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The use of polyamide for thin-layer chromatographic separation of steroids*

There have been many media described for separation using thin-layer chromatography (TLC). Among these, polyamide layers have not been thoroughly investigated. Polyamide was used to separate plant phenol derivatives[1], PENZES *et al.*[2] used polyamide layers to separate 2,4-dinitrophenylhydrazones of 17-ketosteroids. This paper presents results for separation of a number of steroids on a new type of polyamide layer coated on a plastic support available commercially. A comparison with Silica Gel G was made of separation properties and ease of operation of this improved medium.

Materials and methods

Polyamide sheets (5 × 10 cm) were obtained from Toyo Kagaku Sangyo Co., Tokyo, Japan. These sheets were used as obtained, no activation nor pre-treatment was required. Silica Gel G thin layers were obtained from domestic sources.

All solvents were of reagent grade and were redistilled before use. Solvent mixtures as described in the tables were prepared each day and used only on that day. All experiments were performed in the prevailing environmental status.

Steroid solutions made up (in acetone) to contain 0.1 μg/μl were spotted 0.5 cm from one end using a Hamilton microliter syringe. The amount of steroid in the spot was limited to 0.5 μg. The steroids were obtained from commercial sources and used as supplied.

TABLE I

R_F VALUES FOR STEROIDS SEPARATED ON SILICA GEL G AND POLYAMIDE LAYERS

Steroid	*Solvent*	*Silica Gel G*	*Polyamide*
Progesterone	Hexane–acetone (80:20)	0.39	0.56
Dehydroepiandrosterone		0.16	0.55
Androstenediol		0.07	0.33
Pregnenolone		0.21	0.64
Testosterone		0.11	0.37
Estrone		0.67	0.86
Estradiol-17β	Chloroform–acetone (80:20)	0.54	0.66
Estriol-16α-17β		0.12	0.26
2-Hydroxyestrone		0.24	0.29
2-Hydroxyestriol		0.36	0.58
Cortisol		0.13	0.61
Cortisone		0.25	0.71
Corticosterone		0.21	0.81
Aldosterone		0.10	0.73

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Results

Table I shows the R_F values obtained for a representative list of steroids using both the new polyamide sheet and the conventional Silica Gel G plates for TLC. The solvent systems used were selected to give median movement of the steroids for more exact calculation of the migration.

A comparison of the time required indicated that the solvent front movement was approximately the same for both media. There was no time advantage found in the polyamide layer under the conditions used.

As indicated in Table I there was faster movement of the applied compounds, thus comparable separations could be obtained in the shorter R_F distance necessitated by the 10-cm lengths of the polyamide layer. As the solvent moved higher on the 20-cm Silica Gel G layer the rate of movement decreased.

As indicated in Table I using the same solvent system the mobility expressed as R_F on the polyamide layer is greater than on the silica gel. In some instances separation between two compounds is greater than in the example of 2-hydroxyestrone and 2-hydroxyestriol (Table I). Conversely, the separation in some instances was less, as in the case of cortisol and cortisone.

In some of our previous work the separation of estriol epimers has posed a problem. We have only been able to separate two of the epimers on Silica Gel G layers impregnated with ammonium bisulfate[3]. The results of Table II indicate, that the polyamide was no more effective than the Silica Gel G layer.

TABLE II

R_F VALUES FOR ESTRIOL EPIMERS SEPARATED ON SILICA GEL G AND POLYAMIDE LAYERS

Estriol epimer	*Solvent*	R_F *Silica Gel G*	R_F *polyamide*
16α, 17β	Chloroform–acetone (80:20)	0.12	0.25
16β, 17α		0.13	0.31
16α, 17α		0.33	0.45
16β, 17β		0.30	0.45
16α, 17β	Ethanol–benzene (8:92)	0.11	0.18
16β, 17α		0.11	0.16
16α, 17α		0.23	0.30
16β, 17β		0.22	0.34

The polyamide layer provides an additional parameter for both qualitative, as well as quantitative procedures in a wide variety of cases. Elution is more simple than with silica gel since zones can be cut out and dropped into the eluting solvent. Visualization of substances absorbing ultraviolet light can be readily accomplished

in the dark room. Iodine vapor[4] has been used to locate substances which do not absorb in the ultraviolet region. Acidic reagents such as sulfuric acid and phosphomolybdic acid are not applicable.

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1 K. EGGER, in E. STAHL (Editor), *Thin-Layer Chromatography*, Springer-Verlag, New York, 1969, p. 699.
2 L. PENZES, P. MENZEL AND G. OERTEL, *J. Chromatogr.*, 44, (1969) 189.
3 J. C. TOUCHSTONE, T. MURAWEC, M. KASPAROW AND W. WORTMANN, *J. Chromatogr.*, 66 (1972) 172.
4 K. G. KREBS, D. HEUSSER AND H. WIMMER, in E. STAHL (Editor), *Thin-Layer Chromatography*, Springer-Verlag, New York, 1969, p. 141.

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