Application Note

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Optimized GC/MS/MS Analysis for PAHs in Challenging Matrices

Using The Agilent 8890/7000D triple quadrupole GC/MS with JetClean and midcolumn backflush

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Abstract

The Agilent 8890 GC combined with an Agilent 7000D triple quadrupole GC/MS 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 results in excellent linearity across a calibration range of 1–1,000 pg. System precision and robustness are demonstrated with replicate injections of an extract from a high organic content soil. The added selectivity of MS/MS versus MS also simplifies data review.

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, PAHs are monitored as trace contaminants in many food products, ranging from seafood to edible oils to smoked meats. They are also monitored in the environment in 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/MS in MRM mode. A common calibration range is from 1–1,000 pg with an acceptable linearity of R^2 >0.99. Internal standard (ISTD) area reproducibility is typically specified at ±20 % for calibration standards, and ±30 % for samples.

A number of issues arise with the analysis due to the properties of PAHs. They span wide molecular weight and boiling 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 the 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 MRM and data analysis time windows from the resulting retention time shift.

Experimental

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

- **MS/MS:** The added selectivity of MRM mode in GC/MS/MS simplifies data review in high-matrix samples relative to GC/MS by reducing or eliminating interfering responses from matrix. Interfering responses often require manual integration of quantifier or qualifier ions.
- **JetClean:** This option on the 7000D triple quadrupole GC/MS 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 PSD module was 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 allows 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 kept at a minimum of 320 °C.

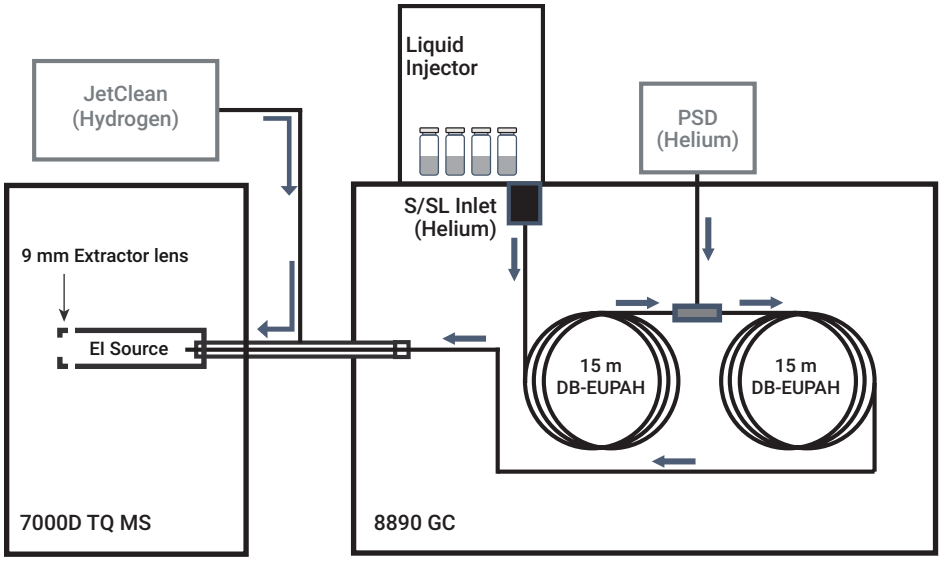

Figure 1. System configuration.

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 for 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, 0.9557 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, 1.1557 mL/min
Inlet connection	PUU
Outlet connection	MSD
Post run flow (backflushing)	12.518 mL/min

7000D triple quadrupole GC/MS	
Source	Inert extractor
Drawout lens	9 mm
Tune file	atunes.ei.tune.xml
Mode	MRM
Solvent delay	4 minutes
EM voltage gain mode	10
Quad temperature (MS1 and MS2)	150 °C
Source temperature	320 °C
Transfer line temperature	320 °C
JetClean mode	Acquire and Clean
JetClean hydrogen flow	0.33 mL/min

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 into the column.

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 pg/µL. Each level also contained 500 pg/µL 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 fold in volume by evaporation. The resulting extract was used for the robustness experiments.

Table 2. MRM transitions used for quantifier and qualifiers.

Name	RT	Quantifier	CE	Qualifier	CE
Napthalene-d8	5.041	136.0 → 136.0	19		
Napthalene	5.067	128.0 → 102.0	22	128.0 → 127.0	20
1-Methylnaphthalene	5.693	142.0 → 115.0	30	142.0 → 141.0	30
2-Methylnaphthalene	5.864	142.0 → 115.0	30	142.0 → 141.0	30
Biphenyl	6.249	154.0 → 152.0	25	154.0 → 153.0	25
2,6-Dimethylnaphthalene	6.285	156.0 → 115.0	30	156.0 → 141.0	30
Acenapthylene	6.986	152.0 → 150.0	40	152.0 → 151.0	40
Acenaphthene-d10	7.095	162.0 → 160.0	19		
Acenapthene	7.149	154.0 → 152.0	40	153.0 → 152.0	40
2,3,5-Trimethylnaphthalene	7.361	170.0 → 155.0	25	170.0 → 153.0	25
Fluorene	7.858	166.0 → 165.0	30	166.0 → 163.0	34
Dibenzothiophene	9.618	184.0 → 139.0	40	184.0 → 152.0	40
Phenanthrene-d10	9.819	188.0 → 188.0	19		
Phenanthrene	9.879	178.0 → 176.0	34	178.0 → 152.0	30
Anthracene	9.940	178.0 → 176.0	34	178.0 → 152.0	30
1-Methylphenanthrene	11.217	192.0 → 191.0	25	192.0 → 165.0	30
Fluoranthene	12.882	202.0 → 200.0	50	202.0 → 201.0	50
Pyrene	13.692	202.0 → 200.0	50	202.0 → 201.0	30
Benzo(a)anthracene	17.145	228.0 → 226.0	38	228.0 → 224.0	38
Chrysene-d12	17.309	240.0 → 236.0	25	118.0 → 116.0	25
Chrysene	17.400	228.0 → 226.0	38	228.0 → 224.0	38
Benzo(b)fluoranthrene	20.379	252.0 → 250.0	42	250.0 → 248.0	40
Benzo(k)fluoranthrene	20.445	252.0 → 250.0	42	250.0 → 248.0	40
Benzo(j)fluoranthrene	20.543	252.0 → 250.0	42	250.0 → 248.0	40
Benzo(e)pyrene	21.412	252.0 → 250.0	40	250.0 → 248.0	40
Benzo(a)pyrene	21.549	252.0 → 250.0	40	250.0 → 248.0	40
Perylene-d12	21.806	264.0 → 260.0	40	264.0 → 236.0	25
Perylene	21.884	252.0 → 250.0	40	250.0 → 248.0	40
Dibenz(a,c)anthracene	24.347	278.0 → 276.0	38	276.0 → 274.0	38
Dibenz(a,h)anthracene	24.474	278.0 → 276.0	38	276.0 → 274.0	38
Indeno(1,2,3,-cd)pyrene	24.504	276.0 → 274.0	42	138.0 → 124.0	30
Benzo(ghi)perylene	25.644	276.0 → 274.0	42	274.0 → 272.0	42

Results and discussion

Initial calibration

Figure 2 shows the MRM TIC of the 100 pg 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 somewhat 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.

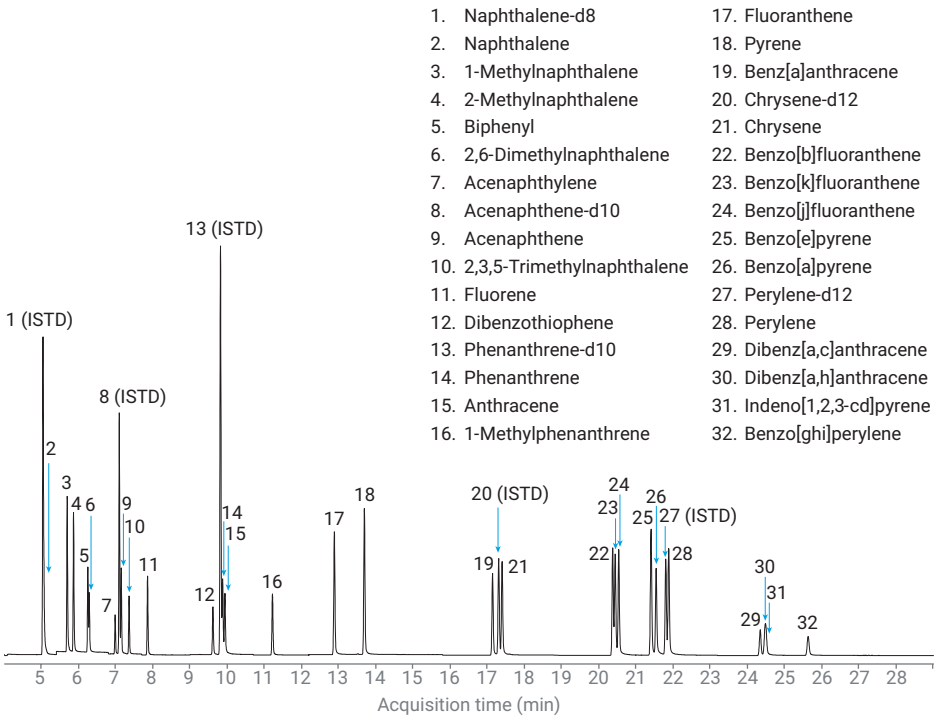

Figure 2. MRM TIC of the 100 pg standard mix.

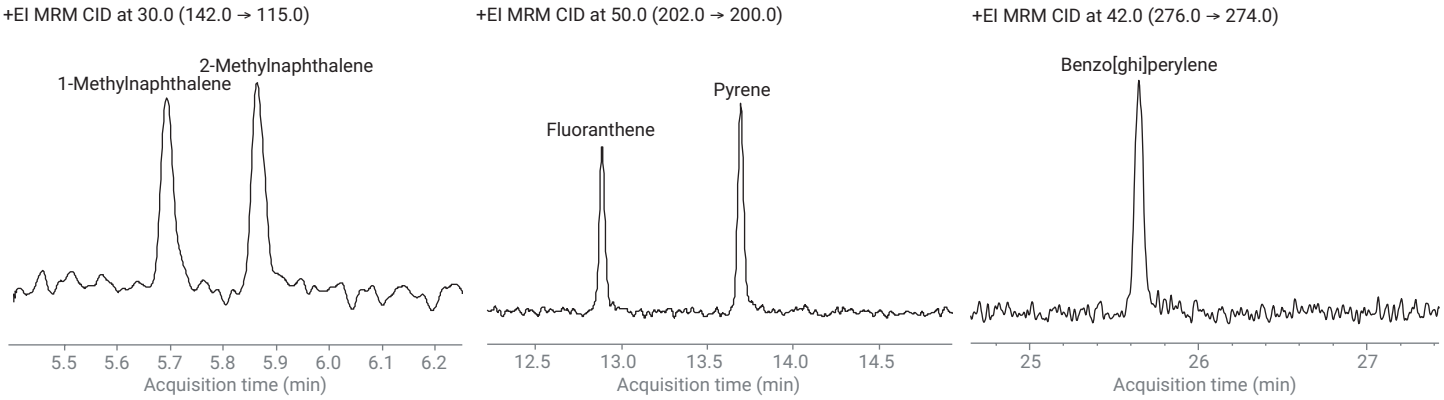

Figure 3. Response at quantifier MRM for select compounds in the lowest calibration standard (1 pg).

Table 3 shows the R^2 values for four ISTD calibrations of the system with seven levels from 1 to 1,000 pg. All analytes show excellent linearity across the entire range. The first two calibrations were the initial ones, and the last two were after 60 runs of the 100 ppb standard. These data demonstrate the linearity and consistency of response with the system as configured here.

Stability of response

Figure 4 shows the calculated concentrations for several analytes in 120 sequential replicate runs of the 100 pg standard. The system exhibits excellent stability of response. The average RSD of the calculated concentration is 1.5 % over 120 injections for all 27 analytes.

The RSDs of the raw areas of the ISTDs over the 120 injections were:

- Naphthalene-d_8 (2.9 %)
- Acenaphthene-d_{10} (3.2 %)
- Phenanthrene-d_{10} (2.9 %)
- Chrysene-d_{12} (4.7%)
- Perylene-d_{12} (5.1 %)

Table 3. R^2 values of seven level ISTD calibration: 1–1,000 pg MRM. Calibrations 1 and 2 are initials; calibrations 3 and 4 were performed after 60 runs of a 100 pg standard.

Compound	Calibration 1	Calibration 2	Calibration 3*	Calibration 4*
Napthalene	0.9999	0.9999	0.9999	0.9999
1-Methylnaphthalene	0.9999	0.9999	0.9999	0.9998
2-Methylnaphthalene	0.9999	0.9999	0.9999	0.9998
Biphenyl	0.9999	0.9998	0.9999	0.9998
2,6-Dimethylnaphthalene	0.9998	0.9998	0.9998	0.9997
Acenapthylene	0.9999	0.9998	0.9999	0.9999
Acenapthene	0.9999	0.9999	0.9999	1.0000
2,3,5-Trimethylnaphthalene	0.9999	0.9999	0.9999	0.9999
Fluorene	0.9999	0.9999	0.9999	0.9999
Dibenzothiophene	0.9998	0.9998	0.9999	0.9999
Phenanthrene	0.9999	0.9999	0.9999	0.9999
Anthracene	0.9997	0.9999	0.9999	0.9999
1-Methylphenanthrene	0.9998	0.9999	0.9999	0.9998
Fluoranthene	0.9997	0.9999	0.9999	0.9999
Pyrene	0.9998	0.9999	0.9998	0.9998
Benzo(a)anthracene	0.9998	0.9998	0.9999	0.9999
Chrysene	0.9999	0.9999	0.9999	0.9999
Benzo(b)fluoranthrene	0.9996	0.9996	0.9996	0.9997
Benzo(k)fluoranthrene	0.9997	0.9996	0.9998	0.9995
Benzo(j)fluoranthrene	0.9992	0.9999	1.0000	0.9985
Benzo(e)pyrene	0.9996	0.9998	0.9998	0.9999
Benzo(a)pyrene	0.9994	0.9996	0.9995	0.9997
Perylene	0.9995	0.9996	0.9995	0.9996
Dibenz(a,c)anthracene	0.9996	1.0000	0.9996	0.9993
Dibenz(a,h)anthracene	0.9994	0.9995	0.9997	0.9996
Indeno(1,2,3,-cd)pyrene	0.9994	0.9994	0.9996	0.9996
Benzo(ghi)perylene	0.9997	0.9998	0.9998	0.9998

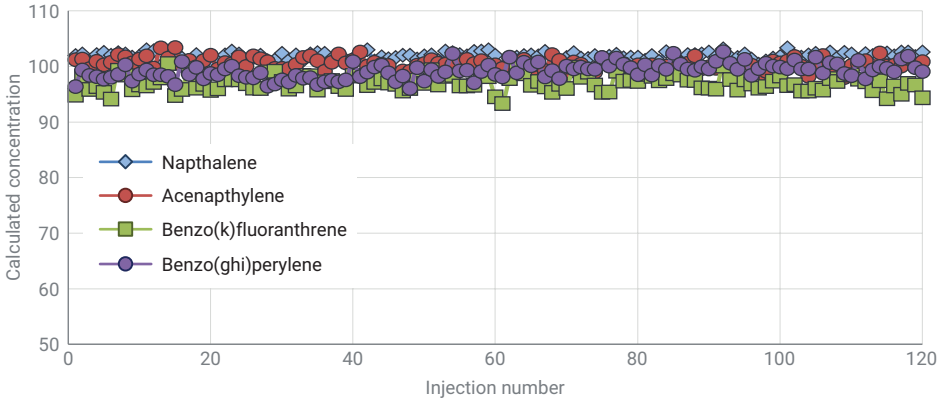

Figure 4. Stability of calculated concentrations over 120 sequential injections for a 100 pg calibration standard.

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 5 shows the scan TIC of the extract spiked with 100 pg PAH standards and 500 pg ISTDs, and that of the 100 pg PAH standard for comparison. 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 prep 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 6 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 4.1 %. For 25 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.

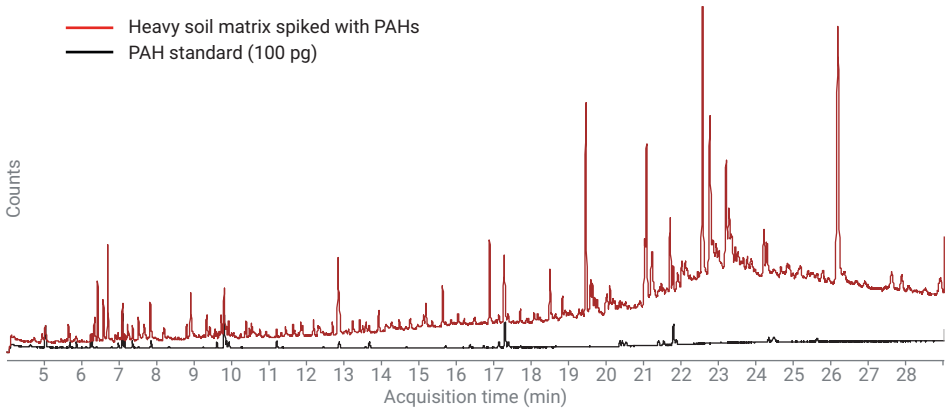

Figure 5. Scan TIC of soil extract and PAH 100 pg standard with 500 pg ISTDs, both drawn in the same scale, showing a large amount of material in the extract.

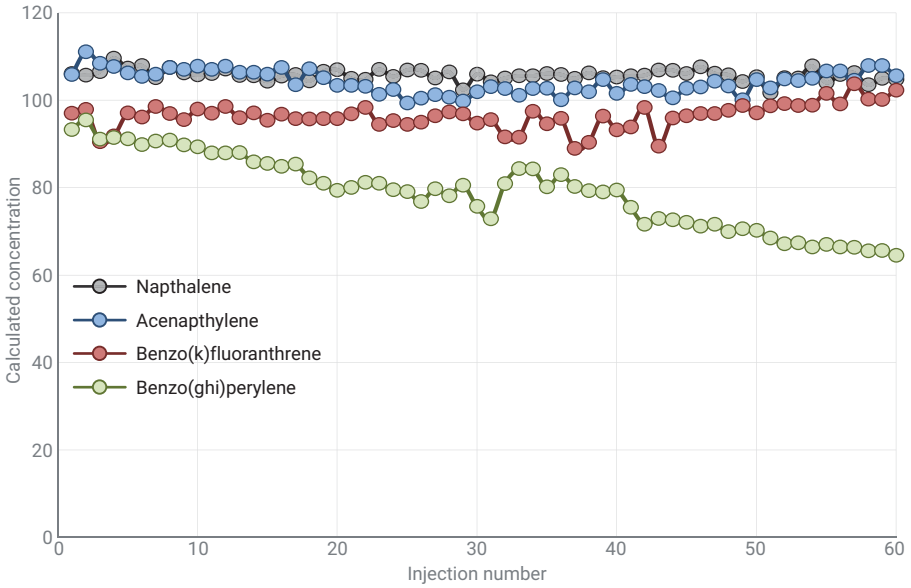

Figure 6. Stability of calculated concentrations over 60 injections of a soil matrix spiked with 100 pg PAH standards and 500 pg ISTDs.

After 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 12 % of the expected concentration. Table 4 displays 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. The use of JetClean and the 9-mm drawout lens greatly reduce the deposits that normally degrade source performance.

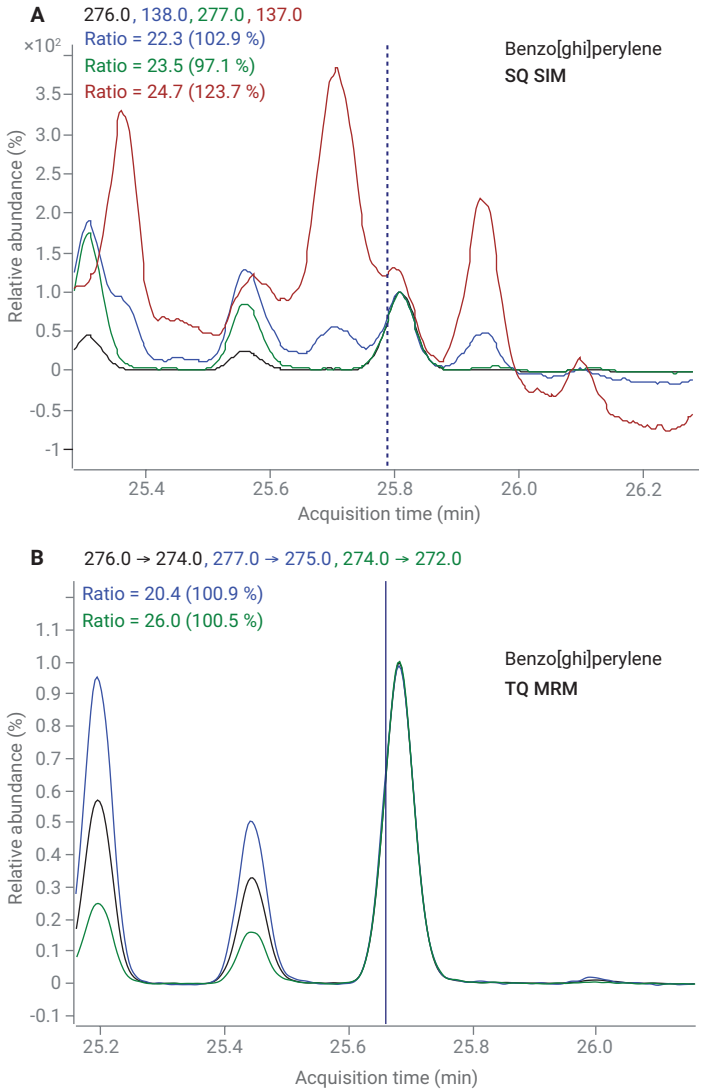

Figure 7. Overlaid quantifier and qualifier chromatograms for 100 ppb PAH spiked into soil extract. A) Benzo[ghi]perylene in SIM mode on single quadrupole GC/MS. B) Same extract in MRM mode on GC/MS/MS.

Selectivity of MRMs

While the analysis of PAHs can often be done successfully[4] with single quadrupole GC/MS, matrices such as soil can make data review difficult due to spectral interferences with the target compounds. These interferences often result in the need for manual integration to account for the effects of the interferences. The use of GC/MS/MS greatly reduces these interferences. This is illustrated in Figure 7. The upper portion shows the quantifier for benzo[ghi]perylene overlaid with three qualifier ions in SQ SIM data, and the bottom shows the same with MRMs on a TQ.

In the GC/MS chromatograms, one of the qualifiers (277) is relatively free of interferences. The other two qualifiers (138 and 137) have significant matrix interferences that would require manual integration. In contrast, the GC/MS/MS MRMs (B) show much greater selectivity, making data review much easier.

Conclusions

This system addresses many of the problems encountered with GC/MS PAH analysis. Use of GC/MS/MS simplifies data review versus GC/MS by providing much higher selectivity over spectral interferences from the matrix. 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 and column trimming provided by JetClean and backflushing, respectively, are welcome productivity improvements for the lab.

Table 4. Calibration check and R^2 values of seven level ISTD calibration: 1–1,000 pg MRM after the system maintenance.

Compound	Calculated concentration of a calibration verification 100 pg standard before recalibrating	Calibration after maintenance
Naphthalene	99	1.0000
1-methylnaphthalene	96	1.0000
2-Methylnaphthalene	98	1.0000
Biphenyl	94	1.0000
2,6-dimethylnaphthalene	93	1.0000
Acenaphthylene	99	1.0000
Acenaphthene	98	1.0000
2,3,5-Trimethylnaphthalene	98	1.0000
Fluorene	97	1.0000
Dibenzothiophene	90	1.0000
Phenanthrene	96	1.0000
Anthracene	105	1.0000
1-methylphenanthrene	96	1.0000
Fluoranthene	97	1.0000
Pyrene	97	1.0000
Benz[a]anthracene	95	0.9999
Chrysene	96	1.0000
Benzo[b]fluoranthene	97	1.0000
Benzo[k]fluoranthene	99	1.0000
Benzo[j]fluoranthene	112	1.0000
Benzo[e]pyrene	94	1.0000
Benzo[a]pyrene	96	1.0000
Perylene	94	1.0000
Dibenz[a,c]anthracene	96	0.9999
Dibenz[a,h]anthracene	94	1.0000
Indeno[1,2,3-cd]pyrene	95	1.0000
Benzo[ghi]perylene	94	1.0000

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