

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)

- Article 1 Member States shall take all necessary steps to ensure that...
Article 2 Member States shall bring into force the laws, regulations or...
Article 3 This Directive is addressed to the Member States.
Signature

ANNEX

IDENTIFICATION AND DETERMINATION OF GLYCEROL 1-(4-AMINOBENZOATE)

A. IDENTIFICATION

1. SCOPE AND FIELD OF APPLICATION
2. PRINCIPLE
3. REAGENTS
 - 3.1. Solvent mixture: cyclohexane/propan-2-ol/stabilized dichloromethane 48/64/9 (v/v/v).
 - 3.2. Development solvent: petroleum ether (40-60)/benzene/acetone/ammonium hydroxide solution (minimum 25 %...
 - 3.3. Developing solution sodium nitrite: 1 g in 100 ml of...
 - 3.4. Standard solutions:
 - 3.5. Silica gel 60 F254 plates, 0,25 mm thick, 200 mm...
4. APPARATUS
 - 4.1. Normal apparatus for thin layer chromatography.
 - 4.2. Ultrasonic vibrator.
 - 4.3. Millipore filter FH 0,5 µm or equivalent.
5. PROCEDURE
 - 5.1. Sample preparation
 - 5.2. Thin layer chromatography
 - 5.3. Development
 - 5.3.1. Observe the plate under 254 nm UV light.
 - 5.3.2. Spray the completely dried plate with the solution 3.3 (a)....

B. DETERMINATION

1. SCOPE AND FIELD OF APPLICATION
2. DEFINITION
3. PRINCIPLE
4. REAGENTS
 - 4.1. Methanol.
 - 4.2. Potassium dihydrogenorthophosphate (KH₂PO₄).
 - 4.3. Zinc di(acetate) (Zn(CH₃COO)₂ · 2H₂O).
 - 4.4. Acetic acid d 20 4 = 1,05 .
 - 4.5. Tetrapotassium hexacyanoferrate, (K₄(Fe(CN)₆) · 3H₂O).
 - 4.6. Ethyl 4-hydroxybenzoate.

- 4.7. Alpha monoglyceryl 4-aminobenzoate.
- 4.8. Ethyl 4-aminobenzoate.
- 4.9. Phosphate buffer solution (0,02 M): dissolve 2,72 g of potassium...
- 4.10. Eluant: phosphate buffer solution (4.9)/methanol (4.1) 61/39(v/v)
- 4.11. Stock solution of alpha-monoglyceryl 4-aminobenzoate: weigh accurately about 40 mg...
- 4.12. Stock solution of ethyl 4-aminobenzoate: weigh accurately about 40 mg...
- 4.13. Internal standard solution: weigh accurately about 50 mg of ethyl...
- 4.14. Standard solutions: prepare four standard solutions by dissolving in 100...
- 4.15. Carrez I solution: dissolve 26,5 g of tetrapotassium hexacyanoferrate (4.5)...
- 4.16. Carrez II solution: dissolve 54,9 g of zinc di(acetate) (4.3)...
- 4.17. Merck Lichrosorb RP-18, or equivalent, with an average particle size...
5. APPARATUS
 - 5.1. The usual laboratory equipment.
 - 5.2. High-performance chromatography equipment with a variable wavelength UV detector and...
 - 5.3. Stainless-steel column: length: 250 mm; internal diameter: 4,6 mm; packing:...
 - 5.4. Ultrasonic bath.
6. PROCEDURE
 - 6.1. Sample preparation
 - 6.1.1. Weigh accurately about 1 g of sample into a 100...
 - 6.1.2. Place the beaker in the ultrasonic bath (5.4) for 20...
 - 6.1.3. With a pipette, transfer 3,0 ml of the filtrate obtained...
 - 6.2. Chromatography
 - 6.2.1. Adjust the flow rate of the mobile phase (4.10) to...
 - 6.2.2. Set the detector (5.2) to 274 nm.
 - 6.2.3. With a microsyringe, inject at least two times 20 µl...
 - 6.3. Calibration curve
 - 6.3.1. Inject 20 µl of each of the standard solutions (4.14)...
 - 6.3.2. For each concentration calculate the ratio between the peak areas...
 - 6.3.3. Proceed in the same manner for ethyl 4-hydroxybenzoate.
7. CALCULATION
 - 7.1. From the calibration curve obtained in 6.3 read off the...
 - 7.2. From the mass ratios obtained in this way calculate the...
8. REPEATABILITY
 - 8.1. For a 5 % (m/m) content of alpha-monoglyceryl 4-aminobenzoate, the...
 - 8.2. For a 1 % (m/m) content of ethyl 4-aminobenzoate the...
9. NOTES
 - 9.1. Before carrying out an analysis, check whether the sample contains...
 - 9.2. In order to check the absence of any interference, repeat...

DETERMINATION OF CHLOROBUTANOL

1. SCOPE AND FIELD OF APPLICATION
2. DEFINITION
3. PRINCIPLE
4. REAGENTS
 - 4.1. Chlorobutanol (1,1,1-trichloro-2-methylpropan-2-ol).
 - 4.2. 2,2,2-Trichloroethanol.
 - 4.3. Absolute ethanol.
 - 4.4. Standard solution of chlorobutanol: 0,025 g in 100 ml ethanol...
 - 4.5. Standard solution of 2,2,2-trichloroethanol: 4 mg in 100 ml ethanol...
5. APPARATUS
 - 5.1. Normal laboratory equipment.
 - 5.2. Gas chromatograph with electron detector, Ni 63.
6. PROCEDURE
 - 6.1. Preparation of sample
 - 6.2. Gas chromatography conditions
 - 6.2.1. The operating conditions must yield a resolution factor $R \geq \dots$
 - 6.2.2. As examples, the following operating conditions provide the required resolution:...
 - 6.3. Standard curve
 - 6.4. Inject 1 μ l of solution obtained in 6.1 and proceed...
7. CALCULATION
 - 7.1. Calculate from the standard curve (6.3) the quantity 'a' expressed...
 - 7.2. The content of chlorobutanol in the sample is calculated according...
8. REPEATABILITY
Note

IDENTIFICATION AND DETERMINATION OF QUININE

- A. IDENTIFICATION
 1. SCOPE AND FIELD OF APPLICATION
 2. PRINCIPLE
 3. REAGENTS
 - 3.1. Silica gel plates, without fluorescence indicators, 0,25 mm thick, 200...
 - 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 20 4 = 1,84)....
 - 3.5. Diethyl ether.
 - 3.6. Developing agent: carefully add 5 ml of sulphuric acid (3.4)...
 - 3.7. Bromine.
 - 3.8. Ammonium hydroxide solution (28 %; d 20 4 = 0,90...
 - 3.9. Quinine, anhydrous.
 - 3.10. Standard solution: weigh accurately about 100,0 mg of anhydrous quinine...
 4. APPARATUS
 - 4.1. Normal equipment for thin layer chromatography.
 - 4.2. Ultrasonic bath.

- 4.3. Millipore filter, FH 0,5 μ m or equivalent with suitable filtration...
5. PROCEDURE
 - 5.1. Preparation of the sample
 - 5.2. Thin layer chromatography
 - 5.3. Development
 - 5.3.1. Dry the plate at room temperature.
 - 5.3.2. Spray with reagent 3.6.
 - 5.3.3. Leave the plate to dry for one hour at room...
 - 5.3.4. Observe under the light from a UV lamp adjusted to...
 - 5.3.5. For further confirmation that quinine is present, the plate is...
- B. DETERMINATION
 1. SCOPE AND FIELD OF APPLICATION
 2. DEFINITION
 3. PRINCIPLE
 4. REAGENTS
 - 4.1. Acetonitrile.
 - 4.2. Potassium dihydrogenorthophosphate (KH₂PO₄).
 - 4.3. Orthophosphoric acid (85 %; d 20 4 = 1,7)....
 - 4.4. Tetramethylammonium bromide.
 - 4.5. Quinine, anhydrous.
 - 4.6. Methanol.
 - 4.7. Orthophosphoric acid solution (0,1 M): weigh 11,53 g of orthophosphoric...
 - 4.8. Potassium dihydrogenorthophosphate solution (0,1 M): weigh 13,6 g of potassium...
 - 4.9. Tetramethylammonium bromide solution: dissolve 15,40 g of tetramethylammonium bromide (4.4)...
 - 4.10. Eluant: orthophosphoric acid (4.7) /potassium dihydrogenorthophosphate (4.8) /tetramethylammonium bromide (4.9)/water/acetonitrile...
 - 4.11. Silica treated with octadecylsilane, 10 μ m.
 - 4.12. Standard solutions: weigh accurately approximately 5,0, 10,0, 15,0 and 20,0...
 5. APPARATUS
 - 5.1. Usual laboratory equipment.
 - 5.2. Ultrasonic bath.
 - 5.3. High-performance liquid chromatography equipment with a variable wavelength detector.
 - 5.4. Column: length: 250 mm; internal diameter: 4,6 mm; filling: silica...
 - 5.5. Millipore filter FH 0,5 μ m, or equivalent, with suitable filtration...
 6. PROCEDURE
 - 6.1. Sample preparation
 - 6.2. Chromatography
 - 6.3. Calibration curve
 7. CALCULATION
 - 7.1. From the calibration curve (6.3) determine the quantity in μ g...
 - 7.2. The concentration of anhydrous quinine in the sample, as a...
 8. REPEATABILITY

IDENTIFICATION AND DETERMINATION OF INORGANIC SULPHITES AND HYDROGEN SULPHITES

- A. IDENTIFICATION
1. PRINCIPLE
 2. REAGENTS
 - 2.1. Hydrochloric acid (4 M).
 - 2.2. Potassium iodate starch paper or other suitable alternative.
 3. APPARATUS
 - 3.1. Normal laboratory equipment.
 - 3.2. Flask (25 ml) fitted with a short reflux condenser.
 4. PROCEDURE
 - 4.1. Place about 2,5 g of sample in the flask (3.2)...
 - 4.2. Mix and heat to boiling.
 - 4.3. Test for the emission of sulphur dioxide either by smell...
- B. DETERMINATION
1. DEFINITION
 2. PRINCIPLE
 3. REAGENTS
 - 3.1. Hydrogen peroxide 0,2 % (m/v). Prepare daily.
 - 3.2. Orthophosphoric acid ($d_{25}^4 = 1,75$).
 - 3.3. Methanol.
 - 3.4. Sodium hydroxide (0,01 M) standardized solution.
 - 3.5. Nitrogen.
 - 3.6. Indicator: mixture 1: 1 (v/v) of methyl red (0,03 %...
 4. APPARATUS
 - 4.1. Normal laboratory equipment.
 - 4.2. Distillation apparatus (see figure).
 5. PROCEDURE
 - 5.1. Weigh accurately about 2,5 g of sample into the distillation...
 - 5.2. Add 60 ml of water and 50 ml of methanol...
 - 5.3. Place 10 ml of hydrogen peroxide (3.1), 60 ml of...
 - 5.4. Repeat 5.3 for the wash bottle E (see figure).
 - 5.5. Assemble the apparatus and adjust the nitrogen (3.5) flow to...
 - 5.6. Run 15 ml of orthophosphoric acid (3.2) from the funnel...
 - 5.7. Heat rapidly to boiling and then simmer gently for a...
 - 5.8. Detach the distillation receiver D. Rinse the tube and then...
 6. CALCULATION
 7. REPEATABILITY
- Sulphur dioxide distillation apparatus according to Tanner

IDENTIFICATION AND DETERMINATION OF CHLORATES OF THE ALKALI METALS

- A. IDENTIFICATION
1. PRINCIPLE
 2. REAGENTS
 - 2.1. Reference solutions: aqueous solutions of potassium chlorate, bromate and iodate...
 - 2.2. Development solvent: ammonia solution (28% m/v) acetone/butanol (60/130/30 v/v/v).
 - 2.3. Potassium iodide, aqueous solution (5 % m/v).
 - 2.4. Starch solution (1 to 5 % m/v).
 - 2.5. Hydrochloric acid (1 M).
 - 2.6. Ready-for-use cellulose thin-layer plates (0,25 mm).

3. APPARATUS
4. PROCEDURE
 - 4.1. Extract about 1 g of the sample with water, filter,...
 - 4.2. Deposit 2 μ l on the plate (2.6) of the solution...
 - 4.3. Place the plate in a tank and develop by ascending...
 - 4.4. Remove from the tank and allow the solvent to evaporate....
 - 4.5. Spray the plate with potassium iodide (2.3) and allow to...
 - 4.6. Spray the plate with starch solution (2.4) and allow to...
 - 4.7. Spray the plate with hydrochloric acid (2.5).
5. EVALUATION
- B. DETERMINATION
 1. DEFINITION
 2. PRINCIPLE
 3. REAGENTS
 - 3.1. Acetic acid, 80 % (m/m).
 - 3.2. Zinc powder.
 - 3.3. Silver nitrate standard solution (0,1 M).
 4. APPARATUS
 - 4.1. Normal laboratory equipment.
 - 4.2. Potentiometer equipped with a silver indicator electrode.
 5. PROCEDURE
 - 5.1. Sample preparation
 - 5.2. Reduction of chlorate
 - 5.3. Determination of chloride
 6. CALCULATION
 7. REPEATABILITY

IDENTIFICATION AND DETERMINATION OF SODIUM IODATE

- A. IDENTIFICATION
 1. PRINCIPLE
 2. REAGENTS
 - 2.1. Reference solutions. Aqueous solutions of potassium chlorate, bromate and iodate...
 - 2.2. Development solvent.
 - 2.3. Potassium iodide aqueous solution (5 % m/v).
 - 2.4. Starch solution (1 to 5 % m/v).
 - 2.5. Hydrochloric acid (1 M).
 3. APPARATUS
 - 3.1. Ready-for-use cellulose thin-layer chromatography (0,25 mm) plates.
 - 3.2. Normal equipment for thin layer chromatography.
 4. PROCEDURE
 - 4.1. Extract about 1 g of the sample with water, filter,...
 - 4.2. Deposit 2 μ l of this solution onto the base line...
 - 4.3. Place the plate in a tank and develop by ascending...
 - 4.4. Remove the plate from the tank and allow the solvent...
 - 4.5. Spray the plate with potassium iodide (2.3) and allow to...
 - 4.6. Spray with starch (2.4) and allow to dry for about...
 - 4.7. Finally spray with hydrochloric (2.5).
 5. EVALUATION
- B. DETERMINATION
 1. DEFINITION
 2. PRINCIPLE

3. REAGENTS
 - 3.1. Hydrochloric acid (4 M).
 - 3.2. Sodium sulphite aq, 5 % m/v.
 - 3.3. Sodium iodate stock solution.
 - 3.4. Potassium dihydrogenorthophosphate.
 - 3.5. Disodium hydrogenorthophosphate · 2H₂O.
 - 3.6. HPLC mobile phase: dissolve 3,88 g potassium dihydrogenorthophosphate (3.4) and...
 - 3.7. Universal indicator paper, pH 1-11.
4. APPARATUS
 - 4.1. Ordinary laboratory apparatus.
 - 4.2. Circular filter paper, diameter 110 mm, Schleicher and Schüll No...
 - 4.3. High-performance liquid chromatograph with a variable wavelength detector.
 - 4.4. Columns: length: 120 mm; internal diameter: 4,6 mm; number: two...
5. PROCEDURE
 - 5.1. Sample preparation
 - 5.1.1. Fluid samples (shampoos)
 - 5.1.2. Solid samples (soap)
 - 5.2. Chromatography
 - 5.3. Calibration
6. CALCULATION
7. REPEATABILITY
8. CONFIRMATION
 - 8.1. Principle
 - 8.2. Procedure

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IP completion day (31 December 2020 11pm) no further amendments will be applied to this version.

- (1) OJ No L 262, 27. 9. 1976, p. 169.
- (2) OJ No L 224, 22. 8. 1985, p. 40.
- (3) OJ No L 383, 31. 12. 1980, p. 27.
- (4) OJ No L 185, 30. 6. 1982, p. 1.
- (5) OJ No L 291, 24. 10. 1983, p. 9.