Journal of Chromatography, 104 (1975) 217–218

CHROM. 7968

Note

In situ spectrofluorimetric estimation of substituted tetrahydroisoquinolines by thin-layer chromatographic separation and oxidation to the dihydroisoquinolinium derivatives

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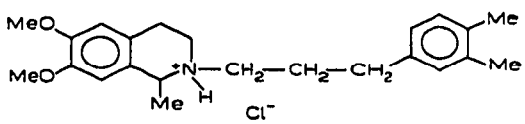
(Received August 6th, 1974)

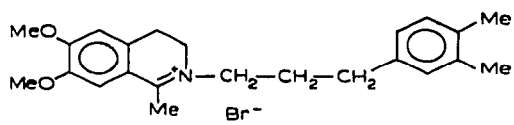
A recent paper on the estimation of tetrahydroisoquinolines in biological fluids reported the use of photochemical oxidation to form the corresponding isoquinolinium derivatives[1]. The drug was separated from the metabolites by thin-layer chromatography (TLC) and samples were eluted from the plate for oxidation and estimation in solution. We have recently been investigating the metabolism of a series of related compounds in a number of species[2] and used essentially the same analytical technique except that the analysis was performed by direct oxidation and estimation *in situ* on the TLC plate. This method has the advantages that: (1) The working time is considerably reduced with little loss of accuracy. (2) An internal standard can be incorporated into the assay to correct for variable recoveries from different samples.

EXPERIMENTAL AND RESULTS

Fluorescence measurements were performed using a Farrand Mark 1 spectrophotofluorimeter which can be purchased with a TLC scanning attachment. The validity of the analysis was determined using Ro 03-3753 —1,2,3,4-tetrahydro-6,7-dimethoxy-1-methyl-2-[3-(3,4-xylyl)-propyl]-isoquinoline hydrochloride— and the corresponding isoquinolinium derivative, Ro 03-4614. Eight standard solutions of



Ro 03-3753



Ro-03-4614

Ro 03-4614 in 0.1 *N* H_2SO_4 (2.0 to 0.01 μg/ml) were prepared and the fluorescence intensities of 1-ml samples were determined (excitation 355 nm and emission 460 nm with 7-54 and 3-73 filters on the excitation and emission monochromators, respectively). A separate sample was dissolved in chloroform and aliquots (2.0–0.01 μg) were spotted onto a Merck Kieselgel G_{60} plate which had been pre-washed in methanol–

triethylamine (95:5) and ruled on a 2-cm grid. After development in benzene–triethylamine (95:5) the fluorescent intensity of the spots was determined *in situ* using the TLC scanning attachment. The same concentrations of Ro 03-3753 were spotted onto a second plate, which was developed in an unlined tank containing cyclohexane–triethylamine (90:10), and the plate was irradiated with ultraviolet light in a Fluotest UV cabinet, using both the 254- and 366-nm lamps for 1 h, then overnight under the longer wavelength.

The results (Table I) were expressed as the fluorescent intensity divided by the weight of the sample. The determinations on the TLC plates gave a slightly higher coefficient of variation with a smaller linear response range when compared to the measurements in solution. There was, however, an improvement in the lower detectable limit, with the result that when the determination of other members of this series of compounds in biological fluids was investigated, drug and/or metabolite levels of 1–2 ng/ml could be measured by using a 4-ml sample of blood or urine[2].

TABLE I

COMPARISON OF THE RESULTS FOR FLUORESCENCE INTENSITIES IN SOLUTION AND ON TLC PLATES

Results are expressed as the ratio of corrected fluorescence to weight of drug.

Weight (μg)	*Sample*		
	Ro 03-4614		*Ro 03-3753 (Oxidation/TLC)*
	Solution	*TLC*	
2.0	11.8	(16.8)**	
1.0	11.6	18.5	7.1
0.5	12.2	20.2	7.4
0.2	12.5	24.0	7.1
0.1	13.5	27.0	6.9
0.05	13.0	24.0	6.4
0.02	ND*	22.5	5.0
0.01	ND	20.0	5.0
Mean ±S.E.	12.4 ± 0.3	22.3 ± 1.1	6.4 ± 0.4

* ND = Reading < twice blank.
** Value outside linear response range, not included in the mean ±S.E.

ACKNOWLEDGEMENTS

The authors are indebted to Dr. G. A. Fothergill (Roche), who prepared the reference compounds used in this study, and to Mr. C. Hallett for statistical analysis.

REFERENCES

1 J. A. F. de Silva, N. Strojny and N. Munno, *J. Pharm. Sci.*, 62 (1973) 1066.
2 J. G. Allen and J. L. Haigh, *Arzneim.-Forsch.*, submitted for publication.