Waters Lab Highlights

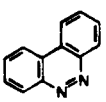
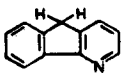
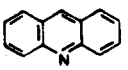
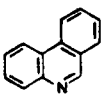
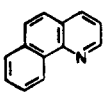
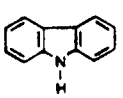
An Internal Communication of Applications and Techniques

LAH 0349 10/87
AN/AC/MD/RA/DS

Waters Nova-Pak™ C_{18} Column: The Best for Azaarene Separations

Azaarenes are nitrogen analogues of polycyclic aromatic hydrocarbons (PAHs); their structures are shown in Figure 1. These compounds are usually found in water in association with sediment [1]; they are believed to be formed during combustion or pyrolysis processes involving fossil fuels. A method for their analysis has recently been described in the literature using a Sep-Pak® C_{18} cartridge and a Radial-Pak™ cartridge with Nova-Pak™ C_{18} material.

Figure 1: Nomenclature and Structures of Azaarenes Selected for Study

formula	named as derivative of polycyclic aromatic hydrocarbon	common name	structure
C_9H_7N	1-azanaphthalene	quinoline	
C_9H_7N	2-azanaphthalene	isoquinoline	
$C_{12}H_8N_2$	9,10-diazaphenanthrene	benzo[*c*]-cinnoline: 5,6-phenanthroline	
$C_{12}H_9N$	4-azafluorene		
$C_{13}H_9N$	10-azaanthracene	acridine: benzo[*b*]-quinoline	
$C_{13}H_9N$	10-azaphenanthrene	phenanthridine: benzo[*c*]-quinoline	
$C_{13}H_9N$	4-azaphenanthrene	benzo[*h*]-quinoline	
$C_{12}H_9N$	9-azafluorene	carbazole	

The trace enrichment procedure and sample clean-up were accomplished by activating a Waters Sep-Pak C_{18} cartridge with 10 mL of acetonitrile, followed by 10 mL of purified water. A filtered water sample was then passed through the cartridge, using air pressure or a 60-mL disposable syringe. Following trace enrichment, the Sep-Pak cartridge was connected to a 2-mL glass Luer-Lock syringe, and the unit was suspended in a small test tube. The apparatus was centrifuged (1500 rpm) to ensure removal of most of the residual water from the cartridge packing. Exactly 2.00 mL of acetonitrile, was pipetted into the syringe barrel, forced through the Sep-Pak cartridge into a clean test tube, and centrifuged as before. The final sample extract was then membrane filtered through a 0.2-µm-nylon filter. A 20-µL aliquot was injected into the LC.

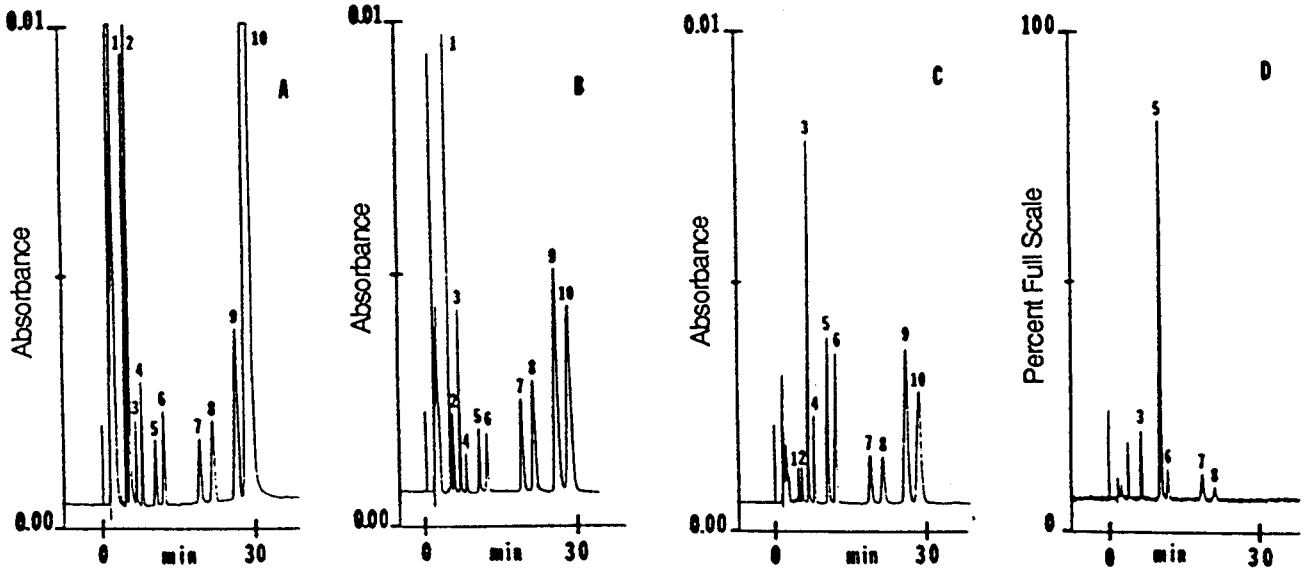
After sample concentration and clean-up, separation was performed with an 8 mm I.D. x 10 cm Radial-Pak cartridge with Nova-Pak C_{18} material held in an RCM-100® radial compression module.

Waters
Division of MILLIPORE 34 Maple Street Milford, MA 01757 508-478-2000

Carmen Santasania

Mobile phase was prepared by adding 1 mL of phosphoric acid to 1 L of water and titrating to pH=6.5 by dropwise addition of 14 M aqueous ammonia. This aqueous portion of the mobile phase was then mixed with acetonitrile in a ratio of 58/42 (ammonium acetate/acetonitrile) (v/v). Equilibration of the column was done by recycling the mobile phase at 1.0 mL/min overnight.

Figures 2,3,4 and 5 show a separation of a standard mixture of azaarenes plus internal standard. The authors report that azaarenes, due to their structures (Figure 1), show peak tailing on most columns that were initially tried. They further reported; "the column that ultimately seemed to provide the best combination of separation efficiency and speed, peak symmetry and column life was the recently introduced radial compression C_{18} Nova-Pak."



Figures 2,3,4, and 5: LC separation on Nova-Pak C_{18} Radial-Pak cartridge of standard mixture containing analytes, internal standard, and PAH reference (0.05 mg/L azaarene, 5.0 mg/L acetone-dinitrophenylhydrazone, 8.0 mg/L naphthalene). 20-µL injection, equivalent to 0.01 µg of azaarene: (A) 214 nm. 0.01 AUFS; (B) 229 nm, 0.01 AUFS: (C)254 nm 0.01 AUFS: (D) fluorescence, excitation 254 nm, emission 425 nm, gain 32X. Peak identification is as follows: (0) injection, (1) quinoline, (2) isoquinoline, (3) benzo[c]cinnoline. (4) 4-azafluorene. (5) acridine, (6) phenanthridine, (7) benzo[h]quinoline, (8) carbazole, (9) acetone-DNPH, (10) naphthalene.

Literature cited.

1. Steinheimer, T.R. and Ondrus, M.G., *Anal. Chem.*, 1986, 58 (8), 1839-1844.

***