THIN-LAYER PARTITION CHROMATOGRAPHY
A QUICK METHOD OF CHROMATOGRAPHY FOR STEROIDS

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Work on thin-layer chromatography of steroid compounds has up to now been chiefly based on adsorption chromatography similar to the classical process of TSWETT[1-9]. Though some of the papers[5-11] have dealt with the separation of more polar steroids, the best results have been obtained with mixtures of lipophilic steroids.

In our work we have based thin-layer chromatography on a pure partition process and have obtained very good results with steroids of widely differing polarities.

A practically inert medium spread on a glass plate was saturated with a liquid stationary phase, after which the steroid mixtures were applied. A mobile phase ascending the plate separated the steroids according to their partition coefficients. The separation of steroids of widely differing polarities, ranging from sterol esters to corticosteroids, was effected with solvent systems commonly used in paper chromatography. Particularly good results were obtained with Zaffaroni's solvent systems based on formamide or glycols as stationary phases.

Reversed-phase systems also gave good results in the fractionation of the least polar steroids.

With classical solvent systems based on water as the stationary phase, "tailing" could not be avoided and the results were unsatisfactory.

It should be emphasized that the unusually short time of development is the greatest advantage of this method. Using Celite No. 545* with an admixture of gypsum as binder and formamide as the stationary phase, chromatograms were obtained whereby the solvent front covered a distance of 10 cm in 3 to 7 minutes depending on the mobile phase used. In spite of so short a time of development, the separation of steroids differing in structure by only one double bond was achieved, *e.g.* methyltestosterone from Δ^1-dehydro-methyltestosterone.

For spot detection all the reagents used for steroids in paper chromatography and also the drastic reagents recommended for thin-layer chromatography are suitable. We used concentrated sulphuric acid, concentrated phosphoric acid, antimony trichloride, triphenyltetrazolium chloride (TTC), tetrazolium blue and isonicotinic acid hydrazide (INH). Concentrated phosphoric acid has proved very useful, giving intense fluorescence and different colours with different steroids. The same reagent used in paper chromatography as 20% solution is much less sensitive.

The amounts of the substances used in the analyses were 0.1 to 10 γ.

* Johns Manville, International Corp., New York.

EXPERIMENTAL

Six plates of mirror glass, 5 mm thick, 100 mm wide, 180 mm long, were covered with a slurry prepared by mixing 7 g of Celite No. 545, 0.4 g of gypsum and 40 ml of water. The Celite and gypsum were both sifted through a DIN 1171 sieve (pore size 0.07 mm). The mixture was spread on each plate with a glass rod; afterwards the plates were shaken by hand for a short time to obtain a uniform layer of Celite. The plates were then dried for an hour in an oven, the temperature being gradually raised from 20° to 120°. The dried plates were kept in a desiccator.

The starting points (1.5 cm from the lower edge of the plate and at distances of 1.5 cm from each other) and a line at a distance of 10 cm from these points, were marked on the plates with a needle. The coating of the plate was saturated with the stationary phase by means of a fine sprayer. The amount of stationary phase on the plate was estimated by weighing the plate before and after spraying. (In routine work one can do this by counting the number of sprays applied.) Immediately after spraying, the mixtures to be analysed and, where necessary, the reference standards, were applied to the starting points by means of a micropipette and the plate was placed in a chamber containing the mobile phase to a depth of 1 cm. Development was carried out until the solvent front had reached the marked line (2–7 min). The chromatogram

TABLE I

R_F VALUES OF SOME STEROIDS ON CELITE NO. 545

Solvent system	*Z-1*	*Z-2*	*Z-3*	*Z-4*	*Z-5*	*GP-1*	*N*	*P-1*	*Detection reagent*
Time (min)	*3*	*4*	*5*	*4*	*5*	*5–6*	*15*	*7–9*	
Amount of stationary phase (g)	*1.3*	*1.4*	*0.6*	*0.4*	*0.5*	*0.2*	*0.15*	*0.3*	
Cholesteryl benzoate							0.11	0.12	H_3PO_4,$SbCl_3$
Cholesteryl acetate							0.30	0.24	H_3PO_4,$SbCl_3$
Cholesterol							0.75	0.87	H_3PO_4,$SbCl_3$
Pregnenolone acetate	0.94						0.91	0.93	H_3PO_4,$SbCl_3$
Androstenolone acetate	0.84						0.93	0.94	H_3PO_4,$SbCl_3$
Androstenedione		0.79				0.75			H_3PO_4,INH
Methyltestosterone		0.70				0.43			H_3PO_4,INH
Δ^1-Dehydro-methyltestosterone		0.36				0.15			H_3PO_4,INH
Testosterone		0.46				0.19			H_3PO_4,INH
Substance S acetate			0.89						H_3PO_4,TTC
Cortisone acetate			0.44						H_3PO_4,TTC
Prednisone acetate			0.41						H_3PO_4,TTC
Cortisol acetate			0.30						H_3PO_4,TTC
Prednisolone acetate			0.17						H_3PO_4,TTC
Substance S				0.77	0.94				H_3PO_4,TTC
Cortisone				0.30	0.78				H_3PO_4,TTC
Prednisone				0.26	0.72				H_3PO_4,TTC
Cortisol				0.13	0.40				H_3PO_4,TTC
Prednisolone				0.09	0.26				H_3PO_4,TTC

Solvent systems:

Z-1 = formamide/*n*-hexane
Z-2 = formamide/*n*-hexane–benzene (1:1)
Z-3 = formamide/benzene
Z-4 = formamide/benzene–chloroform (1:1)
Z-5 = formamide/chloroform
GP-1 = propylene glycol/ligroin
N = petroleum/methanol–*n*-butanol–water (40:30:30)
P-1 = paraffin oil/methanol–water (95:5)

was dried in an oven at 90–100°, until the heavy fumes of formamide or glycols had disappeared. After cooling, the appropriate detection reagent was applied and, if necessary, the plate was heated again.

The solvent systems, R_F values and detection reagents are listed in Table I.

SUMMARY

A method of thin-layer partition chromatography using Celite No. 545 and Zaffaroni's solvent systems is described. This method has the advantage that the time of development is very short (3–7 min). The separation of steroids of widely differing polarities was satisfactory.

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