EPROGEN
INC

Fast Online Analysis of Free Urinary Catecholamines Using MICRA *NPS*® and Restricted Access Affinity Precolumns

MICRA *NPS*® is a break-through 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 HPLC 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 HPLC '95, in Innsbruck, Austria entitled; "Coupled-Column LC-Analysis of Catecholamines in Urine using a MICRA *NPS* Analytical Column" by A. Rudolphi, K.-S. Boos, K. D. Bischoff, and T.J. Barder.

A fast coupled column analysis for routine online determination of free urinary catecholamines has been developed using a MICRA *NPS* column and a Restricted Access Affinity precolumn[1,2]. Selective extraction of the catecholamines from urine was performed using alkaline pH and boronic acid-modified copolymers supports. Analytical separation and quantitation was performed with a 4.6 x 53 mm MICRA 1.5µm *NPS* ODS-I column using ion-pair RP HPLC with no organic modifier in the mobile phase.

Figure 1. Separation on *NPS* 1.5µm

LOD's well below 5 ng/mL urine

LC Conditions[2]:

Mobile phase:	10 mM NaH_2PO_4 + 0.1 mM 1-dodecanesulfonic acid, pH = 2.5 with H_3PO_4
Flow rate:	0.5 mL/min
Detection:	FD; ex = 275 nm, em = 330 nm, time const. = 3 s, 12 µL flow cell
Injection volume:	50 µL
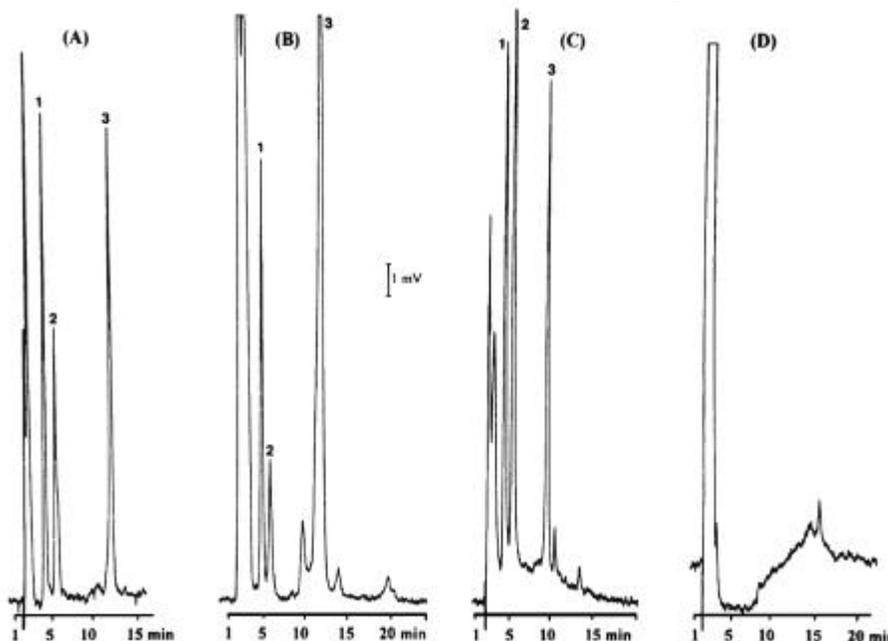
Coupled-column analysis of norepinephrine (1), epinephrine (2) and dopamine (3)
(A)   50 µL aqueous standard solution (1, 2, 3 = 1.99, 1.09, 7.03 nmol/mL)
(B)   50 µL Human urine from healthy person (1,2,3 = 1.77, 0.73, 14.34 nmol/mL)
(C)   50 µL Human urine from pheochromocytoma patient (1,2,3 = 2.16, 2.73, 6.71 nmol/mL)
(D)   50 µL blank urine after oxidative destruction of endogenous catecholamines
         (as described in paper)

Used by permission of K. -S Boos and F. Vieweg and Sohn, <u>Chromatographia</u>, 41(11/12)

METHOD DEVELOPMENT

Think small

Think fast

Think *NPS*®

Table 1. Linearity & Precision

| Analyte | LINEARITY | | PRECISION | |
	Range	corr. coeff.	CV within run	CV within day
	pmol/mL	[r]	(n=10) [%]	(n=10) [%]
NE	48.9 - 9784	0.9994	0.97	1.68
E	54.6 -10916	0.9983	2.25	1.90
DA	105.5 - 8701	0.9997	3.74	2.51

Table 2. LOQ & LOD

Analyte	LOQ	LOD	Mean Recoveries, %
	pmol on column	pmol on column	nmol/mL [%RSD]
NE	5.57	2.43	98.18 [2.8]
E	1.75	0.76	102.00 [1.8]
DA	36.81	15.61	101.12 [1.8]

NE = norepinephrine, E = epinephrine, DA = dopamine

MICRA *NPS* Performance

Combined with the precolumn, the MICRA *NPS* column showed:

1. No degradation in performance over the life of the study (5 months);

2. 50% reduction in run time compared to a typical porous column assay[3];

3. >70% reduction in solvent consumption[3]; and

4. Quantitative recovery of catecholamines.

Eprogen
8205 S. Cass Avenue
Suite #106
Darien, Illinois 60561
USA
Phone: (630) 963-1481
Fax: (630) 963-6432
E-mail: info@eprogen.com
www.eprogen.com

References:
1   Boos, K.-S., et. al., *J. Chromatogr.* (1988) **456**, 83-104
2   Rudolphi, A., et. al., *Chromatographia* (1995) **41**, 645-650
3   K.-S. Boos, et. al., *Chromatographia* (1987) **24**, 363-370