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 QUINOLIN-8-OL AND BIS(8-HYDROXYQUINOLINIUM) SULPHATE

1. SCOPE AND FIELD OF APPLICATION

This method describes the identification and quantitative determination of quinolin-8-ol and its sulphate.

2. DEFINITION

The quinolin-8-ol and bis(8-hydroxyquinolinium) sulphate content of the sample as determined by this method is expressed in percentage by mass of quinolin-8-ol.

3. PRINCIPLE

3.1. ***Identification***

Identification is by thin-layer chromatography.

3.2. ***Determination***

The determination is carried out by spectrophotometry at 410 nm of the complex obtained by reaction with Fehling's solution.

4. REAGENTS

All reagents should be of analytical purity.

4.1. Quinolin-8-ol.

4.2. Benzene. In view of its toxicity great care must be taken when working with benzene.

4.3. Chloroform.

4.4. Aqueous sodiun hydroxide, 50 % (m/m) solution.

4.5. Copper sulphate pentahydrate.

4.6. Potassium sodium tartrate.

4.7. M hydrochloric acid.

4.8. 0,5 M sulphuric acid.

4.9. M sodium hydroxide solution.

4.10. Ethanol.

4.11. Butan-1-ol.

4.12. Glacial acetic acid.

4.13. 0,1 hydrochloric acid.

4.14 ‘Celite 545’or equivalent.

4.15. ***Standard solutions***

4.15.1. Weigh 100 mg of quinolin-8-ol (4.1) into a 100 ml standard flask. Dissolve in a little sulphuric acid (4.8) Make up to the mark with sulphuric acid (4.8).

4.15.2. Weigh 100 mg of quinolin-8-ol into a 100 ml standard flask. Dissolve in ethanol (4.10). Make up to the mark with ethanol (4.10) and mix.

4.16. ***Fehling's solution***

Solution A

Weigh 7 g of copper sulphate pentahydrate (4.5) into a 100 ml standard flask.

Dissolve in a little water. Make up to the mark with water and mix.

Solution B

Weigh 35 g of potassium sodium tartrate (4.6) into a 100 ml standard flask. Dissolve in 50 ml of water. Add 20 ml of sodium hydroxide (4.4). Make up to the mark with water and mix. Immediately before use, pipette 10 ml of solution A and 10 ml of solution B into a 100 ml standard flask. Make up to the mark and mix.

4.17. ***Eluting solvents for thin-layer chromatography***

I : Butan-1-ol (4.11) /acetic acid (4.12) /water (80: 20: 20; v/v/v).
II : Chloroform (4.13) /acetic acid (4.12) (95: 5; v/v).

4.18. 2,6-dichloro-4-(chloroin ino)cyclohexa-2,5-dienone, 1 % (m/v) solution in ethanol (4.10).

4.19. Sodium carbonate, 1 % (m/v) solution in water.

4.20. Ethanol (4.10), 30 % (v/v) solution in water.

4.21. Disodium dihydrogen ethylenediaminetetraacetate, 5 % (m/v) solution in water.

4.22. ***Buffer solution, pH 7***

Weigh 27 g of potassium dihydrogenorthophosphate anhydrous and 70 g of dipotassium hydrogenorthophosphate trihydrate into a one litre standard flask. Make up to the mark with water.

4.23. ***Prepared thin-layer plates***

Ready made thin-layer plates of a thickness of 0,25 mm (e.g. Merck Kieselgel 60 or equivalent). Before use, spray with 10 ml of reagent (4.21) and dry at 80 ^{o}C.

5. APPARATUS

5.1. 100 ml round-bottom flask with ground-glass neck.

5.2. Standard flasks.

5.3. Graduated pipettes, 10 and 5 ml.

5.4. Bulb pipettes, 20, 15, 10 and 5 ml.

5.5. Separating funnels, 100, 50 and 25 ml.

5.6. Pleated filter paper, diameter 90 mm.

5.7. Rotary evaporator.

5.8. Reflux condenser with ground-glass neck.

5.9. Spectrophotometer.

5.10. Optical cells of 10 mm path length.

5.11. Stirrer hotplate.

5.12. Glass chromatography column dimensions: 160 mm long with a diameter of 8 mm, a construction at the lower end containing a glass-wool plug, and an adaptor at the upper end for application of pressure.

6. PROCEDURE

6.1. ***Identification***

6.1.1. *Liquid samples*

6.1.1.1. The pH of part of the test sample is adjusted to 7. 5 and 10 µl are spotted on the starting line of a pretreated silica gel thin-layer plate (4.23).

6.1.1.2. 10 and 30 µl of the standard solution (4.15.2) is spotted on two more points of the starting line after which the plate is developed in one of the two eluents (4.17).

6.1.1.3. When the solvent front has advanced 150 mm, the plate is dried at 110 ^{o}C (for 15 minutes). Under a UV lamp (366 nm) the quinolin-8-ol spots fluoresce yellow.

6.1.1.4. Spray the plate with sodium carbonate solution (4.19). Dry and spray with 2,6-dichloro-4-(chloroimino)cyclohexa-2,5-dienone solution (4.18). The quinolin-8-ol becomes visible as a blue spot.

6.1.2. Solid samples or creams

6.1.2.1. Disperse 1 g of the sample in 5 ml of buffer solution (4.22). Then transfer with 10 ml of chloroform (4.3) into a separating funnel and shake. After separation of the chloroform ayer the aqueous layer is extracted twice more with 10 ml of chloroform (4.3) Evaporate the combined and filtered chloroform extracts almost to dryness in a 100 ml round-bottom flask (5.1) on the rotary evaporator (5.7). Dissolve the res due in 2 ml of chloroform (4.3) and spot 10 and 30µl of the solution obtained on a silica gel, thin-layer plate (4.23) in accordance with the method described in 6.1.1.1 onward.

6.1.2.2. Apply 10 and 30 µl of the standard solution (4.15.2) to the plate and continue as described in 6.1.1.2 to 6.1.1.4.

6.2. ***Determination***

6.2.1. *Liquid samples*

6.2.1.1. Weigh 5 g of the sample into a 100 ml round-bottom flask. Add 1 ml of a suphuric acid solution (4.8) and evaporate the mixture almost to dryness under reduced pressure at 50 ^{o}C.

6.2.1.2. Dissolve this residue in 20 ml of warm water. Transfer into a 100 ml standard flask. Rinse three times with 20 ml of water. Make up to 100 ml with water and mix.

6.2.1.3. Pipette 5 ml of this solution into a 50 ml separating funnel (5.5). Add 10 ml of Fehling's solution (4.16). Extract the quinolin-8-ol copper complex [oxine copper (ISO)] obtained with three times 8 ml of chloroform (4.3).

6.2.1.4. Filter and collect the chloroform layers in a 25 ml standard flask (5.2). Make up to the mark with chloroform (4.3) and shake. Measure the optical density of the yellow solution against chloroform at 410 nm.

6.2.2. *Solid samples or creams*

6.2.2.1. Weigh 0,500 g of the sample into a 100 ml round-bottom flask (4.1). Add 30 ml of benzene (4.2) and 20 ml of hydrochloric acid (4.7). Boil the contents of the flask under reflux, with stirring, for 30 minutes.

6.2.2.2. Transfer the contents of the flask into a 100 ml separating funnel (5.5). Rinse with 5 ml of 1 *N* HC1 (4.7). Transfer the aqueous phase into a round-bottom flask (5.1) and wash the benzene phase with 5 ml of hydrochloric acid (4.7).

6.2.2.3. In the case of emulsions that impede further treatment, mix 0,500 g of the sample with 2 g of Celite 545 (4.14) to form a freely flowing powder. Transfer the mixture in small portions into a glass chromatography column (5.12).

After each addition, tarmp down the column packing. As soon as the whole of the mixture has been transferred into the column, elute with hydrochloric acid (4.13) in such a way that 10 ml of eluate is obtained in approximately 10 minutes (if necessary, this elution can be performed under a slight nitrogen pressure). During the elution it must be ensured that there is always some hydrochloric acid above the column packing. The first 10 ml of eluate is further treated as described in 6.2.2.4.

6.2.2.4. Evaporate the collectec aqueous phases (6.2.2.2) or the eluate (6.2.2.3) almost to dryness in the rotary evaporator under reduced pressure.

6.2.2.5. Dissolve the residue in 6 ml of the sodium hydroxide solution (4.9). Add 20 ml of Fehling's solution (4.16) and transfer the contents of the flask into a 50 ml separating funnel (5.5) Rinse the flask with 8 ml of chloroform (4.3). Shake and filter the chloroform phase into a 50 ml standard flask (5.2).

6.2.2.6. Repeat the extraction three times with 8 ml of chloroform (4.3). Filter the chloroform phases and collect in the 50 ml flask. Make up to the mark with chloroform (4.3) and shake. Measure the optical density of the yellow solution against chloroform (4.3) at 410 nm.

7. STANDARD CURVE

Into four 100 ml round-bottom flasks (5.1), each containing 3 ml of 30 % aqueous ethanol (4.20), pipette 5, 10, 15 and 20 ml portions of the standard solution (4.15.1) corresponding to 5, 10, 15 and 20 mg of quinolin-8-ol. Proceed as described in 6.2.1.

8. CALCULATION

8.1. Liquid samples

$$\text{Quinolin-8-ol content (in \% (m/m))} = \frac{a}{m} \times 100$$

where:

a = milligrams of quinolin-8-ol on the standard curve (7),
m = the mass (in milligrams) of the test portion (6.2.1.1).

8.2. ***Solid samples or creams***

Quinolin-8-ol content (in % (m/m))

$$\frac{2a}{m} \times 100$$

where:

a = milligrams of quinolin-8-ol on the standard curve (7),
m = the mass (in milligrams) of test portion (6.2.2.1).

9. REPEATABILITY[1]

For a content of about 0,3 % quinolin-8-ol, the difference between the results of two determinations carried out in parallel on the same sample should not exceed an absolute value of 0,02 %.

(**1**) Norm ISO 5725.