Agilent Application Solution

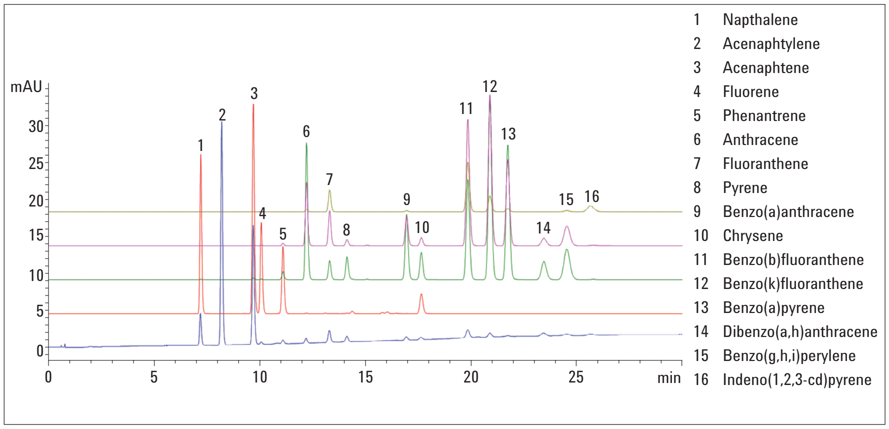
Analysis of PAHs in soil according to EPA 8310 method with UV and fluorescence detection

Application Note

Environmental

Authors

Sonja Volk,
Angelika Gratzfeld-Huesgen
Agilent Technologies, Inc.
Waldbronn, Germany



Abstract

Burning carboniferous materials releases copious amounts of smoke containing polynuclear aromatic hydrocarbons (PAHs). These PAHs were declared by the US Environmental Protection Agency (US EPA) as priority pollutants, suspected to be carcinogenic. This Application Note describes a reproducible method according to EPA 8310 for sample preparation and HPLC analysis of PAHs in soil with good recovery rates, and detection limits that fulfill regulatory requirements.

The HPLC method included both diode array and fluorescence detection modes. The method was developed on an Agilent 1260 Infinity LC system using a 4.6 × 150 mm, 5 µm column and validated including robustness testing. This conventional method was then transferred to an Agilent 1290 Infinity LC System using a 2.1 × 50 mm 1.8 µm column. Both methods were compared regarding precision, limit of detection (LOD) and limit of quantitation (LOQ).

Agilent Technologies

Introduction

Hydrocarbons with multiple ring structures are collectively referred to as polynuclear aromatic hydrocarbons, commonly abbreviated as PNAs or PAHs. This class of compounds is suspected to be mutagenic and/or contain many toxic chemicals. The carcinogenic properties of the PAHs led to legislative restrictions on their release into the environment. PAHs are generally produced by the incomplete combustion of organic material, for example, fossil fuels. Burning carboniferous materials release copious amounts of smoke containing polynuclear aromatic hydrocarbons (PAHs). Via atmospheric deposition PAHs also reach soil and sediments in areas remote from industry, airports and highways. In addition, oil spills due to accidents or careless oil handling are polluting factors.

In the last decades, legislation has introduced maximum permissible levels in many countries which, in turn, has awakened the need for suitable monitoring methods.

The guide values for contaminated sites in Baden-Württemberg, Germany (UM & SM BW, 1993) are classified as follows:

- **H-value - 1-3 mg/kg soil**
  Upper limit for background PAH levels in soil
- **PP-value - 10 mg/kg soil**
  Guidance value for PAH levels in soil for the protection of nutritive/forage crops and animals
- **PM$_1$-value - 5 mg/kg soil**
  Guidance values for PAH levels in soil for children's playgrounds
- **PM$_2$-value - 25 mg/kg soil**
  Guidance values for PAH levels in soil for settlement areas
- **PM$_3$-value - 100 mg/kg soil**
  Guidance values for PAH levels in soil for industrial real estates

Liquid chromatography displays significant advantages for the detection of low abundant analytes in complex matrixes:

- Simplified sample preparation compared to GC/MS analysis techniques
- Less risk of decomposition of high molecular weight analytes at ambient temperatures
- Selectivity and sensitivity of fluorescence and UV detection in series
- Multi-signal acquisition using status of the art fluorescence detectors
- Complementary diode-array UV-visible absorbance detection for the quantification of non-fluorescent compounds (such as Acenaphthylene) and quantification of co-eluting peaks at additional wavelengths.

Experimental

Instruments and Software

An Agilent 1260 Infinity Quaternary LC system consisting of the following modules was used:

- Agilent 1260 Infinity Binary Pump (G1312B)
- Agilent 1260 Infinity Autosampler (G1392B)
- Agilent 1290 Thermostatted Column Compartment (G1316A)
- Agilent 1260 Infinity Diode Array Detector (G4212B) with 10-mm Max-Light flow cell
- Agilent 1260 Infinity Series Fluorescence Detector (G1321A), equipped with standard FLD flow cell

The UHLC analysis was developed an performed using an Agilent 1290 Infinity LC system consisting of the following modules:

- Agilent 1290 Infinity Binary Pump (G4220A)
- Agilent 1290 Infinity Autosampler (G4226A)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity Diode Array Detector (G4212A) with 10-mm Max-Light flow cell

Software:

- Agilent ChemStation B.04.03
- Method translator

Standards and Samples

SS EPA 610 PAH Mix in Methanol/Methylene Chloride (1:1), (Supelco Analytical), containing the following PAHs:

Naphtalene
Acenaphtylene
Acenaphtene
Fluorene
Phenanthrene
Anthracene
Fluoranthene
Pyrene
Benzo(a)anthracene
Chrysene
Benzo(b)fluoranthene
Benzo(k)fluoranthene
Benzo(a)pyrene
Dibenzo(a,h)anthracene
Benzo(g,h,i)perylene
Indeno(1,2,3-cd)pyrene

Minor contaminated soil as blank sample

Acetonitrile, Hexane and Acetone were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak).

Chromatographic conditions

Parameter	**Agilent 1260 Infinity LC system**	**Agilent 1290 Infinity LC system**
Solvents:	A: Water, B: Acetonitrile	A: Water, B: Acetonitrile
Columns:	Agilent ZORBAX Eclipse PAH, 4.6 × 150 mm, 5 µm	Agilent ZORBAX Eclipse PAH Rapid Resolution HT, 2.1 x 50 mm, 1.8 µm
Gradient:	0 min - 40 % B, 20 min - 95 % B	0 min–40 % B, 4 min - 95 % B
Flow rate:	1.5 mL/min	0.85 mL/min
Injection volume:	3 µL	0.3 µL
DAD:	230 nm, reference 400 nm	
FLD:	Multisignal acquisition, set at λ_{ex} = 260 and λ_{em} = 350 (FLD A), 420 (FLD B), 440 (FLD C), 500 (FLD D)	

Sample preparation – Soil samples

5 g homogenated soil was spiked with four amounts of PAH standard mix diluted in 2 mL acetonitrile:

1. 0 µg PAH standard (blank)
2. 5 µg PAH standard (low spike equivalent to 1 mg/kg soil)
3. 50 µg PAH standard (middle spike equivalent to 10 mg/kg soil)
4. 500 µg PAH standard (high spike equivalent to 100 mg/kg soil)

Classic extraction method after EPA 3550C

The PAHs were extracted by adding 15 mL hexane/acetone (4:1) and 3 minutes sonication (repeating this procedure three times). The extracts were combined and centrifuged at 4,000 rpm for 5 minutes. The samples were concentrated and exchanged to acetonitrile with a rotary evaporator to 5 mL total volume and analyzed with HPLC/UHPLC. Blank and low spike samples were analyzed directly, whereas middle spike and high spike samples had to be diluted 1:10 and 1:100, respectively, before HPLC measurement. Thus the peak intensities were within the calibration curve, enabling exact quantification.

Results and Discussion

Linearity

Linearity and precision were determined by analysis of a dilution series of the standard PAH mix in Acetonitrile, ranging from 19.3 ng/µL to 8.8 pg/µL total PAH content in eight dilution steps (1:3 dilution). To cover a broad concentration range, a dilution series of eight PAH concentrations was prepared in ACN and analyzed by HPLC with an Agilent 1260 Infinity LC system. Figure 1 shows the correlation curves of the dilution series analysis. Four of five measured signals are presented in Figure 1: FLD1 A, FLD1 B, FLD1 C ,and DAD1 A. All correlation curves showed high linearity with correlation factors over 0.9999.

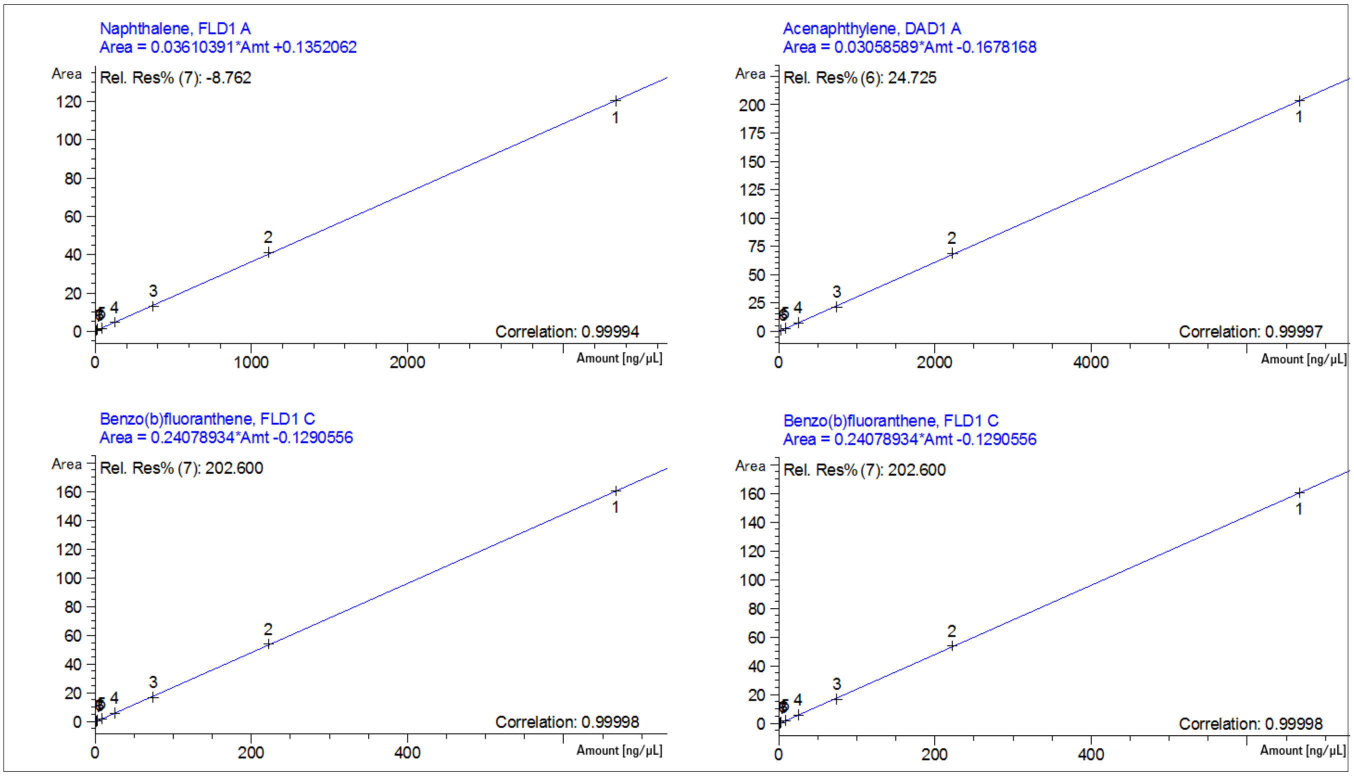


Figure 1
Linearity of the Agilent 1260 Inifnity LC system for PAH analysis, represented from four signals: FLD1 A, FLD1 B, FLD1 C and DAD1 A.

Precision of retention time (RT) and area

The precisions of the retention times, displayed as relative standard deviation in %, are depicted in Figure 2. All RSD values were under 0.2 %.

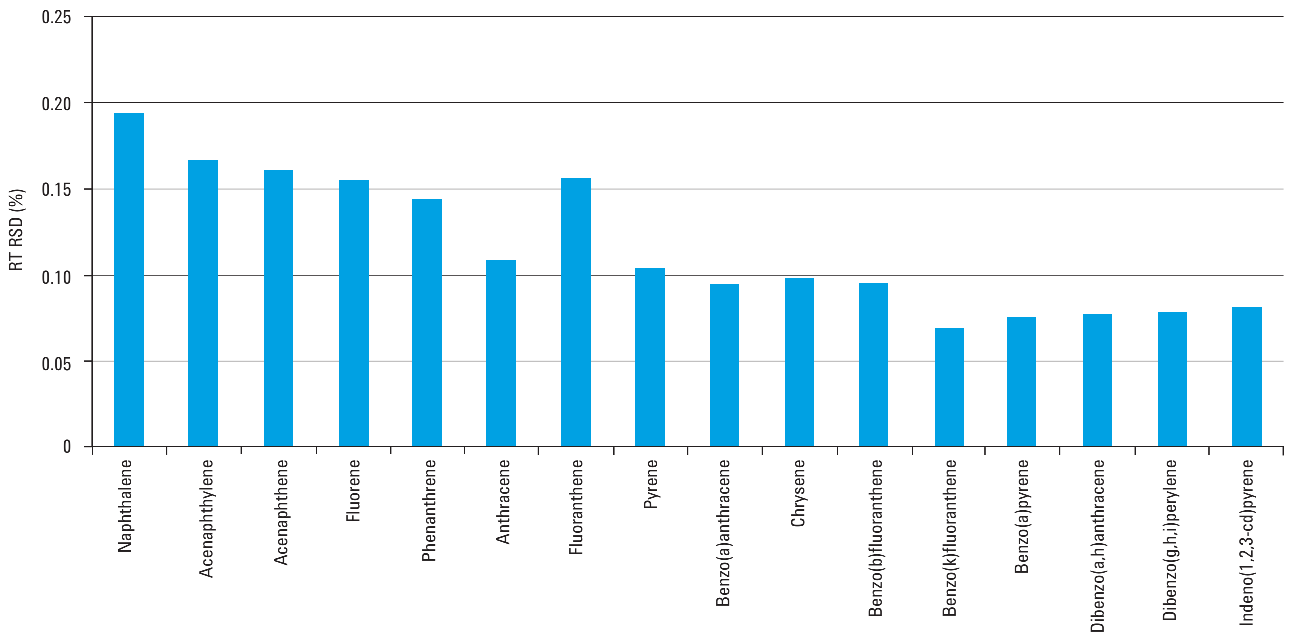


Figure 2
Precision of retention times of the single PAH analyte, displayed as relative standard deviation (RSD), using the Agilent 1260 Infinity LC system .

Precision of area, displayed as relative standard deviation in % were found to be smaller than 2% for the four highest dilutions; see Figure 3.

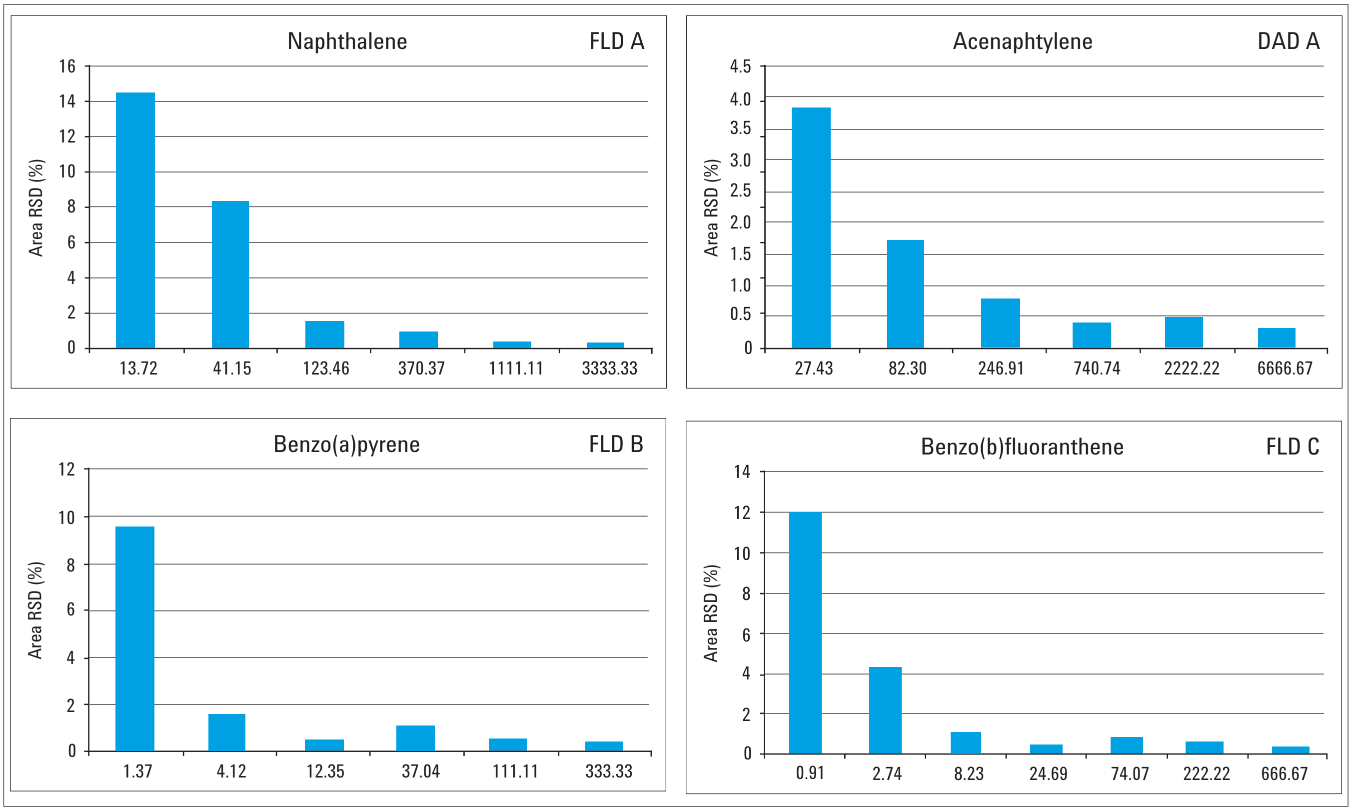


Figure 3
Precision of area, using the Agilent 1260 Infinity LC system.

Limit of Detection (LOD), Limit of Quantification (LOQ), and Resolution

The limits of detection and quantification were evaluated from the concentration of PAHs required to give at least a signal to noise ratio of 3 and 10, respectively. Resolution over 2.0 was considered as required for the efficient separation of the individual peaks.

Robustness

Robustness was evaluated using the highest sample amount (19.3 ng/µL) of the concentration series. The limit for the variations of retention time and amount were clarified at 5%. The evaluation of robustness of the Agilent 1260 Infinity LC method led to the finding, that the parameters injection amount and gradient slope are critical for the exact determination of the sample amount. Table 2 shows the results of the robustness tests for the PAHs Naphthalene and Benzo(a)-anthracene as an example. Values above limits are marked red.

RT	Signal	Compound	Lvl	LOD [pg/µL]	LOQ [pg/µL]	Resolution
7.24	FLD A	Naphthalene	7	17.29	51.86	7.41
8.24	DAD A	Acenaphthylene	6	50.29	150.86	6.32
9.72	FLD A	Acenaphthene	7	15.83	47.49	14.50
10.10	FLD A	Fluorene	6	8.39	25.17	2.05
11.11	FLD A	Phenanthrene	6	6.25	18.76	5.19
12.22	FLD B	Anthracene	7	0.97	2.91	2.62
13.34	FLD C	Fluoranthene	6	7.82	23.47	5.00
14.16	FLD B	Pyrene	5	10.10	30.29	3.44
16.95	FLD B	Benzo(a)anthracene	6	2.97	8.90	4.83
17.63	FLD B	Chrysene	5	7.25	21.74	2.57
19.82	FLD C	Benzo(b)fluoranthene	7	2.26	6.77	7.77
20.86	FLD B	Benzo(k)fluoranthene	7	0.82	2.47	3.53
21.68	FLD B	Benzo(a)pyrene	6	1.74	5.23	2.69
23.37	FLD B	Dibenzo(a,h)anthracene	5	21.68	65.05	4.34
24.47	FLD B	Benzo(g,h,i)perylene	5	18.83	56.49	2.20
25.75	FLD D	Indeno(1,2,3-cd)pyrene	3	25.10	75.29	1.96

Table 1
LOD, LOQ and resolution for the 16 PAHs.

Parameters changed	Changes	% Deviation for RT Naphthalene	% Deviation for RT Benzo(a)anthracene	% Deviation limits	% Deviation of amount Naphthalene	% Deviation of amount Benzo(a)anthracene	% Deviation limits
Flow ± 2% standard: 1.5 mL/min	High: 1.53 mL/min	1.3	0.7	5%	3.8	3.1	5%
	Low: 1.47 mL/min	1.4	0.7	5%	0.6	0.8	5%
TCC ± 5% standard: 25 °C	High: 26.25 °C	1.0	1.5	5%	1.4	0.4	5%
	Low: 23.75 °C	0.9	1.6	5%	2.9	4.0	
Injection ± 5% standard: 3 µL	High: 3.15 µL	0.1	0.01	5%	4.2	3.4	5%
	Low: 2.85 µL	0.1	0.04	5%	6.2	7.0	5%
Gradient slope ± 10%	+ 10%	3.6	6.9	5%	1.7	2.8	5%
	– 10%	0.3	0.6	5%	1.5	2.2	5%
Wavelength ± 3 nm	DAD – 233 nm FLDex – 263 nm	0.02	0.11	5%	3.6	0.9	5%
	DAD – 227 nm FLDex – 257 nm	0.03	0.08	5%	1.9	2.6	5%

Table 2
Robustness tests for Naphtalene and Benzo(a)anthracene.

Method transfer to UHPLC

The HPLC method, conducted with the Agilent 1260 Infinity LC system, was transferred to an Agilent 1290 Infinity LC System using the Method Translator 1.0. The method was manually optimized with regards to resolution and speed. Figure 4 shows the chromatogram of the UHPLC analysis of the PAH standard mix. The UHPLC gradient was only 4 minutes with a total run time of 6 minutes, which resulted in an over 5-fold saving of time and a nearly 10-fold saving of eluent compared to the normal HPLC run using the Agilent 1260 Infinity LC system.

Peak identification

1 Napthalene
2 Acenaphtylene
3 Acenaphtene
4 Fluorene
5 Phenantrene
6 Anthracene
7 Fluoranthene
8 Pyrene
9 Benzo(a)anthracene
10 Chrysene
11 Benzo(b)fluoranthene
12 Benzo(k)fluoranthene
13 Benzo(a)pyrene
14 Dibenzo(a,h)anthracene
15 Benzo(g,h,i)perylene
16 Indeno(1,2,3-cd)pyrene

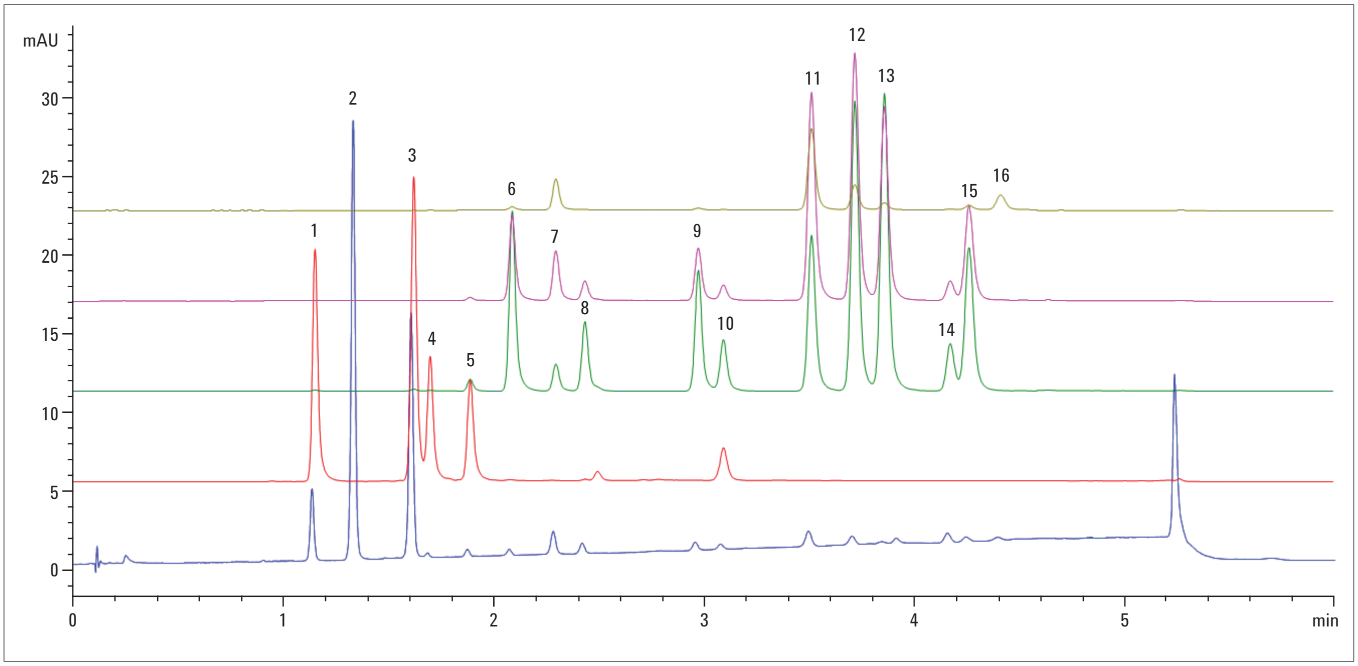


Figure 4

UHPLC analysis of the PAH standard mix using the Agilent 1260 Infinity LC system.

Table 3 displays LOD, LOQ, resolution values, RSD (%) of retention time, and area for the UHPLC method. LOD and LOQ show improved values compared to the standard HPLC method. The resolution was found to be over 2 for most of the compounds. For Fluorene, Chrysene, and Benzo(g,h,i)perylene it was between 1 and 2. The UHPLC method showed very high precision for all PAHs with very low relative standard deviations smaller 0.05 % for retention time and area. Consequently, a transfer to UHPLC is possible and is even the more sensitive and reproducible method based on the improved LOD and LOQ values. Sensitivity is improved by a mean factor over all PAHs of about six. Reproducibility is improved by a factor of up to 100 using the UHPLC method.

RT	Signal	Compound	Lvl	LOD [pg/µL]	LOQ [pg/µL]	Resolution	RSD (%) RT	RSD (%) area
1.64	FLD1 A	Naphthalene	4	6.00	18.01	n.a.	0.0561	0.02
1.35	DAD1 A	Acenaphthylene	4	36.70	110.11	5.59	0.0005	0.04
2.10	FLD1 A	Acenaphthene	4	0.70	2.10	9.43	0.0006	0.02
2.99	FLD1 A	Fluorene	4	1.04	3.12	1.50	0.0007	0.05
3.87	FLD1 A	Phenanthrene	4	0.84	2.51	3.48	0.0006	0.03
3.53	FLD1 B	Anthracene	4	0.70	2.09	2.31	0.0007	0.02
4.28	FLD1 C	Fluoranthene	4	1.71	5.14	3.68	0.0008	0.01
3.73	FLD1 B	Pyrene	4	0.43	1.30	6.04	0.0006	0.02
3.11	FLD1 B	Benzo(a)anthracene	4	2.45	7.34	8.99	0.0004	0.00
4.19	FLD1 B	Chrysene	4	5.27	15.81	1.91	0.0006	0.01
2.31	FLD1 C	Benzo(b)fluoranthene	4	5.58	16.74	6.27	0.0003	0.02
1.71	FLD1 B	Benzo(k)fluoranthene	4	2.77	8.31	3.02	0.0004	0.01
4.41	FLD1 B	Benzo(a)pyrene	4	13.02	39.07	2.03	0.0005	0.03
1.17	FLD1 B	Dibenzo(a,h)anthracene	4	7.79	23.36	4.44	0.0005	0.01
1.90	FLD1 B	Benzo(g,h,i)perylene	4	1.78	5.34	1.20	0.0005	0.02
2.45	FLD1 D	Indeno(1,2,3-cd)pyrene	4	1.80	5.41	3.07	0.0053	0.40

Table 3

Summary UHPLC method using the Agilent 1260 Infinity LC system.

Soil samples

Low contaminated soil from rural areas outside towns or villages was used as sample for the analysis of PAHs. The soil was spiked with three amounts of PAH mix: 1 mg/kg, 10 mg/kg, and 100 mg/kg soil. The spike-in amounts were chosen according to the guide values for contaminated sites in Baden-Württemberg with 1–3 mg/kg soil as a background value for non-contaminated sites up to 100 mg/kg value for industrial real estates. The background, analyzed with blank soil without spike-in is depicted in Figure 5. Except for most of the volatile PAHs, all PAHs are already detectable in the blank sample without spike-in. In the blank soil sample, a total PAH concentration of 0.63 mg/kg soil was found, which indicates very low PAH contaminated soil.

Peak identification

1	Napthalene
2	Acenaphtylene
3	Acenaphtene
4	Fluorene
5	Phenantrene
6	Anthracene
7	Fluoranthene
8	Pyrene
9	Benzo(a)anthracene
10	Chrysene
11	Benzo(b)fluoranthene
12	Benzo(k)fluoranthene
13	Benzo(a)pyrene
14	Dibenzo(a,h)anthracene
15	Benzo(g,h,i)perylene
16	Indeno(1,2,3-cd)pyrene

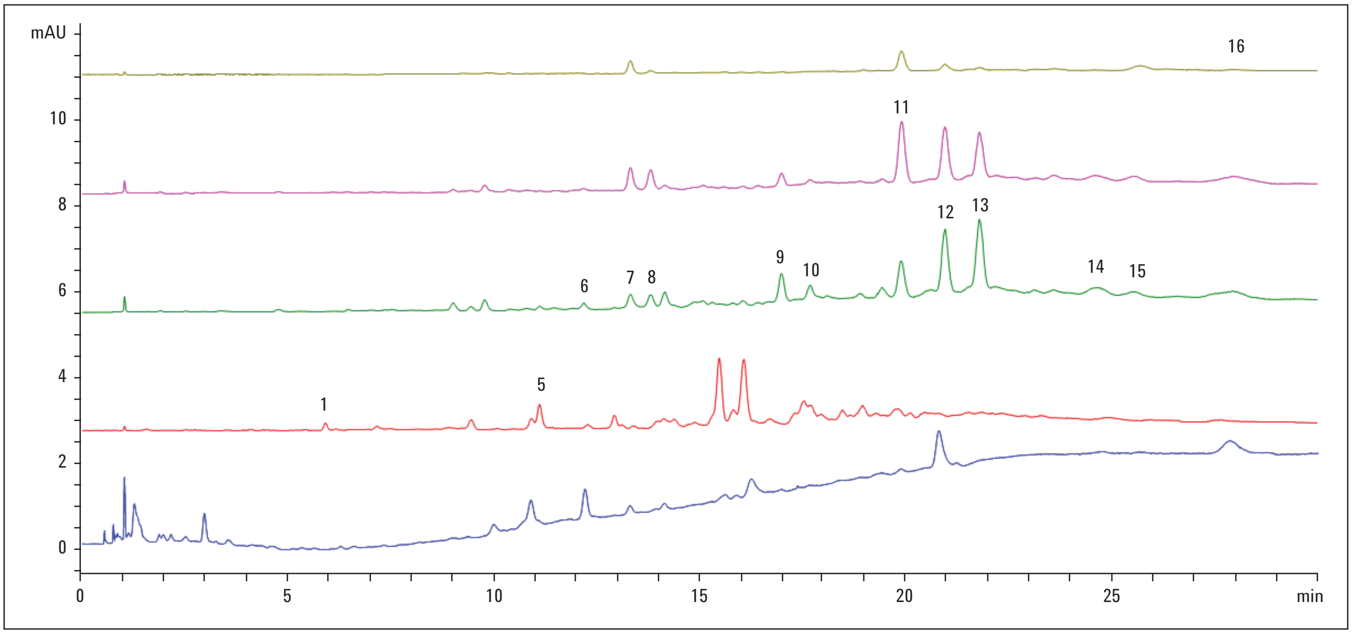


Figure 5
Blank PAH background in soil.

The recoveries for all three spiked samples were around 85 % after the extraction procedure (see Table 4), with a loss of 15 % mostly due to evaporated volatile compounds like Napthalene, Acenaphtylene, Acenaphtene and other small PAH compounds.

Spike-in amount	Recovery
1 mg/kg	87 %
10 mg/kg	85 %
100 mg/kg	84 %

Table 4
Recovery of PAH extraction out of soil.

Conclusion

The Application Note proves the applicability of analyzing PAHs with Agilent 1260 and 1290 Infinity LC systems. Sample preparation according to EPA 3550C (including extraction and concentration using a rotary evaporator) resulted in 85% recovery. Together with the good linearity and precision of the Infinity LC systems, accurate quantification of PAHs in soil is feasible.

References

1.
Stoffverhalten von Gaswerkspezifischen Polycyclischen Aromatischen-Kohlenwasserstoffen (PAK), Landesanstalt für Umweltschutz Baden-Württemberg, Karlsruhe (1997).

2.
K. Theo von der Trenck, Joachim Ruf und Manfred Flittner, “Guide values for contaminated sites in Baden-Württemberg”, ESPR – Environ. Sci. & Pollut. Res. 1 (4) 253-261 (1994).

www.agilent.com/chem

Published in USA, September 1, 2011
Publication Number 5990-8414EN

Agilent Technologies