Journal of Chromatography, 202 (1980) 491–493
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,194

Note

The chromatographic separation of uroporphyrin I and III octamethyl esters

IRENE C. WALKER*, MARY T. GILBERT and KIM STUBBS

Wolfson Liquid Chromatography Unit, Department of Chemistry, University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ (Great Britain)

(Received July 29th, 1980)

Of the four possible isomers of uroporphyrin (designated by the Roman numerals I–IV), the I and III isomers (Fig. 1) are of biochemical interest. In nature, under normal conditions, uroporphyrin I is only encountered in trace amounts but in contrast, in certain disease states considerable quantities of this isomer are produced and excreted. The separation and hence quantification of uroporphyrins I and III is therefore important in clinical diagnosis.

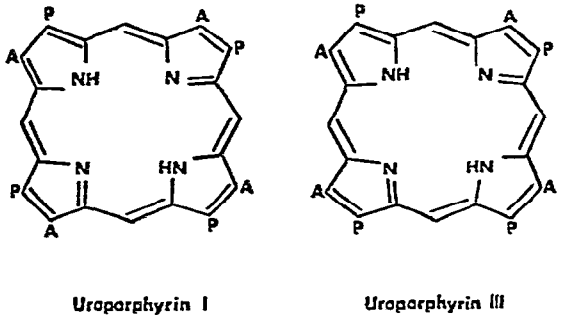

Fig. 1. Structures of uroporphyrin I and III isomers. A = CH_2-COOH; P = CH_2-CH_2-COOH.

Resolution of the octamethyl esters of these compounds by high-performance liquid chromatography (HPLC) was initially reported[1] using a μPorasil column with re-cycling (five cycles, 2 h analysis time). Improved separation was obtained by Nordlöv *et al.*[2] using two μPorasil columns in series with a modified eluent from the previous report but the analysis time was still unacceptably long (3 h). An alternative method of analysis was developed by Wayne *et al.*[3] who recently reported the resolution of the free acids of uroporphyrin I and III isomers by reversed-phase HPLC (15 min analysis time).

The present paper describes a method for resolution of the octamethyl esters of the uroporphyrin I and III isomers using an APS-Hypersil column with considerably reduced analysis time compared with existing methods for the esterified compounds.

0021-9673/80/0000–0000/$02.25

EXPERIMENTAL

Materials

Uroporphyrins I and III were gifts from Professor A. I. Scott. AnalaR butanol (BDH, Poole, Great Britain) and HPLC-grade hexane and ethyl acetate (Rathburn Chemicals, Walkerburn, Great Britain) were used as chromatographic solvents. All other solvents were reagent grade. Before use, the hexane was purified by passing through a silica gel column (60–120 mesh) and stored over molecular sieve type 4A (BDH).

Equipment and operating conditions

Analyses were performed on 125 × 5 mm I.D. and 250 × 5 mm I.D. columns packed with 5 μm APS Hypersil (Batch No. 1/776; Shandon Southern Products, Runcorn, Great Britain). The packing material was slurried in isopropyl alcohol and packed at 6500 p.s.i. using the upward packing method with initially hexane and then isopropyl alcohol as eluent. Before use, the columns were washed with sodium-dried diethyl ether. A Cecil Instruments CE 212 variable-wavelength UV monitor set at 400 nm was employed as detector (Cecil, Cambridge, Great Britain).

RESULTS AND DISCUSSION

Attempts to reproduce the separation obtained by Nordlöv *et al.*[2] using Hypersil were unsuccessful and alternative approaches examined with this column produced only very slight separation of the uroporphyrin I and III isomers. APS-Hypersil was therefore examined and it was found that with this packing material the uroporphyrin I and III isomers were resolved in a single chromatographic separation (approximately 20 min) using hexane–ethyl acetate–butanol–acetic acid (85:12:3:0.1) as eluent (Fig. 2a). Although complete baseline resolution was not obtained, the method was satisfactory for detecting small amounts of one isomer in the presence of the other (Fig. 2b).

Despite numerous attempts to improve this separation by modifying the eluent no increased separation could be obtained. These attempts revealed that the acetic

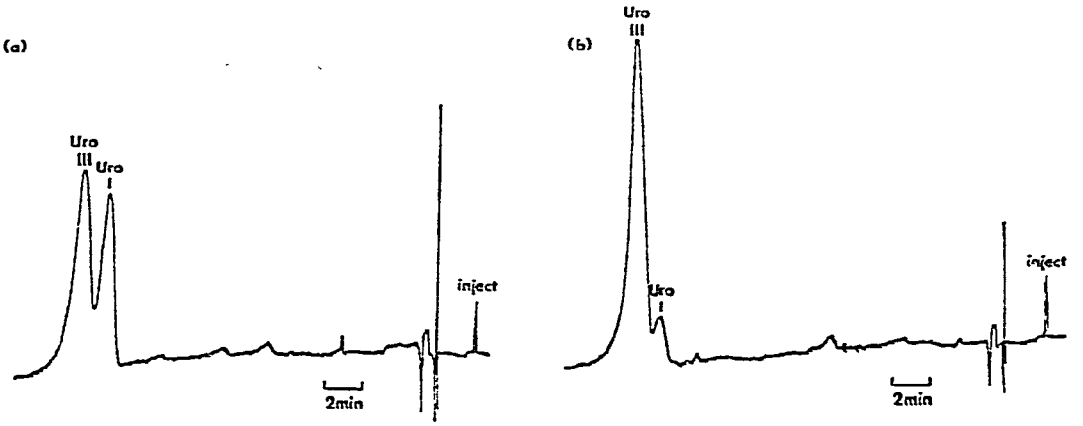

Fig. 2. Chromatograms illustrating the separation of uroporphyrins I and III on a 250-mm column.

acid concentration was a crucial factor in the separation as both its removal and increase in concentration resulted in loss of resolution. It is noteworthy that both the 125- and 250-mm columns initially showed increasing resolution of the isomers with use. They then stabilized which allowed different eluents to be evaluated. Preliminary results using an earlier batch of APS-Hypersil showed this material exhibited similar resolving power although the column gave greater retention and hence a more polar eluent was required.

It was demonstrated that the eluted peaks were indeed the uroporphyrins injected by collecting the eluted peak thought to be uroporphyrin III and showing that its UV spectrum was identical to that of the original solution.

The use of APS-Hypersil has provided a method of separation of the octamethyl esters of uroporphyrin I and III isomers with significantly reduced time of analysis to previously reported methods and is therefore advantageous.

ACKNOWLEDGEMENT

We would like to thank Professor J. H. Knox for helpful discussion during the course of this work.

REFERENCES

1 J. C. Bommer, B. F. Burnham, R. E. Carlson and D. Dolphin, *Anal. Biochem.*, 95 (1979) 444.
2 H. Nordlöv, P. M. Jordan, G. Burton and A. I. Scott, *J. Chromatogr.*, 190 (1980) 221.
3 A. W. Wayne, R. C. Straight, E. E. Wales and E. Englert, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 621.