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Elastomeric parts for aqueous parenteral preparations

Éléments en élastomère pour préparations aqueuses parentérales

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ISO 8871:1990(E)

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 8871 was prepared by Technical Committee ISO/TC 76, *Transfusion, infusion and injection equipment for medical use*.

This second edition cancels and replaces the first edition (ISO 8871:1988), presentation has been modified and clauses D.3.2, E.3.2, F.3.2, G.3, J.3.2 and K.3.2 have been technically revised.

Annexes A, B, C, D, E, F, G, H, J, K, L and M form an integral part of this International Standard.

Introduction

The elastomeric parts described in this International Standard are made from a class of material which is generally called "rubber". The parts are made from various elastomers involving different vulcanization systems, and may vary considerably in their composition with regard to fillers, softeners, pigments and other auxiliary ingredients.

The potency, purity, stability, and safety of a drug during its manufacture, storage and administration can be affected by the nature and performance of an elastomeric part used to seal the drug in its final container.

Elastomeric parts for aqueous parenteral preparations

1 Scope

1.1 This International Standard defines procedures for identifying and classifying elastomeric parts for primary packs and medical devices used in direct contact with aqueous preparations for parenteral use including dry preparations which have to be dissolved before use.

This International Standard specifies a series of comparative test methods for chemical and biological evaluation (see clause 6) and describes the various fields of application for elastomeric parts. Dimensions and functional characteristics are specified in the relevant International Standards. Required properties as specified in this International Standard shall be regarded as minimum requirements.

1.2 This International Standard is applicable for the categories of elastomeric parts given in clause 3; specific requirements, however, are laid down in the relevant International Standards dealing with the items or devices listed in clause 3.

NOTE 1 Elastomeric parts for empty syringes for single use are excluded by definition (see ISO 7886).

1.3 Compatibility studies with the intended preparation have to be performed before the approval for final use can be given; however, this International Standard does not specify procedures for carrying out compatibility studies.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 48:1979, *Vulcanized rubbers — Determination of hardness (Hardness between 30 and 85 IRHD).*

ISO 247:1978, *Rubber — Determination of ash.*

ISO 2781:1988, *Rubber, vulcanized — Determination of density.*

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods.*

3 Classification

Depending on the intended end-use, elastomeric parts exist in various designs and sizes. These parts serve different purposes depending on the item or device into which they are incorporated; elastomeric parts have, therefore, been classified into the following categories:

- elastomeric parts for injection vials (see ISO 8362-2);
- elastomeric parts for infusion bottles (see ISO 8536-2);
- elastomeric parts for prefilled syringes;
- elastomeric parts for medical devices for pharmaceutical use (excluding gloves and probes);
- elastomeric parts for freeze-dried products.

4 Identification

4.1 General

Rubber is a complex material and not generally definable. The only property which all elastomeric materials have in common is a special type of resilience or elasticity. When a strip of rubber is stretched, it will extend up to many times its original length without breaking. On release of the stretching force, it snaps back to its original size and shape virtually unaltered. Similarly one can squeeze it, twist it or distort it in any direction comparatively easily, and it will spring back again to its original shape unchanged.

Owing to its three-dimensional network, achieved by chemical cross-linking of the polymer chains during vulcanization, rubber is practically insoluble in solvents such as tetrahydrofurane, although considerable reversible swelling may occur; this characteristic differentiates rubber from pseudo-elastic materials, such as polyvinylchloride and certain thermoplastic elastomers.

In view of the complexity of rubber, a set of tests is needed for reliable identification and the identity of a given elastomeric material cannot be verified just by a single physical or chemical test. Recommended tests for this purpose are, among others, the following ones:

— determination of density;

— determination of ash;

— ultraviolet spectrometry of extracts;

— infra-red spectrometry of pyrolysates.

The manufacturer shall guarantee that all elastomeric parts of current supplies have been produced from the same formulation and that they exhibit the same characteristics as the samples which have been given to the user first and the suitability of which has been proved.

The tests specified in 4.2 to 4.5 shall be used for identification — especially in tests carried out by the end-user.

4.2 Determination of density

Density shall be measured in accordance with the procedure described in ISO 2781:1988, Method A.

4.3 Determination of ash

The residue of inorganic materials after combustion shall be determined as described in ISO 247:1978, Method B or, if necessary, Method C.

4.4 Ultraviolet spectrometry

The ultraviolet spectrum shall be obtained on an aqueous extract as described in annex A; it shall be compared with a reference spectrum.

4.5 Infra-red spectrometry

The infra-red spectrum shall be obtained on a pyrolysate as described in annex B; it shall be compared with a reference spectrum.

5 Requirements

5.1 Biological requirements

Biological requirements are not specified in this International Standard; biological tests are, however, required by most national pharmacopoeias or related health authority regulations and are mandatory for producers and users in countries where they exist. If this is not the case, reference shall be made to biological tests, e.g. as described in the United States Pharmacopoeia, the European Pharmacopoeia or other pharmacopoeias.

5.2 Chemical requirements

Elastomeric parts shall comply with the chemical requirements specified in the relevant International Standards (see clause 3).

Analytical procedures to compare and evaluate the chemical characteristics of elastomeric parts are described in annex A to annex M.

5.3 Physical requirements

5.3.1 Hardness

The hardness shall be in the specified limits within the "shelf-life" guaranteed by the manufacturer; hardness shall be determined in accordance with ISO 48.

NOTE 2 The "shelf-life" is understood to be a storage period without interference from outside factors, such as drugs, etc.

5.3.2 Resistance to steam sterilization

Elastomeric parts shall not lose the required biological, chemical and physical properties after a twofold sterilization process in saturated steam at 121 °C ± 1 °C for 30 min.

6 Testing

6.1 General

The test methods described in annex A to annex M shall be considered as a means of examining various elastomeric formulations in order to select the appropriate rubber formulation for a specific use. A selection of these test methods may be used for assessing product lot-to-lot reproducibility.

In order to provide a certain degree of protection against misinterpretation in the case of erroneous results, all tests shall be performed in duplicate, unless otherwise stated.

6.2 Sampling

A statistically random sample of elastomeric parts to be examined shall be representative for each supply and shall be provided in their original state. The necessary number of elastomeric parts shall be as specified in the relevant International Standards (see clause 3).

6.3 Apparatus and reagents

6.3.1 Only reagents of recognized analytical grade shall be used.

Purified water, prepared by distillation, by using an ion exchanger or by any other suitable process shall be used.

Its conductivity should be less than 3 µS/cm.

Purified water as specified in various national pharmacopoeias corresponds to grades 1 and 2 water as specified in ISO 3696.

6.3.2 Glassware shall be made from borosilicate glass.

6.4 Preparation of test solutions

6.4.1 Use a number of complete elastomeric parts which correspond to a surface area of at least 150 cm^2 to give a test solution of 1 cm^2 of elastomeric surface area per 2 ml of test solution.

Wash these samples: place them in a suitable glass container, cover with 300 ml of purified water, boil for 5 min and then rinse five times with 300 ml portions of cold purified water.

Place the washed elastomeric parts in a wide-necked flask and add 300 ml of purified water per 150 cm^2 surface area of the samples. Cover the mouth of the flask with aluminium foil or a borosilicate glass beaker. Heat in an autoclave so that a temperature of 121 °C ± 1 °C is reached in the flask within 30 min max. and maintain at this temperature for 30 min. Cool to room temperature over 20 min to 30 min.

Shake and immediately separate this solution S_1 from the elastomeric parts by decantation. Make up to original volume with purified water. Shake solution S_1 before each test.

6.4.2 Blank solution S_0 is prepared in the same way as for solution S_1 except that 300 ml of purified water are used without the elastomeric parts.

6.4.3 Solutions S_1 and S_0 obtained as described in 6.4.1 and 6.4.2 shall be used to carry out the chemical and biological tests.

7 Packaging

The elastomeric parts shall be packaged in a suitable way so that they are protected against contamination and exposure to light.

8 Storage

The elastomeric parts shall be stored at a temperature in the range from 0 °C to 30 °C, they shall be protected against exposure to visible and ultraviolet light.

9 Marking and labelling

The following information relating to the packaged goods shall be marked on the outside packaging:

a) a description of the contents;

b) the month and year of manufacture;

c) the lot number;

d) the manufacturer's trade-mark or name.

Annex A
(normative)

Ultraviolet spectrometry of extracts

A.1 Principle

The ultraviolet (UV) spectrum obtained on extracts of elastomeric materials is primarily a function of the kind of accelerator or antioxidant present in the individual elastomeric formulations. Recording the ultraviolet absorption with a scanning UV spectrometer is extremely useful in distinguishing formulations with different vulcanization and stabilization systems. This type of test is applicable to all vulcanized rubber products and is usually performed using aqueous extracts.

A.2 Procedure

Pass the test solution S_1 through a membrane filter (mesh size: 0,45 µm) to avoid stray light interference. Within 5 h of preparation, place the solution in a scanning UV spectrometer in a 1 cm quartz cell with the blank solution S_0 in the reference cell and obtain the spectrum over the wavelength range from 220 nm to 360 nm.

If a dilution is necessary, the dilution factor shall be noted.

Compare the sample spectrum obtained under standard conditions to the approved reference spectrum for the elastomeric material obtained under the same conditions.

A.3 Expression of results

Report the results as a recorded diagram showing the absorbance (extinction) plotted versus the wavelength.

Annex B

(normative)

Infra-red spectrometry of pyrolysates

B.1 Principle

The infra-red (IR) spectrometry of pyrolysed elastomeric materials is basically considered to be a qualitative identification test for elastomers and certain rubber ingredients. With the exception of silicone rubber it can be applied to any elastomer formulation.

An elastomeric sample is heated, the pyrolytic vapours are condensed and the resulting condensate is analysed by IR spectrometry.

B.2 Procedure

Place 1 g to 2 g of the elastomeric sample into a heat-resistant glass tube (preferred size: 160 mm × ø 16 mm). While holding the tube horizontally, heat moderately over a low bunsen burner flame, passing the flame over the bottom and the sides of the test tube until the water in the sample is driven off. When condensate has formed near the top edge of the test tube, deposit several drops onto a potassium bromide crystal of the transmittance cell. Place the assembled cell in the IR spectrometer and scan between 4 000 cm^{-1} and 600 cm^{-1}.

B.3 Expression of results

Report the results as a recorded diagram showing the absorbance (transmittance) plotted versus the wave number.

Annex C
(normative)

Determination of extracted reducing matter (oxidizables)

C.1 Principle

When subjected to extraction processes in aqueous media and depending on the individual composition, a given elastomeric material may release oxidizable matter. The source for such extractables is not clearly understood and the significance of this class of contaminants is still questionable. However, probably the most common of the possible extractables from elastomeric parts are the vulcanizing agents, the accelerators and their reaction products. This category of materials might include one or more of the following constituents: sulfur, thiurams, sulfenamides, thiazoles, dithiocarbamates, complex organic amines, phenolic resins and/or organic peroxides.

The extraction of reducing matter is performed under conditions which equal the stress factors to which elastomeric parts are usually exposed during sterilization in a steam autoclave.

C.2 Reagents

C.2.1 Sulfuric acid, standard volumetric solution, $c(H_2SO_2) = 1$ mol/l.

C.2.2 Potassium permanganate, standard volumetric solution, $c(KMnO_4) = 2$ mmol/l.

C.2.3 Sodium thiosulfate, standard volumetric solution, $c(Na_2S_2O_3) = 10$ mmol/l.

C.2.4 Starch, 5 g/l solution.

C.3 Procedure

Carry out the test procedure within 5 h of preparing solution S_1. Add 2 ml of sulfuric acid (C.2.1) and 20 ml of potassium permanganate solution (C.2.2) to 20 ml of solution S_1. Boil for 3 min. Cool rapidly. Add 1 g of potassium iodide and titrate immediately with sodium thiosulfate solution (C.2.3) using 0,25 ml of starch solution (C.2.4) as indicator.

Treat 20 ml of blank solution S_0 in the same way.

C.4 Expression of results

Report the results as the difference between the volumes of the potassium permanganate solution consumed by 20 ml of test solution S_1 and the same volume of blank solution S_0.

Annex D

(normative)

Determination of extracted heavy metals

D.1 Principle

The extraction of metallic oxides due to the presence of mineral fillers and certain metal oxides, used as cure boosters, is common. Metals which can be detected in low levels, but which are nevertheless low-level contaminants in other rubber ingredients include, for example, lead. They can be eliminated or controlled by the rubber manufacturer by careful selection of material and thorough inspection of current supplies.

The extraction of heavy metals is performed under conditions which simulate the stress factors to which elastomeric parts are usually exposed during sterilization in a steam autoclave.

D.2 Reagents

D.2.1 Acetate buffer solution, pH = 3,5.

Dissolve 25,0 g of ammonium acetate in 25 ml of water. Add 38,0 ml of a 250 g/l solution of hydrochloric acid. Determine the pH potentiometrically. Adjust, if necessary, by adding hydrochloric acid, $c(\mathrm{HCl}) = 2$ mol/l, or ammonia, $c(\mathrm{NH_3}) = 6$ mol/l. Dilute to 100 ml with water.

D.2.2 Thioacetamide reagent.

Prepare solution A by mixing 15 ml of a standard volumetric solution of sodium hydroxide, $c(\mathrm{NaOH}) = 1$ mol/l, with 5 ml of water and 30 ml of glycerol.

Immediately before use, mix 0,20 ml of a 40 g/l solution of thioacetamide and 1,00 ml of solution A. Heat this mixture in a boiling water bath for 20 s.

D.2.3 Lead(II) nitrate, stock solution, 1,00 mg of $\mathrm{Pb^{2+}}$ per millilitre.

Shortly before use, dissolve 0,160 g of lead(II) nitrate in freshly boiled and cooled water to give a volume of 100 ml.

D.2.4 Lead(II) nitrate, solution 1,0 µg of $\mathrm{Pb^{2+}}$ per millilitre.

Dilute 1,0 ml of the stock solution of lead(II) nitrate (D.2.3) to 1 000 ml with water.

D.3 Procedure

D.3.1 Mix 12,0 ml of test solution S_1 with 2 ml of acetate buffer solution (D.2.1).

Add this mixture to 1,0 ml of thioacetamide reagent (D.2.2) and shake.

D.3.2 Prepare a comparison solution, as described below. The maximum permitted quantity of heavy metals is stipulated in the appropriate specification as n µg $\mathrm{Pb^{2+}}$/10 ml. Take this figure n in the following as V ml.

Mix V ml of lead(II) nitrate solution (D.2.4) with $(10 - V)$ ml of water, 2 ml of acetate buffer solution (D.2.1) and 2 ml of test solution S_1. Add this mixture to 1,0 ml of thioacetamide reagent (D.2.2) and shake.

D.3.3 After 2 min, compare the colour intensity of the two preparations.

D.4 Expression of results

Report the results of the comparison.

Annex E
(normative)

Determination of extracted ammonia

E.1 Principle

The leaching of traces of ammonia is mostly observed with conventional natural elastomeric formulations. It is usually caused by the presence of protein material in the natural isoprene polymer or by amines.

The extraction of ammonia is performed under conditions which simulate the stress factors to which elastomeric parts are usually exposed during sterilization in a steam autoclave.

E.2 Reagents

E.2.1 Sodium hydroxide, standard volumetric solution, $c(\mathrm{NaOH}) = 3$ mol/l.

E.2.2 Nessler's reagent.

Dissolve 11,0 g of potassium iodide and 15,0 g of mercury(II) iodide in water to give a volume of 100 ml.

Shortly before use, mix a volume of this solution with an equal volume of a standard volumetric solution of sodium hydroxide [$c(\mathrm{NaOH}) = 6$ mol/l].

E.2.3 Ammonium chloride, stock solution, 1,00 mg of $\mathrm{NH_4^+}$ per millilitre.

Shortly before use, dissolve 0,300 g of ammonium chloride in water to give a volume of 100 ml.

E.2.4 Ammonium chloride, solution, 2,0 µg of $\mathrm{NH_4^+}$ per millilitre.

Dilute 2,0 ml of the stock solution of ammonium chloride (E.2.3) to 1 000 ml with water.

E.3 Procedure

E.3.1 Mix 10,0 ml of test solution S_1 with 1 ml of sodium hydroxide solution (E.2.1) and 1,0 ml of Nessler's reagent (E.2.2).

E.3.2 Prepare a comparison solution, as described below. The maximum permitted quantity of extracted ammonia is stipulated in the appropriate specification as n µg $\mathrm{NH_4^+}$/10 ml. Take this figure n, multiply by 0,5 and take the result in the following as V ml.

Mix V ml of ammonium chloride solution (E.2.4) with $(10 - V)$ ml of water, 1 ml of sodium hydroxide solution (E.2.1) and 1,0 ml of Nessler's reagent (E.2.2).

E.3.3 After 5 min, compare the colour intensity of the two preparations.

E.4 Expression of results

Report the results of the comparison.

Annex F

(normative)

Determination of extracted halides

F.1 Principle

The extraction of halides is performed under conditions which simulate the stress factors to which elastomeric parts are usually exposed during sterilization in a steam autoclave.

F.2 Reagents

F.2.1 Nitric acid, standard volumetric solution, $c(HNO_3) = 6$ mol/l.

F.2.2 Silver nitrate, standard volumetric solution, $c(AgNO_3) = 0,1$ mol/l.

F.2.3 Sodium chloride, solution, 4,0 µg of Cl^- per millilitre.

Shortly before use, dissolve 0,660 g of sodium chloride in water to give a volume of 1 000 ml.

Dilute 10,0 ml of this solution with water to give a volume of 1 000 ml.

F.3 Procedure

F.3.1 Mix 10,0 ml of filtered test solution S_1 (membrane filter, mesh size: 0,45 µm) with 1,0 ml of nitric acid (F.2.1) and 1,0 ml of silver nitrate solution (F.2.2).

F.3.2 Prepare a comparison solution, as described below. The maximum permitted quantity of extracted halides is stipulated in the appropriate specification as n µg Cl^-/10 ml. Take this figure n, multiply by 0,25 and take the result in the following as V ml.

Mix V ml of sodium chloride solution (F.2.3) with $(10 - V)$ ml of water, 1,0 ml of nitric acid (F.2.1) and 1,0 ml of silver nitrate solution (F.2.2).

F.3.3 After 5 min, compare the turbidity of the two preparations.

F.4 Expression of results

Report the results of the comparison.

Annex G
(normative)

Determination of acidity and alkalinity

G.1 Principle

Depending on their composition, some types of closure formulations contain functional ingredients which may act as acid or alkali. If acidic or alkaline material is extracted, a change in the pH-value, which may adversely affect the stability of a pharmaceutical product, may occur. Such a change in the pH-value can be detected by potentiometric measurement of the aqueous extract. However, the potentiometric determination may lead to misinterpretation when measurements are performed in unbuffered systems in the neighbourhood of a neutral pH-value. Therefore, it is recommended that a titration method is used to determine acidity or alkalinity.

G.2 Reagents

G.2.1 Tashiro indicator.

Dissolve 0,2 g of methyl red and 0,1 g of methylene blue in ethanol [95 % (V/V)] to give a volume of 100 ml.

G.2.2 Sodium hydroxide, standard volumetric solution, $c(\text{NaOH}) = 5$ mmol/l.

G.2.3 Hydrochloric acid, standard volumetric solution, $c(\text{HCl}) = 5$ mmol/l.

G.3 Procedure

Add 0,1 ml of Tashiro indicator solution (G.2.1) to 20 ml of test solution S_1 in a titration flask.

If the colour of the resulting solution is violet, titrate with sodium hydroxide solution (G.2.2); if the colour of the resulting solution is green, titrate with hydrochloric acid (G.2.3), until the appearance of a greyish colour.

With blank solution S_0, the procedure is the same.

G.4 Expression of results

Report the results as millilitres of sodium hydroxide solution (G.2.2) or hydrochloric acid solution (G.2.3) consumed by 20 ml of test solution S_1.

Report the difference between the titration of test solution S_1 and blank solution S_0 in millilitres of sodium hydroxide (G.2.2) or millilitres of hydrochloric acid solution (G.2.3) per 20 ml.

Annex H

(normative)

Determination of extracted non-volatile solids

H.1 Principle

Depending on their composition, various rubber formulations on extraction may release different quantities of non-volatile solids.

The extraction of non-volatile solids is performed under conditions which simulate the stress factors to which elastomeric parts are usually exposed during sterilization in a steam autoclave.

They can be determined by the mass upon evaporation to dryness.

H.2 Procedure

Transfer 100,0 ml of test solution S_1 into a previously tared evaporating dish. Evaporate to dryness at a temperature just below boiling point. Heat to constant mass at 105 °C.

Treat 100,0 ml of blank solution S_0 in the same way.

H.3 Expression of results

Report the results as the difference between the masses of the residues from the test solution and from the blank solution, expressed in milligrams per 100 millilitres.

Annex J
(normative)

Determination of volatile sulfides

J.1 Principle

Conventional cure systems use sulfur or sulfur-containing compounds for cross-linking purposes. Rubber materials based on such a vulcanization system may form volatile sulfides when exposed to aqueous extraction media, especially at acidic pH-values. The sulfides released can be detected visually by reaction with lead acetate paper.

J.2 Reagents

J.2.1 Citric acid, 20 g/l solution, pH ≈ 2.

Dissolve 2,0 g of citric acid monohydrate in water to give a volume of 100 ml.

J.2.2 Lead acetate paper.

Slightly acidulate a convenient quantity of lead(II) acetate solution, $c[Pb(CH_3CO_2)_2] = 0{,}25$ mol/l, by adding acetic acid [30 % (V/V)]. Dip pieces of white filter paper ($\rho_A = 80$ g/m^2) into this solution. Remove the paper from the solution and allow to dry.

Cut the paper in strips having dimensions of approximately 15 mm × 40 mm.

Store in a tightly closed container.

J.2.3 Citric acid, 80 g/l solution.

Dissolve 8,0 g of citric acid monohydrate in water to give a volume of 100 ml.

J.2.4 Sodium sulfide, 10 mg/l solution.

Dissolve 10 mg of sodium sulfide (Na_2S) or the equivalent amount of water containing sodium sulfide (Na_2S) × "X" H_2O in water to give a volume of 1 000 ml.

J.3 Procedure

J.3.1 Place elastomeric parts, cut if necessary, with a total surface area of 20 cm^2 ± 2 cm^2 in a 100 ml Erlenmeyer flask, containing 50 ml of a citric acid solution (J.2.1).

Place a piece of lead acetate paper (J.2.2) on the opening of the flask and hold in position with an inverted glass beaker.

J.3.2 Prepare a comparison piece of lead acetate paper, as described below. The maximum permitted quantity of sulfides is stipulated in the appropriate specification as n µg Na_2S/20 cm^2. Take this figure n, multiply by 0,1 and take the result in the following as V ml.

Place 12,5 ml of citric acid solution (J.2.3) and $(37{,}5 - V)$ ml of water in a 100 ml Erlenmeyer flask.

Add V ml of sodium sulfide solution (J.2.4). Place a piece of lead acetate paper (J.2.2) on the opening of the flask and hold in position with an inverted glass beaker.

J.3.3 Heat both assemblies in an autoclave for 30 min at 121 °C ± 1 °C.

J.3.4 Compare the colour intensity of the two pieces of lead acetate paper.

J.4 Expression of results

Report the results of the comparison.

Annex K
(normative)

Determination of extracted zinc

K.1 Principle

Most rubber formulations require a small portion of zinc oxide to support the vulcanization process and to improve certain physical characteristics. Consequently zinc ions may be extracted by aqueous fluids.

The preferred method for detecting zinc in test solution S_1 is atomic absorption spectrometry, but, if the necessary apparatus is not available, the procedure described in this annex shall be used.

K.2 Reagents

K.2.1 Hydrochloric acid, standard volumetric solution, $c(\mathrm{HCl}) = 2$ mol/l.

K.2.2 Potassium hexacyanoferrate(II), solution.

Dissolve 4,2 g of potassium hexacyanoferrate(II) trihydrate in water to give a volume of 100 ml.

K.2.3 Zinc sulfate, solution, 10 µg of Zn^{2+} per millilitre.

Dissolve 44,0 mg of zinc sulfate heptahydrate ($ZnSO_4.7H_2O$) under addition of 1 ml of ester acid (5 mol/l), in water to give a volume of 1 000 ml.

K.3 Procedure

K.3.1 Mix 10,0 ml of filtered test solution S_1 (membrane filter mesh size: 0,45 µm) with 1 ml of hydrochloric acid (K.2.1) and 3 drops of potassium hexacyanoferrate(II) solution (K.2.2).

K.3.2 Prepare a comparison solution, as described below. The maximum permitted quantity of zinc is stipulated in the appropriate specification as n µg Zn^{2+}/10 ml. Take this figure n, multiply by 0,1 and take the result in the following as V ml.

Mix V ml of zinc sulfate solution (K.2.3) with $(10 - V)$ ml of water, 1 ml of hydrochloric acid (K.2.1) and 3 drops of potassium hexacyanoferrate(II) solution (K.2.2).

K.3.3 After 5 min, compare the colour intensity of the two preparations.

K.4 Expression of results

Report the results of the comparison.