Third Commission Directive 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 (83/514/EEC)

THIRD COMMISSION DIRECTIVE

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

(83/514/EEC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products[(1)], as 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-hydroxyquino-linium) 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 tion 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-hydroxyquinolinium) sulphate,

— determination of ammonia,

— identification and determination of nitro-methane,
— identification and determination of mercapto-acetic 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 organo-mercury 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)[(5)].

5.4. Pressure gas-syringe, 25 or 50 µl (see sampling method 5.4.2.2)[(5)].

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

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***	***II***
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***	***II***
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	15µl

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 = l):

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

then:

$$\%\ (m/m)\ CH_2Cl_2 = \frac{m_e \times A_1 \times k_1 \times 100}{A_e \times M_e}$$

$$\%\ (n/m)\ CH_3CCl_3 = \frac{m_e \times A_2 \times k_2 \times 100}{A_e \times M_e}$$

10. REPEATABILITY[6]

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.

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 sodiun 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-(chloroin ino)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 ^{o}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 construction 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 ^{o}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 ayer 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 res due in 2 ml of chloroform (4.3) and spot 10 and 30µl 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 µl 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 suphuric acid solution (4.8) and evaporate the mixture almost to dryness under reduced pressure at 50 ^{o}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 1 *N* HC1 (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, tarmp 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 collectec 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***

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[7]

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.

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 overnighs in the refrigerator (5 ^{o}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 inillilitres) 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:

$$\text{ammonia \% (m/m)} = \frac{(20\,M_2 - V_1 M_1) \times 17 \times 100}{0{,}4\,m} = \frac{(20\,M_2 - V_1 M_1) \times 4250}{m}$$

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:

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

8. REPEATABILIT Y[8]

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.

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-l-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/EEC of 22 December 1980[9].

5.2.3. Usual laboratory apparatus.

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 above-mentioned 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 mg 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:

Materia: 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 ^{o}C,

Detector: 150 ^{o}C,

Column: between 50 and 80 ^{o}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-dimathylheptane:

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 o[2] the apparatus).

6.2. ***Concentration of nkromethane 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 grans) 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[10]

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 he 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) papes.

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

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 (thioglycollic 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-l,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-l,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 Rf values and the colour of the reference solutions with those of the standards. The mean Rf 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 RF VALUES OBTAINED ON A SILICA GEL LAYER

	Eluting solvents	
	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 (see NB)

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:

$2\ HOOC\text{-}CH_2SH + I_2 \rightarrow (HOOC\text{-}CH_2\text{-}S)_2 + 2\ I + 2\ H^+$

5.1.2. *Reagents*

Iodine, 0,05 M standard solution.

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

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 mercaptoaceric acid content is calculated according to the formula:

$$\% \, (m/m) = \frac{92 \times n \times 100}{1000 \times 10 \times m} = \frac{0{,}92 \, n}{m}$$

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

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_{410} = 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), 27,5 g.

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, 0,05 M standard solution.

5.2.2.11. Diethyl ether.

5.2.2.12. *Diazomethane solution prepared from iV-methyl-AT-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 (5.2.2.6).

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 l-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 flame 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 ^{o}C

Detector: 200 ^{o}C

Column: 90 ^{o}C

5.2.7.6. *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 tempera-ture from 90 to 150 ^{o}C at a rate of 10 ^{o}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 millegrams) in the mixture,
S'_c = its peak area,

then:

$$kt = \frac{m'_t}{m'_c} \times \frac{S'_c}{S'_t}$$

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 mill grams) 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[11]

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-(cMoroimino)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.

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. *Visualization*

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 Rf of approximately 0,5.

7.2. Visualizing agent II (3.6):

Hexachlorophene is revealed as a sky-blue to turquoise coloured spot on a white background and has an Rf 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 63 Ni 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***[12]

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 ^{o}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 ^{o}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)[13].

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 µl of this solution into the chromatograph.

6.4. ***Methylation of the standard***

Cool all reagents and apparatus to between 0 and 4 ^{o}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.

7. **GAS CHROMATOGRAPHY**

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

let:

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

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 mas (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,

then:

$$kh = \frac{m'_h}{m'_s} \times \frac{A'_s}{A'_h}$$

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:

$$\frac{m_e \times k_b \times A_b \times 100}{M \times A_e}$$

9 REPEATABILITY[14]

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.

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 plates, silica gel 60, without fluorescent indicator.

4.8. Potassium permanganace.

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. Photodensitometer.

6. PROCEDURE

6.1. ***Hydrolysis***

Weigh accurate y 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 3000 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 µl 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 µl 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 ^{o}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[15]

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 %.

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 ^{o}C to constant mass.

4.2. Water, double distilled or equivalent quality.

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. Bühler, shaker, type SMB_1 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 ^{o}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 (A_{TEFS}/A_{CH}) 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. ***Calibration 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),
m_1 = the amount of F (in milligrams) read from the calibration graph (6.1.8).

8. REPEATABILITY[16]

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 organo-mercury 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.

A. **IDENTIFICATION**

1. PRINCIPLE

The organomercury compounds are complexed with l,5-diphenyl-3-thiocarba-zone. 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. l,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. ***Separatum 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 mineralization, 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 ^{o}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 ^{o}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 ^{o}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 ^{o}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[17]

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 reagents should be of analytical purity.

4.1. Concentrated hydrochloric acid, d_4^{20} = 1,19 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).

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 flasks (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. CALCULATON

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

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

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[18]

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**) 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.

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

(**6**) Norm ISO 5725.

(**7**) Norm ISO 5725.

(**8**) Norm ISO 5725.

(**9**) OJ No L383, 31.12.1980, p. 27.

(**10**) Norm ISO 5725.

(**11**) Norm ISO 5725.

(**12**) 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 ethyl acetate) etc., could be introduced with agreement of the parties concerned.

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

(**14**) Norm ISO 5725.

(**15**) Norm ISO 5725.

(**16**) Norm ISO 5725.

(**17**) Norm ISO 5725.

(**18**) Norm ISO 5725.