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 MERCAPTOACETIC ACID IN HAIR-WAVING, HAIR-STRAIGHTENING AND DEPILATORY PRODUCTS

5. DETERMINATION (see NB)

The determination should always start with the iodometric procedure.

5.1. ***Iodometry***

5.1.1. *Principle*

The determination is performed by oxidation of the '-SH' group with iodine in an acid medium according to the equation:

$2\ HOOC\text{-}CH_2SH + I_2 \rightarrow (HOOC\text{-}CH_2\text{-}S)_2 + 2\ I + 2\ H^+$

5.1.2. *Reagents*

Iodine, 0,05 M standard solution.

NB: The determination of mercaptoacetic acid must be carried out on unused product from freshly opened containers in order to prevent oxidation.

5.1.3. *Apparatus*

Usual laboratory equipment.

5.1.4. *Procedure*

Accurately weigh out a quantity of between 0,5 and 1 g of the sample into a 150 ml stoppered conical flask containing 50 ml of distilled water. Add 5 ml of hydrochloric acid (4.1.1.2) (pH of solution about 0) and titrate with iodine solution (5.1.2) until a yellow colour appears. Use an indicator (e.g. starch solution or carbon tetrachloride) if desired.

5.1.5. *Calculation*

The mercaptoaceric acid content is calculated according to the formula:

$$\%\ (m/m) = \frac{92 \times n \times 100}{1000 \times 10 \times m} = \frac{0{,}92\ n}{m}$$

where:

m = the mass (in grams) of the test portion,
n = the volume of iodine solution (5.1.2) used.

5.1.6. *Remarks*

If the result, calculated as mercaptoacetic acid, is 0,1 % or more below the authorized maximum concentration, there is no point in carrying out further determinations. If the result is equal to or above the permitted maximum concentration, and the identification has revealed the presence of several reducing agents, it is necessary to carry out a gas chromatographic determination.

5.2. ***Gas chromatograyhy***

5.2.1. Principle

Mercaptoacetic acid is separated from the excipient by precipitation with cadmium di(acetate) solution. After methylation with diazomethane, prepared either *in situ* or in advance in a diethyl

ether solution, the methyl derivative of the mercaptoacetic acid is measured by gas/liquid chromatography, methyl octanoate being used as the internal standard.

5.2.2. *Reagents*

All the reagents must be of analytical quality.

5.2.2.1. Mercaptoacetic acid, 98 %.

5.2.2.2. Hydrochloric acid, d_{410} = 1,19 g/ml.

5.2.2.3. Methanol.

5.2.2.4. Cadmium di(acetate) dihydrate, 10 % (m/v) solution in water.

5.2.2.5. Methyl octanoate, 2 % (m/v) solution in methanol.

5.2.2.6. Acetate buffer solution (pH 5):

Sodium acetate trihydrate, 77 g.

Acetic acid (glacial), 27,5 g.

Demineralized water to give a final volume of one litre.

5.2.2.7. Hydrochloric acid, 3 M solution in methanol (5.2.2.3), freshly prepared.

5.2.2.8. 1-methyl-3-nitro 1 -nitrosoguanidine.

5.2.2.9. Sodium hydroxide, 5 M solution.

5.2.2.10. Iodine, 0,05 M standard solution.

5.2.2.11. Diethyl ether.

5.2.2.12. *Diazomethane solution prepared from iV-methyl-AT-nitrosotoluen-4-sulfonamide (Fieser, Reagents for Organic Synthesis (Wiley), 1967)*

The solution obtained contains about 1,5 g of diazomethane in 100 ml of diethyl ether. As diazomethane is a toxic and very unstable gas, all experiments must be carried out under a powerful hood and the use of ground-glass apparatus must be avoided (there are special kits for this purpose).

5.2.3. *Apparatus*

5.2.3.1. Usual laboratory equipment.

5.2.3.2. Apparatus for the preparation of diazomethane for *in situ* methylation (see Fales, H. M., Jaouni, T. M. and Babashak, J. F., Analyt. Chem. 1973, 45, 2302).

5.2.3.3. Apparatus for the advance preparation of diazomethane (Fieser).

5.2.4. *Preparation of the sample*

Weigh accurately into a 50 ml centrifuge tube enough of the sample to give a presumed quantity of 50 to 70 mg of mercaptoacetic acid. Acidify with a few drops of hydrochloric acid (5.2.2.2) to obtain a pH of about 3.

Add 5 ml of demineralized water and 10 ml of acetate buffer solution (5.2.2.6).

Check with pH paper that the pH value is about 5. Then add 5 ml of cadmium di(acetate) solution (5.2.2.4).

Wait 10 minutes and then centrifuge for at least 15 minutes at 4 000 g. Remove the supernatant liquid which may contain an insoluble fat (in the case of cream products). This fat cannot be confused with the thiols which collects in a compact mass at the bottom of the tube. Check that no precipitation occurs when a few drops of cadmium di(acetate) solution (5.2.2.4) are added to the supernatant.

Where earlier identification revealed no reducing agents other than the thiols, check by iodometry that the thiol present in the supernatant liquid does not exceed 6 to 8 % of the initial quantity.

Introduce 10 ml of methanol (5.2.2.3) into the centrifuge tube containing the precipitate and finely disperse the precipitate with a stirring rod. Centrifuge again for at least 15 minutes at 4 000 g. Pour off the supernatant and check for the absence of thiols.

Wash the precipitate a second time by the same procedure.

Still using the same centrifuge tube, add:
— 2 ml of methyl octanoate solution (5.2.2.5),
— 5 ml of hydrochloric acid in methanol (5.2.2.7).

Completely dissolve the thiols (a little insoluble matter may persist from the excipient). This is solution ‘S’.

With an aliquot of this solution, check iodometrically that the thiols content is at least 90 % of that obtained in 5.1.

5.2.5. *Methylation*

The methylation is carried out either by *in situ* preparation (5.2.5.1) or with previously prepared diazomethane solution (5.2.5.2).

5.2.5.1. *Methylation in situ*

Into the methylation apparatus (5.2.3.2) containing 1 ml of ether (5.2.2.11) introduce 50 µl of solution ‘S’ and methylate by the method (5.2.3.2) with about 300 mg of l-methyl-3 nitro-1-nitrosoguanidine (5.2.2.8). After 15 minutes (the ether solution should be yellow to indicate excess diazomethane) transfer the sample solution to a 2 ml bottle having an airtight stopper. Place in the refrigerator overnight. Methylate two samples simultaneously.

5.2.5.2. *Methylation with the previously prepared diazomethane solution*

Introduce, into a 5 ml stoppered flask, 1 ml of diazomethane solution (5.2.2.12) then 50 µl of solution ‘S’. Leave in the refrigerator overnight.

5.2.6. *Preparation of the standard*

Prepare a standard solution of mercaptoacetic acid (5.2.2.1) of known strength containing about 60 mg of pure mercaptoacetic acid (5.2.2.1) in 2 ml.

This is solution ‘E’.

Precipitate, assay and methylate as described in 5.2.4 and 5.2.5.

5.2.7. *Gas chromatographic conditions*

5.2.7.1. *Column*

Type: stainless steel.

Length: 2 m.

Diameter: 3 mm.

5.2.7.2. *Packing*

20 % didecyl phthalate/chromosorb, WAW 80 to 100 mesh.

5.2.7.3. *Detector*

Flame ionization. A suitable sensitivity setting for the electrometer of the flame ionization detector is 8×10^{-10} A.

5.2.7.4. *Gas supplies*

Carrier gas: nitrogen.

pressure: 2,2 bar,

flow: 35 ml/min.

Auxiliary gas: hydrogen.

pressure: 1,8 bar,

flow: 15 ml/min.

Detector supplies: as specified by the makers of the apparatus.

5.2.7.5. *Temperature conditions*

Injector: 200 °C

Detector: 200 °C

Column: 90 °C

5.2.7.6. *Recorder chart speed*

5 mm/min.

5.2.7.7. *Quantity injected*

3 µl Carry out five injections.

5.2.7.8. The conditions of chromatography are given as a guide. They permit the achievement of a resolution 'R' equal to, or better than, 1,5, where:

$$R = 2\frac{d'(r_2 - r_1)}{W_1 + W_2}$$

let:

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).

It is recommended that chromatography be terminated by regulating the tempera-ture from 90 to 150 °C at a rate of 10 °C per minute so as to eliminate substances liable to interfere with subsequent measurements.

5.2.8. *Calculations*

5.2.8.1. *Coefficient of proportionality for mercaptoacetic acid*

This is calculated with respect to methyl octanoate on the basis of a standard mixture.

If ‘t’ represents mercaptoacetic acid:

let:

k_t = its response factor,
m'_t = its mass (in milligrams) in the mixture,
S'_t = its peak area.

If ‘c’ represents methyl octanoate:

let:

m'_c = its mass (in millegrams) in the mixture,
S'_c = its peak area,

then:

$$kt = \frac{m'_t}{m'_c} \times \frac{S'_c}{S'_t}$$

This coefficient varies according to the apparatus used.

5.2.8.2. *Concentration of mercaptoacetic acid present in the sample*

If ‘t’ represents mercaptoacetic acid:

let:

k_t its response factor,

S_t = its peak area.

If ‘c’ represents methyl octanoate:

let:

m_c = its mass (in mill grams) in the mixture,
S_c = its peak area,
M = the mass (in milligrams) of the initial test portion,

then the % (m/m) mercaptoacetic acid present in the sample is:

$$\frac{m_c}{M} \times \frac{k_t \times S_t}{S_c} \times 100$$