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An improved solvent system for thin-layer chromatography of adrenal steroids

The literature for thin-layer chromatography suggests many solvent systems suitable for the chromatography of steroids[1,2]. We have found that none of these systems are satisfactory for the separation of cortexolone (S) and corticosterone (B) in extracts of biological preparations. However, a solvent system described by TOUCHSTONE *et al.*[3] was found to be effective for the differentiation of S and B from extracts of an hydroxylation media and of pure standards run simultaneously on the same plate. This paper describes the use of these solvents for the separation of eighteen different adrenal steroids and the determination of their R_F values.

Material and methods

A 20% acetone in isopropyl ether solvent system was prepared from freshly redistilled reagent grade solvents. Brinkmann and Analtech Silica Gel G precoated glass plates and Gelman Silica Gel impregnated glass fiber sheets were used. The plates were all of the 20 × 20 cm size. The Brinkmann plates were all fluorescent and contained an organic binder, the Analtech plates were scored in 1 cm channels and contained a gypsum binder.

All steroids with the exception of 6β-hydroxycortisol (courtesy of Dr. SEYMOUR BERNSTEIN, Lederle Laboratories, Pearl River, New York) were standard commercial preparations and were made up in a 1 mg/ml concentration in redistilled methanol.

The standards were applied to the plates with a 10 μl pipette. Two plates were

TABLE I

R_F VALUES FOR STEROIDS ON DIFFERENT PRE-COATED PLATES

Steroids	*R_F values (× 100)*	
	Analtech Brinkmann	*Gelman*
1 Pregnenolone (pregn-5-en-3β-ol-20-one)	63	80
2 Dehydroepiandrosterone (androst-5-en-3-ol-17-one)	59	71
3 Androstenedione (androst-4-en-3,17-dione)	55	68
4 17-Hydroxypregnenolone (pregn-5-en-3β-,17α-diol-20-one)	50	63
5 Epitestosterone (androst-4-en-17α-ol-3-one)	48	62
6 Testosterone (androst-4-en-17β-ol-3-one)	47	61
7 Androsterone (5α-androstan-3α-ol-17-one)	35	50
8 11-Dehydrocorticosterone (pregn-4-en-21-ol-3,11,20-trione)	14	28
9 *d*-Aldosterone (pregn-4-en-11β,21-diol-3,20-dione-18-al)	5	16
10 Cholesterol	81	90
11 Progesterone (pregn-4-en-3,20-dione)	65	77
12 17α-Hydroxyprogesterone (pregn-4-en-17α-ol-3,20-dione)	54	68
13 Deoxycorticosterone (pregn-4-en-21-ol-3,20-dione)	35	54
14 Cortexolone (S) (pregn-4-en-17α,21-diol-3,20-dione)	28	47
15 Corticosterone (B) (pregn-4-en-11β,21-diol-3,20-dione)	15	32
16 Cortisone (pregn-4-en-17α,21-diol-3,11,20-trione)	14	31
17 Hydrocortisone (pregn-4-en-11β,17α,21-triol-3,20-dione)	12	30
18 6β-Hydroxyhydrocortisone (pregn-4-en-6β,11β,17α,21-tetrol-3,20-dione)	27	18

run simultaneously in the same tank; one plate with standards 1–9 (Table I) plus a mixture of these, the other with standards 10–18 plus the unknown extract. As a result of previous studies, compounds 10–18 were anticipated in the unknown extract. The solvent front was allowed to run 15 cm from the spotting point. Most of the spots were visible without any further treatment under an U.V. lamp. All the spots, however, were visualized when the plates were sprayed with 3% vanillin in 0.5% sulfuric acid ethanol solution and heated in an oven at 110°.

Results and discussion

The R_F values of the standards illustrated in Table I were determined in the usual manner and are the average of 4–6 determinations on each of the 3 type plates: Brinkmann, Analtech and Gelman. Although the Brinkmann and Analtech plates gave similar R_F values for each of the compounds, the development time of the Analtech plate was shorter than that of the Brinkmann. The Gelman sheet developed faster than the others and gave higher R_F values although the spots were in the same relative position to each other.

The separation of B and S was of prime importance in the selection of this solvent system since these two steroids appear in extracts from our incubation procedure and normally move so close together as to resemble a single spot. However, as can be noted by the R_F values, the 20% acetone in isopropyl ether separated B and S into two discrete spots. The solvent system was also useful in the separation of desoxycorticosterone and 17α-hydroxyprogesterone which like B and S move as a single spot or very closely overlapping in conventional systems. Although *d*-aldosterone and cortisone were not run on the same plate the R_F values illustrate distinct difference of polarity in the new system. The steroids in our extract that did not separate in 20% acetone isopropyl ether were separated by two-dimensional TLC with chloroform–methanol–water (188:12:1)[4] in the second direction.

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