Chip Seal Phenanthrene Report Jack Bell 7/30/10
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Introduction and background
Polyaromatic hydrocarbons (PAH's), known as the sixteen EPA "priority pollutants," exist in asphalts in significant levels. The petroleum-based asphalts commonly used today contain anywhere from less than one to 64 ppm PAH's depending on the source (2). To what extent are they leaching into the immediate environment, and enhancing PAH's generated by combustion and petroleum sources?

Immunoassays will provide an estimate of total PAH's, but not specific and accurate levels of individual PAH's. For that, HPLC with UV and Fluorescence detection is the standard method of choice approved by the EPA. In 2007 we looked for phenanthrene in fresh asphalt paving and chip seals on Lopez and San Juan Islands using HPLC/UV (1). We found UV peaks in extracts that had the retention time of phenanthrene. However, many other organics with UV absorbance could co-elute at the same retention time.

This summer San Juan Island county roads were coated with "Chip Seal" asphalt. We used this opportunity to revisit PAH's with two detectors providing increased selectivity and sensitivity: an LCMS (mass spectrometer) and a fluorescence detector. This report details our results with a 30-year old Schoeffel Fluorescence detector on my HPLC/UV system at Friday Harbor Labs. The technical detail and calculations are for the benefit of students learning analytical techniques.

Experimental
LC components in flow path sequence: Waters Model 510 pumps A (85% MeOH in MQ water) and B (100% MeOH), Valco 1 mL loop injector, 4.6 X 150 mm Column Engineering 5 micron C18 column, ABI UV detector at 254 nm, and the Schoeffel Fluorescence detector with 249 nm excitation for phenanthrene and in the emission path the pre-filter and 370 nm filters removed for maximum sensitivity. The 1 volt UV output and 15 mV Fluor outputs were connected to an eDAQ data logger (input amplifier range 1 V, low pass filter 1 Hz, digital low pass filter 1 Hz, and sampling rate 100/s).

LC performance QC: At 1 mL/min 85% MeOH the system back pressure was typically 1200 psi, with no increase during this project. Detector baseline noise peak-to-peak was constant at ~ 40 microvolts for the UV detector and ~ 300 microvolts for the Fluor detector at Range 0.01. Replicate peak retention times and height, as a measure of pump, injector and system stability, were typically within +/- 0.3% coefficient of variation.

LC calibration with phenanthrene: Crystalline phenanthrene (Sigma, > 96% by LC) was weighed on a digital balance good to 0.1 mg. To 7.7 mL pesticide grade MeOH in a 20 mL glass vial, 7.7 mg phenanthrene was added, the vial tightly capped, and warmed briefly under hot tap water to dissolve all crystals. This 1 mg/mL stock standard was kept on ice when opened to remove aliquots for dilution. Using a 100 ul LC syringe dedicated to phenanthrene, 10 uL was added to 10.0 mL 85% MeOH in a 20 mL vial = a 1/1000 dilution = 1 ng/uL phenanthrene standard. In the same way a 0.01 ng/uL standard was prepared.

Instrumental Response Factor (RF) and Limit of Detection (LOD) calculations for phenanthrene standards

Detector	Phenanthrene standard injected	Baseline Noise	Peak height of the Signal	Signal-to-Noise (S/N)	Response factor	Instrument Limit of Detection at S/N 3
7/18 Fluorescence run at Range 0.01 (Fig. 1)	10 uL of the 0.01 ng/uL standard = 0.1 ng on-column	0.3 mV peak-to-peak	5.4 mV peak at 4:40 minutes	5.4 mV/0.3 mV = 18 for 0.1 ng	18/0.1 = S/N 180 for 1 ng	3/180 x 1 ng = 0.017 ng on-column
7/18 UV run	10 uL of the 0.01 ng/uL standard = 0.1 ng on-column	0.1 mV peak-to-peak	0.15 mV peak at 4:30 minutes	0.15 mV/0.1 mV = 1.5 for 0.1 ng	1.5/0.1 = S/N 15 for 1 ng	3/15 x 1 ng = 0.2 ng on-column
7/21 UV run	10 uL of the 1 ng/uL standard = 10 ng on-column	0.04 mV peak-to-peak	6.7 mV peak at 3:50 minutes	6.7 mV/0.04 mV = 168 for 10 ng	168/10 = S/N 17 for 1 ng	3/17 x 1 ng = 0.17 ng on-column

Sample preparation: A 20 mL, preweighed, PTFE capped glass vial full of Chip Seal coated road gravel (1 day old) was extracted with 8 mL of diethyl ether (Et2O) by shaking and filtering through a cotton plug into a second vial, followed by a 2 mL Et2O "chaser." The dry vial and gravel was reweighed to obtain 1.58 g tar in ~ 10 mL solution = 158 ug/uL tar solution. To remove particulates that might plug the LC, one mL of this solution was centrifuged 15 minutes at 15,000 rpm in a small fixed-angle rotor (x g) in a Beckman Spinco Model centrifuge.

LC analysis: Ten uL of the clear, dark brown Et2O solution (containing 1.58 mg tar) was injected into the LC column equilibrated in 85% MeOH. At 10 minutes run time Pump A is switched to zero flow rate, and Pump B (100% MeOH) is switched to 1 mL/min to clean to column of co-extracted matrix impurities. At 20 minutes, the pumps are switched back to 85% MeOH and the column re-equilibrated for another 10 minutes or until the back pressure stabilizes in the new mobile phase.

Results and explanation

Phenanthrene standards in fluorescence chromatograms eluted at between 4:40 and 4:00 minutes before and after the day the extract was run. The ether extract of Chip Seal tar produced two peaks in the fluorescence chromatogram where phenanthrene might elute: 4:28 and 5:00 minutes (Figure 2). Unfortunately the fluorescence lamp died before we could do spiking runs to determine which peak was due to phenanthrene. However both fluorescent peaks were of similar size, approximately 0.3 mV at a Range of 0.1 (fluorescence detector saturated at Range 0.01). From the Response Factor of 180 at Range 0.01 above, the RF would be 18/ng at Range 0.1. Baseline noise at Range 0.1 is 0.05 mV. The 0.3 mV peak's S/N is therefore 0.3/0.05 = 60, and 60/18 per ng = 3.3 ng of phenanthrene injected on-column in 10 uL of ether extract. From this there is 3.3 ug of phenanthrene in the 10 mL extract containing 1.58 g tar. The level of phenanthrene in tar is therefore 3.3 ug/1.58g = 2.1 ppm, with an estimated error of +/- 1 ppm due to evaporation of ether (higher results) or peak spreading due to co-extractables on the column (lower results). The average phenanthrene level of twelve U.S. asphalts is 2.4 ppm (2). The Chip Seal used by San Juan County this summer is an anionic emulsion containing 65% petroleum asphalt, and thus somewhere around 1 ppm phenanthrene.

From comparison of the UV and fluorescent LOD's, the vintage fluorescence detector is ten times more sensitive than a typical UV detector, and relatively selective for phenanthrene at its excitation wavelength. The ultimate selectivity will be found on the mass spectrometer.

References

(1) Russel Barsh, Jack Bell, Sam Barr, Katie Taylor, Arielle Wilson, and Rachel Wilbur, "Preliminary evidence for phenanthrene, a polycyclic aromatic hydrocarbon (PAH), in some batches of paving materials used in San Juan County, WA," December 2007.

(2) Kim E. Grosenheider, etal, "A review of the current literature regarding polycyclic aromatic hydrocarbons in asphalt pavement," University of Minnesota under contract with DOT, accessed at www.rmrc.unh.edu/conference/oct2005.

Figure 1: Instrumental limit of detection for phenanthrene. 0.1 ng on-column

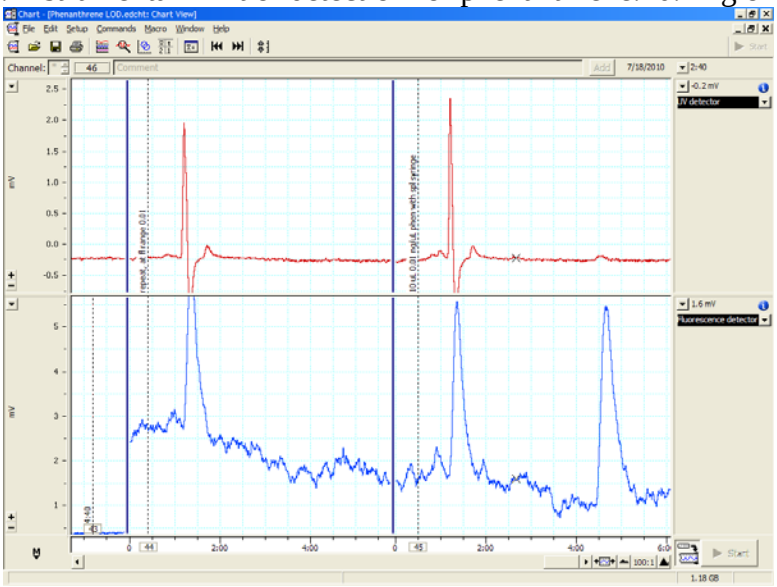

Figure 2: Chromatograms of the Chip Seal ether extract