LAH 002

An Internal Communication
of Applications and Techniques

Waters
Lab Highlights

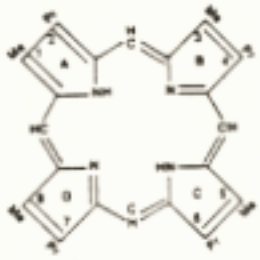
81.600.028.001.002

HPLC DETERMINATION OF BIOLOGICALLY ACTIVE PORPHYRINS

Porphyrias are diseases caused by distrubances of the porphyrin metabolic pathway. These disturbances result from specific genetic disorders where there is defective production of particular enzymes involved in porphyrin biosynthesis. Due to these enzymatic abnormalities, various porphyrins are produced in excess and accumulate in various tissues. These excess porphyrins are later excreted in the urine and feces.

The diagnosis of these pathological conditions requires the analysis, quantitation and differentiation of either individual porphyrin levels or porphyrin profiles in blood, urine and feces. Uroporphyrin I and Coproporphyrin III, shown below, are two such diagnostically important porphyrins.

Uroporphyrin I



Coproporphyrin III

Me = methyl (- CH_3)
Ac = acetic (- CH_2 - COOH)
Vn = vinyl (- CH = CH_2)
Pr = propionic (- CH_2 - CH_2 - COOH)

These two porphyrins have been successfully separated using HPLC (see Figure 1). The chromatographic conditions are:

Column:	Radial-PAK^R CN (8mm x 10cm)
Solvent:	a) CH_3CN/H_2O (0.01 M D4-Reagent), (49/51)
	b) CH_3CN/H_2O (0.01 M D4-Reagent), (56/44)
Flow Rate:	3ml/min.
Detector	405 nm, 0.01 AUFS (Waters M440)
Sample Volume:	25µl

During the development of these separations, it was observed that resolution of these porphyrins could be substantially altered by varying either the acetonitrile concentration or the concentration of Reagent D4. Therefore, similar chromatographic conditions should be adaptable to the separation of other biologically important porphyrins.

Mike Mac Neil

Chromatograms on back of page...

PORPHYRINS

81.600.028.001.002

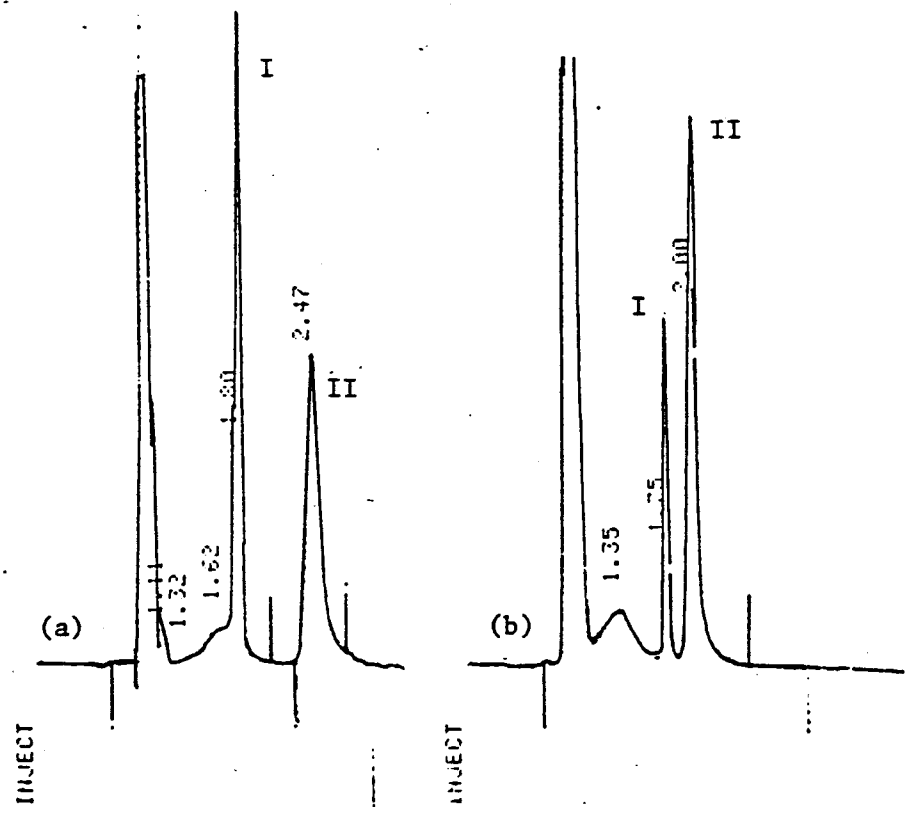


Figure 1

Compound I - Uroporphyrin I

Compound II- Coproporphyrin III