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Note

An artefact in the chromatography of L-3,3',5-triiodothyronine

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The chromatographic artefact showing double spots of the same compound may arise in various ways and has been described in standard texts[1,2]. A specific example occurs in the chromatography of L-3,3',5-triiodothyronine (T3), a compound obtainable labelled with ^{125}I or ^{131}I and vital to the study of thyroid function. The compound is supplied in 50% aqueous propylene glycol and if this solution is applied directly to a chromatogram serious spot splitting occurs in some systems, the glycol eluting as a discrete spot and carrying some of the T3 with it. It is therefore essential to prepare a threefold dilution of the aqueous propylene glycol solution in ethanol before spotting onto the chromatogram.

EXPERIMENTAL AND RESULTS

Two such systems illustrating this effect are: (A) *n*-Butanol saturated with 1 *N* ammonia solution, descending, Whatman No. 1 paper; (B) methyl acetate–isopropanol–water–ammonia (45:35:15:5) on Merck precoated silica gel thin-layer plates.

Table I shows the true and apparent radiochemical purities of a sample of ^{125}I-labelled T3 with and without a threefold dilution in ethanol. Sample spots were superimposed on 25 μg T3 carrier and the chromatograms were analysed by an argon–methane proportional counter scanner.

TABLE I

RADIOCHEMICAL PURITIES OF ^{125}I-LABELLED T3

System	*R_F value*		*True % T3 (ethanol dilution)*	*Apparent % T3 (undiluted aqueous glycol)*
A	T3	0.50	>95%	*ca.* 70–80%
	Glycol	0.70	—	(*ca.* 20–30% T_3 at glycol R_F)
B	T3	0.73	>95%	*ca.* 60%
	Glycol	0.83	—	(*ca.* 40% T_3 at glycol R_F)

CONCLUSION

This artefact is a common source of disagreement between users and manufacturers regarding the radiochemical purity of labelled T3 and may be avoided by a simple ethanol dilution of the spotting solution as indicated.

REFERENCES

1 I. M. Hais and K. Macek, *Paper Chromatography*, Academic Press, New York, 1963, p. 158.
2 I. Smith, *Chromatographic and Electrophoretic Techniques*, Vol. I, Heinemann, London, 3rd ed., 1969, pp. 35–36.