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

FOURTH COMMISSION DIRECTIVE

of 11 October 1985

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

(85/490/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 85/391/EEC[(2)], and in particular Article 8 (1) thereof,

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

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

Whereas the measures provided for in this Directive are in accordance with the opinion of the Committee on the Adaptation of Directive 76/768/EEC to Technical Progress,

HAS ADOPTED THIS DIRECTIVE:

Article 1

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

— identification and determination of glycerol l-(4-aminobenzoate),
— determination of chlorobutanol,
— identification and determination of quinine,
— identification and determination of inorganic sulphites and hydrogen sulphites,
— identification and determination of chlorates of the alkali metals, and
— identification and determination of sodium iodate are performed in accordance with the methods described in the Annex.

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Article 2

Member States shall bring into force the laws, regulations or administrative provisions necessary to comply with this Directive not later than 31 December 1986.

They shall forthwith inform the Commission thereof.

Article 3

This Directive is addressed to the Member States.

Done at Brussels, 11 October 1985.

For the Commission

Stanley CLINTON-DAVIS

Member of the Commission

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ANNEX

IDENTIFICATION AND DETERMINATION OF GLYCEROL l-(4-AMINOBENZOATE)

A. IDENTIFICATION

1. SCOPE AND FIELD OF APPLICATION

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

2. PRINCIPLE

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

3. REAGENTS

All reagents should be of analytical purity.

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

3.2. Development solvent: petroleum ether (40-60)/benzene/acetone/ammonium hydroxide solution (minimum 25 % NH_3): 35/35/35/1 (v/v/v/v).

3.3.

3.4. Standard solutions:
alpha-monoglyceryl 4-aminobenzoate: 0,05 g in 100 ml of mixed solvent 3.1;
ethyl 4-aminobenzoate: 0,05 g in 100 ml of mixed solvent 3.1.

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

4. APPARATUS

4.1. Normal apparatus for thin layer chromatography.

4.2. Ultrasonic vibrator.

4.3. Millipore filter FH 0,5 µm or equivalent.

5. PROCEDURE

5.1. **Sample preparation**

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

5.2. **Thin layer chromatography**

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

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

5.3. **Development**

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5.3.1. Observe the plate under 254 nm UV light.

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

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

Dry the plate in an oven at 60 ^{o}C. The spots appear an orange colour. Alpha-monoglyceryl 4-aminobenzoate: R_F 0,07; ethyl 4-aminobenzoate: R_F 0,55.

B. DETERMINATION

1. SCOPE AND FIELD OF APPLICATION

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

2. DEFINITION

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

3. PRINCIPLE

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

4. REAGENTS

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

4.1. Methanol.

4.2. Potassium dihydrogenorthophosphate (KH_2PO_4).

4.3. Zinc di(acetate) ($Zn(CH_3COO)_2 \cdot 2H_2O$).

4.4. Acetic acid
$\left(d\frac{20}{4} = 1{,}05\right)$

.

4.5. Tetrapotassium hexacyanoferrate, ($K_4(Fe(CN)_6) \cdot 3H_2O$).

4.6. Ethyl 4-hydroxybenzoate.

4.7. Alpha monoglyceryl 4-aminobenzoate.

4.8. Ethyl 4-aminobenzoate.

4.9. Phosphate buffer solution (0,02 M): dissolve 2,72 g of potassium dihydrogenorthophosphate (4.2) in one litre of water.

4.10. Eluant: phosphate buffer solution (4.9)/methanol (4.1) 61/39(v/v)

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

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

where

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

4.11. Stock solution of alpha-monoglyceryl 4-aminobenzoate: weigh accurately about 40 mg of alpha-monoglyceryl 4-aminobenzoate and introduce it into a 100 ml graduated flask. Dissolve in 40 ml of methanol (4.1). Make up to the mark with buffer solution (4.9) and mix.

4.12. Stock solution of ethyl 4-aminobenzoate: weigh accurately about 40 mg of ethyl 4-aminobenzoate and introduce it into a 100 ml graduated flask. Dissolve in 40 ml of methanol (4.1). Make up to the mark with the buffer solution (4.9) and mix.

4.13. Internal standard solution: weigh accurately about 50 mg of ethyl 4-hydroxybenzoate (4.6), transfer to a 100 ml standard flask, dissolve in 40 ml of methanol (4.1), make up to the mark with the buffer solution (4.9) and mix.

4.14. Standard solutions: prepare four standard solutions by dissolving in 100 ml eluant (4.10) according to the following table:

Standard Solution	**Alpha-monoglyceryl 4-aminobenzoate**		**Ethyl 4-aminobenzoate**		**Ethyl 4-hydroxybenzoate**	
	(µg/ml)[a]	**ml (4.11)[a]**	**(µg/ml)[a]**	**ml (4.12)**	**(µg/ml)[a]**	**ml (4.13)**
I	8	2	8	2	50	10
II	16	4	12	3	50	10
III	24	6	16	4	50	10
IV	40	10	20	5	50	10

a These values are given as an indication and correspond to the exact masses of 4.11, 4.12 and 4.13.

NB: These solutions may be prepared in a different way.

4.15 Carrez I solution: dissolve 26,5 g of tetrapotassium hexacyanoferrate (4.5) in water and make up to 250 ml.

4.16. Carrez II solution: dissolve 54,9 g of zinc di(acetate) (4.3) and 7,5 ml of acetic acid (4.4) in water and make up to 250 ml.

4.17. Merck Lichrosorb RP-18, or equivalent, with an average particle size of 5 µm.

5. APPARATUS

5.1. The usual laboratory equipment.

5.2. High-performance chromatography equipment with a variable wavelength UV detector and thermostatted chamber set at 45 ^{o}C.

5.3. Stainless-steel column: length: 250 mm; internal diameter: 4,6 mm; packing: Lichrosorb RP — 18 (4.17).

5.4. Ultrasonic bath.

6. PROCEDURE