# Fourth Commission Directive of 11 October 1985 on the approximation of the laws of the Member States relating to methods of analysis necessary for checking the composition of cosmetic products (85/490/EEC)

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ANNEX

## IDENTIFICATION AND DETERMINATION OF QUININE

A. IDENTIFICATION

1. SCOPE AND FIELD OF APPLICATION

This method is intended to detect the presence of quinine in shampoo and hair lotions.

2. PRINCIPLE

Identification is done by thin layer chromatography on silica gel. Detection of quinine is by the blue fluorescence of quinine in acidic conditions at 360 nm.

For further confirmation, the fluorescence can be eliminated by bromine vapours, and ammonia vapours will cause a yellowish fluorescence to appear.

3. REAGENTS

All reagents should be of analytical purity.

3.1. Silica gel plates, without fluorescence indicators, 0,25 mm thick, 200 mm × 200 mm

3.2. Developing solvent: toluene /diethyl ether /dichloromethane / diethylamine /20/20/20/8 (v/v/v/v).

3.3. Methanol.

3.4. Sulphuric acid (96 %; $d\frac{20}{4} = 1,84$ ).

3.5. Diethyl ether.

3.6. Developing agent: carefully add 5 ml of sulphuric acid (3.4) to 95 ml of diethyl ether (3.5) in a cooled container.

3.7. Bromine.

3.8. Ammonium hydroxide solution (28 %; $d\frac{20}{4} = 0,90$ ).

3.9. Quinine, anhydrous.

3.10. Standard solution: weigh accurately about 100,0 mg of anhydrous quinine (3.9) into a standard flask and dissolve in 100 ml of methanol (3.3).

4. APPARATUS

4.1. Normal equipment for thin layer chromatography.

4.2. Ultrasonic bath.

4.3. Millipore filter, FH 0,5 ƒm or equivalent with suitable filtration equipment.

5. PROCEDURE

5.1. **Preparation of the sample**

Weigh accurately a quantity of the sample which may contain approximately 100 mg of quinine into a 100 ml standard flask, dissolve and make up to the mark with methanol (3.3).

Stopper the flask and leave for one hour at room temperature in an ultrasonic vibrator (4.2). Filter (4.3) and use the filtrate for the chromatography.

5.2. **Thin layer chromatography**

Deposit 1,0 µl of standard solution (3.10) and 1,0 µl of sample solution (5.1) on the silica gel plate (3.1). Develop the chromatogram over a distance of 150 mm using solvent 3.2. in a tank previously saturated with solvent (3.2).

5.3. **Development**

5.3.1. Dry the plate at room temperature.

5.3.21. Spray with reagent 3.6.

5.3.3. Leave the plate to dry for one hour at room temperature.

5.3.4. Observe under the light from a UV lamp adjusted to a wavelength of 360 nm. Quinine appears as a fluorescent intense blue spot.

By way of example the table below gives the values of the $R_F$ of the main alkaloids related to quinine when developed with solvent 3.2.

| **Alkaloid** | **$R_F$** |
|---|---|
| Quinine | 0,20 |
| Quinidine | 0,29 |
| Cinchonine | 0,33 |
| Cinchonidine | 0,27 |
| Hydroquinidine | 0,17 |

5.3.5. For further confirmation that quinine is present, the plate is exposed for approximately one hour to bromine vapour (3.7). The fluorescence disappears. When the same plate is exposed to ammonia vapour (3.8), the spots reappear with a brown colour, and when the plate is again examined under UV light at 360 nm a yellowish fluorescence can be observed.

Detection limit: 0,1 µg of quinine.

B. DETERMINATION

1. SCOPE AND FIELD OF APPLICATION

This method describes the determination of quinine. It may be used to determine the maximum permitted concentration of 0,5 % (m/m) in shampoos and 0,2 % in hair lotions.

2. DEFINITION

The quinine content determined by this method is expressed as a percentage by mass (% m/m) of the product.

3. PRINCIPLE

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After appropriate treatment of the product to be analyzed the determination is done by high-performance liquid chromatography (HPLC).

4. REAGENTS

All reagents should be of analytical purity and suitable for HPLC.

4.1. Acetonitrile.

4.2. Potassium dihydrogenorthophosphate ($KH_2PO_4$).

4.3. Orthophosphoric acid (85 %;
$$d^{20}_{4} = 1{,}7$$
).

4.4. Tetramethylaminium bromide.

4.5. Quinine, anhydrous.

4.6. Methanol.

4.7. Orthophosphoric acid solution (0,1 M): weigh 11,53 g of orthophosphoric acid (4.3) and dissolve in 1 000 nl of water in a graduated flask.

4.8. Potassium dihyrogenorthophosphate solution (0,1 M): weigh 13,6 g of potassium dihydrogenorthophosphate (4.2) and dissolve in 1 000 ml of water in a graduated flask.

4.9. Tetramethylammonium bromide solution: dissolve 15,40 g of tetramethylammonium bromide (4.4) in 1 000 ml of water in a graduated flask.

4.10 Eluant: orthophosphoric acid (4.7) /potassium dihydrogenorthophosphate (4.8) / tetramethylammonium bromide (4.9)/water/acetonitrile (4.1) 10/50/100/340/90 (v/v/v/v/v).

The composition of this mobile phase may be changed in order to achieve a resolution factor R ≥ 1,5.
$$R = 2\frac{d'R_2 - d'R_1}{W_1 + W_2}$$

where

| | | |
|---|---|---|
| $R_1$ and $R_2$ | = | retention times, in minutes, of the peaks, |
| $W_1$ and $W_2$ | = | peak widths at half height, in millimetres, |
| d' | = | the chart speed, in millimetres per minute. |

4.11. Silica treated with octadecylsilane, 10 µm.

4.12. Standard solutions: weigh accurately approximately 5,0, 10,0, 15,0 and 20,0 mg respectively of quinine anhydrous (4.5) into a set of 100 ml standard flasks. Make up to the mark with methanol (4.6) and shake the contents of the flasks until the quinine dissolves. Filter each sample through a 0,5 µm filter.

5. APPARATUS

5.1. Usual laboratory equipment.

5.2. Ultrasonic bath.

5.3. High-performance liquid chromatography equipment with a variable wavelength detector.

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5.4. Column: length: 250 mm; internal diameter: 4,6 mm; filling: silica (4.11).

5.5. Millipore filter FH 0,5 µm, or equivalent, with suitable filtration apparatus.

6. PROCEDURE

6.1. **Sample preparation**

Weigh accurately into a 100 ml standard flask a quantity of the product sufficient to contain 10,0 mg of anhydrous quinine, add 20 ml of methanol (4.6) and place the flask in an ultrasonic bath (5.2) for 20 minutes. Make up to the mark with methanol (4.6). Mix the solution and then filter an aliquot (5.5).

6.2. **Chromatography**

Flowrate: 1,0 ml/min.
Detector wavelength (5.3): 332 nm.
Injection volume: 10 µl of filtered solution (6.1).
Measurement: peak area.

6.3. **Calibration curve**

Inject at least three times 10,0 µl of each reference solution (4.12), measure the area of the peaks, and calculate the average area at each concentration.

Produce the calibration curve and verify that it is rectilinear.

7. CALCULATION

7.1. From the calibration curve (6.3) determine the quantity in µg of anhydrous quinine present in the volume injected (6.2).

7.2. The concentration of anhydrous quinine in the sample, as a percentage by mass (% m/m), is obtained by the following formula:

$$\% \text{ (m/m) of anhydrous quinine} = \frac{B}{A}$$

where

B is the quantity, in micrograms, of anhydrous quinine determined in the 10 microlitres of the filtered solution (6.1).
A is the mass of the sample in grams (6.1).

8. REPEATABILITY[(1)]

For an anhydrous quinine content of 0,5 % (m/m), the difference between the results of two determinations performed in parallel on the same sample must not exceed 0,02 %.

For an anhydrous quinine content of 0,2 % (m/m), the difference between the results of two determinations performed in parallel on the same sample must not exceed 0,01 %.

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(**1**) ISO 5725.