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)

ANNEX

DETERMINATION OF DICHLOROMETHANE AND 1,1,1-TRICHLOROETHANE
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 ^{o}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 ^{o}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***[(1)]

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)[(2)].

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:

$$\frac{m_s \times k_h \times A_h \times 100}{M \times A_s}$$

9 REPEATABILITY[3]

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).

(**1**) 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.

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

(**3**) Norm ISO 5725.