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Note

Simple and fast separation of the iodotyrosines by thin-layer chromatography

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In recent years there have evolved highly efficient and fast separations, both in gas chromatography (GC) and high-performance liquid chromatography (HPLC). To obtain such separations, all factors involved had to be evaluated and optimised, and the effort necessary was often considerable.

Thin-layer chromatography (TLC), paper chromatography and paper electrophoresis have been re-examined only much later and it was realized that here also extremely efficient separations were possible, comparable to those in GC and HPLC, if optimization was aimed at. There are many publications dealing with high-performance TLC (HPTLC) and we have shown recently that paper electrophoresis can also yield excellent separations within 5 min by a very simple scale reduction of the arrangement[1].

In this paper we report another kind of simple separation, which requires no optimized support or elaborate equipment, and separates tyrosine (T), monoiodotyrosine (MIT) and diiodotyrosine (DIT) in 10 min.

While looking for a suitable separation of iodide, MIT, DIT, triiodotyrosine (T_3) and thyroxine (T_4) in relation to a clinical problem, we found that various books and reviews (for example, ref. 2) indicate that there are numerous paper chromatographic and TLC systems which can achieve this separation. However, our attention was held by a very simple technique in which these compounds are separated by paper chromatography with 3% sodium chloride solution[3].

Repeating this work, we found that development took over 30 min and yielded a good separation, but with elongated spots, as was also evident from the original paper[3].

Transferring this separation to cellulose thin layers, we obtained remarkably compact spots and development took only 10 min. In such a short period no problems of saturating the atmosphere, evaporation from the layer, etc., exist with an aqueous solution, so that the development could be carried out in microscope staining jars, simply covered with a glass plate.

EXPERIMENTAL AND RESULTS

Solutions of T, MIT, DIT, T_3 and T_4 were prepared in methanol with a small amount of ammonia and stored in a refrigerator.

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The solutions were spotted on to Polygram Cel 300 layers (80 × 40 mm) with a glass capillary, so as to form spots about 3 mm in diameter.

The layers were developed immediately with the aqueous solvent in a microscope staining jar, covered with a flat piece of glass, for exactly 10 min, in which time the solvent rose 65 mm.

The thin layer was withdrawn, dried in an oven, dipped into the reagent (we used 0.5% ninhydrin in acetone + 1% acetic acid) and heated again in the oven at 110 °C until spots appeared. The average time taken from the spotting to obtaining the finished chromatogram was 15–17 min.

A typical separation is shown in Fig. 1. There is an excellent separation of T–MIT–DIT, while T_3 and T_4 remain at the point of application. Probably a longer development or other "optimization" could also separate T_3 and T_4.

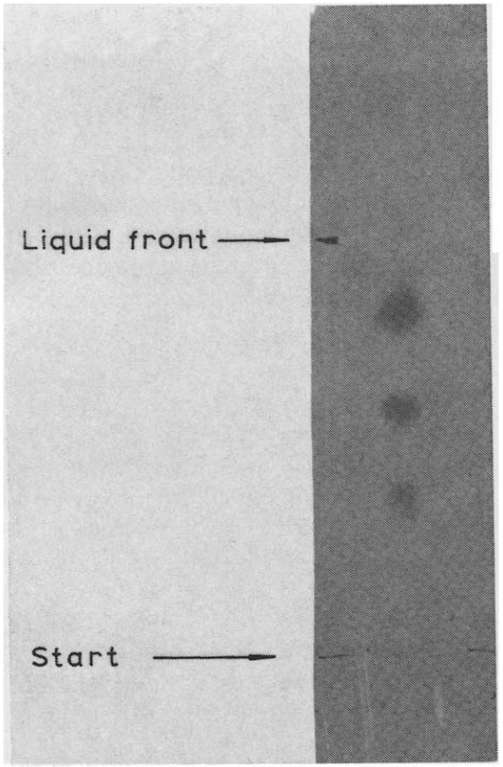

Fig. 1. TLC separation of tyrosine, monoiodotyrosine and diiodotyrosine on cellulose developed with 0.5 *N* NaCl. Length of thin layer, 80 mm.

The separation proved to be extremely reproducible. Changes in sodium chloride concentration or pH or the addition of small amounts of an organic solvent had only a small effect on the R_F values, as shown in Table I. We feel that in this respect it is a considerable improvement over the various separations involving partition systems.

Some comments can be made on the efficiency of the separation. The MIT spot

TABLE I

R_F VALUES OF IODOTYROSINES ON CELLULOSE THIN LAYERS WITH AQUEOUS SOLVENTS

Layer: Polygram Cel 300 (80 × 40 mm). Length of development: 10 min, in which time the solvent moved 55 mm.

Solvent	*R_F values*					
	T	*MIT*	*DIT*	*T_3*	*T_4*	*Iodide*
0.5 *N* NaCl	0.83	0.58	0.37	0	0	0.86
2 *N* NaCl	0.76	0.50	0.29		0	
0.5 *N* NaCl + *n*-propanol (9:1)	0.82	0.66	0.48			
10% Acetic acid	0.85	0.635	0.43	0.09 + tail	0	
0.1 *M* NaH_2PO_4 (pH ~ 3.2)	0.86	0.63	0.42			
0.1 *M* K_2HPO_4	0.86	0.72	0.66			

is 3 mm long and the distance of development is 55 mm. This gives a theoretical plate number of 5000, which compares favourably with TLC, which usually gives 400–3000 theoretical plates and is, according to Halpaap and Ripphahn[4], as good as HPTLC. Perhaps it could also be used as an illustration that for efficient separations a number of factors are important and if some are favourable (*e.g.*, an aqueous solvent with fast equilibrium and fast development) there is no need to resort to micro-scale arrangements and very fine particles of the adsorbent.

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