Improved Data Quality in the Automated HPLC Analysis of PNAs (PAHs)

Application Note

Enviromental

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This note describes an improved method for analyzing polycyclic aromatic hydrocarbons (PNAs) by HPLC. During the investigations the major focus was on improving precision of retention times and peak areas by using vacuum degassing and stable, low ambient temperatures. Thorough degassing prevented quenching effects in fluorescence detection and improved retention-time stability. Thermostatting the column temperature slightly below ambient temperature improved resolution of critical peak pairs. With this method the retention-time stability was within 0.05 – 0.2 % RSD. The RSD for the area in the low ng range was in general below 2 %. An improved diode-array detection system allowed PNAs to be detected at ppb levels and their identity at less than 1 mAUFS confirmed automatically by UV-Visible spectral library search.

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Introduction

Hydrocarbons with multiple-ring structures are collectively referred to as polynuclear aromatic hydrocarbons, commonly abbreviated as PNAs or PAHs (figure 1).

This class of compounds are suspected to be carcinogenic or mutagenic. This has lead to legislative restrictions on their release into the environment. They are mainly formed due to incomplete combustion of organic material, such as fossil fuels.

Analysis by HPLC with UV-Visible diode-array and/or fluorescence detection has become a well-established method for the determination of PNAs in soil, water, sludge air and food. However, despite the common use of this method, degassing techniques and temperature stability still cause problems with qualitative and quantitative evaluation.

The best separation of PNAs is achieved at or slightly below ambient temperatures. Without column cooling this often results in unstable retention times caused by ambient temperature fluctuation. To get optimum sensitivity in fluorescence detection, excitation and emission wavelength switching is required. This implicates not only good resolution of the compounds but also stable retention times.

Traces of oxygen in the mobile phase deteriorate fluorescence sensitivity because of quenching effects. Helium degassing and other conventional techniques however will not give highest sensitivity compared to the vacuum system used in this study.

For samples with higher contamination levels a UV-Visible diode-array detector was used to get spectral information for identification and peak-purity control. A major improvement in the sensitivity of this detector allowed spectra to be taken at ppb levels with identification at less than 1 mAUFS.

Current evaluation software for diode-array detectors focuses on interactive spectral evaluation. This is very time consuming, requires sophisticated operators and is impractical for routine analysis.

Traditionally standard columns of about 4 mm id are used for PNA analysis, requiring high solvent amounts with high purchase and disposal costs. Although narrow-bore columns with 2 mm id are available, ideally suited for use at lower flow rates and give higher mass sensitivity they are still not widely used. Further, much of the currently-available routine HPLC equipment does not give reliable results for gradient operation at low flow rates.

In this study we investigated the Agilent 1100 Series HPLC system to determine to what extent this new system could solve some or all of the problems described.

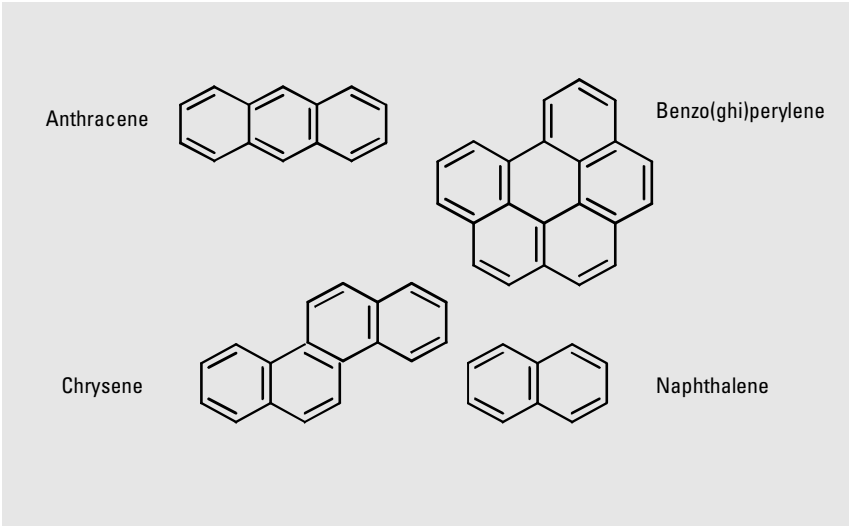


Figure 1
Examples of PNAs (PAHs)

Experimental

The system comprised an Agilent 1100 Series quaternary gradient pump, vacuum degasser, autosampler, thermostatted column compartment, diode-array detector and HP 1046A fluorescence detector. Complete system control and data evaluation was done on the Agilent ChemStation for HPLC. The thermostatted column compar-tment included a Peltier element for precise temperatures above, below and at ambient temperature.

For the separation we used a dedicated PNAs column that allowed the separation of PNA isomers. For economic reasons we used a column with an internal diameter of 2.1 nm that allowed a solvent flow of 0.4 ml/min. For evaluation of separation we used the 16-component EPA standard spiked with some other components that may be present in typical environmental samples and that may chromatographically interfere with the compounds of interest.

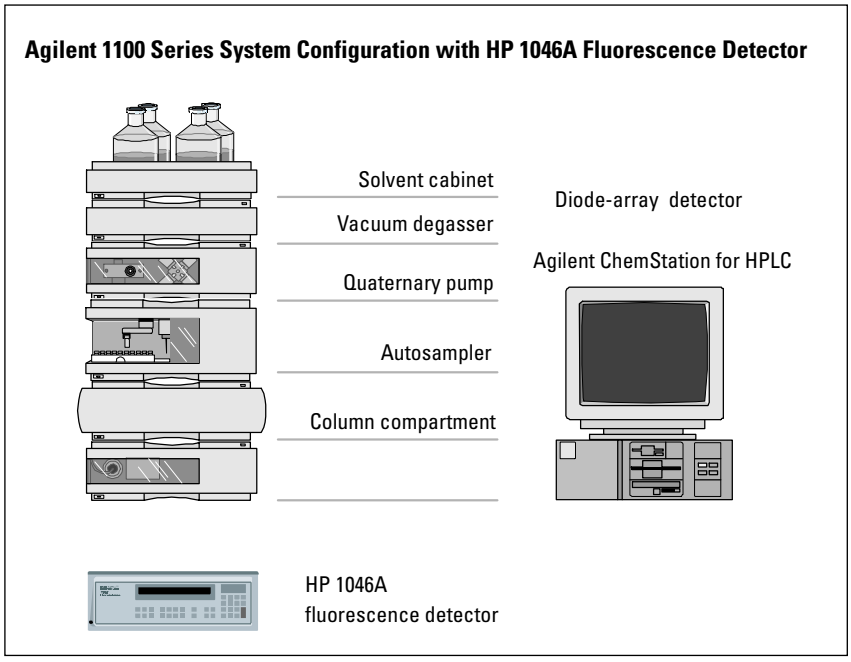


Figure 2
Schematic of instrumentation used

Column	250 _ 2.1 mm PAH column, 5 mm (Agilent part no. 79918PAH-582)
	Buffer A water
	Buffer B acetonitrile
Temperature	18 °C, 25 °C, 30 °C, see figure
Flow rate	0.4 ml/min
Gradient	50 % B to 60 % in 3 min
	to 90 % in 14.5 min
	to 95 % in 22.5 min
Detector	Sample wavelength 270 nm, bandwidth 40 nm
Samples	See table 1

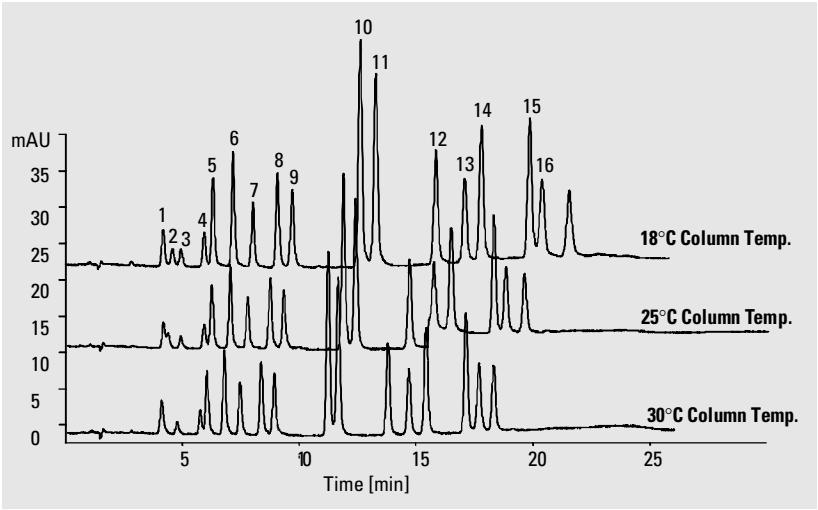


Figure 3
Separation of DIN/EPA standards at different column temperatures

Results

Impact of column temperature on separation

We investigated the impact of column temperature on the separation at three different temperatures: 30, 25 and 18 °C. The 16 EPA compounds could be separated at all temperatures (see figure 3), however a temperature of 18 °C had several advantages:

- The resolution between critical compound pairs such as benzo(ghi)perylene and indeno(1,2,3-cd)pyrene was better. This allowed trouble-free switching of excitation and emission wavelengths when using a programmable fluorescence detector.

- Additional interfering compounds such as anthrachinon could be separated from the PNAs. Figure 3 clearly shows the improved separation of PNAs far below 30 °C.

Peak #	Name of compound	RSD t_R (15 runs)	RSD area (15 runs)
1	Naphthalene	0.12%	1.41
2	Anthrachinon	0.05%	3.70
3	Acenaphthylene	0.10%	3.51
4	Acenaphthene	0.09%	3.71
5	Fluorene	0.08%	1.76
6	Phenanthrene	0.06%	1.72
7	Anthracene	0.05%	1.42
8	Fluoranthene	0.05%	1.40
9	Pyrene	0.05%	1.62
10	Benzo(a)anthracene	0.05%	1.59
11	Chrysene	0.06%	1.60
12	Benzo(b)fluoranthene	0.07%	1.90
13	Benzo(k)fluoranthene	0.08%	1.96
14	Benzo(a)pyrene	0,08%	1.76
15	Dibenzo(a,h)anthracene	0.09%	1.62
16	Benzo(ghi)perylene	0.09%	1.99
17	Indeno(123-cd)pyrene	0.11%	2.50

Table 1
Precision of retention times at 18 °C column temperature, with ambient temperature of 25 °C

Precision of retention times and peak areas

Stable retention times are important for correct identification of complex environmental matrices. When using a time-programmable fluorescence detector stable retention times are also important to avoid wavelength switching during an analyte's elution.

Precision of peak areas is important for obtaining reliable quantitative data.

Table 1 demonstrates typical RT precision of better than 0.2 %, obtained over 15 runs at 7 degrees below ambient. Peak-area precision for the low ng range is below 2 % RSD in general, if the peaks are well separated. It goes up to 4 % RSD if the peaks are not baseline separated.

Impact of vacuum degassing on fluorescence detection

PNAs can be quantified reproducibly down to the low picogram range with the correct fluorescence detection method. Careful selection of excitation and emission wavelengths and the use of mobile phase degassing, ensure high-sensitive PNA analysis. It is well known that the presence of oxygen in the mobile phase deteriorates detection limits because of quenching effects. Therefore thorough degassing is of utmost importance. We investigated the influence of no degas-sing, helium degassing and vacuum degassing on the response of critical compounds. As figure 4 demonstrates, the best results were achieved with vacuum degassing.

Spectral information with diode-array detection

For highly contamin-ated samples UV-Visible absorbance diode-array detection offers additional analytical tools using the spectral domain: peak-identity and peak-purity confirmation. Acenaphthylene (EPA compound) does not fluoresce, so for this compound UV-Visible absorption is the detection method of choice. An ideal detection method for PNAs is the serial detection with fluorescence and UV-Visible diode-array instrumentation. Here highest sensitivity is combined with optimum selectivity and additional identification tools for highly contaminated samples.

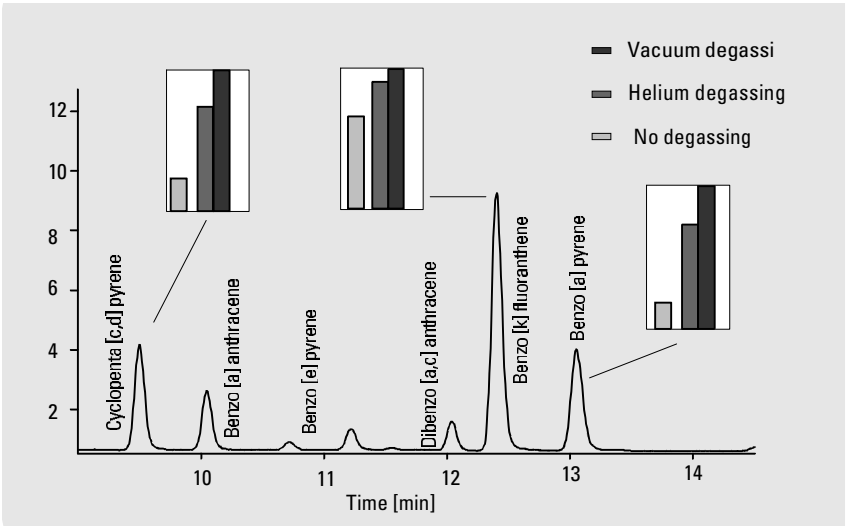


Figure 4
Quenching by dissolved oxygen can be avoided with a suitable mobile phase degassing technique

Automated positive identification of PNAs in soil with diode-array detection

Figure 5 shows the analysis of a soil extract using UV-Visible diode-array detection. The oven temperature was 22 °C. This still gave good resolution for early eluting peaks and allowed sensitive detection of late eluting peaks, for example, coronene.

The Agilent ChemStation's spectral search routine used here compares each spectrum with those stored in a spectral library compiled from analyses of standards run beforehand. The software recognizes those spectra that mach each other closely within the tolerance window specified. In those cases where a retention time has been tagged to the library records, the spectral match can be further qualified before being pronounced as specified. Figure 6 shows spectral overlay of sample spectra with library pyrene spectrum in the low mAU range. Normalization was done on the spectra library's spectra.

The complete method — HPLC separation with data acquisition, data evaluation, quantification, and identification — can be automated for multiple, unattended analyses. The reports that are generated include sample amounts, purity and library identity information.

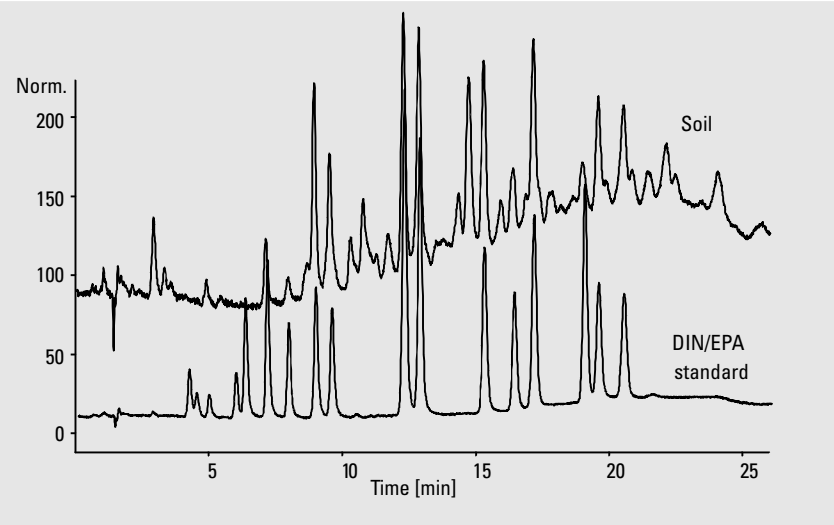
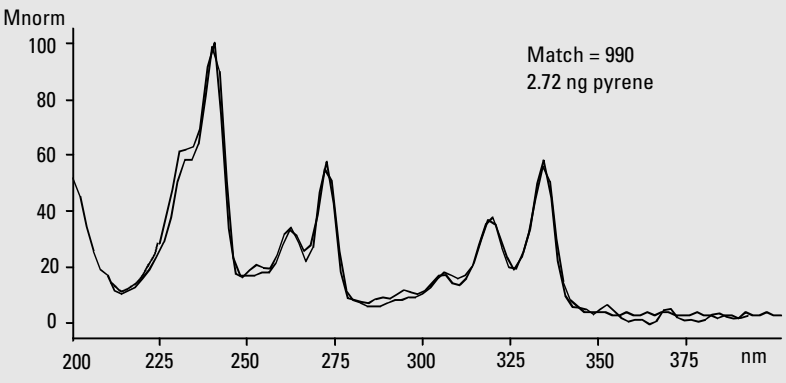


Figure 5
Analysis of PNAs in soil sample (extraction was made with super critical fluid extraction)1



Signal 1: DAD1 B, Sig=270,40 Ref=550,100

Calibrated compounds

Meas. RtTime [min]	Library RtTime [min]	CalTbl RtTime [min]	Sig	Amount [ng/ul]	Purity Factor	#	Library Match		Name
7.08	7.09	7.09	1	0.777	985	1	974		Phenanthrene
8.89	8.91	8.90	1	2.966	970	1	982		Fluoranthene
9.48	9.52	9.51	1	2.719	965	1	990		Pyrene
12.23	12.23	12.22	1	1.411	985	1	967		Benzo(a)anthracene
12.82	12.81	12.81	1	1.791	979	1	970		Chrysene
15.24	15.23	15.23	1	1.976	756	1	973		Benzo(b)fluoranthene
16.35	16.35	16.35	1	1.371	730	1	894	x	Benzo(k)fluoranthene
17.09	17.09	17.08	1	2.157	885	1	908	x	Benzo(a)pyrene
18.94	18.99	18.99	1	0.746	320	1	75	x	Dibenzo(ah)anthracene
19.52	19.50	19.50	1	1.907	920	1	969		Benzo(ghi)perylene
20.47	20.45	20.45	1	2.228	910	1	944	x	Indeno(123-cd)pyrene

Figure 6
Identification of pyrene using spectral library search with report containing quantitative and qualitative results

Conclusion

The new Agilent 1100 Series HPLC systems solved the problems usually associated with the analysis of PNAs.

The Peltier thermostatted column compartment allowed the analysis of PNAs at ambient and subambient column temperatures with high precision. Peltier cooling was preferred to water cooling systems, because no additional equipment was needed and control and setup was easier and convenient.

The vacuum degasser enabled quenching-free fluorescence detection of PNAs at lowest detection limits due to the highly-efficient removal of oxygen from the mobile phase.

The Agilent ChemStation for HPLC gave full automation capabilities starting with complete control of all modules, data acquisition, data evaluation and report presentation with spectrally confirmed *qualitative* and *quantitative* results.

The quaternary gradient pump, vacuum degasser and Peltier thermostatted column compartment provided excellent retention-time stability at a flow of 0.4 ml/min. This saved purchase and disposal costs for solvents and allowed to work with a factor of four lower sample volumes.

References

1
Helmut Schulenberg-Schell, Rainer Schuster (Agilent Technologies, Waldbronn, Germany), unpublished results presented as a poster at the *3rd International Capillary Chromatography Conference*, *Riva del Garda*, **1993**.

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