

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)

- 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

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ANNEX

DETERMINATION OF DICHLOROMETHANE AND 1,1,1-TRICHLOROETHANE

1. SCOPE AND FIELD OF APPLICATION
2. DEFINITION
3. PRINCIPLE
4. REAGENTS
  - 4.1. Chloroform (CHCl<sub>3</sub>).
  - 4.2. Carbon tetrachloride (CCl<sub>4</sub>).
  - 4.3. Dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>).
  - 4.4. 1,1,1-trichloroethane (CH<sub>3</sub>CCl<sub>3</sub>).
  - 4.5. Acetone.
  - 4.6. Nitrogen.
5. APPARATUS
  - 5.1. Usual laboratory apparatus.
  - 5.2. Gas chromatograph fitted with a thermal conductivity detector.
  - 5.3. Transfer bottle, 50 to 100 ml (see sampling method 5.3)....
  - 5.4. Pressure gas-syringe, 25 or 50 µl (see sampling method 5.4.2.2)....
6. PROCEDURE
  - 6.1. Non-pressurized sample: weigh the sample accurately in a stoppered conical...
  - 6.2. Pressurized sample: use the sampling method described in the sampling...
7. CHROMATOGRAPHIC CONDITIONS
  - 7.1. Precolumn
  - 7.2. Column
  - 7.3. As examples the following columns yield the results sought:
8. MIXTURE FOR ESTABLISHING THE RESPONSE FACTORS
9. CALCULATIONS
  - 9.1. Calculating a response factor of a substance 'p' relative to...

- 9.2 Calculate the % (m/m) of dichloromethane and 1,1,1-trichloroethane present in...

## 10. REPEATABILITY

### IDENTIFICATION AND DETERMINATION OF QUINOLIN-8-OL AND BIS(8-HYDROXYQUINOLINIUM) SULPHATE

1. SCOPE AND FIELD OF APPLICATION
2. DEFINITION
3. PRINCIPLE
  - 3.1. Identification
  - 3.2. Determination
4. REAGENTS
  - 4.1. Quinolin-8-ol.
  - 4.2. Benzene. In view of its toxicity great care must be...
  - 4.3. Chloroform.
  - 4.4. Aqueous sodium 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.
  - 4.15. Standard solutions
    - 4.15.1. Weigh 100 mg of quinolin-8-ol (4.1) into a 100 ml...
    - 4.15.2. Weigh 100 mg of quinolin-8-ol into a 100 ml standard...
  - 4.16. Fehling's solution
  - 4.17. Eluting solvents for thin-layer chromatography
  - 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
  - 4.23. Prepared thin-layer plates
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...

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6. PROCEDURE
  - 6.1. Identification
    - 6.1.1. Liquid samples
      - 6.1.1.1. The pH of part of the test sample is adjusted...
      - 6.1.1.2. 10 and 30 µl of the standard solution (4.15.2) is...
      - 6.1.1.3. When the solvent front has advanced 150 mm, the plate...
      - 6.1.1.4. Spray the plate with sodium carbonate solution (4.19). Dry and...
    - 6.1.2. Solid samples or creams
      - 6.1.2.1. Disperse 1 g of the sample in 5 ml of...
      - 6.1.2.2. Apply 10 and 30 µl of the standard solution (4.15.2)...
  - 6.2. Determination
    - 6.2.1. Liquid samples
      - 6.2.1.1. Weigh 5 g of the sample into a 100 ml...
      - 6.2.1.2. Dissolve this residue in 20 ml of warm water. Transfer...
      - 6.2.1.3. Pipette 5 ml of this solution into a 50 ml...
      - 6.2.1.4. Filter and collect the chloroform layers in a 25 ml...
    - 6.2.2. Solid samples or creams
      - 6.2.2.1. Weigh 0,500 g of the sample into a 100 ml...
      - 6.2.2.2. Transfer the contents of the flask into a 100 ml...
      - 6.2.2.3. In the case of emulsions that impede further treatment, mix...
      - 6.2.2.4. Evaporate the collected aqueous phases (6.2.2.2) or the eluate (6.2.2.3)...
      - 6.2.2.5. Dissolve the residue in 6 ml of the sodium hydroxide...
      - 6.2.2.6. Repeat the extraction three times with 8 ml of chloroform...
7. STANDARD CURVE
8. CALCULATION
  - 8.1. Liquid samples
  - 8.2. Solid samples or creams
9. REPEATABILITY

#### DETERMINATION OF AMMONIA

1. SCOPE AND FIELD OF APPLICATION
2. DEFINITION
3. PRINCIPLE
4. REAGENTS
  - 4.1. Methanol.
  - 4.2. Barium chloride dihydrate, 25 % (m/v) solution.
  - 4.3. Orthoboric acid, 4 % (m/v) solution.
  - 4.4. Sulphuric acid, 0,25 M standard solution.
  - 4.5. Anti-foam liquid.
  - 4.6. Sodium hydroxide, 0,5 M standard solution.
  - 4.7. Indicator, if required: mix 5 ml of a 0,1 %...
5. APPARATUS
  - 5.1. Usual laboratory apparatus.
  - 5.2. Centrifuge with stoppered 100 ml bottles.
  - 5.3. Steam distillation apparatus.
  - 5.4. Potentiometer.

- 5.5. Indicating glass electrode and dimercury dichloride (calomel) reference electrode.
6. PROCEDURE
  - 6.1. Weigh into a 100 ml standard flask a mass (m)...
  - 6.2. Add 10 ml of water, 10 ml of methanol (4.1)...
  - 6.3. Mix and leave overnighs in the refrigerator (5 oC).
  - 6.4. Then filter, or centrifuge the still cold solution in closed...
  - 6.5. Pipette 40 ml of this clear solution into the steam...
  - 6.6. Distil and collect 200 ml of distillate in a 250...
  - 6.7. Back titrate the excess acid with standard sodium hydroxide solution...
  - 6.8. NB: For potentiometric determination, collect 200 ml of distillate in...
7. CALCULATIONS
  - 7.1. Calculation in the case of back titration
  - 7.2. Calculation in the case of direct potentiometric titration
8. REPEATABILITY

#### IDENTIFICATION AND DETERMINATION OF NITROMETHANE

1. SCOPE AND FIELD OF APPLICATION
2. DEFINITION
3. PRINCIPLE
4. IDENTIFICATION
  - 4.1. Reagents
    - 4.1.1. Sodium hydroxide, 0,5 M solution.
    - 4.1.2. Folin's reagent
  - 4.2. Procedure
5. DETERMINATION
  - 5.1. Reagents
    - 5.1.1. Chloroform (internal standard 1).
    - 5.1.2. 2,4-dimethylheptane (internal standard 2).
    - 5.1.3. Ethanol, 95 %.
    - 5.1.4. Nitromethane.
    - 5.1.5. Chloroform reference solution
    - 5.1.6. 2,4-dimethylheptane reference solution
  - 5.2. Apparatus
    - 5.2.1. Gas chromatograph with flame ionization detector.
    - 5.2.2. Apparatus for sampling of aerosols (transfer bottle, microsyringe connectors, etc.)...
    - 5.2.3. Usual laboratory apparatus.
  - 5.3. Procedure
    - 5.3.1. Preparation of the sample
    - 5.3.2. Preparation of the standard
    - 5.3.3. Gas chromatographic conditions
      - 5.3.3.1. Column
      - 5.3.3.2. Detector
      - 5.3.3.3. Temperature conditions
      - 5.3.3.4. Suitable gas supplies
6. CALCULATIONS
  - 6.1. Response factor of nitromethane, calculated with reference to the internal...
  - 6.2. Concentration of nkromethane in the sample
7. REPEATABILITY

## IDENTIFICATION AND DETERMINATION OF MERCAPTOACETIC ACID IN HAIR-WAVING, HAIR-STRAIGHTENING AND...

1. SCOPE AND FIELD OF APPLICATION
2. DEFINITION
3. PRINCIPLE
4. IDENTIFICATION
  - 4.1. Identification by spot tests
    - 4.1.1. Reagents
      - 4.1.1.1. Lead di(acetate) papes.
      - 4.1.1.2. Hydrochloric acid solution (one volume of concentrated hydrochloric acid plus...
    - 4.1.2. Procedure
      - 4.1.2.1. Identification of mercaptoacetic acid by means of a colour reaction...
      - 4.1.2.2. Characterization of inorganic sulphides by the formulation of hydrogen sulphide...
      - 4.1.2.3. Characterization of sulphites by the formation of sulphur dioxide upon...
  - 4.2. Identification by thin-layer chromatography
    - 4.2.1. Reagents
      - 4.2.1.1. Mercaptoacetic acid (thioglycollic acid), 98 % minimum purity assayed by...
      - 4.2.1.2. 2,2'-dithiodi(acetic acid), 99 % minimum purity assayed by iodometry.
      - 4.2.1.3. 2-mercaptopropionic acid (thiolactic acid), 95 % minimum purity assayed by...
      - 4.2.1.4. 3-mercaptopropionic acid, 98 % minimum purity assayed by iodometry.
      - 4.2.1.5. 3-mercaptopropane-1,2-diol (1-thioglycerol), 98 % minimum purity assayed by iodometry.
      - 4.2.1.6. Thin-layer plates, silica gel, ready prepared, 0,25 mm thickness.
      - 4.2.1.7. Thin-layer plates, aluminium oxide, Merck F 254 E or equivalent....
      - 4.2.1.8. Hydrochloric acid, concentrated, d<sub>4</sub> 20 = 1,19 g/ml.
      - 4.2.1.9. Ethyl acetate.
      - 4.2.1.10 Chloroform.
      - 4.2.1.11 Diisopropyl ether
      - 4.2.1.12 Carbon tetrachloride.
      - 4.2.1.13 Acetic acid, glacial.
      - 4.2.1.14 Potassium iodide, 1 % (m/v) solution in water.
      - 4.2.1.15 Platinum tetrachloride, 0,1 % (m/v) solution in water.
      - 4.2.1.16 Eluting solvents
        - 4.2.1.16H Ethyl acetate (4.2.1.9), chloroform (4.2.1.10), diisopropyl ether (4.2.1.11), acetic acid...
        - 4.2.1.16Q Chloroform (4.2.1.10), acetic acid (4.2.1.13) (90: 20, by volume).
      - 4.2.1.17 Detection reagents
        - 4.2.1.17M Mix, immediately before use, equal volumes of solution (4.2.1.14) and...
        - 4.2.1.17B Bromine solution 5 % (m/v):
        - 4.2.1.17E Fluorescein solution, 0,1 % (m/v):

- 4.2.1.17 ~~Hexa~~ammonium heptamolybdate, 10 % (m/v) solution in water.
- 4.2.1.18 Reference solutions
  - 4.2.1.18 ~~M~~ercaptoacetic acid (4.2.1.1), 0,4 % (m/v) solution in water.
  - 4.2.1.18 ~~2~~'-dithiodi(acetic) acid (4.2.1.2), 0,4 % (m/v) solution in water.
  - 4.2.1.18 ~~3~~-mercaptopropionic acid (4.2.1.3), 0,4 % (m/v) solution in water.
  - 4.2.1.18 ~~4~~-mercaptopropionic acid (4.2.1.4), 0,4 % (m/v) solution in water.
  - 4.2.1.18 ~~5~~-mercapto propane-1,2-diol (4.2.1.5), 0,4 % (m/v) solution in water.
- 4.2.2. Apparatus
- 4.2.3. Procedure
  - 4.2.3.1. Treatment of samples
  - 4.2.3.2. Elution
  - 4.2.3.3. Detection
  - 4.2.3.4. Interpretation
- 5. DETERMINATION (see NB)
  - 5.1. Iodometry
    - 5.1.1. Principle
    - 5.1.2. Reagents
    - 5.1.3. Apparatus
    - 5.1.4. Procedure
    - 5.1.5. Calculation
    - 5.1.6. Remarks
  - 5.2. Gas chromatography
    - 5.2.1. Principle
    - 5.2.2. Reagents
      - 5.2.2.1. Mercaptoacetic acid, 98 %.
      - 5.2.2.2. Hydrochloric acid, d<sub>4</sub> 10 = 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):
      - 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...
    - 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...
      - 5.2.3.3. Apparatus for the advance preparation of diazomethane (Fieser).
    - 5.2.4. Preparation of the sample

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- 5.2.5. Methylation
  - 5.2.5.1. Methylation in situ
  - 5.2.5.2. Methylation with the previously prepared diazomethane solution
- 5.2.6. Preparation of the standard
- 5.2.7. Gas chromatographic conditions
  - 5.2.7.1. Column
  - 5.2.7.2. Packing
  - 5.2.7.3. Detector
  - 5.2.7.4. Gas supplies
  - 5.2.7.5. Temperature conditions
  - 5.2.7.6. Recorder chart speed
  - 5.2.7.7. Quantity injected
  - 5.2.7.8. The conditions of chromatography are given as a guide. They...
- 5.2.8. Calculations
  - 5.2.8.1. Coefficient of proportionality for mercaptoacetic acid
  - 5.2.8.2. Concentration of mercaptoacetic acid present in the sample

## 6. REPEATABILITY

### IDENTIFICATION AND DETERMINATION OF HEXACHLOROPHENE

#### A. IDENTIFICATION

- 1. SCOPE AND FIELD OF APPLICATION
- 2. PRINCIPLE
- 3. REAGENTS
  - 3.1. Sulphuric acid, 4 M solution.
  - 3.2. Celite AW.
  - 3.3. Ethyl acetate.
  - 3.4. Eluting solvent: Benzene containing 1 % (v/v) of glacial acetic...
  - 3.5. Visualizing agent I:
  - 3.6. Visualizing agent II:
  - 3.7. Reference solution:
- 4. APPARATUS
  - 4.1. Kiesel gel 254 TLC plates, 200 x 200 mm (or...
  - 4.2. Usual TLC equipment.
  - 4.3. Bath thermostatted at 26 oC to hold the chromatography tank....
- 5. PREPARATION OF THE TEST SAMPLE
  - 5.1. Thoroughly mix 1 g of homogenized sample with 1 g...
  - 5.2. Dry at 100 oC for two hours.
  - 5.3. Cool and finely powder the dried residue.
  - 5.4. Extract twice with 10 ml of ethyl acetate (3.3) each...
  - 5.5. Evaporate at 60 oC.
  - 5.6. Dissolve the residue in 2 ml of ethyl acetate (3.3)....
- 6. PROCEDURE
  - 6.1. Place 2 µl of the test sample solution (5.6) and...
  - 6.2. Saturate the tank (4.3) with the eluting solvent (3.4).
  - 6.3. Place the TLC plate in the tank and elute up...
  - 6.4. Remove the TLC plate and dry in a ventilated oven...
  - 6.5. Visualization
    - 6.5.1. Spray the visualizing agent I (3.5) evenly on the plate....

- 6.5.2. Spray the 2,6-dibromo-4-(chloroimino)cyclohexa-2,5-dienone solution of visualizing agent II (3.6) evenly...
7. INTERPRETATION
  - 7.1. Visualizing agent I (3.5):
  - 7.2. Visualizing agent II (3.6):
- B. DETERMINATION
  1. SCOPE AND FIELD OF APPLICATION
  2. DEFINITION
  3. PRINCIPLE
  4. REAGENTS
    - 4.1. Ethyl acetate.
    - 4.2. N -methyl- N -nitroso-p toluenesulphonamide (diazald).
    - 4.3. Diethyl ether.
    - 4.4. Methanol.
    - 4.5. 2-(2-ethoxyethoxy)ethanol (carbitol).
    - 4.6. Formic acid.
    - 4.7. Potassium hydroxide, 50 % (m/m) aqueous solution (prepare fresh daily)....
    - 4.8. Hexane for spectroscopy.
    - 4.9. Bromochlorophene (standard No 1).
    - 4.10. 4,4',6,6'-tetrachloro-2,2'-thiodiphenol (standard No 2).
    - 4.11. 2,4,4'-trichloro- 2-hydroxy-diphenyl ether (standard No 3).
    - 4.12. Acetone.
    - 4.13. 4 M sulphuric acid.
    - 4.14. Celite AW.
    - 4.15. Formic acid/ethyl acetate, 10 % (v/v) solution.
    - 4.16. Hexachlorophene.
  5. APPARATUS
    - 5.1. Usual laboratory glassware.
    - 5.2. Mini-apparatus for the preparation of diazomethane (Analyt. Chem., 1973, 45,...
    - 5.3. Gas chromatograph equipped with a 63 Ni source electron capture...
  6. PROCEDURE
    - 6.1. Preparation of the standard solution
      - 6.1.1. Accurately weigh about 50 mg of standard No 1, 2...
      - 6.1.2. Accurately weigh about 50 mg of standard No 1, 2...
    - 6.2. Preparation of the sample
    - 6.3. Methylation of the sample
    - 6.4. Methylation of the standard
  7. GAS CHROMATOGRAPHY
  8. CALCULATION
    - 8.1. Proportionality coefficient of hexachlorophene
    - 8.2. The amount of hexachlorophene in the sample
  9. REPEATABILITY

~~QUANTITATIVE DETERMINATION OF TOSYLCHLORAMIDE SODIUM (INN)~~

1. SCOPE AND FIELD OF APPLICATION
2. DEFINITION
3. PRINCIPLE
4. REAGENTS
  - 4.1. Tosylchloramide sodium (chloramine-T).

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- 4.2. Standard solution of 4-toluenesulphonamide: 50 mg of 4-toluenesulphonamide in 100...
- 4.3. Hydrochloric acid, 37 % (m/m), d<sub>4</sub> 20 = 1,18 g/ml....
- 4.4. Diethyl ether.
- 4.5. Ethanol, 96 % (v/v).
- 4.6. Development solvent
  - 4.6.1. 1-butanol /ethanol (4.5) /water (40: 4: 9; v/v/v), or
  - 4.6.2. Chloroform /acetone (6: 4; v/v).
- 4.7. Ready prepared thin-layer chromatography plates, silica gel 60, without fluorescent...
- 4.8. Potassium permanganate.
- 4.9. Hydrochloric acid, 15 % (m/m).
- 4.10. Spray reagent: 2-toluidine, 1 % (m/v) solution in ethanol (4.5)....
5. APPARATUS
  - 5.1. Normal laboratory apparatus.
  - 5.2. Usual thin-layer chromatography equipment.
  - 5.3. Photodensitometer.
6. PROCEDURE
  - 6.1. Hydrolysis
  - 6.2. Extraction
    - 6.2.1. Take 30 ml of the filtrate and extract three times...
    - 6.2.2. Take 25 ml of the dried ethereal extract and evaporate...
  - 6.3. Thin-layer chromatography
    - 6.3.1. Spot 20 µl of the ethanolic residue (6.2) on to...
    - 6.3.2. Then allow to develop approximately 150 mm in the development...
    - 6.3.3. After completely evaporating the development solvent, place the plate for...
  - 6.4. Measurement
  - 6.5. Plotting the calibration curves
7. NOTE
8. CALCULATION
9. REPEATABILITY

#### DETERMINATION OF TOTAL FLUORINE IN DENTAL CREAMS

1. SCOPE AND FIELD OF APPLICATION
2. DEFINITION
3. PRINCIPLE
4. REAGENTS
  - 4.1. Sodium fluoride, dried at 120 °C to constant mass.
  - 4.2. Water, double distilled or equivalent quality.
  - 4.3. Hydrochloric acid, d<sub>4</sub> 20 = 1,19 g/ml.
  - 4.4. Cyclohexane (CH).
  - 4.5. Xylene with no peaks in the chromatogram prior to the...
  - 4.6. Chlorotriethylsilane (TECS Merck or an equivalent).
  - 4.7. Fluorine standard solutions
    - 4.7.1. Stock solution, 0,250 mg F-/ml. Weigh accurately 138,1 mg of...
    - 4.7.2. Diluted stock solution, 0,050 mg F-/ml. Transfer by pipette 20...
  - 4.8. Internal standard solution
  - 4.9. Chlorotriethylsilane/internal standard solution
  - 4.10. Perchloric acid, 70 % (m/v).

- 4.11. Perchloric acid, 20 % (m/v) in water (4.2).
5. APPARATUS
- 5.1. Standard laboratory equipment.
  - 5.2. Gas chromatograph fitted with a flame ionization detector.
  - 5.3. Vortex swirl mixer or equivalent.
  - 5.4. Bühler, shaker, type SMB1 or equivalent.
  - 5.5. Volumetric flasks, 100 and 250 ml, made of polypropylene.
  - 5.6. Centrifuge tubes (glass); 20 ml with teflon lined screw-caps, Sovirel...
  - 5.7. Pipettes, adjustable to deliver volumes of 50 to 200 µl,...
  - 5.8. Distillation apparatus, fitted with a three-ball Schneider column or an...
6. PROCEDURE
- 6.1. Sample analysis
    - 6.1.1. Select a dental-cream tube not previously opened, cut open the...
    - 6.1.2. Weigh accurately 150 mg (m) of sample into a centrifuge...
    - 6.1.3. Add 1 ml of xylene (4.5).
    - 6.1.4. Add dropwise 5 ml of hydrochloric acid (4.3) and homogenize...
    - 6.1.5. Add, by pipette, 0,5 ml of chlorotriethylsilane/internal standard solution (4.9)...
    - 6.1.6. Close the tube with the screw-cap (5.6) and mix for...
    - 6.1.7. Centrifuge 10 minutes at such a speed as to produce...  
Remark:
    - 6.1.8. Repeat the injection, calculate the average peak area ratio (ATEFS/ACH)...
    - 6.1.9. Calculate the total fluorine content of the sample (in per...
  - 6.2. Chromatographic conditions
    - 6.2.1. Column: stainless steel.
  - 6.3. Calibration graph
    - 6.3.1. Place, by pipette, into a series of six centrifuge tubes...
    - 6.3.2. Proceed as described under 6.1.3 to 6.1.6 inclusive.
    - 6.3.3. Inject 3 µl of the organic phase on to the...
    - 6.3.4. Repeat the injection and calculate the average peak ratio (ATEFS/ACH)....
    - 6.3.5. Plot a calibration graph correlating the mass of fluorine (in...
7. CALCULATION
8. REPEATABILITY

## IDENTIFICATION AND DETERMINATION OF ORGANOMERCURY COMPOUNDS

### SCOPE AND FIELD OF APPLICATION

#### A. IDENTIFICATION

1. PRINCIPLE
2. REAGENTS
  - 2.1. Sulphuric acid, 25 % (v/v).
  - 2.2. 1,5-diphenyl-3-thiocarbazone (dithizone): 0,8 mg in 100 ml carbon tetrachloride (2.4)....
  - 2.3. Nitrogen.
  - 2.4. Carbon tetrachloride.
  - 2.5. Development solvent: hexane /acetone, 90:10 (v/v).
  - 2.6. Standard solution, 0,001 % in water of:
  - 2.7. Ready prepared silica gel plates (e.g. Merck 5721 or equivalent)....

- 2.8. Sodium chloride.
- 3. APPARATUS
  - 3.1. Normal laboratory equipment.
  - 3.2. Normal TLC apparatus.
  - 3.3. Phase-separating filter.
- 4. PROCEDURE
  - 4.1. Extraction
    - 4.1.1. Dilute 1 g of sample in a centrifuge tube by...
    - 4.1.2. Centrifuge for at least 20 minutes at 4 500 rev/min...
    - 4.1.3. Extract several times with 2 or 3 ml of dithizone...
    - 4.1.4. Filter each organic phase sequentially through a phase-separating filter (3.3)...
    - 4.1.5. Evaporate to dryness in a stream of nitrogen (2.3).
    - 4.1.6. Dissolve with 0,5 ml of carbon tetrachloride (2.4). Apply this...
  - 4.2. Separatum and identification
    - 4.2.1. Apply immediately 50 µl of the carbon tetrachloride solution obtained...
    - 4.2.2. Place the plate in the solvent (2.5) and allow the...
- B. DETERMINATION
  - 1. DEFINITION
  - 2. PRINCIPLE
  - 3. REAGENTS
    - 3.1. Concentrated nitric acid, d4 20 = 1,41 g/ml.
    - 3.2. Concentrated sulphuric acid, d4 20 = 1,84 g/ml.
    - 3.3. Redistilled water.
    - 3.4. Potassium permanganate, 7 % (m/v) solution.
    - 3.5. Hydroxylammonium chloride, 1,5 % (m/v) solution.
    - 3.6. Dipotassium peroxodisulphate, 5 % (m/v) solution.
    - 3.7. Tin dichloride, 10 % (m/v) solution.
    - 3.8. Concentrated hydrochloric acid, d4 20 = 1,18 g/ml.
    - 3.9. Palladium dichloride impregnated glass wool, 1 % (m/m).
  - 4. APPARATUS
    - 4.1. Normal laboratory equipment.
    - 4.2. Apparatus for flameless atomic absorption mercury determination (cold vapour technique),...
  - 5. PROCEDURE
    - 5.1. Breakdown
      - 5.1.1. Weigh accurately 150 mg of the sample (m). Add 10...
      - 5.1.2. After cooling, add 10 ml of sulphuric acid (3.2) and...
      - 5.1.3. Place the flask in an ice bath and add carefully...
      - 5.1.4. Adding 2 ml aliquots of 7 % potassium permanganate solution...
      - 5.1.5. Add 4 ml of dipotassium peroxodisulphate solution (3.6). Continue to...

- 5.1.6. Allow to cool and transfer the contents of the flask...
- 5.2. Determination
  - 5.2.1. Place 10 ml of the test solution (5.1.6) in the...
  - 5.2.2. Place some palladium dichloride impregnated glass wool (3.9) between the...
- 6. CALCULATION
- 7. NOTES
  - 7.1. To improve mineralization it might be necessary to start by...
  - 7.2. If absorption of the mercury by the substrate is suspected,...
- 8. REPEATIBILITY

#### DETERMINATION OF ALKALI AND ALKALINE EARTH SULPHIDES

- 1. SCOPE AND FIELD OF APPLICATION
- 2. DEFINITION
- 3. PRINCIPLE
- 4. REAGENTS
  - 4.1. Concentrated hydrochloric acid,  $d_4^{20} = 1,19$  g/ml.
  - 4.2. Sodium thiosulphate, 0,1 M standard solution.
  - 4.3. Iodine, 0,05 M standard solution.
  - 4.4. Disodium sulphide.
  - 4.5. Cadmium di(acetate).
  - 4.6. Concentrated ammonia,  $d_4^{20} = 0,90$  g/ml.
  - 4.7. Ammoniacal solution of cadmium di(acetate): dissolve 10 g of cadmium...
  - 4.8. Nitrogen.
  - 4.9. Solution of ammonia M.
- 5. APPARATUS
  - 5.1. Usual laboratory equipment.
- 6. PROCEDURE
  - 6.1. Entrainment of the sulphides
    - 6.1.1. Take a package which has not been previously opened. Weigh...
    - 6.1.2. Transfer 50 ml of solution (4.7) to each of the...
    - 6.1.3. Fit a dropping funnel, the dip tube and the outlet...
    - 6.1.4. Pass nitrogen (4.8) through for 15 minutes, at a rate...
    - 6.1.5. Heat the round-bottom flask to  $85 \pm 5$  oC.
    - 6.1.6. Stop the nitrogen (4.8) stream and add 40 ml of...
    - 6.1.7. Turn the nitrogen (4.8) stream on again when nearly all...
    - 6.1.8. Cease heating after 30 minutes. Allow the flask (5.2) to...
  - 6.2. Titration
    - 6.2.1. Filter the cadmium sulphide through a long-stem funnel (5.4).
    - 6.2.2. Rinse the conical flasks (5.3) first with the ammonia solution...
    - 6.2.3. Complete the washing of the precipitate with 100 ml of...
    - 6.2.4. Place the paper filter in the first conical flask that...
    - 6.2.5. Determine the excess iodine using the sodium thiosulphate solution ( $n_2$ )...
- 7. CALCULATON
- 8. REPEATABILITY

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**Status:** EU Directives are being published on this site to aid cross referencing from UK legislation. After IP completion day (31 December 2020 11pm) no further amendments will be applied to this version.

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- (1) OJ No L 262, 27. 9. 1976, p. 169.
- (2) OJ No L 188, 13. 7. 1983, p. 15.
- (3) OJ No L 383, 31. 12. 1980, p. 27.
- (4) OJ No L 185, 30. 6. 1982, p. 1.