

## 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-(chloroimino)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.

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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 R<sub>f</sub> 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 R<sub>f</sub> of approximately 0,5.