

The rules governing cosmetic products
in the European Union

Volume 2

Cosmetics legislation

Cosmetic products

Methods of analysis

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EUROPEAN COMMISSION
Enterprise Directorate-General
Pharmaceuticals and cosmetics

THE RULES GOVERNING COSMETIC PRODUCTS IN THE EUROPEAN UNION

Volume 1 Cosmetics legislation
 Cosmetic products

Volume 2 Methods of analysis
 Cosmetic products

Volume 3 Guidelines
 Cosmetic products

FOREWORD

In the early 1970's, the Member States of the EU decided to harmonise their national cosmetic regulations in order to enable the free circulation of cosmetic products within the Community. As a result of numerous discussions between experts from all Member States, Council Directive 76/768/EEC was adopted on 27 July 1976. The principles laid down in the Cosmetics Directive take into account the needs of the consumer while encouraging commercial exchange and eliminating barriers to trade. For example, if a product is to move freely within the EU, the same labelling, packaging and safety regulations must apply. This is one of the main objectives of the Cosmetics Directive: to give clear guidance on what requirements a safe cosmetic product should fulfil in order to freely circulate within the EU, without pre-market authorisation. The Cosmetics Directive aims to guarantee the safety of cosmetic products for human use. This safety relates to composition, packaging and information and it falls totally under the responsibility of the producer or the importer into the EU who is responsible for the marketing liability. There is no pre-market control for cosmetic products at Member State or EU level. Control of cosmetic products within the EU is assured through the responsibility of the person who places the product on the market, a simple notification of manufacturing/importing site, and an in-market surveillance system.

Volume 2 of the series entitled "The Rules governing cosmetic products in the European Union" incorporates the seven Commission Directives on the approximation of the laws of the Member States relating to methods of analysis necessary for checking the composition of cosmetic products.

Directive 76/768/EEC provides for the official testing of cosmetic products with the aim of ensuring that the conditions prescribed pursuant to Community provisions concerning the composition of the cosmetic products are satisfied. Effective in-market control by Member States ensures that only cosmetic products which conform to the provisions of the Cosmetics Directive and its Annexes are on the market. Inspectors appointed at national level may visit department stores, supermarkets, small shops and market stalls to check the products being sold. If necessary, these inspectors may take any product from the market to official laboratories to be tested for compliance with EU regulations. Article 8 (1) of Directive 76/768/EEC provides for the determination of the methods of analysis necessary for checking the composition of cosmetic products. A certain number of methods of analysis have already been validated at European level and accepted as official methods and described in seven Commission Directives on the approximation of the laws of the Member States relating to methods of analysis necessary for checking the composition of cosmetic products. This means that official testing of cosmetic products by laboratories of any kind (national, control, etc.) have to be carried out in accordance with the European official methods described in these Directives.

Commission Directives 80/1335/EEC and 82/434/EEC have already been amended once respectively by Commission Directive 87/143/EEC and Commission Directive 90/207/EEC. With a view to facilitating consultation, there are set out here in codified form for internal use by the competent Commission departments. These codified texts are available to the public but have no force in law. Where doubts exist, the original texts as published in the Official Journal of the European Communities, should be consulted.

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FIRST COMMISSION DIRECTIVE 80/1335/EEC

First Commission Directive 80/1335/EEC 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

(As amended by Commission Directive 87/143/EEC of 10 February 1987)

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 pursuant 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 phenosulfonic 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,

⁽¹⁾ OJ No L 262, 27. 9. 1976, p. 169.

⁽²⁾ OJ No L 192, 31. 7. 1979, p. 35.

■ **Directive 80/1335/EEC**

— 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.

Done at Brussels, 22 December 1980.

For the Commission

Richard BURKE

Member of the Commission

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 °C.

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 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:
- 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 propellant (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 propellant gases without loss of liquid.

(1) OJ No L 147, 9. 6. 1975, p. 40.

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 °C. 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 °C;

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

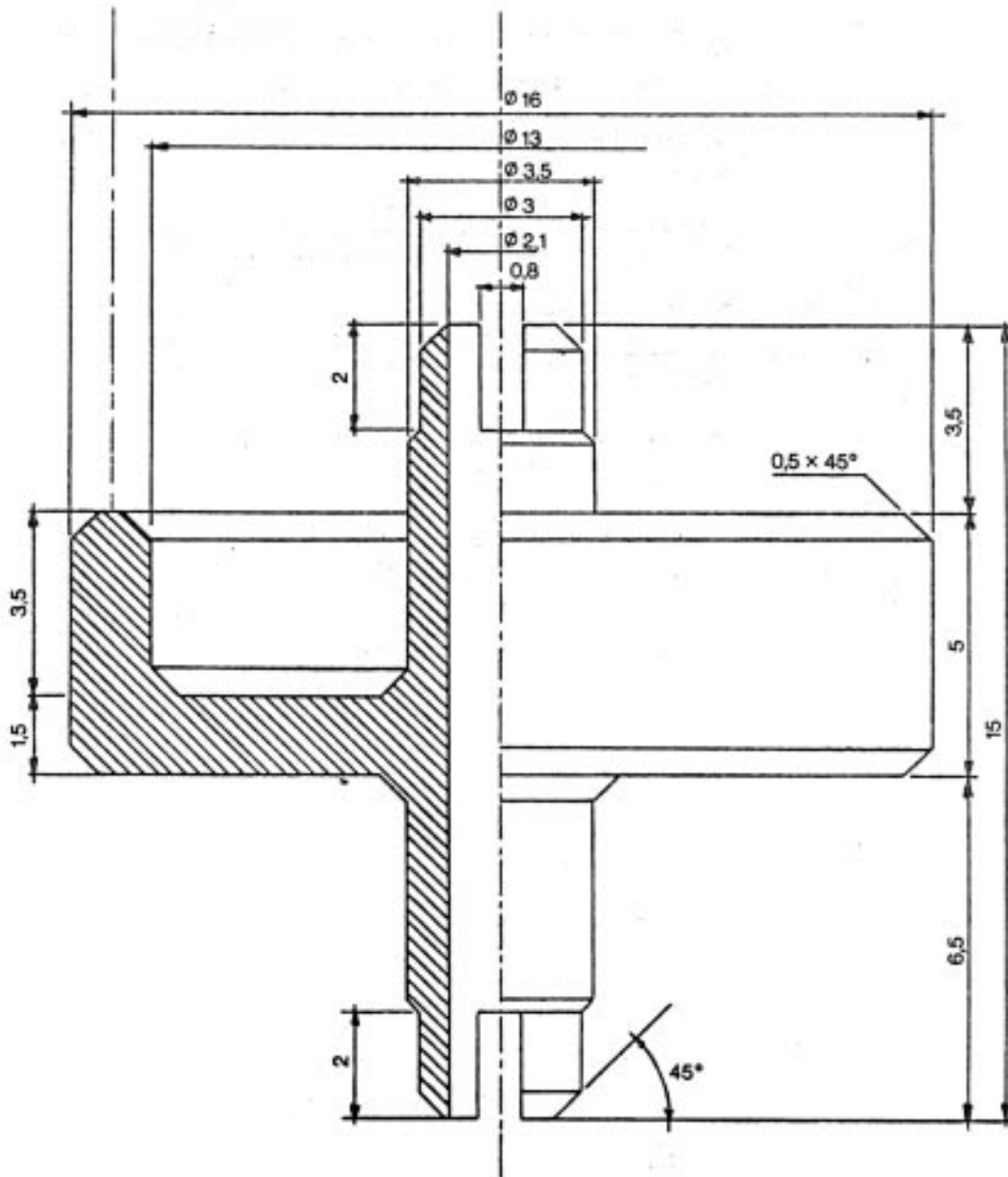


Figure 1
Connector P1

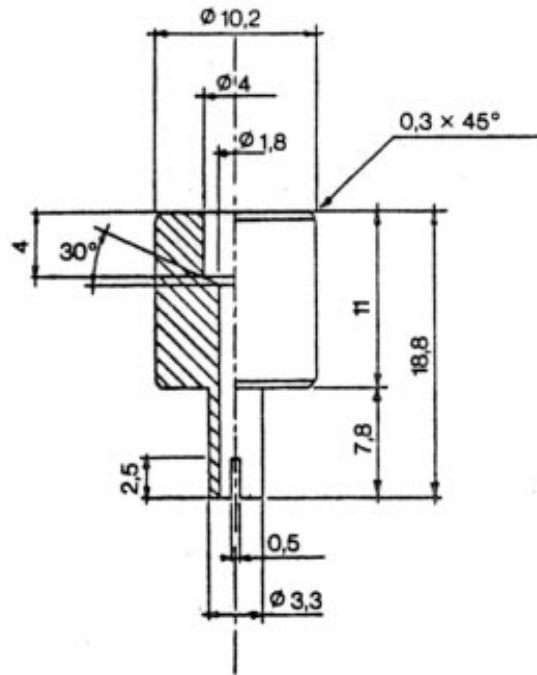


Figure 2
Connector M₂
 for transfer between male and female valves

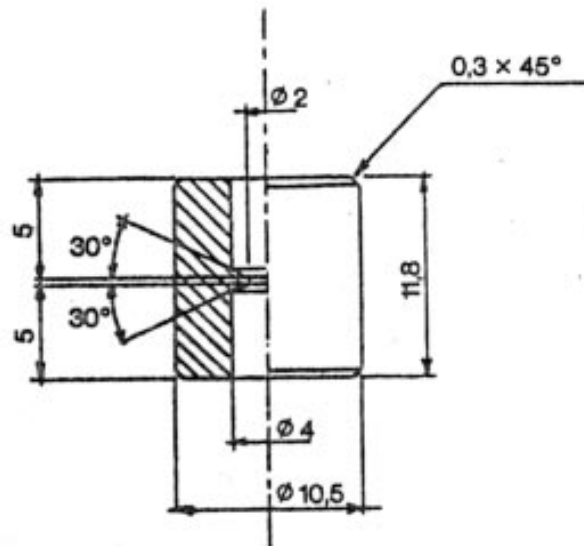


Figure 3
Connector M₁
 for transfer between two male valves



Figure 4
Transfer bottle
Capacity 50 to 100 ml

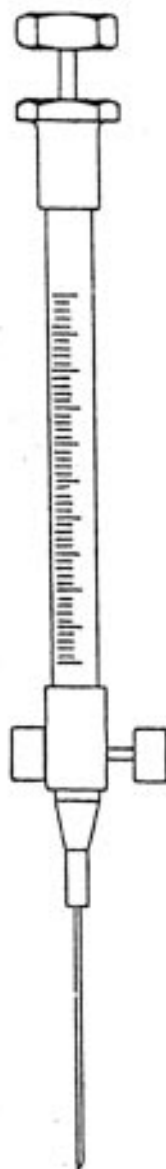


Figure 5
Pressure gas syringe

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 °C: 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

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:

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

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 vacuum 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 isopropanol (5.2.1.3) recording the change in apparent pH (5.2.2.2).

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,

(1) See ISO/DIS 5725.

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 0,1 N 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
- 5.8 Thermometer graduated from 0 to 100 °C
- 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 °C) 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

$$\% \text{ oxalic acid} = \frac{A \times 4,50179 \times 100}{E \times 1000}$$

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,

(1) See ISO/DIS 5725.

which turns colourless and causes the formation of carbon dioxide. When the resultant carbon dioxide is passed through a barium hydroxide solution, a white precipitate (milky) 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

4.6 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 μ l and graduation of 0,1 μ l.

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

5.5 Volumetric flasks, 10 and 50 ml.

5.6 Test tubes, approximately 20 ml, with screw caps, Sovirel France No 20 or equivalent. The crew 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 aid of a vibrator.

6.1.3 Detector, flame ionization: adjust its sensitivity so that when 3 μ l 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 °C

detector block 210 °C

column oven 175 °C.

6.1.6 **Chart speed:**

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_0 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 μ l 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 μ l of solution 4.7 for the determination of this factor. Repeat this operation. Determine the relative response factor daily.

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 x 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:

$$f_s = \frac{A_s \cdot M_i}{M_s \cdot A_i} = \frac{A_s \cdot 1/10 M}{A_i \cdot M_1}$$

in which:

f_s = the relative response factor for chloroform;

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

- A_i = the area of the acetonitrile peak (6.3.5);
- M_s = the quantity of chloroform in mg per 10 ml of the solution referred to in section 6.3.5 (= M_1);
- M_i = 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:

$$\% X = \frac{A_s \cdot M_i}{f_s \cdot M_{sx} \cdot A_i} \cdot 100 \% = \frac{A_s \cdot M}{f_s \cdot A_i \cdot M_0 \cdot 100}$$

in which:

- $\% X$ = the chloroform content of the toothpaste expressed by mass;
- A_s = the area of the chloroform peak (6.3.4);
- A_i = 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 (= $1\ 000 M_0$)
- M_i = 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.

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; $d_{\frac{20}{4}} = 0,91$

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 °C

5.9 Desiccator with a suitable desiccant and humidity indicator. e.g. silica-gel or equivalent

5.10 Drying oven regulated to a temperature 150 ± 2 °C

5.11 pH meter

5.12 Hot plate

5.13 Filter paper, Whatman No 4 or equivalent.

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.
- 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.
- 6.2 For every 10 mg of zinc present in the solution (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 °C (5.12) and add 45 ml of 0,2 M ammonium acetate solution (4.6), stirring constantly.
- 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 °C) and weighed after cooling (M_0 grams), and wash the precipitate with 150 ml of distilled water at 95 °C.
- 6.6 Place the crucible in a drying oven regulated to 150 °C 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:

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

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 %.

(1) ISO/DIS 5725.

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-hydroxybenzene-sulphonic 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 ($d_{\frac{20}{4}} = 1,18$)
- 4.2 Chloroform
- 4.3 Butanol-1-ol
- 4.4 Glacial acetic acid
- 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 °C (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 x 20 cm, thickness of the adsorbent layer 0,25 mm.

5. APPARATUS AND EQUIPMENT

- 5.1 Round-bottomed 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 °C
- 5.9 Water bath equipped with a heating element
- 5.10 Drying oven, well ventilated and regulated at 80 °C
- 5.11 The usual apparatus for carrying out thin-layer chromatography.

6. SAMPLE PREPARATION

In the method described below for the identification 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 °C 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 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 R_f value of about 0,26 whilst a yellow spot with an R_f 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 °C.
- 8.2 Pipette 10,0 ml (V₁ 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 (V₂) 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 °C with the aid of a water bath.
- 8.6 From a burette, add 0,1 N potassium bromate (4.10) until the solution, which is still at 50 °C 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 °C.
- 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 °C. 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 0,1 N 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:

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

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.

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.

SECOND COMMISSION DIRECTIVE 82/434/EEC

Second Commission Directive 82/434/EEC of 14 May 1982 on the approximation of the laws of the Member States relating to methods of analysis necessary for checking the composition of cosmetic products

(As amended by Commission Directive 90/207/EEC of 4 April 1990)

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 laid down by Community provisions concerning the composition of cosmetic products are satisfied;

Whereas all the necessary methods of analysis should be drawn up as quickly as possible; whereas, the first step towards the attainment of this objective having already been taken by the definition of certain methods in Commission Directive 80/1335/EEC ⁽³⁾, the second step is to consist in the definition of methods for identification of some oxidizing agents and determination of hydrogen peroxide in cosmetic hair-care products, identification and semi-quantitative determination of certain oxidation colorants in hair dyes, identification and determination of nitrite, identification and determination of free formaldehyde, determination of resorcinol in shampoos and hair lotions, and determination of methanol in relation to ethanol or propan-2-ol;

Whereas the measures laid down in this Directive are in accordance 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, during official testing of cosmetic products:

- identification of oxidizing agents and determination of hydrogen peroxide in hair-care products,
- identification and semi-quantitative determination of certain oxidation colorants in hair dyes,
- identification and determination of nitrite,
- identification and determination of free formaldehyde,

⁽¹⁾ OJ No L 262, 27. 9. 1976, p. 169.

⁽²⁾ OJ No L 192, 31. 7. 1979, p. 35.

⁽³⁾ OJ No L 383, 31. 12. 1980, p. 27.

■ **Directive 82/434/EEC**

- determination of resorcinol in shampoos and hair lotion,
 - determination of methanol in relation to ethanol or propan-2-ol
- 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 1983.

They shall forthwith inform the Commission thereof.

Article 3

This Directive is addressed to the Member States.

Done at Brussels, 14 May 1982.

For the Commission
Karl-Heinz NARJES
Member of the Commission

ANNEX**I. IDENTIFICATION OF OXIDIZING AGENTS AND DETERMINATION OF HYDROGEN PEROXIDE IN HAIR-CARE PRODUCTS****PURPOSE AND SCOPE**

The iodometric determination of hydrogen peroxide in cosmetics is only possible in the absence of other oxidizing agents that form iodine from iodides. Consequently, before the iodometric determination of hydrogen peroxide it is necessary to detect and identify any other oxidizing agents present. This identification breaks down into two stages; the first covers the persulphates, the bromates and hydrogen peroxide and the second covers barium peroxide.

A. IDENTIFICATION OF PERSULPHATES, BROMATES AND HYDROGEN PEROXIDE**1. PRINCIPLE**

Sodium persulphate, potassium persulphate and ammonium persulphate; potassium bromate, sodium bromate and hydrogen peroxide – whether or not originating from barium peroxide – are identified by means of descending paper chromatography, use being made of two developing solvents.

2. REAGENTS

All reagents should be of analytical purity.

- 2.1 0,5 % (m/v) aqueous reference solutions of the following compounds:
 - 2.1.1 Sodium persulphate
 - 2.1.2 Potassium persulphate
 - 2.1.3 Ammonium persulphate
 - 2.1.4 Potassium bromate
 - 2.1.5 Sodium bromate
 - 2.1.6 Hydrogen peroxide
- 2.2 Developing solvent A, 80 % (v/v) ethanol
- 2.3 Developing solvent B, benzene — methanol — 3-methyl butan-1-ol — water (34:38:18:10 by vol)
- 2.4 Detecting agent A, 10 % (m/v) aqueous solution of potassium iodide
- 2.5 Detecting agent B, 1 % (m/v) aqueous solution of starch
- 2.6 Detecting agent C, 10 % (m/m) hydrochloric acid

2.7 4N hydrochloric acid

3. APPARATUS AND EQUIPMENT

3.1 Chromatography paper (Whatman paper No 3 and No 4 or their equivalents)

3.2 Micropipette 1 µl

3.3 Standard flasks, 100 ml

3.4 Fluted filters

3.5 Apparatus for descending paper chromatography

4. SAMPLE PREPARATION

4.1 Water soluble products

Make two solutions of each sample by dissolving 1 g and 5 g of the product respectively in 100 ml of water. Use 1 µl of each of these solutions for carrying out the paper chromatography described in Section 5.

4.2 Products sparingly soluble in water

4.2.1 Weigh out 1 g and 5 g of the sample and disperse in 50 ml of water, make up to 100 ml with water in each case and mix. Filter the two dispersions over a fluted filter (3.4) and use 1 µl of each of the filtrates in order to carry out the paper chromatography described in Section 5.

4.2.2 Prepare once again two dispersions of each sample by dispersing 1 g and 5 g in 50 ml of water, acidify with dilute hydrochloric acid (2.7), make up with water to 100 ml and mix. Filter the dispersions through a fluted filter (3.4) and use 1 µl of the two filtrates in order to carry out the paper chromatography described in Section 5.

4.3 Creams

Disperse 5 g and 20 g of each product in 100 ml of water and use the dispersions to carry out the paper chromatography described in Section 5.

5. METHOD

5.1 Put an appropriate quantity of solvents A (2.2) and B (2.3) into two separate chromatography tanks in order to carry out the descending paper chromatography. Saturate the chromatography tanks for at least 24 hours with solvent vapour.

5.2 Apply 1 µl of one sample solution and of one reference solution prepared according to Sections 4 and 2.1 to each starting point on a strip of chromatography paper (Whatman No 3 or equivalent) 40 cm long and 20 cm wide (3.1) or another suitable format and evaporate the solution in air.

5.3 Place the chromatography strip (5.2) in the chromatography tank filled with developing solvent A (5.1) and develop until the solvent front has advanced 35 cm (about 15 hours).

5.4 Repeat the procedure described in Sections 5.2 and 5.3, using chromatography paper (Whatman No 4 or equivalent) (3.1) and developing solvent B. Chromatograph until the solvent front has advanced 35 cm (about five hours).

5.5 After development remove the chromatograms and dry them in air.

- 5.6 Reveal the spots in the chromatogram by spraying it successively with:
- 5.6.1 detecting agent A (2.4) followed shortly thereafter by detecting agent B (2.5). The spots of the persulphates will now appear first in the chromatogram and will be followed by the hydrogen peroxide spots. Mark the spots with a pencil;
- 5.6.2 detecting agent C (2.6) on the chromatograms obtained in accordance with Section 5.6.1; the presence of bromates will now be indicated by greyish blue spots in the chromatogram.
- 5.7 Under the abovementioned conditions pertaining to developing solvents A (2.2) and B (2.3), the R_f values of the reference substances (2.1) are approximately as follows:

	<i>Developing solvent A (2.2)</i>	<i>Developing solvent B (2.3)</i>
Sodium persulphate	0,40	0,10
Potassium persulphate	0,40	0,02 + 0,05
Ammonium persulphate	0,50	0,10 + 0,20
Sodium bromate	0,40	0,20
Potassium bromate	0,40	0,10 + 0,20
Hydrogen peroxide	0,80	0,80

B. IDENTIFICATION OF BARIUM PEROXIDE

1. PRINCIPLE

Barium peroxide is identified by the formation of hydrogen peroxide after acidification of the sample (A.4.2), and by the presence of the barium ion:

- in the absence of persulphates (A), by adding dilute sulphuric acid to a portion of the acid sample solution (B.4.1), as a result of which a white precipitate of barium sulphate is formed. The presence of barium ions in the sample (B.4.1) is again confirmed by paper chromatography in the manner described below (B.5),
- where barium peroxide and persulphates are present simultaneously (B.4.2) by digesting the residue from the solution (B.4.2) in an alkali; after dissolution in hydrochloric acid, the presence of barium ions is confirmed in the solution of the melt (B.4.2.3) by paper chromatography and/or by barium sulphate precipitation.

2. REAGENTS

- 2.1 Methanol
- 2.2 36 % (m/m) concentrated hydrochloric acid
- 2.3 6N hydrochloric acid
- 2.4 4N sulphuric acid
- 2.5 Rhodizonic acid disodium salt
- 2.6 Barium chloride ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$)
- 2.7 Anhydrous sodium carbonate

- 2.8 1 % (m/v) aqueous solution of barium chloride
- 2.9 Developing solvent consisting of methanol, concentrated hydrochloric acid (concentration 36 %) and water (80:10:10 by vol)
- 2.10 Detecting agent, 0,1 % (m/v) aqueous solution of rhodizonic acid disodium salt, to be freshly prepared immediately before use.

3. APPARATUS AND EQUIPMENT

- 3.1 Micropipette, 5 µl
- 3.2 Platinum crucibles
- 3.3 Standard flasks, 100 ml
- 3.4 Chromatography paper Schleicher and Schull 2043 b or equivalent. Clean the paper by developing it overnight in a descending chromatography tank (A.3.5) containing developing solvent (B.2.9) and then dry.
- 3.5 Fluted filter paper
- 3.6 The usual apparatus for carrying out ascending paper chromatography

4. SAMPLE PREPARATION

- 4.1 **Products in which persulphates are absent**
- 4.1.1 Disperse 2 g of the product in 50 ml of water and bring the pH of the dispersion to about 1 with hydrochloric acid (B.2.3).
- 4.1.2 Transfer the dispersion with water into a 100-ml standard flask, make up to the mark with water and mix. Use this dispersion for the paper chromatography analysis described in Section 5 and for identification of barium by precipitation of the sulphate.
- 4.2 **Products in which persulphates are present**
- 4.2.1 Disperse 2 g of the product in 100 ml of water and filter.
- 4.2.2 Add to the dried residue seven to 10 times its weight of sodium carbonate (B.2.7), mix and melt the mixture in a platinum crucible (B.3.2) for half an hour.
- 4.2.3 Cool to room temperature, dissolve the melt in 50 ml of water and filter (B.3.5)
- 4.2.4 Dissolve the residue from the melt in hydrochloric acid (B.2.3) and make up to 100 ml with water. Use this solution for the paper chromatography analysis described in Section 5 and for the identification of barium by precipitation of the sulphate.

5. METHOD

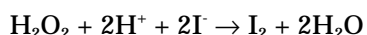
- 5.1 Place an appropriate quantity of developing solvent (B.2.9) in a tank for ascending paper chromatography and saturate the tank for at least 15 hours.
- 5.2 On a piece of chromatography paper – pretreated as described in Section B.3.4 – apply 5 µl of each of the solutions prepared in accordance with Sections B.4.1.2 and B.4.2.4 and reference solution B.2.8 at three starting points.
- 5.3 Dry the sample and reference spots in air. Develop the chromatogram until the solvent front has ascended 30 cm.

- 5.4 Remove the chromatogram from the tank and dry in air.
- 5.5 Reveal the spots on the chromatogram by spraying the paper with detecting agent B.2.10. In the presence of the barium, red spots with an R_f value of about 0,10 appear on the chromatogram.

C. DETERMINATION OF HYDROGEN PEROXIDE

1. PRINCIPLE

The iodometric determination of hydrogen peroxide is based on the following reaction:



This conversion proceeds slowly but can be accelerated by the addition of ammonium molybdate. The iodine formed is determined titrimetrically against sodium thiosulphate and is a measure of the hydrogen peroxide content.

2. DEFINITION

The hydrogen peroxide content measured in the manner described below is expressed as a percentage by mass (% m/m) of the product.

3. REAGENTS

All reagents should be of analytical purity.

- 3.1 2 N sulphuric acid
- 3.2 Potassium iodide
- 3.3 Ammonium molybdate
- 3.4 0,1 N sodium thiosulphate
- 3.5 10 % (m/v) potassium iodide solution, to be freshly prepared immediately before use
- 3.6 20 % (m/v) ammonium molybdate solution
- 3.7 1 % (m/v) starch solution

4. APPARATUS AND EQUIPMENT

- 4.1 Beakers, 100 ml
- 4.2 Burette, 50 ml
- 4.3 Standard flasks, 250 ml
- 4.4 Measuring cylinders, 25 and 100 ml
- 4.5 One-mark pipettes, 10 ml
- 4.6 Conical flasks, 250 ml

5. METHOD

- 5.1 Into a 100-ml beaker weigh out 10 g (m gram) of the product, containing about 0,6 g of hydrogen peroxide. Transfer the contents with water into a 250-ml standard flask, make up to the mark with water and mix.
- 5.2 Pipette 10 ml of the sample solution (5.1) into a 250-ml conical flask (4.6) and add successively 100 ml of 2 N sulphuric acid (3.1), 20 ml of potassium iodide solution (3.5) and three drops of ammonium molybdate solution (3.6).
- 5.3 Titrate the iodine formed immediately against 0,1 N sodium thiosulphate solution (3.4) and just before the end point is reached add a few millilitres of starch solution as indicator (3.7). Record the consumption of 0,1 N sodium thiosulphate (3.4) in millilitres (V).
- 5.4 In the manner described in Sections 5.2 and 5.3, carry out a blank determination, replacing the 10 ml of the sample solution by 10 ml of water. Record the consumption of 0,1 N sodium thiosulphate in the blank determination (Vo ml).

6. CALCULATION

Calculate the hydrogen peroxide content of the product as a percentage by mass (% m/m) with the aid of the following formula:

$$\begin{aligned} \text{\% hydrogen peroxide} &= \frac{(V - V_0) \times 1,7008 \times 250 \times 100}{m \times 10 \times 1000} \\ &= \frac{(V - V_0) \times 4,252}{m} \end{aligned}$$

in which:

- m = the quantity in grams of product analyzed (5.1),
- V₀ = the consumption in millilitres of 0,1 N thiosulphate solution in the blank determination (5.4),
- V = the consumption in millilitres of 0,1 N thiosulphate solution in the titration of the sample solution (5.3).

7. REPEATABILITY (1)

For a product containing about 6 % m/m hydrogen peroxide the difference between the results of two determinations in parallel carried out on the same sample should not exceed an absolute value of 0,2 %.

(1) See Norm ISO 5725.

II. IDENTIFICATION AND SEMI-QUANTITATIVE DETERMINATION OF CERTAIN OXIDATION COLORANTS IN HAIR DYES

1. PURPOSE AND SCOPE

This method is suitable for the identification and semi-quantitative determination of the following substances in hair dyes in cream or liquid form:

Substances	Symbol
<i>Phenylenediamines</i>	
o-Phenylenediamine	(OPD)
m-Phenylenediamine	(MPD)
p-Phenylenediamine (Annex V)	(PPD)
<i>Methylphenylenediamines</i>	
4-Methyl-1,2-phenylenediamine (toluene-3,4-diamine)	(OTD)
4-Methyl-1,3-phenylenediamine (toluene-2,4-diamine)	(MTD)
2-Methyl-1,4-phenylenediamine (toluene-2,5-diamine)	(PTD)
<i>Diaminophenols</i>	
2,4-diaminophenol	(DAP)
<i>Hydroquinone</i>	
1,4 Benzenediol	(H)
<i>α-Naphthol</i>	(α-N)
<i>Pyrogallol</i>	
1,2,3-trihydroxybenzene	(P)
<i>Resorcinol</i>	
1,3-dihydroxybenzene	(R)

2. PRINCIPLE

Oxidation colorants are extracted at pH 10 with 96 % ethanol from dyes in cream or liquid form and identified by thin-layer chromatography, either one- or two-dimensional.

For semi-quantitative determination of these substances, the chromatogram of the samples is compared by means of four developing systems with those for reference substances produced at the same time and under as similar conditions as possible.

3. REAGENTS

All reagents should be of analytical purity.

- 3.1 Ethanol, anhydrous
- 3.2 Acetone
- 3.3 Ethanol, 96 % v/v

- 3.4 Ammonia solution 25 % ($d_4^{20} = 0,91$)
- 3.5 L(+)-ascorbic acid
- 3.6 Chloroform
- 3.7 Cyclohexane
- 3.8 Nitrogen, technical grade
- 3.9 Toluene
- 3.10 Benzene
- 3.11 n-Butanol
- 3.12 Butan-2-ol
- 3.13 Hypophosphorous acid, 50 % v/v solution
- 3.14 Diazo reagent. Either:
- 3-Nitro-1-benzenediazonium chlorobenzenesulphonate, (stabilized salt form) as in Red 2 JN – Francolor,
 - 2-Chloro-4-nitro-1-benzenediazonium naphthalenebenzoate (stabilized salt form) as in NNCD Reagent – reference No 74 150 FLUKA,
- or an equivalent.
- 3.15 Silver nitrate
- 3.16 p-Dimethylaminobenzaldehyde
- 3.17 2,5-Dimethylphenol
- 3.18 Ferric chloride hexahydrate
- 3.19 Hydrochloric acid, 10 % m/v solution
- 3.20 **Reference substances**
- The reference substances are those shown in paragraph 1 'Purpose and scope'. In the case of amine compounds, the reference substance must be either the hydrochloride (mono or di) or the free base.
- 3.21 **Reference solutions 0,5 % (m/v)**
- A 0,5 % (m/v) solution of each of the reference substances in Section 3.20 is prepared.
- Weigh out $50 \text{ mg} \pm 1 \text{ mg}$ of the reference substance in a standard 10-ml flask.
- Add 5 ml of 96% ethanol (3.3) and 250 mg of ascorbic acid (3.5).
- Make the solution alkaline by addition of the ammonia solution (3.4) to give an apparent pH of 10 (test with indicator paper).
- Make up to 10 ml with 96 % ethanol (3.3) and mix.
- The solutions may be kept for a week in a cool place away from the light.
- In certain cases, after the addition of the ascorbic acid and the ammonia, a precipitate may form. It should then be allowed to settle before proceeding.
- 3.22 **Developing solvents**
- 3.22.1 Acetone – chloroform – toluene (35:25:40 by vol)
- 3.22.2 Chloroform – cyclohexane – absolute ethanol – 25 % ammonia (80:10:10:1 by vol)

3.22.3 Benzene – butan-2-ol – water (50:25:25 by vol). Shake well and use the upper phase after separating at room temperature (20 to 25 °C).

3.22.4 n-Butanol – chloroform – reagent M (7:70:23 by vol). Separate carefully at room temperature (20 to 25 °C) and use the lower phase.

Preparation of reagent M

Ammonia solution, 25 % (v/v) 24 volumes

Hypophosphorous acid, 50 % (3.13) 1 volume

Water 75 volumes

Note

Developing solvents containing ammonia must be well shaken immediately before use.

3.23 **Indicator sprays**

3.23.1 *Diazo reagent*

Make a 5 % (m/v) aqueous solution of the chosen reagent (3.14). This Solution must be freshly prepared just before use.

3.23.2 *Ehrlich's reagent*

Dissolve 2 g of p-diamethylaminobenzaldehyde (3.16) in 100 ml of hydrochloric acid 10 % (m/v) aqueous solution (3.19).

3.23.3 *2,5-dimethylphenol – ferric chloride hexahydrate*

Solution 1: Dissolve 1 g of dimethylphenol (3.17) in 100 ml of 96 % ethanol (3.3).

Solution 2: Dissolve 4 g of ferric chloride hexahydrate (3.18) in 100 ml of 96 % ethanol (3.3).

For development, these solutions are sprayed separately, first solution 1 then solution 2.

3.23.4 *Ammoniacal silver nitrate*

25 % ammonia (3.4) is added to 5 % (m/v) aq. solution of silver nitrate (3.15) until the precipitate just dissolves. This reagent must be prepared immediately before use.

Do not keep.

4. APPARATUS

4.1 Usual laboratory equipment for thin-layer chromatography.

4.1.1 Plastics or glass cover so constructed that the chromatographic plate can be surrounded with nitrogen during application of the spots and drying. This precaution is necessary because of the susceptibility to oxidation of certain colorants.

4.1.2 Micro-syringe, 10 µl, graduated in 0,2 µl divisions, with a square cut needle, or, better, a 50 µl repeating dispenser, mounted on a clamp stand in such a way that the plate can be kept under nitrogen.

4.1.3 Silica gel thin layer plates, ready to use, 0,25 mm thick, formed by 20 x 20 cm (Macherey and Nagel, Silica G-HR, which are on plastics support, or equivalent).

4.2 Centrifuge, 4 000 rev./min.

4.3 Centrifuge tubes, 10 ml with PTFE-lined screw caps, or equivalent.

5. PROCEDURE

5.1 Treatment of test samples

Discard the first 2 or 3 cm of cream extruded from the tube.

Put the following into a centrifuge tube (4.3) previously flushed out with nitrogen: 300 mg ascorbic acid with 3 g cream or 3 g homogenized liquid.

Add 25 % ammonia drop-wise (3.4) until the pH is 10. Make up to 10 ml with 96 % ethanol (3.3).

Homogenize under nitrogen (3.8), stopper and then centrifuge at 4 000 rev./min. for 10 minutes.

Use the supernatant liquid.

5.2 Chromatography

5.2.1 Spotting the plates

Under an atmosphere of nitrogen (3.8), apply to a chromatography plate (4.1.3) 1 µl of each of the above-described reference solutions at nine points situated about 1,5 cm apart along a line approximately 1,5 cm from the edge of the plate.

These reference solution spots are arranged as follows:

1	2	3	4	5	6	7	8	9
R	P	H	PPD	DAP	PTD	OPD	OTD	MPD
MTD	α-N							

In addition, apply at points 10 and 11 respectively 2 µl of the test solution samples obtained according to Section 5.1.

Keep the plate under nitrogen (3.8) until the moment when it is chromatographed.

5.2.2 Development

Place the plate in a tank previously flushed out with nitrogen (3.8), saturated with one of the four solvents (3.22) and allow to develop at room temperature (20 to 25 °C) in the dark until the solvent front has moved about 15 cm from the baseline.

Remove the plate and dry under nitrogen (3.8) at room temperature.

5.2.3 Spraying

Spray the plate immediately with one of the four solutions specified in 3.23.

5.2.4 Identification

Compare the R_f value and the colour obtained from the sample with those of the reference substances chromatographed.

Table I gives as examples the R_f values and colours for each substance depending on the solvent and indicator used.

Confirmation of doubtful identification may sometimes be achieved by a spiking method, adding the corresponding reference substance solution to the sample extract.

5.2.5 Semi-quantitative estimation

Compare visually the intensity of the spots for each substance identified in 5.2.4 with an appropriate range of concentrations of the reference substances.

If the concentration of one or more of the substances found in the sample is excessive, dilute the sample extract and repeat the measurement.

TABLE I**Rf values and colours obtained immediately after spraying**

Reference substance (3.20)	Developing solvents				Indicator sprays			
	Rf values				Resultant colours			
	(3.22.1)	(3.22.2)	(3.22.3)	(3.22.4)	Diazo (3.23.1)	Ehrlich (3.23.2)	Dimethyl- phenol (3.23.3)	AgNO (3.23.4)
OPD	0,62	0,60	0,30	0,57	pale brown	–	–	pale brown
MPD	0,40	0,60	0,47	0,48	violet brown (*)	yellow	pale brown	pale brown
PPD	0,20	0,50	0,30	0,48	brown	bright red (*)	violet	grey
OTD	0,60	0,60	0,53	0,60	brown (*)	pale orange	pale brown	greyish brown
MTD	0,40	0,67	0,45	0,60	reddish brown (*)	yellow	brown	black
PTD	0,33	0,65	0,37	0,70	brown	orange	violet (*)	grey
DAP	0,07	–	0	0,05	brown (*)	orange	violet	brown
H	0,50	0,35	0,80	0,20	–	orange	violet	black (*)
α-N	0,90	0,80	0,90	0,75	orange- brown	–	violet (*)	black
P	0,37	–	0,67	0,05	brown	very pale violet	very pale brown	brown (*)
R	0,50	0,37	0,80	0,17	orange (*)	pale violet	very pale brown	pale brown

Note

- OPD is only weakly shown up; the solvent (3.22.3) must be used to separate it clearly from the OTD.
- (*) Indicates the best colour development.

6. EXAMINATION BY TWO-DIMENSIONAL THIN-LAYER CHROMATOGRAPHY

This two-dimensional chromatographic procedure requires the use of additional standards and reagents.

6.1 Additional reference solutions and substances

- 6.1.1 β-naphthol (β-N)
- 6.1.2 2-aminophenol (OAP)
- 6.1.3 3-aminophenol (MAP)
- 6.1.4 4-aminophenol (PAP)
- 6.1.5 2-nitro-1,4-phenylenediamine (2-NPPD)
- 6.1.6 4-nitro-1,2-phenylenediamine (4-NOPD)

Prepare a 0,5 % m/v solution of each of the additional reference substances as described in 3.21.

6.2 Additional developing solvent

- 6.2.1 Ethyl acetate – cyclohexane – ammonia solution, 25 % (65:30:0,5 by vol)

6.3 Additional indication system

Place a glass vessel in a developing tank for thin-layer chromatography, add about 2 g crystallized iodine and close the tank with an adequate lid.

6.4 Chromatography

6.4.1 Draw two lines, as shown in Figure 1, on the absorbent surface of a thin-layer plate (4.1.3).

6.4.2 Under a nitrogen atmosphere (4.1.1), apply 1 to 4 μl extract (5.1) at base point 1 (Figure 1) which is at 2 cm from the two sides. The quantity of extract depends on the intensity of the spots on the chromatograms 5.2.

6.4.3 Divide between points 2 and 3 (Figure 1) the oxidation colorants identified or assumed to be identified by 5.2 (distance between points 1,5 cm). Apply 2 μl of each of the reference solutions – except DAP of which 6 μl must be applied. Conduct the operation under nitrogen (6.4.2).

6.4.4 Repeat the operation in 6.4.3 at base points 4 and 5 (Figure 1) and keep the plate under nitrogen until the moment it is chromatographed (distance between points 1,5 cm).

6.4.5 Flush out a chromatographic tank with nitrogen (3.8) and place in it a suitable quantity of developing solvent 3.22.2. Place the plate (6.4.4) in the tank and develop it in the first elution direction (Figure 1) in the dark.

Elute until the solvent front reaches the line marked on the plate (approximately 13 cm).

6.4.6 Remove the plate from the tank and place it in the chromatography tank previously flushed out with nitrogen to evaporate the elution solvent for at least 60 minutes.

6.4.7 With a graduated test-tube, place a suitable quantity of elution solvent (6.2) in a tank flushed out with nitrogen (3.8), place the plate rotated through 90° in the tank (6.4.6) and chromatograph in the second direction (also in the dark) until the solvent front reaches the line drawn on the absorbent surface. Remove the plate from the tank and evaporate the elution solvent in air.

6.4.8 Place the plate for 10 minutes in the chromatography tank with iodine vapour (6.3) and interpret the two-way chromatogram, using the R_f and colour values of the reference substances chromatographed at the same time (Table II gives a guide to the R_f values and colours).

Note

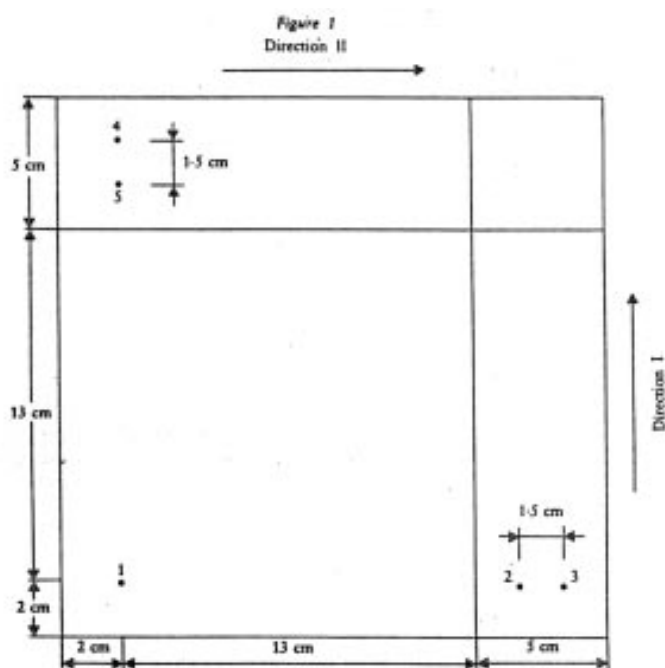
To obtain maximum colouring of the spots leave the chromatogram exposed to the atmosphere for half an hour after developing.

6.4.9 The presence of the oxidation colorants found in 6.4.8 can be definitively confirmed by repeating the operations described in 6.4.1 to 6.4.8 and adding at base point 1 on top of the amount of extract specified in 6.4.2 1 μl of the reference substances identified in 6.4.8. If no other spot is found in comparison with the chromatogram obtained in 6.4.8, the interpretation of chromatogram 6.4.8 is correct.

TABLE II

Colour of the reference substances after chromatography and developing with iodine vapour

Reference substances	Colour after developing with iodine vapour
R	beige
p	brown
α -N	violet
β -N	pale brown
H	violet-brown
MPD	yellowish brown
PPD	violet-brown
MTD	dark brown
PTD	yellowish brown
DAP	dark brown
OAP	orange
MAP	yellowish brown
PAP	violet-brown
2-NPPD	brown
4-NOPD	orange



III. IDENTIFICATION AND DETERMINATION OF NITRITE

A. IDENTIFICATION

1. PURPOSE AND SCOPE

This method is suitable for the identification of nitrite in cosmetic products, particularly creams and pastes.

2. PRINCIPLE

The presence of nitrite is indicated by the formation of coloured derivatives with 2-aminobenzaldehyde phenylhydrazone (Nitrin®).

3. REAGENTS

All reagents should be of analytical purity.

3.1 Dilute sulphuric acid: dilute 2 ml concentrated sulphuric acid ($d_4^{20} = 1,84$) with 11 ml distilled water.

3.2 Dilute hydrochloric acid: dilute 1 ml concentrated hydrochloric acid ($d_4^{20} = 1,19$) with 11 ml distilled water.

3.3 Methanol

3.4 A solution of 2-aminobenzaldehyde phenylhydrazone (Nitrin® reagent) in methanol.

Weigh out 2,0 g of Nitrin® and transfer this quantitatively into a 100-ml standard flask. Add drop-wise 4 ml dilute hydrochloric acid (3.2) and shake. Fill up to the mark with methanol and mix until the solution is completely clear. Store the solution in a brown glass bottle (4.3).

4. APPARATUS

4.1 Beakers, 50 ml

4.2 Standard flask, 100 ml

4.3 Brown glass bottle, 125 ml

4.4 Glass plate, 10 x 10 cm

4.5 Plastics spatula

4.6 Filter paper, 10 x 10 cm

5. PROCEDURE

5.1 Spread part of the sample to be examined evenly over the glass plate (4.4) so as to cover the surface to a thickness of no more than 1 cm.

5.2 Soak a sheet of filter paper (4.6) in distilled water. Lay it on the sample and press the filter paper down with the plastics spatula (4.5).

5.3 Wait about one minute and apply to the centre of the filter paper:

— two drops of dilute sulphuric acid (3.1),

— followed by two drops of the Nitrin[®] solution (3.4).

5.4 After five to 10 seconds, remove the filter paper and examine it against daylight. The presence of nitrite is indicated by a reddish purple coloration.

If the nitrite content is low, the reddish purple colour changes to yellow after five to 15 seconds. This change of colour takes place only after one to two minutes where large quantities of nitrite are present.

6. COMMENT

The intensity of the reddish purple colour and the time that elapses before the change to yellow may give an indication of the nitrite content of the sample.

B. DETERMINATION

1. PURPOSE

The method describes the determination of nitrite in cosmetic products.

2. DEFINITION

The nitrite content of the sample, determined according to this method, is expressed in % by mass of sodium nitrite.

3. PRINCIPLE

After diluting the sample with water and clarifying, the nitrite present is made to react with sulphanilamide and N-1-naphthylethylenediamine and the optical density of the colour obtained is measured at 538 nm.

4. REAGENTS

All reagents should be of analytical quality.

4.1 Clarification reagents: these reagents may not be used more than one week after preparation.

4.1.1 Carrez I reagent:

Dissolve 106 g of potassium cyanoferrate (II) $K_4Fe(CN)_6 \cdot 3H_2O$, in distilled water and dilute with water to 1 000 ml.

4.1.2 Carrez II reagent:

Dissolve 219,5 g of zinc acetate, $Zn(CH_3COO)_2 \cdot 2H_2O$ and 30 ml of glacial acetic acid in distilled water and dilute with water to 1 000 ml.

4.2 Sodium nitrite solution:

Dissolve 0,500 g of sodium nitrite in distilled water in a 1 000-ml volumetric flask and dilute with water to the mark. Dilute 10,0 ml of this stock standard solution to 500 ml; 1 ml of the latter solution = 10 micrograms of $NaNO_2$.

4.3 1N sodium hydroxide solution

4.4 0,2 % sulphanilamide hydrochloride solution:

Dissolve 2,0 g of sulphanilamide in 800 ml of water by warming. Cool and add 100 ml of concentrated hydrochloric acid while stirring. Dilute with water to 1 000 ml.

4.5 5N hydrochloric acid

4.6 N-1-naphthyl reagent:

This solution must be prepared on the day of use. Dissolve 0,1 g of N-1-naphthylethylenediamine dihydrochloride in water and dilute with water to 100 ml.

5. APPARATUS

5.1 Analytical balance

5.2 Volumetric flasks of 100, 250, 500 and 1 000 ml

5.3 Bulb or graduated pipettes

5.4 Measuring cylinders of 100 ml

5.5 Fluted filter papers, free of nitrite, diameter 15 cm

5.6 Water-bath

5.7 Spectrophotometer with optical cells of 1 cm path-length

5.8 pH meter

5.9 Microburette of 10 ml

5.10 Beakers of 250 ml

6. PROCEDURE

6.1 Weigh about 0,5 g (m gram) to a precision of 0,1 mg of the homogenized sample, transfer with hot distilled water quantitatively into a 250-ml beaker (5.10) and bring the volume to approximately 150 ml with hot distilled water. Place the beaker (5.10) in a water-bath (5.6) at 80 °C for half an hour. During this period, shake the contents occasionally.

6.2 Cool to room temperature and add successively, while stirring, 2 ml of the Carrez I reagent (4.1.1) and 2 ml of the Carrez II reagent (4.1.2).

6.3 Add 1 N sodium hydroxide solution (4.3) to bring the pH to 8,3. (Use the pH meter (5.8)). Transfer the contents quantitatively to a 250-ml volumetric flask (5.2) and make up to the mark with distilled water.

6.4 Mix the contents and filter through a fluted filter paper (5.5).

6.5 Pipette (5.3) a suitable aliquot (V ml) of the clear filtrate, but not more than 25 ml, into a 100-ml volumetric flask (5.2) and add distilled water to a volume of 60 ml.

6.6 After mixing, add 10,0 ml of sulphanilamide hydrochloride solution (4.4) and then 6,0 ml of 5N hydrochloric acid (4.5). Mix and allow to stand for five minutes. Add 2,0 ml of N-1-naphthyl reagent (4.6), mix and allow to stand for three minutes. Dilute with water to the mark and mix.

6.7 Prepare a blank test by repeating the operations 6.5 and 6.6 without addition of the N-1-naphthyl reagent (4.6).

- 6.8 Measure (5.7) the optical density at 538 nm of the solution obtained under 6.6 using the blank solution (6.7) as a reference.
- 6.9 Read from the calibration graph (6.10) the sodium nitrite content in micrograms per 100 ml of the solution (m_1 micrograms) that corresponds to the optical density measured in 6.8.
- 6.10 Using the 10 µg per ml sodium nitrite solution (4.2), prepare a calibration graph for concentrations of 0, 20, 40, 60, 80, 100 µg of sodium nitrite per 100 ml.

7. CALCULATION

Calculate the sodium nitrite content of the sample in percent by mass with the aid of the following formula:

$$\% \text{NaNO}_2 = \frac{250}{V} \times m_1 \times 10^{-6} \times \frac{100}{m} = \frac{m_1}{V \times m \times 40}$$

in which:

- m = the mass of the sample in grams taken for analysis (6.1),
- m_1 = the sodium nitrite content in micrograms found in 6.9,
- V = the number of millilitres of the filtrate used for the measurement (6.5).

8. REPEATABILITY (1)

For a content of about 0,2 % m/m of sodium nitrite the difference between the results of two determinations in parallel carried out on the same sample should not exceed an absolute value of 0,005 %.

IV. IDENTIFICATION AND DETERMINATION OF FREE FORMALDEHYDE

1. PURPOSE AND SCOPE

This method describes the identification and two determinations according to the presence or not of formaldehyde donors. It is applicable to all cosmetic products.

1.1 Identification

1.2 General determination by pentane-2,4-dione colorimetry

This method applies when formaldehyde is used alone or with other preservatives that are not formaldehyde donors.

Where this is not the case, and if the result exceeds the maximum permitted concentration, the following method of confirmation must be used.

(1) See Norm ISO 5725.

1.3 Determination in the presence of formaldehyde donors

In the method mentioned above (1.2), during the derivization, the formaldehyde donors split and lead to results that are too high (combined and polymerized formaldehyde).

It is necessary to separate the free formaldehyde by liquid chromatography.

2. DEFINITION

The free formaldehyde content of the sample determined according to this method is expressed as percentage by mass.

3. IDENTIFICATION

3.1 Principle

Free and combined formaldehyde in a sulphuric acid medium turns Schiff's reagent pink or mauve.

3.2 Reagents

All reagents should be of analytical purity and the water has to be demineralized.

3.2.1 Fuchsin;

3.2.2 Sodium sulphite hydrated at 7H₂O;

3.2.3 Concentrated hydrochloric acid (d = 1,19);

3.2.4 Sulphuric acid, about 1M;

3.2.5 Schiff's reagent:

100 mg of fuchsin (3.2.1) is weighed into a beaker and dissolved in 75 ml of water at 80 °C. After cooling, add 2,5 g of sodium sulphite (3.2.2). Make up to 100 ml.

Use within two weeks.

3.3 Procedure

3.3.1 Weigh 2 g of the sample in a 10 ml beaker.

3.3.2 Add two drops of sulphuric acid (3.2.4) and 2 ml of Schiff's reagent (3.2.5). This reagent must be absolutely colourless when it is used.

Shake and leave to stand for five minutes.

3.3.3 If a pink or mauve tint is observed within the five minutes, the formaldehyde is present in excess of 0,01 % and is to be determined by the free and combined method (4) and, if necessary, by procedure (5).

4. GENERAL DETERMINATION BY PENTANE-2,4-DIONE COLORIMETRY

4.1 Principle

Formaldehyde reacts with pentane-2,4-dione in the presence of ammonium acetate to form 3,5-diacetyl-1,4-dihydrolutidine. This is extracted with butan-1-ol and the absorbance of the extract is measured at 410 nm.

4.2 Reagents

All reagents should be of analytical purity and the water has to be demineralized.

4.2.1 Anhydrous ammonium, acetate;

4.2.2 Concentrated acetic acid, $d_4^{20} = 1,05$;

4.2.3 Pentane-2,4-dione freshly distilled under reduced pressure 25 mm Hg 25 ° – it should not exhibit any absorption at 410 nm;

4.2.4 Butan-1-ol;

4.2.5 Hydrochloric acid, 1 M;

4.2.6 Hydrochloric acid, approximately 0,1 M;

4.2.7 Sodium hydroxide, 1 M;

4.2.8 Starch solution freshly prepared according to the European Pharmacopoeia (1 g/50 ml water), 2nd edition 1980, part I-VII-1-1;

4.2.9 37 to 40 % w/v formaldehyde;

4.2.10 Standard iodine solution, 0,05 M;

4.2.11 Standard sodium thiosulphate solution, 0,1 M;

4.2.12 Pentane-2,4-dione reagent:

In a 1 000 ml volumetric flask dissolve:

— 150 g ammonium acetate (4.2.1),

— 2 ml pentane-2,4-dione (4.2.3),

— 3 ml acetic acid (4.2.2).

Make up to 1 000 ml with water (pH of solution about 6,4).

This reagent must be freshly prepared;

4.2.13 Reagent (4.2.12) without pentane-2,4-dione;

4.2.14 Formaldehyde standard: stock solution

Pour 5 g of formaldehyde (4.2.9) into a 1 000 ml volumetric flask and make up to 1 000 ml with water.

Determine the strength of this solution as follows:

Remove 10,00 ml; add 25,00 ml of a standard iodine solution (4.2.10) and 10,00 ml of sodium hydroxide solution (4.2.7).

Allow to stand for five minutes.

Acidify with 11,00 ml of HCl (4.2.5) and determine the excess iodine with a standard sodium thiosulphate solution (4.2.11), using starch solution (4.2.8) as indicator.

1 ml of 0,05 M iodine (4.2.10) consumed is equivalent to 1,5 mg formaldehyde;

4.2.15 Formaldehyde standard: diluted solution

Dilute the formaldehyde stock solution successively 1/20 and then 1/100 with water.

1 ml of this solution contains about 1 µg of formaldehyde.

Calculate the exact content.

4.3 **Apparatus**

- 4.3.1 Standard laboratory apparatus;
- 4.3.2 Phase separation filter, Whatman 1 PS (or equivalent);
- 4.3.3 Centrifuge;
- 4.3.4 Waterbath set at 60 °C;
- 4.3.5 Spectrophotometer;
- 4.3.6 Glass cells with an optical path of 1 cm.

4.4 **Procedure**

4.4.1 **Sample solution**

Into a 100-ml volumetric flask weigh to within 0,001 g a quantity (in g) of the test sample corresponding to a presumed quantity of formaldehyde of about 150 µg.

Make up to 100 ml with water and mix (solution S).

(Check that the pH is close to 6; if not, dilute in the hydrochloric acid solution (4.2.6).)

To a 50-ml Erlenmeyer flask add:

- 10,00 ml of the solution S,
- 5,00 ml pentane-2,4-dione reagent (4.2.12),
- demineralized water to a final volume of 30 ml.

4.4.2 **Reference solution**

Possible interference due to background colour in the test sample is eliminated by the use of this reference solution:

To a 50-ml Erlenmeyer flask add:

- 10,00 ml S solution,
- 5,00 ml reagent (4.2.13),
- demineralized water to a final volume of 30 ml.

4.4.3 **Blank test**

To a 50-ml Erlenmeyer flask add:

- 5,0 ml pentane-2,4-dione reagent (4.2.12),
- demineralized water to a final volume of 30 ml.

4.4.4 **Determination**

- 4.4.4.1 Shake the mixtures from 4.4.1, 4.4.2 and 4.4.3. Immerse the Erlenmeyer flasks in a water bath at 60 °C for exactly 10 minutes. Allow to cool for two minutes in a bath of iced water.
- 4.4.4.2 Transfer into 50-ml separating funnels containing 10 ml of butan-1-ol (4.2.4). Rinse each flask with 3 to 5 ml of water. Shake the mixture vigorously for exactly 30 seconds. Allow it to separate.
- 4.4.4.3 Filter the butan-1-ol phase into the measurement cells (4.3.2) through a phase separation filter. Centrifuging (3 000 g for five minutes) may also be used.
- 4.4.4.4 Measure the absorbance A_1 at 410 nm of the extract of the sample solution from 4.4.1 against the extract of the reference solution 4.4.2.

4.4.4.5 Similarly measure the absorbance A_2 of the extract of the blank solution from 4.4.3 against butan-1-ol.

NB: All these operations must be carried out within 25 minutes from the moment when the Erlenmeyer flasks are placed in the water bath at 60 °C.

4.4.5 **Calibration curve**

4.4.5.1 Into a 50-ml Erlenmeyer flask place:

- 5,00 ml of the diluted standard solution from 4.2.15,
- 5,00 ml of the pentane-2,4-dione reagent (4.2.12),
- demineralized water to a final volume of 30 ml.

4.4.5.2 Continue as described in 4.4.4 and measure the absorbance against butan-1-ol (4.2.4).

4.4.5.3 Repeat the procedure with 10, 15, 20 and 25 ml of the diluted standard solution (4.2.15).

4.4.5.4 To obtain the zero value (corresponding to the colouration of the reagents) proceed as in 4.4.4.5.

4.4.5.5 Construct the calibration curve after subtraction of the zero value from each of the absorbances obtained in 4.4.5.1 and 4.4.5.3. Beer's Law is valid up to 30 µg formaldehyde.

4.5 **Calculations**

4.5.1 Subtract A_2 from A_1 and read off from the calibration curve (4.4.5.5) the amount C , in µg, of formaldehyde in the sample solution (4.4.1).

4.5.2 Calculate the formaldehyde content of sample (% m/m) with the aid of the following formula:

$$\text{formaldehyde content in \%} = \frac{C}{10^3 \cdot m}$$

where:

m = mass of the test portion in g.

4.6 **Repeatability** ⁽¹⁾

For a formaldehyde content of 0,2 % the difference between the results of two determinations in parallel carried out on the same sample should not exceed 0,005 % for determination by pentane-2,4-dione colorimetry.

If the determination of free formaldehyde leads to results greater than the maximum concentrations provided for in Directive 76/768/EEC, i.e.:

- (a) between 0,05 % and 0,2 % in a non-labelled product;
- (b) greater than 0,2 % in the product, whether or not labelled

the procedure described in 5 below must be applied.

⁽¹⁾ See Norm ISO 5725.

5. DETERMINATION IN THE PRESENCE OF FORMALDEHYDE DONORS

5.1 Principle

The separate formaldehyde is transformed into a yellow lutidinic derivative by a reaction with the pentane-2,4-dione in a post-column reactor and the derivative obtained is detected by absorbance at 420 nm.

5.2 Reagents

All reagents should be of analytical purity and the water has to be demineralized.

5.2.1 HPLC grade water or water of equivalent quality;

5.2.2 Anhydrous ammonium, acetate;

5.2.3 Concentrated acetic acid;

5.2.4 Pentane-2,4-dione (kept at 4 °C);

5.2.5 Anhydrous disodium phosphate;

5.2.6 85 % orthophosphoric acid (d = 1,7);

5.2.7 HPLC grade methanol;

5.2.8 Dichloromethane;

5.2.9 37 to 40 % w/v formaldehyde;

5.2.10 Sodium hydroxide, 1 M;

5.2.11 Hydrochloric acid, 1 M;

5.2.12 Hydrochloric acid, 0,002 M;

5.2.13 Starch solution freshly prepared according to the European Pharmacopoeia (see 4.2.8);

5.2.14 Standard iodine solution, 0,05 M;

5.2.15 Standard sodium thiosulphate solution, 0,1 M;

5.2.16 **Mobile phase:**

Aqueous solution of disodium phosphate (5.2.5), 0,006 M adjusted to pH 2,1 with orthophosphoric acid (5.2.6);

5.2.17 **Postcolumn reagent:**

In a 1 000 ml volumetric flask dissolve:

- 62,5 g ammonium acetate (5.2.2),
- 7,5 ml acetic acid (5.2.3),
- 5 ml pentane-2,4-dione (5.2.4).

Make up to 1 000 ml with water (5.2.1).

Keep this reagent away from the light.

Conservation time: maximum three days at 25 °C.

No change in colour should be observed;

5.2.18 **Formaldehyde standard: stock solution**

Pour 10 g of formaldehyde (5.2.9) into a 1 000 ml volumetric flask and make up to 1 000 ml with water.

Determine the strength of this solution as follows:

Remove 5,00 ml; add 25,00 ml of the standard iodine solution (5.2.14) and 10,00 ml of the sodium hydroxide solution (5.2.10).

Allow to stand for five minutes.

Acidify with 11,00 ml of HCl (5.2.11) and titrate the excess standard iodine solution with standard sodium thiosulphate solution (5.2.15), using starch solution (5.2.13) as indicator.

1 ml of iodine solution (5.2.14) is equivalent to 1,5 mg formaldehyde;

5.2.19 **Formaldehyde standard: diluted solution**

Dilute the stock solution to 1/100th of its initial strength in the mobile phase (5.2.16).

1 ml of this solution contains about 37 mg formaldehyde.

Calculate the exact content.

5.3 **Apparatus**

5.3.1 Standard laboratory apparatus;

5.3.2 HPLC pump, pulsation-free;

5.3.3 Low-pressure pulsation-free pump for the reagent (or a second HPLC pump);

5.3.4 Injection valve with a 10 µl loop;

5.3.5 Post-column reactor with the following components:

- + one 1-litre three-neck flask,
- + one 1-litre flask heater,
- + two Vigreux columns with a minimum of 10 plates, two air-cooled,
- + stainless steel tube (for heat exchange) 1,6 mm – internal diameter 0,23 mm, length = 400 mm,
- + Teflon tube 1,6 mm – internal diameter 0,30 mm, length 5 m (French knitting) see Appendix 1),
- + one T-piece without any dead volume (Valco or equivalent),
- + three unions without any dead volume

Or: one post-column module Applied Biosystems PCRS 520 or equivalent fitted with a 1-ml reactor;

5.3.6 Membrane filter, pore size 0,45 µm;

5.3.7 SEP-PAK^R C₁₈ cartridge or equivalent;

5.3.8 **Ready-to-use columns:**

- Bischoff hypersil RP 18 (type NC reference C 25.46 1805)
(5 µm, length = 250 mm, internal diameter = 4,6 mm),
- or Dupont, Zorbax ODS
(5 µm, length = 250 mm, internal diameter = 4,6 mm),

- or Phase SEP, spherisorb ODS 2
(5 µm, length = 250 mm, internal diameter = 4,6 mm);

5.3.9 **Pre-column**

Bischoff K₁ hypersil RP 18 (reference K1 G 6301 1805)
(5 µm, length = 10 mm, or equivalent).

5.3.10 The column and precolumn are connected by means of an Ecotube system (reference A 15020508 Bischoff) or equivalent.

5.3.11 Assemble the apparatus (5.3.5) as shown in the block diagram in Appendix 2.

The connections after the injection valve must be kept as short as possible. In this case, the stainless-steel tube between the reactor outlet and the detector inlet is intended to cool the mixture prior to detection and the temperature in the detector is unknown but constant;

5.3.12 UV visible detector;

5.3.13 Recorder;

5.3.14 Centrifuge;

5.3.15 Ultrasonic bath;

5.3.16 Vibrating stirrer (vortex or equivalent).

5.4 **Procedure**

5.4.1 ***Calibration curve***

This is produced by plotting peak heights as a function of the concentration of formaldehyde standard: diluted.

Prepare the standard solutions by diluting the formaldehyde reference solution (5.2.19) with the mobile phase (5.2.16):

- 1,00 ml of solution (5.2.19) diluted to 20,00 ml (about 185 µg/100 ml)
- 2,00 ml of solution (5.2.19) diluted to 20,00 ml (about 370 µg/100 ml)
- 5,00 ml of solution (5.2.19) diluted to 25,00 ml (about 740 µg/100 ml)
- 5,00 ml of solution (5.2.19) diluted to 20,00 ml (about 925 µg/100 ml)

The standard solutions are kept for one hour at laboratory temperature and must be freshly prepared.

The linearity of the calibration curve is good for concentrations between 1,00 and 15,00 µg/ml.

5.4.2 ***Preparation of the samples***

5.4.2.1 *Emulsions (creams, make-up base, eyeliners)*

Into a stoppered 100-ml flask weigh to the nearest 0,001 g a quantity of test sample (m g) corresponding to a presumed quantity of 100 µg of formaldehyde. Add 20,00 ml dichloromethane (5.2.8) and 20,00 ml hydrochloric acid (5.2.12), accurately measured. Mix with the vibrating stirrer (5.3.16) and by means of the ultrasonic bath (5.3.15). Separate the two phases by centrifuging (3 000 g for two minutes). Meanwhile, wash a cartridge (5.3.7) with 2 ml methanol (5.2.7), then condition with 5 ml water (5.2.1).

Pass 4 ml of the aqueous phase of the extract through the conditioned cartridge, discard the first 2 ml and recover the following fraction.

5.4.2.2 *Lotions, shampoos*

Weigh into a stoppered 100-ml flask to the nearest 0,001 g a quantity of test sample (m g) corresponding to a presumed quantity of about 500 µg of formaldehyde.

Make up to 100 ml with the mobile phase (5.2.16).

Filter the solution through a filter (5.3.6) and inject or pass it through a cartridge (5.3.7) conditioned as before (5.4.2.1). All the solutions must be injected immediately after preparation.

5.4.3 **Chromatographic conditions**

- Flowrate of the mobile phase: 1 ml/min,
- Reagent flowrate: 0,5 ml/min,
- Total flowrate at the detector outlet: 1,5 ml/min,
- Injected volume: 10 µl,
- Elution temperature: in the case of difficult separations, immerse the column in a bath of melting ice: wait for the temperature to stabilize (15-20 min),
- Temperature of post-column reaction: 100 °C,
- Detection: 420 nm.

NB: The entire chromatographic system and post-column must be flushed out with water after use (5.2.1). Where the system is not used for more than two days, this flushing must be followed by flushing with methanol (5.2.7). Before reconditioning the system pass water through it to avoid recrystallization.

5.5 **Calculation**

Emulsions: (5.4.2.1):

Formaldehyde content in % (m/m):

$$\frac{C \cdot 10^{-6} \cdot 100}{5m} = \frac{C \cdot 10^{-4}}{5m}$$

Lotions, shampoos:

In this case the formula becomes:

$$\frac{C \cdot 10^{-6} \cdot 100}{m} = \frac{C \cdot 10^{-4}}{m}$$

where:

m = mass of the sample analysed in g (5.4.2.1),

C = formaldehyde concentration in µg/100 ml read off from the calibration curve (5.4.1).

5.6 **Repeatability** ⁽¹⁾

For a content of 0,05 % of formaldehyde the difference between the results of two determinations in parallel carried out on the same sample should not exceed 0,001 %.

For a content of 0,2 % of formaldehyde the difference between the results of two determinations in parallel carried out on the same sample should not exceed 0,005 %.

⁽¹⁾ See Norm ISO 5725

APPENDIX 1

INTRODUCTION FOR “FRENCH KNITTING”

ACCESSORIES REQUIRED

- One wooden bobbin:
external diameter 5 cm with a hole of 1,5 cm diameter made through the centre. Insert four steel nails (as shown in Figures 1 and 2). The distance between two nails must be 1,8 cm and they must be 0,5 cm from the hole,
- one rigid needle (of the crotchet-hook type) to loop the Teflon tube,
- 5 m of 1,6 mm Teflon tube, internal diameter 0,3 mm.

PROCEDURE

To start off the “French knitting”, the Teflon tube must be threaded from the top of the bobbin to the bottom via the central hole (leaving around 10 cm of tube protruding from the bottom of the bobbin, enabling the chain to be pulled through during the knitting process); then wind the tube around the four nails in turn as shown in Figure 3.

The top and bottom of the French knitting will be protected by metal rings and compression screws; take care not to crush the Teflon when pulling tight. Wind the tube around each nail for a second turn and make the ‘stitches’ as follows:

- lift the lower tube over the upper tube with the hook (see Figure 4). Repeat this process on each of the nails in order (1, 2, 3, 4 in an anti-clockwise direction), until 5 m or the desired length of knitting is produced.

Leave around 10 cm of tube to close the chain. Thread the tube through each of the four loops and pull gently, to close up the end of the chain.

NB: French knitting manufactured for post column reactors is available on the market (Supelco).

SCHEMATIC DIAGRAM OF THE BOBBIN

Figure 1

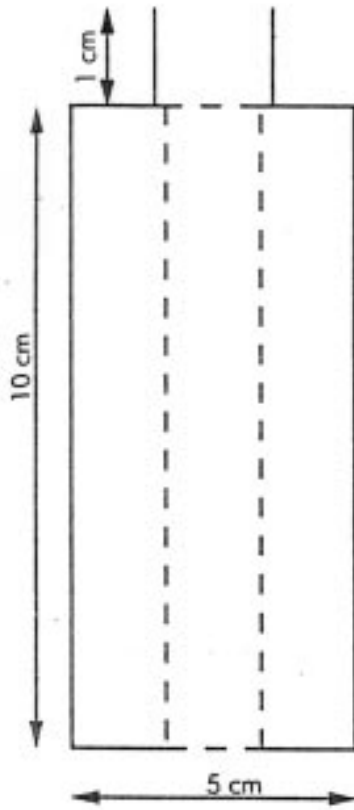


Figure 2

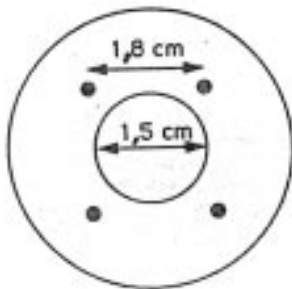
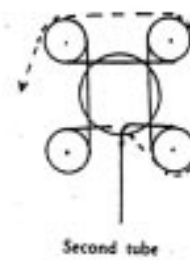


Figure 3



First tube

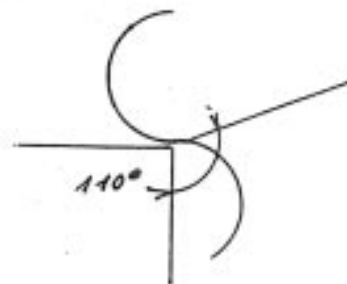
Figure 4



Second tube

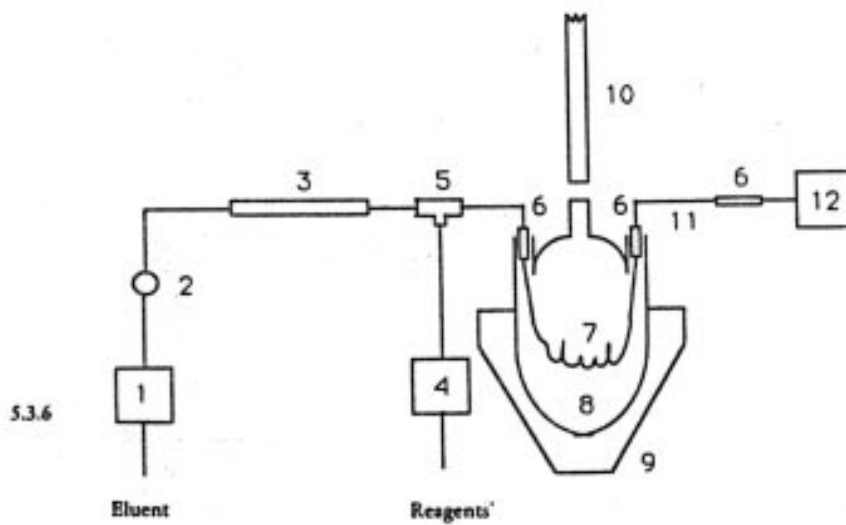
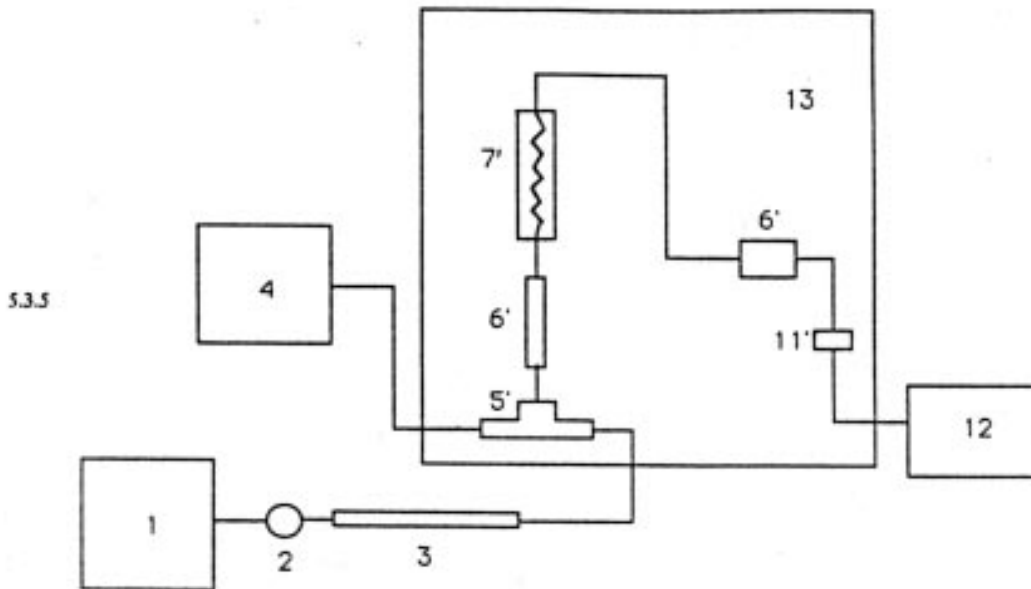
To form a "stitch", lift the lower tube (unbroken line) up over the second tube (dotted line)

Figure 5



APPENDIX 2

- 1 = HPLC pump
- 2 = Injection valve
- 3 = Column with pre-column
- 4 = Reagent pump
- 5 = T-piece without dead volume
- 5' = T-piece (Vortex)
- 6-6' = Union without dead volume
- 7 = 'French knitting'
- 7' = Reactor
- 8 = Three-neck flask with boiling water
- 9 = Flask heater
- 10 = Coolant
- 11 = Stainless steel heat-exchanger tube
- 11' = Heat exchanger
- 12 = Visible UV detector
- 13 = PCRS 520 post-column module



V. DETERMINATION OF RESORCINOL IN SHAMPOOS AND HAIR LOTIONS

1. PURPOSE AND SCOPE

This method specifies the gas chromatographic determination of resorcinol in shampoos and hair lotions. The method is suitable for concentrations of 0,1 up to 2,0 % by mass in the sample.

2. DEFINITION

The content of resorcinol in the sample as determined by this method is expressed as percentage by mass.

3. PRINCIPLE

Resorcinol and 3,5-dihydroxytoluene, (5-methylresorcinol) added as an internal standard, are separated from the sample by thin-layer chromatography. Both compounds are isolated by scraping their spots from the thin-layer plate and extracting with methanol. Finally the extracted compounds are dried, silylated and determined by gas chromatography.

4. REAGENTS

All reagents must be of analytical grade.

4.1 Hydrochloric acid 25 % (m/m)

4.2 Methanol

4.3 Ethanol 96 % (v/v)

4.4 Ready made silica gel TLC sheets (plastic or aluminium) with fluorescent indicator. Deactivate as follows: spray ordinary pre-coated silica sheets with water until glazed. Allow the sprayed plates to dry in air at room temperature for one to three hours.

Note

If plates are not deactivated losses of resorcinol can occur by irreversible adsorption on silica.

4.5 Developing solvent; acetone – chloroform – acetic acid (20:75:5 by volume).

4.6 Resorcinol standard solution; dissolve 400 mg resorcinol in 100 ml of 96 % ethanol (4.3) (1 ml corresponds to 4 000 µg resorcinol).

4.7 Internal standard solution; dissolve 400 mg 3,5-dihydroxytoluene (DHT) in 100 ml of 96 % ethanol (4.3) (1 ml corresponds to 4 000 µg DHT).

4.8 Standard mixture; mix 10 ml of solution 4.6 and 10 ml of solution 4.7 in a 100-ml volumetric flask, make up to the mark with 96 % ethanol (4.3) and mix (1 ml corresponds to 400 µg resorcinol and 400 µg DHT).

4.9 Silylating agents:

4.9.1 N, O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA)

4.9.2 Hexamethyldisilazane (HMDS)

4.9.3 Trimethylchlorosilane (TMCS)

5. APPARATUS

- 5.1 Usual thin-layer and gas chromatography equipment
- 5.2 Glassware

6. PROCEDURE

6.1 Preparation of the sample

- 6.1.1 Weigh accurately into a 150-ml beaker a test portion (m grams) of the product which contains approximately 20 to 50 mg resorcinol.
- 6.1.2 Acidify with hydrochloric acid (4.1) until the mixture is acid (approximately 2 to 4 ml are needed), add 10 ml (40 mg DHT) of the internal standard solution (4.7) and mix. Transfer to a 100-ml volumetric flask with ethanol (4.3) make up to the mark with ethanol and mix.
- 6.1.3 Apply 250 µl of the solution (6.1.2) to a deactivated silica sheet (4.4) as a continuous line of approximately 8 cm length. Take care to get the line as narrow as possible.
- 6.1.4 Apply 250 µl of the standard mixture (4.8) to the same plate in the same way (6.1.3).
- 6.1.5 Spot on two points of the starting line 5 µl of each of the solutions 4.6 and 4.7 to aid localizing after plate development.
- 6.1.6 Develop the plate in an unlined (unsaturated) tank filled with developing solvent 4.5 until the solvent front has reached a line 12 cm from the starting line; usually this takes about 45 minutes. Air-dry the plate and localize the resorcinol/DHT-zone under short wave UV-light (254 nm). The two compounds have approximately the same R_f values. Mark the bands with a pencil at 2 mm distance from the outside dark borderline of the bands. Remove these zones and collect the adsorbent of each band in a 10-ml bottle.
- 6.1.7 Extract the adsorbent containing the sample and the adsorbent containing the standard mixture each in the following way:
add 2 ml methanol (4.2) and extract for one hour with continuous stirring. Filter the mixture and repeat the extraction for another 15 minutes with 2 ml methanol.
- 6.1.8 Combine the extracts and evaporate the solvent by drying overnight in a vacuum desiccator filled with a suitable desiccant. Do not apply any heat.
- 6.1.9 Silylate the residues (6.1.8) either as indicated under 6.1.9.1 or 6.1.9.2.
- 6.1.9.1 Add 200 µl BSTFA (4.9.1) with a microsyringe and leave the mixture in a closed vessel for 12 hours at room temperature.
- 6.1.9.2 Add successively 200 µl HMDS(4.9.2) 100 µl TMCS (4.9.3) with a microsyringe and beat the mixture for 30 minutes at 60 °C in a closed vessel. Cool the mixture.

6.2 Gas chromatography

6.2.1 Chromatographic conditions

The column must yield a resolution, R, equal to or better than 1,5, where:

$$R = \frac{2d'(r_2 - r_1)}{w_1 + w_2}$$

in which:

r_1 and r_2 = retention times in minutes of two peaks,

w_1 and w_2 = the same peaks widths at half height in mm,

d' = the chart speed in mm per minute.

The following column and gas chromatographic conditions have been found suitable:

Column material:	stainless steel
length:	200 cm
internal diameter:	~ 3 mm
filling:	10 % OV-17 on Chromosorb WAW 100 to 120 mesh

Flame ionization detector

Temperatures:

column: 185 °C (isothermal)

detector: 250 °C

injection port: 250 °C

Carrier gas: nitrogen

flow: 45 ml/min.

for settings of hydrogen and air flow follow the manufacture's instructions.

- 6.2.2 Inject 1 to 3 μ l of the solutions obtained under 6.1.9 in the gas chromatograph. Carry out five injections for each solution (6.1.9), measure the peak areas, average these and calculate the peak area ratio: $S = \text{peak area resorcinol} / \text{peak area DHT}$.

7. CALCULATION

The concentration of resorcinol in the sample, expressed as % by mass (% m/m), is given by:

$$\% \text{ resorcinol} = \frac{4}{M} \times \frac{S_{\text{sample}}}{S_{\text{standard mixture}}}$$

in which:

M = test portion in grams (6.1.1),

S_{sample} = the average peak area ratio according to 6.2.2 for the sample solution,

$S_{\text{standard mixture}}$ = the average peak area ratio according to 6.2.2 for the standard mixture.

8. REPEATABILITY (1)

For a resorcinol content of about 0,5 % the difference between the results of two determinations in parallel carried out on the same sample should not exceed an absolute value of 0,025 %.

(1) See Norm ISO 5725.

VI. DETERMINATION OF METHANOL IN RELATION TO ETHANOL OR PROPAN-2-OL

1. PURPOSE AND SCOPE

This method describes the gas chromatographic analysis of methanol in all kinds of cosmetic products (including aerosols).

Relative levels of 0 to 10 % can be determined.

2. DEFINITION

The methanol content determined according to this method is expressed in % by mass of methanol in relation to ethanol or propan-2-ol.

3. PRINCIPLE

The determination is carried out by gas chromatography.

4. REAGENTS

Use analytical grade reagents.

4.1 Methanol

4.2 Ethanol absolute

4.3 Propan-2-ol

4.4 Chloroform, freed from alcohols by washing with water

5. APPARATUS

5.1 Gas chromatograph:

with Katharometer detector for aerosol samples,

with flame ionization detector for non-aerosol samples.

5.2 Volumetric flasks, 100 ml

5.3 Pipettes, 2 ml, 20 ml, 0 to 1 ml

5.4 Microsyringes 0 to 100 μ l and 0 to 5 μ l

and (only for aerosol samples) special gas-tight syringe with sliding valve, (see sampling procedure Figure 5 ⁽¹⁾).

6. PROCEDURE

6.1 Sample preparation

6.1.1 Aerosol products are sampled according to Chapter II of the Annex to Commission Directive 80/1335/EEC of 22 December 1980 ⁽¹⁾ and then analyzed by gas chromatography under the conditions of 6.2.1.

⁽¹⁾ OJ No L 383, 31. 12. 1980, p. 27.

6.1.2 Non-aerosol products sampled according to the abovementioned Chapter II are diluted with water to a level of 1 to 2 % ethanol or propan-2-ol, and then analyzed by gas chromatography under the conditions of 6.2.2.

6.2 Gas chromatography

6.2.1 For aerosol samples, the katharometer detector is used.

6.2.1.1 The column is filled with 10 % Hallcomid M18 on Chromosorb WAW 100 to 200 mesh.

6.2.1.2 The column must yield a resolution, R, equal to or better than 1,5, where:

$$R = 2 \frac{d' r_2 - d' r_1}{w_1 + w_2}$$

in which:

r_1 and r_2 = retention times in minutes of two peaks,

w_1 and w_2 = the same peaks widths at half height in mm,

d' = the chart speed in mm per minute.

6.2.1.3 The following conditions allow this resolution to be achieved:

Column material:	stainless steel
length:	3,5 metres
diameter:	3 mm

Katharometer bridge current:	150 mA
------------------------------	--------

Carrier gas:	helium
pressure:	2,5 bar
flow:	45 ml/min.

Temperatures

injection port:	150 °C
detector:	150 °C
column oven:	65 °C

Peak area measurements can be improved by electronic integration.

6.2.2 For non-aerosol samples:

6.2.2.1 The column is filled with Chromosorb 105 or Porapak QS and the flame ionization detector is used.

6.2.2.2 The column must yield a resolution, R, equal to or better than 1,5, where:

$$R = 2 \frac{d' r_2 - d' r_1}{w_1 + w_2}$$

r_1 and r_2 = retention times in minutes of two peaks,

w_1 and w_2 = the same peaks widths at half height in mm,

d' = the chart speed in mm per minute.

6.2.2.3 This resolution has been achieved using the following conditions:

Column material:	stainless steel
length:	2 metres
diameter:	3 mm

Electrometer sensitivity: $8 \times 10^{-10} \text{A}$

Gases:

carrier: nitrogen
 pressure: 2,1 bar
 flow: 40 ml/min.

Auxiliary gas: hydrogen
 pressure: 1,5 bar
 flow: 20 ml/min.

Temperatures:

injection port: 150 °C
 detector: 230 °C
 column oven 120 to 130 °C

7. STANDARD GRAPH

7.1 For the gas chromatography procedure 6.2.1 (Hallcomid M18 column) use the following standard mixtures. Prepare these mixtures by measuring with pipettes, but find the exact amount by immediate weighing of the pipette or flask after each addition.

Relative strength (m/m %)	Methanol (ml)	Ethanol or propan-2-ol (ml)	Chloroform added to a volume of
approximately 2,5 %	0,5	20	100 ml
approximately 5,0 %	1,0	20	100 ml
approximately 7,5 %	1,5	20	100 ml
approximately 10,0 %	2,0	20	100 ml

Inject 2 to 3 μl into the chromatograph using conditions of 6.2.1.

Calculate peak areas ratio (methanol/ethanol) or (methanol/propan-2-ol) of each mixture.

Plot standard graph using:

X-axis: % methanol in relation to ethanol or propan-2-ol,

Y-axis: peak area ratio (methanol/ethanol) or (methanol/propan-2-ol).

7.2 For the gas chromatography procedure 6.2.2 (Porapak QS or Chromosorb 105) use the following standard mixtures. Prepare these mixtures by measuring with microsyringe and pipette, but find the exact amount by immediate weighing of the pipette or flask after each addition.

Relative strength (m/m %)	Methanol (μl)	Ethanol or propan-2-ol (ml)	Water added to a volume of
approximately 2,5%	50	2	100 ml
approximately 5,0 %	100	2	100 ml
approximately 7,5%	150	2	100 ml
approximately 10,0%	200	2	100 ml

Inject 2 to 3 μl into the chromatograph using conditions of 6.2.2.

Calculate peak area ratio (methanol/ethanol) or (methanol/propan-2-ol) of each mixture.

Plot standard graph using:

X-axis: % methanol in relation to ethanol or propan-2-ol,

Y-axis: peak area ratio (methanol/ethanol) or (methanol/propan-2-ol).

7.3 The standard graph must be a straight line.

8. REPEATABILITY (1)

For a methanol content of 5 % relative to ethanol or propan-2-ol, the difference between the results of two determinations in parallel carried out on the same sample should not exceed 0,25 %.

(1) See Norm ISO 5725.

THIRD COMMISSION DIRECTIVE 83/514/EEC

Third Commission Directive 83/514/EEC of 27 September 1983 on the approximation of the laws of the Member States relating to methods of analysis necessary for checking the composition of cosmetic products

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 last amended by Directive 83/341/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 laid down by Community provisions concerning the composition of cosmetic products are satisfied;

Whereas all the necessary methods of analysis should be laid down as quickly as possible; whereas two steps towards the attainment of this objective having already been taken through the definition of certain methods in Commission Directives 80/1335/EEC ⁽³⁾ and 82/434/EEC ⁽⁴⁾, the third step is to consist in the definition of methods for the determination of dichloromethane and 1,1,1-trichloroethane, the identification and determination of quinolin-8-ol and bis(8-hydroxyquinolinium) sulphate, the determination of ammonia, the identification and determination of nitromethane, the identification and determination of mercaptoacetic acid in hair-waving, hair-straightening and depilatory products, the identification and determination of hexachlorophene (INN), the determination of tosylchloramide sodium (INN), the determination of total fluorine in dental creams, the identification and determination of organomercury compounds, the determination of alkali and alkaline earth sulphides;

Whereas the measures provided for in this Directive are in accordance 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 during official testing of cosmetic products:

- determination of dichloromethane and 1,1,1-trichloroethane,
- identification and determination of quinolin-8-ol and bis(8-hydroxy-quinolinium) sulphate,
- determination of ammonia,

⁽¹⁾ OJ No L 262, 27. 9. 1976, p. 169.

⁽²⁾ OJ No L 188, 13. 7. 1983, p. 15.

⁽³⁾ OJ No L 383, 31. 12. 1980, p. 27.

⁽⁴⁾ OJ No L 185, 30. 6. 1982, p. 1.

- identification and determination of nitromethane,
- identification and determination of mercaptoacetic acid in hair-waving, hair-straightening and depilatory products,
- identification and determination of hexachlorophene (INN),
- determination of tosylchloramide sodium (INN),
- determination of total fluorine in dental creams,
- identification and determination of organomercury compounds,
- determination of alkali and alkaline earth sulphides,

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

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 1984.

They shall forthwith inform the Commission thereof.

Article 3

This Directive is addressed to the Member States.

Done at Brussels, 27 September 1983.

For the Commission
Frans ANDRIESSEN
Member of the Commission

ANNEX**DETERMINATION OF DICHLOROMETHANE AND
1,1,1-TRICHLOROETHANE****1. SCOPE AND FIELD OF APPLICATION**

This method describes the determination of dichloromethane (methylene chloride) and 1,1,1-trichloroethane (methyl chloroform) in all cosmetic products likely to contain these solvents.

2. DEFINITION

The dichloromethane and 1,1,1-trichloroethane content of the sample determined according to this method are expressed in percentage by mass.

3. PRINCIPLE

The method uses gas chromatography with chloroform as internal standard.

4. REAGENTS

All reagents must be of analytical quality.

- 4.1 Chloroform (CHCl_3).
- 4.2 Carbon tetrachloride (CCl_4).
- 4.3 Dichloromethane (CH_2Cl_2).
- 4.4 1,1,1-trichloroethane (CH_3CCl_3).
- 4.5 Acetone.
- 4.6 Nitrogen.

5. APPARATUS

- 5.1 Usual laboratory apparatus.
- 5.2 Gas chromatograph fitted with a thermal conductivity detector.
- 5.3 Transfer bottle, 50 to 100 ml (see sampling method 5.3) ⁽¹⁾.
- 5.4 Pressure gas syringe, 25 or 50 μl (see sampling method 5.4.2.2) ⁽¹⁾.

6. PROCEDURE

- 6.1 Non-pressurized sample: weigh the sample accurately in a stoppered conical flask. Introduce an accurately weighed quantity of chloroform (4.1) as internal standard

⁽¹⁾ OJ No L 383, 31. 12. 1980, p. 27.

equivalent to the presumed quantity of dichloromethane and 1,1,1-trichloroethane contained in the sample. Mix thoroughly.

- 6.2 Pressurized sample: use the sampling method described in the sampling chapter, but with the following refinements:
- 6.2.1 After transferring a sample into a transfer bottle (5.3), further introduce into the transfer bottle a volume of chloroform (4.1) as internal standard equivalent to the presumed quantity of dichloromethane and/or 1,1,1-trichloroethane contained in the sample. Mix thoroughly. Rinse the dead volume of the valve with 0,5 ml of carbon tetrachloride (4.2). After drying, determine accurately the added mass of the internal standard by difference.
- 6.2.2 After filling the syringe with the sample, the nozzle of the syringe should be purged with nitrogen (4.6) so that no residue remains before injection into the chromatograph.
- 6.2.3 After each sample is taken, the surface of the valve and of the transfer piece should be rinsed several times with acetone (4.5) (using as required a hypodermic syringe) and then dried thoroughly with nitrogen (4.6).
- 6.2.4 For each analysis, take measurements using two different transfer bottles and five measurements per bottle.

7. CHROMATOGRAPHIC CONDITIONS

7.1 Precolumn

Tubing: stainless steel.

Length: 300 mm.

Diameter: 3 or 6 mm.

Packing: same material as used for the analytical column packing.

7.2 Column

The stationary phase is made of Hallcomid M 18 on chromosorb. The column must yield a resolution 'R' equal to, or better than, 1,5, where:

$$R = 2 \frac{d' (r_2 - r_1)}{W_1 + W_2}$$

let:

r_1 and r_2 = retention times (in minutes),

W_1 and W_2 = peak widths at half height (in millimetres),

d' = the chart speed (in millimetres per minute).

7.3 As examples the following columns yield the results sought:

Column:	<i>I</i>	<i>II</i>
Material:	Stainless steel tubing	Stainless steel tubing
Length:	350 cm	400 cm
Diameter:	3 mm	6 mm

Support:		
chromosorb:	WAW	WAW-DMCS-HP
sieve analysis:	100 to 120 mesh	60 to 80 mesh
Stationary phase:	Hallcomid M 18, 10 %	Hallcomid M 18, 20 %
Temperature conditions may vary as a function of the apparatus. In the examples they have been set as follows:		
Column:	<i>I</i>	<i>II</i>
Temperatures:		
column:	65 °C	75 °C
injector:	150 °C	125 °C
detector:	150 °C	200 °C
Carrier gas:		
helium flow rate:	45 ml/min	60 ml/min
inlet pressure:	2,5 bar	2 bar
Injection:	15 µl	15 µl

8. MIXTURE FOR ESTABLISHING THE RESPONSE FACTORS

Make up the following accurately weighed mixture in a stoppered conical flask:

Dichloromethane (4.3), 30 % (m/m).

1,1,1-trichloroethane (4.4), 35 % (m/m).

Chloroform (4.1), 35 % (m/m).

9. CALCULATIONS

9.1 Calculating a response factor of a substance 'p' relative to a substance 'a' selected as an internal standard

Let the first substance be 'p', where:

k_p = its response factor,

m_p = its mass in the mixture,

A_p = its peak area.

Let the second substance be 'a', where:

k_a = its response factor (made equal to unity),

M_a = its mass in the mixture,

A_a = its peak area,

then:

$$k_p = \frac{m_p \times A_a}{M_a \times A_p}$$

As examples the following response factors have been obtained (for chloroform: $k = 1$):

Dichloromethane: $k_1 = 0,78 \pm 0,03$

1,1,1-trichloroethane: $k_2 = 1,00 \pm 0,03$

9.2 Calculate the % (m/m) of dichloromethane and 1,1,1-trichloroethane present in the sample to be analyzed

Let:

m_a = the mass (in grams) of chloroform introduced,

M_s = the mass (in grams) of the sample to be analyzed,

A_a = the area of the chloroform peak,

A_1 = the area of the dichloromethane peak,

A_2 = the area of the 1,1,1-trichloroethane peak,

then:

$$\% \text{ (m/m) CH}_2\text{Cl}_2 = \frac{m_a \times A_1 \times k_1 \times 100}{A_a \times M_s}$$

$$\% \text{ (m/m) CH}_3\text{CCl}_3 = \frac{m_a \times A_2 \times k_2 \times 100}{A_a \times M_s}$$

10. REPEATABILITY (1)

For a dichloromethane and/or 1,1,1-trichloroethane content of 25 % (m/m), the difference between the results of two determinations carried out in parallel on the same sample should not exceed an absolute value of 2,5 % (m/m).

IDENTIFICATION AND DETERMINATION OF QUINOLIN-8-OL AND BIS(8-HYDROXYQUINOLINIUM) SULPHATE

1. SCOPE AND FIELD OF APPLICATION

This method describes the identification and quantitative determination of quinolin-8-ol and its sulphate.

2. DEFINITION

The quinolin-8-ol and bis(8-hydroxyquinolinium) sulphate content of the sample as determined by this method is expressed in percentage by mass of quinolin-8-ol.

3. PRINCIPLE

3.1 Identification

Identification is by thin-layer chromatography.

3.2 Determination

The determination is carried out by spectrophotometry at 410 nm of the complex obtained by reaction with Fehling's solution.

(1) Norm ISO 5725.

4. REAGENTS

All reagents should be of analytical purity.

- 4.1 Quinolin-8-ol.
- 4.2 Benzene. In view of its toxicity great care must be taken when working with benzene.
- 4.3 Chloroform.
- 4.4 Aqueous sodium hydroxide, 50 % (m/m) solution.
- 4.5 Copper sulphate pentahydrate.
- 4.6 Potassium sodium tartrate.
- 4.7 M hydrochloric acid.
- 4.8 0,5 M sulphuric acid.
- 4.9 M sodium hydroxide solution.
- 4.10 Ethanol.
- 4.11 Butan-1-ol.
- 4.12 Glacial acetic acid.
- 4.13 0,1 hydrochloric acid.
- 4.14 'Celite 545' or equivalent.
- 4.15 **Standard solutions**
- 4.15.1 Weigh 100 mg of quinolin-8-ol (4.1) into a 100 ml standard flask. Dissolve in a little sulphuric acid (4.8). Make up to the mark with sulphuric acid (4.8).
- 4.15.2 Weigh 100 mg of quinolin-8-ol into a 100 ml standard flask. Dissolve in ethanol (4.10). Make up to the mark with ethanol (4.10) and mix.
- 4.16 **Fehling's solution**
- Solution A*
- Weigh 7 g of copper sulphate pentahydrate (4.5) into a 100 ml standard flask. Dissolve in a little water. Make up to the mark with water and mix.
- Solution B*
- Weigh 35 g of potassium sodium tartrate (4.6) into a 100 ml standard flask. Dissolve in 50 ml of water. Add 20 ml of sodium hydroxide (4.4). Make up to the mark with water and mix. Immediately before use, pipette 10 ml of solution A and 10 ml of solution B into a 100 ml standard flask. Make up to the mark and mix.
- 4.17 **Eluting solvents for thin-layer chromatography**
- I: Butan-1-ol (4.11) / acetic acid (4.12) / water (80:20:20; v/v/v).
- II: Chloroform (4.13) / acetic acid (4.12) (95:5; v/v).
- 4.18 2,6-dichloro-4-(chloroimino)cyclohexa-2,5-dienone, 1 % (m/v) solution in ethanol (4.10).
- 4.19 Sodium carbonate, 1 % (m/v) solution in water.
- 4.20 Ethanol (4.10), 30 % (v/v) solution in water.
- 4.21 Disodium dihydrogen ethylenediaminetetraacetate, 5 % (m/v) solution in water.

4.22 **Buffer solution, pH 7**

Weigh 27 g of potassium dihydrogenorthophosphate anhydrous and 70 g of dipotassium hydrogenorthophosphate trihydrate into a one litre standard flask. Make up to the mark with water.

4.23 **Prepared thin-layer plates**

Ready made thin-layer plates of a thickness of 0,25 mm (e.g. Merck Kieselgel 60 or equivalent). Before use, spray with 10 ml of reagent (4.21) and dry at 80 °C.

5. APPARATUS

5.1 100 ml round-bottom flask with ground-glass neck.

5.2 Standard flasks.

5.3 Graduated pipettes, 10 and 5 ml.

5.4 Bulb pipettes, 20, 15, 10 and 5 ml.

5.5 Separating funnels, 100, 50 and 25 ml.

5.6 Pleated filter paper, diameter 90 mm.

5.7 Rotary evaporator.

5.8 Reflux condenser with ground-glass neck.

5.9 Spectrophotometer.

5.10 Optical cells of 10 mm path length.

5.11 Stirrer hotplate.

5.12 Glass chromatography column dimensions: 160 mm long with a diameter of 8 mm, a constriction at the lower end containing a glass-wool plug, and an adaptor at the upper end for application of pressure.

6. PROCEDURE

6.1 **Identification**

6.1.1 *Liquid samples*

6.1.1.1 The pH of part of the test sample is adjusted to 7. 5 and 10 µl are spotted on the starting line of a pretreated silica gel thin-layer plate (4.23).

6.1.1.2 10 and 30 µl of the standard solution (4.15.2) is spotted on two more points of the starting line after which the plate is developed in one of the two eluents (4.17).

6.1.1.3 When the solvent front has advanced 150 mm, the plate is dried at 110 °C (for 15 minutes). Under a UV lamp (366 nm) the quinolin-8-ol spots fluoresce yellow.

6.1.1.4 Spray the plate with sodium carbonate solution (4.19). Dry and spray with 2,6-dichloro-4-(chloroimino)cyclohexa-2,5-dienone solution (4.18). The quinolin-8-ol becomes visible as a blue spot.

6.1.2 *Solid samples or creams*

6.1.2.1 Disperse 1 g of the sample in 5 ml of buffer solution (4.22). Then transfer with 10 ml of chloroform (4.3) into a separating funnel and shake. After separation of the chloroform layer the aqueous layer is extracted twice more with 10 ml of chloroform

(4.3). Evaporate the combined and filtered chloroform extracts almost to dryness in a 100 ml round-bottom flask (5.1) on the rotary evaporator (5.7). Dissolve the residue in 2 ml of chloroform (4.3) and spot 10 and 30 μ l of the solution obtained on a silica gel, thin-layer plate (4.23) in accordance with the method described in 6.1.1.1 onward.

6.1.2.2 Apply 10 and 30 μ l of the standard solution (4.15.2) to the plate and continue as described in 6.1.1.2 to 6.1.1.4.

6.2 Determination

6.2.1 *Liquid samples*

6.2.1.1 Weigh 5 g of the sample into a 100 ml round-bottom flask. Add 1 ml of a sulphuric acid solution (4.8) and evaporate the mixture almost to dryness under reduced pressure at 50 °C.

6.2.1.2 Dissolve this residue in 20 ml of warm water. Transfer into a 100 ml standard flask. Rinse three times with 20 ml of water. Make up to 100 ml with water and mix.

6.2.1.3 Pipette 5 ml of this solution into a 50 ml separating funnel (5.5). Add 10 ml of Fehling's solution (4.16). Extract the quinolin-8-ol copper complex [oxine copper (ISO)] obtained with three times 8 ml of chloroform (4.3).

6.2.1.4 Filter and collect the chloroform layers in a 25 ml standard flask (5.2). Make up to the mark with chloroform (4.3) and shake. Measure the optical density of the yellow solution against chloroform at 410 nm.

6.2.2 *Solid samples or creams*

6.2.2.1 Weigh 0,500 g of the sample into a 100 ml round-bottom flask (4.1). Add 30 ml of benzene (4.2) and 20 ml of hydrochloric acid (4.7). Boil the contents of the flask under reflux, with stirring, for 30 minutes.

6.2.2.2 Transfer the contents of the flask into a 100 ml separating funnel (5.5). Rinse with 5 ml of 1N HCl (4.7). Transfer the aqueous phase into a round-bottom flask (5.1) and wash the benzene phase with 5 ml of hydrochloric acid (4.7).

6.2.2.3 In the case of emulsions that impede further treatment, mix 0,500 g of the sample with 2 g of Celite 545 (4.14) to form a freely flowing powder. Transfer the mixture in small portions into a glass chromatography column (5.12).

After each addition, tamp down the column packing. As soon as the whole of the mixture has been transferred into the column, elute with hydrochloric acid (4.13) in such a way that 10 ml of eluate is obtained in approximately 10 minutes (if necessary, this elution can be performed under a slight nitrogen pressure). During the elution it must be ensured that there is always some hydrochloric acid above the column packing. The first 10 ml of eluate is further treated as described in 6.2.2.4.

6.2.2.4 Evaporate the collected aqueous phases (6.2.2.2) or the eluate (6.2.2.3) almost to dryness in the rotary evaporator under reduced pressure.

6.2.2.5 Dissolve the residue in 6 ml of the sodium hydroxide solution (4.9). Add 20 ml of Fehling's solution (4.16) and transfer the contents of the flask into a 50 ml separating funnel (5.5). Rinse the flask with 8 ml of chloroform (4.3). Shake and filter the chloroform phase into a 50 ml standard flask (5.2).

6.2.2.6 Repeat the extraction three times with 8 ml of chloroform (4.3). Filter the chloroform phases and collect in the 50 ml flask. Make up to the mark with chloroform (4.3) and shake. Measure the optical density of the yellow solution against chloroform (4.3) at 410 nm.

7. STANDARD CURVE

Into four 100 ml round-bottom flasks (5.1), each containing 3 ml of 30 % aqueous ethanol (4.20), pipette 5, 10, 15 and 20 ml portions of the standard solution (4.15.1) corresponding to 5, 10, 15 and 20 mg of quinolin-8-ol. Proceed as described in 6.2.1.

8. CALCULATION

8.1 Liquid samples

$$\text{Quinolin-8-ol content (in \% (m/m))} = \frac{a}{m} \times 100$$

where:

a = milligrams of quinolin-8-ol on the standard curve (7),

m = the mass (in milligrams) of the test portion (6.2.1.1).

8.2 Solid samples or creams

$$\text{Quinolin-8-ol content (in \% (m/m))} = \frac{2a}{m} \times 100$$

where:

a = milligrams of quinolin-8-ol on the standard curve (7),

m = the mass (in milligrams) of test portion (6.2.2.1).

9. REPEATABILITY (1)

For a content of about 0,3 % quinolin-8-ol, the difference between the results of two determinations carried out in parallel on the same sample should not exceed an absolute value of 0,02 %.

DETERMINATION OF AMMONIA

1. SCOPE AND FIELD OF APPLICATION

This method describes the determination of free ammonia in cosmetic products.

2. DEFINITION

The ammonia content of the sample determined in accordance with this method is expressed in percentage by mass of ammonia.

3. PRINCIPLE

Barium chloride solution is added to a test portion of the cosmetic product diluted in an aqueous methanol medium. Any precipitate which may form is filtered or centrifuged off. This procedure avoids the loss of ammonia, during steam distillation, from certain ammonium salts such as the carbonate and hydrogencarbonate and those of the fatty acids, with the exception of ammonium acetate.

(1) Norm ISO 5725.

The ammonia is steam distilled from the filtrate or supernatant and is determined by potentiometric or other titration.

4. REAGENTS

All reagents should be of analytical purity.

- 4.1 Methanol.
- 4.2 Barium chloride dihydrate, 25 % (m/v) solution.
- 4.3 Orthoboric acid 4 % (m/v) solution.
- 4.4 Sulphuric acid, 0,25 M standard solution.
- 4.5 Anti-foam liquid.
- 4.6 Sodium hydroxide, 0,5 M standard solution.
- 4.7 Indicator, if required: mix 5 ml of a 0,1 % (m/v) methyl red solution in ethanol with 2 ml of 0,1 % (m/v) methylene blue solution in water.

5. APPARATUS

- 5.1 Usual laboratory apparatus.
- 5.2 Centrifuge with stoppered 100 ml bottles.
- 5.3 Steam distillation apparatus.
- 5.4 Potentiometer.
- 5.5 Indicating glass electrode and dimercury dichloride (calomel) reference electrode.

6. PROCEDURE

- 6.1 Weigh into a 100 ml standard flask a mass (m) of the sample corresponding to 150 mg maximum of ammonia.
- 6.2 Add 10 ml of water, 10 ml of methanol (4.1) and 10 ml of barium chloride solution (4.2). Make up to 100 ml with methanol (4.1).
- 6.3 Mix and leave overnight in the refrigerator (5 °C).
- 6.4 Then filter, or centrifuge the still cold solution in closed tubes for 10 minutes, so as to obtain a clear filtrate or supernatant layer.
- 6.5 Pipette 40 ml of this clear solution into the steam distillation apparatus (5.3), followed by 0,5 ml of antifoam liquid (4.5), where appropriate.
- 6.6 Distil and collect 200 ml of distillate in a 250 ml beaker containing 10 ml of standard sulphuric acid (4.4) and 0,1 ml of indicator (4.7).
- 6.7 Back titrate the excess acid with standard sodium hydroxide solution (4.6).
- 6.8 *NB:* For potentiometric determination, collect 200 ml of distillate in a 250 ml beaker containing 25 ml of orthoboric acid solution (4.3) and titrate with standard sulphuric acid (4.4), recording the neutralization curve.

7. CALCULATIONS

7.1 Calculation in the case of back titration

Let:

V_1 = the volume (in millilitres) of the sodium hydroxide solution (4.6) used,

M_1 = its actual molarity (4.6),

M_2 = the actual molarity factor of the sulphuric acid solution (4.4),

m = the mass (in milligrams) of the test portion (6.1) taken,

then:

$$\text{ammonia \% (m/m)} = \frac{(20M_2 - V_1M_1) \times 17 \times 100}{0,4m} = \frac{(20M_2 - V_1M_1) \times 4250}{m}$$

7.2 Calculation in the case of direct potentiometric titration

Let:

V_2 = the volume (in millilitres) of the sulphuric acid solution (4.4) used,

M_2 = its actual molarity (4.4),

m = the mass (in milligrams) of the test portion (6.1) taken,

then:

$$\text{ammonia \% (m/m)} = \frac{V_2 \times M_2 \times 17 \times 100}{0,4m} = \frac{4250 V_2 M_2}{m}$$

8. REPEATABILITY (1)

For a content of about 6 % ammonia, the difference between the results of two determinations carried out in parallel on the same sample should not exceed an absolute value of 0,6 %.

IDENTIFICATION AND DETERMINATION OF NITROMETHANE

1. SCOPE AND FIELD OF APPLICATION

This method is suitable for the identification and determination of nitromethane at up to about 0,3 % in cosmetic products packed in aerosol dispensers.

2. DEFINITION

The nitromethane content of the sample determined according to this method is expressed in percentage by mass of nitromethane, in the total aerosol dispenser content.

(1) Norm ISO 5725.

3. PRINCIPLE

The nitromethane is identified by colour reaction. Nitromethane is determined gas chromatographically after addition of an internal standard.

4. IDENTIFICATION

4.1 Reagents

All reagents should be of analytical purity.

4.1.1 Sodium hydroxide, 0,5 M solution.

4.1.2 *Folin's reagent*

Dissolve 0,1 g of sodium 3,4-dihydro-3,4-dioxonaphthalene-1-sulphonate in water and dilute to 100 ml.

4.2 Procedure

To 1 ml of sample add 10 ml of 4.1.1 and 1 ml of 4.1.2. A violet coloration indicates the presence of nitromethane.

5. DETERMINATION

5.1 Reagents

All reagents must be of analytical quality.

5.1.1 Chloroform (internal standard 1).

5.1.2 2,4-dimethylheptane (internal standard 2).

5.1.3 Ethanol, 95 %

5.1.4 Nitromethane.

5.1.5 *Chloroform reference solution*

Into a tared 25 ml volumetric flask, introduce about 650 mg of chloroform (5.1.1). Accurately reweigh the flask and contents. Make up to 25 ml with 95 % ethanol (5.1.3). Weigh and calculate the percentage by mass of chloroform in this solution.

5.1.6 *2,4-dimethylheptane reference solution*

Make up in a similar manner to the chloroform reference solution but weigh 270 mg of 2,4-dimethylheptane (5.1.2) into the 25 ml volumetric flask.

5.2 Apparatus

5.2.1 Gas chromatograph with flame ionization detector.

5.2.2 Apparatus for sampling of aerosols (transfer bottle, microsyringe connectors, etc.) as described in Chapter II of the Annex to Commission Directive 80/1335/BEC of 22 December 1980 (1).

5.2.3 Usual laboratory apparatus.

(1) OJ No L 383, 31. 12. 1980, p. 27.

5.3 **Procedure**

5.3.1 *Preparation of the sample*

Into a 100 ml tared transfer bottle, purged or evacuated according to the procedure described in 5.4 of Chapter II of the abovementioned Directive, introduce about 5 ml of either of the internal standard solutions (5.1.5 or 5.1.6). Use a 10 or 20 ml glass syringe, without needle, adapted to the transfer piece following the technique described in paragraph 5 of Chapter II of the abovementioned Commission Directive. Reweigh to determine the quantity introduced. Using the same technique, transfer into this bottle about 50 g of the contents of the aerosol dispenser sample. Again reweigh to determine the quantity of sample transferred. Mix well.

Inject about 10 µl using the specified microsyringe (5.2.2). Make five injections.

5.3.2 *Preparation of the standard*

Into a 50 ml volumetric flask, accurately weigh about 500 mg of nitromethane (5.1.4) and either 500 mg of chloroform (5.1.1) or 210 mg of 2,4-dimethylheptane (5.1.2). Make up to volume with 95 % ethanol (5.1.3). Mix well. Place 5 ml of this solution into a 20 ml volumetric flask. Make up to volume with 95 % ethanol (5.1.3).

Inject about 10 µl using the specified microsyringe (5.2.2). Make five injections.

5.3.3 *Gas chromatographic conditions*

5.3.3.1 **Column**

This is in two parts, the first containing didecyl phthalate on Gas Chrom Q as packing, the second having Ucon 50 HB 280X on Gas Chrom Q as packing. The prepared combined column must yield a resolution 'R' equal to, or better than, 1,5, where:

$$R = 2 \frac{d' (r_2 - r_1)}{W_1 + W_2}$$

let:

r_1 and r_2 = retention times (in minutes),

W_1 and W_2 = peak widths at half height (in millimetres),

d' = the chart speed (in millimetres per minute).

As examples the following two parts yield the required resolution:

Column A:

Material: stainless steel.

Length: 1,5 m.

Diameter: 3 mm.

Packing: 20 % didecyl phthalate on Gas Chrom Q (100 to 120 mesh).

Column B:

Material: stainless steel.

Length: 1,5 m.

Diameter: 3 mm.

Packing: 20 % Ucon 50 HB 280X on Gas Chrom Q (100 to 120 mesh).

5.3.3.2 Detector

A suitable sensitivity setting for the electrometer of the flame ionization detector is 8×10^{-10} A.

5.3.3.3 Temperature conditions

The following have been found suitable:

Injection port: 150 °C,

Detector: 150 °C,

Column: between 50 and 80 °C depending upon individual columns and apparatus.

5.3.3.4 Suitable gas supplies

Carrier gas: nitrogen.

Pressure: 2,1 bar.

Flow: 40 ml/min.

Detector supplies: as specified by the makers of the detector.

6. CALCULATIONS

6.1 Response factor of nitromethane, calculated with reference to the internal standard used

If 'n' represents nitromethane:

let:

k_n = its response factor,

m'_n = its mass (in grams) in the mixture,

S'_n = its peak area.

If 'c' represents the internal standard, chloroform or 2,4-dimethylheptane:

let:

m'_c = its mass (in grams) in the mixture,

S'_c = its peak area

then:

$$k_n = \frac{m'_n}{m'_c} \times \frac{S'_c}{S'_n}$$

(k_n is a function of the apparatus).

6.2 Concentration of nitromethane in the sample

If 'n' represents nitromethane:

let:

k_n = its response factor,

S'_n = its peak area.

If 'c' represents the internal standard, chloroform or 2,4-dimethylheptane:

let:

m_c = its mass (in grams) in the mixture,

S_c = its peak area,

M

= the mass (in grams) of the aerosol transferred,

then the % (m/m) nitromethane in the sample is:

$$\frac{m_c}{M} \times \frac{k_n \times S_n}{S_c} \times 100$$

7. REPEATABILITY (1)

For a nitromethane content of about 0,3 % (m/m), the difference between the results of two determinations carried out in parallel on the same sample should not exceed an absolute value of 0,03 % (m/m).

IDENTIFICATION AND DETERMINATION OF MERCAPTOACETIC ACID IN HAIR-WAVING, HAIR-STRAIGHTENING AND DEPILATORY PRODUCTS

1. SCOPE AND FIELD OF APPLICATION

This method describes the identification and determination of mercaptoacetic acid in hair-waving, hair-straightening and depilatory products in which other reducing agents may be present.

2. DEFINITION

The mercaptoacetic acid content of the sample determined according to this method is expressed in percentage by mass of mercaptoacetic acid.

3. PRINCIPLE

Mercaptoacetic acid is identified by spot tests and by thin-layer chromatography and is determined by iodometry or gas chromatography.

4. IDENTIFICATION

4.1 Identification by spot tests

4.1.1 *Reagents*

All reagents should be of analytical purity.

4.1.1.1 Lead di(acetate) paper.

4.1.1.2 Hydrochloric acid solution (one volume of concentrated hydrochloric acid plus one volume of water).

(1) Norm ISO 5725.

4.1.2 *Procedure*

4.1.2.1 Identification of mercaptoacetic acid by means of a colour reaction with lead di(acetate)

Place a drop of the sample to be analyzed on lead di(acetate) paper (4.1.1.1). If an intense yellow colour appears, mercaptoacetic acid is probably present.

Sensitivity: 0,5 %.

4.1.2.2 Characterization of inorganic sulphides by the formulation of hydrogen sulphide on acidification

Introduce, into a test tube, a few milligrams of the sample to be studied. Add 2 ml of distilled water and 1 ml of hydrochloric acid (4.1.1.2). Hydrogen sulphide, recognizable by its smell, is evolved and a black lead sulphide precipitate forms on the lead di(acetate) paper (4.1.1.1).

Sensitivity: 50 ppm.

4.1.2.3 Characterization of sulphites by the formation of sulphur dioxide upon acidification

Proceed as described in 4.1.2.2. Bring to the boil. The sulphur dioxide is recognizable by its smell and by its reducing properties in respect, for example, of permanganate ions.

4.2 ***Identification by thin-layer chromatography***

4.2.1 *Reagents*

All reagents, except where otherwise stated, should be of analytical purity.

4.2.1.1 Mercaptoacetic acid (thioglycolic acid), 98 % minimum purity assayed by iodometry.

4.2.1.2 2,2-dithiodi(acetic acid), 99 % minimum purity assayed by iodometry.

4.2.1.3 2-mercaptopropionic acid (thiolactic acid), 95 % minimum purity assayed by iodometry.

4.2.1.4 3-mercaptopropionic acid, 98 % minimum purity assayed by iodometry.

4.2.1.5 3-mercaptopropane-1,2-diol (1-thioglycerol), 98 % minimum purity assayed by iodometry.

4.2.1.6 Thin-layer plates, silica gel, ready prepared, 0,25 mm thickness.

4.2.1.7 Thin-layer plates, aluminium oxide, Merck F 254 E or equivalent.

4.2.1.8 Hydrochloric acid, concentrated, $d_4^{20} = 1,19$ g/ml.

4.2.1.9 Ethyl acetate.

4.2.1.10 Chloroform.

4.2.1.11 Diisopropyl ether.

4.2.1.12 Carbon tetrachloride.

4.2.1.13 Acetic acid, glacial.

4.2.1.14 Potassium iodide, 1 % (m/v) solution in water.

4.2.1.15 Platinum tetrachloride, 0,1 % (m/v) solution in water.

4.2.1.16 Eluting solvents

4.2.1.16.1 Ethyl acetate (4.2.1.9), chloroform (4.2.1.10), diisopropyl ether (4.2.1.11), acetic acid (4.2.1.13) (20: 20: 10: 10, by volume).

4.2.1.16.2 Chloroform (4.2.1.10), acetic acid (4.2.1.13) (90 : 20, by volume).

4.2.1.17 Detection reagents

4.2.1.17.1 Mix, immediately before use, equal volumes of solution (4.2.1.14) and solution (4.2.1.15).

4.2.1.17.2 Bromine solution, 5 % (m/v):

Dissolve 5 g of bromine in 100 ml of carbon tetrachloride (4.2.1.12).

4.2.1.17.3 Fluorescein solution, 0,1 % (m/v):

Dissolve 100 mg of fluorescein in 100 ml of ethanol.

4.2.1.17.4 Hexaammonium heptamolybdate, 10 % (m/v) solution in water.

4.2.1.18 Reference solutions

4.2.1.18.1 Mercaptoacetic acid (4.2.1.1), 0,4 % (m/v) solution in water.

4.2.1.18.2 2,2'-dithiodi(acetic) acid (4.2.1.2), 0,4 % (m/v) solution in water.

4.2.1.18.3 2-mercaptopropionic acid (4.2.1.3), 0,4 % (m/v) solution in water.

4.2.1.18.4 3-mercaptopropionic acid (4.2.1.4), 0,4 % (m/v) solution in water.

4.2.1.18.5 3-mercaptopropane-1,2-diol (4.2.1.5), 0,4 % (m/v) solution in water.

4.2.2 *Apparatus*

Usual apparatus for thin-layer chromatography.

4.2.3 *Procedure*

4.2.3.1 Treatment of samples

Acidify to pH 1 with a few drops of hydrochloric acid (4.2.1.8) and filter if necessary.

In certain cases it may be advisable to dilute the sample. If so acidify it with hydrochloric acid before dilution.

4.2.3.2 Elution

Place on the plate 1 µl of sample solution (4.2.3.1) and one litre of each of the five reference solutions (4.2.1.18). Dry carefully in a gentle current of nitrogen and elute the plate with solvents (4.2.1.16.1 or 4.2.1.16.2). Dry the plate as quickly as possible to minimize oxidation of the thiols.

4.2.3.3 Detection

Spray the plate with one of the three reagents (4.2.1.17.1, 4.2.1.17.3 or 4.2.1.17.4). If the plate is sprayed with reagent (4.2.1.17.3), further treat it with bromine vapour (e.g. in a tank containing a small beaker of the reagent (4.2.1.17.2)) until the spots are visible. Detection with the spray reagent (4.2.1.17.4) will be satisfactory only if the drying time for the thin layer has not exceeded 30 minutes.

4.2.3.4 Interpretation

Compare the R_f values and the colour of the reference solutions with those of the standards. The mean R_f values given below as a rough guide have only a comparative value. They depend upon:

— the state of activation of the thin layer at the time of chromatographing,

— the temperature of the chromatography tank.

Examples of R_f values on a silica gel layer

	Eluting solutions	
	4.2.1.16.1	4.2.1.16.2
Mercaptoacetic acid	0,25	0,80
2-mercaptopropionic acid	0,40	0,95
2,2'-dithiodi(acetic) acid	0,00	0,35
3-mercaptopropionic acid	0,45	0,95
3-mercaptopropane-1,2-diol	0,45	0,35

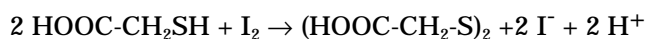
5. DETERMINATION (*)

The determination should always start with the iodometric procedure.

5.1 Iodometry

5.1.1 Principle

The determination is performed by oxidation of the '-SH' group with iodine in an acid medium according to the equation:



5.1.2 Reagents

Iodine, 0,05 M standard solution.

5.1.3 Apparatus

Usual laboratory equipment.

5.1.4 Procedure

Accurately weigh out a quantity of between 0,5 and 1 g of the sample into a 150 ml stoppered conical flask containing 50 ml of distilled water. Add 5 ml of hydrochloric acid (4.1.1.2) (pH of solution about 0) and titrate with iodine solution (5.1.2) until a yellow colour appears. Use an indicator (e.g. starch solution or carbon tetrachloride) if desired.

5.1.5 Calculation

The mercaptoacetic acid content is calculated according to the formula:

$$\% (\text{m/m}) = \frac{92 \times n \times 100}{1\,000 \times 10 \times m} = \frac{0,92 \, n}{m}$$

where:

m = the mass (in grams) of the test portion,

n = the volume of iodine solution (5.1.2) used.

5.1.6 Remarks

If the results, calculated as mercaptoacetic acid, is 0,1 % or more below the authorized maximum concentration, there is no point in carrying out further

(*) The determination of mercaptoacetic acid must be carried out on unused product from freshly opened containers in order to prevent oxidation.

determinations. If the result is equal to or above the permitted maximum concentration, and the identification has revealed the presence of several reducing agents, it is necessary to carry out a gas chromatographic determination.

5.2 Gas chromatography

5.2.1 Principle

Mercaptoacetic acid is separated from the excipient by precipitation with cadmium di(acetate) solution. After methylation with diazomethane, prepared either *in situ* or in advance in a diethyl ether solution, the methyl derivative of the mercaptoacetic acid is measured by gas/liquid chromatography, methyl octanoate being used as the internal standard.

5.2.2 Reagents

All the reagents must be of analytical quality.

5.2.2.1 Mercaptoacetic acid, 98 %.

5.2.2.2 Hydrochloric acid, $d_4^{20} = 1,19$ g/ml.

5.2.2.3 Methanol.

5.2.2.4 Cadmium di(acetate) dihydrate, 10 % (m/v) solution in water.

5.2.2.5 Methyl octanoate, 2 % (m/v) solution in methanol.

5.2.2.6 Acetate buffer solution (pH 5):

Sodium acetate trihydrate, 77 g.

Acetic acid (glacial), 27,5g.

Demineralized water to give a final volume of one litre.

5.2.2.7 Hydrochloric acid, 3 M solution in methanol (5.2.2.3), freshly prepared.

5.2.2.8 1-methyl-3-nitro-1-nitrosoguanidine.

5.2.2.9 Sodium hydroxide, 5 M solution.

5.2.2.10 Iodine, 0,05M standard solution.

5.2.2.11 Diethyl ether.

5.2.2.12 Diazomethane solution prepared from N-methyl-N-nitrosotoluen-4-sulfonamide (Fieser, Reagents for Organic Synthesis (Wiley), 1967).

The solution obtained contains about 1,5 g of diazomethane in 100 ml of diethyl ether. As diazomethane is a toxic and very unstable gas, all experiments must be carried out under a powerful hood and the use of ground-glass apparatus must be avoided (there are special kits for this purpose).

5.2.3 Apparatus

5.2.3.1 Usual laboratory equipment.

5.2.3.2 Apparatus for the preparation of diazomethane for *in situ* methylation (see Fales, H. M., Jaouni, T. M. and Babashak, J. F., *Analyt. Chem.* 1973, 45, 2302).

5.2.3.3 Apparatus for the advance preparation of diazomethane (Fieser).

5.2.4 **Preparation of the sample**

Weigh accurately into a 50 ml centrifuge tube enough of the sample to give a presumed quantity of 50 to 70 mg of mercaptoacetic acid. Acidify with a few drops of hydrochloric acid (5.2.2.2) to obtain a pH of about 3.

Add 5 ml of demineralized water and 10 ml of acetate buffer solution (3.2.2.6).

Check with pH paper that the pH value is about 5. Then add 5 ml of cadmium di(acetate) solution (5.2.2.4).

Wait 10 minutes and then centrifuge for at least 15 minutes at 4 000 g. Remove the supernatant liquid which may contain an insoluble fat (in the case of cream products). This fat cannot be confused with the thiols which collects in a compact mass at the bottom of the tube. Check that no precipitation occurs when a few drops of cadmium di(acetate) solution (5.2.2.4) are added to the supernatant.

Where earlier identification revealed no reducing agents other than the thiols, check by iodometry that the thiol present in the supernatant liquid does not exceed 6 to 8 % of the initial quantity.

Introduce 10 ml of methanol (5.2.2.3) into the centrifuge tube containing the precipitate and finely disperse the precipitate with a stirring rod. Centrifuge again for at least 15 minutes at 4 000 g. Pour off the supernatant and check for the absence of thiols.

Wash the precipitate a second time by the same procedure.

Still using the same centrifuge tube, add:

- 2 ml of methyl octanoate solution (5.2.2.5),
- 5 ml of hydrochloric acid in methanol (5.2.2.7).

Completely dissolve the thiols (a little insoluble matter may persist from the excipient). This is solution 'S'.

With an aliquot of this solution, check iodometrically that the thiols content is at least 90 % of that obtained in 5.1.

5.2.5 **Methylation**

The methylation is carried out either by *in situ* preparation (5.2.5.1) or with previously prepared diazomethane solution (5.2.5.2).

5.2.5.1 Methylation *in situ*

Into the methylation apparatus (5.2.3.2) containing 1 ml of ether (5.2.2.11) introduce 50 µl of solution 'S' and methylate by the method (5.2.3.2) with about 300 mg of 1-methyl-3-nitro-1-nitrosoguanidine (5.2.2.8). After 15 minutes (the ether solution should be yellow to indicate excess diazomethane) transfer the sample solution to a 2 ml bottle having an airtight stopper. Place in the refrigerator overnight. Methylate two samples simultaneously.

5.2.5.2 Methylation with the previously prepared diazomethane solution

Introduce, into a 5 ml stoppered flask, 1 ml of diazomethane solution (5.2.2.12) then 50 µl of solution 'S'. Leave in the refrigerator overnight.

5.2.6 **Preparation of the standard**

Prepare a standard solution of mercaptoacetic acid (5.2.2.1) of known strength containing about 60 mg of pure mercaptoacetic acid (5.2.2.1) in 2 ml.

This is solution 'E'.

Precipitate, assay and methylate as described in 5.2.4 and 5.2.5.

5.2.7 ***Gas chromatographic conditions***

5.2.7.1 Column

Type: stainless steel.

Length: 2 m.

Diameter: 3 mm.

5.2.7.2 Packing

20 % didecyl phthalate/chromosorb, WAW 80 to 100 mesh.

5.2.7.3 Detector

Flame ionization. A suitable sensitivity setting for the electrometer of the name ionization detector is 8×10^{-10} A.

5.2.7.4 Gas supplies

Carrier gas: nitrogen.

pressure: 2,2 bar,

flow: 35 ml/min.

Auxiliary gas: hydrogen.

pressure: 1,8 bar,

flow: 15 ml/min.

Detector supplies: as specified by the makers of the apparatus.

5.2.7.5 Temperature conditions

Injector: 200 °C

Detector: 200 °C

Column: 90 °C.

3.2.7.6 Recorder chart speed

5 mm/min.

5.2.7.7 Quantity injected

3 µl. Carry out five injections.

5.2.7.8 The conditions of chromatography are given as a guide. They permit the achievement of a resolution 'R' equal to, or better than, 1.5, where:

$$R = 2 \frac{d' (r_2 - r_1)}{W_1 + W_2}$$

let:

r_1 and r_2 = retention times (in minutes),

W_1 and W_2 = peak widths at half height (in millimetres),

d' = the chart speed (in millimetres per minute).

It is recommended that chromatography be terminated by regulating the temperature from 90 to 150 °C at a rate of 10 °C per minute so as to eliminate substances liable to interfere with subsequent measurements.

5.2.8 *Calculations*

5.2.8.1 Coefficient of proportionality for mercaptoacetic acid

This is calculated with respect to methyl octanoate on the basis of a standard mixture.

If 't' represents mercaptoacetic acid:

let:

k_t = its response factor,

m_t' = its mass (in milligrams) in the mixture,

S_t' = its peak area.

If 'c' represents methyl octanoate:

let:

m_c' = its mass (in milligrams) in the mixture,

S_c' = its peak area,

then:

$$k_1 = \frac{m_t'}{m_c'} \times \frac{S_c'}{S_t'}$$

This coefficient varies according to the apparatus used.

5.2.8.2 Concentration of mercaptoacetic acid present in the sample

If 't' represents mercaptoacetic acid:

let:

k_t = its response factor.

S_t = its peak area.

If 'c' represents methyl octanoate:

let:

m_c = its mass (in milligrams) in the mixture,

S_c = its peak area,

M = the mass (in milligrams) of the initial test portion,

then the % (m/m) mercaptoacetic acid present in the sample is:

$$\frac{m_c}{M} \times \frac{k_t \times S_t}{S_c} \times 100$$

6. REPEATABILITY (1)

For a mercaptoacetic acid content of 8 % (m/m), the difference between the results of two determinations carried out in parallel on the same sample should not exceed an absolute value of 0,8 % (m/m).

IDENTIFICATION AND DETERMINATION OF HEXACHLOROPHENE

A. IDENTIFICATION

1. SCOPE AND FIELD OF APPLICATION

This method is suitable for all cosmetic products.

2. PRINCIPLE

Hexachlorophene in the sample is extracted with ethyl acetate and identified by thin-layer chromatography.

3. REAGENTS

All reagents should be of analytical purity.

3.1 Sulphuric acid, 4 M solution.

3.2 Celite AW.

3.3 Ethyl acetate.

3.4 Eluting solvent: Benzene containing 1 % (v/v) of glacial acetic acid.

3.5 Visualizing agent I:

Rhodamine B solution: dissolve 100 mg of Rhodamine B in a mixture of 150 ml of diethyl ether, 70 ml of absolute ethanol and 16 ml of water.

3.6 Visualizing agent II:

2,6-dibromo-4-(chloroimino)cyclohexa-2,5-dienone solution: dissolve 400 mg of 2,6-dibromo-4-(chloroimino)cyclohexa-2,5-dienone in 100 ml of methanol (prepare fresh daily).

Sodium carbonate solution: dissolve 10 g of sodium carbonate in 100 ml of demineralized water.

3.7 Reference solution:

Hexachlorophene, 0,05 % (m/v) solution in ethyl acetate.

4. APPARATUS

4.1 Kiesel gel 254 TLC plates, 200 x 200 mm (or equivalent).

4.2 Usual TLC equipment.

(1) Norm ISO 5725.

4.3 Bath thermostatted at 26 °C to hold the chromatography tank.

5. PREPARATION OF THE TEST SAMPLE

5.1 Thoroughly mix 1 g of homogenized sample with 1 g of Celite AW (3.2) and 1 ml of sulphuric acid (3.1).

5.2 Dry at 100 °C for two hours.

5.3 Cool and finely powder the dried residue.

5.4 Extract twice with 10 ml of ethyl acetate (3.3) each time, centrifuge after each extraction and combine the ethyl acetate layers.

5.5 Evaporate at 60 °C.

5.6 Dissolve the residue in 2 ml of ethyl acetate (3.3).

6. PROCEDURE

6.1 Place 2 µl of the test sample solution (5.6) and 2 µl of the reference solution (3.7) on a TLC plate (4.1).

6.2 Saturate the tank (4.3) with the eluting solvent (3.4).

6.3 Place the TLC plate in the tank and elute up to 150 mm.

6.4 Remove the TLC plate and dry in a ventilated oven at a temperature of about 105 °C.

6.5 Visualisation

Hexachlorophene spots on the thin-layer plate are visualized as indicated under 6.5.1 or 6.5.2.

6.5.1 Spray the visualizing agent I (3.5) evenly on the plate. After 30 minutes examine the plate under UV light at 254 nm.

6.5.2 Spray the 2,6-dibromo-4-(chloroimino)cyclohexa-2,5-dienone solution of visualizing agent II (3.6) evenly on the plate. Subsequently spray the plate with sodium carbonate solution (3.6). Examine the plate in daylight after 10 minutes drying at room temperature.

7. INTERPRETATION

7.1 Visualizing agent I (3.5):

Hexachlorophene is revealed as a bluish spot on a yellow-orange fluorescent background and has an R_f of approximately 0,5.

7.2 Visualising agent II (3.6):

Hexachlorophene is revealed as a sky-blue to turquoise coloured spot on a white background and has an R_f of approximately 0,5.

B. DETERMINATION

1. SCOPE AND FIELD OF APPLICATION

This method applies to all cosmetic products.

2. DEFINITION

The hexachlorophene content of the sample determined according to this method is expressed in percentage by mass of hexachlorophene.

3. PRINCIPLE

Hexachlorophene is determined, after conversion to the methyl derivative, gas chromatographically with an electron capture detector.

4. REAGENTS

All reagents should be of analytical purity.

- 4.1 Ethyl acetate.
- 4.2 N-methyl-N-nitroso-p-toluenesulphonamide (diazald).
- 4.3 Diethyl ether.
- 4.4 Methanol.
- 4.5 2-(2-ethoxyethoxy)ethanol (carbitol).
- 4.6 Formic acid.
- 4.7 Potassium hydroxide, 50 % (m/m) aqueous solution (prepare fresh daily).
- 4.8 Hexane for spectroscopy.
- 4.9 Bromochlorophene (standard No 1).
- 4.10 4,4',6,6'-tetrachloro-2,2'-thiodiphenol (standard No 2).
- 4.11 2,4,4'-trichloro-2-hydroxy-diphenyl ether (standard No 3).
- 4.12 Acetone.
- 4.13 4 M sulphuric acid.
- 4.14 Celite AW.
- 4.15 Formic acid/ethyl acetate, 10 % (v/v) solution.
- 4.16 Hexachlorophene.

5. APPARATUS

- 5.1 Usual laboratory glassware.
- 5.2 Mini-apparatus for the preparation of diazomethane (Analyt. Chem., 1973, 45, 2302-2).
- 5.3 Gas chromatograph equipped with a ⁶³Ni source electron capture detector.

6. PROCEDURE

6.1 Preparation of the standard solution

The standard is chosen so that it does not interfere with any substance contained in the excipient of the product being analyzed. Usually standard No 1 is most suitable (4.9).

6.1.1 Accurately weigh about 50 mg of standard No 1, 2 or 3 (4.9, 4.10 or 4.11) and 50 mg of hexachlorophene (4.16) into a 100 ml volumetric flask. Make up to volume with ethyl acetate (4.1) (solution A). Dilute 10 ml of solution A to 100 ml with ethyl acetate (4.1) (solution B).

6.1.2 Accurately weigh about 50 mg of standard No 1, 2 or 3 (4.9, 4.10 or 4.11) into a 100 ml volumetric flask. Make up to volume with ethyl acetate (4.1) (solution C).

6.2 **Preparation of the sample** ⁽¹⁾

Accurately weigh 1 g of homogenized sample and mix thoroughly with 1 ml of sulphuric acid (4.13), 15 ml of acetone (4.12) and 8 g of Celite AW (4.14). Air dry the mixture for 30 minutes on a steam bath, then dry for one-and-a-half hours in a ventilated oven. Cool, finely powder the residue and transfer to a glass column.

Elute with ethyl acetate (4.1) and collect 100 ml. Add 2 ml of internal standard solution (solution C) (6.1.2).

6.3 **Methylation of the sample**

Cool all reagents and apparatus to between 0 and 4 °C for two hours. Into the external compartment of the diazomethane apparatus place 1,2 ml of the solution obtained in 6.2 and 0,1 ml of methanol (4.4). Place about 200 mg of diazald (4.2) in the central reservoir, add 1 ml of carbitol (4.5) and 1 ml of diethyl ether (4.3) and dissolve. Assemble the apparatus, half immerse the apparatus in a bath at 0 °C and introduce by syringe about 1 ml of cooled potassium hydroxide solution (4.7) into the central reservoir. Ensure that the yellow colour formed from the formation of diazomethane persists. If the yellow colour does not persist, repeat the methylation with a further 200 mg of diazald (4.2) ⁽²⁾.

The apparatus is removed from the bath after 15 minutes then left closed at ambient temperature for 12 hours. Open the apparatus, react the excess diazomethane by adding a few drops of a 10 % (v/v) solution of formic acid in ethyl acetate (4.15) and transfer the organic solution to a 25 ml volumetric flask. Make up to volume with hexane (4.8).

Inject 1,5 ml of this solution into the chromatograph.

6.4 **Methylation of the standard**

Cool all reagents and apparatus to between 0 and 4 °C for two hours. Into the external compartment of the diazomethane apparatus introduce:

0,2 ml of solution B (6.1.1),

1 ml of ethyl acetate (4.1),

0,1 ml of methanol (4.4).

Continue the methylation as described in 6.3. Inject 1,5 µl of the resultant solution into the chromatograph.

⁽¹⁾ Because of the wide range of product types in which hexachlorophene could be present, it is important to first check recovery of hexachlorophene from the sample by this procedure before recording results. If recoveries are low, modifications, such as change of solvent (benzene instead of ethylacetate) etc., could be introduced with agreement of the parties concerned.

⁽²⁾ The persistence of this yellow coloration indicates an excess of diazomethane, which is necessary to ensure a complete methylation of the sample.

7. GAS CHROMATOGRAPHY

The column must yield a resolution 'R' equal to, or better than, 1,5, where:

$$R = 2 \frac{d' (r_2 - r_1)}{W_1 + W_2}$$

let:

r_1 and r_2 = retention times (in minutes),

W_1 and W_2 = peak widths at half height (in millimetres),

d' = the chart speed (in millimetres per minute).

The following gas chromatographic conditions have been found suitable:

Column: stainless steel.

Length: 1,7 m.

Diameter: 3 mm.

Support:

chromosorb: WAW

sieve analysis: 80 to 100 mesh.

Stationary phase: 10 % OV 17.

Temperatures:

column: 280 °C,

injector: 280 °C,

detector: 280 °C.

Carrier gas: oxygen-free nitrogen.

Pressure: 2,3 bar.

Flow: 30 ml/min.

8. CALCULATION

8.1 Proportionality coefficient of hexachlorophene

This is calculated with respect to the chosen standard in relation to the standard mixture.

Let:

h = the hexachlorophene,

k_h = its proportionality coefficient,

m'_h = its mass (in grams) in the mixture,

A'_h = its peak area,

s = the chosen standard,

m_s = its mass (in grams) in the mixture,

A_s = its peak area,

$$\text{then: } k_h = \frac{m_h}{m_s} \times \frac{A_s}{A_h}$$

8.2 The amount of hexachlorophene in the sample

Let:

- h = the hexachlorophene,
- k_h = its proportionality coefficient,
- A_h = its peak area,
- s = the chosen standard,
- m_s = its mass (in grams) in the mixture.
- A_s = its peak area,
- M = the mass (in grams) of the sample taken,

then % (m/m) of hexachlorophene in the sample is: $\frac{m_s \times k_h \times A_h \times 100}{M \times A_s}$

9. REPEATABILITY (1)

For a content of hexachlorophene of 0,1 % (m/m), the difference between the results of two determinations carried out in parallel on the sample should not exceed an absolute value of 0,005 % (m/m).

QUANTITATIVE DETERMINATION OF TOSYLCHLORAMIDE SODIUM (INN) (CHLORAMINE-T)

1. SCOPE AND FIELD OF APPLICATION

This method relates to the quantitative thin-layer chromatographic determination of tosylchloramide sodium (chloramine-T) in cosmetic products.

2. DEFINITION

The chloramine-T content of the sample, as determined by this method, is expressed as a percentage by mass (m/m).

3. PRINCIPLE

Chloramine-T is completely hydrolyzed to 4-toluenesulphonamide by boiling with hydrochloric acid.

The amount of 4-toluenesulphonamide formed is determined photo-densitometrically by thin-layer chromatography.

(1) Norm ISO 5725.

4. REAGENTS

All reagents should be of analytical purity.

- 4.1 Tosylchloramide sodium (chloramine-T).
- 4.2 Standard solution of 4-toluenesulphonamide: 50 mg of 4-toluenesulphonamide in 100 ml of ethanol (4.5).
- 4.3 Hydrochloric acid, 37 % (m/m), $d_4^{20} = 1,18$ g/ml.
- 4.4 Diethyl ether.
- 4.5 Ethanol, 96 % (v/v).
- 4.6 **Development solvent**
 - 4.6.1 1-butanol / ethanol (4.5) / water (40:4:9; v/v/v), or
 - 4.6.2 Chloroform / acetone (6:4; v/v).
- 4.7 Ready prepared thin-layer chromatography plate, silica gel 60, without fluorescent indicator.
- 4.8 Potassium permanganate.
- 4.9 Hydrochloric acid, 15 % (m/m).
- 4.10 Spray reagent: 2-toluidine, 1 % (m/v) solution in ethanol (4.5).

5. APPARATUS

- 5.1 Normal laboratory apparatus.
- 5.2 Usual thin-layer chromatography equipment.
- 5.3 Photo-densitometer.

6. PROCEDURE

6.1 Hydrolysis

Weigh accurately into a 50 ml round-bottom flask approximately 1 g of the sample (m). Add 5 ml of water and 5 ml of hydrochloric acid (4.3) and boil for one hour, using a reflux condenser. Immediately transfer the hot suspension with water into a 50 ml graduated flask. Allow to cool and make up to the mark with water. Centrifuge at at least 3 000 rpm for five minutes and pass the supernatant liquid through a filter.

6.2 Extraction

- 6.2.1 Take 30 ml of the filtrate and extract three times with 15 ml of diethyl ether (4.4). If necessary dry the ethereal phases and collect them in a 50 ml graduated flask. Make up with diethyl ether (4.4).
- 6.2.2 Take 25 ml of the dried ethereal extract and evaporate to dryness in a nitrogen stream. Redissolve the residue with 1 ml of ethanol (4.5).

6.3 Thin-layer chromatography

- 6.3.1 Spot 20 μ l of the ethanolic residue (6.2) on to a thin-layer chromatography plate (4.7).
At the same time and in the same manner, apply 8, 12, 16 and 20 μ l of the standard solution of 4-toluenesulphonamide (4.2).

6.3.2 Then allow to develop approximately 150 mm in the development solvent (4.6.1 or 4.6.2).

6.3.3 After completely evaporating the development solvent, place the plate for two to three minutes in an atmosphere of chlorine vapour, which is produced by pouring about 100 ml of hydrochloric acid (4.9) over about 2 g of potassium permanganate (4.8) in a closed vessel. Remove the excess chlorine by heating the plate to 100 °C for five minutes. Then spray the plate with the reagent (4.10).

6.4 **Measurement**

After approximately one hour, measure the violet spots by means of a photodensitometer at 525 nm.

6.5 **Plotting the calibration curves**

Plot the maximum peak height values ascertained for the four 4-toluenesulphonamide spots against the corresponding quantities of 4-toluenesulphonamide (i.e. 4, 6, 8, 10 µg of 4-toluenesulphonamide per spot).

7. **NOTE**

The method may be controlled by using a solution of 0,1 or 0,2 % (m/v) of chloramine-T (4.1) treated in the same way as the sample (6).

8. **CALCULATION**

The chloramine-T content of the sample, expressed as a percentage by mass, is calculated as follows:

$$\% \text{ (m/m) Tosylchloramide sodium} = \frac{1,33 \times a}{60 \times m}$$

where:

1,33 = the 4-toluenesulphonamide-chloramine-T conversion factor,

a = the quantity (in µg) of 4-toluenesulphonamide in the sample as read from the calibration curves,

m = the mass (in grams) of the sample taken.

9. **REPEATABILITY (1)**

For a chloramine-T content of about 0,2 % (m/m), the difference between the results of two determinations carried out in parallel on the same sample should not exceed an absolute value of 0,03 % (m/m).

DETERMINATION OF TOTAL FLUORINE IN DENTAL CREAMS

1. SCOPE AND FIELD OF APPLICATION

This method is designed for the determination of total fluorine in dental creams. It is suitable for levels not in excess of 0,25 %.

(1) Norm ISO 5725.

2. DEFINITION

The fluorine content of the sample determined according to this method is expressed as a percentage by mass.

3. PRINCIPLE

The determination is carried out by gas chromatography. The fluorine from the fluorine containing compounds is converted to triethylfluorosilane (TEFS) by direct reaction with chlorotriethylsilane (TECS) in acid solution and simultaneously extracted with xylene containing cyclohexane as internal standard.

4. REAGENTS

All reagents should be of analytical purity.

4.1 Sodium fluoride, dried at 120 °C to constant mass.

4.2 Water, double distilled, or equivalent quantity.

4.3 Hydrochloric acid, $d_4^{20} = 1,19$ g/ml.

4.4 Cyclohexane (CH).

4.5 Xylene with no peaks in the chromatogram prior to the solvent peak when chromatographed under the same conditions as the sample (6.1). If necessary purify by distillation (5.8).

4.6 Chlorotriethylsilane (TECS Merck or an equivalent).

4.7 Fluorine standard solutions

4.7.1 Stock solution, 0,250 mg F⁻/ml. Weigh accurately 138,1 mg of sodium fluoride (4.1) and dissolve in water (4.2). Quantitatively transfer the solution into a 250 ml volumetric flask (5.5). Dilute to the mark with water (4.2) and mix.

4.7.2 Diluted stock solution, 0,050 mg F⁻/ml. Transfer by pipette 20 ml of the stock solution (4.7.1) into a 100 ml volumetric flask (5.5). Dilute to the mark with water and mix.

4.8 Internal standard solution

Mix 1 ml of cyclohexane (4.4) and 5 ml of xylene (4.5).

4.9 Chlorotriethylsilane/internal standard solution

Transfer, by pipette (5.7), 0,6 ml of TECS (4.6) and 0,12 ml of the internal standard solution (4.8) into a 10 ml volumetric flask. Dilute with xylene (4.5) to the mark and mix. Prepare fresh daily.

4.10 Perchloric acid, 70 % (m/v).

4.11 Perchloric acid, 20 % (m/v) in water (4.2).

5. APPARATUS

5.1 Standard laboratory equipment.

5.2 Gas chromatograph fitted with a flame ionization detector.

5.3 Vortex swirl mixer or equivalent.

5.4 Buhler, shaker, type SMB₁ or equivalent.

- 5.5 Volumetric flasks, 100 and 250 ml, made of polypropylene.
- 5.6 Centrifuge tubes (glass); 20 ml with teflon lined screw-caps, Sovirel type 611-56 or equivalent. Clean tubes and screw-caps by leaching several hours in perchloric acid (4.11), followed by five subsequent rinsings with water (4.2), and finally dry at 100 °C.
- 5.7 Pipettes, adjustable to deliver volumes of 50 to 200 µl, with disposable plastics tips.
- 5.8 Distillation apparatus, fitted with a three-ball Schneider column or an equivalent Vigreux column.

6. PROCEDURE

6.1 Sample analysis

- 6.1.1 Select a dental-cream tube not previously opened, cut open the tube and remove the whole contents. Transfer to a plastics container, mix thoroughly and store under conditions avoiding deterioration.
- 6.1.2 Weigh accurately 150 mg (m) of sample into a centrifuge tube (5.6), add 5 ml of water (4.2) and homogenize (5.3).
- 6.1.3 Add 1 ml of xylene (4.5).
- 6.1.4 Add dropwise 5 ml of hydrochloric acid (4.3) and homogenize (5.3).
- 6.1.5 Add, by pipette, 0,5 ml of chlorotriethylsilane/internal standard solution (4.9) into the centrifuge tube (5.6).
- 6.1.6 Close the tube with the screw-cap (5.6) and mix for 45 minutes thoroughly on a shaker (5.4) set at 150 strokes per minute.
- 6.1.7 Centrifuge 10 minutes at such a speed as to produce a clear separation of the phases, uncap the tube, withdraw the organic layer and inject 3 µl of the organic phase on to the column of the gas chromatograph (5.2).

Remark:

It takes about 20 minutes before all components are eluted.

- 6.1.8 Repeat the injection, calculate the average peak area ratio (ATEFS/ACH) and read the corresponding amount of fluorine (in milligrams (m_1)) from the calibration graph (6.3).
- 6.1.9 Calculate the total fluorine content of the sample (in per cent by mass of fluorine) as indicated in paragraph 7.

6.2 Chromatographic conditions

- 6.2.1 Column: stainless steel.

Length: 1,8 m.

Diameter: 3 mm.

Support: Gaschrom Q 80 to 100 mesh.

Stationary phase: silicon oil DC 200 or equivalent, 20 %. Condition the column overnight at 100 °C (carrier gas flow at 25 ml nitrogen per minute) and repeat every night. After each fourth or fifth injection recondition the column by heating for 30 minutes at 100 °C.

Temperatures:

column: 70 °C,

injector: 150 °C,

detector: 250 °C.

Gas flow carrier: 35 ml of nitrogen per minute.

6.3 Calibrate graph

6.3.1 Place, by pipette, into a series of six centrifuge tubes (5.6), 0, 1, 2, 3, 4 and 5 ml of the diluted fluoride standard solution (4.7.2). Make up the volume in each tube to 5 ml with water (4.2).

6.3.2 Proceed as described under 6.1.3 to 6.1.6 inclusive.

6.3.3 Inject 3 µl of the organic phase on to the column of the gas chromatograph (5.2).

6.3.4 Repeat the injection and calculate the average peak ratio (ATEFS/ACH).

6.3.5 Plot a calibration graph correlating the mass of fluorine (in milligrams) in the standard solutions (6.3.1) and the peak area ratio (ATEFS/ACH) measured under 6.3.4. Connect the points of the graph with the best fitting straight line calculated by regression analysis.

7. CALCULATION

The concentration of the total fluorine content of the sample (in per cent by mass of fluorine) (% (m/m) F) is given by:

$$\% F = \frac{m_1}{m} \times 100 \%$$

where:

m = the test portion (in milligrams) (6.1.2),

m₁ = the amount of F (in milligrams) read from the calibration graph (6.1.8).

8. REPEATABILITY (1)

For a fluorine content of about 0,15 % (m/m), the difference between the results of two determinations carried out in parallel on the same sample should not exceed an absolute value of 0,012 % (m/m).

IDENTIFICATION AND DETERMINATION OF ORGANOMERCURY COMPOUNDS

SCOPE AND FIELD OF APPLICATION

The method described below can be used to identify and determine organomercury derivatives employed as preservatives in cosmetic products for the eyes. It is applicable to thiomersal (INN) (sodium 2-(ethylmercuriothio)benzoate) and phenylmercury and its salts.

(1) Norm ISO 5725.

A. IDENTIFICATION

1. PRINCIPLE

The organomercury compounds are complexed with 1,5-diphenyl-3-thiocarbazone. After extraction of the dithizonate with carbon tetrachloride, silica gel, thin-layer chromatography is carried out. The spots of the dithizonates appear as an orange colour.

2. REAGENTS

All the reagents should be of analytical purity.

- 2.1 Sulphuric acid, 25 % (v/v).
- 2.2 1,5-diphenyl-3-thiocarbazone (dithizone): 0,8 mg in 100 ml carbon tetrachloride (2.4).
- 2.3 Nitrogen.
- 2.4 Carbon tetrachloride.
- 2.5 Development solvent: hexane / acetone, 90:10 (v/v).
- 2.6 Standard solution, 0,001 % in water of:
sodium 2-(ethylmercuriothio)benzoate,
ethylmercury chloride or methylmercury chloride,
phenylmercury nitrate or phenylmercury acetate,
mercury dichloride or mercury di(acetate).
- 2.7 Ready prepared silica gel plates (e.g. Merck 5721 or equivalent).
- 2.8 Sodium chloride.

3. APPARATUS

- 3.1 Normal laboratory equipment.
- 3.2 Normal TLC apparatus.
- 3.3 Phase-separating filter.

4. PROCEDURE

4.1 Extraction

- 4.1.1 Dilute 1 g of sample in a centrifuge tube by titration with 20 ml of distilled water. Obtain the maximum dispersion and warm to 60 °C in a water bath. Add 4 g of sodium chloride (2.8). Shake. Allow to cool.
- 4.1.2 Centrifuge for at least 20 minutes at 4 500 rev/min in order to separate the greater part of the solid from the liquid. Filter into a separating funnel and add 0,25 ml of sulphuric acid solution (2.1).
- 4.1.3 Extract several times with 2 or 3 ml of dithizone solution (2.2) until the last organic phase remains green.
- 4.1.4 Filter each organic phase sequentially through a phase-separating filter (3.3).
- 4.1.5 Evaporate to dryness in a stream of nitrogen (2.3).

4.1.6 Dissolve with 0,5 ml of carbon tetrachloride (2.4). Apply this solution immediately as indicated in 4.2.1.

4.2 Separation and identification

4.2.1 Apply immediately 50 µl of the carbon tetrachloride solution obtained in 4.1.6 on to a silica gel plate (2.7). Treat simultaneously 10 ml of standard solution (2.6) as in 4.1 and apply 50 µl of the solution obtained in 4.1.6 on the same plate.

4.2.2 Place the plate in the solvent (2.5) and allow the latter to rise 150 mm. The organomercury compounds appear as coloured spots whose colour is stable, provided the plate is covered by a glass plate immediately the solvent evaporates.

For example, the following Rf values are obtained:

	Rf	Colour
Thiomersal	0,33	Orange
Ethylmercury chloride	0,29	Orange
Methylmercury chloride	0,29	Orange
Phenylmercury salts	0,21	Orange
Mercury (II) salts	0,10	Orange
Mercury di(acetate)	0,10	Orange
1,5-diphenyl-3-thiocarbazone	0,09	Pink

B. DETERMINATION

1. DEFINITION

The content of organomercurial compounds determined by this method is expressed as the percentage by mass (m/m) as mercury in the sample.

2. PRINCIPLE

The method consists in measuring the quantity of total mercury present. It is thus necessary to have first made sure that no mercury in an inorganic state is present and to have identified the organomercurial derivative contained in the sample. After mineralisation, the mercury liberated is measured by flameless atomic absorption.

3. REAGENTS

All the reagents should be of analytical purity.

3.1 Concentrated nitric acid, $d_4^{20} = 1,41$ g/ml.

3.2 Concentrated sulphuric acid, $d_4^{20} = 1,84$ g/ml.

3.3 Redistilled water.

3.4 Potassium permanganate, 7 % (m/v) solution.

3.5 Hydroxylammonium chloride, 1,5 % (m/v) solution.

3.6 Dipotassium peroxodisulphate, 5 % (m/v) solution.

- 3.7 Tin dichloride, 10 % (m/v) solution.
- 3.8 Concentrated hydrochloric acid, $d_4^{20} = 1,18$ g/ml.
- 3.9 Palladium dichloride impregnated glass wool, 1 % (m/m).

4. APPARATUS

- 4.1 Normal laboratory equipment.
- 4.2 Apparatus for flameless atomic absorption mercury determination (cold vapour technique), including the necessary glassware. Path length of the cell at least 100 mm.

5. PROCEDURE

Take all normal precautions for trace mercury analysis.

5.1 Breakdown

- 5.1.1 Weigh accurately 150 mg of the sample (m). Add 10 ml of nitric acid (3.1) and leave to digest for three hours in an airtight flask in a water bath at 55 °C, shaking at regular intervals. At the same time, carry out a blank test on the reagents.
- 5.1.2 After cooling, add 10 ml of sulphuric acid (3.2) and return to the water bath at 55 °C for 30 minutes.
- 5.1.3 Place the flask in an ice bath and add carefully 20 ml of water (3.3).
- 5.1.4 Adding 2 ml aliquots of 7 % potassium permanganate solution (3.4) until the solution remains coloured. Return to the water bath at 55 °C for a further 15 minutes.
- 5.1.5 Add 4 ml of dipotassium peroxodisulphate solution (3.6). Continue to warm in the water bath at 55 °C for 30 minutes.
- 5.1.6 Allow to cool and transfer the contents of the flask into a 100 ml standard flask. Rinse the flask with 5 ml of hydroxylammonium chloride (3.5) and then rinse four times with 10 ml of water (3.3). The solution should be completely decolorized. Make up to the mark with water (3.3).

5.2 Determination

- 5.2.1 Place 10 ml of the test solution (5.1.6) in the glass vessel for the cold vapour mercury determination (4.2). Dilute with 100 ml of water (3.3) and subsequently 5 ml of sulphuric acid (3.2) and 5 ml of tin dichloride solution (3.7). Mix after each addition. Wait 30 seconds to reduce all ionic mercury to the metallic state and take a reading (n).
- 5.2.2 Place some palladium dichloride impregnated glass wool (3.9) between the mercury reduction vessel and the flow cell of the instrument (4.2). Repeat 5.2.1 and record the reading. If the reading is not zero mineralization was incomplete and analysis must be repeated.

6. CALCULATION

Let:

m = the mass (in milligrams) of the test sample.

n = the quantity of mercury (in µg) read on the instrument.

The quantity of mercury, expressed as mercury, as percentage by mass, is calculated by the formula:

$$\% \text{ mercury} = \frac{n}{m}$$

7. NOTES

- 7.1 To improve mineralization it might be necessary to start by diluting the sample.
- 7.2 If absorption of the mercury by the substrate is suspected, a quantitative determination by the method of standard additions should be done.

8. REPEATIBILITY (1)

In the case of mercury concentrations of 0,007 % the difference between the results of two determinations carried out in parallel on the sample should not exceed an absolute value of 0,00035 %.

DETERMINATION OF ALKALI AND ALKALINE EARTH SULPHIDES

1. SCOPE AND FIELD OF APPLICATION

This method describes the determination of sulphides present in cosmetic products. The presence of thiols or other reducing agents (including sulphites) does not interfere.

2. DEFINITION

The concentration of sulphides determined by this method is expressed as a percentage of sulphur by mass.

3. PRINCIPLE

After acidification of the medium, hydrogen sulphide is entrained by a stream of nitrogen and then fixed in the form of cadmium sulphide. The latter is filtered and rinsed and then determined by iodometry.

4. REAGENTS

All the reagents should be of analytical purity.

- 4.1 Concentrated hydrochloric acid, $d_4^{20} = 1,19 \text{ g/ml}$.
- 4.2 Sodium thiosulphate, 0,1 M standard solution.
- 4.3 Iodine, 0,05 M standard solution.
- 4.4 Disodium sulphide.
- 4.5 Cadmium di(acetate).

(1) Norm ISO 5725.

- 4.6 Concentrated ammonia, $d_4^{20} = 0,90$ g/ml.
- 4.7 Ammoniacal solution of cadmium di(acetate): dissolve 10 g of cadmium di(acetate) (4.5) in approximately 50 ml of water. Add ammonia (4.6) until the precipitate redissolves (i.e. approximately 20 ml). Make up to the 100 ml mark with water.
- 4.8 Nitrogen.
- 4.9 Solution of ammonia M.

5. APPARATUS

- 5.1 Usual laboratory equipment.
- 5.2 100 ml round-bottom flask with three standard ground-glass necks.
- 5.3 Two 150 ml conical flasks with ground-glass necks, fitted with a device comprising a dip tube and a side outlet tube for releasing the entraining gas.
- 5.4 One long-stem tunnel.

6. PROCEDURE

6.1 Entrainment of the sulphides

- 6.1.1 Take a package which has not been previously opened. Weigh accurately a mass (m) (expressed in grams) of the product corresponding to not more than 30 mg of sulphide ions in the round-bottom flask (5.2). Add 60 ml of water and two drops of an anti-foaming liquid.
- 6.1.2 Transfer 50 ml of solution (4.7) to each of the two conical flasks (5.3).
- 6.1.3 Fit a dropping funnel, the dip tube and the outlet tube on to the round-bottom flask (5.2). Connect the outlet tube to the conical flask (5.3) set up in series by means of PVC tubing.
- NB:* The entraining apparatus must pass the following leak-tightness test: simulating the test conditions, replace the product to be determined by 10 ml of a sulphide solution (prepared from 4.4) containing 'X mg' of sulphide (iodometrically determined). Let 'Y' be the number of milligrams of sulphide found at the end of this operation. The difference between quantity 'X' and quantity 'Y' must not exceed 3 %.
- 6.1.4 Pass nitrogen (4.8) through for 15 minutes, at a rate of two bubbles per second, in order to expel the air contained in the round-bottom flask (5.2).
- 6.1.5 Heat the round-bottom flask to 85 ± 5 °C.
- 6.1.6 Stop the nitrogen (4.8) stream and add 40 ml of hydrochloric acid (4.1) drop by drop.
- 6.1.7 Turn the nitrogen (4.8) stream on again when nearly all the acid has been transferred, leaving a minimum liquid seal to prevent leakage of hydrogen sulphide.
- 6.1.8 Cease heating after 30 minutes. Allow the flask (5.2) to cool and continue to pass the nitrogen (4.8) stream through for at least one-and-a-half hours.

6.2 Titration

- 6.2.1 Filter the cadmium sulphide through a long-stem funnel (5.4).

- 6.2.2 Rinse the conical flasks (5.3) first with the ammonia solution (4.9) and pour on the filter. Then rinse with distilled water and use the water to wash the precipitate retained by the filter.
- 6.2.3 Complete the washing of the precipitate with 100 ml of water.
- 6.2.4 Place the paper filter in the first conical flask that contained the precipitate. Add 25 ml (n_1) of the iodine solution (4.3), approximately 20 ml of hydrochloric acid (4.1) and 50 ml of distilled water.
- 6.2.5 Determine the excess iodine using the sodium thiosulphate solution (n_2) (4.2).

7. CALCULATION

The sulphide content of the sample, expressed as sulphur, as percentage by mass, is calculated by the following formula:

$$\% \text{ sulphur} = \frac{32 (n_1 x_1 - n_2 x_2)}{20m}$$

where:

- n_1 = the number (in millilitres) of iodine standard solution (4.3) used,
 x_1 = the molarity of this solution,
 n_2 = the number (in millilitres) of the sodium thiosulphate standard solution (4.2),
 x_2 = the molarity of this solution,
 m = the mass (in grams) of the test sample.

8. REPEATABILITY (1)

For a sulphide content of about 2 % (m/m), the difference between the results of two determinations carried out in parallel on the same sample should not exceed an absolute value of 0,2 % (m/m).

(1) Norm ISO 5725.

FOURTH COMMISSION DIRECTIVE 85/490/EEC

Fourth Commission Directive 85/490/EEC of 11 October 1985 on the approximation of the laws of the Member States relating to methods of analysis necessary for checking the composition of cosmetic products

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 last amended by Directive 85/391/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 laid down by Community provisions concerning the composition of cosmetic products are satisfied;

Whereas all the necessary methods of analysis should be laid down as quickly as possible; whereas three steps towards the attainment of this objective having already been taken through the definition of certain methods in Commission Directive 80/1335/EEC (3), 82/434/EEC (4) and 83/514/EEC (5), the fourth step is to consist in the definition of methods for the identification and determination of glycerol 1-(4-aminobenzoate), the determination of chlorobutanol, the identification and determination of quinine, the identification and determination of inorganic sulphites and hydrogen sulphites, the identification and determination of chlorates of the alkali metals and the identification and determination of sodium iodate;

Whereas the measures provided for in this Directive are in accordance 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 during official testing of cosmetic products:

- identification and determination of glycerol 1-(4-aminobenzoate),
- determination of chlorobutanol,
- identification and determination of quinine,
- identification and determination of inorganic sulphites and hydrogen sulphites,
- identification and determination of chlorates of the alkali metals, and

(1) OJ No L 262, 27. 9. 1976, p. 169.

(2) OJ No L 224, 22. 8. 1985, p. 40.

(3) OJ No L 383, 31. 12. 1980, p. 27.

(4) OJ No L 185, 30. 6. 1982, p. 1.

(5) OJ No L 291, 24. 10. 1983, p. 9.

— identification and determination of sodium iodate
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 1986.

They shall forthwith inform the Commission thereof.

Article 3

This Directive is addressed to the Member States.

Done at Brussels, 11 October 1985.

For the Commission
Stanley CLINTON-DAVIS
Member of the Commission

ANNEX**IDENTIFICATION AND DETERMINATION OF GLYCEROL
1-(4-AMINO BENZOATE)****A. IDENTIFICATION****1. SCOPE AND FIELD OF APPLICATION**

This method will detect alpha-monoglyceryl 4-aminobenzoate (glycerol 1-(4-aminobenzoate)). It will also detect ethyl 4-aminobenzoate (benzocaine INN) which may be present as an impurity.

2. PRINCIPLE

This identification is done by thin layer chromatography on silica gel with a fluorescent indicator and detection of the free primary amine group by formation of a diazo dye on the plate.

3. REAGENTS

All reagents should be of analytical purity.

3.1 Solvent mixture: cyclohexane/propan-2-ol/stabilized dichloromethane 48/64/9 (v/v/v).

3.2 Development solvent: petroleum ether (40-60) / benzene / acetone / ammonium hydroxide solution (minimum 25 % NH₃): 35/35/35/1 (v/v/v/v).

3.3 Developing solution: (a) sodium nitrite: 1 g in 100 ml of 1 M hydrochloric acid (prepared immediately before use);
(b) 2-naphthol: 0,2 g in 100 ml of 1 M potassium hydroxide.

3.4 Standard solutions:

Alpha-monoglyceryl 4-aminobenzoate: 0,05 g in 100 ml of mixed solvent 3.1;

ethyl 4-aminobenzoate: 0,05 g in 100 ml of mixed solvent 3.1.

3.5 Silica gel 60 F254 plates, 0,25 mm thick, 200 mm x 200 mm.

4. APPARATUS

4.1 Normal apparatus for thin layer chromatography.

4.2 Ultrasonic vibrator.

4.3 Millipore filter FH 0,5 µm or equivalent.

5. PROCEDURE

5.1 Sample preparation

Weigh 1,5 g of the product to be analyzed in a 10 ml stoppered graduated flask. Make up to the mark with the solvent 3.1. Stopper and leave for one hour at room temperature in an ultrasonic vibrator (4.2). Filter through a Millipore filter (4.3) and use the filtrate for chromatography.

5.2 Thin layer chromatography

Deposit 10 µl of sample solution (5.1) and of each standard solution (3.4) on the plate (3.5).

Develop the chromatogram to a height of 150 mm in a tank previously saturated with solvent 3.2. Allow the plate to dry at ambient temperature.

5.3 Development

5.3.1 Observe the plate under 254 nm UV light.

5.3.2 Spray the completely dried plate with the solution 3.3 (a).

Allow to dry at room temperature for 1 minute and immediately spray with the solution 3.3 (b).

Dry the plate in an oven at 60 °C. The spots appear an orange colour. Alpha-monoglyceryl 4-aminobenzoate: Rf 0,07; ethyl 4-aminobenzoate: Rf 0,55.

B. DETERMINATION

1. SCOPE AND FIELD OF APPLICATION

This method determines alpha monoglyceryl 4-aminobenzoate. It will also determine ethyl 4-aminobenzoate. It cannot determine more than 5 % (m/m) of alpha monoglyceryl 4-aminobenzoate and 1 % (m/m) of ethyl 4-aminobenzoate.

2. DEFINITION

The alpha monoglyceryl 4-aminobenzoate and ethyl 4-aminobenzoate contents measured by this method are expressed as percentage by mass (% m/m) of the product.

3. PRINCIPLE

The product to be analyzed is suspended in methanol and after appropriate treatment of the sample it is determined by high-performance liquid chromatography (HPLC).

4. REAGENTS

All reagents should be of analytical purity and should be suitable for HPLC where appropriate.

4.1 Methanol.

4.2 Potassium dihydrogenorthophosphate (KH₂PO₄).

- 4.3 Zinc di(acetate) ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$).
- 4.4 Acetic acid ($d \frac{20}{4} = 1,05$).
- 4.5 Tetrapotassium hexacyanoferrate, ($\text{K}_4(\text{Fe}(\text{CN})_6) \cdot 3\text{H}_2\text{O}$).
- 4.6 Ethyl 4-hydroxybenzoate.
- 4.7 Alpha monoglyceryl 4-aminobenzoate.
- 4.8 Ethyl 4-aminobenzoate.
- 4.9 Phosphate buffer solution (0,02 M): dissolve 2,72 g of potassium dihydrogenorthophosphate (4.2) in one litre of water.
- 4.10 Eluant: phosphate buffer solution (4.9)/methanol (4.1) 61/39(v/v)

The composition of the mobile phase may be changed in order to achieve a resolution factor $R \geq 1,5$.

$$R = 2 \frac{d' R_2 - d' R_1}{W_1 + W_2}$$

where:

- R_1 and R_2 = retention times, in minutes, of the peaks,
 W_1 and W_2 = peak widths at half height, in millimetres,
 d' = the chart speed, in millimetres per minute.

- 4.11 Stock solution of alpha-monoglyceryl 4-aminobenzoate: weigh accurately about 40 mg of alpha-monoglyceryl 4-aminobenzoate and introduce it into a 100 ml graduated flask. Dissolve in 40 ml of methanol (4.1). Make up to the mark with buffer solution (4.9) and mix.
- 4.12 Stock solution of ethyl 4-aminobenzoate: weigh accurately about 40 mg of ethyl 4-aminobenzoate and introduce it into a 100 ml graduated flask. Dissolve in 40 ml of methanol (4.1). Make up to the mark with the buffer solution (4.9) and mix.
- 4.13 Internal standard solution: weigh accurately about 50 mg of ethyl 4-hydroxybenzoate (4.6), transfer to a 100 ml standard flask, dissolve in 40 ml of methanol (4.1), make up to the mark with the buffer solution (4.9) and mix.
- 4.14 Standard solutions: prepare four standard solutions by dissolving in 100 ml eluant (4.10) according to the following table:

Standard solution	Alpha- monoglyceryl 4-aminobenzoate		Ethyl 4-aminobenzoate		Ethyl 4-hydroxybenzoate	
	($\mu\text{g}/\text{ml}$) (*)	ml (4.11)	($\mu\text{g}/\text{ml}$) (*)	ml (4.12)	($\mu\text{g}/\text{ml}$) (*)	ml (4.13)
I	8	2	8	2	50	10
II	16	4	12	3	50	10
III	24	6	16	4	50	10
IV	40	10	20	5	50	10

(*) These values are given as an indication and correspond to the exact masses of 4.11, 4.12 and 4.13.
 NB: These solutions may be prepared in a different way.

- 4.15 Carrez I solution: dissolve 26,5 g of tetrapotassium hexacyanoferrate (4.5) in water and make up to 250 ml.
- 4.16 Carrez II solution: dissolve 54,9 g of zinc di(acetate) (4.3) and 7,5 ml of acetic acid (4.4) in water and make up to 250 ml.
- 4.17 Merck Lichrosorb RP-18, or equivalent, with an average particle size of 5 µm.

5. APPARATUS

- 5.1 The usual laboratory equipment.
- 5.2 High-performance chromatography equipment with a variable wavelength UV detector and thermostatted chamber set at 45 °C.
- 5.3 Stainless-steel column: length: 250 mm; internal diameter: 4,6 mm; packing: Lichrosorb RP – 18 (4.17).
- 5.4 Ultrasonic bath.

6. PROCEDURE

6.1 Sample preparation

- 6.1.1 Weigh accurately about 1 g of sample into a 100 ml beaker and add 10 ml of methanol (4.1).
- 6.1.2 Place the beaker in the ultrasonic bath (5.4) for 20 minutes to produce a suspension. Transfer the suspension thus obtained quantitatively into a 100 ml standard flask with not more than 75 ml of eluant (4.10).

Add in succession 1 ml of Carrez I solution (4.15) and 1 ml of Carrez II solution (4.16) and mix after each addition. Make up to the mark with eluant (4.10), re-mix and filter through a pleated filter paper.

- 6.1.3 With a pipette, transfer 3,0 ml of the filtrate obtained in 6.1.2 and 5,0 ml of the internal standard solution (4.13) into a 50 ml standard flask. Make up to the mark with eluant (4.10) and mix. Use the solution thus obtained for carrying out the chromatography analysis described in 6.2.

6.2 Chromatography

- 6.2.1 Adjust the flow rate of the mobile phase (4.10) to 1,2 ml/min and set the column temperature to 45 °C.
- 6.2.2 Set the detector (5.2) to 274 nm.
- 6.2.3 With a microsyringe inject at least two times 20 µl of solution (6.1.3) into the chromatograph and measure the areas of the peaks.

6.3 Calibration curve

- 6.3.1 Inject 20 µl of each of the standard solutions (4.14) and measure the peak area.
- 6.3.2 For each concentration calculate the ratio between the peak areas of alpha-monoglyceryl 4-aminobenzoate and the peak areas of the internal standard. Plot this ratio on the abscissa and on the ordinate the ratio of the corresponding masses.
- 6.3.3 Proceed in the same manner for ethyl 4-hydroxybenzoate.

7. CALCULATION

7.1 From the calibration curve obtained in 6.3 read off the mass ratios (RP1, RP2) corresponding to the ratios between the areas of the peaks calculated in 6.2.3 where

RP1 = mass of alpha-monoglyceryl 4-aminobenzoate / mass of ethyl 4-hydroxybenzoate,

RP2 = mass of ethyl 4-aminobenzoate / mass of ethyl 4-hydroxybenzoate.

7.2 From the mass ratios obtained in this way calculate the alpha-monoglyceryl 4-aminobenzoate and ethyl 4-aminobenzoate contents, as mass percentages (% m/m) with the formulae:

$$R_p \% \text{ (m/m) alpha-monoglyceryl 4-aminobenzoate} = RP1 \times \frac{q}{6p}$$

$$R_p \% \text{ (m/m) ethyl 4-aminobenzoate} = RP2 \times \frac{q}{6p}$$

q = quantity of ethyl 4-hydroxybenzoate (internal standard) weighed, in milligrams, in 4.12,

p = quantity of sample, in grams, weighed in 6.1.1.

8. REPEATABILITY (1)

8.1 For a 5 % (m/m) content of alpha-monoglyceryl 4-aminobenzoate, the difference between the results of two parallel determinations carried out on the same sample must not exceed 0,25 %.

8.2 For a 1 % (m/m) content of ethyl 4-aminobenzoate the difference between the results of two parallel determinations carried out on the same sample must not exceed 0,10 %.

9. NOTES

9.1 Before carrying out an analysis, check whether the sample contains substances likely to overlap with the peak of the internal standard (ethyl 4-aminobenzoate) on the chromatogram.

9.2 In order to check the absence of any interference, repeat the determination by changing the proportion of methanol in the mobile phase by 10 % relative.

DETERMINATION OF CHLOROBUTANOL

1. SCOPE AND FIELD OF APPLICATION

This method is suitable for the determination of chlorobutanol (INN) up to a maximum concentration of 0,5 % (m/m) in any cosmetic product, except aerosols.

(1) ISO 5725.

2. DEFINITION

The content of chlorobutanol measured by this method is expressed as percentage by mass (% m/m) of product.

3. PRINCIPLE

After appropriate treatment of the product to be analyzed the determination is done by gas chromatography using 2,2,2-trichloroethanol as the internal standard.

4. REAGENTS

All the reagents should be of analytical purity.

4.1 Chlorobutanol (1,1,1-trichloro-2-methylpropan-2-ol).

4.2 2,2,2-Trichloroethanol.

4.3 Absolute ethanol.

4.4 Standard solution of chlorobutanol: 0,025 g in 100 ml ethanol (4.3) (m/v).

4.5 Standard solution of 2,2,2-trichloroethanol: 4 mg in 100 ml ethanol (4.3) (m/v).

5. APPARATUS

5.1 Normal laboratory equipment.

5.2 Gas chromatograph with electron detector, Ni 63.

6. PROCEDURE

6.1 Preparation of sample

Weigh accurately between 0,1 and 0,3 g (p g) of the sample. Place in 100 ml volumetric flask. Dissolve it in ethanol (4.3), add 1 ml of the internal standard solution (4.5) and make up to the mark with ethanol (4.3).

6.2 Gas chromatography conditions

6.2.1 The operating conditions must yield a resolution factor $R \geq 1,5$.

$$R = 2 \frac{d'R_2 - d'R_1}{W_1 + W_2}$$

Where

R_1 and R_2 = retention times, in minutes, of the peaks,

W_1 and W_2 = peak widths at half height, in millimetres,

d' = the chart speed, in millimetres per minute.

6.2.2 As examples, the following operating conditions provide the required resolution:

<i>Column</i>	<i>I</i>	<i>II</i>
Material	Glass	Stainless steel
Length	1,80 m	3 m
Diameter	3 mm	3 mm
Stationary phase	10 % Carbowax 20 M TPA on Gaschrom Q 80-100 mesh	5 % OV 17 on Chromosorb WAW DMCS 80-100 mesh
Conditioning	2 to 3 days at 190 °C	
Temperature:		
— injector	200 °C	150 °C
— column	150 °C	100 °C
— detector	200 °C	150 °C
Carrier gas	Nitrogen	Argon/methane (95/5 v/v)
Flowrate	35 ml/min	35 ml/min

6.3 Standard curve

Using five 100 ml volumetric flasks, add 1 ml of the standard solution (4.5) and 0,2, 0,3, 0,4, 0,5, and 0,6 ml of solution 4.4 respectively, and make up to the mark with ethanol (4.3) and mix. Inject 1 µl of each of these solutions into the chromatograph in accordance with the operating conditions described in 6.2.2 and construct a calibration curve by plotting as the abscissa the ratio of the mass of chlorobutanol to that of 2,2,2-trichloroethanol and as the ordinate the ratio of the corresponding peak areas.

6.4 Inject 1 µl of solution obtained in 6.1 and proceed according to the conditions described in 6.2.2.

7. CALCULATION

7.1 Calculate from the standard curve (6.3) the quantity 'a' expressed as µg of chlorobutanol, in the solution 6.1.

7.2 The content of chlorobutanol in the sample is calculated according to the formula:

$$\% \text{ chlorobutanol (m/m)} = \frac{a \times 10^2}{p \times 10^6} = \frac{a}{p \times 10^4}$$

8. REPEATABILITY (1)

For a chlorobutanol content of 0,5 % (m/m) the difference between the results of two determinations in parallel carried out on the same sample should not exceed 0,01 %.

Note

If the result is equal to or exceeds the maximum permitted concentration it is necessary to check the absence of interferences.

(1) ISO 5725.

IDENTIFICATION AND DETERMINATION OF QUININE

A. IDENTIFICATION

1. SCOPE AND FIELD OF APPLICATION

This method is intended to detect the presence of quinine in shampoo and hair lotions.

2. PRINCIPLE

Identification is done by thin layer chromatography on silica gel. Detection of quinine is by the blue fluorescence of quinine in acidic conditions at 360 nm.

For further confirmation, the fluorescence can be eliminated by bromine vapours, and ammonia vapours will cause a yellowish fluorescence to appear.

3. REAGENTS

All reagents should be of analytical purity.

- 3.1 Silica gel plates, without fluorescence indicators, 0,25 mm thick, 200 mm x 200 mm.
- 3.2 Developing solvent: toluene / diethyl ether / dichloromethane / diethylamine / 20/20/20/8 (v/v/v/v).
- 3.3 Methanol.
- 3.4 Sulphuric acid (96 %; $d_{4}^{20} = 1,84$).
- 3.5 Diethyl ether.
- 3.6 Developing agent: carefully add 5 ml of sulphuric acid (3.4) to 95 ml of diethyl ether (3.5) in a cooled container.
- 3.7 Bromine.
- 3.8 Ammonium hydroxide solution (28 %; $d_{4}^{20} = 0,90$).
- 3.9 Quinine, anhydrous.
- 3.10 Standard solution: weigh accurately about 100,0 mg of anhydrous quinine (3.9) into a standard flask and dissolve in 100 ml of methanol (3.3).

4. APPARATUS

- 4.1 Normal equipment for thin layer chromatography.
- 4.2 Ultrasonic bath.
- 4.3 Millipore filter, FH 0,5 μm or equivalent with suitable filtration equipment.

5. PROCEDURE

5.1 Preparation of the sample

Weigh accurately a quantity of the sample which may contain approximately 100 mg of quinine into a 100 ml standard flask, dissolve and make up to the mark with methanol (3.3).

Stopper the flask and leave for one hour at room temperature in an ultrasonic vibrator (4.2). Filter (4.3) and use the filtrate for the chromatography.

5.2 Thin layer chromatography

Deposit 1,0 µl of standard solution (3.10) and 1,0 µl of sample solution (5.1) on the silica gel plate (3.1). Develop the chromatogram over a distance of 150 mm using solvent 3.2 in a tank previously saturated with solvent (3.2).

5.3 Development

5.3.1 Dry the plate at room temperature.

5.3.2 Spray with reagent 3.6.

5.3.3 Leave the plate to dry for one hour at room temperature.

5.3.4 Observe under the light from a UV lamp adjusted to a wavelength of 360 nm. Quinine appears as a fluorescent intense blue spot.

By way of example the table below gives the values of the R_f of the main alkaloids related to quinine when developed with solvent 3.2.

Alkaloid	R _f
Quinine	0,20
Quinidine	0,29
Cinchonine	0,33
Cinchonidine	0,27
Hydroquinidine	0,17

5.3.5 For further confirmation that quinine is present, the plate is exposed for approximately one hour to bromine vapour (3.7). The fluorescence disappears. When the same plate is exposed to ammonia vapour (3.8), the spots reappear with a brown colour, and when the plate is again examined under UV light at 360 nm a yellowish fluorescence can be observed.

Detection limit: 0,1 µg of quinine.

B. DETERMINATION

1. SCOPE AND FIELD OF APPLICATION

This method describes the determination of quinine. It may be used to determine the maximum permitted concentration of 0,5 % (m/m) in shampoos and 0,2 % in hair lotions.

2. DEFINITION

The quinine content determined by this method is expressed as a percentage by mass (% m/m) of the product.

3. PRINCIPLE

After appropriate treatment of the product to be analyzed the determination is done by high-performance liquid chromatography (HPLC).

4. REAGENTS

All reagents should be of analytical purity and suitable for HPLC.

4.1 Acetonitrile.

4.2 Potassium dihydrogenorthophosphate (KH_2PO_4).

4.3 Orthophosphoric acid (85 %; $d_{4}^{20} = 1,7$).

4.4 Tetramethylammonium bromide.

4.5 Quinine, anhydrous.

4.6 Methanol.

4.7 Orthophosphoric acid solution (0,1 M): weigh 11,53 g of orthophosphoric acid (4.3) and dissolve in 1 000 ml of water in a graduated flask.

4.8 Potassium dihydrogenorthophosphate solution (0,1 M): weigh 13,6 g of potassium dihydrogenorthophosphate (4.2) and dissolve in 1 000 ml of water in a graduated flask.

4.9 Tetramethylammonium bromide solution: dissolve 15,40 g of tetramethyl-ammonium bromide (4.4) in 1 000 ml of water in a graduated flask.

4.10 Eluant: orthophosphoric acid (4.7) / potassium dihydrogenorthophosphate (4.8) / tetramethylammonium bromide (4.9) / water / acetonitrile (4.1) 10/50/100/340/90 (v/v/v/v/v).

The composition of this mobile phase may be changed in order to achieve a resolution factor $R \geq 1,5$.

$$R = 2 \frac{d'R_2 - d'R_1}{W_1 + W_2}$$

where

R_1 and R_2 = retention times, in minutes, of the peaks,

W_1 and W_2 = peak widths at half height, in millimetres,

d' = the chart speed, in millimetres per minute.

4.11 Silica treated with octadecylsilane, 10 μm .

4.12 Standard solutions: weigh accurately approximately 5,0, 10,0, 15,0 and 20,0 mg respectively of quinine anhydrous (4.5) into a set of 100 ml standard flasks. Make up to the mark with methanol (4.6) and shake the contents of the flasks until the quinine dissolves. Filter each sample through a 0,5 μm filter.

5. APPARATUS

- 5.1 Usual laboratory equipment.
- 5.2 Ultrasonic bath.
- 5.3 High-performance liquid chromatography equipment with a variable wavelength detector.
- 5.4 Column: length: 250 mm; internal diameter: 4,6 mm; filling: silica (4.11).
- 5.5 Millipore filter FH 0,5 µm, or equivalent, with suitable filtration apparatus.

6. PROCEDURE

6.1 Sample preparation

Weigh accurately into a 100 ml standard flask a quantity of the product sufficient to contain 10,0 mg of anhydrous quinine, add 20 ml of methanol (4.6) and place the flask in an ultrasonic bath (5.2) for 20 minutes. Make up to the mark with methanol (4.6). Mix the solution and then filter an aliquot (5.5).

6.2 Chromatography

Flowrate: 1,0 ml/min.

Detector wavelength (53): 332 nm.

Injection volume: 10 µl of filtered solution (6.1).

Measurement: peak area.

6.3 Calibration curve

Inject at least three times 10,0 µl of each reference solution (4.12), measure the area of the peaks, and calculate the average area at each concentration.

Produce the calibration curve and verify that it is rectilinear.

7. CALCULATION

- 7.1 From the calibration curve (6.3) determine the quantity in µg of anhydrous quinine present in the volume injected (6.2).

- 7.2 The concentration of anhydrous quinine in the sample, as a percentage by mass (% m/m), is obtained by the following formula:

$$\% \text{ (m/m) of anhydrous quinine} = \frac{B}{A}$$

where

B is the quantity, in micrograms, of anhydrous quinine determined in the 10 microlitres of the filtered solution (6.1).

A is the mass of the sample in grams (6.1).

8. REPEATABILITY (1)

For an anhydrous quinine content of 0,5 % (m/m), the difference between the results of two determinations performed in parallel on the same sample must not exceed 0,02 %.

For an anhydrous quinine content of 0,2 % (m/m), the difference between the results of two determinations performed in parallel on the same sample must not exceed 0,01 %.

IDENTIFICATION AND DETERMINATION OF INORGANIC SULPHITES AND HYDROGEN SULPHITES

SCOPE AND FIELD OF APPLICATION

The method describes the identification and determination of inorganic sulphites and hydrogen sulphites in cosmetic products. It is only applicable to products that have an aqueous or alcoholic phase and for concentrations up to 0,2 % sulphur dioxide.

A. IDENTIFICATION

1. PRINCIPLE

The sample is heated in hydrochloric acid, and sulphur dioxide liberated is identified either by its odour or its effect on an indicator paper.

2. REAGENTS

All reagents should be of analytical purity.

2.1 Hydrochloric acid (4 M).

2.2 Potassium iodate starch paper or other suitable alternative.

3. APPARATUS

3.1 Normal laboratory equipment.

3.2 Flask (25 ml) fitted with a short reflux condenser.

4. PROCEDURE

4.1 Place about 2,5 g of sample in the flask (3.2) with 10 ml of hydrochloric acid (2.1).

4.2 Mix and heat to boiling.

4.3 Test for the emission of sulphur dioxide either by smell or indicator paper (2.2).

(1) ISO 5725.

B. DETERMINATION

1. DEFINITION

The sulphite or hydrogen sulphite content of the sample as determined by the method is expressed as percentage by mass of sulphur dioxide.

2. PRINCIPLE

After acidification of the sample, sulphur dioxide liberated is distilled into a solution of hydrogen peroxide. Sulphuric acid formed is titrated against a standardized sodium hydroxide solution.

3. REAGENTS

All reagents should be of analytical purity.

3.1 Hydrogen peroxide 0,2 % (m/v). Prepare daily.

3.2 Orthophosphoric acid $d_{4}^{25} = 1,75$.

3.3 Methanol.

3.4 Sodium hydroxide (0,01 M) standardized solution.

3.5 Nitrogen.

3.6 Indicator: mixture 1:1 (v/v) of methyl red (0,03 % m/v in ethanol) and methylene blue (0,05 % m/v in ethanol). Filter the solution.

4. APPARATUS

4.1 Normal laboratory equipment.

4.2 Distillation apparatus (see figure).

5. PROCEDURE

5.1 Weigh accurately about 2,5 g of sample into the distillation flask A (see figure).

5.2 Add 60 ml of water and 50 ml of methanol (3.3) and mix.

5.3 Place 10 ml of hydrogen peroxide (3.1), 60 ml of water and a few drops of indicator (3.6) in the distillation receiver D (see figure). Add a few drops of sodium hydroxide (3.4) until the indicator turns green.

5.4 Repeat 5.3 for the wash bottle E (see figure).

5.5 Assemble the apparatus and adjust the nitrogen (3.5) flow to about 60 bubbles per minute.

5.6 Run 15 ml of orthophosphoric acid (3.2) from the funnel into the distillation flask A.

5.7 Heat rapidly to boiling and then simmer gently for a total time of 30 minutes.

5.8 Detach the distillation receiver D. Rinse the tube and then titrate with sodium hydroxide solution (3.4) until the indicator turns green (3.6).

6. CALCULATION

Calculate the content of sulphite or hydrogen sulphite by mass in the sample by the expression:

$$\% \text{ m/m of sulphur dioxide} = \frac{3,2 MV}{m}$$

where

M = molar concentration of sodium hydroxide solution (3.4),

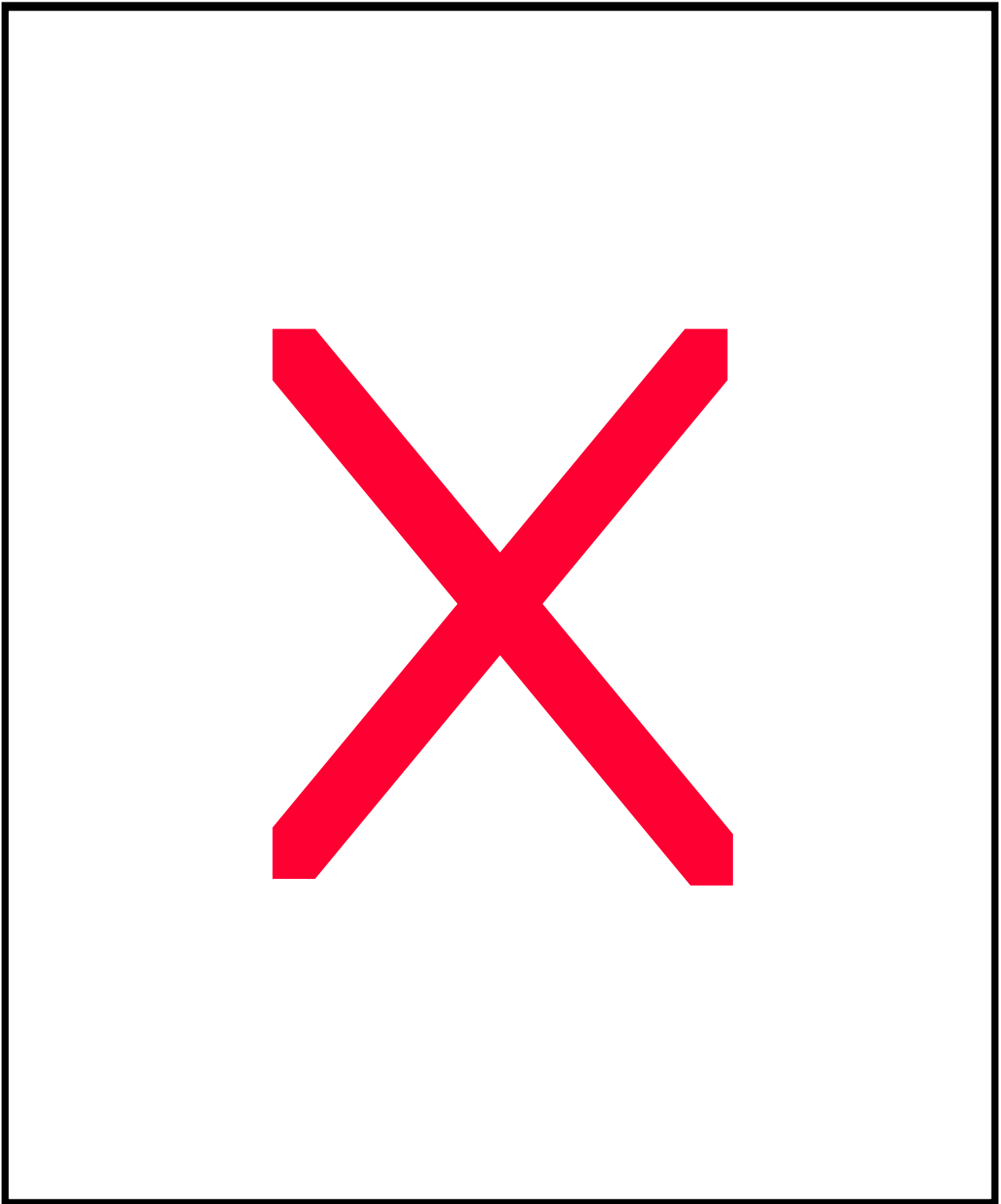
V = volume of sodium hydroxide (3.4) required for titration (5.8) in millilitres,

m = mass of sample (5.1) in grams.

7. REPEATABILITY (1)

For a content of 0,2 % m/m of sulphur dioxide the difference between two parallel determinations done on the same sample should not be greater than 0,006 %.

(1) ISO 5725.



IDENTIFICATION AND DETERMINATION OF CHLORATES OF THE ALKALI METALS

SCOPE AND FIELD OF APPLICATION

The method described the identification and determination of chlorates in toothpastes and other cosmetic products.

A. IDENTIFICATION

1. PRINCIPLE

Chlorates are separated from other halates by thin layer chromatography and identified by the oxidation of iodide to form iodine.

2. REAGENTS

All reagents should be of analytical purity.

- 2.1 Reference solutions: aqueous solutions of potassium chlorate, bromate and iodate (0,2 % m/v) prepared freshly.
- 2.2 Development solvent: ammonia solution (23 % m/v) acetone/butanol (60/130/30 v/v/v).
- 2.3 Potassium iodide, aqueous solution (5 % m/v).
- 2.4 Starch solution (1 to 5 % m/v).
- 2.5 Hydrochloric acid (1 M).
- 2.6 Ready-for-use cellulose thin-layer plates (0,25 mm).

3. APPARATUS

Normal equipment for thin layer chromatography.

4. PROCEDURE

- 4.1 Extract about 1 g of the sample with water, filter, and dilute to about 25 ml.
- 4.2 Deposit 2 µl on the plate (2.6) of the solution (4.1) together with 2 µl aliquots of each of the three reference solutions (2.1).
- 4.3 Place the plate in a tank and develop by ascending chromatography about three-quarters of the length of the plate (2.6) with solvent 2.2.
- 4.4 Remove from the tank and allow the solvent to evaporate. (NB: This may take up to two hours.)
- 4.5 Spray the plate with potassium iodide (2.3) and allow to dry for about five minutes
- 4.6 Spray the plate with starch solution (2.4) and allow to dry for about five minutes.
- 4.7 Spray the plate with hydrochloric acid (2.5).

5. EVALUATION

If the chlorate is present a blue spot (possibly a brown spot) will appear after half an hour with Rf value approximately 0,7 to 0,8.

Halates	Rf
Iodate	0 to 0,2
Bromate	0,5 to 0,6
Chlorate	0,7 to 0,8

It should be noted that bromates and iodates give immediate reaction. Care should be taken not to confuse spots from bromates and chlorates.

B. DETERMINATION

1. DEFINITION

The chlorate content of the sample determined by this method is expressed as percentage by mass of chlorate.

2. PRINCIPLE

Chlorate is reduced by zinc powder under acid conditions. The formed chloride is measured by potentiometric titration using a silver nitrate solution. A similar determination before reduction permits the possible presence of halides.

3. REAGENTS

All reagents should be of analytical purity.

- 3.1 Acetic acid, 80 % (m/m).
- 3.2 Zinc powder.
- 3.3 Silver nitrate standard solution (0,1 M).

4. APPARATUS

- 4.1 Normal laboratory equipment.
- 4.2 Potentiometer equipped with a silver indicator electrode.

5. PROCEDURE

5.1 Sample preparation

Weigh accurately a quantity 'm' of approximately 2 g in centrifuge tube. Add about 15 ml acetic acid (3.1) and mix carefully. Wait 30 minutes and centrifuge for 15 minutes at 2 000 rev/min. Transfer the supernatant solution to a 50 ml volumetric flask. Repeat centrifuging twice by adding 15 ml acetic acid (3.1) to the residue. Collect the solution containing chlorate in the same volumetric flask. Fill to the mark with acetic acid (3.1).

5.2 Reduction of chlorate

Take 20 ml of solution 5.1 and add 0,6 g of zinc powder (3.2). Bring to the boil in a flask fitted with a condenser tube. After 30 minutes boiling, cool and filter. Rinse the flask with water. Filter and combine the filtrate with the rises.

5.3 Determination of chloride

Titrate 20 ml solution 5.2 with silver nitrate (3.3) by using the potentiometer (4.2). Titrate in the same way 20 ml of solution 5.1 with silver nitrate (3.3).

NB: If the product contains bromine or iodine derivatives which can release bromides or iodides after reduction, the titration curve will have several inflexion points. In this case the volume of the titrated solution (3.3) corresponding to chloride is the difference between the last and the penultimate inflexion points.

6. CALCULATION

The content of chlorate of the sample (% m/m) is calculated by the formula:

$$\text{Chlorate (ClO}_3^-) \% \text{ m/m} = \frac{20,9 (V - V') M}{m}$$

where

V = volume in millilitres, of silver nitrate solution (3.3) used to titrate solution 5.2,

V' = volume, in millilitres, of silver nitrate solution (3.3) used to titrate 20 millilitres of solution 5.1,

M = molarity of silver nitrate standard solution (3.3),

m = mass of sample, in grams.

7. REPEATABILITY (1)

For a chlorate content of 3 to 5 % m/m the difference between the results of two determinations carried out in parallel on the same sample should not exceed 0,07 % m/m.

IDENTIFICATION AND DETERMINATION OF SODIUM IODATE

SCOPE AND FIELD OF APPLICATION

The method describes the procedure for identifying and determining rinse of cosmetic products containing sodium iodate.

(1) ISO 5725.

A. IDENTIFICATION

1. PRINCIPLE

Sodium iodate is separated from other halates by thin layer chromatography and identified by the oxidation of iodide to form iodine.

2. REAGENTS

All reagents should be of analytical purity.

2.1 Reference solutions. Aqueous solutions of potassium chlorate, bromate and iodate (0,01 % m/v) prepared freshly.

2.2 Development solvent.

Ammonia solution (28 % m/v) / acetone / butanol (60/130/30 v/v/v).

2.3 Potassium iodide aqueous solution (5 % m/v).

2.4 Starch solution (1 to 5 % m/v).

2.5 Hydrochloric acid (1 M).

3. APPARATUS

3.1 Ready-for-use cellulose thin-layer chromatography (0,25 mm) plates.

3.2 Normal equipment for thin layer chromatography.

4. PROCEDURE

4.1 Extract about 1 g of the sample with water, filter, and dilute to about 10 ml.

4.2 Deposit 2 µl of this solution onto the base line of the plate (3.1) together with 2 µl aliquots of each of the three reference solutions (2.1).

4.3 Place the plate in a tank and develop by ascending chromatography about three-quarters of the length of the plate with solvent (2.2).

4.4 Remove the plate from the tank and allow the solvent to evaporate at ambient temperature (*NB*: this may take up to two hours).

4.5 Spray the plate with potassium iodide (2.3) and allow to dry for about five minutes.

4.6 Spray with starch (2.4) and allow to dry for about five minutes.

4.7 Finally spray with hydrochloric (2.5).

5. EVALUATION

If iodate is present a blue spot (the colour may be brown or become brown on standing) will appear immediately with an R_f value approximately 0 to 0,2.

It should be noted that bromates give immediate reactions at R_f values approximately at 0,5 to 0,6 and chlorates after about 30 minutes, at R_f values of 0,7 to 0,8 respectively.

B. DETERMINATION

1. DEFINITION

The sodium iodate content as determined by this method is expressed as a percentage by mass of sodium iodate.

2. PRINCIPLE

Sodium iodate is dissolved in water and determined by means of high-performance liquid chromatography, using in series, a reverse-phase C18 column and an anion-exchange column.

3. REAGENTS

All reagents should be of analytical purity and especially suitable for high-performance liquid chromatography (HPLC).

3.1 Hydrochloric acid (4 M).

3.2 Sodium sulphite aq, 5 % m/v.

3.3 Sodium iodate stock solution.

Prepare a stock solution containing 50 mg sodium iodate per 100 ml water.

3.4 Potassium dihydrogenorthophosphate.

3.5 Disodium hydrogenorthophosphate · 2H₂O.

3.6 HPLC mobile phase: dissolve 3,88 g potassium dihydrogenorthophosphate (3.4) and 1,19 g disodium hydrogenorthophosphate · 2H₂O (3.5) in 1 litre water.

The pH of the resulting solution is 6,2.

3.7 Universal indicator paper, pH 1-11.

4. APPARATUS

4.1 Ordinary laboratory apparatus.

4.2 Circular filter paper, diameter 110 mm, Schleicher and Schüll No 575, or equivalent.

4.3 High-performance liquid chromatograph with a variable wavelength detector.

4.4 Columns: length 120 mm; internal diameter: 4,6 mm number: two connected in series; first column – Nucleosil[®] 5 C18 or equivalent; second column – Vydac[™] – 301 – SB or equivalent.

5. PROCEDURE

5.1 Sample preparation

5.1.1 *Fluid samples (shampoos)*

Weigh accurately a test portion of approximately 1,0 g sample in a 10 ml glass stoppered calibrated tube or measuring flask.

Fill up to the mark with water and mix.

If necessary, filter the solution.

Determine the iodate in the solution by means of HPLC as described in section 5.2.

5.1.2 **Solid samples (soap)**

Finely divide part of the sample and weigh accurately a test portion of approximately 1,0 g into a 100 ml glass stoppered measuring cylinder. Fill up to 50 ml with water and shake vigorously for one minute. Centrifuge and filter through a filter paper (4.1) or allow the mixture to stand for at least one night.

Shake the jellylike solution vigorously and filter it through a filter paper (4.1).

Determine the iodate in the filtrate by means of HPLC as described in section 5.2.

5.2 **Chromatography**

Flowrate: 1 ml/min.

Detector wavelength (4.2): 210 nm.

Injection volume: 10 µl.

Measurement: peak area

5.3 **Calibration**

Pipette respectively 1,0, 2,0, 5,0, 10,0 and 20,0 ml of the sodium iodate stock solution (3.3) into 50 ml volumetric flasks. Fill to the mark and mix.

The solutions thus obtained, contain 0,01, 0,02, 0,05, 0,10 and 0,20 mg sodium iodate per ml respectively.

Inject a 10 µl portion of each standard iodate solution into the liquid chromatograph (4.2) and obtain a chromatogram. Determine the peak area for iodate and plot a curve relating the peak area to the sodium iodate concentration.

6. **CALCULATION**

Calculate the sodium iodate content, in percentage by mass (% m/m), using the formula:

$$\% \text{ (m/m) of sodium iodate} = \frac{Vc}{10 m}$$

where

m is the mass, in grams, of the test portion (5.1),

V is the total volume of the sample solution, in millilitres, obtained as described in 5.1,

c is the concentration, in milligrams per millilitre of sodium iodate, obtained from the calibration curve (5.3).

7. **REPEATABILITY (1)**

For a sodium iodate content of 0,1 % (m/m) the difference between the results of two parallel determinations carried out on the same sample must not exceed 0,002 %.

(1) ISO 5725.

8. CONFIRMATION

8.1 Principle

In an acidified solution of a cosmetic product, iodate (IO_3^-) is reduced to iodide (I^-) by sulphite and the resulting solution is investigated by means of HPLC. If a peak having a retention time corresponding to the retention time of iodate disappears after treatment with sulphite, the original peak can most probably be attributed to iodate.

8.2 Procedure

Pipette a 5 ml portion of the sample solution obtained as described in section 5.1 into a conical flask.

Adjust the pH of the solution to a value of 3 or lower with hydrochloric acid (3.1); universal indicator paper (3.7).

Add three drops of sodium sulphite solution (3.2) and mix.

Inject a 10 μl portion of the solution into the liquid chromatograph (4.2).

Compare this chromatogram with the chromatogram obtained as described in paragraph 5 for the same sample.

FIFTH COMMISSION DIRECTIVE 93/73/EEC

Fifth Commission Directive 93/73/EEC of 9 September 1993 on the methods of analysis necessary for checking composition of cosmetic products

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 last amended by Directive 93/35/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 laid down by Community provisions concerning the composition of cosmetic products are satisfied;

Whereas all the necessary methods of analysis should be laid down as quickly as possible; whereas four steps have already been taken by Commission Directive 80/1335/EEC ⁽³⁾ as amended by Directive 87/143/EEC ⁽⁴⁾, Commission Directive 82/434/EEC ⁽⁵⁾, as amended, by Directive 90/207/EEC ⁽⁶⁾ and Commission Directives 83/514/EEC ⁽⁷⁾ and 85/490/EEC ⁽⁸⁾; whereas the identification and determination of silver nitrate, the identification and determination of selenium disulphide in antidandruff shampoos, the determination of soluble barium and soluble strontium in pigments in the form of salts or lakes, the identification and determination of benzyl alcohol, the identification of zirconium, and the determination of zirconium, aluminium and chlorine in non-aerosol antiperspirants and the identification and determination of hexamidine, dibromohexamidine, dibromopamidine and chlorhexidine, constitute a fifth step;

Whereas the measures provided for in this Directive are in accordance 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 during official testing of cosmetic products, the:

- identification and determination of silver nitrate,
- identification and determination of selenium disulphide in anti-dandruff shampoos,

⁽¹⁾ OJ No L 262, 27. 9. 1976, p. 169.

⁽²⁾ OJ No L 151, 23. 6. 1993, p. 32.

⁽³⁾ OJ No L 383, 31. 12. 1980, p. 27.

⁽⁴⁾ OJ No L 57, 27. 2. 1987, p. 56.

⁽⁵⁾ OJ No L 185, 30. 6. 1982, p. 1.

⁽⁶⁾ OJ No L 108, 28. 4. 1990, p. 92.

⁽⁷⁾ OJ No L 291, 24. 10. 1983, p.9.

⁽⁸⁾ OJ No L 295, 7. 11. 1985, p. 30.

- determination of soluble barium and soluble strontium in pigments in the form of salts or lakes,
- identification and determination of benzyl alcohol,
- identification of zirconium, and determination of zirconium, aluminium and chlorine in non-aerosol antiperspirants,
- identification and determination of hexamidine, dibromohexamidine, dibromopropamidine and chlorhexidine,

shall be carried out in accordance with the methods described in the Annex.

Article 2

1. Member States shall bring into force the laws, regulations or administrative provisions needed to comply with this Directive no later than 30 September 1994. They shall forthwith inform the Commission thereof.

When Member States adopt these provisions, these shall contain a reference to this Directive or shall be accompanied by such reference at the time of their official publication. The procedure for such reference shall be adopted by Member States.

2. Member States shall communicate to the Commission the provisions of national law which they adopt in the field covered by this Directive.

Article 3

This Directive is addressed to the Member States.

Done at Brussels, 9 September 1993.

For the Commission
Christiane SCRIVENER
Member of the Commission

ANNEX**IDENTIFICATION AND DETERMINATION OF SILVER NITRATE IN COSMETIC PRODUCTS****A. IDENTIFICATION****1. SCOPE AND FIELD OF APPLICATION**

This method describes the identification of silver nitrate as silver in aqueous cosmetic products.

2. PRINCIPLE

Silver is identified by the characteristic white precipitate formed with chloride ions.

3. REAGENTS

All reagents must be of analytical purity.

3.1 Hydrochloric acid solution, 2 M.

3.2 Ammonia solution: dilute concentrated ammonium hydroxide solution ($d_{20} = 0,88$ g/ml) with an equal quantity of water and mix.

3.3 Nitric acid solution, 2 M.

4. APPARATUS

4.1 Normal laboratory equipment

4.2 Centrifuge

5. PROCEDURE

5.1 To about 1 g of sample in a centrifuge tube add 2 M hydrochloric acid solution (3.1) dropwise until precipitation is complete; mix and centrifuge.

5.2 Discard the supernatant liquid and wash the precipitate once with five drops of cold water. Reject the washings.

5.3 Add a quantity of water equal to the bulk of precipitate in the centrifuge tube. Heat to boiling and stir.

5.4 Centrifuge hot; discard the supernatant liquid.

5.5 To the precipitate add a few drops of ammonia solution (3.2); mix and centrifuge.

5.6 To one drop of the supernatant liquid on a glass slide add a few drops of 2 M nitric acid solution (3.3).

5.7 A white precipitate indicates the presence of silver.

B. DETERMINATION

1. SCOPE AND FIELD OF APPLICATION

This method is suitable for the determination of silver nitrate as silver in cosmetic products intended to dye eyelashes or eyebrows.

2. PRINCIPLE

Silver is determined in the product by atomic absorption spectrometry.

3. REAGENTS

All reagents must be of analytical purity.

3.1 Nitric acid solution, 0,02 M.

3.2 Silver standard solutions

3.2.1 Stock silver standard solution, 1 000 µg/ml in 0,5 M nitric acid solution ('Spectrosol' or equivalent)

3.2.2 Silver standard solution, 100 µg/ml: transfer by pipette 10 ml of the stock silver standard solution (3.2.1) into a 100-ml volumetric flask. Make up to volume with 0,02 M nitric acid solution (3.1) and mix. This standard solution should be freshly prepared and stored in a dark-coloured glass bottle.

4. APPARATUS

4.1 Normal laboratory equipment

4.2 Atomic absorption spectrophotometer equipped with a silver hollow-cathode lamp

5. PROCEDURE

5.1 Sample preparation

Weigh accurately approximately 0,1 g (m gram) of an homogenous sample of the product. Transfer quantitatively into a one-litre volumetric flask and make up to volume with 0,02 M nitric acid solution (3.1) and mix.

5.2 Conditions for atomic absorption spectrometry

Flame: air-acetylene

Wavelength: 338,3 nm

Background correction: yes

Fuel condition: lean; for maximum absorbance, optimization of burner height and fuel conditions will be necessary.

5.3 Calibration

5.3.1 Into a series of 100-ml volumetric flasks transfer by pipette 1,0, 2,0, 3,0, 4,0 and 5,0 ml of the silver standard solution (3.2.2). Make up each flask to volume with 0,02 M nitric acid solution (3.1) and mix. These solutions contain 1,0, 2,0, 3,0, 4,0 and 5,0 µg silver per millilitre, respectively.

5.3.2 Measure the absorbance of a 0,02 M nitric acid solution (3.1) and use the value obtained as the zero silver concentration for the calibration curve. Measure the absorbance of each silver calibration standard (5.3.1). Plot a calibration curve relating absorbance values to silver concentration.

5.4 **Determination**

Measure the absorbance of the sample solution (5.1). From the calibration curve read off the concentration of silver corresponding to the absorbance value obtained for the sample solution.

6. **CALCULATION**

Calculate the silver nitrate content of the sample, in percentage by mass (% m/m), using the formula:

$$\% \text{ (m/m) of silver nitrate} = \frac{1,5748 \times c}{10 \times m}$$

in which:

m = mass in grams of the sample taken for analysis (5.1);

and

c = concentration of silver in the sample solution (5.1), in micrograms per millilitre, obtained from the calibration curve.

7. **REPEATABILITY (1)**

For a silver nitrate content of 4 % (m/m) the difference between the results of two determinations carried out in parallel on the same sample should not exceed 0,05 % (m/m).

IDENTIFICATION AND DETERMINATION OF SELENIUM DISULPHIDE IN ANTI-DANDRUFF SHAMPOOS

A. IDENTIFICATION

1. SCOPE AND FIELD OF APPLICATION

This method describes the identification of selenium disulphide as selenium in anti-dandruff shampoos.

2. PRINCIPLE

Selenium is identified by the characteristic yellow to orange colour produced on reaction with urea and potassium iodide.

3. REAGENTS

All reagents must be of analytical purity.

(1) ISO 5725.

- 3.1 Nitric acid, concentrated = ($d_{20} = 1,42$ g/ml)
- 3.2 Urea
- 3.3 Potassium iodide solution, 10% (m/v): dissolve 10 g of potassium iodide in 100 ml of water

4. APPARATUS

- 4.1 Normal laboratory equipment
- 4.2 Digestion tube, 100-ml capacity
- 4.3 Heated-block digester
- 4.4 Filter paper (Whatman No 42 or equivalent) or a 0,45 μ m membrane filter

5. PROCEDURE

- 5.1 To approximately 1 g of shampoo in a digestion tube (4.2) add 2,5 ml of concentrated nitric acid (3.1) and digest at 150 °C for 30 minutes on a heated-block digester (4.3).
- 5.2 Dilute the digested sample to 25 ml with water and filter through a filter paper or a 0,45 μ m membrane filter (4.4).
- 5.3 To 2,5 ml of the filtrate add 5 ml water, 2,5 g urea (3.2) and boil. Cool and add 1 ml of potassium iodide solution (3.3).
- 5.4 A yellow to orange colour which darkens rapidly on standing indicates the presence of selenium.

B. DETERMINATION

1. SCOPE AND FIELD OF APPLICATION

This method is suitable for the determination of selenium disulphide as selenium in anti-dandruff shampoos containing up to 4,5 % (m/m) selenium disulphide.

2. PRINCIPLE

The sample is digested with nitric acid and the selenium in the resultant digest determined by means of atomic absorption spectrometry.

3. REAGENTS

All reagents must be of analytical purity.

- 3.1 Nitric acid, concentrated ($d_{20} = 1,42$ g/ml)
- 3.2 Nitric acid solution, 5 % (v/v): add 50 ml concentrated nitric acid (3.1) to 500 ml of water in a beaker, stirring continuously. Transfer this solution to a one-litre volumetric flask and make up to volume with water.
- 3.3 Stock selenium standard solution, 1 000 μ g/ml in 0,5 M nitric acid solution ('SpectrosoL' or equivalent)

4. APPARATUS

- 4.1 Normal laboratory equipment
- 4.2 Digestion tube, 100-ml capacity
- 4.3 Heated-block digester
- 4.4 Filter paper (Whatman No 42 or equivalent) or a 0,45 µm membrane filter
- 4.5 Atomic absorption spectrophotometer equipped with a selenium hollow-cathode lamp

5. PROCEDURE

5.1 Sample Preparation

- 5.1.1 Weigh accurately approximately 0,2 g (m gram) of an homogenous sample of shampoo into a digestion tube (4.2).
- 5.1.2 Add 5 ml of concentrated nitric acid (3.1) and digest at 150 °C for one hour on a heated-block digester (4.3).
- 5.1.3 Allow solution to cool and dilute to 100 ml with water. Filter through a filter paper or a 0,45 µm membrane filter (4.4) and retain the filtered solution for the determination.

5.2 Conditions for atomic absorption spectrometry

Flame: air-acetylene

Wavelength: 196,0 nm

Background correction: yes

Fuel condition: lean; for maximum absorbance, optimization of burner height and fuel conditions will be necessary.

5.3 Calibration

- 5.3.1 Into a series of 100-ml volumetric flasks, transfer by pipette 1,0, 2,0, 3,0, 4,0 and 5,0 ml of the stock selenium standard solution (3.3). Make up each flask to volume with 5 % (v/v) nitric acid solution (3.2) and mix. These solutions contain 10, 20, 30, 40 and 50 µg selenium per millilitre, respectively.
- 5.3.2 Measure the absorbance of a 5 % (v/v) nitric acid solution (3.2) and use the value obtained as the zero selenium concentration for the calibration curve. Measure the absorbance of each selenium calibration standard (5.3.1). Plot a calibration curve relating absorbance values to selenium concentration.

5.4 Determination

Measure the absorbance of the sample solution (5.1.3). From the calibration curve read off the concentration of selenium corresponding to the absorbance value obtained for the sample solution.

6. CALCULATION

Calculate the selenium disulphide content of the sample, in percentage by mass (% m/m), using the formula:

$$\% \text{ (m/m) of selenium disulphide} = \frac{1,812 \times c}{100 \times m}$$

in which

m = mass in grams of the sample taken for analysis (5.1.1);
and

c = concentration of selenium in the sample solution (5.1.3), in
micrograms per millilitre, obtained from the calibration curve.

7. REPEATABILITY (1)

For a selenium disulphide content of 1 % (m/m) the difference between the results of two determinations carried out in parallel on the same sample should not exceed 0,05 % (m/m).

DETERMINATION OF SOLUBLE BARIUM AND STRONTIUM IN PIGMENTS IN THE FORM OF SALTS OR LAKES

A. DETERMINATION OF SOLUBLE BARIUM

1. SCOPE AND FIELD OF APPLICATION

This method describes the procedure for extracting and determining soluble barium from pigments in the form of salts or lakes.

2. PRINCIPLE

The pigment is extracted with 0,07 M hydrochloric acid solution under defined conditions and the amount of barium in the extractant determined by atomic absorption spectrometry.

3. REAGENTS

All reagents must be of analytical purity.

3.1 Ethanol, absolute

3.2 Hydrochloric acid solution, 0,07 M

3.3 Hydrochloric acid solution, 0,5 M

3.4 Potassium chloride solution, 8 % (m/v): dissolve 16 g of potassium chloride in 200 ml of 0,07 M hydrochloric acid solution (3.2).

3.5 Barium standard solutions

3.5.1 Stock barium standard solution, 1 000 µg/ml in 0,5 M nitric acid solution ('SpectrosoL' or equivalent)

3.5.2 Barium standard solution, 200 µg/ml: transfer by pipette 20,0 ml of the stock barium standard solution (3.5.1) into a 100-ml volumetric flask. Make up to volume with 0,07 M hydrochloric acid solution (3.2) and mix.

(1) ISO 5725.

4. APPARATUS

- 4.1 Normal laboratory equipment
- 4.2 pH meter with an accuracy of $\pm 0,02$ units
- 4.3 Wrist-action flask-shaker
- 4.4 Membrane filter with a pore size of $0,45 \mu\text{m}$
- 4.5 Atomic absorption spectrophotometer equipped with a barium hollow-cathode lamp

5. PROCEDURE

5.1 Sample preparation

- 5.1.1 Weigh accurately approximately 0,5 g (m gram) of pigment into conical flask. To ensure sufficient volume for effective agitation a flask of capacity less than 150-ml shall not be used.
- 5.1.2 Add by pipette 1,0 ml of ethanol (3.1) and rotate the flask to ensure thorough wetting of the pigment. Add from a burette the exact quantity of 0,07 M hydrochloric acid solution (3.2) required to give a volume-of-acid to mass-of-pigment ratio of exactly 50 millilitres per gram. Let the total volume of extractant including the ethanol be V ml. Swirl the contents of the flask for five seconds to ensure thorough mixing of the contents.
- 5.1.3 Using a pH meter (4.2) measure the pH of the resultant suspension and, if it is above 1,5, add 0,5 M hydrochloric acid solution (3.3) dropwise until in the range 1,4 to 1,5.
- 5.1.4 Stopper and immediately shake the flask for 60 minutes using a wrist-action flask-shaker (4.3). The shaker must be operated at a sufficiently high speed to produce a foam. Filter through a $0,45 \mu\text{m}$ membrane filter (4.4) and collect the filtrate. Do not centrifuge the extract before filtering. Transfer by pipette 5,0 ml of the filtrate to a 50-ml volumetric flask; make up to volume with 0,07 M hydrochloric acid solution (3.2) and mix. This solution is also used for the determination of strontium (Part B).
- 5.1.5 Into a 100-ml volumetric flask transfer by pipette 5,0 ml potassium chloride solution (3.4) and an aliquot (W_{Ba} ml) of the diluted filtrate (5.1.4) to give an expected concentration of between 3 and $10 \mu\text{g}$ barium per millilitre. (An aliquot of 10 ml should be a satisfactory starting point.) Make up to volume with 0,07 M hydrochloric acid solution (3.2) and mix.
- 5.1.6 Determine the barium concentration of the solution (5.1.5) by atomic absorption spectrometry on the same day.

5.2 Conditions for atomic absorption spectrometry

Flame: nitrous oxide/acetylene

Wavelength: 553,5 nm

Background correction: no

Fuel condition: lean; for maximum absorbance, optimization of burner height and fuel conditions will be necessary.

5.3 Calibration

- 5.3.1 Into a series of 100-ml volumetric flasks transfer by pipette 1,0, 2,0, 3,0, 4,0 and 5,0 ml of the barium standard solution (3.5.2). To each flask transfer by pipette 5,0

ml potassium chloride solution (3.4); make up to volume with 0,07 M hydrochloric acid solution (3.2) and mix. These solutions contain 2,0, 4,0, 6,0, 8,0 and 10,0 µg barium per millilitre, respectively.

Similarly, prepare a blank solution omitting the barium standard solution.

5.3.2 Measure the absorbance of the blank solution (5.3.1) and use the value obtained as the zero barium concentration for the calibration curve. Measure the absorbance of each barium calibration standard (5.3.1). Plot a calibration curve relating absorbance values to barium concentration.

5.4 **Determination**

Measure the absorbance of the sample solution (5.1.5). From the calibration curve read off the concentration of barium corresponding to the absorbance value obtained for the sample solution.

6. **CALCULATION**

The soluble barium content (% m/m) of the pigment is given by the formula:

$$\% \text{ (m/m) of soluble barium} = \frac{c \times V}{10W_{\text{Ba}} \times m}$$

in which

m = mass in grams of the sample taken for analysis (5.1.1);

c = concentration of barium in the sample solution (5.1.5), in micrograms per millilitre, obtained from the calibration curve;

V = total volume of extractant in millilitres (5.1.2);

and

W_{Ba} = volume of extract, in millilitres, taken in 5.1.5.

7. **REPEATABILITY**

The best available estimate of the repeatability (ISO 5725) for this method is 0,3 % for a soluble barium content of 2 % (m/m).

8. **REMARKS**

8.1 Under certain conditions the barium absorbance can be enhanced by the presence of calcium. This can be countered by the addition of magnesium ion at a concentration of 5 g per litre ⁽¹⁾.

8.2 The use of inductively-coupled plasma - optical emission spectrometry is permitted as an alternative to flame atomic absorption spectrometry.

⁽¹⁾ 'Magnesium as modifier for the determination of barium by flame atomic emission spectrometry'. Jerrow M. et al, Analytical Proceedings, 1991, 28, 40.

B. DETERMINATION OF SOLUBLE STRONTIUM

1. SCOPE AND FIELD OF APPLICATION

This method describes the procedure for extracting and determining soluble strontium from pigments in the form of salts or lakes.

2. PRINCIPLE

The pigment is extracted with 0,07 M hydrochloric acid solution under defined conditions and the amount of strontium in the extractant determined by atomic absorption spectrometry.

3. REAGENTS

All reagents must be of analytical purity.

3.1 Ethanol, absolute

3.2 Hydrochloric acid solution, 0,07 M

3.3 Potassium chloride solution, 8 % (m/v): dissolve 16 g of potassium chloride in 200 ml of 0,07 M hydrochloric acid solution (3.2).

3.4. Strontium standard solutions

3.4.1 Stock strontium solution 1,000 µg/ml in 0,5 M nitric acid solution ('SpectrosoL' or equivalent)

3.4.2 Strontium standard solution, 100 µg/ml: transfer by pipette 10,0 ml of the stock strontium standard solution (3.4.1) into a 100-ml volumetric flask. Make up to volume with 0,07 M hydrochloric acid solution (3.2) and mix.

4. APPARATUS

4.1 Normal laboratory equipment

4.2 Membrane filter with a pore size of 0,45 µm

4.3 Atomic absorption spectrophotometer equipped with a strontium hollow-cathode lamp

5. PROCEDURE

5.1 Sample preparation

The solution prepared in A.5.1.4 is used to determine the soluble strontium content.

5.1.1 Into a 100-ml volumetric flask transfer by pipette 5,0 ml potassium chloride solution (3.3) and an aliquot (W_{Sr} ml) of the diluted filtrate (A.5.1.4) to give an expected concentration of between 2 and 5 µg strontium per millilitre. (An aliquot of 25 ml should be a satisfactory starting point.) Make up to volume with 0,07 M hydrochloric acid solution (3.2) and mix.

5.1.2 Determine the strontium concentration of the solution (5.1.1) by atomic absorption spectrometry on the same day.

5.2 Conditions for atomic absorption spectrometry

Flame: nitrous oxide/acetylene

Wavelength: 460,7 nm

Background correction: no

Fuel condition: lean; for maximum absorbance, optimization of burner height and fuel conditions will be necessary.

5.3 Calibration

5.3.1 Into a series of 100-ml volumetric flasks transfer by pipette 1,0, 2,0, 3,0, 4,0 and 5,0 ml of the strontium standard solution (3.4.2). To each flask transfer by pipette 5,0 ml potassium chloride solution (3.3); make up to volume with 0,07 M hydrochloric acid solution (3.2) and mix. These solutions contain 1,0, 2,0, 4,0, and 5,0 µg strontium per millilitre, respectively.

Similarly, prepare a blank solution omitting the strontium standard solution.

5.3.2 Measure the absorbance of the blank solution (5.3.1) and use the value obtained as the zero strontium concentration for the calibration curve. Measure the absorbance of each strontium calibration standard (5.3.1). Plot a calibration curve relating peak absorbance values to strontium concentration.

5.4 Determination

Measure the absorbance of the sample solution (5.1.1). From the calibration curve read off the concentration of strontium corresponding to the absorbance value obtained for the sample solution.

6. CALCULATION

The soluble strontium content (% m/m) of the pigment is given by the formula:

$$\% \text{ (m/m) of soluble strontium} = \frac{c \times V}{100W_{Sr} \times m}$$

in which:

m = mass in grams of the sample taken for analysis (A.5.1.1);

c = concentration of strontium in the sample solution in micrograms per millilitre, obtained from the calibration curve;

V = volume of extractant in millilitres (A.5.1.2);

and

W_{Sr} = volume of extract, in millilitres, taken in 5.1.1.

7. REPEATABILITY

The best available estimate of the repeatability (ISO 5725) for this method is 0,09 % for a soluble strontium content of 0,6 % (m/m).

8. REMARK

The use of inductively-coupled plasma – optical emission spectrometry is permitted as an alternative to flame atomic absorption spectrometry.

IDENTIFICATION AND DETERMINATION OF BENZYL ALCOHOL IN COSMETIC PRODUCTS**A. IDENTIFICATION****1. SCOPE AND FIELD OF APPLICATION**

This method describes the identification of benzyl alcohol in cosmetic products.

2. PRINCIPLE

Benzyl alcohol is identified by means of thin-layer chromatography on silica gel plates.

3. REAGENTS

All reagents must be of analytical purity.

- 3.1 Benzyl alcohol
- 3.2 Chloroform
- 3.3 Ethanol, absolute
- 3.4 n-Pentane
- 3.5 Development solvent: diethyl ether
- 3.6 Standard solution of benzyl alcohol: weigh 0,1 g of benzyl alcohol (3.1) into a 100-ml volumetric flask, make up to volume with ethanol (3.3) and mix.
- 3.7 Thin-layer chromatography plates, glass, 100 x 200 mm or 200 x 200 mm, coated with a 0,25 mm layer of silica gel 60 F₂₅₄.
- 3.8 Visualizing agent: 12-molybdophosphoric acid, 10 % (m/v) in ethanol (3.3).

4. APPARATUS

- 4.1 Normal apparatus for thin-layer chromatography
- 4.2 Chromatography tank, double trough chamber, overall dimensions of approximately 80 mm x 230 mm x 240 mm
- 4.3 Chromatography paper: Whatman, or equivalent
- 4.4 Ultra-violet lamp, wavelength 254 nm.

5. PROCEDURE

5.1 Sample preparation

Weigh 1,0 g of the product to be analysed into a 10-ml volumetric flask. Add 3 ml of chloroform (3.2) and shake vigorously until the product has dispersed. Make up to volume with ethanol (3.3) and shake vigorously to produce a clear, or almost clear, solution.

5.2 Thin-layer chromatography

5.2.1 Saturate the chromatography tank (4.2) with n-pentane (3.4) as follows: line the wall of the chamber adjacent to the back through with chromatography paper (4.3), ensuring that the lower edge of the paper is in the trough. Transfer 25 ml of n-pentane (3.4) into the back trough by pouring this solvent over the exposed surface of the chromatography paper lining. Immediately replace the lid and allow the tank to stand for 15 minutes.

5.2.2 Deposit 10 µl of the sample solution (5.1) and 10 µl of the benzyl alcohol standard solution (3.6) at suitable points on the start line of a thin-layer chromatography plate (3.7). Allow to dry.

5.2.3 Pipette 10 ml of diethyl ether (3.5) into the front through of the tank and immediately afterwards place the plate (5.2.2) into the same trough. Quickly replace the lid of the tank, and develop the plate over a distance of 150 mm. Remove the plate from the chromatography tank and allow it to dry at room temperature.

5.2.4 Observe the plate under ultra-violet light and mark the position of the violet spots. Spray the plate with the visualizing agent (3.8) and then heat the plate at 120 °C for about 15 minutes. Benzyl alcohol appears as a dark blue spot.

5.2.5 Calculate the R_f value obtained from the benzyl alcohol standard solution. A dark blue spot with the same R_f value obtained from the sample solution indicates the presence of benzyl alcohol.

Detection limit: 0,1 µg benzyl alcohol.

B. DETERMINATION

1. SCOPE AND FIELD OF APPLICATION

This method describes the determination of benzyl alcohol in cosmetic products.

2. DEFINITION

The amount of benzyl alcohol determined by this method is expressed as a percentage by mass (% m/m).

3. PRINCIPLE

The sample is extracted with methanol and the amount of benzyl alcohol in the extract determined by high-performance liquid chromatography (HPLC).

4. REAGENTS

All reagents must be of analytical purity and suitable for HPLC, where appropriate.

4.1 Methanol

4.2 4-Ethoxyphenol

4.3 Benzyl alcohol

4.4 Mobile phase: methanol (4.1)/water (45:55; v/v)

4.5 Benzyl alcohol stock solution: weigh accurately approximately 0,1 g of benzyl alcohol (4.3) into a 100-ml volumetric flask. Make up to volume with methanol (4.1) and mix.

4.6 Internal standard stock solution: weigh accurately approximately 0,1 g of 4-ethoxyphenol (4.2) into a 100-ml volumetric flask. Make up to volume with methanol (4.1) and mix.

4.7 Standard solutions: into a series of 25-ml volumetric flasks, transfer by pipette amounts of benzyl alcohol stock solution (4.5) and internal standard stock solution (4.6) according to the table set out below. Make up to volume with methanol (4.1) and mix.

Standard solution	Benzyl alcohol concentration		4-ethoxyphenol concentration	
	ml (4.5) added	µg/ml(*)	ml (4.6) added	µg/ml (*)
I	0,5	20	2,0	80
II	1,0	40	2,0	80
III	2,0	80	2,0	80
IV	3,0	120	2,0	80
V	5,0	200	2,0	80

(*) These values are given as an indication and correspond to the concentrations of standard solutions prepared using solutions of benzyl alcohol (4.5) and 4-ethoxyphenol (4.6) which contain exactly 0,1 % (m/v) benzyl alcohol and exactly 0,1 % (m/v) 4-ethoxyphenol, respectively.

5. APPARATUS

5.1 Normal laboratory equipment

5.2 High-performance chromatography equipment with a variable wavelength ultra-violet detector and 10 µl injection loop

5.3 Analytical column: 250 mm x 4,6 mm stainless steel column packed with 5 µm Spherisorb ODS, or equivalent.

5.4 Water-bath

5.5 Ultrasonic bath

5.6 Centrifuge

5.7 Centrifuge tubes, 15-ml capacity

6. PROCEDURE

6.1 Sample preparation

- 6.1.1 Weigh accurately approximately 0,1 g (m gram) of sample into a centrifuge tube (5.7) and add 5 ml methanol (4.1).
- 6.1.2 Heat for 10 minutes in a water-bath (5.4) maintained at 50 °C, then place the tube in an ultrasonic bath (5.5) until the sample is thoroughly dispersed.
- 6.1.3 Cool, then centrifuge at 3 500 rpm for five minutes.
- 6.1.4 Transfer the supernatant liquid to a 25-ml volumetric flask.
- 6.1.5 Re-extract the sample with a further 5 ml methanol (4.1). Combine the extracts in the 25-ml volumetric flask.
- 6.1.6 Transfer by pipette 2,0 ml of internal standard stock solution (4.6) into the 25-ml volumetric flask. Make up to volume with methanol (4.1) and mix. This solution is used in the determination stage of the analysis described in 6.4.

6.2 Chromatography

- 6.2.1 Set up the high-performance liquid chromatography equipment (5.2) in the usual manner. Adjust the flow rate of the mobile phase (4.4) to 2,0 ml per minute.
- 6.2.2 Set the wavelength of the UV detector (5.2) to 210 nm.

6.3 Calibration

- 6.3.1 Inject 10 µl of each of the benzyl alcohol standard solutions (4.7) and measure the areas of the benzyl alcohol and the 4-ethoxyphenol peaks.
- 6.3.2 For each benzyl alcohol standard solution (4.7) calculate the peak-area ratio of benzyl alcohol to 4-ethoxyphenol. Plot a calibration curve using these ratios as the ordinate and the corresponding concentrations of benzyl alcohol in µg per millilitre as abscissa.

6.4 Determination

- 6.4.1 Inject 10 µl of the sample solution (6.1.6) and measure the areas of the benzyl alcohol and the 4-ethoxyphenol peaks. Calculate the peak-area ratio of benzyl alcohol to 4-ethoxyphenol. Repeat this process with further 10 µl aliquots of the sample solution until consistent results are obtained.
- 6.4.2 From the calibration curve (6.3.2) read off the concentration of benzyl alcohol corresponding to the peak area ratio of benzyl alcohol to 4-ethoxyphenol.

7. CALCULATION

Calculate the benzyl alcohol content of the sample, as a percentage by mass, using the formula:

$$\% \text{ (m/m) of benzyl alcohol} = \frac{c}{400 \times m}$$

in which:

m = mass in grams of the sample taken for analysis (6.1.1);
and

c = concentration of benzyl alcohol in the sample solution (6.1.6), in micrograms per millilitre, obtained from the calibration curve.

8. REPEATABILITY (1)

For a benzyl alcohol content of 1 % (m/m) the difference between the results of two determinations carried out in parallel on the same sample should not exceed 0,10 %.

IDENTIFICATION OF ZIRCONIUM, AND DETERMINATION OF ZIRCONIUM, ALUMINIUM AND CHLORINE IN NON-AEROSOL ANTIPERSPIRANTS

The method comprises five stages:

- A. Identification of zirconium
- B. Determination of zirconium
- C. Determination of aluminium
- D. Determination of chlorine
- E. Calculation of the ratios of aluminium atoms to zirconium atoms, and of aluminium plus zirconium atoms to chlorine atoms

A. IDENTIFICATION OF ZIRCONIUM

1. SCOPE AND FIELD OF APPLICATION

The method describes the identification of zirconium in non-aerosol antiperspirant cosmetic products. No attempt has been made to describe methods suitable for the identification of the aluminium zirconium chloride hydroxide complex $[Al_xZr(OH)_yCl_z \cdot nH_2O]$.

2. PRINCIPLE

Zirconium is identified by the characteristic red-violet precipitate produced with alizarin red S under strongly acidic conditions.

3. REAGENTS

All reagents must be of analytical purity.

- 3.1 Hydrochloric acid, concentrated ($d_{20} = 1,18$ g/ml)
- 3.2 Alizarin red S (CI 58005) solution: 2 % (m/v) aqueous sodium alizarin sulphonate.

4. APPARATUS

- 4.1 Normal laboratory equipment

(1) ISO 5725.

5. PROCEDURE

- 5.1 To about 1 g of sample in a test tube add 2 ml of water. Stopper and shake.
- 5.2 Add three drops of alizarin red S solution (3.2) followed by 2 ml of concentrated hydrochloric (3.1). Stopper and shake.
- 5.3 Leave to stand for approximately two minutes.
- 5.4 A red-violet coloured supernatant solution and precipitate indicates the presence of zirconium.

B. DETERMINATION OF ZIRCONIUM

1. SCOPE AND FIELD OF APPLICATION

This method is suitable for the determination of zirconium in aluminium zirconium chloride hydroxide complexes up to a maximum concentration of 7,5 % (m/m) zirconium in non-aerosol antiperspirants.

2. PRINCIPLE

Zirconium is extracted from the product under acidic conditions and determined by flame atomic absorption spectrometry.

3. REAGENTS

All reagents must be of analytical purity

- 3.1 Hydrochloric acid, concentrated ($d_{20} = 1,18$ g/ml)
- 3.2 Hydrochloric acid solution, 10 % (v/v): add 100 ml concentrated hydrochloric acid (3.1) to 500 ml of water in a beaker, stirring continuously. Transfer this solution to a one-litre volumetric flask and make up to volume with water.
- 3.3 Stock zirconium standard solution, 1 000 µg/ml in 0,5 M hydrochloric acid solution ('SpectrosoL' or equivalent).
- 3.4 Aluminium chloride (hydrated) $[\text{AlCl}_3 \cdot 6\text{H}_2\text{O}]$ reagent: dissolve 22,6 g of aluminium chloride hexahydrate in 250 ml of 10 % (v/v) hydrochloric acid solution (3.2).
- 3.5 Ammonium chloride reagent: dissolve 5,0 g of ammonium chloride in 250 ml of 10 %, (v/v) hydrochloric acid solution (3.2).

4. APPARATUS

- 4.1 Normal laboratory equipment
- 4.2 Heater with magnetic stirrer
- 4.3 Filter paper (Whatman No 41 or equivalent)
- 4.4 Atomic absorption spectrophotometer equipped with a zirconium hollow-cathode lamp

5. PROCEDURE

5.1 Sample preparation

5.1.1 Weigh accurately approximately 1,0 g (m gram) of an homogeneous sample of the product into a 150-ml beaker. Add 40 ml of water and 10 ml of concentrated hydrochloric acid (3.1).

5.1.2 Place the beaker on a heater with a magnetic stirrer (4.2). Commence stirring and heat to boiling. To prevent rapid drying place a watch-glass on top of the beaker. Boil for five minutes, remove beaker from heat and cool to room temperature.

5.1.3 Using the filter paper (4.3), filter the contents of the beaker into a 100-ml volumetric flask. Rinse the beaker with two 10-ml portions of water and add the washings after filtration to the flask. Make up to volume with water and mix. This solution is also used for the determination of aluminium (Part C).

5.1.4 Into a 50-ml volumetric flask transfer by pipette 20,00 ml of the sample solution (5.1.3), 5,00 ml of the aluminium chloride reagent (3.4) and 5,00 ml of the ammonium chloride reagent (3.5). Make up to volume with 10 % (v/v) hydrochloric acid solution (3.2) and mix.

5.2 Conditions for atomic absorption spectrometry

Flame: nitrous oxide/acetylene

Wavelength: 360,1 nm

Background correction: no

Fuel condition: rich; for maximum absorbance, optimization of burner height and fuel conditions will be necessary.

5.3 Calibration

5.3.1 Into a series of 50-ml volumetric flasks transfer by pipette 5,00, 10,00, 15,00, 20,00 and 25,00 ml of the stock zirconium standard solution (3.3). To each volumetric flask transfer by pipette 5,00 ml of the aluminium chloride reagent (3.4) and 5,00 ml of the ammonium chloride reagent (3.5). Make up to volume with 10 % (v/v) hydrochloric acid solution (3.2) and mix. These solutions contain 100, 200, 300, 400 and 500 µg of zirconium per millilitre respectively.

Similarly, prepare a blank solution omitting the zirconium standard solution.

5.3.2 Measure the absorbance of the blank solution (5.3.1) and use the value obtained as the zero zirconium concentration for the calibration curve. Measure the absorbance of each zirconium calibration standard (5.3.1). Plot a calibration curve relating absorbance values to zirconium concentration.

5.4 Determination

Measure the absorbance of the sample solution (5.1.4). From the calibration curve read off the concentration of zirconium corresponding to the absorbance value obtained for the sample solution.

6. CALCULATION

Calculate the zirconium content of the sample, in percentage by mass, using the formula:

$$\% \text{ (m/m) of zirconium} = \frac{c}{40 \times m}$$

in which

m = mass in grams of the sample taken for analysis (5.1.1);
and

c = concentration of zirconium in the sample solution (5.1.4), in micrograms per millilitre, obtained from the calibration curve.

7. REPEATABILITY (1)

For a zirconium content of 3,00 % (m/m) the difference between the results of two determinations carried out in parallel on the same sample should not exceed 0,10 % (m/m).

8. REMARK

The use of inductively-coupled plasma - optical emission spectrometry is permitted as an alternative to flame atomic absorption spectrometry.

C. DETERMINATION OF ALUMINIUM

1. SCOPE AND FIELD OF APPLICATION

This method is suitable for the determination of aluminium present in aluminium zirconium chloride hydroxide complexes up to a maximum concentration of 12 % (m/m) aluminium in non-aerosol anti-perspirants.

2. PRINCIPLE

Aluminium is extracted from the product under acidic conditions and determined by flame atomic absorption spectrometry.

3. REAGENTS

All reagents must be of analytical purity.

3.1 Hydrochloric acid, concentrated ($d_{20} = 1,18 \text{ g/ml}$)

3.2 Hydrochloric acid solution, 1 % (v/v): add 10 ml concentrated hydrochloric acid (3.1) to 500 ml of water in a beaker, stirring continuously. Transfer this solution to a one litre volumetric flask and make up to volume with water.

3.3 Stock aluminium standard solution, 1 000 µg/ml in 0,5 M nitric acid solution ('SpectrosoL' or equivalent).

(1) ISO 5725.

- 3.4 Potassium chloride reagent: dissolve 10,0 g of potassium chloride in 250 ml of 1 % (v/v) hydrochloric acid solution (3.2).

4. APPARATUS

- 4.1 Normal laboratory equipment
- 4.2 Atomic absorption spectrophotometer equipped with an aluminium hollow-cathode lamp.

5. PROCEDURE

5.1 Sample preparation

The solution prepared in B.5.1.3 is used to determine the aluminium content.

- 5.1.1 Into a 100-ml volumetric flask transfer by pipette 5,00 ml of the sample solution (B.5.1.3) and 10,00 ml of the potassium chloride reagent (3.4). Make up to volume with 1 % (v/v) hydrochloric acid solution (3.2) and mix.

5.2 Conditions for atomic absorption spectrometry

Flame: nitrous oxide/acetylene

Wavelength: 309,3 nm

Background correction: no

Fuel condition: rich; for maximum absorbance, optimization of burner height and fuel conditions will be necessary.

5.3 Calibration

- 5.3.1 Into a series of 100 ml volumetric flasks transfer by pipette 1,00, 2,00, 3,00, 4,00 and 5,00 ml of the stock aluminium standard solution (3.3). To each volumetric flask transfer by pipette 10,00 ml of the potassium chloride reagent (3.4) and make up to volume with 1 % (v/v) hydrochloric acid solution (3.2) and mix. These solutions contain 10, 20, 30, 40 and 50 µg of aluminium per millilitre.

Similarly, prepare a blank solution omitting the aluminium standard solution.

- 5.3.2 Measure the absorbance of the blank solution (5.3.1) and use the value obtained as the zero aluminium concentration for the calibration curve. Measure the absorbance of each aluminium calibration standard. Plot a calibration curve relating absorbance valued to aluminium concentration.

5.4 Determination

Measure the absorbance of the sample solution (5.1.1). From the calibration curve read off the concentration of aluminium corresponding to the absorbance value obtained for the sample solution.

6. CALCULATION

Calculate the aluminium content of the sample, in percentage by mass, using the formula:

$$\% \text{ (m/m) of aluminium} = \frac{c}{5 \times m}$$

in which:

m = mass in grams of the sample taken for analysis (B.5.1.1);
and

c = concentration of aluminium in the sample solution (B.5.1.1), in
micrograms per millilitre, obtained from the calibration curve.

7. REPEATABILITY (1)

For an aluminium content of 3,5 % (m/m) the difference between the results of two determinations carried out in parallel on the same sample should not exceed 0,10 % (m/m).

8. REMARK

The use of inductively-coupled plasma – optical emission spectrometry is permitted as an alternative to flame atomic absorption spectrometry.

D. DETERMINATION OF CHLORINE

1. SCOPE AND FIELD OF DETERMINATION

This method is suitable for the determination of chlorine present as chloride ion in aluminium zirconium chloride hydroxide complexes in non-aerosol anti-perspirants.

2. PRINCIPLE

Chloride ion in the product is determined by potentiometric titration against standard silver nitrate solution.

3. REAGENTS

All reagents must be of analytical purity.

3.1 Nitric acid, concentrated ($d_{20} = 1,42$ g/ml)

3.2 Nitric acid solution, 5 % (v/v): add 25 ml concentrated nitric acid (3.1) to 250 ml of water in a beaker, stirring continuously. Transfer this solution to a 500-ml volumetric flask and make up to volume with water.

3.3 Acetone

3.4 Silver nitrate, 0,1 M volumetric solution ('AnalaR' or equivalent).

4. APPARATUS

4.1 Normal laboratory equipment

4.2 Heater with magnetic stirrer

4.3 Silver electrode

4.4 Calomel reference electrode

(1) ISO 5725.

4.5 pH/millivolt meter suitable for potentiometric titration

5. PROCEDURE

5.1 Sample preparation

5.1.1 Weigh accurately into a 250-ml beaker approximately 1,0 g (m gram) of an homogenous sample of the product. Add 80 ml of water and 20 ml of 5 % (v/v) nitric acid solution (3.2).

5.1.2 Place the beaker on a heater with a magnetic stirrer (4.2). Commence stirring and heat to boiling. To prevent rapid drying, place a watch-glass on top of the beaker. Boil for five minutes, remove beaker from heat and cool to room temperature.

5.1.3 Add 10 ml of acetone (3.3), dip electrodes (4.3 and 4.4) below surface of solution and commence stirring. Titrate potentiometrically against 0,1 M silver nitrate solution (3.4) and plot a differential curve to determine the endpoint (V ml).

6. CALCULATION

Calculate the chlorine content of the sample, in percentage by mass, using the formula:

$$\% \text{ (m/m) of chlorine} = \frac{0,3545 \times V}{m}$$

in which:

m = mass in grams of the sample taken for analysis (5.1.1)

and

v = volume of 0,1 M silver nitrate, in millilitres, titrated at the endpoint (5.1.3).

7. REPEATABILITY (1)

For a chlorine content of 4 % (m/m) the difference between the results of two determinations carried out in parallel on the same sample should not exceed 0,10 % (m/m).

E. CALCULATION OF THE RATIOS OF ALUMINIUM ATOMS TO ZIRCONIUM ATOMS, AND OF ALUMINIUM PLUS ZIRCONIUM ATOMS TO CHLORINE ATOMS

1. CALCULATION OF RATIO OF ALUMINIUM ATOMS TO ZIRCONIUM ATOMS

Calculate the Al : Zr ratio using the formula:

$$\text{Al : Zr ratio} = \frac{\text{Al \% (m/m)} \times 91,22}{\text{Zr \% (m/m)} \times 26,98}$$

(1) ISO 5725.

2. CALCULATION OF THE RATIO OF ALUMINIUM PLUS ZIRCONIUMS ATOMS TO CHLORINE ATOMS

Calculate the (Al + Zr) : Cl ratio using the formula:

$$(Al + Zr) : Cl \text{ ratio} = \frac{\frac{Al \% (m/m)}{26,98} + \frac{Zr \% (m/m)}{91,22}}{\frac{Cl \% (m/m)}{35,45}}$$

IDENTIFICATION AND DETERMINATION OF HEXAMIDINE, DIBROMOHEXAMIDINE, DIBROMOPROPAMIDINE AND CHLORHEXIDINE

1. SCOPE AND FIELD OF APPLICATION

This method describes the qualitative and quantitative determination of:

- hexamidine and its salts, including the isethionate and the 4-hydroxybenzoate,
- dibromohexamidine and its salts, including the isethionate,
- dibromopropamidine and its salts, including the isethionate,
- chlorhexidine diacetate, digluconate and dihydrochloride in cosmetic products.

2. DEFINITION

The concentrations of hexamidine, dibromohexamidine, dibromopropamidine and chlorhexidine determined by this method are expressed as a percentage by mass (% m/m).

3. PRINCIPLE

The identification and determination is carried out by ion-pair, reversed-phase high-performance liquid chromatography (HPLC) followed by ultra-violet spectrophotometric detection. Hexamidine, dibromohexamidine, dibromopropamidine and chlorhexidine are identified by their retention times on the chromatographic column.

4. REAGENTS

All reagents must be of analytical purity and suitable for HPLC, where appropriate.

- 4.1 Methanol
- 4.2 1-Heptanesulphonic acid, sodium salt, monohydrate
- 4.3 Acetic acid, glacial ($d_{20} = 1,05 \text{ g/ml}$)
- 4.4 Sodium chloride
- 4.5 Mobile phases

4.5.1 Solvent I: 0,005 M solution of 1-heptanesulphonic acid, sodium salt, monohydrate (4.2) in methanol (4.1), adjusted to an apparent pH of 3,5 with glacial acetic acid (4.3).

4.5.2 Solvent II: 0,005 M solution of 1-heptanesulphonic acid, sodium salt, monohydrate (4.2) in water, adjusted to a pH of 3,5 with glacial acetic acid (4.3).

Note:

If necessary to improve the shape of the peaks, the mobile phases may be modified and prepared as follows:

— solvent I: dissolve 5,84 g sodium chloride (4.4) and 1,1013 g of 1-heptanesulphonic acid, sodium salt, monohydrate (4.2) in 100 ml water. Add 900 ml methanol (4.1) and adjust to an apparent pH of 3,5 with glacial acetic acid (4.3),

— solvent II: dissolve 5,84 g sodium chloride (4.4) and 1,1013 g of 1-heptanesulphonic acid, sodium salt, monohydrate (4.2) in one litre of water and adjust to a pH of 3,5 with glacial acetic acid (4.3).

4.6 Hexamidine diisethionate [C₂₀H₂₆N₄O₂·2C₂H₆O₄S]

4.7 Dibromohexamidine diisethionate [C₂₀H₂₄Br₂N₄O₂·2C₂H₆O₄S]

4.8 Dibromopropamide diisethionate [C₁₇H₁₈Br₂N₄O₂·2C₂H₆O₄S]

4.9 Chlorhexidine diacetate [C₂₂H₃₀Cl₂N₁₀·2C₂H₄O₂]

4.10 Reference solutions: prepare 0,05 % (m/v) solutions of each of the four preservatives (4.6 to 4.9) in solvent I (4.5.1).

4.11 3,4,4'-Trichlorocarbanilide (triclocarban)

4.12 4,4'-Dichloro-3-(trifluoromethyl)carbanilide (halocarban)

5. APPARATUS

5.1 Normal laboratory equipment

5.2 High-performance liquid chromatograph with variable-wavelength UV detector

5.3 Analytical column: stainless steel, length 30 cm, internal diameter 4 mm, packed with μ -Bondapack C₁₈, 10 μ m, or equivalent

5.4 Ultrasonic bath

6. IDENTIFICATION

6.1 Sample preparation

Weigh approximately 0,5 g of sample into a 10-ml volumetric flask and make up to volume with solvent I (4.5.1). Place the flask in an ultrasonic bath (5.4) for 10 minutes. Filter or centrifuge the solution. Collect the filtrate or supernatant for chromatography.

6.2 Chromatography

6.2.1 Mobile-phase gradient

Time (min)	Solvent I (% v/v) (4.5.1)	Solvent II (% v/v) (4.5.2)
------------	------------------------------	-------------------------------

0	50	50
15	65	35
30	65	35
45	50	50

- 6.2.2 Adjust the flow rate of the mobile phase (6.2.1) to 1,5 ml/min and the column temperature to 35 °C.
- 6.2.3 Set the detector wavelength to 264 nm.
- 6.2.4 Inject 10 µl of each of the reference solutions (4.10) and record their chromatograms.
- 6.2.5 Inject 10 µl of the sample solution (6.1) and record its chromatogram.
- 6.3 Identify whether hexamidine, dibromohexamidine, dibromopropamidine or chlorhexidine is present by comparing the retention time(s) of the peak(s) recorded in 6.2.5 with those obtained from the reference solutions in 6.2.4.

7. DETERMINATION

7.1 Determination

Preparation of standard solutions.

Use one of the preservatives (4.6 to 4.9) which is absent from the sample as an internal standard. If this is not possible, triclocarban (4.11), or halocarban (4.12), may be used.

- 7.1.1 A 0,05 % (m/v) stock solution in solvent I (4.5.1) of the preservative identified in 6.3.
- 7.1.2 A 0,05 % (m/v) stock solution in solvent I (4.5.1) of the preservative chosen as in internal standard.
- 7.1.3 For each identified preservative, prepare four standard solutions by transferring into a series of 10-ml volumetric flasks amounts of the stock solution of the identified preservative (7.1.1) and appropriate amounts of the stock solution of the internal standard (7.1.2) according to the table set out below. Make each flask up to volume with solvent I (4.5.1) and mix.

Standard solution	Internal standard stock solution	Identified preservative stock solution	
	ml (7.1.2) added	ml (7.1.1) added	µg/ml (*)
I	1,0	0,5	25
II	1,0	1,0	50
III	1,0	1,5	75
IV	1,0	2,0	100

(*) These values are given as in indication and correspond to the concentrations of the identified preservative in standard solutions prepared using a stock solution which contains exactly 0,05 % of the identified preservative.

7.2 Sample preparation

- 7.2.1 Weigh accurately approximately 0,5 g (p gram) of sample into a 10-ml volumetric flask, add 1,0 ml of the internal standard solution (7.1.2) and 6 ml of solvent I (4.5.1) and mix.

7.2.2 Place the flask in an ultrasonic bath (5.4) for 10 minutes. Cool. Make up to volume with solvent I and mix. Centrifuge or filter through a folded filter paper. Collect the supernatant or the filtrate, as the case may be, for chromatography.

7.3 **Chromatography**

7.3.1 Adjust the mobile-phase gradient, flow rate, column temperature and detector wavelength of the HPLC equipment (5.2) to the conditions as required in the identification stage (6.2.1 to 6.2.3).

7.3.2 Inject 10 µl of the sample solution (7.2.2) and measure the peak areas. Repeat this process with further 10 µl aliquots of the sample solution until consistent results are obtained. Calculate the ratio of the peak area produced by the compound to be analysed to the peak area produced by the internal standard.

7.4 **Calibration**

7.4.1 Inject 10 µl of each of the standard solutions (7.1.3) and measure the peak areas.

7.4.2 For each standard solution (7.1.3), calculate the ratio of the hexamidine, dibromohexamidine, dibromopropamidine or chlorhexidine peak area to the internal standard peak area. Plot a calibration curve using these ratios as the ordinate and the corresponding concentrations of the identified preservative in the standard solutions, in micrograms per millilitre, as the abscissa.

7.4.3 From the calibration curve (7.4.2) read off the concentration of the identified preservative corresponding to the peak area ratio calculated in 7.3.2.

8. **CALCULATION**

8.1 Calculate the hexamidine, dibromohexamidine, dibromopropamidine or chlorhexidine content of the sample, as a percentage by mass, using the formula:

$$\% \text{ (m/m)} = \frac{c}{1000 \times p} \times \frac{MW_1}{MW_2}$$

in which

p = mass in grams of the sample taken for analysis (7.2.1);

c = concentration of the preservative in the sample solution, in micrograms per millilitre, obtained from the calibration curve;

MW₁ = molecular weight of the basic form of the preservative present
and

MW₂ = molecular weight of the corresponding salt (see point 10).

9. **REPEATABILITY (1)**

For a hexamidine, dibromohexamidine, dibromopropamidine or chlorhexidine concentration of 0,1 % (m/m) the difference between the results of two determinations carried out in parallel on the same sample should not exceed 0,005 %.

(1) ISO 5725.

10. TABLE OF FORMULA WEIGHTS

Hexamidine	$C_{20}H_{26}N_4O_2$	354,45
Hexamidine diisethionate	$C_{20}H_{26}N_4O_2 \cdot 2C_2H_6O_4S$	606,72
Hexamidine di-p-hydroxybenzoate	$C_{20}H_{26}N_4O_2 \cdot 2C_7H_6O_3$	630,71
Dibromohexamidine	$C_{20}H_{24}Br_2N_4O_2$	512,24
Dibromohexamidine diisethionate	$C_{20}H_{24}Br_2N_4O_2 \cdot 2C_2H_6O_4S$	764,51
Dibromopropamidine	$C_{17}H_{18}Br_2N_4O_2$	470,18
Dibromopropamidine diisethionate	$C_{17}H_{18}Br_2N_4O_2 \cdot 2C_2H_6O_4S$	722,43
Chlorhexidine	$C_{22}H_{30}Cl_2N_{10}$	505,45
Chlorhexidine diacetate	$C_{22}H_{30}Cl_2N_{10} \cdot 2C_2H_4O_2$	625,56
Chlorhexidine digluconate	$C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$	897,76
Chlorhexidine dihydrochloride	$C_{22}H_{30}Cl_2N_{10} \cdot 2HCl$	578,37

SIXTH COMMISSION DIRECTIVE 95/32/EC

Sixth Commission Directive 95/32/EC of 7 July 1995 relating to methods of analysis necessary for checking the composition of cosmetic products

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European 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 last amended by Commission Directive 94/32/EC ⁽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 laid down by Commission provisions concerning the composition of cosmetic products are satisfied;

Whereas all the necessary methods of analysis should be laid down as quickly as possible; whereas certain methods have already been adopted in Commission Directives 80/1335/EEC ⁽³⁾, as amended by Directive 87/143/EEC ⁽⁴⁾, 82/434/EEC ⁽⁵⁾, as amended by Directive 90/207/EEC ⁽⁶⁾, 83/514/EEC ⁽⁷⁾, 85/490/EEC ⁽⁸⁾ and 93/73/EEC ⁽⁹⁾;

Whereas the identification and determination of benzoic acid, 4-hydroxybenzoic acid, sorbic acid, salicylic acid and propionic acid in cosmetic products and the identification and determination of hydroquinone, hydroquinone monomethylether, hydroquinone monoethylether and hydroquinone monobenzylether in cosmetic products constitute a sixth step;

Whereas the measures provided for in this Directive are in accordance 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 the necessary steps to ensure that during official testing of cosmetic products:

- identification and determination of benzoic acid, 4-hydroxybenzoic acid, sorbic acid, salicylic acid and propionic acid,

⁽¹⁾ OJ No L 262, 27. 9. 1976, p. 169.

⁽²⁾ OJ No L 181, 15. 7. 1994, p. 31.

⁽³⁾ OJ No L 383, 31. 12. 1980, p. 27.

⁽⁴⁾ OJ No L 57, 27. 2. 1987, p. 56.

⁽⁵⁾ OJ No L 185, 30. 6. 1982, p. 1.

⁽⁶⁾ OJ No L 108, 28. 4. 1990, p. 92.

⁽⁷⁾ OJ No L 291, 24. 10. 1983, p. 9.

⁽⁸⁾ OJ No L 295, 7. 11. 1985, p. 30.

⁽⁹⁾ OJ No L 231, 14. 9. 1993, p. 34.

— identification and determination of hydroquinone, hydroquinone monomethylether, hydroquinone monoethylether and hydroquinone monobenzylether, shall be carried out in accordance with the methods described in the Annex.

Article 2

1. Member States shall bring into force the laws, regulations and administrative provisions necessary to comply with this Directive no later than 30 September 1996. They shall forthwith inform the Commission thereof.

When Member States adopt these provisions, these shall contain a reference to this Directive or shall be accompanied by such reference at the time of their official publication. The procedure for such reference shall be adopted by Member States.

2. Member States shall communicate to the Commission the provisions of national law which they adopt in the field covered by this Directive.

Article 3

This Directive shall enter into force on the 20th day following its publication in the *Official Journal of the European Communities*.

Article 4

This Directive is addressed to the Member States.

Done at Brussels, 7 July 1995.

For the Commission
Emma BONINO
Member of the Commission

ANNEX**I. IDENTIFICATION AND DETERMINATION OF BENZOIC ACID, 4-HYDROXYBENZOIC ACID, SORBIC ACID, SALICYLIC ACID AND PROPIONIC ACID IN COSMETIC PRODUCTS****1. SCOPE AND FIELD OF APPLICATION**

The method is applicable to the identification and determination of benzoic acid, 4-hydroxybenzoic acid, sorbic acid, salicylic acid and propionic acid in cosmetic products. Separate procedures describe the identification of these preservatives; the determination of propionic acid; and the determination of 4-hydroxybenzoic acid, salicylic acid, sorbic acid and benzoic acid.

2. DEFINITION

The amounts of benzoic acid, 4-hydroxybenzoic acid, salicylic acid, sorbic acid and propionic acid determined by this method are expressed as percentage by mass of the free acids.

A. IDENTIFICATION**1. PRINCIPLE**

Following acid/base extraction of the preservatives, the extract is analysed by thin layer chromatography (TLC) employing on-date derivatization. Depending on the results, the identification is confirmed by high performance liquid chromatography (HPLC) or, in the case of propionic acid, by gas chromatography (GC).

2. REAGENTS**2.1 General**

All reagents must be of analytical purity. Water used must be distilled water, or water of at least equivalent purity.

2.2 Acetone

2.3 Diethyl ether

2.4 Acetonitrile

2.5 Toluene

2.6 n-Hexane

2.7 Paraffin, liquid

2.8 Hydrochloric acid, 4 M

2.9 Potassium hydroxide, aqueous, 4 M

2.10 Calcium chloride, CaCl₂·2H₂O

2.11 Lithium carbonate, Li_2CO_3

2.12 2-Bromo-2'-acetonaphthone

2.13 4-Hydroxybenzoic acid

2.14 Salicylic acid

2.15 Benzoic acid

2.16 Sorbic acid

2.17 Propionic acid

2.18 **Reference solutions**

Prepare 0,1 % (m/v) solutions (100 mg/100 ml) of each of the five preservatives (2.13 to 2.17) in diethyl ether

2.19 **Derivatization reagent**

0,5 % (m/v) solution of 2-bromo-2'-acetonaphthone (2.12) in acetonitrile (2.4) (50 mg/10 ml). This solution should be prepared weekly and stored in a refrigerator

2.20 **Catalyst solution**

0,3 % (m/v) solution of lithium carbonate (2.11) in water (300 mg/100 ml). This solution should be freshly prepared

2.21 **Development solvent**

Toluene (2.5)/Acetone (2.2) (20:0,5, v/v)

2.22 Liquid paraffin (2.7)/n-hexane (2.6) (1:2, v/v)

3. APPARATUS

Ordinary laboratory equipment

3.1 Water bath, capable of maintaining a temperature of 60 °C

3.2 Developing tank

3.3 Ultraviolet light source, 254 and 366 nm

3.4 Thin layer plates, Kieselgel 60, without fluorescence indicator, 20 x 20 cm, layer thickness 0,25 mm with concentrating zone 2,5 x 20 cm (Merck 11845, or equivalent)

3.5 Microsyringe, 10 μl

3.6 Microsyringe, 25 μl

3.7 Oven, capable of maintaining temperatures up to 105 °C

3.8 50-ml glass tubes with screw cap

3.9 Filter paper, diameter 90 mm, Schleicher & Schull, Weissband No 5892, or equivalent

3.10 Universal pH indicator paper, pH 1-11

3.11 5-ml glass sample vials

3.12 Rotating film evaporator (Rotavapor or equivalent)

3.13 Hot plate

4. PROCEDURE

4.1 Sample preparation

Weigh approximately 1 g of the sample into a 50-ml glass tube with screw cap (3.8). Add four drops of hydrochloric acid 4 M (2.8) and 40 ml acetone (2.2). For strongly basic products such as toilet soap, 20 drops of hydrochloric acid 4 M (2.8) should be added. Check that the pH is approximately two, using indicator paper (3.10). Close the tube and shake vigorously for one minute.

If necessary to facilitate the extraction of the preservatives into the acetone phase, beat the mixture gently to about 60 °C to melt the liquid phase.

Cool the solution to room temperature and filter through a filter paper (3.9) into a conical flask.

Transfer 20 ml of the filtrate to a 200-ml conical flask, add 20 ml water and mix. Adjust the pH of the mixture to approximately 10 with potassium hydroxide 4 M (2.9), using indicator paper (3.10) to measure the pH.

Add 1 g calcium chloride (2.10) and shake vigorously. Filter through a filter paper (3.9) into a 250-ml separating funnel containing 75 ml diethyl ether (2.3) and shake vigorously for one minute. Allow to separate and draw off the aqueous layer into a 250 ml conical flask. Discard the ether layer. Using indicator paper (3.10), adjust the pH of the aqueous solution to approximately two with hydrochloric acid 4 M (2.8). Add 10 ml diethyl ether (2.3), stopper the flask and shake vigorously for one minute. Allow to separate and transfer the ethyl layer to a rotating film evaporator (3.12). Discard the aqueous layer.

Evaporate the ether layer almost to dryness and redissolve the residue in 1 ml of diethyl ether (2.3). Transfer the solution to a sample vial (3.11).

4.2 Thin layer chromatography

For each of the references and the samples to be chromatographed, apply approximately 3 µl lithium carbonate solution (2.20) and with a syringe (3.5) at equal distances on the start line in the concentration zone of a TLC plate (3.4) and dry in a stream of cold air.

Transfer the TLC plate to a hot plate (3.13), heated at 40 °C, in order to keep the spots as small as possible. With a microsyringe (3.5) apply 10 µl of each of the reference solutions (2.18) and the sample solution (4.1) to the start line of the plate, on the exact spots where the lithium carbonate solution has been applied.

Finally apply approximately 15 µl derivatization reagent (2.19) (2-bromo-2'-acetonaphthone solution), again on the exact spots where the reference/sample solutions and the lithium carbonate solution have been applied.

Heat the TLC plate in an oven (3.7) at 80 °C for 45 minutes. After cooling, develop the plate in a tank (3.2), that has equilibrated for 15 minutes (without the use of filter paper lining), using development solvent 2.21 (toluene/acetone), until the solvent front has reached a distance of 15 cm (this may take approximately 80 minutes).

Dry the plate in a stream of cold air and examine the spots obtained under UV light (3.3). To enhance the fluorescence of the weak spots, the TLC plate may be dipped in liquid paraffin/n-hexane (2.22).

5. IDENTIFICATION

Calculate the R_f for each spot.

Compare the R_f and the behaviour under UV radiation obtained for the sample with that obtained for the reference solutions.

Draw a preliminary conclusion about the presence and identity of the preservatives present. Perform the HPLC described in Section B, or, when it appears that propionic acid is present, the GC described in Section C. Compare the retention times obtained with those of the reference solutions.

Combine the results from the TLC and HPLC or GC and base the final identification of the preservatives present in the sample on the combined results.

B. DETERMINATION OF BENZOIC ACID, 4-HYDROXYBENZOIC ACID, SORBIC ACID AND SALICYLIC ACID

1. PRINCIPLE

After acidification, the sample is extracted with a mixture of ethanol and water. Following filtration the preservatives are determined by high performance liquid chromatography (HPLC).

2. REAGENTS

2.1 All reagents must be of analytical purity, and suitable for HPLC where appropriate. Water used must be distilled water, or water of at least equivalent purity.

2.2 Ethanol, absolute

2.3 4-Hydroxybenzoic acid

2.4 Salicylic acid

2.5 Benzoic acid

2.6 Sorbic acid

2.7 Sodium acetate, (CH₃COONa·3H₂O)

2.8 Acetic acid, $d_4^{20} = 1,05$ g/ml

2.9 Acetonitrile

2.10 Sulfuric acid, 2 M

2.11 Potassium hydroxide, aqueous, 0,2 M

2.12 2-Methoxybenzoic acid

2.13 Ethanol/water mixture

Mix nine volumes of ethanol (2.2) and one volume of water (2.1).

2.14 Internal standard solution

Prepare a solution containing approximately 1 g 2-methoxybenzoic acid (2.12) in 500 ml ethanol/water mixture (2.13).

- 2.15 Mobile phase for HPLC
- 2.15.1 Acetate buffer: to 1 l of water add 6,35 g sodium acetate (2.7) and 20,0 ml acetic acid (2.8) and mix.
- 2.15.2 Prepare the mobile phase by mixing nine volumes acetate buffer (2.15.1) and one volume acetonitrile (2.9).
- 2.16 Preservative stock solution
- Accurately weigh approximately 0,05 g 4-hydroxybenzoic acid (2.3), 0,2 g salicylic acid (2.4), 0,2 g benzoic acid (2.5) and 0,05 g sorbic acid (2.6) in a 50-ml volumetric flask and make up to volume with ethanol/water mixture (2.13). Store this solution in a refrigerator. The solution is stable for one week.
- 2.17 Standard preservative solutions
- Transfer respectively 8,00, 4,00, 2,00, 1,00 and 0,50 ml of the stock solution (2.16) into a series of 20-ml volumetric flasks. To each flask, add 10,00 ml internal standard solution (2.14) and 0,5 ml sulfuric acid 2 M (2.10). Make up to volume with ethanol/water mixture (2.13). These solutions must be freshly prepared.

3. APPARATUS

Usual laboratory equipment not otherwise specified, and:

- 3.1 Water bath, set at 60 °C
- 3.2 High performance liquid chromatograph with variable-wavelength UV detector and 10- μ l injection loop
- 3.3 Analytical column
- Stainless steel, length 12,5 to 25 cm, internal diameter 4,6 mm, packed with Nucleosil 5C18, or equivalent
- 3.4 Filter paper, diameter: 90 mm, Schleicher and Schull, Weissband No 5892, or equivalent
- 3.5 50-ml glass tubes with screw cap
- 3.6 5-ml glass sample vials
- 3.7 Boiling chips, carborundum, size 2 to 4 mm, or equivalent

4. PROCEDURE

4.1 Sample preparation

4.1.1 Sample preparation without addition of internal standard

Weigh 1 g of the sample in a 50-ml glass tube with screw cap (3.5). Pipette 1,00 ml sulfuric acid 2 M (2.10) and 40,0 ml ethanol/water mixture (2.13) into the tube. Add approximately 1 g of boiling chips (3.7), close the tube and shake vigorously for at least one minute until a homogeneous suspension is obtained. To facilitate the extraction of the preservatives into the ethanol phase, place the tube for exactly five minutes in a water bath (3.1) kept at 60 °C.

Cool the tube immediately in a stream of cold water and store the extract at 5 °C for one hour.

Filter the extract through a filter paper (3.4). Transfer approximately 2 ml of the extract into a sample vial (3.6). Store the extract at 5 °C and perform the HPLC determination within 24 hours of preparation.

4.1.2 Sample preparation including addition of internal standard

Weigh to three decimal places $1 \pm 0,1$ g (a grams) of the sample in a 50-ml glass tube with screw cap (3.5). Add by pipette 1,00 ml sulfuric acid 2 M (2.10) and 30,0 ml ethanol/water mixture (2.13). Add approximately 1 g of boiling chips (3.7) and 10,00 ml internal standard solution (2.14). Close the tube and shake vigorously for at least one minute until an homogeneous suspension is obtained. To facilitate extraction of the preservatives into the ethanol phase place the tube for exactly five minutes in a water bath (3.1) kept at 60 °C.

Cool the tube immediately in a stream of cold water and store the extract at 5 °C for one hour.

Filter the extract through a filter paper (3.4). Transfer approximately 2 ml of the filtrate into a sample vial (3.6). Store the filtrate at 5 °C and perform the HPLC-determination within 24 hours of preparation.

4.2 High performance liquid chromatography

Mobile phase: acetonitrile/acetate buffer (2.15).

Adjust the flow rate of mobile phase through the column to $2,0 \pm 0,5$ ml/minute. Set the detector wavelength to 240 nm.

4.2.1 Calibration

Inject 10 µl portions of each of the standard preservative solutions (2.17) into the liquid chromatography (3.2). For each solution determine the ratios of the peak heights of the investigated preservatives to the height of the internal standard peak obtained from the chromatograms. Plot a graph for each preservative relating the peak height ratio to the concentration of each standard solution.

Ascertain that a linear response is obtained for the standard solutions in the calibration procedure.

4.2.2 Determination

Inject 10 µl of the sample extract (4.1.1) into the liquid chromatograph (3.2) and record the chromatogram. Inject 10 µl of a standard preservative solution (2.17) and record the chromatogram. Compare the chromatograms obtained. If in the chromatogram of the sample extract (4.1.1) no peak appears to be present having approximately the same retention time as 2-methoxybenzoic acid (recommended internal standard), inject 10 µl sample extract with added internal standard (4.1.2) into the liquid chromatograph and record the chromatogram.

If an interfering peak is observed in the chromatogram of the sample extract (4.1.1) having the same retention time as 2-methoxybenzoic acid, another appropriate internal standard should be selected. (If one of the preservatives under investigation is absent from the chromatogram, this preservative can be used as the internal standard.)

Ascertain whether the chromatograms obtained for a standard solution and the sample solution meet the following requirements:

- the peak separation of the worst separated pair shall be at least 0,90. (For definition of peak separation, see Figure 1).

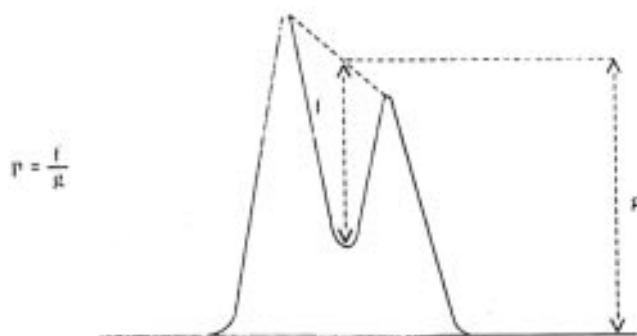


Figure 1: Peak separation

If the required separation is not achieved, either a more efficient column should be used, or the mobile phase composition should be adjusted until the requirement is met.

- The asymmetry factor A_s , of all peaks obtained shall range between 0,9 to 1,5. (For definition of the peak asymmetry factor, see Figure 2). To record the chromatogram for the determination of the asymmetry factor a chart speed of at least 2 cm/minute is recommended.

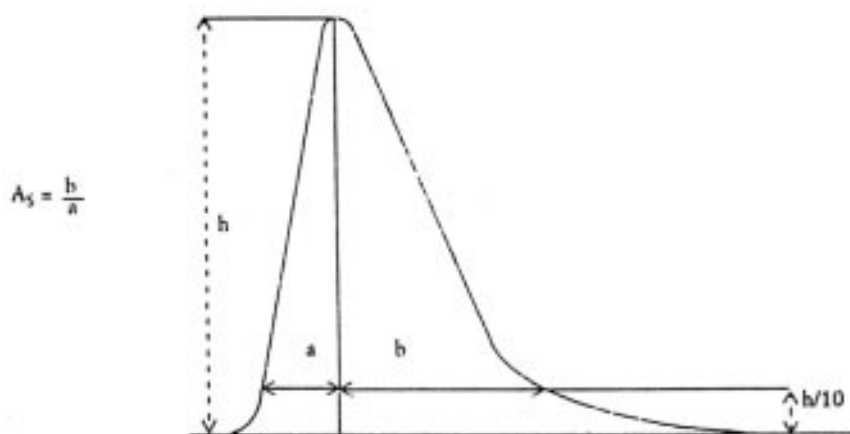


Figure 2: Peak asymmetry factor

- A steady baseline shall be obtained.

5. CALCULATION

Use the ratios of the heights of the peaks of the investigated preservatives to the height of the 2-methoxybenzoic acid (internal standard) peak and the calibration graph to calculate the concentration of the acid preservatives in the sample solution. Calculate the benzoic acid, 4-hydroxybenzoic acid, sorbic acid or salicylic acid content of the sample, as a percentage by mass (x_i), using the formula:

$$x_i \% (m/m) = \frac{100 \cdot 20 \cdot b}{10^6 \cdot a} = \frac{b}{500 \cdot a}$$

in which:

a = the mass (g) of the test portion (4.1.2),

- b = the concentration ($\mu\text{g/ml}$) of the preservative in the sample extract (4.1.2) obtained from the calibration graph.

6. REPEATABILITY (1)

For a 4-hydroxybenzoic acid content of 0,40 % the difference between the results of two determinations in parallel carried out on the sample should not exceed an absolute value of 0,035 %.

For a benzoic acid content of 0,50 % the difference between the results of two determinations in parallel carried out on the same sample should not exceed an absolute value of 0,050 %.

For a salicylic acid content of 0,50 % the difference between the results of two determinations in parallel carried out on the same sample should not exceed an absolute value of 0,045 %.

For a sorbic acid content of 0,60 % the difference between the results of two determinations in parallel carried out on the same sample should not exceed an absolute value of 0,035 %.

7. REMARKS

- 7.1 Results of a ruggedness test performed on the method indicated that the amount of sulfuric acid added to extract the acids from the sample is critical, and the limits for the amount of sample worked up should be kept within the prescribed boundaries.
- 7.2 If desired, an appropriate guard column can be used.

C. DETERMINATION OF PROPIONIC ACID

1. SCOPE AND FIELD OF APPLICATION

This method is suitable for the determination of propionic acid, maximum concentration 2 % (m/m) in cosmetic products.

2. DEFINITION

The concentration of propionic acid measured by this method is expressed as a percentage by mass (% m/m) of the product.

3. PRINCIPLE

Following extraction of propionic acid from the product determination is performed by means of gas chromatography with the use of 2-methylpropionic acid as internal standard.

4. REAGENTS

All the reagents must be of analytical purity; distilled water or water of equivalent quality must be used.

(1) ISO 5725.

- 4.1 Ethanol 96 % (v/v)
- 4.2 Propionic acid
- 4.3 2-Methylpropionic acid
- 4.4 Orthophosphoric acid, 10 % (m/v)
- 4.5 Propionic acid solution
- Accurately weigh approximately 1,00 g (p grams) of propionic acid into a 50-ml volumetric flask and make up to volume with ethanol (4.1)
- 4.6 Internal standard solution
- Accurately weigh approximately 1,00 g (e grams) of 2-methylpropionic acid into a 50-ml volumetric flask and make up to volume with ethanol (4.1)

5. APPARATUS

- 5.1 Usual laboratory equipment, and:
- 5.2 Gas chromatograph with flame-ionization detector
- 5.3 Glass tube (20 x 150 mm) with screw cap
- 5.4 Water bath at 60 °C
- 5.5 10 ml glass syringe with filter membrane (pore diameter: 0,45 µm)

6. PROCEDURE

6.1 Sample preparation

6.1.1 Sample preparation without the internal standard

Into a glass tube (5.3), weigh approximately 1 g of the sample. Add 0,5 ml of phosphoric acid (4.4) and 9,5 ml of ethanol (4.1).

Close the tube and shake vigorously. If necessary, place the tube in a water bath heated at 60 °C (5.4) for five minutes in order to completely dissolve the lipid phase. Cool rapidly under running water. Filter part of the solution through a membrane filter (5.5). Chromatograph the filtrate the same day.

6.1.2 Sample preparation with the internal standard

Weigh to three decimal places $1 \pm 0,1$ g (a grams) of the sample into a glass tube (5.3). Add 0,5 ml of phosphoric acid (4.4), 0,50 ml of the internal standard solution (4.6) and 9 ml of ethanol (4.1).

Close the tube and shake vigorously. If necessary, place the tube in a water bath heated to 60 °C (5.4) for five minutes in order to dissolve the lipid phase. Cool rapidly under running water. Filter part of the solution through a membrane filter (5.5). Chromatograph the filtrate the same day.

6.2 Conditions for gas chromatography

The following operation conditions are recommended:

Column

Type	Stainless steel
Length	2 m

Diameter	1/8 "
Packing	10 % SP TM 1000 (or equivalent) + 1 % H ₃ PO ₄ on Chromosorb WAW 100 to 120 mesh
<i>Temperature</i>	
Injector	200 °C
Column	120 °C
Detector	200 °C
Carrier gas	nitrogen
Flow rate	25 ml/min

6.3 Chromatography

6.3.1 Calibration

Into a series of 20-ml volumetric flasks, transfer by pipette 0,25, 0,50, 1,00, 2,00 and 4,00 ml respectively of the propionic acid solution (4.5). To each volumetric flask transfer by pipette 1,00 ml of the internal standard solution (4.6); make up to volume with ethanol (4.1) and mix. The solutions prepared in this way contain e mg/ml of 2-methylpropionic acid as internal standard (that is to say, 1 mg/ml if e = 1 000) and p/4, p/2, p, 2p, 4p mg/ml propionic acid (that is to say, 0,25, 0,50, 1,00, 2,00, 4,00 mg/ml if p = 1 000).

Inject 1 µl of each of these solutions and obtain the calibration curve by plotting the ratio of the propionic acid/2-methylpropionic acid mass on the x-axis and the ratio of the corresponding peak areas on the y-axis.

Make three injections of each solution and calculate the average peak area ratio.

6.3.2 Determination

Inject 1 µl of the sample filtrate 6.1.1. Compare the chromatogram with that of one of the standard solutions (6.3.1). If a peak has approximately the same retention time as 2-methylpropionic acid, change the internal standard. If no interference is observed, inject 1 µl of the sample filtrate 6.1.2 and measure the areas of the propionic acid peak and the internal standard peak.

Make three injections of each solution and calculate the average peak area ratio.

7. CALCULATIONS

7.1 From the calibration curve obtained in 6.3.1, obtain the ratio of mass (K) corresponding to the peak area ratio calculated in 6.3.2.

7.2 From the ratio of mass thus obtained calculate the propionic acid content of the sample (X) as percentage by mass using the formula:

$$x \% (m/m) = K \frac{0,5 \cdot 100 \cdot e}{50 \cdot a} = K \frac{e}{a}$$

in which:

K = the ratio calculated in 7.1,

e = mass in grams of the internal standard weighed in 4.6,

a = mass in grams of the sample weighed in 6.1.2.

Round off results to one decimal place.

8. REPEATABILITY (1)

For a propionic acid content of 2 % (m/m) the difference between the results of two determinations in parallel carried out on the same sample should not exceed 0,12 %.

II. IDENTIFICATION AND DETERMINATION OF HYDROQUINONE, HYDROQUINONE MONOMETHYLETHER, HYDROQUINONE MONOETHYLETHER AND HYDROQUINONE MONOBENZYLETHER IN COSMETIC PRODUCTS

A. IDENTIFICATION

1. SCOPE AND FIELD OF APPLICATION

The method describes the detection and identification of hydroquinone, hydroquinone monomethylether, hydroquinone monoethylether and hydroquinone monobenzylether (monobenzene) in cosmetic products for lightening the skin.

2. PRINCIPLE

Hydroquinone and its ethers are identified by thin layer chromatography (TLC).

3. REAGENTS

All reagents must be of analytical grade.

- 3.1 Ethanol 96 % (v/v)
- 3.2 Chloroform
- 3.3 Diethyl ether
- 3.4 Developing solvent:
Chloroform/Diethyl ether, 66:33 (v/v)
- 3.5 Ammonia, 25 % (m/m) ($d_4^{20} = 0,91$ g/ml)
- 3.6 Ascorbic acid
- 3.7 Hydroquinone
- 3.8 Hydroquinone monomethylether
- 3.9 Hydroquinone monoethylether
- 3.10 Hydroquinone monobenzylether (monobenzene)
- 3.11 Reference solutions

The following reference solutions should be freshly prepared, and are stable for one day.

(1) ISO 5725.

- 3.11.1 Weigh 0,05 g hydroquinone (3.7) into a 10-ml graduated test tube. Add 0,250 g of ascorbic acid (3.6) and 5 ml of ethanol (3.1). Add ammonia (3.5) until the pH is 10 and make up to a volume of 10 ml with ethanol (3.1).
- 3.11.2 Weigh 0,05 g hydroquinone monomethylether (3.8) into a 10-ml graduated test tube. Add 0,250 g of ascorbic acid (3.6) and 5 ml of ethanol (3.1). Add ammonia (3.5) until the pH is 10 and make up to a volume of 10 ml with ethanol (3.1).
- 3.11.3 Weigh 0,05 g hydroquinone monoethylether (3.9) into a 10-ml graduated test tube. Add 0,250 g of ascorbic acid (3.6) and 5 ml of ethanol (3.1). Add ammonia (3.5) until the pH is 10 and make up to a volume of 10 ml with ethanol (3.1).
- 3.11.4 Weigh 0,05 g hydroquinone monobenzylether (3.10) into a 10-ml graduated test tube. Add 0,250 g of ascorbic acid (3.6) and 5 ml of ethanol (3.1). Add ammonia (3.5) until the pH is 10 and make up to a volume of 10 ml with ethanol (3.1).
- 3.12 Silver nitrate
- 3.13 12-Molybdophosphoric acid
- 3.14 Potassium ferricyanide hexahydrate
- 3.15 Ferric chloride, hexahydrate
- 3.16 Spray reagents
- 3.16.1 To a 5 % (m/v) aqueous solution of silver nitrate (3.12), add ammonia (3.5) until the precipitate that forms dissolves.
- Warning:*
the solution becomes explosively unstable on standing and should be discarded after use.
- 3.16.2 10 % (m/v) solution of 12-molybdophosphoric acid (3.13) in ethanol (3.1).
- 3.16.3 Prepare a 1 % (m/v) aqueous solution of potassium ferricyanide (3.14) and a 2 % (m/v) solution of ferric chloride (3.15). Mix equal parts of both solutions just before use.

4. APPARATUS

Normal laboratory equipment, and:

- 4.1 Usual TLC equipment
- 4.2 TLC plates, ready for use: silica gel GHR/UV₂₅₄; 20 x 20 cm (Machery, Nagel, or equivalent). Layer thickness 0,25 mm
- 4.3 Ultrasonic bath
- 4.4 Centrifuge
- 4.5 UV lamp, 254 nm

5. PROCEDURE

- 5.1 Preparation of the sample

Weigh 3,0 g of sample into a 10-ml graduated tube. Add 0,250 g of ascorbic acid (3.6) and 5 ml of ethanol (3.1). Adjust the pH of the solution to 10, using ammonia (3.5). Make up to a volume of 10 ml with ethanol (3.1). Close the tube with a stopper and homogenize in an ultrasonic bath for 10 minutes. Filter through a filter paper or centrifuge at 3 000 rpm.

5.2 TLC

5.2.1 Saturate a chromatographic tank with developing solvent (3.4).

5.2.2 Deposit on a plate 2 µl of the reference solutions (3.11) and 2 µl of the sample solution (5.1). Develop in the dark at ambient temperature until the solvent front has migrated 15 cm from the start.

5.2.3 Remove the plate and allow to dry at room temperature.

5.3 Detection

5.3.1 Observe the plate under UV light at 254 nm, and mark the position of the spots.

5.3.2 Spray the plate with:

- silver nitrate reagent (3.16.1), or
- 12-molybdophosphoric acid reagent (3.16.2); heat to approximately 120 °C, or
- potassium ferricyanide solution and ferric chloride solution (3.16.3).

6. IDENTIFICATION

Calculate the R_f value for each spot.

Compare the spots obtained for the sample solution with those for the reference solutions with respect to: their R_f values; the colour of the spots under UV radiation; and the colours of the spots after visualization with the spray reagent.

Perform the HPLC described in the following section (B), and compare the retention times obtained for the sample peak(s) with those for the reference solutions.

Combine the results from TLC and HPLC to identify the presence of hydroquinone and/or its ethers.

7. REMARKS

Under the conditions described, the following R_f values were observed:

hydroquinone	0,32
hydroquinone monomethylether	0,53
hydroquinone monoethylether	0,55
hydroquinone monobenzylether	0,58

B. DETERMINATION**1. SCOPE AND FIELD OF APPLICATION**

This method specifies a procedure for the determination of hydroquinone, hydroquinone monomethylether, hydroquinone monoethylether and hydroquinone monobenzylether in cosmetic products for lightening the skin.

2. PRINCIPLE

The sample is extracted with a water/methanol mixture under gentle heating to melt any lipid material. Determination of the analytes in the resulting solution is performed by reversed phase liquid chromatography with UV detection.

3. REAGENTS

3.1 All reagents must be of analytical quality. Water used must be distilled water, or water of at least equivalent purity

3.2 Methanol

3.3 Hydroquinone

3.4 Hydroquinone monomethylether

3.5 Hydroquinone monethylether

3.6 Hydroquinone monobenzylether (monobenzone)

3.7 Tetrahydrofuran, HPLC grade

3.8 Water/methanol mixture 1:1 (v/v). Mix one volume of water and one volume of methanol (3.2)

3.9 Mobile phase: tetrahydrofuran/water mixture 45:55 (v/v). Mix 45 volumes of tetrahydrofuran (3.7) and 55 volumes of water

3.10 Reference solution

Weigh 0,06 g of hydroquinone (3.3), 0,08 g hydroquinone monomethylether (3.4), 0,10 g hydroquinone monoethylether (3.5) and 0,12 g hydroquinone monobenzyl-ether (3.6) into a 50-ml volumetric flask. Dissolve and make up to volume with methanol (3.2). Prepare the reference solution by diluting 10,00 ml of this solution to 50,00 ml with water/methanol mixture (3.8). These solutions must be freshly prepared.

4. APPARATUS

Normal laboratory equipment and:

4.1 Water bath, capable of maintaining a temperature of 60 °C

4.2 High-performance liquid chromatograph with a variable-wavelength UV detector and 10- μ l injection loop

4.3 Analytical column

Stainless steel chromatographic column, length 250 mm, internal diameter 4,6 mm, packed with Zorbax phenyl (chemically bonded phenethylsilane on Zorbax SIL, end-capped with trimethylchlorosilane), particle size 6 μ m, or equivalent. Do not use a guard column, except phenyl guard, or equivalent

4.4 Filter paper, diameter 90 mm, Schleicher and Schull, Weissband No 5892, or equivalent

5. PROCEDURE

5.1 Sample preparation

Weigh to three decimal places $1 \pm 0,1$ g (a gram) of sample into a 50-ml volumetric flask. Disperse the sample in 25 ml water/methanol mixture (3.8). Close the flask and shake vigorously until a homogeneous suspension is obtained. Shake for at least one minute. Place the flask in a water bath (4.1) kept at 60 °C to enhance the extraction. Cool the flask, and make up to volume with water/methanol (3.8). Filter the extract using a filter paper (4.4). Perform the HPLC determination within 24 hours of preparing the extract.

5.2 High performance liquid chromatography

5.2.1 Adjust the flow rate of the mobile phase (3.9) to 1,0 ml/min and set the detector wavelength to 295 nm.

5.2.2 Inject 10 µl of the sample solution obtained as described in section 5.1, and record the chromatogram. Measure the peak areas. Perform a calibration as described under 5.2.3. Compare the chromatograms obtained for sample and standard solutions. Use the peak areas and the response factors (RF) calculated under 5.2.3 to calculate the concentration of the analytes in the sample solution.

5.2.3 Calibration

Inject 10 µl of the reference solution (3.10) and record the chromatogram. Inject several times until a constant peak area is obtained.

Determine the response factor RF_i :

$$RF_i = \frac{p_i}{c_i}$$

in which:

p_i = peak area for hydroquinone, hydroquinone monomethylether, hydroquinone monoethylether or hydroquinone monobenzylether, and

c_i = concentration (g/50 ml) in the reference solution (3.10) of hydroquinone, hydroquinone monomethylether, hydroquinone monoethylether or hydroquinone monobenzylether.

Ascertain whether the chromatograms obtained for a standard solution and the sample solution meet the following requirements:

- the peak separation of the worst separated pair shall be at least 0,90. (For definition of peak separation, see Figure 1).

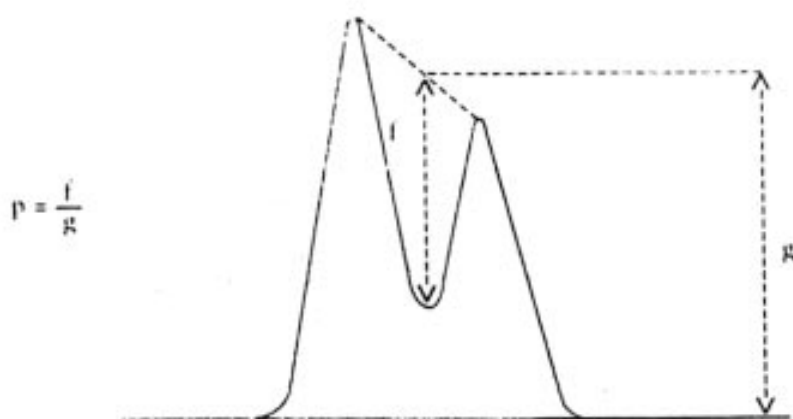


Figure 1: Peak separation

If the required separation is not achieved, either a more efficient column should be used, or the mobile phase composition should be adjusted until the requirement is met.

- The asymmetry factor A_s of all peaks obtained shall range between 0,9 to 1,5. (For definition of the peak asymmetry factor, see Figure 2.) To record the chromatogram for the determination of the asymmetry factor a chart speed of at least 2 cm/min is recommended.

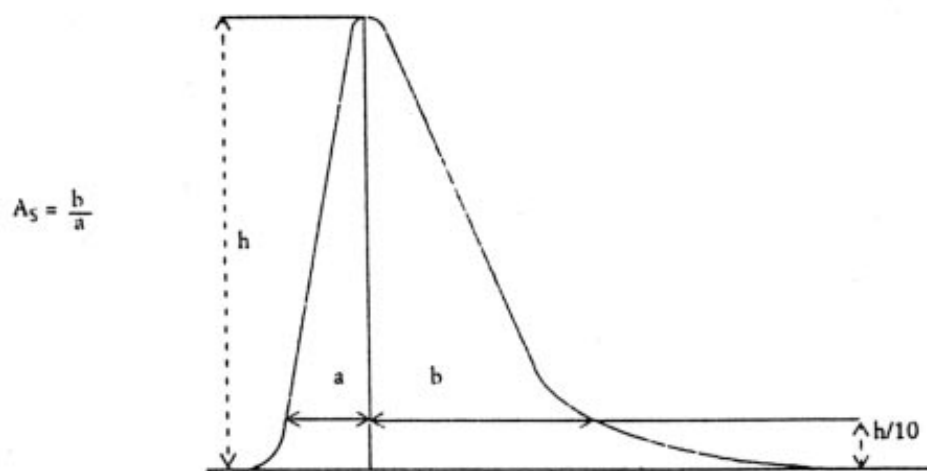


Figure 2: Peak asymmetry factor

- A steady baseline shall be obtained.

6. CALCULATION

Use the areas of the analyte peaks to calculate the concentration(s) of the analyte(s) in the sample. Calculate the analyte concentration in the sample, as a percentage by mass, (x_i) using the formula:

$$x_i \% (m/m) = \frac{b_i \cdot 100}{RF_i \cdot a}$$

in which:

- a = mass of the sample in grams, and
b_i = peak area of analyte in the sample.

7. REPEATABILITY (1)

- 7.1 For a hydroquinone content of 2,0 % the difference between the results of two determinations carried out in parallel on the same sample should not exceed an absolute value of 0,13 %.
- 7.2 For a hydroquinone monomethylether content of 1,0 % the difference between the results of two determinations carried out in parallel on the same sample should not exceed an absolute value of 0,1 %.
- 7.3 For a hydroquinone monoethylether content of 1,0 % the difference between the results of two determinations carried out in parallel on the same sample should not exceed an absolute value of 0,11 %.
- 7.4 For a hydroquinone monobenzylether content of 1,0 % the difference between the results of two determinations carried out in parallel on the same sample should not exceed an absolute value of 0,11 %.

8. REPRODUCIBILITY (1)

- 8.1 For a hydroquinone content of 2,0 % the difference between the results of two determinations carried out on the same sample under different conditions (different laboratories, different operators, different apparatus and/or different time) should not exceed an absolute value of 0,37 %.
- 8.2 For a hydroquinone monomethylether content of 1,0 % the difference between the results of two determinations carried out on the same sample under different conditions (different laboratories, different operators, different apparatus and/or different time) should not exceed an absolute value of 0,21 %.
- 8.3 For a hydroquinone monoethylether content of 1,0 %, the difference between the results of two determinations carried out on the same sample under different conditions (different laboratories, different operators, different apparatus and/or different time) should not exceed an absolute value of 0,19 %.
- 8.4 For a hydroquinone monobenzylether content of 1,0 % the difference between the results of two determinations carried out on the same sample under different conditions (different laboratories, different operators, different apparatus and/or different time) should not exceed an absolute value of 0,11 %.

9. REMARKS

- 9.1 When a hydroquinone content considerably higher than 2 % is found and an accurate estimate of the content is required, the sample extract (5.1) should be diluted to a similar concentration as would be obtained from a sample containing 2 % hydroquinone, and the determination repeated.

(In some instruments the absorbance may be out of the linear range of the detector for high hydroquinone concentrations.)

(1) ISO 5725.

9.2 Interferences

The method described above allows the determination of hydroquinone and its ethers in a single isocratic run. The use of the phenyl column assures sufficient retention for hydroquinone, which cannot be guaranteed when a C18 column is used with the mobile phase described.

However, this method is prone to interferences by a number of parabens. In such cases the determination should be repeated employing a different mobile phase/stationary phase system. Suitable methods may be found in references 1 and 2, namely:

Column: Zorbax ODS, 4,6 mm x 25 mm, or equivalent:

temperature: 36 °C

flow: 1,5 ml/min

mobile phase:

for hydroquinone: methanol/water 5/95 (V/V)

for hydroquinone monomethylether: methanol/water 30/70 (V/V)

for hydroquinone monobenzylether: methanol/water 80/20 (V/V) (1).

Column: Spherisorb S5-ODS, or equivalent:

mobile phase: water/methanol 90/10 (V/V)

flow: 1,5 ml/min.

These conditions are suitable for hydroquinone (2).

(1) M. Herpol-Borremans et M.-O. Masse, Identification et dosage de l'hydroquinone et de ses éthers méthylique et benzylique dans les produits cosmétiques pour blanchir la peau. *Int. J. Cosmet. Sci.* 8-203-214 (1986).

(2) J. Firth and I. Rix, Determination of hydroquinone in skin toning creams, *Analyst* (1986), 111, p. 129.

SEVENTH COMMISSION DIRECTIVE 96/45/EC

Seventh Commission Directive 96/45/EC of 2 July 1996 relating to methods of analysis necessary for checking the composition of cosmetic products

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European 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 last amended by Commission Directive 95/34/EC ⁽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 laid down by Community provisions concerning the composition of cosmetic products are satisfied;

Whereas all the necessary methods of analysis should be laid down as quickly as possible; whereas certain methods have already been adopted in Commission Directives 80/1335/EEC ⁽³⁾, as amended by Directive 87/143/EEC ⁽⁴⁾, 82/434/EEC ⁽⁵⁾, as amended by Directive 90/207/EEC ⁽⁶⁾, and by Commission Directives 83/514/EEC ⁽⁷⁾, 85/490/EEC ⁽⁸⁾, 93/73/EEC ⁽⁹⁾ and 95/32/EC ⁽¹⁰⁾;

Whereas the identification and determination of 2-phenoxyethanol, 1-phenoxypropan-2-ol, methyl, ethyl, propyl, butyl and benzyl 4-hydroxybenzoate in cosmetic products constitute a seventh step;

Whereas the measures provided for in this Directive are in accordance 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 the necessary steps to ensure that during official testing of cosmetic products, the identification and determination of 2-phenoxyethanol, 1-phenoxypropan-2-ol, methyl, ethyl, propyl, butyl and benzyl 4-hydroxybenzoate shall be carried out in accordance with the method described in the Annex.

⁽¹⁾ OJ No L 262, 27. 9. 1976, p. 169.

⁽²⁾ OJ No L 167, 18. 7. 1995, p. 19.

⁽³⁾ OJ No L 383, 31. 12. 1980, p. 27.

⁽⁴⁾ OJ No L 57, 27. 2. 1987, p. 56.

⁽⁵⁾ OJ No L 185, 30. 6. 1982, p. 1.

⁽⁶⁾ OJ No L 108, 28. 4. 1990, p. 92.

⁽⁷⁾ OJ No L 291, 24. 10. 1983, p. 9.

⁽⁸⁾ OJ No L 295, 7. 11. 1985, p. 30.

⁽⁹⁾ OJ No L 231, 14. 9. 1993, p. 34.

⁽¹⁰⁾ OJ No L 178, 28. 7. 1995, p. 20.

Article 2

1. Member States shall bring into force the laws, regulations and administrative provisions needed to comply with this Directive no later than 30 September 1997. They shall forthwith inform the Commission thereof.
2. When Member States adopt these provisions, these shall contain a reference to this Directive or shall be accompanied by such reference at the time of their official publication. The procedure for such reference shall be adopted by the Member States.
3. Member States shall communicate to the Commission the provisions of national law which they adopt in the field covered by this Directive.

Article 3

This Directive shall enter into force on the 20th day following its publication in the *Official Journal of the European Communities*.

Article 4

This Directive is addressed to the Member States.

Done at Brussels, 2 July 1996.

For the Commission
Emma BONINO
Member of the Commission

ANNEX**IDENTIFICATION AND DETERMINATION OF 2-PHENOXY-ETHANOL, 1-PHENOXYPROPAN-2-OL, METHYL, ETHYL, PROPYL, BUTYL AND BENZYL 4-HYDROXYBENZOATE IN COSMETIC PRODUCTS****A. IDENTIFICATION****1. SCOPE AND FIELD OF APPLICATION**

This method specifies a TLC procedure that, in combination with the determination method described in Section B, allows the identification of 2-phenoxyethanol, 1-phenoxypropan-2-ol, methyl 4-hydroxybenzoate, ethyl 4-hydroxybenzoate, propyl 4-hydroxybenzoate, butyl 4-hydroxybenzoate and benzyl 4-hydroxybenzoate in cosmetic products.

2. PRINCIPLE

The preservatives are extracted from the acidified cosmetic sample with acetone. After filtration, the acetone solution is mixed with water, and in an alkaline medium the fatty acids are precipitated as their calcium salts. The alkaline acetone/water mixture is extracted with diethylether to remove lipophilic substances. After acidification the preservatives are extracted with diethylether. An aliquot of the diethylether extract is spotted on a silica-gel coated thin-layer plate. After development of the plate, the chromatogram obtained is observed under UV light and visualized using Millon's reagent.

3. REAGENTS**3.1 General**

All reagents used shall be of analytical purity. Water shall be distilled water, or water of at least equal purity.

3.2 Acetone**3.3 Diethylether****3.4 n-Pentane****3.5 Methanol****3.6 Acetic acid, glacial****3.7 Hydrochloric acid solution, $c(\text{HCl}) = 4 \text{ mol/l}$** **3.8 Potassium hydroxide solution, $c(\text{KOH}) = 4 \text{ mol/l}$** **3.9 Calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$)****3.10 Detection reagent: Millon's reagent**

Millon's reagent (Mercury (II) nitrate) is a ready-made solution which is commercially available (Fluka 69820).

- 3.11 2-Phenoxyethanol
- 3.12 1-Phenoxypropan-2-ol
- 3.13 Methyl 4-hydroxybenzoate (methylparaben)
- 3.14 Ethyl 4-hydroxybenzoate (ethylparaben)
- 3.15 n-Propyl 4-hydroxybenzoate (propylparaben)
- 3.16 n-Butyl 4-hydroxybenzoate (butylparaben)
- 3.17 Benzyl 4-hydroxybenzoate (benzylparaben)
- 3.18 Reference solutions
- Prepare 0,1 % (m/v) solutions of each of the reference substances 3.11, 3.12, 3.13, 3.14, 3.15, 3.16 and 3.17 in methanol.
- 3.19 Development solvent
- Mix 88 volumes of n-pentane (3.4) with 12 volumes of glacial acetic acid (3.6).

4. APPARATUS

Normal laboratory equipment, and:

- 4.1 Waterbath, capable of maintaining a temperature of 60 °C
- 4.2 Developing tank (not lined with filter paper)
- 4.3 Ultraviolet light source, 254 nm
- 4.4 Thin-layer plates, 20 cm x 20 cm, precoated with 0,25 mm silica gel 60F₂₅₄, with concentrating zone (Merck No 11798, Darmstadt, or equivalent)
- 4.5 Oven, capable of maintaining up to 105 °C
- 4.6 Hot-air hair dryer
- 4.7 Woollen paint roller, length approximately 10 cm, outside diameter approximately 3,5 cm. The thickness of the wool-layer shall be 2 to 3 mm. Trim the wool if necessary.
- See note under 5.2
- 4.8 50-ml glass tubes with screw cap
- 4.9 Electric heating plate, with thermostatic temperature controller. Temperature setting: about 80 °C. The hot plate shall be covered with an aluminium plate of 20 cm x 20 cm and a thickness of about 6 mm, to obtain a uniform heat distribution.

5. PROCEDURE

5.1 Sample preparation

Weigh approximately 1 g of sample in a 50-ml glass tube with screw cap (4.8). Add four drops of hydrochloric acid solution (3.7) and 40 ml of acetone.

For strongly basic cosmetic products, such as toilet-soap, 20 drops of hydrochloric acid solution shall be added. Close the tube, gently heat the mixture to approximately 60 °C to facilitate the extraction of the preservatives into the acetone phase and shake vigorously for one minute.

Measure the pH of the solution with pH indicator paper and adjust the pH of the solution ≤ 3 with hydrochloric acid solution. Shake vigorously again for one minute.

Cool the solution to room temperature and filter through a filter paper into a conical flask. Transfer 20 ml of the filtrate into a 200-ml conical flask, add 60 ml water and mix. Adjust the pH of the mixture to approximately 10 with potassium hydroxide (3.8), using pH indicator paper.

Add 1 g calcium chloride dihydrate (3.9) and shake vigorously. Filter the solution through a filter paper into a 250-ml separating funnel containing 75 ml diethylether and shake vigorously for one minute. Allow the phases to separate and collect the aqueous layer in a 200-ml conical flask. Adjust the pH of the solution to approximately 2 with hydrochloric acid solution, using pH indicator paper. Subsequently, add 10 ml diethylether and shake vigorously for one minute. Allow the phases to separate and transfer approximately 2 ml of the diethylether layer into a 5-ml sample vial.

5.2 Thin-layer chromatography (TLC)

Place a TLC plate (4.4) on the heated aluminium plate (4.9). Apply 10 μl of each of the reference solutions (3.18) and 100 μl of the sample solution(s) (5.1) on a start line in the concentration zone of the TLC plate.

If desired, a stream of air can be used to facilitate evaporation of the solvent. Remove the TLC plate from the heating plate and allow to cool to room temperature. Transfer 100 ml of the development solvent (3.19) into a developing tank (4.2).

Place the TLC plate immediately in the unsaturated chamber and develop at room temperature until the solvent front has run about 15 cm from the base line. Remove the plate from the development tank and dry in a stream of hot air by means of a hot-air hair dryer.

Examine the plate under UV light (4.3) and mark the position of the spots. Heat the plate for 30 minutes in an oven (4.5) at 100 °C to remove excess acetic acid. Visualize the preservatives in the chromatogram with Millon's reagent (3.10), by dipping the paint roller (4.7) into the reagent and rolling over the TLC-plate until evenly wetted.

Note: Alternatively, the spots may be visualized by the careful application of a drop of Millon's reagent on each of the spots marked under UV light.

Esters of 4-hydroxybenzoic acid appear as red spots, 2-phenoxyethanol and 1-phenoxypropan-2-ol as yellow spots. Note, however, that 4-hydroxybenzoic acid itself, which may be present in the samples as a preservative or decomposition product of the parabens, will also appear as a red spot. See 7.3 and 7.4.

6. IDENTIFICATION

Calculate the R_f value for each spot. Compare the spots obtained from the sample solution with those of the reference solutions with respect to their R_f values, their behaviour under UV radiation and the colour after visualization. Draw preliminary conclusions about the identity of the preservatives.

If parabens appear to be present, the HPLC procedure described in Section B should be performed. Combine the results from TLC and high-performance liquid chromatography (HPLC) to confirm the presence of the 2-phenoxyethanol, 1-phenoxypropan-2-ol and the parabens.

7. REMARKS

7.1 Because of the toxicity of Millon's reagent this reagent is best applied by one of the procedures described. Spraying is not recommended.

7.2 Other compounds containing hydroxyl groups may also give colours with Millon's reagent. A table of colours and Rf values obtained for a number of preservatives using this TLC procedure may be found in: N. de Kruijf, M. A. H. Rijk, L. A. Pranato-Soetardhi and A. Schouten (1987): Determination of preservatives in cosmetic products I: Thin-layer chromatographic procedure for the identification of preservatives in cosmetic products (*J. Chromatography* 410, 395-411).

7.3 The Rf value listed in the following table serve as an indication of the values that may be obtained:

Compound	hRf	Colour
4-hydroxybenzoic acid	11	red
methylparaben	12	red
ethylparaben	17	red
propylparaben	21	red
butylparaben	26	red
benzylparaben	16	red
2-phenoxyethanol	29	yellow
1-phenoxypropan-2-ol	50	yellow

7.4 No separation is obtained for 4-hydroxybenzoic acid and methylparaben, or for benzylparaben and ethylparaben. Identification of these compounds should be confirmed by performing the HPLC method described under Section B and comparing the retention times obtained from the sample with those of standards.

B. DETERMINATION

1. SCOPE AND FIELD OF APPLICATION

This method specifies a procedure for the determination of 2-phenoxyethanol, 1-phenoxypropan-2-ol, methyl 4-hydroxybenzoate, ethyl 4-hydroxybenzoate, propyl 4-hydroxybenzoate, butyl 4-hydroxybenzoate and benzyl 4-hydroxybenzoate in cosmetic products.

2. DEFINITION

The amounts of the preservatives determined by this method are expressed as percentage by mass.

3. PRINCIPLE

The sample is acidified by adding sulfuric acid and then suspended in a mixture of ethanol and water. After gently heating the mixture to melt the lipid phase to promote quantitative extraction, the mixture is filtered.

The preservatives in the filtrate are determined by reversed phase HPLC using isopropyl 4-hydroxybenzoate as the internal standard.

4. REAGENTS

4.1 General

All reagents must be of analytical purity and suitable for HPLC where appropriate. Water shall be distilled water, or water of at least equal purity.

4.2 Ethanol, absolute

4.3 2-Phenoxyethanol

4.4 1-Phenoxypropan-2-ol

4.5 Methyl 4-hydroxybenzoate (methylparaben)

4.6 Ethyl 4-hydroxybenzoate (ethylparaben)

4.7 n-Propyl 4-hydroxybenzoate (propylparaben)

4.8 Isopropyl 4-hydroxybenzoate (isopropylparaben)

4.9 n-Butyl 4-hydroxybenzoate (butylparaben)

4.10 Benzyl 4-hydroxybenzoate (benzylparaben)

4.11 Tetrahydrofuran

4.12 Methanol

4.13 Acetonitrile

4.14 Sulfuric acid solution $c(\text{H}_2\text{SO}_4) = 2 \text{ mol/l}$

4.15 Ethanol/water mixture

Mix nine volumes of ethanol (4.2) and one volume of water.

4.16 Internal standard solution

Accurately weigh approximately 0,25 g isopropylparaben (4.8), transfer to a 500-ml volumetric flask, dissolve and make up to volume with ethanol/water mixture (4.15).

4.17 Mobile phase: tetrahydrofuran/water/methanol/acetonitrile mixture

Mix 5 volumes of tetrahydrofuran, 60 volumes of water, 10 volumes of methanol and 25 volumes of acetonitrile.

4.18 Preservative stock solution

Accurately weigh approximately 0,2 g 2-phenoxyethanol, 0,2 g 1-phenoxypropan-2-ol, 0,05 g methylparaben, 0,05 g ethylparaben, 0,05 g propylparaben, 0,05 g butylparaben and 0,025 g benzylparaben in a 100-ml volumetric flask, dissolve and make up to volume with ethanol/water mixture.

Kept in a refrigerator the solution is stable for one week.

4.19 Standard preservative solutions

From the stock solution (4.18) transfer respectively 20,00 ml, 10,00 ml, 5,00 ml, 2,00 ml and 1,00 ml into 50-ml volumetric flasks. To each flask, add 10,00 ml internal standard solution (4.16) and 1,0 ml sulfuric acid solution (4.14) and make up to volume with ethanol/water mixture. These solutions should be freshly prepared.

5. APPARATUS

Normal laboratory equipment, and:

- 5.1 Waterbath, capable of maintaining a temperature of $60\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$
- 5.2 High performance liquid chromatograph with a UV-detector, wavelength 280 nm
- 5.3 Analytical column:
Stainless steel, 25 cm x 4,6 mm i.d. (or 12,5 cm x 4,6 mm i.d.) packed with Nucleosil 5C18, or equivalent (see 10.1)
- 5.4 100-ml glass tubes with screw cap
- 5.5 Boiling chips, carborundum, size 2 to 4 mm, or equivalent

6. PROCEDURE

6.1 Sample preparation

6.1.1 Sample preparation without addition of the internal standard

Weigh approximately 1,0 g of sample in a 100-ml glass tube with screw cap. Pipette 1,0 ml sulfuric acid solution (4.14) and 50,0 ml ethanol/water mixture (4.15) into the tubes. Add approximately 1 g of boiling chips (5.5), close the tube and shake vigorously until a homogeneous suspension is obtained. Shake for at least one minute. Place the tube for five minutes in a waterbath (5.1) kept at $60\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ to facilitate the extraction of the preservatives into the ethanol phase.

Immediately cool the tube in a stream of cold water and store the extract in the refrigerator for one hour. Filter the extract using a filter paper. Transfer approximately 2 ml of the filtrate into a 5 ml sample vial. Store the extracts in the refrigerator and perform the HPLC determination within 24 hours.

6.1.2 Sample preparation including addition of internal standard

Weigh to three decimal places $1,0\text{ g} \pm 0,1\text{ g}$ of sample in a 100-ml glass tube with screw cap.

Pipette 1,0 ml sulfuric acid solution and 40,0 ml ethanol/water mixture into the tube. Add approximately 1 g of boiling chips (5.5) and exactly 10,00 ml internal standard solution. Close the tube and shake vigorously until a homogeneous suspension is obtained. Shake for at least one minute. Place the tube for 5 minutes in a waterbath kept at $60\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ to facilitate extraction of the preservatives into the ethanol phase.

Immediately cool the tube in a stream of cold tap water and store the extract in the refrigerator for one hour. Filter the extract using a filter paper.

Transfer approximately 2 ml of the filtrate into a 5-ml sample vial (test solution). Store the extract in the refrigerator and perform the HPLC determinations within 24 hours.

6.2 High-performance liquid chromatography (HPLC)

6.2.1 Chromatographic conditions

- Mobile phase: tetrahydrofuran/water/methanol/acetonitrile mixture (4.17)
- Flow rate: 1,5 ml /minute
- Detection wavelength: 280 nm

6.2.2 Calibration

Inject 10 µl of each of the standard preservative solutions (4.19). From the chromatograms obtained determine the ratios of the peak heights of the standard preservative solutions to the peak height of the internal standard. Plot a curve for each preservative relating these ratios to the concentrations of the standard solutions.

6.2.3 Determination

Inject 10 µl of the sample solution without internal standard (6.1.1) into the chromatograph and record the chromatogram.

Inject 10 µl of one of the standard preservative solutions (4.19) and record the chromatogram. Compare the chromatograms obtained.

If, in the chromatogram of the sample extract (6.1.1), no peak is present having approximately the same retention time as isopropylparaben (recommended internal standard), continue by injecting 10 µl sample solution with internal standard (6.1.2). Record the chromatogram and measure the peak heights.

If an interfering peak is observed in the chromatogram of the sample solution having approximately the same retention time as isopropylparaben, another internal standard should be selected.

If one of the preservatives under examination is absent in the chromatogram of the sample, this preservative can be used as an alternative internal standard.

Calculate the ratios of the peak heights of the investigated preservatives to the peak height of the internal standard.

Ascertain that for the standard solutions used in the calibration procedure a linear response is obtained.

Ascertain whether the chromatograms obtained for a standard solution and the sample solution meet the following requirements:

- the peak separation of the worst separated pair shall be at least 0,90. (For definition of peak separation, see Figure 1).

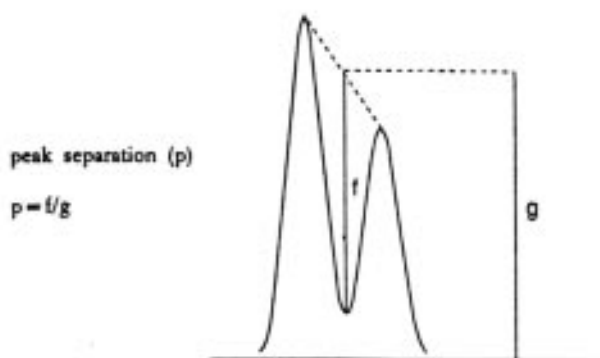


Figure 1: Peak separation

If the required separation is not achieved, either a more efficient column should be used, or the mobile phase composition should be adjusted until the requirement is met.

- the asymmetry factor A_S of all peaks obtained shall range between 0,9 to 1,5. (For definition of the peak asymmetry factor, see Figure 2.) To record the

chromatogram for the determination of the asymmetry factor a chart speed of at least 2 cm/minute is recommended.

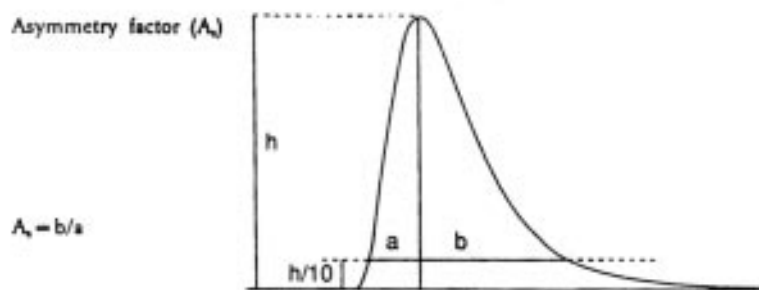


Figure 2: Peak asymmetry factor

— A steady baseline shall be obtained.

7. CALCULATION

Use the calibration curve (6.2.2) and the ratios of the peak heights of the investigated preservatives to the peak height of the internal standard to calculate the concentration of the preservatives in the sample solution. Calculate the 2-phenoxyethanol, 1-phenoxypropan-2-ol, methyl 4-hydroxybenzoate, ethyl 4-hydroxybenzoate, propyl 4-hydroxybenzoate, butyl 4-hydroxybenzoate and benzyl 4-hydroxybenzoate contents, w_i , as percentage by weight (% m/m), using the formula:

$$\% w_i \text{ (m/m)} = \frac{b_i}{200 \times a}$$

in which:

b_i = the concentration ($\mu\text{g/ml}$) of preservative i in the test solution as read from the calibration curve; and

a = the mass (g) of the test position.

8. REPEATABILITY (1)

See remarks, 10.5.

9. REPRODUCIBILITY (1)

See remarks, 10.5.

10. REMARKS

10.1 Stationary phase

The retention behaviour of the solutes in HPLC determinations is strongly dependent on the type, the brand and the history of the stationary phase. Whether a column can be used for the separation of the preservatives under examination, can be concluded from the results obtained for standard solutions (see remarks 6.2.3). In addition to the proposed column packing material, Hypersil ODS and Zorbax ODS were also found to be suitable.

(1) ISO 5725.

Alternatively, the recommended mobile phase composition can be optimized in order to obtain the required separation.

10.2 **Detection wavelength**

A ruggedness test on the described method has shown that a slight change in the detection wavelength can have a significant effect on the results of the determination.

Therefore, this parameter must be controlled carefully during the analysis.

10.3 **Interferences**

Under the conditions described in this method many other compounds, such as preservatives and cosmetic additives, are eluted as well. Retention times of a large number of preservatives mentioned in Annex VI to the Council Directive regarding cosmetic products are listed in: N. de Kruijf, M.A.H. Rijk, L. A. Pranato-Soetardhi and A. Schouten, (1989). Determination of preservatives in cosmetic products II. High-performance liquid chromatographic identification (*J. Chromatography* 469, 317-398).

10.4 To project the analytical column an appropriate guard column may be used.

10.5 The method has been investigated in a collaborative trial in which nine laboratories participated. Three samples were analyzed. The following table lists, for each of the three samples, the means in % m/m (m), repeatabilities (r), reproducibilities (R) found for the analytes they contained:

Sample		2-Phenoxy-ethanol	1-Phenoxy-propan-2-ol	Methyl-paraben	Ethyl-paraben	Propyl-paraben	Butyl-paraben	Benzyl-paraben
Vitamin cream	m	1,124		0,250	0,0628	0,031	0,0906	
	r	0,016		0,018	0,0035	0,0028	0,0044	
	R	0,176		0,030	0,0068	0,0111	0,0034	
Vanishing cream	m	1,196		0,266	0,076			
	r	0,040		0,003	0,002			
	R	0,147		0,022	0,004			
Massage cream	m		0,806			0,180	0,148	0,152
	r		0,067			0,034	0,013	0,015
	R		0,112			0,078	0,012	0,016