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SHORT COMMUNICATION

Thin-layer chromatography of corticosteroids

A. HALL

PAPER chromatography used routinely for examining corticosteroids is time consuming and relatively insensitive. Thin-layer chromatography is simple and useful for handling a number of samples rapidly. In conjunction with 2,5-diphenyl-3(4-styrylphenyl)tetrazolium (Brooks & others, 1958) as a spray reagent, it is capable of detecting less than 1 μg of corticosteroid (0·25 μg of cortisone acetate).

Two solvent systems are described which allow the separation of closely related substances. Two analyses using these systems suffice to identify and separate the corticoids in accordance with the results listed in Table 1.

APPARATUS

Spread thin-layer chromatography plates 20 × 20 cm with a 0·25 mμ layer of Kieselgel G (Macherey Nagel) and dry for 1 hr at 110°.

Line two tanks with Whatman No. 4 filter paper, one containing approximately 1½ cm of solvent system 1 and the other of solvent system 2. 1 μl disposable pipettes.

REAGENTS

2,5-*Diphenyl*-3(4-*styrylphenyl*)*tetrazolium chloride* 0·5% *w/v in ethanol.* Dilute 5 ml of the above solution to 50 ml with 2N sodium hydroxide immediately before using as spray reagent.

Solvent system 1. Shake together dichloroethane (100 ml), methyl acetate (50 ml) and water (50 ml). Allow to separate and run the lower layer through a filter paper into one tank.

Solvent system 2. Shake together methylene chloride (100 ml), dioxan (50 ml) and water (50 ml). Allow to separate and run the lower layer through a filter paper into the second tank.

Saturation of the solvent systems with water reduces the size of the spot.

METHOD

Dissolve 50 mg ± 1 mg of the sample in 10 ml of chloroform: methanol mixture 1 : 1. Select 2 plates and mark off at 1·5 cm intervals, 3 cm from

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the end. Apply 1 μl of the solution of the substance under test to the first mark on each plate. To subsequent marks apply as standards 1 μl of a 0·5% solution of the authentic specimen, 1 μl of a 0·005% and 1 μl of a 0·01% solution of both hydrocortisone and cortisone acetate (equivalent to 1% and 2% impurity). Where impurities other than hydrocortisone and cortisone acetate are possible, these may be used instead provided one substance of low Rf value and one substance of high Rf are used.

Place one plate in each tank with the spots at the lower end and allow the solvent system to rise to 3 cm from the top of the plates.

Allow the plates to dry at room temperature for 5 min, heat at 105° for 5 min, then spray with alkaline 2,5-diphenyl-3(4-styrylphenyl)tetrazolium solution. The sample should give a spot on each plate of the same intensity and in the same position as the authentic specimen. By comparison with the standards the amount of any impurities shown as subsidiary spots can be estimated and may possibly be identified.

RESULTS

These are summarised in Table 1. One point deserves mention. Under the conditions used, dexamethasone and betamethasone could not be separated and the separation of dexamethasone acetate from betamethasone acetate was marginal. However, on plates spread with alumina (Fluka), separation of the latter pair of substances was possible using solvent system 1.

TABLE 1. Rf VALUES OF CORTICOSTEROIDS IN SOLVENT SYSTEMS 1 AND 2

Sample	Rf value of sample: Rf of cortisone acetate in system 1	Sample	Rf value of sample: Rf of hydrocortisone alcohol in system 2
Hydrocortisone	0·19	Hydrocortisone hydrogen succinate	0·18
6-Methylhydrocortisone	0·19	Triamcinolone	0·59
Betamethasone	0·26	Prednisolone	0·83
Triamcinolone acetonide	0·50	Methylprednisolone	0·89
Fluocinolone acetonide	0·53	Dexamethasone	1·0
Prednisolone acetate	0·53	Betamethasone	1·0
Hydrocortisone acetate	0·66	6-Methylhydrocortisone	1·05
Betamethasone 17-valerate	0·71	Prednisone	1·21
Betamethasone acetate	0·76	Cortisone 21-alcohol	1·37
Dexamethasone acetate	0·79	Triamcinolone acetonide	1·39
Prednisone acetate	0·79	Fluocinolone acetonide	1·40
Fludrocortisone acetate	0·87	Cortisone acetate	1·88
Prednisolone trimethyl acetate	1·10		
Betamethasone 21-valerate	1·34		
Deoxycortone acetate	1·92		
Deoxycortisone trimethylacetate	2·22		

Reference

Brooks, S. G., Evans, R. M., Green, G. F. H., Hunt, J. S., Long, A. G., Mooney, B. & Wyman, L. J. (1958). *J. chem. Soc.*, 4614.

The paper was presented by THE AUTHOR.