#AP06

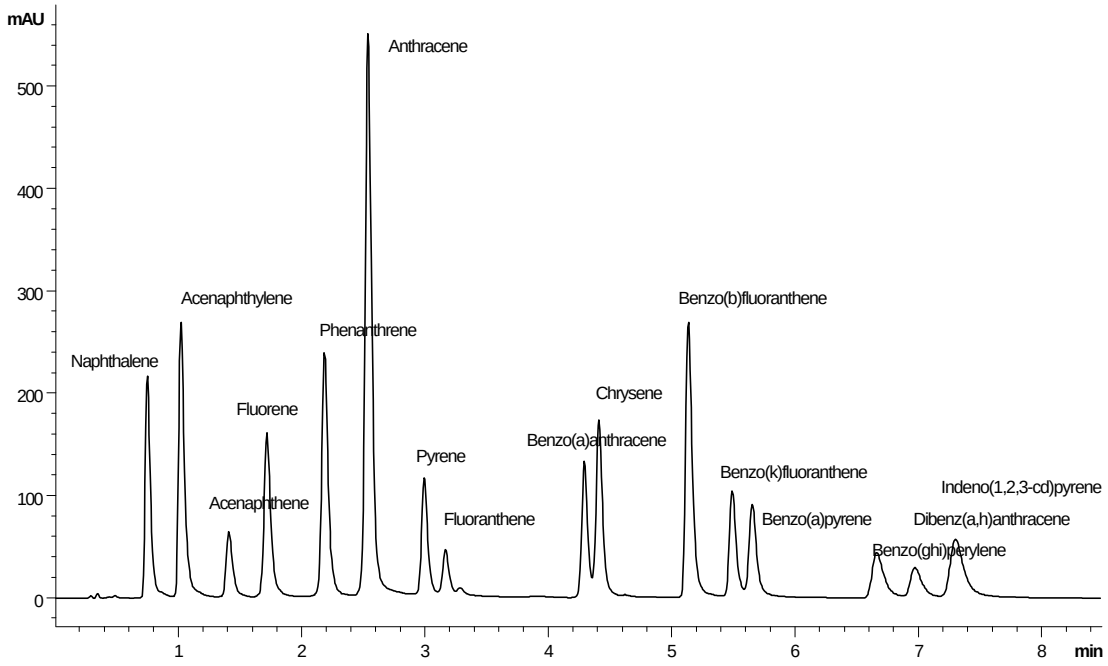
EPROGEN INC

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.

MICRA *NPS*® LC Analytical Column
Fast Separation of Polynuclear Aromatic Hydrocarbons

Polynuclear aromatic hydrocarbons (PAH's) are formed through incomplete combustion and are monitored by the U.S. Environmental Protection Agency (U. S. EPA) in drinking water, waste water and soil using U.S. EPA methods 610, SW-846, and 8310. The U.S. EPA classifies 16 PAH's as priority pollutants and mandates their routine monitoring for regulatory administration. The U.S. EPA method uses reversed phase HPLC with UV detection at 254 nm. This application note outlines a fast, reproducible and rugged analytical method using a MICRA 1.5µ *NPS* ODS-I column.

Figure 1. Analysis on MICRA 1.5µ *NPS* ODS-I, 4.6mm I.D.



Analytical Conditions

Mobile Phase	30% CH_3CN/ 70% H_20
Gradient	4 min to 55% CH_3CN. Hold to end (8.5 min)
Flow Rate	1.0 mL/min
Column Support	MICRA *NPS* ODS-I, 1.5µ, 33mm length Part # 0446PAH101.5
Detection	uv= 254nm
Flow Cell	8µL Flow Cell, 6mm Pathlength
Injection Volume	1µL
Sample	EPA 610 Calibration Standard. Supelco Cat# 4-8743

Think small

Think fast

Think *NPS*®

PEAK IDENTIFICATION AND REPRODUCIBILITY STUDIES

To verify the elution order, each analyte was injected separately onto the column. Except for the reversal of Dibenz(a,h)anthracene and Benzo(ghi)perylene, the elution order was found to be consistent with published methods utilizing porous 3µm and 5µm reverse phase columns. This was reconfirmed with the analysis of the pure substances, as well as by spiking the standard PAH sample with a known amount of Benzo(ghi)perylene.

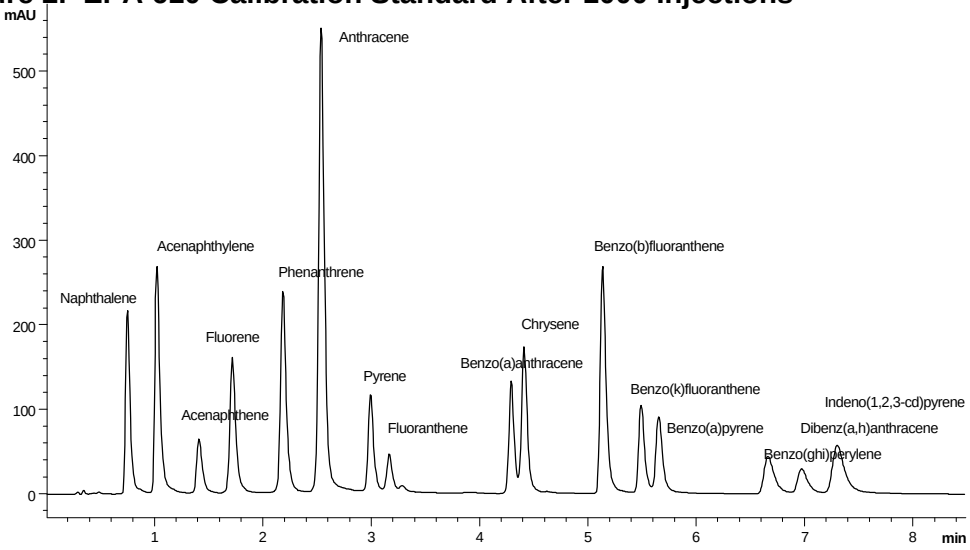
To evaluate column ruggedness and reproducibility, the PAH standard solution was injected 1000 times. Method precision was calculated on retention time (RT) and area counts by determining the relative standard deviation (%RSD) of 10 consecutive injections. Table 2 lists the statistical data acquired. Chromatogram 2 shows the analysis of the EPA 610 Calibration Standard after 1000 injections.

Table 1. %RSD for Retention Time, Area Count and USP Tailing Factor

Analyte	Retention Time %RSD	Area Count %RSD	Tailing Factor*, t (Average)
NAPHTHALENE	0.262	0.985	1.84
ACENAPHTHYLENE	0.339	1.075	2.00
ACENAPHTHENE	0.339	1.313	1.65
FLUORENE	0.364	1.387	1.43
PHENANTHRENE	0.323	1.523	1.33
ANTHRACENE	0.314	1.279	1.25
FLUORANTHENE	0.274	2.987	1.04
PYRENE	0.282	3.646	1.35
BENZO(a)ANTHRACENE	0.262	4.876	1.08
CHRYSENE	0.278	3.649	1.44
BENZO(b)FLUORANTHENE	0.268	0.268	1.46
BENZO(k)FLUORANTHENE	0.329	3.360	1.04
BENZO(a)PYRENE	0.336	0.919	2.30
BENZO(ghi)PERYLENE	0.440	4.207	1.29
DIBENZ(a,h)ANTHRACENE	0.548	3.972	1.27
INDENO (1,2,3-cd)PYRENE	0.577	2.290	1.40

*USP Tailing Factor - Symmetric peak is t=1.00

Figure 2. EPA 610 Calibration Standard After 1000 Injections



Eprogen
HPLC Columns
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