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Analysis of PAHs Using GC/MS with Hydrogen Carrier Gas and the Agilent HydroInert Source

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Abstract

The Agilent 8890 GC and the Agilent 5977C GC/MSD were used with hydrogen carrier gas and a new source optimized for hydrogen operation. The Agilent HydroInert source, when used with the method described in this application note, provides excellent peak shape, sensitivity, and linearity across a calibration range of 0.25 to 1,000 pg for the analysis of polycyclic aromatic hydrocarbons (PAHs). By proper selection of instrument configuration and operating conditions, the system with hydrogen carrier gas can generate results comparable to or better than those with helium. System precision and robustness are demonstrated with replicate injections of an extract from a high organic content soil.

Introduction

PAHs are a group of chemical compounds that are composed of at least two or more fused conjugated benzene rings with a pair of carbon atoms shared between rings in their molecules. Because PAHs originate from multiple sources, they are widely distributed as contaminants throughout the world. 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 in air, water, and soil. PAHs have been analyzed by multiple techniques including HPLC/UV, GC/FID, GC/MS, and GC/MS/MS.

This application note focuses on GC/MS in selected ion monitoring (SIM) mode using hydrogen as the GC carrier gas. While helium is generally considered the best carrier gas for GC/MS analysis, its reoccurring shortages have increased demand for applications using hydrogen as the carrier gas. When adopting hydrogen for GC/MS analysis, there are several things to consider.

First, hydrogen is a reactive gas, and may potentially cause chemical reactions in the inlet, column, and sometimes the mass spectrometer electron ionization (EI) source that can change analysis results. It is important to ensure that there are no chemical reaction problems between analytes and hydrogen gas at elevated temperatures in the GC/MS.

Second, it is essential to use a reliable source of clean hydrogen gas. For long-term use, generators with a >99.9999% specification and low individual specs on water and oxygen are recommended. Moisture filters are recommended for use with hydrogen

generators. For short-term use, cylinders with chromatographic or research-grade hydrogen are acceptable. It is also recommended that anyone working with flammable or explosive gases take a lab safety course covering proper gas handling and use.

Additionally, for GC/MS applications, hardware changes in the gas chromatograph and mass spectrometer may be required when switching to hydrogen carrier gas. The Agilent EI GC/MS Instrument Helium to Hydrogen Carrier Gas Conversion user guide[1] describes in detail the steps for conversion. These steps include selection of the inlet liner, column, vacuum pump, and EI source. Chromatographic conditions and injection solvent may also need to be adjusted.

One of the advantages observed with hydrogen carrier gas is a reduced need for EI source cleaning. A similar improvement is observed when using Agilent JetClean technology, which uses a low continuous flow of hydrogen into the source during the analysis.[2,3,4] A second advantage often observed with hydrogen carrier gas is the ability to decrease the analysis time while maintaining chromatographic resolution.

PAHs are relatively durable compounds and therefore can be analyzed with hydrogen carrier gas when using the optimized method and following the recommendations described in this application note. Other challenges with PAH analysis addressed in this work include peak tailing, often seen for late-eluting analytes, and ISTD response inconsistency across the calibration range. With the optimized method using the HydroInert source, excellent linearity of $R^2 \geq 0.999$ was observed for all 27 analytes over their respective

calibration ranges. Of the 27 analytes, 18 had a calibration range from 0.1 to 1,000 pg, eight from 0.25 to 1,000 pg, and one from 0.5 to 1,000 pg. Method detection limits (MDL) ranged from 0.03 to 0.19 pg, with an average of 0.09 pg.

Experimental

The system used in this experiment was configured to minimize the potential problems with hydrogen carrier gas in PAH analysis. The important parameters used were:

- **Hydrogen gas:** In-house hydrogen with 99.9999% purity specification and low individual specs on water and oxygen was used as a carrier gas.

- **Pulsed splitless injection:** Used to maximize transfer of the PAHs, especially the heavy ones, into the column.

- **Column dimensions:** An Agilent J&W DB-EUPAH column (20 m × 0.18 mm id, 0.14 µm) was used to maintain optimal gas flow and inlet pressure.

- **Inlet liner:** The Agilent universal Ultra Inert mid-frit liner (part number 5190-5105) was found to give good peak shape, inertness, and longevity with the soil extracts.

- **HydroInert EI source:** PAHs present unique challenges with respect to the MS EI source, even with helium as the carrier gas.[2] With hydrogen carrier gas, the performance of PAHs is generally improved, especially with the HydroInert source, which is optimized for use with hydrogen. The 9 mm extractor lens is the default included with the HydroInert source and the best choice for PAH analysis, as it provides the best calibration linearity, precision of response, and peak shape.

Figure 1 shows the system configuration used in this study.

The instrument operating parameters are listed in Tables 1 and 2. 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 is operated at 320 °C.

Pulsed splitless injections are used to maximize transfer of the PAHs, especially the heavy ones, into the column. The Ultra Inert mid-frit liner works well for this application. The frit 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.

PAH calibration standards were diluted from Agilent PAH analyzer calibration kit (part number 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. Twelve calibration levels were prepared: 0.1, 0.25, 0.5, 1, 2, 10, 20, 100, 200, 400, 750, and 1,000 ng/mL. Each level also contained 500 ng/mL of the ISTDs. See Table 2 and Figure 2 for compound identifications. All quantitative measurements were performed with Agilent MassHunter Quantitative Analysis software version 11.1.

A sample of commercial topsoil (Weaver Mulch, Coatesville, PA, USA) was dried at 120 °C overnight. Five grams of the dried soil were extracted with 30 mL dichloromethane/acetone (1:1 v/v) with agitation overnight. 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.
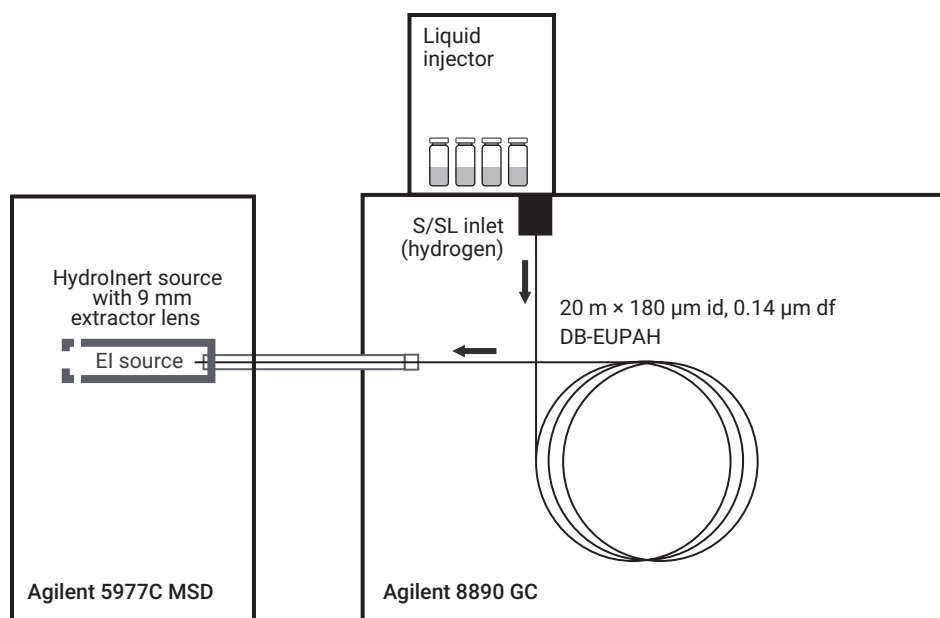

Figure 1. System configuration.

Table 1. GC and MS conditions for PAH analysis.

Method Parameters	
Inlet	EPC split/splitless
Mode	Pulsed splitless
Injection Pulse Pressure	40 psi until 0.70 min
Purge Flow to Split Vent	50 mL/min at 0.75 min
Injection Volume	1 µL
Inlet Temperature	320 °C
Inlet Liner	Agilent Universal Ultra Inert mid-frit liner (p/n 5190-5105)
Column	Agilent J&W DB-EUPAH 20 m × 0.18 mm, 0.14 µm
Column Temperature Program	60 °C (1 min hold) 25 °C/min to 200 °C 10 °C/min to 335 °C (6 min hold)
Carrier Gas and Flow Rate	H_2, 0.9 mL/min constant flow
MSD	HydroInert 9 mm
Transfer Line Temperature	320 °C
Ion Source Temperature	320 °C
Quadrupole Temperature	150 °C
EM Voltage Gain Mode	3
Mode	SIM
Tune	ETUNE.U

The soil extract used for the robustness test was deliberately chosen to have a high matrix content to challenge the system. Note that for soils with this level of organic content, further sample cleanup should be considered for routine analysis. The sample preparation used in this study was for test purposes only. Also, the extraction solvent (1:1 v/v dichloromethane/acetone) is not recommended for routine analysis with hydrogen carrier gas. Halogenated solvents such as dichloromethane may react with hydrogen in the hot injection port, forming low levels of HCl, which can degrade the liner and column head over time.

Table 2. SIM ions used for quantifier and qualifiers.

Name	RT (min)	Quantifier	Qualifier 1	Qualifier 2	Qualifier 3
Naphthalene-d$_8$	4.068	136	134	108	
Naphthalene	4.089	128	127	129	102
1-Methylnaphthalene	4.681	142	141	115	139
2-Methylnaphthalene	4.833	142	141	115	143
Biphenyl	5.215	154	153	76	155
2,6-Dimethylnaphthalene	5.236	156	141	155	115
Acenaphthylene	5.761	152	151	153	76
Acenaphthene-d$_{10}$	5.851	164	80		
Acenaphthene	5.889	153	154	151	155
2,3,5-Trimethylnaphthalene	6.075	170	155	169	153
Fluorene	6.380	166	165	163	167
Dibenzothiophene	7.424	184	185	139	152
Phenanthrene-d$_{10}$	7.552	188	189		
Phenanthrene	7.585	178	179	177	152
Anthracene	7.625	178	179	177	152
1-Methylphenanthrene	8.438	192	191	193	190
Fluoranthene	9.529	202	203	201	101
Pyrene	10.060	202	203	201	101
Benz[a]anthracene	12.611	228	226	229	114
Chrysene-d$_{12}$	12.731	240	236		
Chrysene	12.794	228	226	229	114
Benzo[b]fluoranthene	15.058	252	126		
Benzo[k]fluoranthene	15.114	252	126		
Benzo[j]fluoranthene	15.181	252	126		
Benzo[e]pyrene	15.821	252	253	126	250
Benzo[a]pyrene	15.927	252	253	250	126
Perylene-d$_{12}$	16.133	264	260		
Perylene	16.191	252	253	126	250
Dibenz[a,c]anthracene	18.020	278	279	139	138
Dibenz[a,h]anthracene	18.093	278	279	139	138
Indeno[1,2,3-cd]pyrene	18.093	276	138	277	137
Benzo[ghi]perylene	18.655	276	138	277	137

Results and discussion

Initial calibration

1. Naphthalene-d8
2. Naphthalene
3. 1-Methylnaphthalene
4. 2-Methylnaphthalene
5. Biphenyl
6. 2,6-Dimethylnaphthalene
7. Acenaphthylene
8. Acenaphthene-d10
9. Acenaphthene
10. 2,3,5-Trimethylnaphthalene
11. Fluorene
12. Dibenzothiophene
13. Phenanthrene-d10
14. Phenanthrene
15. Anthracene
16. 1-Methylphenanthrene

17. Fluoranthene
18. Pyrene
19. Benz[a]anthracene
20. Chrysene-d$_{12}$
21. Chrysene
22. Benzo[b]fluoranthene
23. Benzo[k]fluoranthene
24. Benzo[j]fluoranthene
25. Benzo[e]pyrene
26. Benzo[a]pyrene
27. Perylene-d$_{12}$
28. Perylene
29. Dibenz[a,c]anthracene
30. Indeno[1,2,3-cd]pyrene
31. Dibenz[a,h]anthracene
32. Benzo[ghi]perylene

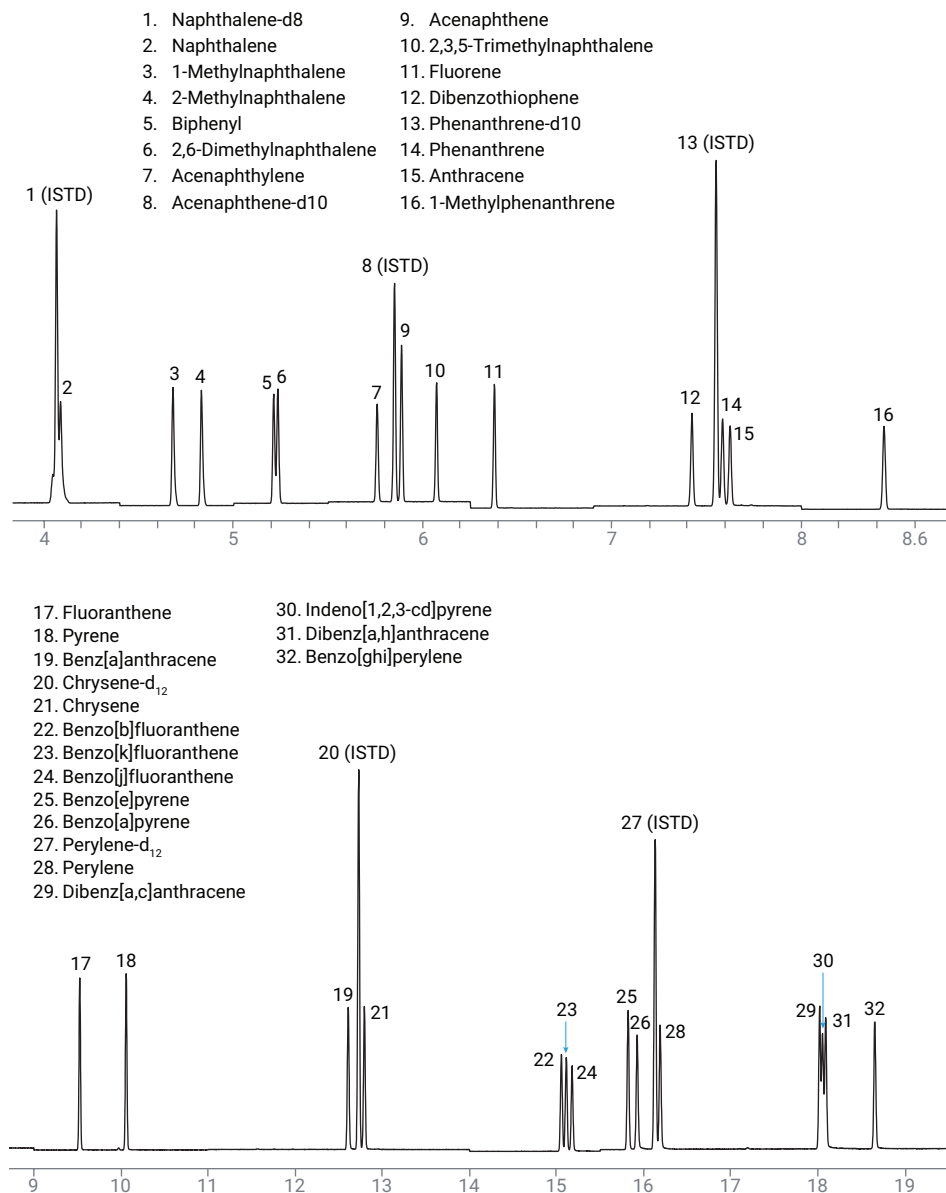**Figure 2.** SIM TIC of the 100 pg/µL calibration standard with 500 pg/µL ISTDs.

Figure 2 shows the SIM TIC of the 100 pg/µL calibration standard with 500 pg/µL ISTDs. With the parameters used in this study, the peak shapes for PAHs, especially the later-eluting compounds, are excellent. The only exceptions are the first two peaks, naphthalene-d8 and naphthalene, which are somewhat distorted from the use of a pulsed injection. In general, the HydroInert source provides the best peak shapes for PAHs when using hydrogen carrier gas. The chromatographic resolution obtained with the current setup is also better than that obtained with helium.[2] Due to the combination of hydrogen carrier and a smaller diameter column, the run time with the current method is 20 minutes compared to 26 minutes for the helium method. The run time with the current method could have been reduced further and still maintain the same resolution as with the helium method. However, the 20-minute method conditions were chosen, as they give the best resolution of dibenz[a,c] anthracene, indeno[1,2,3-cd]pyrene, and dibenz[a,h]anthracene.

Using the HydroInert source also resulted in excellent signal-to-noise ratios, allowing the calibration range to be extended to sub-picogram levels, as shown in Figure 3.

Eighteen of the 27 analytes had sufficient signal for calibration from 0.1 to 1,000 pg. Eight were calibrated from 0.25 to 1,000 pg. Only 1 compound, 2-methylnaphthalene, required calibration from 0.5 to 1,000 pg. Table 3 shows the calibration results of the system with 12 levels from 0.1 to 1,000 pg. All analytes show excellent linearity across the entire range.

If necessary, the relative standard error (RSE) value was used to guide removal of the lowest calibration points to achieve an RSE value of <20%. All calibrations had an R^2 value of 0.999 or greater.
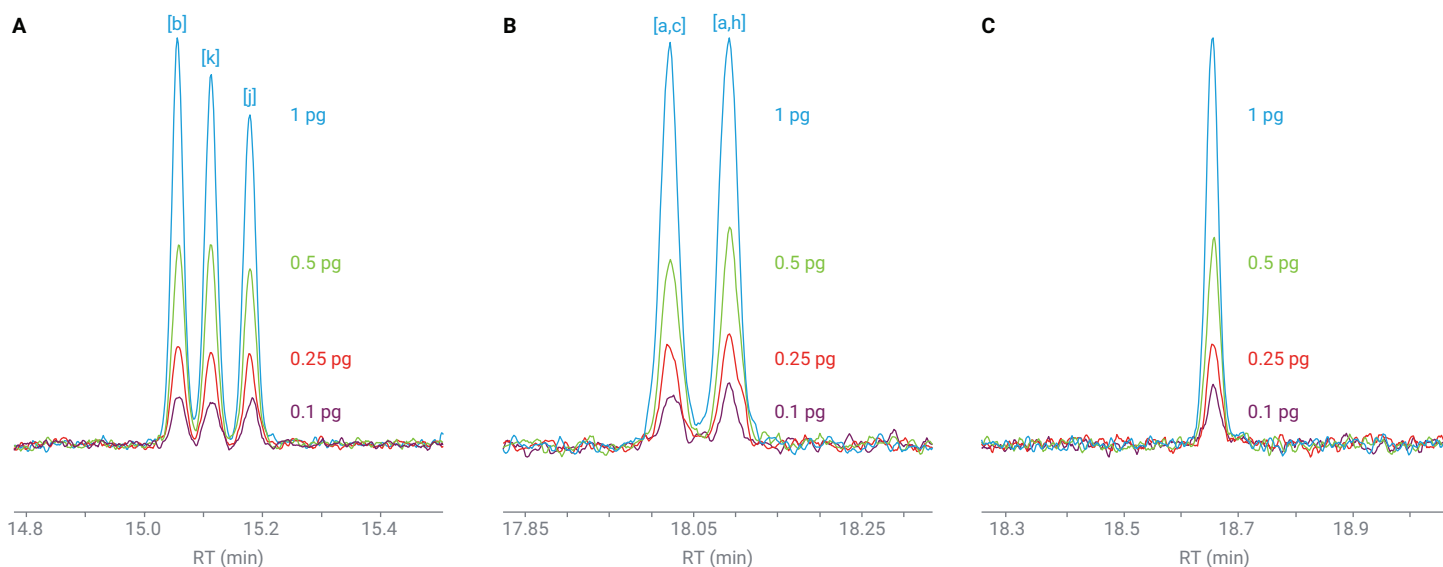
Figure 3. Response at quantifier ion for select compounds at the lowest calibration levels. (A) The [b], [k], and [j] isomers of benzofluoranthene. (B) The [a,c] and [a,h] isomers of dibenzanthracene. (C) Benzo[ghi]perylene.

Method detection limits

An MDL study was performed after completion of the initial calibration. Eight trials were performed with the 0.25 pg calibration standard. The calculated MDLs were obtained by applying the formula shown in Equation 1. For compounds with higher reporting limits, eight trials were performed at the concentration of 0.5 pg. Table 3 lists the calculated MDLs. The MDLs for the 27 compounds ranged from 0.03 to 0.2 pg.

Equation 1. Formula for MDL calculations.

MDL = s × t(n − 1, 1 − alpha = 99) = s × 2.998

Where:

t(n − 1, 1 − alpha) = t value for the 99% confidence level with n − 1 degrees of freedom

n = number of trials (8)

s = standard deviation of the eight trials

Stability of ISTD response over calibration range

One of the problems encountered when using helium carrier gas and the standard 3 mm extractor lens for the analysis of PAHs is the response of ISTDs climbing with increasing concentration of the analytes. This effect can cause the response of perylene-d_{12} to increase by as much as 60% over the calibration range and cause significant errors in quantitation. This problem has been addressed previously by using JetClean and a 9 mm extractor lens.[3,4] With JetClean, helium is used as the carrier gas, but hydrogen is continuously added to the source at a flow rate typically in the range of 0.2 to 0.35 mL/min. This approach greatly reduces the creeping ISTD effect and results in excellent quantitation.

Figure 4 shows the ISTD response stability over the calibration range with the current method. As demonstrated in Figure 4, the use of hydrogen carrier gas with the HydroInert source and 9 mm extractor lens also eliminates the creeping ISTD response problem. The %RSD for the raw area responses across the calibration range are all 3.3% or less. This is an important factor in achieving the excellent calibration linearity shown in Table 3.

Table 3. Results for 12 level SIM mode ISTD calibration over a range of 0.1 to 1,000 pg. All calibrations were linear fit with 1/x weighting.

Name	RT (min)	CF Limit Low (pg)	CF Limit High (pg)	CF R^2	Relative Standard Error	Avg. RF RSD	Conc. for MDL (pg)	MDL (pg)
Naphthalene-d$_8$ [ISTD]	4.068				ISTD			
Naphthalene	4.089	0.1	1,000	0.9998	5.7	23.0	0.50	0.19
1-Methylnaphthalene	4.681	0.25	1,000	0.9992	11.4	4.8	0.50	0.06
2-Methylnaphthalene	4.833	0.5	1,000	0.9992	10.9	7.6	0.50	0.07
Biphenyl	5.215	0.25	1,000	0.9991	11.1	9.7	0.50	0.18
2,6-Dimethylnaphthalene	5.236	0.25	1,000	0.9989	12.8	5.4	0.50	0.07
Acenaphthylene	5.761	0.25	1,000	0.9999	6.6	4.3	0.50	0.06
Acenaphthene-d$_{10}$ [ISTD]	5.851				ISTD			
Acenaphthene	5.889	0.25	1,000	0.9995	8.7	7.4	0.25	0.14
2,3,5-Trimethylnaphthalene	6.075	0.25	1,000	0.9997	13.8	12.6	0.50	0.20
Fluorene	6.380	0.25	1,000	0.9996	10.0	5.0	0.25	0.05
Dibenzothiophene	7.424	0.1	1,000	0.9998	7.6	16.9	0.25	0.09
Phenanthrene-d$_{10}$ [ISTD]	7.552				ISTD			
Phenanthrene	7.585	0.25	1,000	0.9998	6.2	5.3	0.25	0.10
Anthracene	7.625	0.1	1,000	0.9998	15.1	17.3	0.25	0.16
1-Methylphenanthrene	8.438	0.1	1,000	0.9996	8.5	4.9	0.25	0.10
Fluoranthene	9.529	0.1	1,000	0.9994	11.6	10.7	0.25	0.03
Pyrene	10.060	0.1	1,000	0.9993	11.3	16.4	0.25	0.06
Benz[a]anthracene	12.611	0.1	1,000	0.9998	8.3	19.8	0.25	0.03
Chrysene-d$_{12}$ [ISTD]	12.731				ISTD			
Chrysene	12.794	0.1	1,000	0.9999	6.2	19.3	0.25	0.06
Benzo[b]fluoranthene	15.058	0.1	1,000	0.9990	11.4	16.2	0.25	0.05
Benzo[k]fluoranthene	15.114	0.1	1,000	0.9993	13.1	18.5	0.25	0.06
Benzo[j]fluoranthene	15.181	0.1	1,000	0.9994	10.6	18.4	0.25	0.06
Benzo[e]pyrene	15.821	0.1	1,000	0.9996	9.3	16.2	0.25	0.09
Benzo[a]pyrene	15.927	0.1	1,000	0.9998	8.2	4.7	0.25	0.05
Perylene-d$_{12}$ [ISTD]	16.133				ISTD			
Perylene	16.191	0.1	1,000	0.9999	5.2	55.6	0.25	0.12
Dibenz[a,c]anthracene	18.020	0.1	1,000	0.9997	6.3	14.3	0.25	0.06
Dibenz[a,h]anthracene	18.093	0.1	1,000	0.9997	7.0	11.6	0.25	0.07
Indeno[1,2,3-cd]pyrene	18.093	0.1	1,000	0.9993	10.9	9.2	0.25	0.08
Benzo[ghi]perylene	18.655	0.1	1,000	0.9997	9.4	11.0	0.25	0.14

Stability of response with soil extract

Figure 5 shows the scan TIC of the soil extract and that of the solvent blank for comparison. As can be seen, the soil extract has a very high level of matrix and was chosen to challenge the system. For soils with this level of organic content, further sample cleanup would be recommended for routine analysis. The sample preparation used in this study was for test purposes only. Also, the extraction solvent (1:1 v/v dichloromethane/acetone) is not recommended for routine analysis with hydrogen carrier gas. Halogenated solvents like dichloromethane may react with hydrogen in the hot injection port, forming low levels of HCl, which can degrade the liner and column head over time.
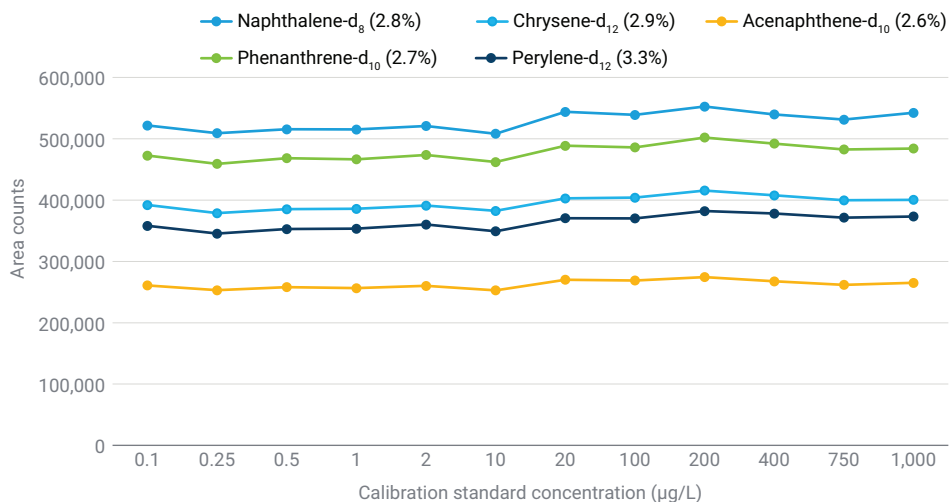

Figure 4. ISTD response stability over the calibration range.
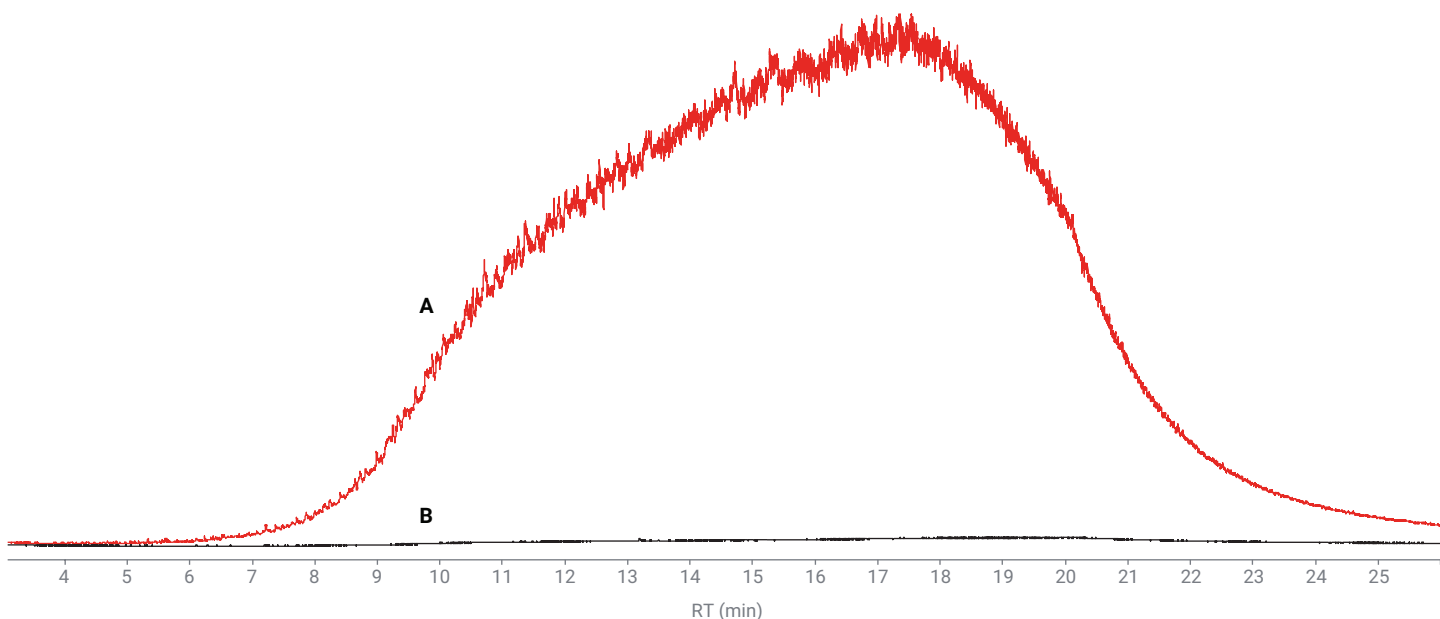

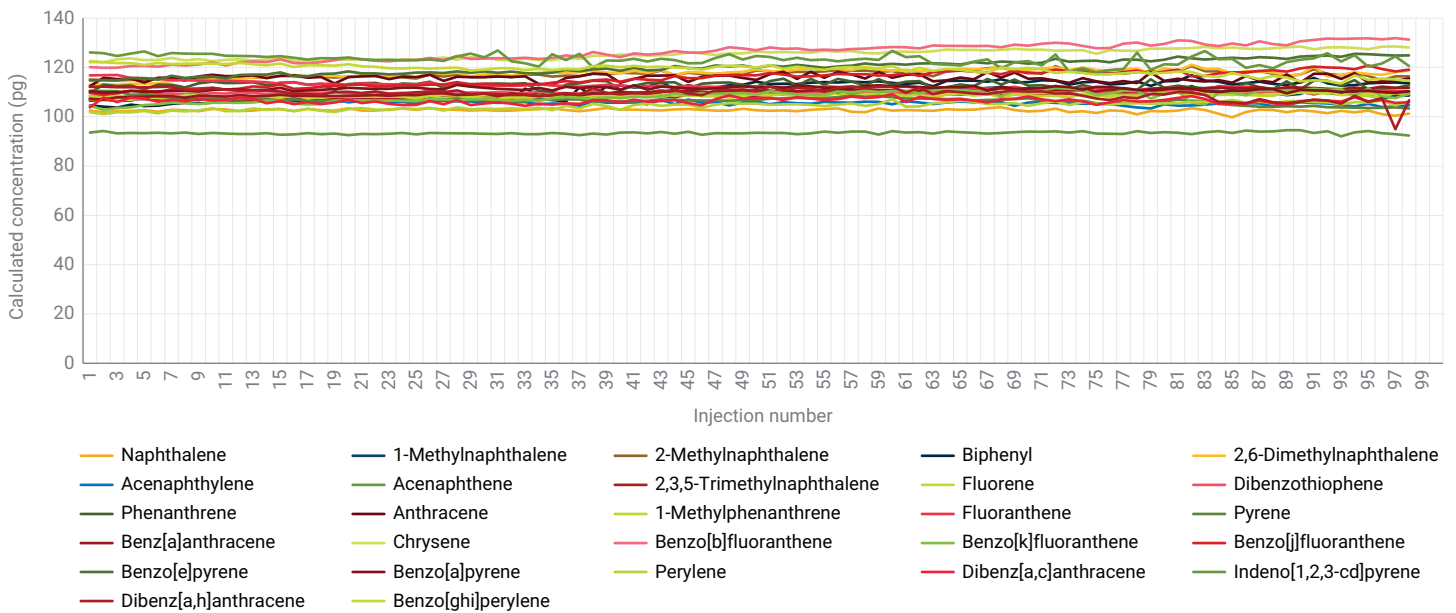**Figure 5.** (A) Scan TIC of the soil extract. (B) Scan TIC of solvent blank for comparison.

Figure 6 shows the SIM TIC for the soil extract spiked with 100 ppb of the PAH standard and 500 ppb of the ISTDs. As see in the SIM TIC, the soil matrix contributes a significant background signal in some of the time segments, the worst being the last segment. The extracted SIM 276 ion (quantifier for benzo[g,h,i]perylene) is also plotted to show that it is still suitable for quantitation.

Figure 7 shows the response stability of the measured concentrations for the PAHs in the spiked soil extract. No GC inlet or column maintenance was required during the 100 injection test. The gain curve for the electron multiplier was updated automatically every 15 runs during the sequence using a keyword. As seen in Figure 7, the results were stable over the 100 injections. The measured concentrations for all compounds fell within the range 92 to 131 pg.
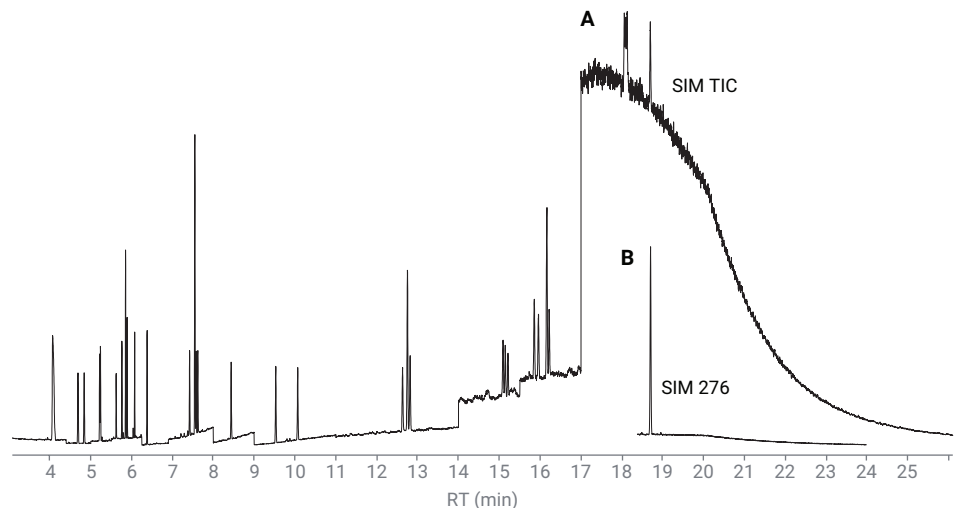
Figure 6. Soil extract spiked with 100 ppb PAH standard and 500 ppb ISTDs. (A) SIM TIC. (B) SIM 276 quantifier for benzo[g,h,i]perylene.

— Naphthalene	— 1-Methylnaphthalene	— 2-Methylnaphthalene	— Biphenyl	— 2,6-Dimethylnaphthalene
— Acenaphthylene	— Acenaphthene	— 2,3,5-Trimethylnaphthalene	— Fluorene	— Dibenzothiophene
— Phenanthrene	— Anthracene	— 1-Methylphenanthrene	— Fluoranthene	— Pyrene
— Benz[a]anthracene	— Chrysene	— Benzo[b]fluoranthene	— Benzo[k]fluoranthene	— Benzo[j]fluoranthene
— Benzo[e]pyrene	— Benzo[a]pyrene	— Perylene	— Dibenz[a,c]anthracene	— Indeno[1,2,3-cd]pyrene
— Dibenz[a,h]anthracene	— Benzo[ghi]perylene			

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

Conclusion

The described GC/MS method using hydrogen carrier gas and an Agilent HydroInert source demonstrated several improvements over previous helium methods:

– Excellent chromatographic peak shape with little or no tailing

– MDL and linearity comparable to or better than obtained with helium

– Better chromatographic resolution with a shorter run time

– ISTD response was stable across four orders of calibration

– Excellent linearity over <1 to 1,000 pg

– Average MDL ~0.1 pg

– Stable performance over 100 injections of a challenging soil extract

For laboratories looking to change their PAH analysis to the more sustainable hydrogen carrier gas, the HydroInert source with the 9 mm extractor lens enables the transition with equivalent or better performance.

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