#AP16

EPROGEN INC

ANALYSIS OF EPIRUBICIN AND METABOLITES IN HUMAN PLASMA

MICRA *NPS*® is a breakthrough in fast HPLC. *NPS* is ultra-pure, highly uniform non porous silica spheres which provide the LC chromatographer greatly improved mass transfer and lower detection limits. Coupled with enhanced stability and dramatically reduced solvent usage, *NPS* is the ideal column to meet the ever increasing demands placed on today's analytical labs - Improved productivity at a lower cost.

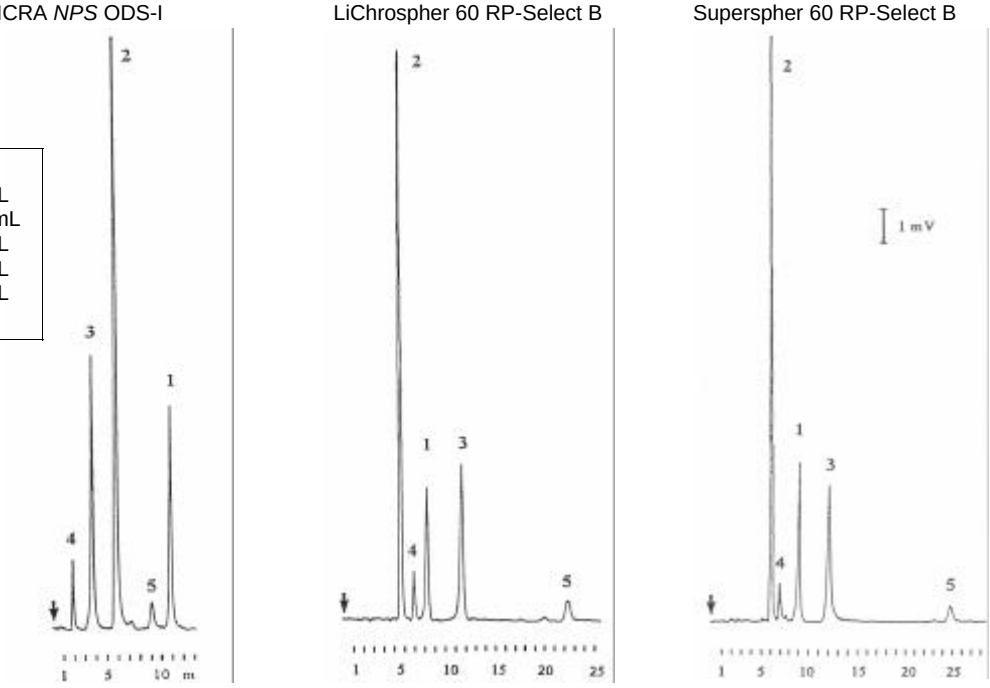
This paper was originally presented at the 5th International Symposium on Pharmaceutical and Biomedical Analysis, in Stockholm, Sweden, September 21-24, 1994 by Professor K.S. Boos and Anne Rudolphi (Institute fur Klinische Chemie Ludwig-Maximillians-Universitat Munchen).

Injection of biological samples, such as serum or plasma, onto an analytical column packed with porous or non-porous RP-bonded silica increases back pressure and causes a loss of retentive power. This is due to irreversible adsorption and denaturization of matrix proteins on the silica surface. This denaturization by the macromolecules of biological samples can be avoided by a coupled-column HPLC system consisting of a pre-column and the analytical column investigated. The pre-column serves as an on-line sample processing unit that serves two major functions:
(a) dynamic extraction and concentration of the analytes of interest by hydrophobic interaction, and (b) eliminates the matrix proteins by size-exclusion. The advantages of using the MICRA *NPS* 1.5µ ODS-I analytical column are a reduction in analysis time, improved LOQ and LOD, and enhanced efficiency.

Figure 1. Single-column Separation of Epirubicin and Metabolites

20 µL Aqueous Solution:	
Epirubicin (1)	62 ng/mL
Epirubicinol-Aglycon (2)	164 ng/mL
Epirubicinol (3)	56 ng/mL
Epirubicin-Aglycon (4)	89 ng/mL
7-Deoxy-epirubicinol-Aglycon (5)	40 ng/mL

MICRA *NPS* ODS-I | LiChrospher 60 RP-Select B | Superspher 60 RP-Select B



Operating Conditions - Fluorescence Detector; ex: 445nm, em: 560nm

	MICRA *NPS* ODS-I	**LiChrospher**	**Superspher**
Column	33x4.6mm ID	250x4mm ID	250x4mm ID
Aqueous/Organic	85/15	70/30	70/30
Flow Rate	0.7 mL/min	1.0mL/min	1.0mL/min
N-alkyl-ligand	C-18	C-8	C-8
Injection Volume (all)	20uL		
Mobile Phase (all)	H_2O (0.1% TEA, pH 2.0 w/ TCA), Acetonitrile (85/15, v/v)		

Think small

Think fast

Think *NPS*®

Compared to Porous Column Supports, *NPS* Showed:

- **2x Increased Efficiency**
- **40% Higher LOD & LOQ**
- **50% Reduction in Analysis Time**

Figure 2. Structures of Epirubicin and Metabolites

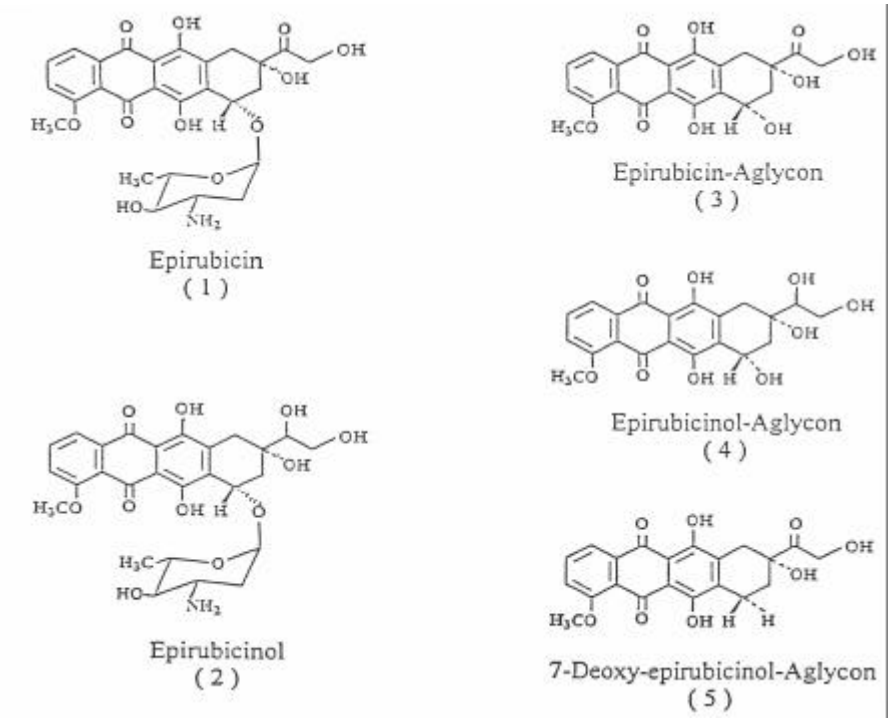
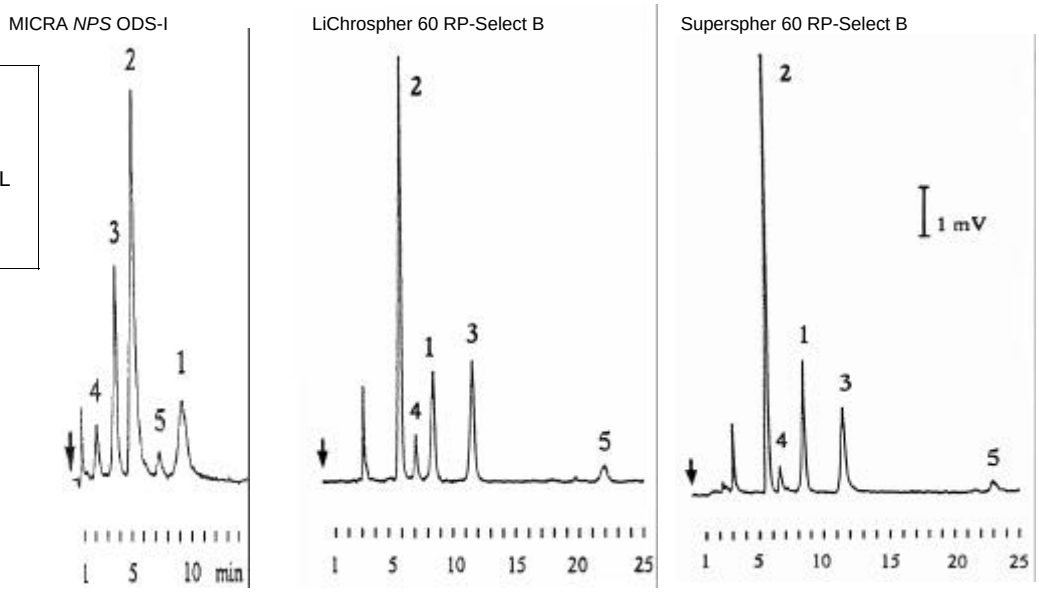


Figure 3. Coupled-column Separation of Spiked Human Plasma

20 µL Human Plasma Spiked with:

Epirubicin (1)	31 ng/mL
Epirubicinol-Aglycon (2)	82 ng/mL
Epirubicinol (3)	28 ng/mL
Epirubicin-Aglycon (4)	44.5 ng/mL
7-Deoxy-epirubicinol-Aglycon (5)	20 ng/mL



Coupled-column HPLC analysis consisting of a restricted access “Alkyl-Diol” silica (ADS) pre-column followed by the analytical column. Further information regarding this analysis may be obtained by contacting Eprogen, Inc.

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