

Application

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Separate and Monitor Porphyrin Isomers by Reversed Phase HPLC

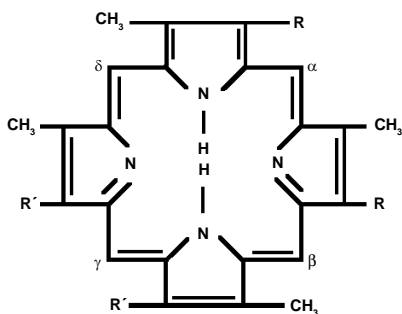
A 3.3cm x 4.6mm SUPELCOSIL LC-18 column containing a 3µm silica-based octadecylsilyl packing effectively separates uroporphyrins I and III and coproporphyrins I and III. Modifications of the mobile phase program sharpen the peaks, but reduce resolution.

Key Words:

• porphyrins • carcinoma • cancer detection

Porphyrins are biologically important nitrogen-containing molecules comprised of four pyrrole rings (Figure A). A metal atom often is incorporated in the center of the molecule (e.g., iron in hemin, the oxygen-transporting molecule; magnesium in chlorophyll). Because certain carcinomas apparently cause changes in porphyrin type and distribution in the human body, there is interest in the possibility of using these complex molecules as indicators for detecting carcinomas in early stages of development.

Figure A. Porphyrin Structure



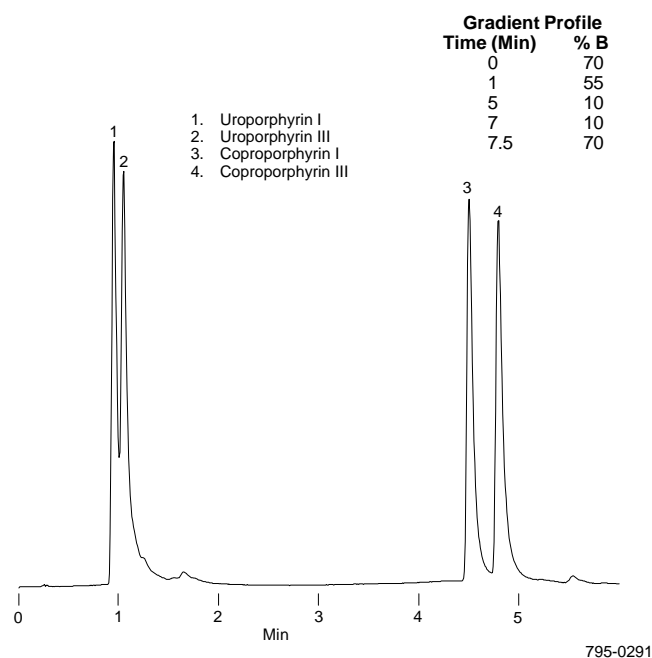
795-0290

We attempted to duplicate a published method for separating porphyrin isomers (1), using a 3.3cm x 4.6mm SUPELCOSIL™ LC-18 column containing a 3µm silica-based octadecylsilyl packing. We conditioned the column with the initial mobile phase for 5-8 minutes prior to sample injection, and flushed the column with methanol when the injections were completed.

A separation of two pairs of porphyrins, uroporphyrins I and III and coproporphyrins I and III, is shown in Figure B and is summarized in Table 1. Overall, separation of the four isomers was similar to that in the published report. Modifications of the

Figure B. Porphyrin Isomers Resolved

Column: SUPELCOSIL LC-18, 3.3cm x 4.6mm, 3µm particles (w/ 0.5µm in-line filter)
Cat. No.: 58977
Mobile Phase: A - methanol
B - 1M ammonium acetate, pH 5.2 w/ glacial acetic acid
Flow Rate: 2mL/min
Temp.: ambient
Det.: UV, 406nm
Inj.: 5µL, 10µg/mL each analyte in 1M HCl



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Table 1. Porphyrin Separation Data

Analyte	t _R (Min)	AF ₁₀	Resolution
Figure B			
Uroporphyrin I	0.95	1.61	
Uroporphyrin III	1.05	2.60	0.93
Coproporphyrin I	4.50	2.21	
Coproporphyrin III	4.79	2.47	2.33
Figure C			
Uroporphyrin I	1.78	1.25	
Uroporphyrin III	1.85	2.01	0.74
Coproporphyrin I	3.85	1.34	
Coproporphyrin III	3.99	1.74	1.64

AF₁₀ = peak symmetry at 10% of peak height

mobile phase program and other conditions sharpened the peaks, but also reduced resolution (Figure C and Table 1). These results indicate that a short SUPELCOSIL LC-18 column of 3µm packing, used with the conditions developed by Kennedy and Maslen (1), provides effective separations of porphyrin isomers. If, for a particular sample, peak symmetry is more important than resolution, the modified conditions in Figure C might be more suitable.

Ordering Information:

Description	Cat. No.
SUPELCOSIL LC-18 Column	
3.3cm x 4.6mm, 3µm particles	58977

Reference

1. Kennedy, S.W. and A.L. Maslen, *J. Chromatogr. Biomed. Apps.* **493**: 53-62 (1989).

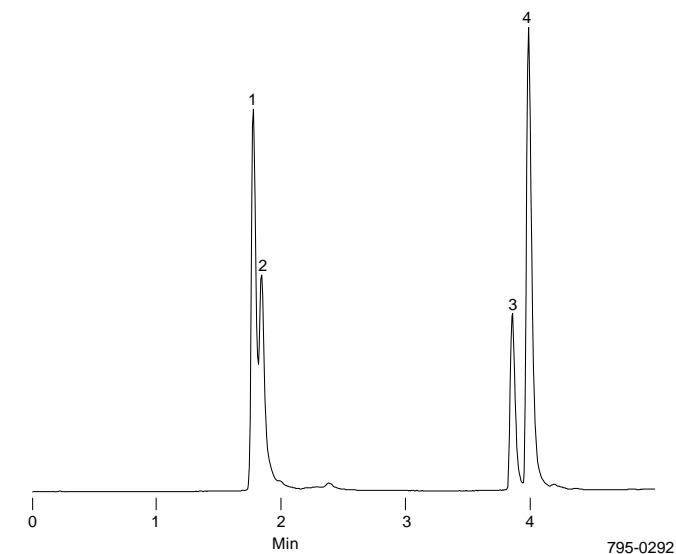
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Figure C. Modified Gradient Program Improves Peak Symmetry, But Reduces Resolution

Column: **SUPELCOSIL LC-18, 3.3cm x 4.6mm, 3µm particles** (w/ 0.5µm in-line filter)
 Cat. No.: **58977**
 Mobile Phase: A - methanol
 B - 1M ammonium acetate, pH 5.2 w/ glacial acetic acid
 Flow Rate: 2mL/min
 Temp.: ambient
 Det.: UV, 406nm
 Inj.: 5µL, 10µg/mL each analyte in 1M HCl

Gradient Profile	
Time (Min)	% B
0	70
1	63
2	55
5	10
7	10
7.5	70

1. Uroporphyrin I
2. Uroporphyrin III
3. Coproporphyrin I
4. Coproporphyrin III



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