II

*(Acts whose publication is not obligatory)*

# COMMISSION

## 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 silver nitrate, the identification and determination of selenium disulphide in anti-dandruff 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, dibromopropamidine 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,

— 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.

(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.

*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. The 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 :

$$\%\ (m/m)\ \text{of silver nitrate} = \frac{1{,}5748 \times c}{10 \times m}$$

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.

(1) ISO 5725.

3. *Reagents*

All reagents must be of analytical purity.

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 digestor

4.4. Filter paper (Whatman No 42 or equivalent) or a 0,45 µm 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 digestor (4.3).

5.2. Dilute the digested sample to 25 ml with water and filter through a filter paper or a 0,45 µm 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 µg/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 digestor

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 digestor (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.

(1) ISO 5725.

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.

4. *Apparatus*

4.1. Normal laboratory equipment

4.2. pH meter with an accuracy of ± 0,02 units

4.3. Wrist-action flask-shaker

4.4. Membrane filter with a pore size of 0,45 µm

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 a 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 µm 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 µg 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:

$$\% \text{ (m/m) of soluble barium} = \frac{c \times V}{10 W_{Ba} \times m}$$

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.

## 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).

(1) 'Magnesium as modifier for the determination of barium by flame atomic emission spectrometry'. Jerrow, M. *et al., Analytical Proceedings*, 1991, 28, 40.

3.4. Strontium standard solutions

3.4.1. Stock strontium standard 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 :

$$\% \text{ (m/m) of soluble strontium} = \frac{c \times V}{10 W_{Sr} \times m}$$

in which :

m = mass in grams of the sample taken for analysis (A.5.1.1);

c = concentration of strontium in the sample solution (5.1.1), 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 × 200 mm or 200 × 200 mm, coated with a 0,25 mm layer of silica gel 60 $F_{254}$.

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 × 230 mm × 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 (5.2.3) 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. 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 × 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 %.

(1) ISO 5725.

## 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 $[Al_xZr(OH)_yCl_z.nH_2O]$.

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

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) [$AlCl_3.6H_2O$] : 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 sepctrometry.

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, 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).

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

Wavelenght : 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.

(1) ISO 5725.

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 (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

4.5. pH/millivolt meter suitable for potentiometric titration

(1) ISO 5725.

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:

$$\text{Al : Zr ratio} = \frac{\text{Al \% (m/m)} \times 91{,}22}{\text{Zr \% (m/m)} \times 26{,}98}$$

2. *Calculation of the ratio of aluminium plus zirconium atoms to chlorine atoms*

Calaculate the (Al + Zr) : Cl ratio using the formula:

$$\text{(Al + Zr) : Cl ratio} = \frac{\frac{\text{Al \% (m/m)}}{26{,}98} + \frac{\text{Zr \% (m/m)}}{91{,}22}}{\frac{\text{Cl \% (m/m)}}{35{,}45}}$$

## 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, dibromopropramidine and chlorhexidine are identified by their retention times on the chromatographic column.

(1) ISO 5725.

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 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 [$C_{20}H_{26}N_4O_2.2C_2H_6O_4S$]

4.7. Dibromohexamidine diisethionate [$C_{20}H_{24}Br_2N_4O_2.2C_2H_6O_4S$]

4.8. Dibromopropamidine diisethionate [$C_{17}H_{18}Br_2N_4O_2.2C_2H_6O_4S$]

4.9. Chlorhexidine diacetate [$C_{22}H_{30}Cl_2N_{10}.2C_2H_4O_2$]

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 $C_{18}$, 10 μm, 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 I (% 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 an 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:

$$\% \quad (m/m) = \frac{c}{1000 \times p} \times \frac{MW_1}{MW_2}$$

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;

$MW_1$ = molecular weight of the basic form of the preservative present;
and

$MW_2$ = 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 %.

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 |

(1) ISO 5725.