Application Note

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Optimized GC/MS Analysis for PAHs in Challenging Matrices

Using the Agilent 5977 Series GC/MSD with JetClean and midcolumn backflush

Authors

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Abstract

The Agilent 8890 GC combined with an Agilent 5977 Series MSD system was used for the analysis of polycyclic aromatic hydrocarbons (PAHs). By proper selection of instrument configuration and operating conditions, the system provides a robust means of analyzing PAHs in difficult matrices. Midcolumn backflushing, continuous hydrogen source cleaning (JetClean), and use of an alternative drawout lens result in excellent linearity across a calibration range of 1 to 1,000 pg. System precision and robustness are demonstrated with replicate injections of an extract from high organic content soil.

Introduction

PAHs are toxic to aquatic life, and are suspected human carcinogens. Because they originate from multiple sources, they are widely distributed as contaminants throughout the world.

PAHs originate from three sources:

- **Petrogenic:** Derived from petroleum inputs associated with fossil fuels
- **Pyrogenic:** Derived from combustion sources
- **Biogenic:** Formed from natural biological processes

Given their ubiquitous nature, they are monitored as trace contaminants in many different food products ranging from seafood to edible oils to smoked meats. They are also monitored in the environment including air, water, and soil. PAHs have been analyzed by multiple techniques including HPLC/UV, GC/FID, GC/MS, or GC/MS/MS.

This Application Note focuses on GC/MS in SIM mode. A common calibration range is from 1 to 1,000 pg with an acceptable linearity of R^2 >0.99. Internal standard (ISTD) area reproducibility is typically specified at ±20 % with calibration standards, and ±30 % with samples.

A number of issues arise with the analysis due to the properties of PAHs. They span wide molecular weight and boiling temperature ranges. Although not considered active or subject to degradation, they are sticky, and readily adhere to surfaces. PAHs are subject to desublimation (deposition), and are difficult to vaporize. High temperatures and minimizing surface contact are important. Peak tailing is often seen on the later eluters, resulting in manual integration and extending data review. In some cases, the ISTD response is inconsistent across the calibration range, and can lead to problems with linearity of the method.

In addition to the PAH-related challenges, there are often matrix-related problems with the analysis. For example, in food and soil analyses, high boiling matrix contaminants that elute after the analytes can require extended bakeout times to prevent ghost peaks in subsequent runs. The highest boiling contaminants can deposit in the head of the column, requiring more frequent column trimming and adjustment of SIM and data analysis time windows from the resulting retention time shift.

Experimental

This system was configured to minimize the potential problems with the analysis of PAHs in high-matrix samples. The important techniques used were:

- **Agilent JetClean:** This option on the 5977 Series GC/MSD system provides a low continuous flow of hydrogen (0.33 mL/min) into the source during the analysis. Continuous cleaning of the source with hydrogen has been demonstrated[1-3] to significantly improve calibration linearity and precision of response over time for PAH analysis. The need for manual source cleaning, especially with high-matrix samples, is substantially reduced.
- **9 mm extractor lens:** The Agilent extractor source provides additional flexibility to meet the specific needs of different analytical challenges. For the analysis of PAHs, a 9 mm extraction lens provides a good choice to minimize the surfaces available for deposition of the PAHs, and contributes, with JetClean, to providing better linearity, precision, and peak shapes.
- **Midcolumn backflushing:** Backflushing is a technique where the carrier gas flow is reversed after the last analyte has exited the column. After the MS data are collected, the oven is held at the final temperature in post run mode, and the carrier gas flow through the first column is reversed. This reversed flow carries any high boilers that were in the column at the end of data collection out of the head of the column and into the split vent trap. The capability to reverse the flow is provided by the Agilent Purged Ultimate Union (PUU). The PUU is a tee inserted, in this case, between two identical 15 m columns. During the analysis, a small makeup flow of carrier gas from the 8890 pneumatic switching device (PSD) module is used to sweep the connection. During backflushing, the makeup flow from the PSD is raised to a much higher value, sweeping high boilers backwards out of the first of column and forwards from the second. For this configuration, the backflushing time was 1.5 minutes.
- **8890 PSD module:** The PSD is an 8890 pneumatics module optimized for backflushing applications. During backflushing, it significantly reduces the flow of helium used compared to previous configurations. The PSD provides for seamless pulsed injections and simpler setup of backflush.

Figure 1 shows the system configuration used.

Tables 1 and 2 list the instrument operating parameters. Instrument temperatures must be kept high enough to prevent deposition of the highest boiling PAHs. The inlet and MSD transfer line are maintained at 320 °C. The MS source should be a minimum of 320 °C.

Pulsed splitless injections are used to maximize transfer of the PAHs, especially the heavy ones, into the column. The straight bore 4 mm liner with glass wool is a must. The wool transfers heat to the PAHs and blocks the line of sight to the inlet base. If the PAHs condense on the inlet base, they are difficult to vaporize, and sweep back into the column.

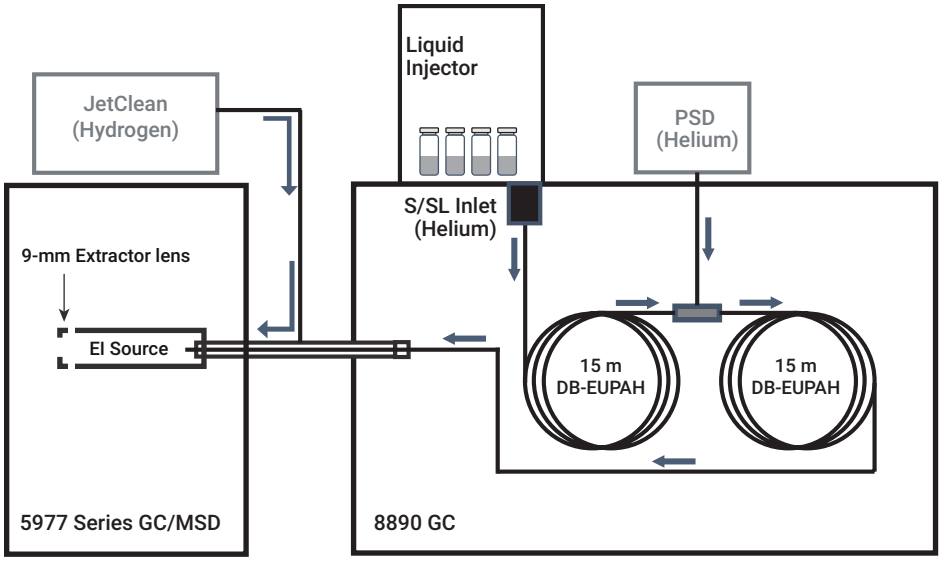

Figure 1. System configuration.

Table 1. GC and MS conditions for the PAH analysis.

8890 GC with fast oven, autoinjector, and tray	
Inlet	EPC Split/splitless
Mode	Pulsed Splitless
Injection pulse pressure	50 psi until 0.7 minutes
Purge flow to split vent	50 mL/min at 0.75 minutes
Septum purge flow mode	Standard
Injection volume	1.0 µL
Inlet temperature	320 °C
Carrier gas	Helium
Inlet liner	Agilent 4 mm single taper, with glass wool (p/n 5190-2293)
Oven	80 °C for 1 minute, 25 °C/min to 200 °C, 8 °C/min to 335 °C, hold 6.325 minutes Total run time: 29 minutes Post run time: 1.5 minutes Equilibration time: 0.5 minutes
Column 1	DB-EUPAH, 0.25 mm × 15 m, 0.25 µm (custom ordered)
Control mode	Constant flow, 0.9272 mL/min
Inlet connection	Split/Splitless
Outlet connection	PSD (PUU)
Post run flow (backflushing)	–12.027 mL/min
Column 2	DB-EUPAH, 0.25 mm × 15 m, 0.25 µm (custom ordered)
Control mode	Constant flow, 1.1272 mL/min
Inlet connection	PUU
Outlet connection	MSD
Post run flow (backflushing)	12.518 mL/min

5977 Series GC/MSD	
Source	Inert Extractor
Drawout lens	9 mm
Vacuum pump	Performance turbo
Tune file	Atune.U
Mode	SIM
Solvent delay	4 minutes
EM voltage gain mode	1.0
TID	on
Quadrupole temperature	150 °C
Source temperature	320 °C
Transfer line temperature	320 °C
JetClean mode	Acquire and Clean
JetClean hydrogen flow	0.33 mL/min

PAH calibration standards were diluted from an Agilent PAH Analyzer calibration kit (p/n G3440-85009) using isooctane. The kit contains a stock solution of 27 PAHs at 10 µg/mL and a stock solution of five ISTDs at 50 µg/mL. Seven calibration levels were prepared: 1, 2, 10, 20, 100, 200, and 1,000 ng/mL. Each level also contained 500 ng/mL of the ISTDs. See Table 2 and Figure 2 for compound identifications.

A sample of sedge peat (Garden Magic, Michigan Peat Company, Houston, TX) was dried at 120 °C overnight. Five grams of the dried peat were extracted overnight with 30 mL of dichloromethane/acetone (1:1 v:v) with agitation. The extract was filtered, and the filtrate was reduced 7.5 times in volume by evaporation. The resulting extract was used for the robustness experiments.

Table 2. SIM ions used for quantifier and qualifiers.

Compound	RT (min)	Quantifier	Qualifier 1	Qualifier 2	Qualifier 3
Naphthalene-d_8	5.126	136	134	108	
Naphthalene	5.149	128	127	129	102
1-Methylnaphthalene	5.758	142	141	115	139
2-Methylnaphthalene	5.926	142	141	115	143
Biphenyl	6.304	154	153	76	155
2,6-Dimethylnaphthalene	6.346	156	141	155	115
Acenaphthylene	7.042	152	151	153	76
Acenaphthene-d_{10}	7.150	164	80		
Acenaphthene	7.204	153	154	151	155
2,3,5-Trimethylnaphthalene	7.416	170	155	169	153
Fluorene	7.912	166	165	163	167
Dibenzothiophene	9.675	184	185	139	152
Phenanthrene-d_{10}	9.881	188	189		
Phenanthrene	9.935	178	179	177	152
Anthracene	10.002	178	179	177	152
1-Methylphenanthrene	11.282	192	191	193	190
Fluoranthene	12.952	202	203	201	101
Pyrene	13.764	202	203	201	101
Benz[a]anthracene	17.215	228	226	229	114
Chrysene-d_{12}	17.381	240	236		
Chrysene	17.474	228	226	229	114
Benzo[b]fluoranthene	20.461	252	126		
Benzo[k]fluoranthene	20.528	252	126		
Benzo[j]fluoranthene	20.624	252	126		
Benzo[e]pyrene	21.494	252	253	126	250
Benzo[a]pyrene	21.631	252	253	250	126
Perylene-d_{12}	21.889	264	260		
Perylene	21.966	252	253	126	250
Dibenz[a,c]anthracene	24.460	278	279	139	138
Dibenz[a,h]anthracene	24.588	278	279	139	138
Indeno[1,2,3-cd]pyrene	24.622	276	138	277	137
Benzo[ghi]perylene	25.778	276	138	277	137

Results and discussion

Initial calibration

Figure 2 shows the SIM TIC of the 100 pg/µL calibration standard. With the parameters chosen, the peak shapes for all PAHs, especially the latest ones, are very good.

The use of the 9 mm lens and continuous hydrogen cleaning often results in a reduced signal-to-noise ratio (S/N), so it is important to check the lowest desired calibration level. As an example, Figure 3 shows the response at the quantifier ion for several of the compounds at the 1 pg level. All analytes at the 1 pg level had sufficient signal for calibration.

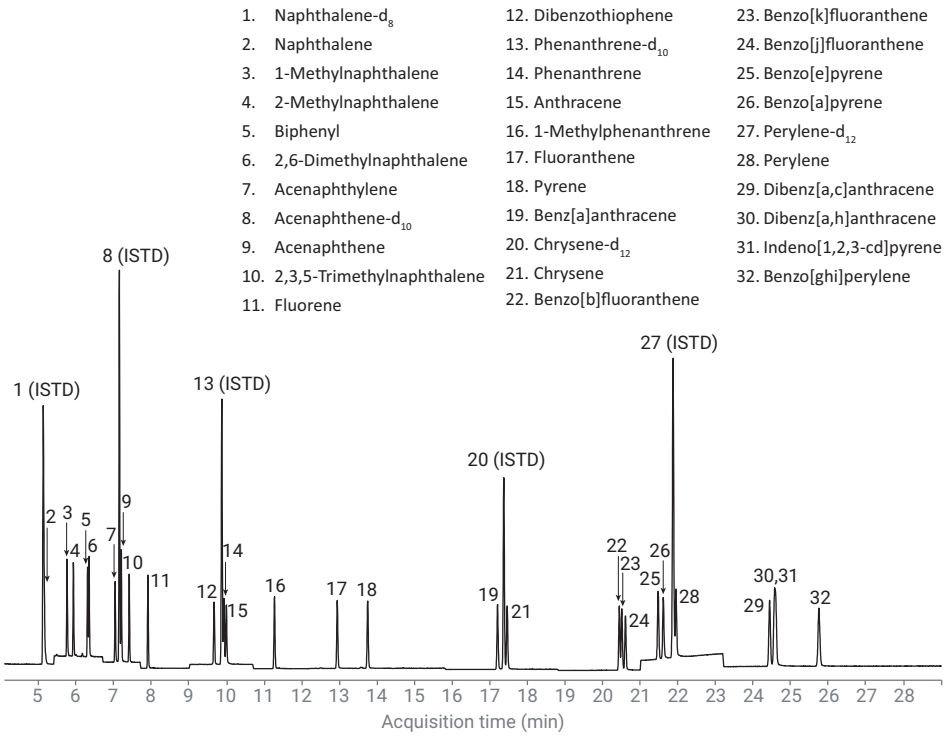

Figure 2. SIM TIC of the 100 pg/µL calibration standard.

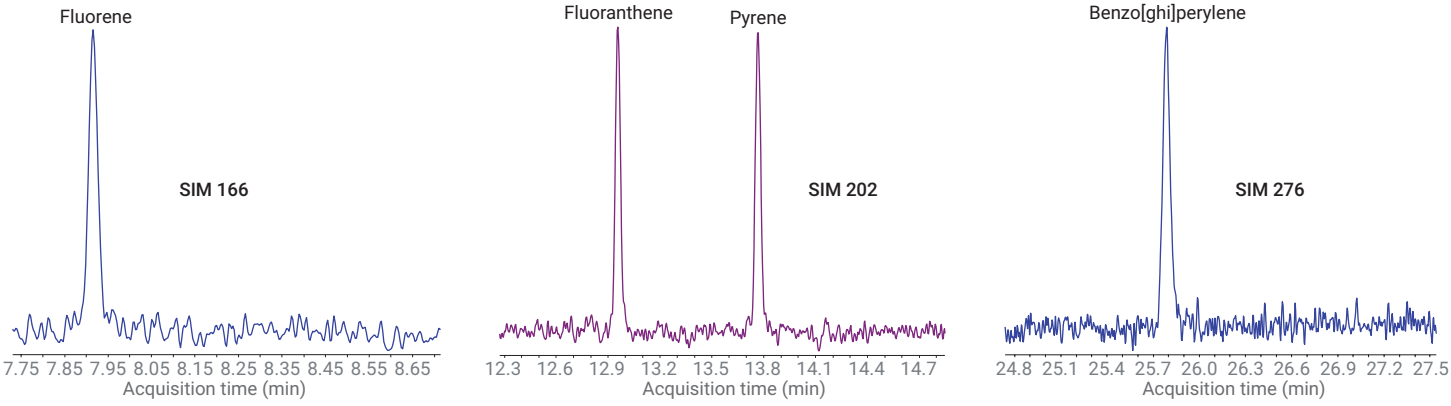

Figure 3. Response at quantifier ion for select compounds in the lowest calibration standard (1 pg).

Table 3 shows the R^2 values for three ISTD calibrations of the system with seven levels from 1 to 1,000 pg. All analytes show excellent linearity across the entire range.

Stability of response

Figure 4 shows the precision of ISTD peak responses for 60 sequential replicate injections of the 100 pg standard. The RSDs of the ISTD areas were:

- Naphthalene-d_8 (3.3 %)
- Acenaphthene-d_{10} (3.2 %)
- Phenanthrene-d_{10} (3.4 %)
- Chrysene-d_{12} (2.7 %)
- Perylene-d_{12} (2.0 %)

Table 3. R^2 values of three seven-level ISTD calibrations, 1 to 1,000 pg SIM.

Compound	RT (min)	Calibration 1	Calibration 2	Calibration 3
Naphthalene	5.149	1.0000	1.0000	1.0000
1-methylnaphthalene	5.758	1.0000	1.0000	1.0000
2-Methylnaphthalene	5.926	1.0000	1.0000	1.0000
Biphenyl	6.304	0.9998	0.9998	0.9998
2,6-dimethylnaphthalene	6.346	1.0000	1.0000	1.0000
Acenaphthylene	7.042	1.0000	1.0000	1.0000
Acenaphthene	7.204	1.0000	1.0000	1.0000
2,3,5-trimethylnaphthalene	7.416	1.0000	1.0000	1.0000
Fluorene	7.912	1.0000	1.0000	1.0000
Dibenzothiophene	9.675	1.0000	1.0000	1.0000
Phenanthrene	9.935	1.0000	1.0000	1.0000
Anthracene	10.002	1.0000	1.0000	1.0000
1-methylphenanthrene	11.282	1.0000	1.0000	1.0000
Fluoranthene	12.952	1.0000	0.9999	1.0000
Pyrene	13.764	1.0000	0.9999	1.0000
Benz[a]anthracene	17.215	1.0000	1.0000	1.0000
Chrysene	17.474	1.0000	1.0000	1.0000
Benzo[b]fluoranthene	20.461	1.0000	1.0000	0.9999
Benzo[k]fluoranthene	20.528	1.0000	1.0000	1.0000
Benzo[j]fluoranthene	20.624	1.0000	0.9999	0.9999
Benzo[e]pyrene	21.494	1.0000	1.0000	1.0000
Benzo[a]pyrene	21.631	1.0000	1.0000	1.0000
Perylene	21.966	1.0000	1.0000	1.0000
Dibenz[a,c]anthracene	24.460	1.0000	1.0000	0.9999
Dibenz[a,h]anthracene	24.588	0.9999	0.9999	0.9997
Indeno[1,2,3-cd]pyrene	24.622	1.0000	1.0000	0.9998
Benzo[ghi]perylene	25.778	1.0000	1.0000	1.0000

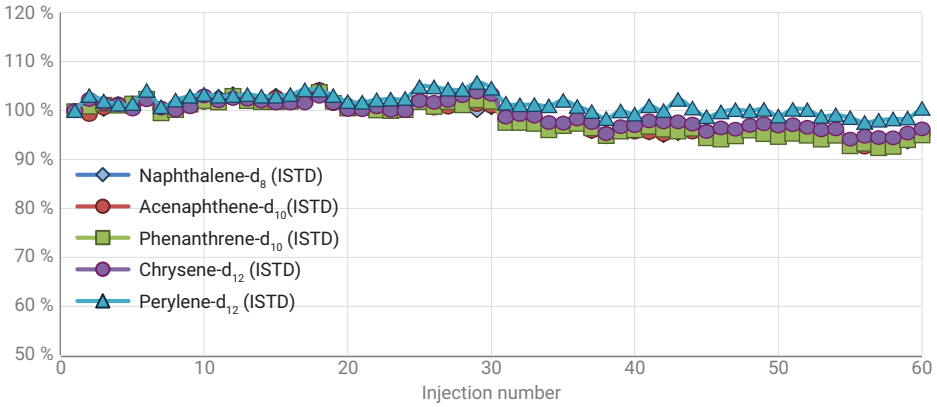

Figure 4. ISTD response stability over 60 injections for a 100 pg calibration standard. Areas are normalized to that of the first injection.

Figure 5 shows the calculated concentration for several analytes in the 60 sequential replicate runs of the 100 pg standard. The system exhibits excellent stability of response. The average RSD of the calculated concentrations for all 27 analytes is 1.1 %.

Stability of response with soil extracts

The soil extract used for the robustness test was deliberately chosen to have a high-matrix content to challenge the system. Figure 6 compares the scan TIC of the extract to that of the 100 pg PAH standard. The soil extract has a very high level of matrix. Note that, for soils with this level of organic content, further sample cleanup should be considered for routine analysis. The sample preparation used was for test purposes only.

To test the robustness of the system, the soil extract was spiked with 100 pg each of the 27 analytes and 500 pg each of the ISTDs. The spiked extract was then injected 60 times. The PAHs were quantitated against the solvent-based calibration curve for each run, and the resulting calculated concentrations were plotted. Figure 7 shows the calculated concentrations for several of the analytes. Naphthalene and benzo[ghi]perylene both show measured concentrations higher than the spiked 100 pg level. These compounds were found to be present in the soil at levels roughly corresponding to the offset in Figure 7. Perylene (not shown) was found at almost 200 pg in the soil.

The average RSD for the calculated concentrations of all 27 analytes was 4.4 %. For 22 of the 27 analytes, the calculated concentration was within 20 % after 60 soil shots, compared to the first injection in the soil. As expected, the heaviest analytes, such as benzo[ghi]perylene, lost response quickest.

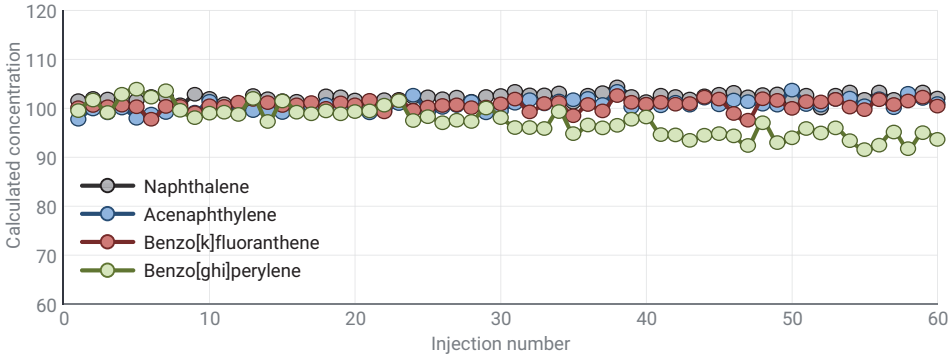

Figure 5. Stability of calculated concentrations over 60 sequential injections for a 100 pg calibration standard.

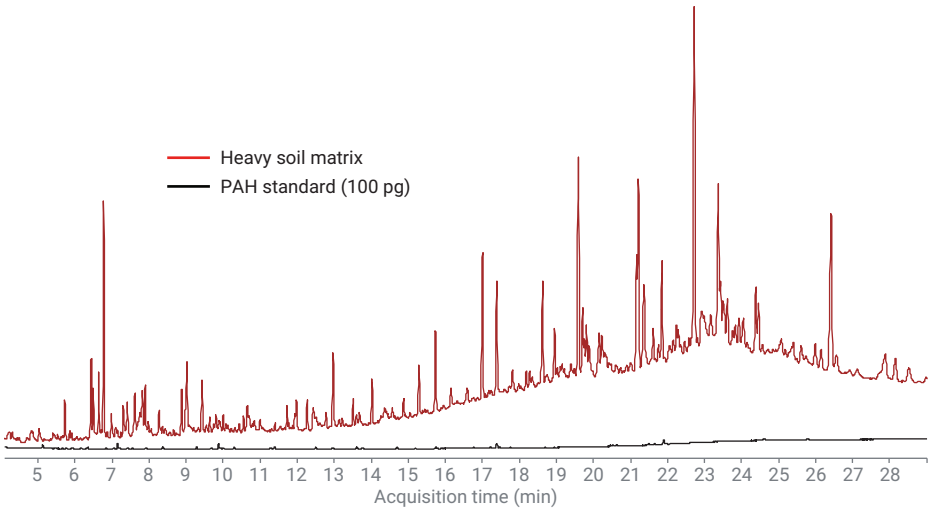

Figure 6. Scan TIC of soil extract and PAH 100 pg standard with 500 pg ISTDs, both drawn in the same scale, showing a large amount of material in the extract.

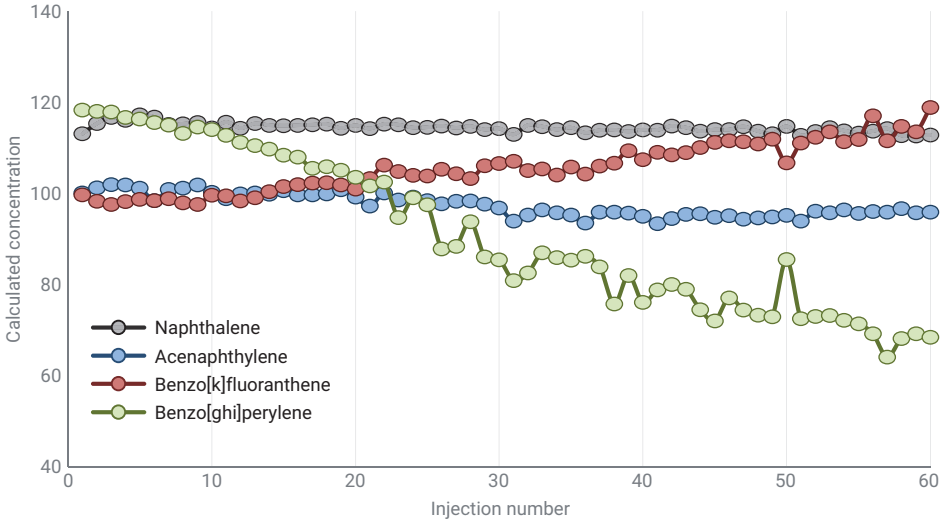

Figure 7. Stability of calculated concentrations over 60 injections of a soil matrix spiked with 100 pg PAH standards and 500 pg ISTDs.

After the 60 injections of soil extract, inlet maintenance was performed. This consisted of changing the septum, inlet liner, and gold seal, and removing 30 cm from the head of column 1. While the liner and gold seal were out, the inlet was cleaned with cotton swabs saturated with methanol. After maintenance, the 100 ppb calibration standard was run and quantitated using the original calibration curve generated before both of the replicate studies. Table 4 shows the measured concentrations. All analytes were within 7 % of the expected concentration. Table 4 presents the R^2 values for a full calibration after inlet maintenance. The data in Table 4 demonstrate that the degradation in system performance with the soil is limited to the inlet and column head, as expected.

The source did not require cleaning, as is often the case with matrix levels such as those used here. The use of JetClean and the 9 mm drawout lens greatly reduce the deposits that normally degrade source performance.

Table 4. Calibration check and R^2 values of 7 level ISTD-calibration 1 to 1,000 pg SIM after the system maintenance.

Compound	RT (min)	Calculated concentration of a calibration verification 100 pg standard after maintenance	R^2 of calibration after maintenance
Naphthalene	5.141	100	1.0000
1-mMethylnaphthalene	5.752	100	1.0000
2-Methylnaphthalene	5.920	102	1.0000
Biphenyl	6.298	99	1.0000
2,6-Dimethylnaphthalene	6.340	100	1.0000
Acenaphthylene	7.031	96	1.0000
Acenaphthene	7.193	98	1.0000
2,3,5-Trimethylnaphthalene	7.408	99	1.0000
Fluorene	7.904	98	1.0000
Dibenzothiophene	9.663	97	1.0000
Phenanthrene	9.923	96	1.0000
Anthracene	9.991	97	1.0000
1-Methylphenanthrene	11.268	97	1.0000
Fluoranthene	12.943	94	1.0000
Pyrene	13.752	95	1.0000
Benz[a]anthracene	17.210	95	1.0000
Chrysene	17.465	95	1.0000
Benzo[b]fluoranthene	20.455	96	1.0000
Benzo[k]fluoranthene	20.519	96	1.0000
Benzo[j]fluoranthene	20.615	95	0.9999
Benzo[e]pyrene	21.485	93	1.0000
Benzo[a]pyrene	21.622	93	1.0000
Perylene	21.957	94	1.0000
Dibenz[a,c]anthracene	24.452	95	1.0000
Dibenz[a,h]anthracene	24.574	95	1.0000
Indeno[1,2,3-cd]pyrene	24.614	94	1.0000
Benzo[ghi]perylene	25.766	93	1.0000

Conclusions

This system addresses many of the problems encountered with GC/MS PAH analysis. The use of JetClean, the 9 mm drawout lens, higher zone temperatures, and the appropriate liner result in substantial improvements in linearity, peak shape, and system robustness. The greatly reduced need for manual source cleaning provided by JetClean is a welcome productivity improvement for the lab.

For labs analyzing high volumes of samples containing significant matrix interferences, the Agilent 8890/7000D triple quadrupole GC/MS with JetClean and midcolumn backflush offers all the advantages demonstrated here plus the much higher specificity of MS/MS[4]. Use of GC/MS/MS simplifies the data review versus GC/MS by providing much higher selectivity over spectral interferences from the matrix.

References

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