Separation of ^{131}I-labelled monoiodotyrosine and diiodotyrosine by thin-layer chromatography

In the course of work on the preparation of iodinated tyrosines labelled with ^{131}I at very high specific activity, it was necessary to find a method for separating ^{131}I-labelled monoiodotyrosine (MIT) from ^{131}I-labelled diiodotyrosine (DIT).

When iodinated tyrosines are prepared, the separation of ^{131}I-MIT, ^{131}I-DIT, iodide ions and unreacted tyrosine may be performed by paper chromatography, according to the method described by LEMMON *et al.*[1].

In order to carry out a faster separation on a preparative scale of MIT and DIT, we have developed a thin-layer chromatographic method.

Thin-layer plates (5 × 20 cm) were prepared in the usual way with silicagel "G" Merck, using the Desaga apparatus, and dried at 120° for 20 min. Several elution mixtures were employed and the results are reported in Table I.

TABLE I

R_F VALUES FOR MIXTURES OF TYROSINE, IODINATED TYROSINES AND IODIDE IONS

Solvents: a = phenol–water (75:15); development time = 7 h; b = *n*-butanol–acetic acid–water (10:1:1); development time = 5 h; c = *n*-butanol–acetic acid–water (4:1:5); development time = 5 h; d = *n*-butanol–methanol–NH_4OH, 20% (8:2:2); development time = 6 h.

Substance	$R_F \times 10^2$			
	a	*b*	*c*	*d*
MIT	52	41	64	54
DIT	77	56	75	45
Tyrosine	40	23	36	26
I^-	14	70	28	27

The spots of ^{131}I-labelled MIT, DIT, and I^- were identified by scanning the plate. The scanning apparatus consists of a mechanical device, which causes the silica gel plate to slide under a collimated thin-window G. M. counter. The chromatographic pattern is obtained by means of a ratemeter–recorder system.

The tyrosine spot was identified by spraying a 0.2% ninhydrin solution in dry acetone.

Standards of pure ^{131}I-labelled MIT and DIT were prepared by the method of LEMMON *et al.*[1].

The chromatographic separation method described above may be usefully employed to isolate ^{131}I-labelled MIT and DIT on a preparative scale. The iodinated mixture (0.4 ml) containing ^{131}I-MIT (50 μg), ^{131}I-DIT (50 μg), I^- (30 μg) and unreacted tyrosine (100 μg) are spotted on the plate at a distance of about 2 cm from the edge.

n-Butanol–acetic acid–water (4:1:5) is used as elution mixture (see Fig. 1). After development (5 h), the spots corresponding to MIT and DIT are removed from the plate. The silica gel is then collected in centrifuge tubes, and 0.1 *N* NH_4OH in 10% isopropanol solution (0.4 ml) is added. The mixture is shaken for a short time and centrifuged, and the supernatant liquid is pipetted into a small test tube. After three extractions, about 90% of both MIT and DIT is recovered.

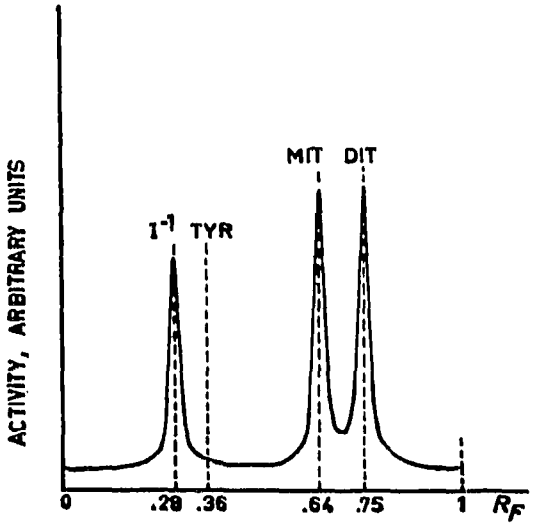

Fig. 1. Thin-layer radiochromatography of a mixture of MIT, DIT, I^-, eluted with *n*-butanol–acetic acid–water (4:1:5).

The yield was evaluated by counting of the supernatant.

When compared with separation methods on paper, thin-layer chromatography has the advantage of reducing the operation time, making this technique suitable for routine controls in the production of labelled MIT and DIT.

In addition, the higher adsorption capacity of silica gel in plate chromatography is very useful for preparative purposes.

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Thin-layer chromatography of metabolic derivatives of tryptophan

Paper chromatography is routinely used in our laboratory for the identification and determination of several metabolic derivatives of tryptophan of the so-called "via kynurenine", in urine of normal and pathological subjects. This method, which is suitable for quantitative analyses, is, however, time-consuming when used for qualitative purposes[1].

Therefore, an attempt was made to apply thin-layer chromatography (TLC) to the separation of tryptophan metabolites. The technique described here not only has the usual advantages of TLC, but several others as well, particularly as regards speed, in which it surpasses the previous method.

DIAMANTSTEIN AND EHRHART[2] were the first to apply TLC on silica gel to the fractionation of tryptophan, indole, indican, anthranilic and quinolinic acids. We