II

(Acts whose publication is not obligatory)

COMMISSION

SECOND COMMISSION DIRECTIVE

of 14 May 1982

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

(82/434/EEC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

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

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

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

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

HAS ADOPTED THIS DIRECTIVE:

Article 1

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

— identification of oxidizing agents and determination of hydrogen peroxide in hair-care products,

— identification and semi-quantitative determination of certain oxidation colorants in hair dyes,

— identification and determination of nitrite,

— identification and determination of free formaldehyde,

[1] OJ No L 262, 27. 9. 1976, p. 169.
[2] OJ No L 192, 31. 7. 1979, p. 35.
[3] OJ No L 383, 31. 12. 1980, p. 27.

— determination of resorcinol in shampoos and hair lotion,

— determination of methanol in relation to ethanol or propan-2-ol

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

Article 2

Member States shall bring into force the laws, regulations or administrative provisions necessary to comply with this Directive not later than 31 December 1983.

They shall forthwith inform the Commission thereof.

Article 3

This Directive is addressed to the Member States.

Done at Brussels, 14 May 1982.

For the Commission

Karl-Heinz NARJES

Member of the Commission

ANNEX

I. IDENTIFICATION OF OXIDIZING AGENTS AND DETERMINATION OF HYDROGEN PEROXIDE IN HAIR-CARE PRODUCTS

PURPOSE AND SCOPE

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

A. IDENTIFICATION OF PERSULPHATES, BROMATES AND HYDROGEN PEROXIDE

1. PRINCIPLE

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

2. REAGENTS

All reagents should be of analytical purity.

2.1. 0·5 % (m/v) aqueous reference solutions of the following compounds:

2.1.1. Sodium persulphate

2.1.2. Potassium persulphate

2.1.3. Ammonium persulphate

2.1.4. Potassium bromate

2.1.5. Sodium bromate

2.1.6. Hydrogen peroxide

2.2. Developing solvent A, 80 % (v/v) ethanol

2.3. Developing solvent B, benzene — methanol — 3-methyl butan-1-ol — water (34:38:18:10 by vol)

2.4. Detecting agent A, 10 % (m/v) aqueous solution of potassium iodide

2.5. Detecting agent B, 1 % (m/v) aqueous solution of starch

2.6. Detecting agent C, 10 % (m/m) hydrochloric acid

2.7. 4N hydrochloric acid

3. APPARATUS AND EQUIPMENT

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

3.2. Micropipette, 1 µl

3.3. Standard flasks, 100 ml

3.4. Fluted filters

3.5. Apparatus for descending paper chromatography

4. SAMPLE PREPARATION

4.1. **Water soluble products**

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

4.2. **Products sparingly soluble in water**

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

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

4.3. **Creams**

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

5. METHOD

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

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

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

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

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

5.6. Reveal the spots in the chromatogram by spraying it successively with:

5.6.1. detecting agent A (2.4) followed shortly thereafter by detecting agent B (2.5). The spots of the persulphates will now appear first in the chromatogram and will be followed by the hydrogen peroxide spots. Mark the spots with a pencil;

5.6.2. detecting agent C (2.6) on the chromatograms obtained in accordance with Section 5.6.1; the presence of bromates will now be indicated by greyish blue spots in the chromatogram.

5.7. Under the abovementioned conditions pertaining to developing solvents A (2.2) and B (2.3), the Rf values of the reference substances (2.1) are approximately as follows:

	Developing solvent A (2.2)	*Developing solvent B (2.3)*
Sodium persulphate	0·40	0·10
Potassium persulphate	0·40	0·02 + 0·05
Ammonium persulphate	0·50	0·10 + 0·20
Sodium bromate	0·40	0·20
Potassium bromate	0·40	0·10 + 0·20
Hydrogen peroxide	0·80	0·80

B. IDENTIFICATION OF BARIUM PEROXIDE

1. PRINCIPLE

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

— in the absence of persulphates (A), by adding dilute sulphuric acid to a portion of the acid sample solution (B.4.1), as a result of which a white precipitate of barium sulphate is formed. The presence of barium ions in the sample (B.4.1) is again confirmed by paper chromatography in the manner described below (B.5),

— where barium peroxide and persulphates are present simultaneously (B.4.2) by digesting the residue from the solution (B.4.2) in an alkali; after dissolution in hydrochloric acid, the presence of barium ions is confirmed in the solution of the melt (B.4.2.3) by paper chromatography and/or by barium sulphate precipitation.

2. REAGENTS

2.1. Methanol

2.2. 36 % (m/m) concentrated hydrochloric acid

2.3. 6N hydrochloric acid

2.4. 4N sulphuric acid

2.5. Rhodizonic acid disodium salt

2.6. Barium chloride ($BaCl_2 \cdot 2H_2O$)

2.7. Anhydrous sodium carbonate

2.8. 1 % (m/v) aqueous solution of barium chloride

2.9. Developing solvent consisting of methanol, concentrated hydrochloric acid (concentration 36 %) and water (80 : 10 : 10 by vol)

2.10. Detecting agent, 0·1 % (m/v) aqueous solution of rhodizonic acid disodium salt, to be freshly prepared immediately before use.

3. APPARATUS AND EQUIPMENT

3.1. Micropipette, 5 µl

3.2. Platinum crucibles

3.3. Standard flasks, 100 ml

3.4. Chromatography paper Schleicher and Schull 2043 b or equivalent. Clean the paper by developing it overnight in a descending chromatography tank (A.3.5) containing developing solvent (B.2.9) and then dry.

3.5. Fluted filter paper

3.6. The usual apparatus for carrying out ascending paper chromatography

4. SAMPLE PREPARATION

4.1. **Products in which persulphates are absent**

4.1.1. Disperse 2 g of the product in 50 ml of water and bring the pH of the dispersion to about 1 with hydrochloric acid (B.2.3).

4.1.2. Transfer the dispersion with water into a 100-ml standard flask, make up to the mark with water and mix. Use this dispersion for the paper chromatography analysis described in Section 5 and for identification of barium by precipitation of the sulphate.

4.2. **Products in which persulphates are present**

4.2.1. Disperse 2 g of the product in 100 ml of water and filter.

4.2.2. Add to the dried residue seven to 10 times its weight of sodium carbonate (B.2.7), mix and melt the mixture in a platinum crucible (B.3.2) for half an hour.

4.2.3. Cool to room temperature, dissolve the melt in 50 ml of water and filter (B.3.5)

4.2.4. Dissolve the residue from the melt in hydrochloric acid (B.2.3) and make up to 100 ml with water. Use this solution for the paper chromatography analysis described in Section 5 and for the identification of barium by precipitation of the sulphate.

5. METHOD

5.1. Place an appropriate quantity of developing solvent (B.2.9) in a tank for ascending paper chromatography and saturate the tank for at least 15 hours.

5.2. On a piece of chromatography paper — pretreated as described in Section B.3.4 — apply 5 µl of each of the solutions prepared in accordance with Sections B.4.1.2 and B.4.2.4 and reference solution B.2.8 at three starting points.

5.3. Dry the sample and reference spots in air. Develop the chromatogram until the solvent front has ascended 30 cm.

5.4. Remove the chromatogram from the tank and dry in air.

5.5. Reveal the spots on the chromatogram by spraying the paper with detecting agent B.2.10. In the presence of the barium, red spots with an RF value of about 0·10 appear on the chromatogram.

C. DETERMINATION OF HYDROGEN PEROXIDE

1. PRINCIPLE

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

$$H_2O_2 + 2H^+ + 2I^- \longrightarrow I_2 + 2H_2O$$

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

2. DEFINITION

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

3. REAGENTS

All reagents should be of analytical purity.

3.1. 2N sulphuric acid

3.2. Potassium iodide

3.3. Ammonium molybdate

3.4. 0·1N sodium thiosulphate

3.5. 10 % (m/v) potassium iodide solution, to be freshly prepared immediately before use

3.6. 20 % (m/v) ammonium molybdate solution

3.7. 1 % (m/v) starch solution

4. APPARATUS AND EQUIPMENT

4.1. Beakers, 100 ml

4.2. Burette, 50 ml

4.3. Standard flasks, 250 ml

4.4. Measuring cylinders, 25 and 100 ml

4.5. One-mark pipettes, 10 ml

4.6. Conical flasks, 250 ml

5. METHOD

5.1. Into a 100-ml beaker weigh out 10 g (m gram) of the product, containing about 0·6 g of hydrogen peroxide. Transfer the contents with water into a 250-ml standard flask, make up to the mark with water and mix.

5.2. Pipette 10 ml of the sample solution (5.1) into a 250-ml conical flask (4.6) and add successively 100 ml of 2N sulphuric acid (3.1), 20 ml of potassium iodide solution (3.5) and three drops of ammonium molybdate solution (3.6).

5.3. Titrate the iodine formed immediately against 0·1N sodium thiosulphate solution (3.4) and just before the end point is reached add a few millilitres of starch solution as indicator (3.7). Record the consumption of 0·1N sodium thiosulphate (3.4) in millilitres (V).

5.4. In the manner described in Sections 5.2 and 5.3, carry out a blank determination, replacing the 10 ml of the sample solution by 10 ml of water. Record the consumption of 0·1N sodium thiosulphate in the blank determination (Vo ml).

6. CALCULATION

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

$$\% \text{ hydrogen peroxide} = \frac{(V - Vo) \times 1{\cdot}7008 \times 250 \times 100}{m \times 10 \times 1\,000}$$

$$= \frac{(V - Vo) \times 4{\cdot}252}{m}$$

in which:

m = the quantity in grams of product analyzed (5.1),

Vo = the consumption in millilitres of 0·1N thiosulphate solution in the blank determination (5.4),

V = the consumption in millilitres of 0·1N thiosulphate solution in the titration of the sample solution (5.3).

7. REPEATABILITY (1)

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

(1) See Norm ISO 5725.

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

1. PURPOSE AND SCOPE

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

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

2. PRINCIPLE

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

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

3. REAGENTS

All reagents should be of analytical purity.

3.1. Ethanol, anhydrous

3.2. Acetone

3.3. Ethanol, 96 % v/v

3.4. Ammonia solution, 25 % ($d_4^{20} = 0{\cdot}91$)

3.5. L(+)-ascorbic acid

3.6. Chloroform

3.7. Cyclohexane

3.8. Nitrogen, technical grade

3.9. Toluene

3.10. Benzene

3.11. n-Butanol

3.12. Butan-2-ol

3.13. Hypophosphorous acid, 50 % v/v solution

3.14. Diazo reagent. Either:

— 3-Nitro-1-benzenediazonium chlorobenzenesulphonate, (stabilized salt form) as in Red 2 JN — Francolor,

— 2-Chloro-4-nitro-1-benzenediazonium naphthalenebenzoate (stabilized salt form) as in NNCD Reagent — reference No 74 150 FLUKA,

or an equivalent.

3.15. Silver nitrate

3.16. p-Dimethylaminobenzaldehyde

3.17. 2,5-Dimethylphenol

3.18. Ferric chloride hexahydrate

3.19. Hydrochloric acid, 10 % m/v solution

3.20. **Reference substances**

The reference substances are those shown in paragraph 1 'Purpose and scope'. In the case of amine compounds, the reference substance must be either the hydrochloride (mono or di) or the free base.

3.21. **Reference solutions 0·5 % (m/v)**

A 0·5 % (m/v) solution of each of the reference substances in Section 3.20 is prepared.

Weigh out 50 mg ± 1 mg of the reference substance in a standard 10-ml flask.

Add 5 ml of 96 % ethanol (3.3) and 250 mg of ascorbic acid (3.5).

Make the solution alkaline by addition of the ammonia solution (3.4) to give an apparent pH of 10 (test with indicator paper).

Make up to 10 ml with 96 % ethanol (3.3) and mix.

The solutions may be kept for a week in a cool place away from the light.

In certain cases, after the addition of the ascorbic acid and the ammonia, a precipitate may form. It should then be allowed to settle before proceeding.

3.22. **Developing solvents**

3.22.1. Acetone — chloroform — toluene (35 : 25 : 40 by vol)

3.22.2. Chloroform — cyclohexane — absolute ethanol — 25 % ammonia (80 : 10 : 10 : 1 by vol)

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

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

Preparation of reagent M

Ammonia solution, 25 % (v/v)	24 volumes
Hypophosphorous acid, 50 % (3·13)	1 volume
Water	75 volumes

Note

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

3.23. **Indicator sprays**

3.23.1. *Diazo reagent*

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

3.23.2. *Ehrlich's reagent*

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

3.23.3. *2,5-dimethylphenol — ferric chloride hexahydrate*

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

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

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

3.23.4. *Ammoniacal silver nitrate*

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

Do not keep.

4. APPARATUS

4.1. Usual laboratory equipment for thin-layer chromatography.

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

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

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

4.2. Centrifuge, 4 000 rev./min.

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

5. PROCEDURE

5.1. **Treatment of test samples**

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

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

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

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

Use the supernatant liquid.

5.2. **Chromatography**

5.2.1. *Spotting the plates*

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

These reference solution spots are arranged as follows:

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

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

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

5.2.2. *Development*

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

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

5.2.3. *Spraying*

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

5.2.4. *Identification*

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

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

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

5.2.5. *Semi-quantitative estimation*

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

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

TABLE I

R_f values and colours obtained immediately after spraying

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

Note

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

6. EXAMINATION BY TWO-DIMENSIONAL THIN-LAYER CHROMATOGRAPHY

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

6.1. **Additional reference solutions and substances**

6.1.1. β-naphthol (β-N)

6.1.2. 2-aminophenol (OAP)

6.1.3. 3-aminophenol (MAP)

6.1.4. 4-aminophenol (PAP)

6.1.5. 2-nitro-1,4-phenylenediamine (2-NPPD)

6.1.6. 4-nitro-1,2-phenylenediamine (4-NOPD)

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

6.2. **Additional developing solvent**

6.2.1. Ethyl acetate — cyclohexane — ammonia solution, 25 % (65 : 30 : 0·5 by vol)

6.3. **Additional indication system**

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

6.4. **Chromatography**

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

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

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

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

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

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

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

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

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

Note

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

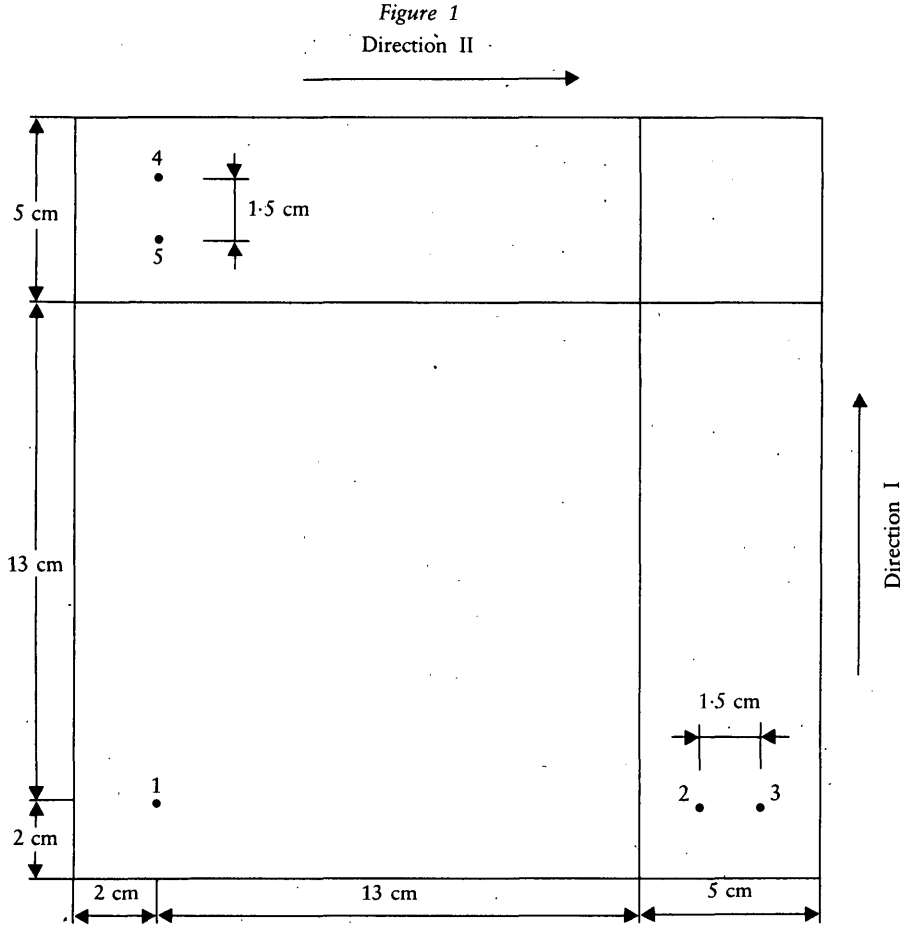
6.4.9. The presence of the oxidation colorants found in 6.4.8 can be definitively confirmed by repeating the operations described in 6.4.1 to 6.4.8 and adding at base point 1 on top of the amount of extract specified in 6.4.2 1 µl of the reference substances identified in 6.4.8. If no other spot is found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

TABLE II

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

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

Figure 1



III. IDENTIFICATION AND DETERMINATION OF NITRITE

A. IDENTIFICATION

1. PURPOSE AND SCOPE

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

2. PRINCIPLE

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

3. REAGENTS

All reagents should be of analytical purity.

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

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

3.3. Methanol

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

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

4. APPARATUS

4.1. Beakers, 50 ml

4.2. Standard flask, 100 ml

4.3. Brown glass bottle, 125 ml

4.4. Glass plate, 10 × 10 cm

4.5. Plastics spatula

4.6. Filter paper, 10 × 10 cm

5. PROCEDURE

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

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

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

— two drops of dilute sulphuric acid (3.1),

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

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

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

6. COMMENT

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

B. DETERMINATION

1. PURPOSE

The method describes the determination of nitrite in cosmetic products.

2. DEFINITION

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

3. PRINCIPLE

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

4. REAGENTS

All reagents should be of analytical quality.

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

4.1.1. Carrez I reagent:

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

4.1.2. Carrez II reagent:

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

4.2. Sodium nitrite solution:

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

4.3. 1N sodium hydroxide solution

4.4. 0·2 % sulphanilamide hydrochloride solution:

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

4.5. 5N hydrochloric acid

4.6. N-1-naphthyl reagent:

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

5. APPARATUS

5.1. Analytical balance

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

5.3. Bulb or graduated pipettes

5.4. Measuring cylinders of 100 ml

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

5.6. Water-bath

5.7. Spectrophotometer with optical cells of 1 cm path-length

5.8. pH meter

5.9. Microburette of 10 ml

5.10. Beakers of 250 ml

6. PROCEDURE

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

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

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

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

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

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

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

6.8. Measure (5.7) the optical density at 538 nm of the solution obtained under 6.6 using the blank solution (6.7) as a reference.

6.9. Read from the calibration graph (6.10) the sodium nitrite content in micrograms per 100 ml of the solution (m_1 micrograms) that corresponds to the optical density measured in 6.8.

6.10. Using the 10 µg per ml sodium nitrite solution (4.2), prepare a calibration graph for concentrations of 0, 20, 40, 60, 80, 100 µg of sodium nitrite per 100 ml.

7. CALCULATION

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

$$\% \ NaNO_2 = \frac{250}{V} \times m_1 \times 10^{-6} \times \frac{100}{m} = \frac{m_1}{V \times m \times 40}$$

in which:

m = the mass of the sample in grams taken for analysis (6.1),

m_1 = the sodium nitrite content in micrograms found in 6.9,

V = the number of millilitres of the filtrate used for the measurement (6.5).

8. REPEATABILITY (1)

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

IV. IDENTIFICATION AND DETERMINATION OF FREE FORMALDEHYDE

1. PURPOSE AND SCOPE

This method describes the identification and the determination of free formaldehyde. It is applicable to all cosmetic products and comprises three parts:

1.1. Identification

1.2. Determination by pentane-2,4-dione colorimetry

This method is inadequate when the formaldehyde is combined or polymerized, as in the case of formaldehyde donors. If the result exceeds the maximum permitted concentration, the following method must be used.

1.3. Determination with bisulphite

With this method, formaldehyde in most combined or polymeric compounds is not taken into account. However, certain unstable combinations (hexamethylene tetramine, for example) are determined. Moreover, the alkalinity measurement is difficult in the presence of a buffer solution.

2. DEFINITION

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

3 PRINCIPLE

3.1. **Part I — Identification**

Formaldehyde in a sulphuric acid medium turns Schiff's reagent pink or mauve.

3.2. **Part II — Determination by pentane-2,4-dione**

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

(1) See Norm ISO 5725.

3.3. **Part III — Determination with bisulphite**

Formaldehyde reacts with the sulphite in an acid medium at 0 °C to form an addition compound. The excess protons are titrated with sodium hydroxide. The protons consumed form the basis of the calculation for determining the quantity of formaldehyde. A blank test without sulphite enables the alkalinity or acidity of the medium to be measured.

4. REAGENTS

All the reagents should be of analytical purity.

4.1. Glacial acetic acid

4.2. Anhydrous ammonium acetate

4.3. Butan-1-ol

4.4. Sulphuric acid, about 2N

4.5. Freshly prepared 0·1 M sodium sulphite solution

4.6. Schiff's reagent 100 mg of fuchsine is weighed into a beaker and dissolved in 75 ml of water at 80 ° C.

After cooling, add 2·5 g of sodium sulphite heptahydrate ($Na_2SO_3 \cdot 7H_2O$) and 1·5 ml of concentrated hydrochloric acid ($d_4^{20} = 1{\cdot}19$). Make up to 100 ml.

(This reagent is unsuitable for use after two weeks.)

4.7. Pentane-2,4-dione reagent

In a 1 000-ml volumetric flask dissolve:

150 g ammonium acetate (4·2),

2 ml pentane-2,4-dione (freshly distilled under reduced pressure — it should not exhibit any absorption at 410 nm),

3 ml glacial acetic acid (4·1).

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

This reagent must be freshly prepared.

4.8. Standard sulphuric acid solution, 0·1N

4.9. Standard sodium hydroxide solution, 0·1N

4.10. Iodine solution, 0·1N

4.11. Sodium thiosulphate, 0·1N

4.12. Formaldehyde stock solution

Pour 5 g of 37 to 40 % formaldehyde solution into 1 000-ml volumetric flask and make up to 1 000 ml.

Determine the strength of this solution as follows: Remove 10·00 ml; add 25·00 ml of a standard 0·1N iodine solution (4.10) and 10 ml of a 1N sodium hydroxide solution.

Allow to stand for five minutes.

Add 11 ml of 1N HCl and titrate the excess standard 0·1N iodine solution (4.10) with standard 0·1N sodium thiosulphate solution (4.11), using starch solution as indicator.

1 ml of 0·1N iodine solution (4.10) is equivalent to 1·5 mg formaldehyde.

4.13. Formaldehyde reference solution

Pipette 5·00 ml of stock solution (4.12) into a 100-ml graduated flask and make up to 100 ml with demineralized water.

Pipette 5·00 ml of the above solution into a 500-ml graduated flask and make up to 500 ml with demineralized water.

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

Calculate the exact content.

4.14. Thymolphthalein solution, 0·1 g/100 ml of 50 % ethanol

4.15. Reference reagent solution: as reagent 4.7 but without the pentane-2,4-dione

5. APPARATUS

5.1. Standard laboratory apparatus

5.2. Phase separation filter, Whatman 1 PS (or equivalent)

5.3. Centrifuge

5.4. Spectrophometer

5.5. Glass cells with an optical path of 1 cm

5.6. Potentiometer with chart recorder

5.7. Glass/calomel electrodes (it is recommended that special low-temperature electrodes be used).

6. PROCEDURE

6.1. **Identification**

6.1.1. Weigh 2 g of the test sample into a 10-ml beaker.

6.1.2. Add two drops of 2N sulphuric acid (4.4) and 2 ml of Schiff's reagent (4.6) (this reagent must be absolutely colourless when it is used).

Shake and leave to stand for five minutes.

6.1.3. If a pink or mauve tint is observed within the five minutes, the formaldehyde is present in excess of 0·01 % and is to be determined by the procedure 6.2 and, if necessary, by procedure 6.3.

6.2. **Determination by pentane-2,4-dione colorimetry**

6.2.1. *Sample solution*

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

6.2.1.2. Make up to 100 ml with demineralized water and mix.

6.2.1.3. To a 50-ml Erlenmeyer flask add:
10·00 ml solution from 6.2.1.2,
5·00 ml pentane-2,4-dione reagent (4.7),
demineralized water to a final volume of 30 ml.

6.2.2. *Reference solution*

Possible interference due to background colour in the test sample is eliminated by the use of this reference solution.
To a 50-ml Erlenmeyer flask add:
10·00 ml solution from 6.2.1.2,
5·00 ml reference reagent solution (4.15),
demineralized water to a final volume of 30 ml.

6.2.3. *Blank solution*

To a 50-ml Erlenmeyer flask add:
5·00 ml pentane-2,4-dione reagent (4.7),
demineralized water to a final volume of 30 ml.

6.2.4. *Determination*

6.2.4.1. Shake the flasks from 6.2.1.3, 6.2.2 and 6.2.3 and immerse in a water-bath at 60 °C for exactly 10 minutes. Allow to cool for two minutes in a bath of iced water.

6.2.4.2. Transfer into 50-ml separating funnels containing 10·00 ml of butan-1-ol (4.3). Rinse each flask with 3 to 5 ml of water and add the rinsings to the funnels. Shake the mixture vigorously for exactly 30 seconds. Allow it to separate.

6.2.4.3. Filter into the measurement cells through a phase-separation filter. Centrifuging (5 000 rev./min. for five minutes) is less practical and takes longer.

6.2.4.4. Measure the optical density A_1 at 410 nm of the extract of the sample solution from 6.2.1.3 against the extract of the reference solution 6.2.2.

6.2.4.5. Similarly measure the extract of the blank solution from 6.2.3 against butan-1-ol (A_2).

Note

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

6.2.5. *Calibration curve*

6.2.5.1. Into a 50-ml Erlenmeyer flask place:
5·00 ml of the standard solution (4.13),
5·00 ml of the pentane-2,4-dione reagent (4.7),
demineralized water to a final volume of 30 ml.

6.2.5.2. Continue as described in Section 6.2.4.5, measure the optical density against butan-1-ol (4.3).

6.2.5.3. Repeat the procedure with 10, 15, 20 and 25 ml of the standard solution.

6.2.5.4. To obtain the zero value proceed as in 6.2.4.5.

6.2.5.5. Construct the calibration curve after subtraction of the zero value (6.2.4.5) from each of the optical densities obtained in 6.2.5.2 and 6.2.5.3. Beers Law is valid up to 30 µg of formaldehyde.

6.3. **Determination with bisulphite**

6.3.1. *Preparation of the test sample*

6.3.1.1. For the test

Into a tared beaker weigh to the nearest 0·001 g a quantity of test sample (m grams) corresponding to a presumed quantity of between 3 and 20 mg of formaldehyde.

6.3.1.2. For the reference test

In a similar manner, weigh a reference test sample (m grams).

6.3.2. *Determination*

6.3.2.1. Place 50·00 ml of 0·1M sodium sulphite (4.5) in a 100-ml beaker and add 10·00 ml of 0·1N sulphuric acid (4.8). Shake.

6.3.2.2. Immerse the beaker in a mixture of ice and salt for the purpose of maintaining the temperature of the whole at +2 °C. Pour in test sample 6.3.1.1.

6.3.2.3. Titrate rapidly by potentiometry with 0·1N sodium hydroxide (4.9), shaking continuously and maintaining the temperature between +2 and +4 °C (the neutral point lies between pH 9 and 11). Let V_1 be the volume of 0·1N sodium hydroxide (4.9) taken.

6.3.3. *Blank test*

Nitrate an additionally prepared solution as in 6.3.2.1 under the conditions described in 6.3.2.

Let V_2 be the volume of 0·1N sodium hydroxide (4.9) taken.

6.3.4. *Reference test*

Determine the acidity or alkalinity of the sample by potentiometric titration with 0·1N sodium hydroxide (4.9) or 0·1N sulphuric acid (4.8) in test sample m′. Let v′ be the volume of 0·1N sodium hydroxide taken or sulphuric acid 0·1N.

6.3.5. *Notes*

Strict observance of the test conditions is important.

It is possible to perform the determination in the presence of thymolphthalein (4.14) as indicator.

7. PRESENTATION OF THE RESULTS

7.1. Calculation for the colorimetric method

7.1.1. Subtract A_2 from A_1 and read off from the calibration curve (6.2.5.5) the amount C, in micrograms, of formaldehyde in the test solution (6.2.1.3).

7.1.2. Calculate the formaldehyde content of the sample as a percentage by mass (% m/m) with the aid of the following formula:

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

7.2. Calculation for the bisulphite titration method

Relate the volume of 0·1N sodium hydroxide (4.9) or 0·1N sulphuric acid (4.8) taken in the reference test to the mass m:

$$v = \frac{v' \cdot m}{m'}$$

For a neutral product v is, of course, zero.

7.2.1. In the case of an acid product:

$$\text{formaldehyde content in \%} = \frac{0{\cdot}30\,(V_2 - V_1 + v)}{m}$$

7.2.2. In the case of an alkaline product:

$$\text{formaldehyde content in \%} = \frac{0{\cdot}30\,(V_2 - V_1 - v)}{m}$$

7.3. Remark

If the results of the two methods differ, only the lower figure is to be taken.

8. REPEATABILITY (1)

For a formaldehyde content of 0·2 % the difference between the results of two determinations in parallel carried out on the same sample should not exceed 0·005 % for the colorimetric method and 0·05 % for the bisulphite method.

(1) See Norm ISO 5725.

V. DETERMINATION OF RESORCINOL IN SHAMPOOS AND HAIR LOTIONS

1. PURPOSE AND SCOPE

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

2. DEFINITION

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

3. PRINCIPLE

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

4. REAGENTS

All reagents must be of analytical grade.

4.1. Hydrochloric acid 25 % (m/m)

4.2. Methanol

4.3. Ethanol 96 % (v/v)

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

Note

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

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

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

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

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

4.9 Silylating agents:

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

4.9.2. Hexamethyldisilazane (HMDS)

4.9.3. Trimethylchlorosilane (TMCS)

5. APPARATUS

5.1. Usual thin-layer and gas chromatography equipment

5.2. Glassware

6. PROCEDURE

6.1. **Preparation of the sample**

6.1.1. Weigh accurately into a 150-ml beaker a test portion (m grams) of the product which contains approximately 20 to 50 mg resorcinol.

6.1.2. Acidify with hydrochloric acid (4.1) until the mixture is acid (approximately 2 to 4 ml are needed), add 10 ml (40 mg DHT) of the internal standard solution (4.7) and mix. Transfer to a 100-ml volumetric flask with ethanol (4.3) make up to the mark with ethanol and mix.

6.1.3. Apply 250 µl of the solution (6.1.2) to a deactivated silica sheet (4.4) as a continuous line of approximately 8 cm length. Take care to get the line as narrow as possible.

6.1.4. Apply 250 µl of the standard mixture (4.8) to the same plate in the same way (6.1.3).

6.1.5. Spot on two points of the starting line 5 µl of each of the solutions 4.6 and 4.7 to aid localizing after plate development.

6.1.6. Develop the plate in an unlined (unsaturated) tank filled with developing solvent 4.5 until the solvent front has reached a line 12 cm from the starting line; usually this takes about 45 minutes. Air-dry the plate and localize the resorcinol/DHT-zone under short wave UV-light (254 nm). The two compounds have approximately the same R_f values. Mark the bands with a pencil at 2 mm distance from the outside dark borderline of the bands. Remove these zones and collect the adsorbent of each band in a 10-ml bottle.

6.1.7. Extract the adsorbent containing the sample and the absorbent containing the standard mixture each in the following way:

add 2 ml methanol (4.2) and extract for one hour with continuous stirring. Filter the mixture and repeat the extraction for another 15 minutes with 2 ml methanol.

6.1.8. Combine the extracts and evaporate the solvent by drying overnight in a vacuum desiccator filled with a suitable desiccant. Do not apply any heat.

6.1.9. Silylate the residues (6.1.8) either as indicated under 6.1.9.1 or 6.1.9.2.

6.1.9.1. Add 200 µl BSTFA (4.9.1) with a microsyringe and leave the mixture in a closed vessel for 12 hours at room temperature.

6.1.9.2. Add successively 200 µl HMDS (4.9.2) and 100 µl TMCS (4.9.3) with a microsyringe and heat the mixture for 30 minutes at 60 °C in a closed vessel. Cool the mixture.

6.2. **Gas chromatography**

6.2.1. *Chromatographic conditions*

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

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

in which:

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

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

d' = the chart speed in mm per minute.

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

Column	material:	stainless steel
	length:	200 cm
	internal diameter:	∽ 3 mm
	filling:	10 % OV-17 on Chromosorb WAW 100 to 120 mesh
Flame ionization detector		
Temperatures:		
column:		185 °C (isothermal)
detector:		250 °C
injection port:		250 °C
Carrier gas:		nitrogen
flow:		45 ml/min.

For settings of hydrogen and air flow follow the manufacturer's instructions.

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

7. CALCULATION

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

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

in which:

M = test portion in grams (6.1.1),

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

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

8. REPEATABILITY (1)

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

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

1. PURPOSE AND SCOPE

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

Relative levels of 0 to 10 % can be determined.

2. DEFINITION

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

3. PRINCIPLE

The determination is carried out by gas chromatography.

(1) See Norm ISO 5725.

4. REAGENTS

Use analytical grade reagents.

4.1. Methanol

4.2. Ethanol absolute

4.3. Propan-2-ol

4.4. Chloroform, freed from alcohols by washing with water

5. APPARATUS

5.1. Gas chromatograph:
with Katharometer detector for aerosol samples,
with flame ionization detector for non-aerosol samples.

5.2. Volumetric flasks, 100 ml

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

5.4. Microsyringes 0 to 100 µl and 0 to 5 µl
and (only for aerosol samples) special gas-tight syringe with sliding valve, (see sampling procedure Figure 5 [1].

6. PROCEDURE

6.1. **Sample preparation**

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

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

6.2. **Gas chromatography**

6.2.1. For aerosol samples, the katharometer detector is used.

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

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

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

in which:

r_1 and r_2 = retention times in minutes of two peaks,
w_1 and w_2 = the same peaks widths at half height in mm,
d' = the chart speed in mm per minute.

6.2.1.3. The following conditions allow this resolution to be achieved:

Column	material:	stainless steel
	length:	3·5 metres
	diameter:	3 mm
Katharometer bridge current:		150 mA

[1] OJ No L 383, 31. 12. 1980, p. 27.

Carrier gas: helium
pressure: 2·5 bar
flow: 45 ml/min.
Temperatures:
injection port: 150 °C
detector: 150 °C
column oven: 65 °C

Peak area measurements can be improved by electronic integration.

6.2.2. For non-aerosol samples:

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

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

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

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

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

d' = the chart speed in mm per minute.

6.2.2.3. This resolution has been achieved using the following conditions:

Column material: stainless steel
length: 2 metres
diameter: 3 mm
Electrometer sensitivity: 8×10^{-10}A
Gases:
carrier: nitrogen
pressure: 2·1 bar
flow: 40 ml/min.
Auxiliary gas: hydrogen
pressure: 1·5 bar
flow: 20 ml/min.
Temperatures:
injection port: 150 °C
detector: 230 °C
column oven 120 to 130 °C

7. STANDARD GRAPH

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

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

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

Calculate peak areas ratio (methanol/ethanol) or (methanol/propan-2-ol) of each mixture. Plot standard graph using:

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

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

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

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

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

Calculate peak area ratio (methanol/ethanol) or (methanol/propan-2-ol) of each mixture. Plot standard graph using:

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

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

7.3. The standard graph must be a straight line.

8. REPEATABILITY (1)

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

(1) See Norm ISO 5725.