based on the extraction of the plastic material.

3.2.1 Aqueous extractives obtained at 70°C

The tests described below are primarily recommended to be carried out on plastic containers made of polyolefin (low-density and high-density polyethylene and polypropylene) and of poly(vinyl chloride).
3.2.1.1 Procedure for extraction

The extracting medium, oven, extraction vessels, cleaning of equipment, and preparation of test material are as described in section 2.2.1.1.

3.2.1.2 Test for non-volatile residue

Carry out the test as described in section 2.2.1.2, using the extract and the blank as described in section 2.2.1.1. The permissible difference between the quantity obtained from the extract and that obtained from the blank depends on the type of ophthalmic preparation.

3.2.1.3 Test for buffering capacity

Carry out the test as described in section 2.2.1.4, using the extracts and the blank as described in section 2.2.1.1.

3.2.2 Aqueous extractives obtained at 120°C

It is recommended that the test for reducing substances be carried out on plastic containers made of polyolefins (low-density and high-density polyethylene and polypropylene) and of poly(vinyl chloride).

3.2.2.1 Procedure for extraction

The procedure is as given in section 2.2.2.1.

3.2.2.2 Test for reducing substances

Carry out the test as described in section 2.2.2.2.

3.3 Biological test

3.3.1 General

The following biological procedure is designed to test the suitability of plastic materials for use in containers for aqueous ophthalmic preparations. The procedure tests the reaction of living animal tissue and of normal animals to extracts prepared from plastic materials. General observations on the selection of material for testing and on extraction procedures are given in section 2.1. The temperature and duration of extraction should depend on the heat resistance of the plastic.

Aseptic handling and storage of the extracts following extraction are important. It is essential to exercise care in the preparation of the materials in order to prevent contamination with microorganisms and other foreign matter.
3.3.2 Extractives obtained with standard extraction media

The test for eye irritation (section 3.3.2.3) is designed to determine the response of animals to plastics by the instillation into the eye of an extract prepared from a test material.

3.3.2.1 Test animals

Rabbits. Select healthy albino rabbits with no eye defects or visible eye irritation and hitherto unused for an eye irritation test. The animal facilities should be designed and maintained so as to exclude sawdust, wood chips, or other extraneous materials that might produce eye irritation. Both eyes of each animal should be examined before testing.

3.3.2.2 Procedure for extraction

Proceed as described in section 2.4.2.2, but using only sodium chloride injection TS as an extracting medium.

3.3.2.3 Test for eye irritation (in rabbits)

Use three or more albino rabbits for each test. Restrain the animals firmly but gently until quiet. Gently pull the lower lid away from the eyeball to form a cup and instil about 0.2 ml of the blank. Hold the lid together for about 30 seconds. Instil into the other eye 0.2 ml of the extract (section 3.3.2.2). Repeat the instillation twice more on two following days using 0.2 ml of the extract or the blank each time. Examine the eyes before each instillation and 24, 48, and 72 hours after the last instillation. Examination is facilitated by the use of a binocular magnifier and a hand slit-lamp.

During the observation period none of the animals should show a significantly greater irritation in the eye treated with the extract than in that treated with the blank. If irritation is observed in either eye of any rabbit, the test must be repeated using an equal number of rabbits. On the repeated test, the requirements are met if none of the animals shows any irritation in either eye.

Appendix

LIST OF REAGENTS, TEST SOLUTIONS, AND VOLUMETRIC SOLUTIONS

The reagents mentioned in Annex 3 are described below. The designations of reagents and of reagent solutions used in the second edition of the International Pharmacopoeia (World Health Organization, Geneva, 1967 and Supplement 1971) have been modified for the expression of concentration to conform to the Système international d'Unités (SI). Previous designations are given in square brackets. The designation VS denotes volumetric solutions described in Appendix 2 of the International Pharmacopoeia, or solutions that are similarly standardized. The reference to SRIP indicates Specifications for Reagents mentioned in the International Pharmacopoeia (World Health Organization, Geneva, 1963). The designation d denotes the relative density $d_20^{\circ}$, i.e., measured in air at 20°C in relation to water at 20°C.

43
Acetic acid, glacial. $\text{C}_2\text{H}_4\text{O}_2$ (SRIP, 1963, p. 25); $d \sim 1.048$.

Acetic acid (300 g/l) TS [acetic acid R]. A solution of acetic acid, glacial, R containing about 300 g/l of $\text{C}_2\text{H}_4\text{O}_2$ (approximately 5 mol/l); $d \sim 1.037$.

Acetic acid (60 g/l) TS [acetic acid, dilute, R]. Acetic acid (300 g/l) TS diluted to contain about 60 g/l of $\text{C}_2\text{H}_4\text{O}_2$ (approximately 1 mol/l); $d \sim 1.008$.

Acetic acid (60 g/l) PBTS [solution of acetic acid, dilute, PbT]. Acetic acid (60 g/l) TS that complies with the following test. Evaporate 20 ml of acetic acid (60 g/l) TS on a water-bath almost to dryness, add 25 ml of water, and carry out the test for heavy metals. The heavy metals limit is 3 µg/ml.

Acetone R. $\text{C}_3\text{H}_6\text{O}$ (SRIP, 1963, p. 27).

Acetonitrile R. Methyl cyanide, $\text{C}_2\text{H}_3\text{N}$.

Description. A clear, colourless liquid.

Miscibility. Freely miscible with water.

Acetonitrile (400 g/l) TS. Mix one volume of acetonitrile R with one volume of water. The resulting solution contains about 400 g/l of $\text{C}_2\text{H}_3\text{N}$.

Ammonia (260 g/l) TS [ammonia, strong, R]. (SRIP, 1963, p. 31); $d \sim 0.894$.

Ammonia (100 g/l) TS [ammonia, dilute, TS]. Ammonia (260 g/l) TS, diluted to contain about 100 g/l of $\text{NH}_3$ (approximately 6 mol/l); $d \sim 0.956$.

Ammonia (100 g/l) PBTS [solution of ammonia PbT]. Ammonia (100 g/l) TS that complies with the following test. Evaporate 5 ml of ammonia (100 g/l) TS on a water-bath to dryness, add to the residue 1 ml of hydrochloric acid (70 g/l) TS, and evaporate to dryness. Dissolve the residue in 2 ml of acetic acid (60 g/l) PBTS, dilute with water to 25 ml and carry out the test for heavy metals. Prepare the blank in a similar way. The heavy metals limit is 2 µg/ml.

Ammonium thiocyanate R. $\text{NH}_4\text{SCN}$ (SRIP, 1963, p. 40).

Ammonium thiocyanate (75 g/l) TS [ammonium thiocyanate TS]. A solution of ammonium thiocyanate R containing about 75 g/l of $\text{NH}_4\text{SCN}$ (approximately 1 mol/l).

Barium, dilute, TS. Dilute 1 ml of barium chloride (1.5 g/l) TS with sufficient water to produce 100 ml; 1 ml of this solution contains 10 µg of Ba.

Barium chloride R. $\text{BaCl}_2\cdot2\text{H}_2\text{O}$ (SRIP, 1963, p. 45).

Barium chloride (76 g/l) TS. A solution of barium chloride R containing 88.9 g/l of $\text{BaCl}_2\cdot2\text{H}_2\text{O}$.

Barium chloride (1.5 g/l) TS. A solution of barium chloride R containing 1.78 g/l of $\text{BaCl}_2\cdot2\text{H}_2\text{O}$.

Barium chloride, ethanolic, TS. Dilute 1.0 ml of barium chloride (76 g/l) TS with 5 ml of water and sufficient ethanol (950 g/l) TS to produce 100 ml.

Barium oxide R. $\text{BaO}$.

Description. White to yellowish-white lumps or powder. Absorbs moisture and carbon dioxide on exposure to air.

Storage. Store in tight containers.

Bromine R. $\text{Br}_2$ (SRIP, 1963, p. 51).
Bromine AsTS. Dissolve 30 g of potassium bromide R in 40 ml of water, add 30 g of bromine R and dilute with sufficient water to produce 100 ml. The solution must comply with the following test. Evaporate 10 ml on a water-bath nearly to dryness, add 50 ml of water, 10 ml of hydrochloric acid (250 g/l) AsTS, and sufficient stannous chloride AsTS to reduce the remaining bromine, and apply the general test for arsenic. The stain produced must not be deeper than that of a 1-ml standard stain, showing that the proportion of arsenic present does not exceed 1 μg/ml.

Ceric ammonium nitrate R. Ce(NO₃)₂₂NH₄NO₃.
Description. Small orange-red monoclinic crystals.
Solubility. Very soluble in water.
Assay. Dissolve 2.5 g, accurately weighed and previously dried at 85°C for 24 hours, in 10 ml of sulfuric acid (190 g/l) TS and add 40 ml of water. Add a few drops of o-phenanthroline TS and titrate with ferrous sulfate (0.1 mol/l) VS. Each ml of ferrous sulfate (0.1 mol/l) VS is equivalent to 0.0548 g of Ce(NO₃)₂₂NH₄NO₃.
Insoluble matter. To 5 g, accurately weighed, add 10 ml of sulfuric acid (1760 g/l) TS, stir, and cautiously add 90 ml of water to dissolve. Heat to boiling and digest in a covered beaker on a water-bath for 1 hour. Filter through a tared filtering crucible, wash thoroughly, and dry at 105°C. The weight of the residue should not exceed 2.5 mg.

Ceric ammonium nitrate TS. Dissolve 6.25 g of ceric ammonium nitrate R in 10 ml of nitric acid (0.25 mol/l) VS. Use within 3 days.

Chloralose R. C₈H₁₃Cl₃O₆.
Description. Colourless crystalline powder.
Melting temperature. About 187°C.
Specific optical rotation. [α]D₂⁰₅⁰ ethanol = +19° (use ethanol, dehydrated, R).

Chromic acid TS. Dissolve 84 g of chromium trioxide R in 700 ml of water and add slowly, while stirring, 400 ml of sulfuric acid (1760 g/l) TS.

Chromium trioxide R. CrO₃ (SRIP, 1963, p. 68).

Cottonseed oil R. A refined fixed oil obtained from the seeds of cultivated plants of various varieties of Gossypium hirsutum or other species of Gossypium.

Dichloromethane R. Methylene chloride, CH₂Cl₂.
Description. A clear colourless mobile liquid.
Miscibility. One millilitre miscible with 50 ml of water; freely miscible with ethanol (950 g/l) TS and with ether R.
Boiling range. Not less than 95% distils between 39 and 41°C.
Residue on evaporation. Leaves, after evaporation on a water-bath and drying at 105°C, not more than 0.5 mg/ml.

Diethylene glycol R. C₄H₁₀O₃.
Description. A colourless to faintly yellow liquid having a mild odour.
Miscibility. Freely miscible with water, ethanol (950 g/l) TS, ether R, and acetone R.
Mass density (ρ₂₀). 1.117–1.120 g/ml.
Boiling range. Between 240 and 250°C.
Acidity. Transfer 60 g to a 250-ml conical flask, add phenolphthalein (10 g/l), ethanolic, TS, and titrate with potassium hydroxide, ethanolic (0.02 mol/l) VS, to a pink colour that is stable for at least 15 seconds. Not more than 2.5 ml should be consumed.

Diphenyl ether R. Phenyl ether (C₈H₇O₃).

Description. A colourless liquid.

Miscibility. Immiscible with water; freely miscible with acetic acid, glacial, R, and with most organic solvents.

Boiling range. About 259°C.

Melting range. Between 26 and 28°C.

Ethanol, dehydrated, R. C₂H₅OH (SRIP, 1963, p. 85).

Ethanol (950 g/l) TS [ethanol (95 per cent) R]. (SRIP, 1963, p. 84.)

Ethanol (900 g/l) TS [ethanol (90 per cent) R]. Dilute 947 ml of ethanol (950 g/l) TS with water to 1000 ml.

Ethanol (500 g/l) TS [ethanol (50 per cent) R]. Dilute 526 ml of ethanol (950 g/l) TS with water to 1000 ml.

Ethanol (200 g/l) TS [ethanol (20 per cent) R]. Dilute 210 ml of ethanol (950 g/l) TS with water to 1000 ml.


Ferrous sulfate R. FeSO₄·7H₂O (SRIP, 1963, p. 90).

Ferrous sulfate (0.1 mol/l) VS. Dissolve 2.8 g of ferrous sulfate R in 90 ml of freshly boiled and cooled water, and add sufficient sulfuric acid (1760 g/l) TS to produce 100 ml. Standardize the solution with potassium permanganate (0.02 mol/l) VS. The solution must be freshly prepared.

Heparinized human blood TS. Thoroughly mix 10 ml of freshly taken blood from a healthy human being with 0.10 ml of heparin injection 5000 IU/ml without preservative agent. This solution should be freshly prepared.

Hexane R. The hexane fraction from petroleum.

Description. A colourless mobile highly inflammable liquid.

Boiling range. Not less than 95% distils between 67 and 70°C.

Mass density (ρₐ). 0.670-0.677 g/ml.

Residue on evaporation. When evaporated on a water-bath and dried to constant weight at 105°C, the residue is not more than 0.1 mg/ml.

Hydrochloric acid (~420 g/l) TS [hydrochloric acid, saturated, R]. (SRIP, 1963, p. 96); d ~ 1.18.

Hydrochloric acid (~250 g/l) TS [hydrochloric acid R]. A solution of hydrochloric acid (~420 g/l) TS in water, containing about 250 g/l of HCl; d ~ 1.12.

Hydrochloric acid (~250 g/l) AS TS [hydrochloric acid AS]. Hydrochloric acid (~250 g/l) TS that complies with the following tests.

(1) Dilute 10 ml with sufficient water to produce 50 ml, add 5 ml of ammonium thiocyanate (75 g/l) TS and stir immediately. No colour should be produced.

(2) To 50 ml add 0.2 ml of bromine AS TS, evaporate on a water-bath until reduced to 16 ml, adding more bromine AS TS if necessary to ensure that an excess,
as indicated by the colour, is present throughout the evaporation. Add 50 ml of water and 5 drops of stannous chloride AsTS and apply the general test for arsenic. The stain produced must not be deeper than 0.2 ml standard stain prepared with the same acid, showing that the proportion of arsenic present does not exceed 0.05 μg/ml.

**Hydrochloric acid (~70 g/l) TS** [hydrochloric acid, dilute, R]. Mix 260 ml of hydrochloric acid (~250 g/l) TS with sufficient water to produce 1000 ml (approximately 2 mol/l); d ~ 1.035.

**Hydrochloric acid (1 mol/l) VS** [hydrochloric acid, N]. Hydrochloric acid (~250 g/l) TS, diluted with water to contain 36.47 g of HCl in 1000 ml.

**Hydrochloric acid (0.1 mol/l) VS** [hydrochloric acid, 0.1 N]. Hydrochloric acid (~250 g/l) TS, diluted with water to contain 3.647 g of HCl in 1000 ml.

**Hydrochloric acid (0.01 mol/l) VS** [hydrochloric acid, 0.01 N]. Hydrochloric acid (~250 g/l) TS, diluted with water to contain 0.3647 g of HCl in 1000 ml.

**Hydrogen peroxide (500 g/l) TS.**

_**Description.**_ A clear colourless liquid.

_**Caution.**_ Hydrogen peroxide (500 g/l) TS is highly reactive; care should be taken to protect the skin and eyes.

_**Assay.**_ Dilute 3 g to 500 ml with water; to 20 ml of the dilution, add 10 ml of sulfuric acid (100 g/l) TS, and titrate with potassium permanganate (0.02 mol/l) VS. Each ml of potassium permanganate (0.02 mol/l) VS is equivalent to 0.001701 g of H₂O₂.

_**Acidity.**_ 10 ml diluted with 100 ml of water requires for neutralization not less than 0.1 ml and not more than 0.5 ml of sodium hydroxide (0.1 mol/l) VS, methyl red TS being used as indicator.

_**Residue on evaporation.**_ Allow 25 ml to decompose in a platinum dish, cooling if necessary; the decomposed solution, when evaporated on a water-bath, leaves not more than 1 mg/ml.

**Hydrogen peroxide (330 g/l) TS** [hydrogen peroxide (30 per cent) R]. (SRIP, 1963, p. 97).


**Hydrogen sulfide TS.** A saturated solution of hydrogen sulfide R in cold water. Hydrogen sulfide TS should be freshly prepared.

**Lead, strong, PbTS** [solution of lead, strong, PbT]. Dissolve 0.1598 g of lead nitrate R in 5 ml of nitric acid (1000 g/l) TS and sufficient water to produce 1000 ml. This solution contains 100 μg of lead in 1 ml.

**Lead, dilute, PbTS** [solution of lead, dilute, PbT]. Dilute 10 ml of lead, strong, PbTS with sufficient water to produce 100 ml. This solution contains 10 μg of lead in 1 ml. It should be freshly prepared.


**Macrogol 400 R.** Polyethylene glycol 400. Macrogol 400 R is a polymer of ethylene oxide and water, represented by the formula H(OCH₂CH₂)nOH, in which the average value of n lies between 8.2 and 9.1.

47
Description. Clear colourless (or practically colourless) viscous liquid having a slight characteristic odour. Is slightly hygroscopic.

Average molecular weight. Transfer to a pressure flask 2.1 g of macrogel 400 R, accurately weighed, and 25.0 ml of phthalic anhydride–pyridine TS. Insert the stopper in the flask, wrap the flask securely with cloth, and immerse it in a water-bath maintained at 96–100°C, to the same depth as the mixture in the flask, for 1 hour. Remove the flask, retaining the cloth wrapping, and allow to cool in air to room temperature. To the contents of the flask add 50.0 ml of sodium hydroxide (0.5 mol/l) VS and 5 drops of phenolphthalein (10 g/l) in pyridine TS. Titrate with sodium hydroxide (0.5 mol/l) VS to a pink end-point that remains for not less than 15 seconds. Perform a blank determination in a similar manner. Calculate the average molecular weight by multiplying by 4000 the weight, in grams, of the tested substance and dividing the result by the difference between the volume, in millilitres, of sodium hydroxide (0.5 mol/l) VS consumed for the tested substance and the blank determination. The average molecular weight is between 380 and 420.

Mass density (ρ95). 1.110–1.140 g/ml.

Congealing point. Between 4 and 8°C, the congealing point being the average of 4 consecutive temperature readings, the highest and lowest of which differ by not more than 0.4°C.

pH. Between 4.5 and 7.5, in a 50 mg/ml solution.

Acidity or alkalinity. Dissolve 5 g, weighed accurately to the nearest 0.1 g, in 50 ml of water. Add a few drops of phenol red TS. If the solution turns yellow, titrate with sodium hydroxide (0.01 mol/l) VS; if the solution turns red, titrate with hydrochloric acid (0.01 mol/l) VS. Not more than 2.0 ml of titrant should be required in either case.

Sulphated ash. Not more than 10 mg/g.

Heavy metals. Mix 4 g, accurately weighed, with 1 ml of hydrochloric acid (70 g/l) TS and dilute with water to 25 ml. The limit is 50 μg/g.

Limit of monophenylene and diethylene glycols. Dissolve 50 g in 75 ml of diphenyl ether R in a 250-ml distillation flask. Slowly distil at a pressure of 100–250 Pa (1–2 mm Hg) into a receiver that is graduated to 100 ml in 1-ml subdivisions, until 25 ml of distillate has been collected. Add 25.0 ml of water to the distillate, shake vigorously, and allow the layers to separate. Cool the container in an ice bath to solidify and facilitate the removal of the layer of diphenyl ether R. Filter the water layer through filter paper into a glass-stoppered, 50-ml graduated cylinder. To the filtrate add an equal volume of freshly distilled acetonitrile R, and shake until solution is complete. Pipette 10 ml of the solution into 15 ml of ceric ammonium nitrate TS, mix, and within 2–5 minutes determine the absorbance of the resulting solution at about 525 nm. Use a blank consisting of 15 ml of ceric ammonium nitrate TS and 10 ml of acetonitrile (400 g/l) TS. Prepare a standard solution by mixing 10 ml of acetonitrile (400 g/l) TS to which 30 mg of diethylene glycol R has been added, and 15 ml of ceric ammonium nitrate TS and determine the absorbance within 2–5 minutes at about 525 nm, using the same blank as above. The absorbance of the test solution should not exceed that of the standard solution.

Methyl red TS. Warm 0.025 g of methyl red R with 0.95 ml of sodium hydroxide (0.05 mol/l) VS and 5 ml of ethanol (950 g/l) TS. After solution has been effected, add a sufficient quantity of ethanol (500 g/l) TS to produce 250 ml.

Nitric acid (~1000 g/l) TS [nitric acid R]. (SRIP, 1963, p. 125); \( d \sim 1.41 \).

Nitric acid (~130 g/l) TS [nitric acid, dilute, R]. Mix 130 ml of nitric acid (~1000 g/l) TS with sufficient water to produce 1000 ml (approximately 2 mol/l); \( d \sim 1.07 \).

Nitric acid (0.25 mol/l) VS. Nitric acid (~1000 g/l) TS, diluted with water to contain 15.75 g of HNO₃ in 1000 ml.

α-Phenanthroline R. 1,10-Phenanthroline, C₁₂H₈N₂O₂ (SRIP, 1963, p. 138).

α-Phenanthroline TS. Dissolve 0.7 g of ferrous sulfate R in about 70 ml of water and add about 1.5 g of α-phenanthroline R and sufficient water to produce 100 ml.


Phenolphthalein (10 g/l), ethanolic, TS. Dissolve 1.0 g of phenolphthalein R in sufficient ethanol (950 g/l) TS to produce 100 ml.

Phenolphthalein (10 g/l) in pyridine TS. Dissolve 1.0 g of phenolphthalein R in sufficient pyridine R to produce 100 ml.


Phenol red TS. Warm 0.05 g of phenol red R with 2.85 ml of sodium hydroxide (0.05 mol/l) VS and 5 ml of ethanol (900 g/l) TS. After solution has been effected, add a sufficient quantity of ethanol (200 g/l) TS to produce 250 ml.

Phthalic anhydride R. C₆H₄O₃.

Description. White lustrous needles.

Melting temperature. About 130°C.

Solubility. Slightly soluble in water, more soluble in hot water. Soluble in ethanol (950 g/l) TS and ether R.

Phthalic anhydride-pyridine TS. Add 42 g of phthalic anhydride R, accurately weighed, to 300 ml of freshly distilled pyridine R (refluxed with barium oxide R) containing less than 1 mg/ml of water in a glass-stoppered 1-litre flask. Use a dark flask or otherwise prevent exposure to light. Shake vigorously until complete solution is effected, and allow to stand overnight for completion of the reaction. Phthalic anhydride-pyridine TS should be freshly prepared.


Potassium chromate (100 g/l) TS [potassium chromate TS]. A solution of potassium chromate R containing about 97 g/l of K₂CrO₄ (approximately 0.5 mol/l).

Potassium hydroxide R. KOH (SRIP, 1963, p. 159).

Potassium hydroxide, ethanolic (0.02 mol/l) VS. Potassium hydroxide R, dissolved in ethanol (900 g/l) TS to contain 1.122 g of KOH in 1000 ml.


Potassium permanganate (0.02 mol/l) VS [potassium permanganate, 0.1 N]. Potassium permanganate R, dissolved to contain 3.161 g of KMnO₄ in 1000 ml.

Potassium permanganate (0.002 mol/l) VS [potassium permanganate, 0.01 N]. Potassium permanganate R, dissolved to contain 0.3161 g of KMnO₄ in 1000 ml.


Silver nitrate R. AgNO₃ (SRIP, 1963, p. 173).

Silver nitrate (0.1 mol/l) VS [silver nitrate, 0.1 N]. Silver nitrate R, dissolved to contain 16.99 g of AgNO₃ in 1000 ml.

Sodium biphosphate R. Na₂H₄PO₄·H₂O (SRIP, 1963, p. 178).


Sodium chloride injection TS. Sodium chloride injection, as described in the International Pharmacopoeia.

Sodium chloride injection with ethanol TS. Dilute 5 ml of ethanol (950 g/l) TS with sufficient sodium chloride injection TS to produce 1000 ml.

Sodium hydroxide R. NaOH (SRIP, 1963, p. 185).

Sodium hydroxide (10 g/l) TS. A solution of sodium hydroxide R containing about 10 g/l of NaOH (approximately 0.25 mol/l).

Sodium hydroxide (0.5 mol/l) VS [sodium hydroxide, 0.5 N]. Sodium hydroxide R, dissolved to contain 20.00 g of NaOH in 1000 ml.

Sodium hydroxide (0.1 mol/l) VS [sodium hydroxide, 0.1 N]. Sodium hydroxide R, dissolved to contain 4.001 g of NaOH in 1000 ml.

Sodium hydroxide (0.05 mol/l) VS [sodium hydroxide, 0.05 N]. Sodium hydroxide R, dissolved to contain 2.000 g of NaOH in 1000 ml.

Sodium hydroxide (0.01 mol/l) VS [sodium hydroxide, 0.01 N]. Sodium hydroxide R, dissolved to contain 0.4001 g of NaOH in 1000 ml.

Sodium laurilsulfate R. Sodium laurilsulfate R is a mixture of sodium alkyl sulfates consisting chiefly of sodium laurilsulfate, CH₃(CH₂)₁₃CH₃OSO₃Na. The combined content of sodium chloride and sodium sulfate is not more than 80 mg/g of the product.

**Description.** Small white or light yellow crystals having a slight characteristic odour.

**Total alcohols.** Transfer 5 g, accurately weighed, to an 800-ml long-necked flask and add 150 ml of water, 50 ml of hydrochloric acid (250 g/l) TS, and a few boiling chips. Attach a reflux condenser to the long-necked flask, heat carefully to avoid excessive frothing, and then boil for about 4 hours. Cool the flask, rinse the condenser with ether R, collecting the ether R in the flask, and transfer the contents to a 500-ml separator, rinsing the flask twice with ether R and adding the washings to the separator. Extract the solution with two 75-ml portions of ether R, evaporate the combined ether R extracts in a tared beaker on a water-bath, dry the residue at 105°C for 30 minutes, cool and weigh. The residue represents the total alcohols and must be not less than 59% of the weight of sodium laurilsulfate taken.

**Unsulfated alcohols.** Dissolve 10 g, accurately weighed, in 100 ml of water, and add 100 ml of ethanol (950 g/l) TS. Transfer the solution to a separator and extract with three 50-ml portions of hexane R. If an emulsion forms,
sodium chloride R may be added to promote separation of the two layers. Wash the combined hexane R extracts with three 50-ml portions of water and dry with sodium sulfate, anhydrous, R. Filter the hexane R extract into a tared beaker, evaporate on a water-bath until the odour of hexane R is no longer perceptible, dry the residue at 105°C for 30 minutes, cool, and weigh. The weight of the residue must be not more than 4% of the weight of the sodium laurylsulfate taken.

**Sodium chloride.** Dissolve 5 g, accurately weighed, in about 50 ml of water. Neutralize the solution with nitric acid (130 g/l) TS using litmus paper R as the indicator, add 2 ml of potassium chromate (100 g/l) TS, and titrate with silver nitrate (0.1 mol/l) VS. Each ml of silver nitrate (0.1 mol/l) VS is equivalent to 5.844 mg of NaCl.

**Sodium sulfate.** Transfer 1 g, accurately weighed, to a 400-ml beaker, add 10 ml of water, heat the mixture, and stir until completely dissolved. To the hot solution add 100 ml of ethanol (950 g/l) TS, cover, and digest at a temperature just below the boiling point for 2 hours. Filter, while hot, through a filtering crucible, and wash the precipitate with 100 ml of hot ethanol (950 g/l) TS. Dissolve the precipitate in the crucible by washing with about 150 ml of water, collecting the washings in a beaker. Acidify with 10 ml of hydrochloric acid (250 g/l) TS, heat to boiling, add 40 ml of barium chloride (76 g/l) TS, and allow to stand overnight. Collect the precipitate of barium sulfate on a tared filtering crucible, wash until free from chloride, dry, ignite, and weigh. The weight of barium sulfate so obtained, multiplied by 0.6086, represents the weight of Na₂SO₄ present in the 1 g sample.

**Alkalinity.** Dissolve 1 g, accurately weighed, in 100 ml of water, add phenol red TS, and titrate with hydrochloric acid (0.1 mol/l) VS. Not more than 0.6 ml is required for neutralization.

**Heavy metals.** Dissolve 1 g in water, adjust the pH of the solution to 3–4 with acetic acid (60 g/l) PBT, and carry out the test for heavy metals. The heavy metals limit is 20 µg/g.

**Arsenic.** Prepare the sample by a wet-combustion method using sulfuric acid (1760 g/l) AS and hydrogen peroxide (330 g/l) TS, and carry out the test for arsenic. The arsenic limit is 3 µg/g.

**Sodium laurylsulfate (10 g/l) TS.** Dissolve 1 g of sodium laurylsulfate R in sufficient water to produce 100 ml. The solution should be freshly prepared.

**Sodium phosphate R.** Na₅HPO₄.12H₂O (SRIP, 1963, p. 192).

**Sodium phosphate/sodium chloride buffer TS.** Dissolve 90.0 g of sodium chloride R, 27.5 g of sodium phosphate R and 2.17 g of sodium biphosphate R in sufficient water to produce 1000 ml. Store in a cold place. Any crystals produced should be dissolved by warming. Shake the solution before use.

**Sodium sulfate, anhydrous, R.** Na₂SO₄ (SRIP, 1963, p. 195).

**Sodium thiocyanate R.** Na₂S₂O₃·3H₂O (SRIP, 1963, p. 197).

**Sodium thiosulfate (0.01 mol/l) VS.** Sodium thiosulfate R, dissolved to contain 1.582 g of Na₂S₂O₃ in 1000 ml.

**Stannous chloride R.** SnCl₂·2H₂O (SRIP, 1963, p. 198).

**Stannous chloride TS.** Dissolve 330 g of stannous chloride R in 100 ml of hydrochloric acid (250 g/l) TS and sufficient water to produce 1000 ml.
Stannous chloride AsTS [solution of stannous chloride AsT]. Prepare from stannous chloride TS by boiling an equal volume of hydrochloric acid (250 g/l) TS, boiling down to the original volume, and filtering through a fine-grained filter-paper. The solution so obtained must comply with the following test. To 10 ml of it add 6 ml of water and 10 ml of hydrochloric acid (250 g/l) AsTS, and distil 16 ml. To the distillate add 50 ml of water and two drops of stannous chloride AsTS, and apply the general test for arsenic. The stain produced must not be deeper than a 1-ml standard stain, showing that the proportion of arsenic present does not exceed 1 µg/ml.

Starch R. Potato starch or corn starch (SRIP, 1963, p. 199).


Starch TS. Mix 0.5 g of starch R or of starch, soluble, R with 5 ml of water and add this, with constant stirring, to sufficient water to produce about 100 ml. Boil for a few minutes, cool, and filter. Starch TS must be freshly prepared.

Sulfuric acid (≈1760 g/l) TS. Sulfuric acid (≈1760 g/l) AsTS [sulfuric acid AsT]. Sulfuric acid (≈1760 g/l) TS that complies with the following test. Dilute 10 g with 50 ml of water, add 0.2 ml of stannous chloride AsTS, and apply the general test for arsenic. No visible stain should be produced.

Sulfuric acid (≈1760 g/l) PbTS. Sulfuric acid (≈1760 g/l) TS that complies with the following test. Add 5 g to 20 ml of water, adjust the pH with ammonia (100 g/l) PbTS to 3–4, dilute with water, and carry out the test for heavy metals. The heavy metals limit is 3 µg/ml.

Sulfuric acid (≈360 g/l) TS. Mix 2 volumes of sulfuric acid (≈1760 g/l) TS and 8 volumes of water, and cool. The resulting solution contains about 360 g/l of H₂SO₄; d ~ 1.22.

Sulfuric acid (≈190 g/l) TS. Mix 1 volume of sulfuric acid (≈1760 g/l) TS and 9 volumes of water, and cool. The resulting solution contains about 190 g/l of H₂SO₄; d ~ 1.12.

Sulfuric acid (≈100 g/l) TS [sulfuric acid, dilute, R]. Add 57 ml of sulfuric acid (≈1760 g/l) TS to sufficient water to produce 1000 ml (approximately 1 mol/l); d ~ 1.065.

Sulfuric acid (1 mol/l) VS [sulfuric acid, 2N]. Sulfuric acid (1760 g/l) TS, diluted to contain 98.08 g of H₂SO₄ in 1000 ml.


Tin, strong, TS. Dissolve 0.100 g of tin R in 20 ml of sulfuric acid (1760 g/l) TS by heating until fumes are evolved. Cool, cautiously add 150 ml of water, and cool again. Add 65 ml of sulfuric acid (1760 g/l) TS, cool, and dilute with sufficient water to produce 500 ml.

Tin, dilute, TS. Dilute 2.5 ml of tin, strong, TS with sufficient water to produce 100 ml. A millilitre of this solution contains 5 µg of Sn.

Vegetable oil R. Sesame oil, as described in the International Pharmacopoeia, or cottonseed oil R. The oil should preferably be freshly refined and should meet
the following requirement. Using three test rabbits, as directed in section 2.4.2.4, inject 0.2 ml intracutaneously into each of 10 sites on each animal, and examine the sites of injection 24, 48, and 72 hours after the injections. No site should show a greater reaction than oedema or erythema over an area of 0.5 cm in diameter.

**Water for injection R.** Water for injection, as described in the International Pharmacopoeia.

**Zinc dithiol R.** Zinc complex of toluene-3,4-dithiol.

**Zinc dithiol TS.** Dissolve 0.2 g of zinc dithiol R in sodium hydroxide (10 g/l) TS to which a few drops of ethanol (950 g/l) TS have previously been added. Add 1 ml of thioglycolic acid R and sufficient sodium hydroxide (10 g/l) TS to produce 100 ml.