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FIRST COMMISSION DIRECTIVE

of 22 December 1980

on the approximation of the laws of the Member States relating to methods of analysis necessary for checking the composition of cosmetic products

(80/1335/EEC)

(OJ L 383, 31.12.1980, p. 27)

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►**M1** Commission Directive 87/143/EEC of 10 February 1987	L 57	56	27.2.1987

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on the approximation of the laws of the Member States relating to methods of analysis necessary for checking the composition of cosmetic products

(80/1335/EEC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products [1], as amended by Directive 79/661/EEC [2], and in particular Article 8 (1) thereof,

Whereas Directive 76/768/EEC provides for the official testing of cosmetic products with the aim of ensuring that the conditions prescribed pursant to Community provisions concerning the composition of the cosmetic products are satisfied;

Whereas all the necessary methods of analysis must be established as soon as possible; whereas the laying down of methods for the sampling, laboratory preparation, identification and determination of free sodium and potassium hydroxides, the identification and determination of oxalic acid and alkaline salts thereof in hair care products, the determination of chloroform in toothpastes and of zinc, and the identification and determination of phenolsulfonic acid constitutes a first step in this direction;

Whereas the measures laid down in the present Directive are in conformity with the opinion of the Committee on the adaptation of Directive 76/768/EEC to technical progress,

HAS ADOPTED THIS DIRECTIVE:

Article 1

Member States shall take all necessary steps to ensure that, in the official testing of cosmetic products:

— the sampling,
— the laboratory preparation of test samples,
— the identification and determination of free sodium and potassium hydroxides,
— the identification and determination of oxalic acid and alkaline salts in hair-care products,
— the determination of chloroform in toothpastes,
— the determination of zinc,
— the identification and determination of phenolsulfonic acid

are performed in accordance with the methods described in the Annex.

Article 2

Member States shall bring into force the laws, regulations or administrative provisions necessary to comply with this Directive not later than 31 December 1982.

They shall forthwith inform the Commission thereof.

Article 3

This Directive is addressed to the Member States.

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[1] OJ No L 262, 27. 9. 1976, p. 169.
[2] OJ No L 192, 31. 7. 1979, p. 35.

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ANNEX

I. SAMPLING OF COSMETIC PRODUCTS

1. SCOPE AND FIELD OF APPLICATION

The procedure for the sampling of cosmetic products is described with a view to their analysis in the various laboratories.

2. DEFINITIONS

2.1. *Basic sample:*

a unit taken from a batch offered for sale.

2.2. *Total sample:*

the sum of all the basic samples having the same batch number.

2.3. *Laboratory sample:*

a representative fraction of the total sample that is to be analyzed in the individual laboratories.

2.4. *Test portion:*

a representative portion of the laboratory sample that is required for one analysis.

2.5. *Container:*

the article that contains the product and is in continuous direct contact with it.

3. SAMPLING PROCEDURE

3.1. Cosmetic products shall be sampled in their original containers and forwarded to the analytical laboratory unopened.

3.2. For cosmetic products which are placed on the market in bulk or retailed in a container different from the original manufacturer's pack, appropriate instructions for sampling at the point of use or sale should be issued.

3.3. The number of basic samples required for the preparation of the laboratory sample shall be determined by the analytical method and the number of analyses to be performed by each laboratory.

4. SAMPLE IDENTIFICATION

4.1. Samples shall be both sealed where taken and identified, in accordance with the rules in force in the relevant Member State.

4.2. Each basic sample taken shall be labelled with the following information:

— name of the cosmetic product,
— date, time and place of sampling,
— name of the person responsible for taking the sample,
— name of the inspectorate.

4.3. A report on the sampling shall be drawn up in accordance with the rules in force in the relevant Member State.

5. STORAGE OF SAMPLES

5.1. Basic samples must be stored in accordance with the manufacturer's instructions appearing on the label if any.

5.2. Unless other conditions are specified, laboratory samples shall be stored in the dark at between 10 and 25 ºC.

5.3. Basic samples must not be opened until the analysis is about to begin.

II. LABORATORY PREPARATION OF TEST PORTIONS

1. GENERAL

1.1. Where possible the analysis shall be carried out on each basic sample. If the basic sample is too small, the minimum number of basic samples

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should be used. They should first be mixed together thoroughly before taking the test portion.

1.2. Open the container, under an inert gas if so specified in the analytical method and withdraw the number of test portions required as quickly as possible. The analysis should then proceed with the least possible delay. If the sample has to be preserved the container should be resealed under an inert gas.

1.3. Cosmetic products may be prepared in liquid or solid forms or in a semi-solid form. If separation of an initially homogeneous product occurs it should be re-homogenized before taking the test portion.

1.4. If the cosmetic product is put up for sale in a special way, as a result of which it cannot be treated in accordance with these instructions, and if no provision is made for the relevant methods of examination an original procedure may be adopted, provided that it is set out in writing as part of the analysis report.

2. LIQUIDS

2.1. These may occur in the form of products such as solutions in oil, in alcohol, and in water, toilet waters, lotions or milks, and may be packed in flasks, bottles, ampoules or tubes.

2.2. **Withdrawal of the test portion:**

— shake the container vigorously before opening,
— open the container,
— pour a few millilitres of the liquid into a test-tube for visual examination of its character for the purpose of taking the test-portion,
— reseal the container, or
— withdraw the required test portions,
— reseal the container carefully.

3. SEMI-SOLIDS

3.1. These may occur in the form of products such as pastes, creams, stiff emulsions and gels and may be packed in tubes, plastic bottles or jars.

3.2. **Withdrawal of the test portion, *either:***

3.2.1. narrow-necked containers. Expel at least the first centimetre of the product. Extrude the test portion and reseal the container immediately.

3.2.2. wide-necked containers. Scrape the surface evenly to remove the top layer. Take out the test portion and reseal the container immediately.

4. SOLIDS

4.1. These may occur in the form of products such as loose powders, compacted powders, sticks and may be packed in a wide variety of containers.

4.2. Withdrawal of the test portion, either:

4.2.1. loose powder — shake vigorously before unstoppering or opening. Open and remove the test portion.

4.2.2. Compact powder or stick — remove the surface layer by even scraping. Take the test portion from underneath.

5. PRODUCTS IN PRESSURIZED PACKAGES *('aerosol dispensers')*

5.1. These products are defined in Article 2 of Council Directive 75/324/EEC of 20 May 1975 [1].

5.2. **Test portion:**

After vigorous shaking, a representative quantity of the contents of the aerosol dispenser are transferred with the aid of a suitable connector (see for example Figure 1: in specific cases the analytical method may require the use of other connectors) into a plastic-coated glass bottle (Figure 4) fitted with an aerosol valve but not fitted with a dip tube. During the transfer the bottle is held valve downwards. This transfer renders the contents clearly visible corresponding to one of the following four cases:

[1] OJ No L 147, 9. 6. 1975, p. 40.

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5.2.1. An aerosol product in the form of a homogeneous solution for direct analysis.

5.2.2. An aerosol product consisting of two liquid phases. Each phase can be analyzed after the lower phase has been separated into a second transfer bottle. In this case the first transfer bottle is held valve downwards. In such a case this lower phase is often aqueous and devoid of propellent (e.g. butane/water formulation).

5.2.3. An aerosol product containing a powder in suspension. The liquid phase can be analyzed after removal of the powder.

5.2.4. A foam or cream product. First weigh exactly into the transfer bottle 5 to 10 g of 2-methoxyethanol. This substance prevents foam from forming during the degassing operation and it is then possible to expel the propellent gases without loss of liquid.

5.3. **Accessories**

The connector (Figure 1) is made of duralumin or brass. It is designed to fit to different valve systems via a polyethylene adaptor. It is given as an example: other connectors may be used. (See Figures 2 and 3).

The transfer bottle (Figure 4) is made of white glass coated on the outside with a protective layer of transparent plastic material. It holds 50 to 100 ml. It is fitted with an aerosol valve without a dip tube.

5.4. **Method**

In order that enough of the sample may be transferred, the transfer bottle must be purged of air. For this purpose, introduce through the connector about 10 ml of dichlorodifluoromethane or butane (depending on the aerosol product to be examined) and then degas completely until the liquid phase disappears, holding the transfer bottle with the valve uppermost. Remove the connector. Weigh the transfer bottle ('a' grams). Vigorously shake the aerosol dispenser from which the sample is to be taken. Attach the connector to the valve on the sample aerosol container (valve upwards), fit the transfer flask (neck downwards) to the connector and press. Fill the transfer bottle to about two thirds full. If the transfer ceases prematurely owing to pressure equalization, it can be resumed by chilling the transfer bottle. Remove the connector, weigh the filled bottle ('b' grams) and determine the weight of aerosol sample transferred, m_1 (m_1 = b — a).

The sample thus obtained can be used:

1. for a normal chemical analysis;

2. for an analysis of the volatile constituents by gas chromatography.

5.4.1. *Chemical analysis*

Holding the transfer bottle valve upwards, proceed as follows:

— degas. If the degassing operation gives rise to foaming, use a transfer bottle into which an exactly-weighed quantity (5 to 10 g) of 2-methoxyethanol has previously been introduced with a syringe through the connector,

— complete the removal of the volatile constituents without loss by shaking in a waterbath maintained at 40 ºC. Detach the connector,

— reweigh the transfer bottle ('c' grams) in order to determine the weight of the residue, m_2 (m_2 = c — a).

(*NB:*

When calculating the weight of the residue, deduct the weight of any 2-methoxyethanol used.)

— open the transfer bottle by removing the valve,

— dissolve the residue completely in a known quantity of an appropriate solvent,

— perform the desired determination on an aliquot.

Formulas for the calculation are:

$$R = \frac{r \times m_2}{m_1} \text{ and } Q = \frac{R \times P}{100},$$

where:

m_1 = mass of aerosol taken into the transfer bottle;

m_2 = mass of residue after heating at 40 ºC;

r = percentage of the particular substance in m_2 (determined according to the appropriate method);

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R = percentage of the particular substance in the aerosol as received;

Q = total mass of the particular substance in the aerosol dispenser;

p = net mass of initial aerosol dispenser (basic sample).

5.4.2. *Analysis of volatile constituents by gas chromatography*

5.4.2.1. Principle

Using a gas-chromatography syringe, remove an appropriate quantity from the transfer bottle. Then inject the contents of the syringe into the gas chromatograph.

5.4.2.2. Accessories

Series A2 'precision sampling' gas-chromatography syringe 25 µl or 50 µl (Figure 5) or equivalent. This syringe is equipped with a slide valve at the needle end. The syringe is connected to the transfer bottle by a connector at the bottle and a polyethylene tube (length 8 mm, internal diameter 2 · 5 mm) at the syringe.

5.4.2.3. Method

After an appropriate quantity of aerosol product has been taken into the transfer bottle, fit the conical end of the syringe to the transfer bottle as described in 5.4.2.2. Open the valve and aspirate a suitable quantity of liquid. Eliminate gas bubbles by operating the plunger several times (chill the syringe if necessary). Close the valve when the syringe contains the appropriate quantity of bubble-free liquid and detach the syringe from the transfer bottle. Fit the needle, insert the syringe into the gas chromatograph injector, open the valve and inject.

5.4.2.4. Internal standard

If an internal standard is required, it is introduced into the transfer bottle (by means of an ordinary glass syringe using a connector).

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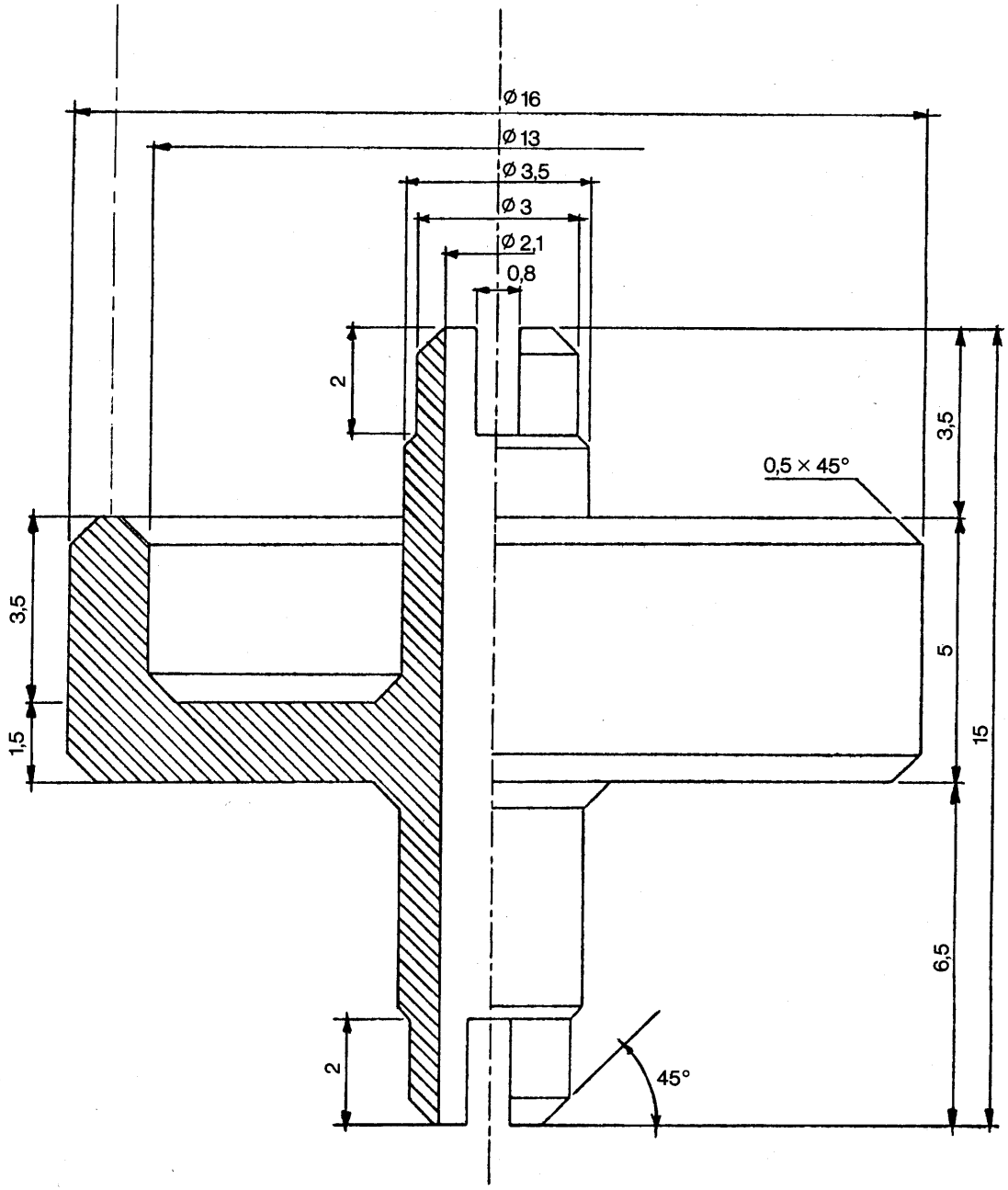

Figure 1

Connector P1

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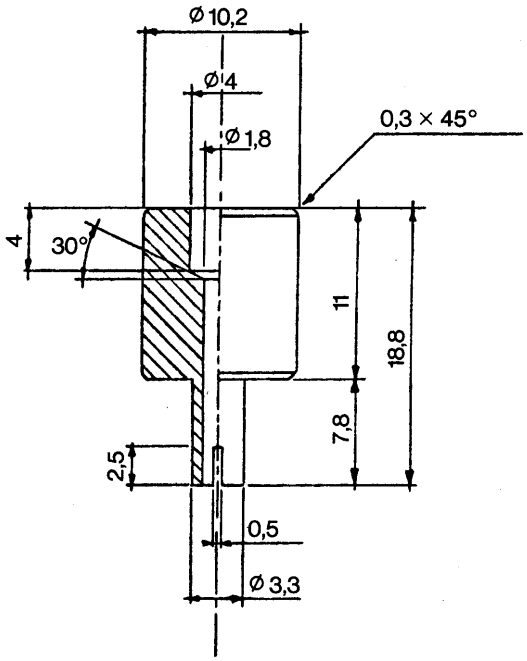

Figure 2

Connector M_2

for transfer between male and female valves

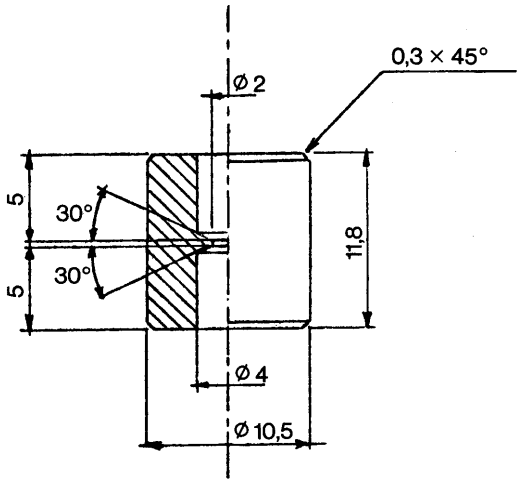

Figure 3

Connector M_1

for transfer between two male vales

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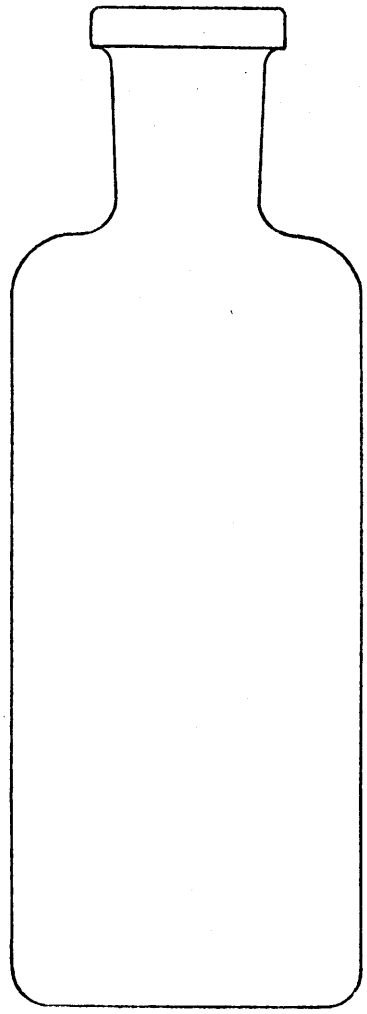

Figure 4

Transfer bottle

Capacity 50 to 100 ml

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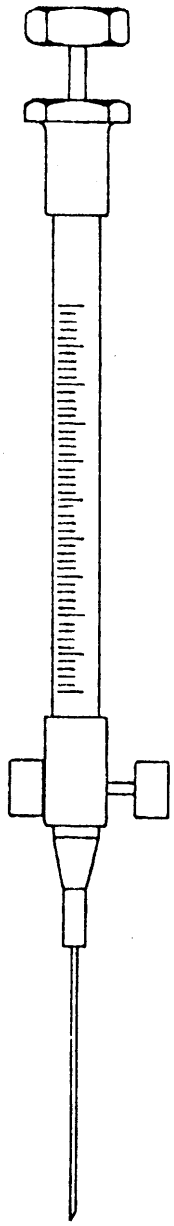

Figure 5

Pressure gas syringe

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III. DETERMINATION AND IDENTIFICATION OF FREE SODIUM AND POTASSIUM HYDROXIDES

1. SCOPE AND FIELD OF APPLICATION

The method specifies the procedure for identifying cosmetic products containing significant amounts of free sodium and/or potassium hydroxides and for the determination of such free sodium and/or potassium hydroxides in hair straightener preparations and nail cuticle solvent preparations.

2. DEFINITION

The free sodium and potassium hydroxide is defined by the volume of standard acid required to neutralize the product under specified conditions, the resulting quantity being expressed as % m/m free sodium hydroxide.

3. PRINCIPLE

The sample is dissolved or dispersed in water and titrated with standard acid. The pH value is recorded concurrently with addition of acid: for a simple solution of sodium or potassium hydroxides the end point is a clear cut maximum rate of change of recorded pH value.

The simple titration curve may be obscured by the presence of:

(a) ammonia and other weak organic bases, which have themselves a rather flat titration curve. Ammonia is removed in the method by evaporation at reduced pressure but at room temperature;

(b) salts of weak acids, which may give rise to a titration curve with several points of inflection. In such cases only the first part of the curve to the first of these points of inflection corresponds to the neutralization of hydroxyl ion coming from free sodium or potassium hydroxide.

An alternative procedure for titration in alcohol is given where excessive interference from salts of weak inorganic acids is indicated.

Whilst the theoretical possibility exists that other soluble strong bases, e.g. lithium hydroxide, quaternary ammonium hydroxide, could be present giving rise to the high pH, the presence of these in this type of a cosmetic product is highly unlikely.

4. IDENTIFICATION

4.1. **Reagents**

4.1.1. Standard alkaline buffer solution pH 9·18 at 25 ºC: 0·05 M sodium tetraborate decahydrate.

4.2. **Apparatus**

4.2.1. Usual laboratory glassware

4.2.2. pH meter

4.2.3. Glass membrane electrode

4.2.4. Standard calomel reference electrode.

4.3. **Procedure**

Calibrate the pH meter with the electrodes using the standard buffer solution.

Prepare a 10 % solution or dispersion of the product to be analyzed, in water, and filter. Measure the pH. If the pH is 12 or over a quantitative determination must be carried out.

5. DETERMINATION

5.1. **Titration in aqueous medium**

5.1.1. *Reagent*

5.1.1.1 Standard 0·1 N hydrochloric acid

5.1.2. *Apparatus*

5.1.2.1. Usual laboratory glassware

5.1.2.2. pH meter preferably with recorder

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5.1.2.3. Glass membrane electrode

5.1.2.4. Standard calomel reference electrode.

5.1.3. *Procedure*

Weigh accurately into a 150-ml beaker a test portion of between 0·5 and 1·0 g. If ammonia is present add a few anti-bumping granules, place the beaker in a vacuum desiccator, evacuate using a water pump until the odour of ammonia is no longer detectable (about three hours).

Add 100 ml water, dissolve or disperse the residue and titrate with the 0·1 N hydrochloric acid solution (5.1.1.1) recording the change in pH (5.1.2.2).

5.1.4. *Calculation*

Identify the points of inflection on the titration curves. Where the first point of inflection occurs at a pH below 7 the sample is free of sodium or potassium hydroxide.

Where there are two or more points of inflection in the curve only the first is relevant.

Note the volume of titrant to this first point of inflection.

Let V represent this volume of titrant, in ml,

M represent the weight of the test portion, in grams.

The content of sodium and/or potassium hydroxides in the sample expressed as % m/m of sodium hydroxide is calculated using the formula:

$$\% = 0 \cdot 4 \ \frac{V}{M}$$

The situation may arise in which, despite indications of the presence of a significant quantity of sodium and/or potassium hydroxides, the titration curve fails to show a distinct point of inflection. In such a case the determination should be repeated in isopropanol.

5.2. **Titration in isopropanol**

5.2.1. *Reagents*

5.2.1.1. Isopropanol

5.2.1.2. Standard 1 · 0 N aqueous hydrochloric acid

5.2.1.3. 0 · 1 N hydrochloric acid in isopropanol prepared immediately before use by diluting the 1 · 0 N aqueous hydrochloric acid with isopropanol.

5.2.2. *Apparatus*

5.2.2.1. Usual laboratory glassware

5.2.2.2. pH meter preferably with recorder

5.2.2.3. Glass membrane electrode

5.2.2.4. Standard calomel reference electrode.

5.2.3. *Procedure*

Weigh accurately into a 150-ml beaker a test portion of between 0 · 5 and 1 · 0 g. If ammonia is present add a few antibumping granules, place the beaker in a vaccum desiccator, evacuate using a water pump until the odour of ammonia is no longer detectable (about three hours).

Add 100 ml isopropanol, dissolve or disperse the residue and titrate with the 0 · 1 N hydrochloric acid in ispropanol (5.2.1.3) recording the change in apparent pH (5.2.2.2).

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5.2.4. *Calculation*

As in 5.1.4. The first point of inflection is at an apparent pH of about 9.

5.3. **Repeatability** (1)

For a sodium or potassium hydroxide content in the range of 5% m/m as sodium hydroxide, the difference between the results of two determinations carried out in parallel on the same sample should not exceed an absolute value of 0 · 25%.

IV. DETERMINATION AND IDENTIFICATION OF OXALIC ACID AND ITS ALKALINE SALTS IN HAIR-CARE PRODUCTS

1. SCOPE AND FIELD OF APPLICATION

The method described below is suitable for the determination and identification of oxalic acid and its alkaline salts in hair-care products. It can be used for colourless aqueous/alcoholic solutions and lotions which contain about 5% of oxalic acid or an equivalent quantity of alkaline oxalate.

2. DEFINITION

The content in oxalic acid and/or its alkaline salts determined by this method is expressed as a percentage by mass ($^{m}/_{m}$) of free oxalic acid in the sample.

3. PRINCIPLE

After removal of any anionic surface-active agents present with p-toluidine hydrochloride, the oxalic acid and/or the oxalates are precipitated as calcium oxalate, whereupon the solution is filtered. The precipitate is dissolved in sulphuric acid and titrated against potassium permanganate.

4. REAGENTS

All reagents should be of analytical purity

4.1. 5% ($^{m}/_{m}$) ammonium acetate solution

4.2. 10% ($^{m}/_{m}$) calcium chloride solution

4.3. 95% (V/V) ethanol

4.4. carbon tetrachloride

4.5. diethyl ether

4.6. 6 · 8% ($^{m}/_{m}$) p-toluidine dihydrochloride solution

4.7. 0 · 1N potassium permanganate solution

4.8. 20% ($^{m}/_{m}$) sulphuric acid

4.9. 10% ($^{m}/_{m}$) hydrochloric acid

4.10. Sodium acetate trihydrate

4.11. Acetic acid glacial

4.12. Sulphuric acid (1:1)

4.13. Saturated barium hydroxide solution.

5. APPARATUS

5.1. Separating funnels, 500 ml

5.2. Beakers, 50 ml and 600 ml

5.3. Glass filter crucibles, G-4

5.4. Measuring cylinders, 25 ml and 100 ml

5.5. Pipettes, 10 ml

5.6. Suction flasks, 500 ml

5.7. Water-jet pump

(1) See ISO/DIS 5725.

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5.8. Thermometer graduated from 0 to 100 ºC

5.9. Magnetic stirrer with heating element

5.10. Magnetic stirring rods, teflon-coated

5.11. Burette, 25 ml

5.12. Conical flasks, 250 ml

6. PROCEDURE

6.1. Weigh out 6 to 7 g of the sample into a 50-ml beaker, bring the pH to 3 with dilute hydrochloric acid (4.9) and wash into a separating funnel with 100 ml of distilled water. Add successively 25 ml of ethanol (4.3), 25 ml of p-toluidine dihydrochloride solution (4.6) and 25 to 30 ml of carbon tetrachloride (4.4) and shake the mixture vigorously.

6.2. After separation of the phases, remove the lower (organic) phase repeat the extraction, using the reagents mentioned in 6.1, and again remove the organic phase.

6.3. Wash the aqueous solution into a 600-ml beaker and remove any carbon tetrachloride still present by boiling the solution.

6.4. Add 50 ml of ammonium acetate solution (4.1), bring the solution to the boil (5.9) and stir 10 ml of hot calcium chloride solution (4.2) into the boiling solution; allow the precipitate to settle.

6.5. Check that precipitation is complete by adding a few drops of calcium chloride solution (4.2), allow to cool to room temperature and then stir in 200 ml of ethanol (4.3); (5.10) leave to stand for 30 minutes.

6.6. Filter the liquid through a glass filter crucible (5.3), transfer the precipitate with a small quantity of hot water (50 to 60 ºC) into the filter crucible and wash the precipitate with cold water.

6.7. Wash the precipitate five times with a little ethanol (4.3) and then five time with a little diethyl ether (4.5) and dissolve the precipitate in 50 ml of hot sulphuric acid (4.8) by drawing the latter through the filter crucible under reduced pressure.

6.8. Transfer the solution without loss into a conical flask (5.11) and titrate against potassium permanganate solution (4.7) until a light pink colouration occurs.

7. CALCULATION

The content of the sample expressed as oxalic acid percentage by mass is calculated from the formula

$$\% \text{ oxalic acid} = \frac{A \times 4 \cdot 50179 \times 100}{E \times 1\,000}$$

in which:

A is the consumption of 0 · 1 N potassium permanganate measured in accordance with 6.8;

E is the test quantity of sample in grams (6.1);

4·50179 is the conversion factor for oxalic acid.

8. REPEATABILITY [1]

For an oxalic acid content of about 5% the difference between the results of two determinations in parallel carried out on the same sample should not exceed an absolute value of 0 · 15%.

9. IDENTIFICATION

9.1. **Principle**

Oxalic acid and/or oxalates are precipitated as calcium oxalate and dissolved in sulphuric acid. To the solution is added a little potassium permanganate solution, which turns colourless and causes the formation of carbon dioxide. When the resultant carbon dioxide is passed through

[1] See ISO/DIS 5725.

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a barium hydroxide solution, a white precipitate (milkiness) of barium carbonate is formed.

9.2. **Procedure**

9.2.1. Treat a portion of the sample to be analyzed as described in section 6.1 to 6.3; this will remove any detergents present.

9.2.2. Add a spatula tipful of sodium acetate (4.10) to about 10 ml of the solution obtained in accordance with 9.2.1 and acidify the solution with a few drops of glacial acetic acid (4.11).

9.2.3. Add 10% calcium chloride solution (4.2) and filter. Dissolve the calcium oxalate precipitate in 2 ml of sulphuric acid (1:1) (4.12).

9.2.4 Transfer the solution into a test tube and add drop-wise about 0·5 ml of 0·1 N potassium permanganate solution (4.7). If oxalate is present, the solution loses colour at first gradually and then rapidly.

9.2.5. Immediately after adding the potassium permanganate, place an appropriate glass tube with stopper over the test tube, heat the contents slightly and collect the carbon dioxide formed in a saturated barium hydroxide solution (4.13). The appearance, after three to five minutes, of a milky cloud of barium carbonate indicates the presence of oxalic acid.

V. DETERMINATION OF CHLOROFORM IN TOOTHPASTE

1. SCOPE AND FIELD OF APPLICATION

This method is used for the determination of chloroform in toothpaste by gas chromatography. This method is suitable for the determination of chloroform at levels of 5 % or less.

2. DEFINITION

The chloroform content determined by this method is expressed as a percentage by mass of the product.

3. PRINCIPLE

The toothpaste is suspended in a dimethylformamide/methanol mixture to which is added a known quantity of acetonitrile as internal standard. After centrifuging, a portion of the liquid phase is subjected to gas chromatography and the chloroform content calculated.

4. REAGENTS

All reagents should be of analytical purity.

4.1. Porapak Q, Chromosorb 101 or equivalent, 80 to 100 mesh

4.2. Acetonitrile

4.3. Chloroform

4.4. Dimethylformamide

4.5. Methanol

4.6. Internal standard solution.

Pipette 5 ml of dimethylformamide (4.4) into a 50-ml standard flask and add about 300 mg (M mg) of acetonitrile, accurately weighed. Make up to the mark with dimethylformamide and mix.

4.7 Solution for the determination of relative response factor. Pipette exactly 5 ml of internal standard solution (4.6) into a 10-ml standard flask and add about 300 mg (M_1 mg) of chloroform, accurately weighed. Make up to the mark with dimethylformamide and mix.

5. APPARATUS AND EQUIPMENT

5.1. Analytical balance.

5.2. Gas chromatograph, with flame ionization detector

5.3. Micro-syringe with a capacity of 5 to 10 µl and graduation of 0 · 1 µl.

5.4. Bulb pipettes with capacities of 1, 4 and 5 ml.

5.5. Volumetric flasks, 10 and 50 ml.

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5.6. Test tubes, approximately 20 ml, with screw caps, Sovirel France No 20 or equivalent. The screw cap has an internal sealing plate coated on one side with teflon.

5.7. Centrifuge.

6. PROCEDURE

6.1. **Suitable gas chromatography conditions**

6.1.1. Column material: glass

length: 150 cm

internal diameter: 4 mm

external diameter: 6 mm.

6.1.2. Pack the column with Porapak Q, Chromosorb 101 or equivalent 80 to 100 mesh (4.1) with the acid of a vibrator.

6.1.3. Detector, flame ionization: adjust its sensitivity so that when 3 µl of solution 4.7 is injected, the height of the acetonitrile peak is about three quarters full-scale deflection.

6.1.4. *Gases:*

Carrier, nitrogen, flow rate 65 ml/min.

Auxiliary: adjust the flow of gases to the detector so that the flow of air or oxygen is five to 10 times that of the hydrogen.

6.1.5. *Temperatures:*

injector block	210 ºC
detector block	210 ºC
column oven	175 ºC.

6.1.6. *Chart speed:*

about 100 cm per hour.

6.2. **Sample preparation**

Take the sample for analysis from an unopened tube. Remove one third of the contents, replace the cap on the tube, mix carefully in the tube and then take the test portion.

6.3. **Determination**

6.3.1. Weigh out, into a screw-capped tube (5.6) to the nearest 10 mg, 6 to 7 g (M_o g) of the toothpaste prepared in accordance with section 6.2, and add three small glass beads.

6.3.2. Pipette exactly 5 ml of the internal standard solution (4.6), 4 ml of dimethylformamide (4.4) and 1 ml of methanol (4.5) into the tube, close the tube and mix.

6.3.3. Shake for half an hour with a mechanical shaker and centrifuge the closed tube for 15 minutes, at such a speed as to produce a clear separation of the phases.

Note:

It occasionally happens that the liquid phase is still cloudy after centrifuging. Some improvement can be obtained by adding 1 to 2 g of sodium chloride to the liquid phase, allowing to settle and recentrifuging.

6.3.4. Inject 3 µl of this solution (6.3.3) under the conditions described in section 6.1. Repeat this operation. For the conditions described above, the following retention times can be given as guide values:

methanol	approximately one minute
acetonitrile	approximately 2 · 5 minutes
chloroform	approximately six minutes
dimethylformamide	> 15 minutes

6.3.5. *Determination of the relative response factor*

Inject 3 µl of solution 4.7 for the determination of this factor. Repeat this operation. Determine the relative response factor daily.

▼B

7. CALCULATIONS

7.1. **Calculation of the relative response**

7.1.1. Measure the height and the width at half height of the acetonitrile and chloroform peaks and calculate the area of both peaks, using the formula: height × width at half height.

7.1.2. Determine the area of the acetonitrile and chloroform peaks in the chromatograms obtained in accordance with section 6.3.5 and calculate the relative response f_s with the aid of the following formula:

$$f_s = \frac{\text{As. Mi}}{\text{Ms. Ai}} = \frac{\text{As. } {}^1/_{10}\text{ M}}{\text{Ai. } M_1}$$

in which:

f_s = the relative response factor for chloroform;

As = the area of the chloroform peak (6.3.5);

Ai = the area of the acetonitrile peak (6.3.5);

Ms = the quantity of chloroform in mg per 10 ml of the solution referred to in section 6.3.5 (= M_1);

Mi = the quantity of acetonitrile in mg per 10 ml of the solution referred to in section 6.3.5 (= 1/10 M).

Calculate the mean of the readings obtained.

7.2 **Calculation of the chloroform content**

7.2.1. Calculate in accordance with item 7.1.1 the area of the chloroform and acetonitrile peaks of the chromatograms obtained by the procedure described in section 6.3.4.

7.2.2. Calculate the chloroform content in the toothpaste with the aid of the following formula:

$$\%\ X = \frac{\text{As. Mi}}{f_s .\ M_{sx} .\ \text{Ai}} \cdot 100\ \% = \frac{\text{As. M}}{f_s .\ \text{Ai} .\ M_o .\ 100}$$

in which:

% X = the chloroform content of the toothpaste expressed by mass;

As = the area of the chloroform peak (6.3.4);

Ai = the area of the acetonitrile peak (6.3.4);

M_{sx} = the mass in mg of the sample referred to in section 6.3.1 (= 1 000 . M_o)

Mi = the quantity of acetonitrile in mg per 10 ml of the solution obtained in accordance with section 6.3.2 (1/10 M).

Calculate the mean of the levels found and express the result to an accuracy of within 0·1 %.

8. REPEATABILITY ([1])

For a chloroform content of about 3 %, the difference between the results of two determinations in parallel carried out on the same sample should not exceed an absolute value of 0·3 %.

VI. **DETERMINATION OF ZINC**

1. SCOPE AND FIELD OF APPLICATION

This method is suitable for the determination of zinc present as chloride, sulphate or 4-hydroxybenzenesulphonate, or as an association of several of these zinc salts, in cosmetics.

2. DEFINITION

The zinc content of the sample is determined gravimetrically as the bis(2-methyl-8-quinolyl oxide) and is expressed as percentage by mass of zinc in the sample.

([1]) See ISO/DIS 5725.

▼B

3. PRINCIPLE

Zinc present in solution is precipitated in an acid medium as zinc bis(2-methyl-8-quinolyl oxide). After filtration the precipitate is dried and weighed.

4. REAGENTS

All reagents should be of analytical purity.

4.1. 25 % ($^{m}/_{m}$) concentrated ammonia; d $\frac{20}{4} = 0 \cdot 91$

4.2. Glacial acetic acid

4.3. Ammonium acetate

4.4. 2-Methylquinolin-8-ol

4.5. 6 % ($^{m}/_{v}$) ammonia solution

Transfer 240 g of concentrated ammonia (4.1) into a 1 000-ml standard flask, make up to the mark with distilled water and mix.

4.6. 0 · 2 M ammonium acetate solution

Dissolve 15 · 4 g of ammonium acetate (4.3) in distilled water, make up to the mark in a 1 000-ml standard flask and mix.

4.7. 2-Methylquinolin-8-ol solution

Dissolve 5 g of 2-methylquinolin-8-ol in 12 ml of glacial acetic acid and transfer with distilled water into a 100-ml standard flask. Make up to the mark with distilled water and mix.

5. APPARATUS AND EQUIPMENT

5.1. Standard flasks, 100 and 1 000 ml

5.2. Beakers, 400 ml

5.3. Measuring cylinders, 50 and 150 ml

5.4. Graduated pipettes, 10 ml

5.5. Glass filter crucibles G-4

5.6. Vacuum flasks, 500 ml

5.7. Water-jet pump

5.8. Thermometer graduated from 0 to 100 ºC

5.9. Desiccator with a suitable desiccant and humidity indicator, e.g. silicagel or equivalent

5.10. Drying oven regulated to a temperature 150± 2 ºC

5.11. pH meter

5.12. Hot plate

▼M1

5.13. Filter paper, Whatman No 4 or equivalent.

▼B

6. PROCEDURE

6.1. Weigh out into a 400-ml beaker, 5 to 10 g (M grams), containing about 50 to 100 mg of zinc, of the sample to be analyzed add 50 ml of distilled water and mix.

▼M1

6.1.1. Filter, with the aid of a vacuum pump if necessary, and retain the filtrate.

6.1.2. Repeat the extraction step with a further 50 ml of distilled water. Filter and combine the filtrates.

▼B

6.2. For every 10 mg of zinc present in the solution (►M1 6.1.2 ◄) add 2 ml of the 2-methylquinolin-8-ol solution (4.7) and mix.

6.3. Dilute the mixture with 150 ml of distilled water, bring the temperature of the mixture up to 60 ºC (5.12) and add 45 ml of 0·2M ammonium acetate solution (4.6), stirring constantly.

▼B

6.4. Adjust the pH of the solution to 5·7 to 5·9, with 6 % ammonia solution (4.5), stirring constantly; use a pH meter to measure the pH of the solution,

6.5. Allow the solution to stand for 30 minutes. Filter with the aid of a water-jet pump through a G-4 filter crucible which has been dried beforehand (150 ºC) and weighed after cooling (M_0 grams), and wash the precipitate with 150 ml of distilled water at 95 ºC.

6.6. Place the crucible in a drying oven regulated to 150 ºC and dry for one hour.

6.7. Remove the crucible from the drying oven, place it in a desiccator (5.9) and, when it has cooled to room temperature, determine the mass (M_1 grams).

7. CALCULATION

Calculate the zinc content of the sample as a percentage by mass (% $^m/_m$) with the aid of the following formula:

$$\% \text{ zinc} = \frac{(M_1 - M_0) \times 17 \cdot 12}{M}$$

in which

M = the mass in grams of the sample taken in accordance with 6.1;

M_0 = the mass in grams of the empty and dry filter crucible (6.5);

M_1 = the mass in grams of the filter crucible with precipitate (6.7).

8. REPEATABILITY ([1])

For a zinc content of about 1 % ($^m/_m$), the difference between the results of two determinations in parallel on the same sample should not exceed an absolute value of 0 · 1 %.

VII. DETERMINATION AND IDENTIFICATION OF 4-HYDROXYBENZENESULPHONIC ACID

1. SCOPE AND FIELD OF APPLICATION

This method is suitable for the identification and determination of 4-hydroxybenzenesulphonic acid in cosmetic products such as aerosols and face lotions.

2. DEFINITION

The 4-hydroxybenzenesulphonic acid content determined in accordance with this method is expressed as a percentage by mass of anhydrous zinc 4-hydroxybenzenesulphonate in the product.

3. PRINCIPLE

The test portion is concentrated under reduced pressure, dissolved in water and purified by chloroform extraction. The determination of 4-hydroxybenzenesulphonic acid is carried out iodometrically on an aliquot of the filtered aqueous solution.

4. REAGENTS

All reagents should be of analytical purity.

4.1. 36 % ($^m/_m$) concentrated hydrochloric acid

$$\left(d\ \frac{20}{4} = 1 \cdot 18\right)$$

4.2. Chloroform

4.3. Butanol-1-ol

4.4. Glacial acetic acid

([1]) ISO/DIS 5725.

▼B

4.5. Potassium iodide

4.6. Potassium bromide

4.7. Sodium carbonate

4.8. Sulphanilic acid

4.9. Sodium nitrite

4.10. 0 · 1 N potassium bromate

4.11. 0 · 1 N sodium thiosulphate solution

4.12. 1 % ($^{m}/_{v}$) aqueous solution of starch

4.13. 2 % ($^{m}/_{v}$) aqueous solution of sodium carbonate

4.14. 4 · 5 % ($^{m}/_{v}$) aqueous solution of sodium nitrite

4.15. 0 · 05 % ($^{m}/_{v}$) solution of dithizone in chloroform

4.16. Developing solvent: butan-1-ol/glacial acetic acid/water (4: 1: 5 parts by volume); after mixing in the separating funnel, discard the lower phase.

4.17. Pauly reagent

Dissolve 4 · 5 g of sulphanilic acid (4.8) in 45 ml of concentrated hydrochloric acid (4.1), while heating, and dilute the solution with water to 500 ml. Cool 10 ml of the solution in a dish with ice-water and add, while stirring, 10 ml of cold sodium nitrite solution (4.14). Allow the solution to stand for 15 minutes at 0 ºC (at this temperature the solution remains stable for one to three days) and immediately before spraying (7.5) add 20 ml of sodium carbonate solution (4.13).

4.18. Ready-prepared cellulose plates for thin-layer chromatography; format 20 × 20 cm, thickness of the adsorbent layer 0 · 25 mm;

5. APPARATUS AND EQUIPMENT

5.1. Round-bottoned flasks with ground glass stopper, 100 ml

5.2. Separating funnel, 100 ml

5.3. Conical flask with ground glass stopper, 250 ml

5.4. Burette, 25 ml

5.5. Bulb pipettes, 1, 2 and 10 ml

5.6. Graduated pipette, 5 ml

5.7. Micro-syringe, 10 µl with 0·1 µl graduations

5.8. Thermometer graduated from 0 to 100 ºC

5.9. Water bath equipped with a heating element

5.10 Drying oven, well ventilated and regulated at 80 ºC

5.11. The usual apparatus for carrying out thin-layer chromatography.

6. SAMPLE PREPARATION

In the method described below for the identfication and determination of hydroxybenzenesulphonic acid in aerosols use is made of the residue obtained by releasing from the aerosol can the solvents and propellants that vaporize at normal pressure.

7. IDENTIFICATION

7.1. With the aid of a micro-syringe (5.7) apply 5 µl of the residue (6) or sample at each of six points on the starting line at a distance of 1 cm from the lower edge of the thin-layer plate (4.18).

7.2. Place the plate in a developing tank which already contains the developing solvent (4.16) and develop until the solvent front has reached 15 cm from the starting line.

7.3. Remove the plate from the bath and dry at 80 ºC until no acetic acid vapour is perceptible. Spray the plate with sodium carbonate solution (4.13) and dry in air.

7.4. Cover one half of the plate with a glass plate and spray the uncovered part with 0 · 05 % dithizone solution (4.15). The appearance of

▼B

purplish-red spots in the chromatogram indicates the presence of zinc ions.

7.5. Cover the sprayed half of the plate with a glass plate and spray the other half with Pauly reagent (4.17). The presence of 4-hydroxybenzenesulphonic acid is indicated by the appearance of a yellowish-brown spot with an Rf value of about 0 · 26 whilst a yellow spot with an Rf value of about 0 · 45 in the chromatogram indicates the presence of 3-hydroxybenzenesulphonic acid.

8. DETERMINATION

8.1. Weigh out 10 g of the sample or residue (6) into a 100-ml round-bottomed flask and evaporate almost to dryness under vacuum in a rotary evaporator over a water bath kept at 40 ºC.

8.2. Pipette 10·0 ml (V_1 ml) water into the flask and dissolve the evaporation residue (8.1) by heating.

8.3. Quantitatively transfer the solution into a separating funnel (5.2) and extract the aqueous solution twice with 20-ml portions of chloroform (4.2). After each extraction discard the chloroform phase.

8.4. Filter the aqueous solution through a fluted filter. Depending on the expected hydroxybenzenesulphonic acid content, pipette 1 · 0 or 2 · 0 ml (V_2) of the filtrate into a 250-ml conical flask (5.3) and dilute to 75 ml with water.

8.5. Add 2·5 ml of 36 % hydrochloric acid (4.1) and 2 · 5 g of potassium bromide (4.6), mix and bring the temperature of the solution up to 50 ºC with the aid of a water bath.

8.6. From a burette, add 0·1N potassium bromate (4.10) until the solution, which is still at 50 ºC turns yellow.

8.7. Add a further 3 · 0 ml of potassium bromate solution (4.10), stopper the flask and allow to stand for 10 minutes in a water bath at 50 ºC.

If after 10 minutes the solution loses its colour, add another 2 · 0 ml of potassium bromate solution (4.10), stopper the flask and heat for 10 minutes over a water bath kept at 50 ºC. Record the total quantity of potassium bromate solution added (a).

8.8. Cool the solution to room temperature, add 2 g of potassium iodide (4.5) and mix.

8.9. Titrate the iodine formed against 0·1N sodium thiosulphate solution (4.11). Towards the end of the titration add a few drops of starch solution (4.12) as indicator. Record the quantity of sodium thiosulphate used (b).

9. CALCULATION

Calculate the zinc hydroxybenzenesulphonate content of the sample or residue (6) as a percentage by mass (% $^{m}/_{m}$) with the aid of the following formula:

$$\%\ {}^{m}/_{m}\ \text{zinc hydroxybenzenesulphonate} = \frac{(a - b) \times V_1 \times 0 \cdot 00514 \times 100}{m \times V_2}$$

in which:

a = the total quantity in millilitres of 0 · 1 N potassium bromate solution added (8.7),

b = the quantity in millilitres of 0 · 1 N sodium thiosulphate solution used for the back-titration (8.9),

m = the quantity of product or residue analyzed, expressed in milligrams (8.1),

V_1 = the volume of the solution obtained in accordance with 8.2, expressed in millilitres,

V_2 = the volume of the dissolved evaporation residue used for the analysis (8.4), expressed in millilitres.

Note:

In the case of aerosols, the measurement result in % ($^{m}/_{m}$) of the residue (6) must be expressed in terms of original product. For the purpose of this conversion, reference is made to the rules for the sampling of aerosols.

▼B

10. REPEATABILITY (1)

For a content of about 5 % zinc hydroxybenzenesulphonate, the difference between the results of two determinations carried out in parallel on the same sample should not exceed an absolute value of 0 · 5 %.

11. INTERPRETATION OF THE RESULTS

Under Council Directive 76/768/EEC relating to cosmetic products, the maximum authorized concentration of zinc 4-hydroxybenzenesulphonate in face lotions and deodorants is 6 % ($^{m}/_{m}$). This formulation means that besides the hydroxybenzenesulphonic acid content, the zinc content must be determined. Multiplication of the calculated zinc hydroxybenzenesulphonate content (9) by a factor of 0·1588 yields the minimum zinc content in % ($^{m}/_{m}$) that must theoretically be present in the product in view of the measured hydroxybenzenesulphonic acid content. The zinc content as actually measured gravimetrically (see the relevant provisions) may, however, be higher, because zinc chloride and zinc sulphate may also be used in cosmetic products.

(1) See ISO/DIS 5725.