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

4.2. 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.

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-1,2-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*

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