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.*

ANNEX

DETERMINATION OF DICHLOROMETHANE AND 1,1,1-TRICHLOROETHANE IDENTIFICATION AND DETERMINATION OF MERCAPTOACETIC ACID IN HAIR-WAVING, HAIR-STRAIGHTENING AND DEPILATORY PRODUCTS

4. IDENTIFICATION

4.1. ***Identification by spot tests***

4.1.1. *Reagents*

All reagents should be of analytical purity.

4.1.1.1. Lead di(acetate) papes.

4.1.1.2. Hydrochloric acid solution (one volume of concentrated hydrochloric acid plus one volume of water)

4.1.2. Procedure

4.1.2.1. *Identification of mercaptoacetic acid by means of a colour reaction with lead di(acetate)*

Place a drop of the sample to be analyzed on lead di(acetate) paper (4.1.1.1). If an intense yellow colour appears, mercaptoacetic acid is probably present.

Sensitivity: 0,5 %.

4.1.2.2. *Characterization of inorganic sulphides by the formulation of hydrogen sulphide on acidification*

Introduce, into a test tube, a few milligrams of the sample to be studied. Add 2 ml of distilled water and 1 ml of hydrochloric acid (4.1.1.2). Hydrogen sulphide, recognizable by its smell, is evolved and a black lead sulphide precipitate forms on the lead di(acetate) paper (4.1.1.1).

Sensitivity: 50 ppm.

4.1.2.3. *Characterization of sulphites by the formation of sulphur dioxide upon acidification*

Proceed as described in 4.1.2.2. Bring to the boil. The sulphur dioxide is recognizable by its smell and by its reducing properties in respect, for example, of permanganate ions.

4.2. Identification by thin-layer chromatography

4.2.1. *Reagents*

All reagents, except where otherwise stated, should be of analytical purity.

4.2.1.1. Mercaptoacetic acid (thioglycollic acid), 98 % minimum purity assayed by iodometry.

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 iodometry.

4.2.1.4. 3-mercaptopropionic acid, 98 % minimum purity assayed by iodometry.

4.2.1.5. 3-mercaptopropane-l,2-diol (1-thioglycerol), 98 % minimum purity assayed by iodometry.

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.*

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_4^{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*

4.2.1.16.1 Ethyl acetate (4.2.1.9), chloroform (4.2.1.10), diisopropyl ether (4.2.1.11), acetic acid (4.2.1.13) (20: 20: 10: 10, by volume).

4.2.1.16.2 Chloroform (4.2.1.10), acetic acid (4.2.1.13) (90: 20, by volume).

4.2.1.17. *Detection reagents*

4.2.1.17.1 Mix, immediately before use, equal volumes of solution (4.2.1.14) and solution (4.2.1.15).

4.2.1.17.2 Bromine solution 5 % (m/v):

Dissolve 5 g of bromine in 100 ml of carbon tetrachloride (4.2.1.12).

4.2.1.17.3 Fluorescein solution, 0,1 % (m/v):

Dissolve 100 mg of fluorescein in 100 ml of ethanol.

4.2.1.17.4 Hexaammonium heptamolybdate, 10 % (m/v) solution in water.

4.2.1.18. *Reference solutions*

4.2.1.18.1 Mercaptoacetic acid (4.2.1.1), 0,4 % (m/v) solution in water.

4.2.1.18.2 2,2'-dithiodi(acetic) acid (4.2.1.2), 0,4 % (m/v) solution in water.

4.2.1.18.3 2-mercaptopropionic acid (4.2.1.3), 0,4 % (m/v) solution in water.

4.2.1.18.4 3-mercaptopropionic acid (4.2.1.4), 0,4 % (m/v) solution in water.

4.2.1.18.5 3-mercaptopropane-l,2-diol (4.2.1.5), 0,4 % (m/v) solution in water.

4.2.2. *Apparatus*

Usual apparatus for thin-layer chromatography.

4.2.3. *Procedure*

4.2.3.1. *Treatment of samples*

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.*

Acidify to pH 1 with a few drops of hydrochloric acid (4.2.1.8) and filter if necessary.

In certain cases it may be advisable to dilute the sample. If so acidify it with hydrochloric acid before dilution.

4.2.3.2. *Elution*

Place on the plate 1 µl of sample solution (4.2.3.1) and one litre of each of the five reference solutions (4.2.1.18). Dry carefully in a gentle current of nitrogen and elute the plate with solvents (4.2.1.16.1 or 4.2.1.16.2). Dry the plate as quickly as possible to minimize oxidation of the thiols.

4.2.3.3. *Detection*

Spray the plate with one of the three reagents (4.2.1.17.1, 4.2.1.17.3 or 4.2.1.17.4). If the plate is sprayed with reagent (4.2.1.17.3), further treat it with bromine vapour (e.g. in a tank containing a small beaker of the reagent (4.2.1.17.2)) until the spots are visible. Detection with the spray reagent (4.2.1.17.4) will be satisfactory only if the drying time for the thin layer has not exceeded 30 minutes.

4.2.3.4. *Interpretation*

Compare the Rf values and the colour of the reference solutions with those of the standards. The mean Rf values given below as a rough guide have only a comparative value. They depend upon:

— the state of activation of the thin layer at the time of chromatographing,

— the temperature of the chromatography tank.

EXAMPLES OF RF VALUES OBTAINED ON A SILICA GEL LAYER

	Eluting solvents	
	4.2.1.16.1	**4.2.1.16.2**
Mercaptoacetic acid	0,25	0,80
2-mercaptopropionic acid	0,40	0,95
2,2'-dithiodi(acetic) acid	0,00	0,35
3-mercaptopropionic acid	0,45	0,95
3-mercaptopropane-1,2 diol	0,45	0,35