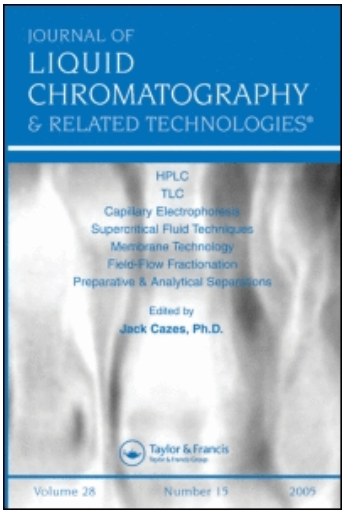
This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

HPLC Isolation and Characterization of Pentacarboxylic Porphyrins Derived from Uroporphyrinogen III

C. K. Lim[a]; J. M. Rideout[a]
[a] Division of Clinical Chemistry MRC Clinical Research Centre, United Kingdom

To cite this Article Lim, C. K. and Rideout, J. M.(1983) 'HPLC Isolation and Characterization of Pentacarboxylic Porphyrins Derived from Uroporphyrinogen III', Journal of Liquid Chromatography & Related Technologies, 6: 11, 1969 — 1976
To link to this Article: DOI: 10.1080/01483918308066552
URL: http://dx.doi.org/10.1080/01483918308066552

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(11), 1969-1976 (1983)

HPLC ISOLATION AND CHARACTERIZATION OF PENTACARBOXYLIC PORPHYRINS DERIVED FROM UROPORPHYRINOGEN III

C. K. Lim and J. M. Rideout
Division of Clinical Chemistry
MRC Clinical Research Centre
Watford Road, Harrow
Middlesec HA1 3UJ, United Kingdom.

Downloaded At: 17:29 24 January 2011

ABSTRACT

A reversed-phase HPLC system with 22% (v/v) acetonitrile in 1 M ammonium acetate buffer pH 5.1 as mobile phase on an ODS-Hypersil column is developed for the analysis, isolation and characterization of the pentacarboxylic porphyrins derived from uroporphyrinogen III. The results proved conclusively that enzymic decarboxylation of uroporphyrinogen III does not always begin at the ring D acetic acid group and proceeds in a clockwise manner as currently believed.

INTRODUCTION

Uroporphyrinogen III is the first cyclic tetrapyrrole formed in the biosynthesis of haem (1). It is converted into coproporphyrinogen III by stepwise decarboxylation of the side chain acetic acid groups and is catalysed by the enzyme uroporphyrinogen decarboxylase. It was proposed (2) that enzyme decarboxylation starts at the ring D acetic acid group and proceeds in a clockwise fashion through the acetic acid groups of ring A, B and C. To support this argument pentacarboxylic porphyrins in the urine of normal subjects and patients with porphyria cutanea tarda (PCT) were analysed by HPLC (3). The authors could not detect the pentacarboxylic porphyrin with the ring D acetic acid group intact (Fig.1) and concluded that the clockwise decarboxylation sequence must be correct.

Copyright © 1983 by Marcel Dekker, Inc. 0148-3919/83/0611-1969$3.50/0

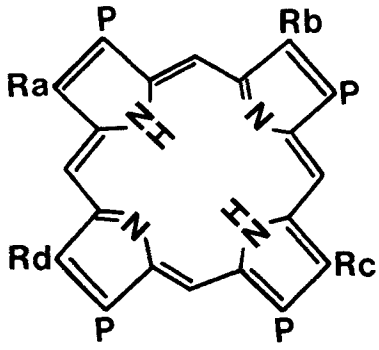


FIGURE 1. Structure of type III pentacarboxylic porphyrin isomers.
5 abc (Ra = Rb = Rc = Me, Rd = CH_2COOH);
5 abd (Ra = Rb = Rd = Me, Rc = CH_2COOH);
5 acd (Ra = Rc = Rd = Me, Rb = CH_2COOH);
5 bcd (Rb = Rc = Rd = Me, Ra = CH_2COOH).

Downloaded At: 17:29 24 January 2011

This paper describes a highly efficient HPLC system for the detail analysis of pentacarboxylic porphyrins (Fig.1) dervied from uroporphyrinogen III. The results provide unequivocal evidences for the presence of naturally occurring pentacarboxylic porphyrin with the ring D acetic acid group unaffected by enzymic decarboxylation and therefore the existence of decarboxylation pathways other than that (2) currently believed.

EXPERIMENTAL

Materials and reagents

Type III pentacarboxylic porphyrin isomers and type I pentacarboxylic porphyrin were prepared by heating uroporphyrin III and I respectively in 0.3 M HCl at 160° for 2 h. Uroporphyrin I and III octamethyl esters were from Sigma Chemical Co., Poole, Dorset, U.K. The esters were hydrolysed in 25% (w/v) HCl at room temperature in the dark for 96 h.

Ammonium acetate, acetic acid, ethylenediamine tetraacetic acid (EDTA) ethyl acetate and hydrochloric acid were AnalaR grade from BDH Chemicals, Poole, Dorset, U.K.

Acetonitrile was HPLC grade from Rathburn Chemicals, Walkerburn, Peebleshire, U.K.

Extraction of porphyrins from urine

The porphyrins were extracted onto talc and esterified as previously described (4). The methyl esters were then hydrolysed in 25% (w/v) HCl for HPLC analysis.

High-performance liquid chromatography

A Varian Associates (Walnut Creek, CA, U.S.A.) model 5000 liquid chromatograph was used. Injection was via a Rheodyne 7125 injector fitted with a 100 µl loop. A variable-wavelength U.V. detector (Varian UV-50) set at 404 nm or an LS-3 fluorescence detector (Perkin-Elmer, Beaconsfield, Bucks., U.K.) set at an excitation and an emission wavelength of 404 and 618 nm respectively, was used for detection.

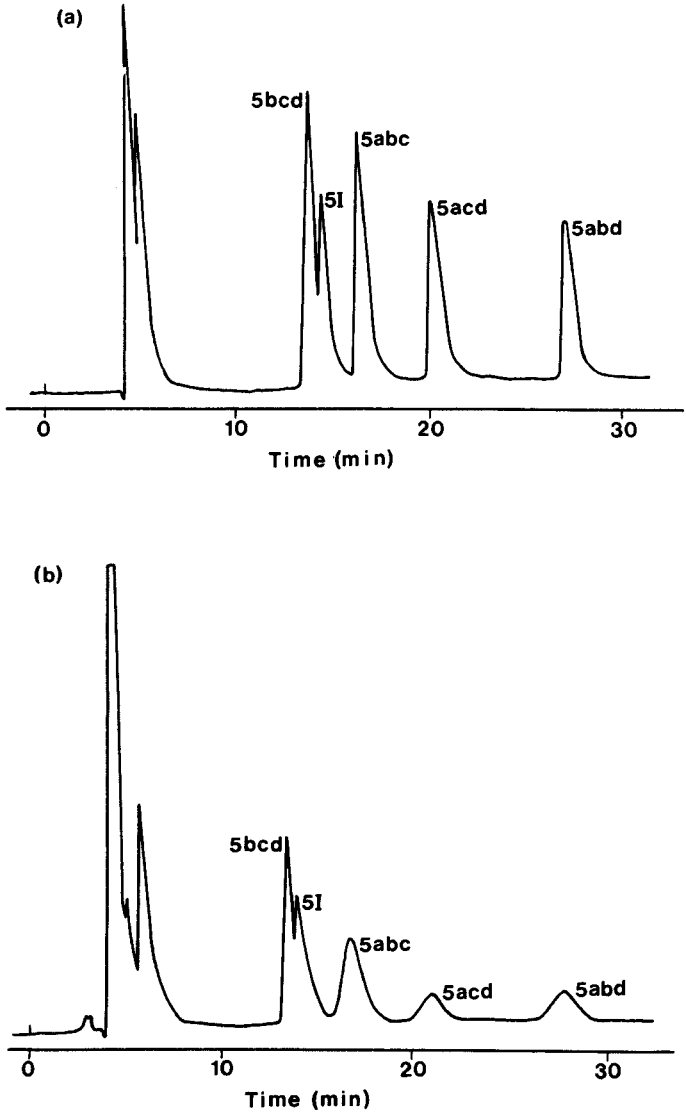
Downloaded At: 17:29 24 January 2011

The analytical separation was carried out on a 25 cm x 5 mm ODS-Hypersil column (Shandon Southern Ltd., Runcorn, Cheshire, U.K.) with 22% (v/v) acetonitrile in 1 M ammonium acetate buffer pH 5.16 as eluent. The mobile phase flow rate was 1 ml/min. Preparative HPLC was carried out on a 25 cm x 8 mm ODS-Hypersil column using the same mobile phase but with EDTA (100 ml/l) added to prevent the formation of metalloporphyrins. The flow rate was 3 ml/min. The isolated porphyrin fractions were washed with ethyl acetate. The aqueous solutions were adjusted to pH 3.0-3.2 and the porphyrins were extracted into ethyl acetate and were recovered by evaporation of the solvent.

RESULTS AND DISCUSSION

There are four possible type III pentacarboxylic porphyrins (Fig.1) that can be produced by enzymic decarboxylation of uroporphyrinogen III or by heating uroporphyrin III in acid. The separation of a standard mixture and of the corresponding porphyrins in the urine

Downloaded At: 17:29 24 January 2011



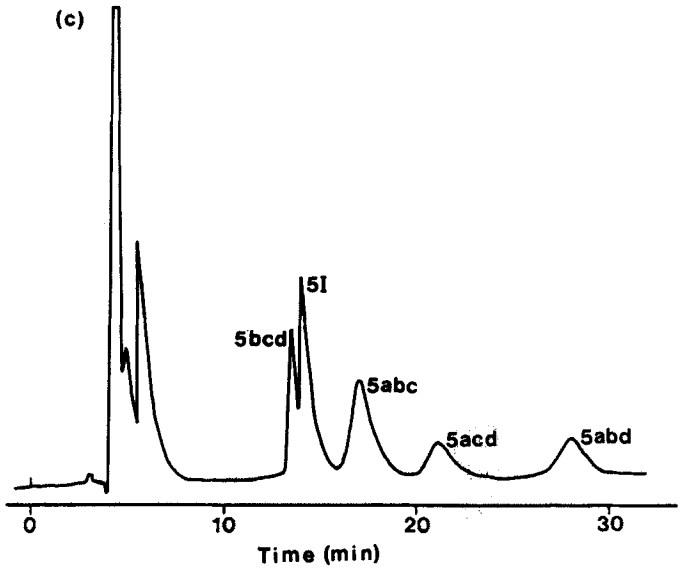


FIGURE 2. HPLC separation of pentacarboxylic porphyrin isomers. (a) Standard mixture; (b) normal urine extract; (c) PCT urine extract. Column, ODS-Hypersil; mobile phase, 22% (v/v) acetonitrile in 1 M ammonium acetate buffer pH 5.16; flow rate, 1 ml/min. 5 I is type I pentacarboxylic porphyrin. For other peak identification see FIGURE 1.

Downloaded At: 17:29 24 January 2011

of normal subjects and PCT patients is shown in Fig.2(a,b and c). The detection of the pentacarboxylic porphyrin 5 abc in urine clearly demonstrates that porphyrins with the ring D acetic acid intact are definitely natural products. The enzymic decarboxylation of uroporphyrinogen III in man is therefore able to begin at the acetic acid groups of either ring A, B, C or D and is not restricted to ring D as reported (2). The most obvious reason for the different results obtained is that our HPLC system is highly efficient and is able to completely resolve the four type III isomers and the type I isomer. This was not possible with the system reported for the separation of pentacarboxylic porphyrin methyl esters (3).

The pentacarboxylic porphyrins in the urine were isolated by preparative HPLC and on decarboxylation by heating in 0.3 HCl at

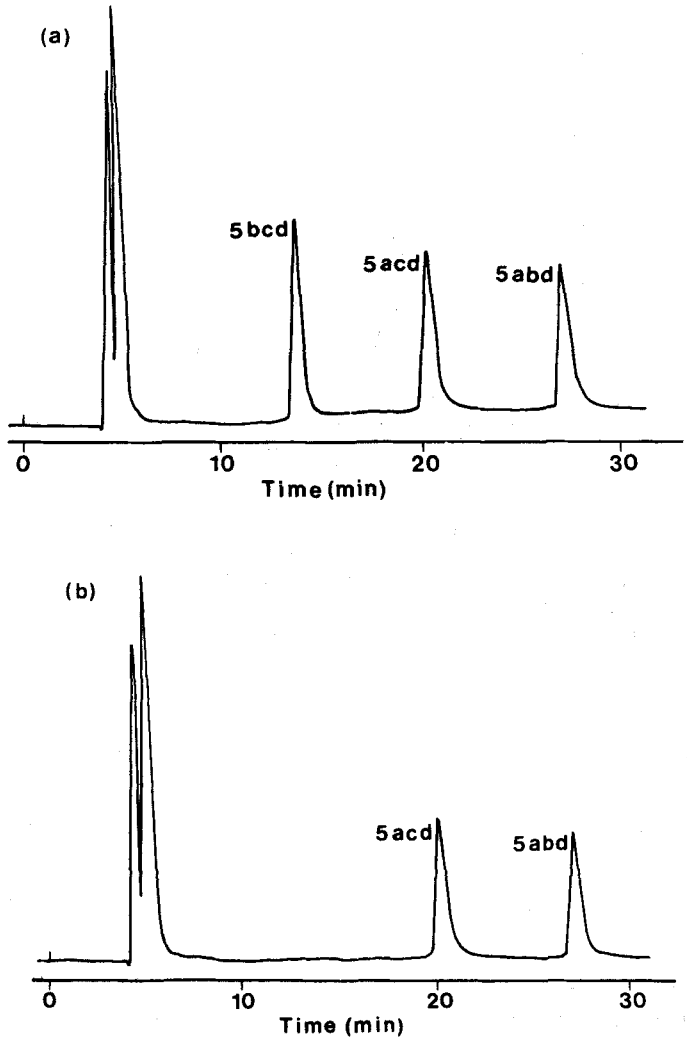


FIGURE 3. HPLC separation of pentacarboxylic porphyrins formed by decarboxylation of type III (a) Hepta- and (b) hexa-carboxylic porphyrins isolated from PCT urine. HPLC conditions as in FIGURE 2. For peak identification see FIGURE 1.

Downloaded At: 17:29 24 January 2011

200° for 2 h each gave the same product, coproporphyrin III. They are therefore isomers. The porphyrin 5 abc can be positively identified by comparing the partial decarboxylation products formed by heating uroporphyrin III and the heptacarboxylic porphyrin III isolated from PCT urine which is known to be a pure compound with the ring D acetic acid decarboxylated (5). On partial decarboxylation of uroporphyrin III in 0.3 M HCl at 160° for 2 h all four possible pentacarboxylic isomers were formed (Fig.2a). The decarboxylation of the heptacarboxylic porphyrin, on the other hand, produced only 5 bcd, 5 acd and 5 abd (Fig.3a). The 5 abc peak is therefore easily identified.

The porphyrin 5 bcd can be similarly identified by partial decarboxylation of the hexacarboxylic porphyrin with the ring A and D acetic acid groups decarboxylated. This compound can be isolated from PCT urine or faeces by preparative HPLC (5) and on heating in 0.3 M HCl at 160° for 1 h gave 5 abd and 5 acd (Fig.3b), thus allowing the assignment of the 5 bcd peak.

The remaining 2 peaks, 5 abd and 5 acd, were assigned by their chromatographic behaviours. 5 abd was more strongly retained than 5 acd because the three side chain Me-groups are closer to each other and this imparts a stronger hydrophobic interaction with the C_{18} stationary phase.

The partial decarboxylation of uro-, hepta- and hexa-carboxylic porphyrins followed by HPLC separation and isolation is a simple way of obtaining pure pentacarboxylic porphyrin isomers important for biochemical studies. It is common for the enzyme uroporphyrinogen decarboxylase to be assayed using synthetic 5 abd as the substrate (6). The availability of this compound, however, is limited to a few chemical laboratories interested in its synthesis. The present system is thus invaluable for the preparative isolation of 5 abd and its isomers.

Downloaded At: 17:29 24 January 2011

REFERENCES

1. Battersby, A.R., Fookes, C.J.R., Gustafson-Potter, K.E., McDonald, E. and Matcham, G.W.J., J. Chem. Soc.Perkin I., 2427, 1982.

2. Jackson, A.H., Sancovich, H.A., Ferramola, A.M., Evans, N., Games, D.E., Matlin, S.A., Elder, G.H. and Smith, S.G., Phil. Trans. R. Soc., Lond. Ser. B, 273, 191, 1976.

3. Smith, S.G., Rao, K.R.N. and Jackson, A.H., Int. J. Biochem., 12, 1081, 1980.

4. Dowdle, E.B., Goldswain, P., Spong, N. and Eales, L., Clin. Sci., 39, 147, 1970.

5. Lim, C.K., Rideout, J.M. and Wright, D.J., Paper presented at the VIIth International Symposium on Column Liquid Chromatography, Baden-Baden, May 3-6, 1983.

6. De Verneuil, H., Sassa, S. and Kappas, A., J. Biol. Chem., 258, 2454, 1983.

Downloaded At: 17:29 24 January 2011