CHROM. 5862

A separation of purine derivatives by thin-layer chromatography on silica gel plates suitable for metabolic studies*

Although silica gel plates are generally used in thin-layer chromatography (TLC) for the separation of many types of compounds, it has not often been used for the separation of purine derivatives. This is a result of the low R_F values, especially for nucleotides, which are not separated on plates coated with silica gel in the usual solvent systems[1,2].

Solvent systems, however, that contain alcoholic components, water and a high proportion of ammonia enable the purine derivatives to be separated satisfactorily on silica gel, even the nucleotides of adenosine and inosine. Contrary to the results for cellulose, the spots are sharp and free of tailing. The present paper de-

TABLE I

THIN-LAYER CHROMATOGRAPHY OF PURINE DERIVATIVES ON SILICA GEL GLASS PLATES AND ALUMINIUM FOIL

Solvent: *n*-propanol–methanol–ammonia (33 %)–water (45 : 15 : 30 : 10).

Compound	*R_F value*					
	Merck				*Riedel de Haen Al plates*[a]	
	Glass plates		*Al foil*			
	1 separation	*2 separations*	*1 separation*	*2 separations*	*2 separations*	*3 separations*
Adenine	0.65	0.81				
Adenosine	0.61	0.77				
Hypoxanthine	0.58	0.74				
Inosine	0.53	0.68				
Guanine	smeared over					
Guanosine	0.52	0.66				
Xanthine	0.54	0.70				
Xanthosine	0.53	0.68				
Cyclic AMP	0.58	0.74				
N^6-methyl-AMP	0.42					
N^6-dimethyl-AMP	0.49					
AMP	0.30	0.40	0.34	0.53	0.43	0.48
ADP	0.08	0.15	0.10	0.24	0.29	0.32
ATP	0.02	0.04	0.02	0.08	0.12	0.16
IMP	0.21	0.30	0.24	0.37	0.35	0.39
IDP	0.07	0.13	0.09	0.21	0.21	0.24
ITP	0.02	0.04	0.20	0.07	0.10	0.13
GMP	0.16	0.26				
GDP	0.04	0.09				
GTP	0.02	0.03				
XMP	0.02	0.30				

[a] Testing a new charge of silica gel plates from E. Merck with a different composition (F_{254}, charge number ≧ 1376637b) we obtained nearly the same R_F values as on the aluminium plates from Riedel de Haen.

* These studies were supported by the Deutsche Forschungsgemeinschaft.

scribes a method that has been used successfully in our laboratory to separate nucleotides in metabolism studies of erythrocytes and heart cells[3].

Methods and results

The separations were performed on Silica Gel F_{254} (5115) TLC plates, Silica Gel F_{254} (5562) TLC aluminium foil from E. Merck, Darmstadt, G.F.R., and on thin-layer plates SIF Kieselgel with fluorescence indicator (37350) from Riedel de Haen (G.F.R.). The purine derivatives were kindly supplied by Waldhof AG, Mannheim, G.F.R.

The solvent systems used were: I, *n*-propanol–methanol–ammonia (33 %)–water (45:15:30:10), with a running time of 5–6 h; and II, *n*-butanol–acetone–ammonia (33 %)–water (50:40:3:15), with a running time of 3–4 h.

The spots were visualized with ultraviolet light.

The results of the chromatographic separation of purine derivatives, studied on silica gel glass plates and aluminium plates using solvent system I, are shown in Table I. It can be seen that in all cases the separation of the phosphates of adenosine or inosine can be achieved. To distinguish between the diphosphates of inosine and adenosine derivatives, a second development is necessary. The triphosphates can be adequately separated by a two- or three-fold development on the aluminium plates from Riedel de Haen. The reason of the better separation on these plates is unknown.

Increasing the proportion of methanol (*n*-propanol– methanol–ammonia–water, 35:25:30:15) gave higher R_F values but had no effect on the separation relationships of the compounds.

Table II shows that by using solvent system II better separation of different bases and nucleotides can be obtained than with solvent system I. In this case there is no difference in the migration rates of the three plates tested. The nucleotides

TABLE II

THIN-LAYER CHROMATOGRAPHY OF PURINE BASES AND NUCLEOTIDES ON GLASS PLATES[a]

Solvent: *n*-butanol–acetone–ammonia (33 %)–water (50:40:3:15).

Compound	R_F *value*	
	1 separation	*2 separations*
Adenine	0.46	0.63
Adenosine	0.39	0.55
Hypoxanthine	0.28	0.41
Inosine	0.16	0.26
Guanine	0.00	0.00
Guanosine	0.17	0.27
Xanthine	0.21	0.31
Xanthosine	0.15	0.24
Cycl. AMP	0.22	0.30
N^6-methyladenosine	0.43	
N^6-dimethyladenosise	0.46	

[a] The three plates tested showed the same R_F values.

remain at the start. Therefore, the use of solvent system II followed by solvent system I represents a suitable combination for studying certain problems of nucleotide metabolism.

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Received October 19th, 1971

CHROM. 5836

Simultaneous separation of common mammalian Δ^4-3-oxosteroids and oestrogens using two-dimensional thin-layer chromatography

Many paper and thin-layer chromatographic (TLC) systems are available for separating particular groups of Δ^4-3-oxosteroids and oestrogens[1–3]. However, the literature does not appear to contain a simple, rapid and effective method for simultaneous separation of the common Δ^4-3-oxosteroids and oestrogens of adrenal, testicular, ovarian, and placental origin. The method described in this paper was first developed to permit fractionation of such steroids produced in *in vitro* incubations with ^{14}C-labelled 3β-hydroxysteroid substrates, after the Δ^4-3-oxosteroids (and oestrogens) had been isolated (from 3β-hydroxysteroids) as a group using the method of TAYLOR[4].

The system involves the two-dimensional development of a 20 × 20 cm TLC plate. Fig. 1 depicts the separation (visualized under ultraviolet light at 254 nm) of thirteen steroids obtained using thin layers, 0.5 mm thick, of silica gel, "Kieselgel GF_{254} nach Stahl" (Merck, Darmstadt). A similar pattern is obtained on 0.1-mm layers of "Kieselgel $HF_{254+366}$ nach Stahl" (Merck, Darmstadt) prepared, using starch as a binder, according to the method of TAYLOR[4].

The 0.5-mm layers readily permit separation of 10-μg quantities of each of the thirteen steroids listed. This occurs even when these quantities are applied together with the ethereal phase from an ether/water partition of an 80% aqueous alcoholic extract of 1 g human foetal adrenal tissue. If a large amount of tissue is extracted, the simple expedient of using several plates to fractionate the extract overcomes problems arising from the large volume of unwanted material present. Even in this circumstance, the system allows rapid primary fractionation without need for prior "cleaning up" procedures.