SIXTH COMMISSION DIRECTIVE 95/32/EC

of 7 July 1995

relating to methods of analysis necessary for checking the composition of cosmetic products

(Text with EEA relevance)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products [1], as last amended by Commission Directive 94/32/EC [2], and in particular Article 8 (1) thereof,

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

Whereas all the necessary methods of analysis should be laid down as quickly as possible; whereas certain methods have already been adopted in Commission Directives 80/1335/EEC [3], as amended by Directive 87/143/EEC [4], 82/434/EEC [5], as amended by Directive 90/207/EEC [6], 83/514/EEC [7], 85/490/EEC [8] and 93/73/EEC [9];

Whereas the identification and determination of benzoic acid, 4-hydroxybenzoic acid, sorbic acid, salicylic acid and propionic acid in cosmetic products and the identification and determination of hydroquinone, hydroquinone monomethylether, hydroquinone monoethylether and hydroquinone monobenzylether in cosmetic products constitute a sixth step;

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

HAS ADOPTED THIS DIRECTIVE:

Article 1

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

— identification and determination of benzoic acid, 4-hydroxybenzoic acid, sorbic acid, salicylic acid and propionic acid,

— identification and determination of hydroquinone, hydroquinone monomethylether, hydroquinone monoethylether and hydroquinone monobenzylether,

shall be carried out in accordance with the methods described in the Annex.

Article 2

1. Member States shall bring into force the laws, regulations and administrative provisions necessary to comply with this Directive no later than 30 September 1996. They shall forthwith inform the Commission thereof.

When Member States adopt these provisions, these shall contain a reference to this Directive or shall be accompanied by such reference at the time of their official publication. The procedure for such reference shall be adopted by Member States.

2. Member States shall communicate to the Commission the provisions of national law which they adopt in the field covered by this Directive.

Article 3

This Directive shall enter into force on the 20th day following its publication in the *Official Journal of the European Communities*.

Article 4

This Directive is addressed to the Member States.

Done at Brussels, 7 July 1995.

For the Commission

Emma BONINO

Member of the Commission

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[1] OJ No L 262, 27. 9. 1976, p. 169.
[2] OJ No L 181, 15. 7. 1994, p. 31.
[3] OJ No L 383, 31. 12. 1980, p. 27.
[4] OJ No L 57, 27. 2. 1987, p. 56.
[5] OJ No L 185, 30. 6. 1982, p. 1.
[6] OJ No L 108, 28. 4. 1990, p. 92.
[7] OJ No L 291, 24. 10. 1983, p. 9.
[8] OJ No L 295, 7. 11. 1985, p. 30.
[9] OJ No L 231, 14. 9. 1993, p. 34.

ANNEX

I. IDENTIFICATION AND DETERMINATION OF BENZOIC ACID, 4-HYDROXYBENZOIC ACID, SORBIC ACID, SALICYLIC ACID AND PROPIONIC ACID IN COSMETIC PRODUCTS

1. Scope and field of application

The method is applicable to the identification and determination of benzoic acid, 4-hydroxybenzoic acid, sorbic acid, salicylic acid and propionic acid in cosmetic products. Separate procedures describe the identification of these preservatives; the determination of propionic acid; and the determination of 4-hydroxybenzoic acid, salicylic acid, sorbic acid and benzoic acid.

2. Definition

The amounts of benzoic acid, 4-hydroxybenzoic acid, salicylic acid, sorbic acid and propionic acid determined by this method are expressed as percentage by mass of the free acids.

A. IDENTIFICATION

1. Principle

Following acid/base extraction of the preservatives, the extract is analysed by thin layer chromatography (TLC) employing on-date derivatization. Depending on the results, the identification is confirmed by high performance liquid chromatography (HPLC) or, in the case of propionic acid, by gas chromatography (GC).

2. Reagents

2.1. General

All reagents must be of analytical purity. Water used must be distilled water, or water of at least equivalent purity

2.2. Acetone

2.3. Diethyl ether

2.4. Acetonitrile

2.5. Toluene

2.6. n-Hexane

2.7. Paraffin, liquid

2.8. Hydrochloric acid, 4 M

2.9. Potassium hydroxide, aqueous, 4 M

2.10. Calcium chloride, $CaCl_2.2H_2O$

2.11. Lithium carbonate, Li_2CO_3

2.12. 2-Bromo-2'-acetonaphthone

2.13. 4-Hydroxybenzoic acid

2.14. Salicylic acid

2.15. Benzoic acid

2.16. Sorbic acid

2.17. Propionic acid

2.18. Reference solutions

Prepare 0,1 % (m/v) solutions (100 mg/100 ml) of each of the five preservatives (2.13 to 2.17) in diethyl ether

2.19. Derivatization reagent

0,5 % (m/v) solution of 2-bromo-2'-acetonaphthone (2.12) in acetonitrile (2.4) (50 mg/10 ml). This solution should be prepared weekly and stored in a refrigerator

2.20. Catalyst solution

0,3 % (m/v) solution of lithium carbonate (2.11) in water (300 mg/100 ml). This solution should be freshly prepared

2.21. Development solvent

Toluene (2.5)/Acetone (2.2) (20:0,5, v/v)

2.22. Liquid paraffin (2.7)/n-hexane (2.6) (1:2, v/v)

3. Apparatus

Ordinary laboratory equipment

3.1. Water bath, capable of maintaining a temperature of 60 °C

3.2. Developing tank

3.3. Ultraviolet light source, 254 and 366 nm

3.4. Thin layer plates, Kieselgel 60, without fluorescence indicator, 20 × 20 cm, layer thickness 0,25 mm with concentrating zone 2,5 × 20 cm (Merck 11845, or equivalent)

3.5. Microsyringe, 10 µl

3.6. Microsyringe, 25 µl

3.7. Oven, capable of maintaining temperatures up to 105 °C

3.8. 50-ml glass tubes with screw cap

3.9. Filter paper, diameter 90 mm, Schleicher & Schull, Weissband No 5892, or equivalent

3.10. Universal pH indicator paper, pH 1—11

3.11. 5-ml glass sample vials

3.12. Rotating film evaporator (Rotavapor or equivalent)

3.13. Hot plate

4. Procedure

4.1. Sample prepartion

Weigh approximately 1 g of the sample into a 50-ml glass tube with screw cap (3.8). Add four drops of hydrochloric acid 4 M (2.8) and 40 ml acetone (2.2). For strongly basic products such as toilet soap, 20 drops of hydrochloric acid 4 M (2.8) should be added. Check that the pH is approximately two, using indicator paper (3.10). Close the tube and shake vigorously for one minute.

If necessary to facilitate the extrraction of the preservatives into the acetone phase, heat the mixture gently to about 60 °C to melt the liquid phase.

Cool the solution to room temperature and filter through a filter paper (3.9) into a conical flask.

Transfer 20 ml of the filtrate to a 200-ml conical flask, add 20 ml water and mix. Adjust the pH of the mixture to approximately 10 with potassium hydroxide 4 M (2.9), using indicator paper (3.10) to measure the pH.

Add 1 g calcium chloride (2.10) and shake vigorously. Filter through a filter paper (3.9) into a 250-ml separating funnel containing 75 ml diethyl ether (2.3) and shake vigorously for one minute. Allow to separate and draw off the aqueous layer into a 250 ml conical flask. Discard the ether layer. Using indicator paper (3.10), adjust the pH of the aqueous solution to approximately two with hydrochloric acid 4 M (2.8). Add 10 ml diethyl ether (2.3), stopper the flask and shake vigorously for one minute. allow to separate and transfer the ether layer to a rotating film evaporator (3.12). Discard the aqueous layer.

Evaporate the ether layer almost to dryness and redissolve the residue in 1 ml of diethyl ether (2.3). Transfer the solution to a sample vial (3.11).

4.2. Thin layer chromatography

For each of the references and the samples to be chromatographed, apply approximately 3 μl lithium carbonate solution (2.20) and with a syringe (3.5) at equal distances on the start line in the concentration zone of a TLC plate (3.4) and dry in a stream of cold air.

Transfer the TLC plate to a hot plate (3.13), heated at 40 °C, in order to keep the spots as small as possible. With a microsyringe (3.5) apply 10 μl of each of the reference solutions (2.18) and the sample solution (4.1) to the start line of the plate, on the exact spots where the lithium carbonate solution has been applied.

Finally apply approximately 15 μl derivatization reagent (2.19) (2-bromo-2'-acetonaphthone solution), again on the exact spots where the reference/sample solutions and the lithium carbonate solution have been applied.

Heat the TLC plate in an oven (3.7) at 80 °C for 45 minutes. After cooling, develop the plate in a tank (3.2), that has equilibrated for 15 minutes (without the use of filter paper lining), using development solvent 2.21 (toluene/acetone), until the solvent front has reached a distance of 15 cm (this may take approximately 80 minutes).

Dry the plate in a stream of cold air and examine the spots obtained under UV light (3.3). To enhance the fluorescence of the weak spots, the TLC plate may be dipped in liquid paraffin/n-hexane (2.22).

5. **Identification**

Calculate the *Rf* for each spot.

Compare the *Rf* and the behaviour under UV radiation obtained for the sample with that obtained for the reference solutions.

Draw a preliminary conclusion about the presence and identity of the preservatives present. Perform the HPLC described in Section B, or, when it appears that propionic acid is present, the GC described in Section C. Compare the retention times obtained with those of the reference solutions.

Combine the results from the TLC and HPLC or GC and base the final identification of the preservatives present in the sample on the combined results.

B. DETERMINATION OF BENZOIC ACID, 4-HYDROXYBENZOIC ACID, SORBIC ACID AND SALICYLIC ACID

1. **Principle**

After acidification, the sample is extracted with a mixture of ethanol and water. Following filtration the preservatives are determined by high performance liquid chromatography (HPLC).

2. **Reagents**

2.1. All reagegnts must be of analytical purity, and suitable for HPLC where appropriate. Water used must be distilled water, or water of at least equivalent purity

2.2. Ethanol, absolute

2.3. 4-Hydroxybenzoic acid

2.4. Salicylic acid

2.5. Benzoic acid

2.6. Sorbic acid

2.7. Sodium acetate, ($CH_3COONa.3H_2O$)

2.8. Acetic acid, $(\alpha)_4^{20}$= 1,05 g/ml

2.9. Acetonitrile

2.10. Sulfuric acid, 2 M

2.11. Potassium hydroxide, aqueous, 0,2 M

2.12. 2-Methoxybenzoic acid

2.13. Ethanol/water mixture

Mix nine volumes of ethanol (2.2) and one volume of water (2:1)

2.14. Internal standard solution

Prepare a solution containing approximately 1 g 2-methoxybenzoic acid (2.12) in 500 ml ethanol/water mixture (2.13)

2.15. Mobile phase for HPLC

2.15.1. Acetate buffer: to 1 l of water add 6,35 g sodium acetate (2.7) and 20,0 ml acetic acid (2.8) and mix

2.15.2. Prepare the mobile phase by mixing nine volumes acetate buffer (2.15.1) and one volume acetonitrile (2.9)

2.16. Preservative stock solution

Accurately weigh approximately 0,05 g 4-hydroxybenzoic acid (2.3), 0,2 g salicylic acid (2.4), 0,2 g benzoic acid (2.5) and 0,05 g sorbic acid (2.6) in a 50-ml volumetric flask and make up to volume with ethanol/water mixture (2.13). Store this solution in a refrigerator. The solution is stable for one week

2.17. Standard preservative solutions

Transfer respectively 8,00, 4,00, 2,00, 1,00 and 0,50 ml of the stock solution (2.16) into a series of 20-ml volumetric flasks. To each flask, add 10,00 ml internal standard solution (2.14) and 0,5 ml sulfuric acid 2 M (2.10). Make up to volume with ethanol/water mixture (2.13). These solutions must be freshly prepared

3. **Apparatus**

Usual laboratory equipment not otherwise specified, and:

3.1. Water bath, set at 60 °C

3.2. High performance liquid chromatograph with variable-wavelength UV detector and 10-μl injection loop

3.3. Analytical column

Stainless steel, length 12,5 to 25 cm, internal diameter 4,6 mm, packed with Nucleosil 5C18, or equivalent

3.4. Filter paper, diameter: 90 mm, Schleicher and Schull, Weissband No 5892, or equivalent

3.5. 50-ml glass tubes with screw cap