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)

ANNEX

IDENTIFICATION AND DETERMINATION OF GLYCEROL l-(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...

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).

(**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.