

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 ORGANOMERCURY COMPOUNDS

#### SCOPE AND FIELD OF APPLICATION

The method described below can be used to identify and determine organo-mercury derivatives employed as preservatives in cosmetic products for the eyes. It is applicable to thiomersal (INN) (sodium 2-(ethylmercuriothio)benzoate) and phenylmercury and its salts.

#### A. IDENTIFICATION

##### 1. PRINCIPLE

The organomercury compounds are complexed with 1,5-diphenyl-3-thiocarbazonone. After extraction of the dithizonate with carbon tetrachloride, silica gel, thin-layer chromatography is carried out. The spots of the dithizonates appear as an orange colour.

##### 2. REAGENTS

All the reagents should be of analytical purity.

- 2.1. Sulphuric acid, 25 % (v/v).
- 2.2. 1,5-diphenyl-3-thiocarbazonone (dithizone): 0,8 mg in 100 ml carbon tetrachloride (2.4).
- 2.3. Nitrogen.
- 2.4. Carbon tetrachloride.
- 2.5. Development solvent: hexane /acetone, 90:10 (v/v).
- 2.6. Standard solution, 0,001 % in water of:  
sodium 2-(ethylmercuriothio)benzoate,  
ethylmercury chloride or methylmercury chloride,  
phenylmercury nitrate or phenylmercury acetate,  
mercury dichloride or mercury di(acetate).
- 2.7. Ready prepared silica gel plates (e.g. Merck 5721 or equivalent).
- 2.8. Sodium chloride.

##### 3. APPARATUS

- 3.1. Normal laboratory equipment.
- 3.2. Normal TLC apparatus.
- 3.3. **Phase-separating filter.**

##### 4. PROCEDURE

###### 4.1. *Extraction*

- 4.1.1. Dilute 1 g of sample in a centrifuge tube by titration with 20 ml of distilled water. Obtain the maximum dispersion and warm to 60 °C in a water bath. Add 4 g of sodium chloride (2.8). Shake. Allow to cool.
- 4.1.2. Centrifuge for at least 20 minutes at 4 500 rev/min in order to separate the greater part of the solid from the liquid. Filter into a separating funnel and add 0,25 ml of sulphuric acid solution (2.1).
- 4.1.3. Extract several times with 2 or 3 ml of dithizone solution (2.2) until the last organic phase remains green.
- 4.1.4. Filter each organic phase sequentially through a phase-separating filter (3.3).
- 4.1.5. Evaporate to dryness in a stream of nitrogen (2.3).
- 4.1.6. Dissolve with 0,5 ml of carbon tetrachloride (2.4). Apply this solution immediately as indicated in 4.2.1.
- 4.2. **Separatum and identification**
- 4.2.1. Apply immediately 50 µl of the carbon tetrachloride solution obtained in 4.1.6 on to a silica gel plate (2.7). Treat simultaneously 10 ml of standard solution (2.6) as in 4.1 and apply 50 µl of the solution obtained in 4.1.6 on the same plate.
- 4.2.2. Place the plate in the solvent (2.5) and allow the latter to rise 150 mm. The organomercury compounds appear as coloured spots whose colour is stable, provided the plate is covered by a glass plate immediately the solvent evaporates.

For example, the following Rf values are obtained:

	<b>Rf</b>	<b>Colour</b>
Thiomersal	0,33	Orange
Ethylmercury chloride	0,29	Orange
Methylmercury chloride	0,29	Orange
Phenylmercury salts	0,21	Orange
Mercury (II) salts	0,10	Orange
Mercury di(acetate)	0,10	Orange
1,5-diphenyl-3-thiocarbazon	0,09	Pink

## B. DETERMINATION

### 1. DEFINITION

The content of organomercurial compounds determined by this method is expressed as the percentage by mass (m/m) as mercury in the sample.

### 2. PRINCIPLE

The method consists in measuring the quantity of total mercury present. It is thus necessary to have first made sure that no mercury in an inorganic state is present and to have identified the organomercurial derivative contained in the sample. After mineralization, the mercury liberated is measured by flameless atomic absorption.

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### 3. REAGENTS

All the reagents should be of analytical purity.

- 3.1. Concentrated nitric acid,  $d_4^{20} = 1,41$  g/ml.
- 3.2. Concentrated sulphuric acid,  $d_4^{20} = 1,84$  g/ml.
- 3.3. Redistilled water.
- 3.4. Potassium permanganate, 7 % (m/v) solution.
- 3.5. Hydroxylammonium chloride, 1,5 % (m/v) solution.
- 3.6. Dipotassium peroxodisulphate, 5 % (m/v) solution.
- 3.7. Tin dichloride, 10 % (m/v) solution.
- 3.8. Concentrated hydrochloric acid,  $d_4^{20} = 1,18$  g/ml.
- 3.9. Palladium dichloride impregnated glass wool, 1 % (m/m).

### 4. APPARATUS

- 4.1. Normal laboratory equipment.
- 4.2. Apparatus for flameless atomic absorption mercury determination (cold vapour technique), including the necessary glassware. Path length of the cell at least 100 mm.

### 5. PROCEDURE

Take all normal precautions for trace mercury analysis.

#### 5.1. **Breakdown**

- 5.1.1. Weigh accurately 150 mg of the sample (m). Add 10 ml of nitric acid (3.1) and leave to digest for three hours in an airtight flask in a water bath at 55 °C, shaking at regular intervals. At the same time, carry out a blank test on the reagents.
- 5.1.2. After cooling, add 10 ml of sulphuric acid (3.2) and return to the water bath at 55 °C for 30 minutes.
- 5.1.3. Place the flask in an ice bath and add carefully 20 ml of water (3.3).
- 5.1.4. Adding 2 ml aliquots of 7 % potassium permanganate solution (3.4) until the solution remains coloured. Return to the water bath at 55 °C for a further 15 minutes.
- 5.1.5. Add 4 ml of dipotassium peroxodisulphate solution (3.6). Continue to warm in the water bath at 55 °C for 30 minutes.
- 5.1.6. Allow to cool and transfer the contents of the flask into a 100 ml standard flask. Rinse the flask with 5 ml of hydroxylammonium chloride (3.5) and then rinse four times with 10 ml of water (3.3). The solution should be completely decolorized. Make up to the mark with water (3.3).

#### 5.2. **Determination**

- 5.2.1. Place 10 ml of the test solution (5.1.6) in the glass vessel for the cold vapour mercury determination (4.2). Dilute with 100 ml of water (3.3) and subsequently 5 ml of sulphuric acid (3.2) and 5 ml of tin dichloride solution (3.7). Mix after each addition. Wait 30 seconds to reduce all ionic mercury to the metallic state and take a reading (n).
- 5.2.2. Place some palladium dichloride impregnated glass wool (3.9) between the mercury reduction vessel and the flow cell of the instrument (4.2). Repeat 5.2.1 and record the reading. If the reading is not zero mineralization was incomplete and analysis must be repeated.

## 6. CALCULATION

Let:

- m = the mass (in milligrams) of the test sample.  
n = the quantity of mercury (in µg) read on the instrument.

The quantity of mercury, expressed as mercury, as percentage by mass, is calculated by the formula:

$$\% \text{ mercury} = \frac{n}{m}$$

## 7. NOTES

- 7.1. To improve mineralization it might be necessary to start by diluting the sample.
- 7.2. If absorption of the mercury by the substrate is suspected, a quantitative determination by the method of standard additions should be done.

## 8. REPEATIBILITY<sup>(1)</sup>

In the case of mercury concentrations of 0,007 %, the difference between the results of two determinations carried out in parallel on the sample should not exceed an absolute value of 0,00035 %.

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- (1) Norm ISO 5725.