Nota applicativa

Converting Quantitative Analysis of Polycyclic Aromatic Hydrocarbons in Environmental Matrices From Electron Ionization GC-HRMS Using Helium Carrier Gas to Atmospheric Pressure Ionization GC-MS/MS Using Nitrogen Carrier Gas

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Questa relazione è un Application Brief e non contiene una sezione dettagliata sull'esperimento.

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are common, often high abundance, contaminants in environmental matrices such as sediment, water, and biota. This is due to the fact that they are created through a number of widespread, natural, and anthropogenic processes such as wildfires and the burning of fossil fuels for power generation, heating and transportation. This constant supply of PAHs combines with long-range transport through the environment resulting in their presence in samples collected for such varied purposes as climate change related studies and potential impacts on the food web and human health. Since certain PAHs are associated with various negative health effects, there is the need to monitor for them in environmental samples and to characterize their distribution in order to make informed decisions about how to most effectively protect

both urban and remote populations.[1,2]

Due to the complexity of matrices encountered when monitoring the fate, transport, and occurrence of these compounds, it is necessary to employ techniques with high sensitivity, specificity, and robustness for these studies. In the past this has required the use of complex sample extraction and clean-up combined with electron ionization (EI) and high resolution mass spectrometry (HRMS) using magnetic sector instruments. However, in recent years the performance of gas chromatography atmospheric pressure ionization tandem quadrupole mass spectrometry (GC-APCI-MS/MS) has demonstrated equal or better performance than the classic technique.[3,4]

In this work the Atmospheric Pressure Gas Chromatography (APGC™) ionization source on a Xevo™ TQ-XS Tandem Quadrupole System was used with both helium (He) and nitrogen (N_2) carrier gas to analyze sediment extracts for the presence of 52 PAHs. Performance characteristics and sample quantification results for APGC-MS/MS were compared with results for the same aliquots run using a GC-EI-HRMS reference method.

Benefits

- The sensitivity, specificity and robustness of APGC-MS/MS provides a more compact and lower cost approach to magnetic sector GC-EI-HRMS methods
- Operator training for APGC-MS/MS is reduced to weeks from the months required for EI-GC-HRMS
- Atmospheric pressure ionization readily adapts to the use of nitrogen carrier gas providing more sustainable, reliable and less expensive day-to-day operation
- APGC-MS/MS demonstrates the ability to replicate quantitative results for complex environmental extracts previously requiring GC-EI-HRMS methods

Introduction

The creation of PAHs from many different processes combined with their worldwide distribution in environmental media such as air, water, sediment and living organisms leads to a high likelihood of exposure to them for virtually the entire world's population. While their potential for causing adverse human health effects is often studied using a list of just 16 analytes this only represents a small portion of the total chemical space they define and is unlikely to be sufficient to serve the purpose of advising comprehensive and effective environmental and health guidelines related to minimizing exposure.[5] Therefore, efforts to expand the number of analytes monitored and increase the inclusion of PAHs into multi-class analyses are required. Previous studies expanding the characterization of PAHs in air to include hydroxy-polyaromatic hydrocarbons (OHPAHs) and in multi-residue analysis that includes pesticides, polychlorinated biphenyls and flame retardants in food matrices have been performed using APGC-MS/MS.[6,7] As analyte lists in methods such as these increase, the use of tandem quadrupole (TQ) mass spectrometers rather than magnetic sector becomes an important element in method improvement given that TQs are able to perform at full sensitivity at acquisition rates that are an order of magnitude faster than those achievable by magnetic sectors.

However, due to the exceptional performance characteristics of EI-GC-HRMS this technique was, in the past, frequently employed in applications combining complex matrices with the need for high specificity, sensitivity and dynamic range. Therefore, it became widely adopted for environmental analyses despite the technique presenting challenges in many labs where the facilities requirements for installation and operator expertise are beyond the means of many organizations. Furthermore, tandem quadrupole (TQ) mass spectrometers made steady improvements across time and eventually began to match and surpass magnetic sectors in various measures of performance until they became the new gold standard for quantification of trace analytes in complex matrices.[8] A current trend in recent years has been the migration of GC-HRMS methods to GC-MS/MS as it better meets the needs of modern day laboratories while maintaining data of quality consistent with classic approaches.

To further improve the scope of applications being successfully transitioned to GC-APCI-MS/MS, this study used a well characterized EI-GC-HRMS method for the quantification of an expanded list of PAHs in environmental matrices. Standard reference materials (SRMs), spiked sediments, and standards were analyzed first using helium (He) carrier gas for method development and direct comparison to the prior method and then using nitrogen (N_2) to compare performance with a more sustainable, less costly alternative carrier gas.

Experimental

Sample Preparation

Sample preparation was performed at Environment and Climate Change Canada’s (ECCC) Quebec Laboratory for Environmental Testing (QLET). Humid samples are dried to a constant weight and finely ground using a glass mortar and pestle. 5 g dried sediment was weighed into cellulose extraction thimbles and surrogates added. Extraction was via soxhlet in toluene for 16 hours. Samples were then concentrated to 1–2 mL and transferred to hexane. Next, copper was directly added to the boiling flask to remove sulfur followed by transfer to 15 mL tubes and nitrogen evaporation to 1 mL. This was then cleaned-up using a silica:alumina column of 300 mm x 12 mm length and inner diameter with a 14/35 reservoir. 5 g of silica:alumina (2:1) deactivated with 3% water was weighed out and topped off with decontaminated sodium sulfate. Elution fractions were F1=10 mL hexane which is discarded; F2=25 mL of hexane:dichloromethane (1:1) followed by; F3=50 mL dichloromethane. F2 and F3 were collected in a boiling flask, concentrated to 1–2 mL, transferred into 15 mL tubes and nitrogen evaporated to 1 mL. Isooctane was used as a keeper and internal standards were added at this step. Final volume was 1 mL.

Xevo TQ XS Tandem Quadrupole Mass Spectrometer

Ionization:	APGC+, dry source charge exchange
Column:	Restek Rxi 35Sil MS 30 m x 0.25 mm ID, 0.25 µm
Carrier gas:	N_2 at 0.80 mL/min, He at 1.7 mL/min
Injection:	1 µL, Split/Splitless injector using 10:1 split
MS acquisition:	MRM

Autospec™ Premier Magnetic Sector Mass Spectrometer

Ionization:	EI+ at 35 eV
Column:	Agilent DB35-MS 30 m x 0.25 mm x 0.25 µm film
Carrier gas:	He at 1.0 mL/min
Injection:	1 µL, Cool-on-Column injector
MS acquisition:	HR SIR

Ramp (°C/min)	Temp (°C)	Hold (min)
Initial	95	0
5	155	0
25	275	0
2.5	335	8
He 48.8 min runtime		

Table 1. He Temperature Program

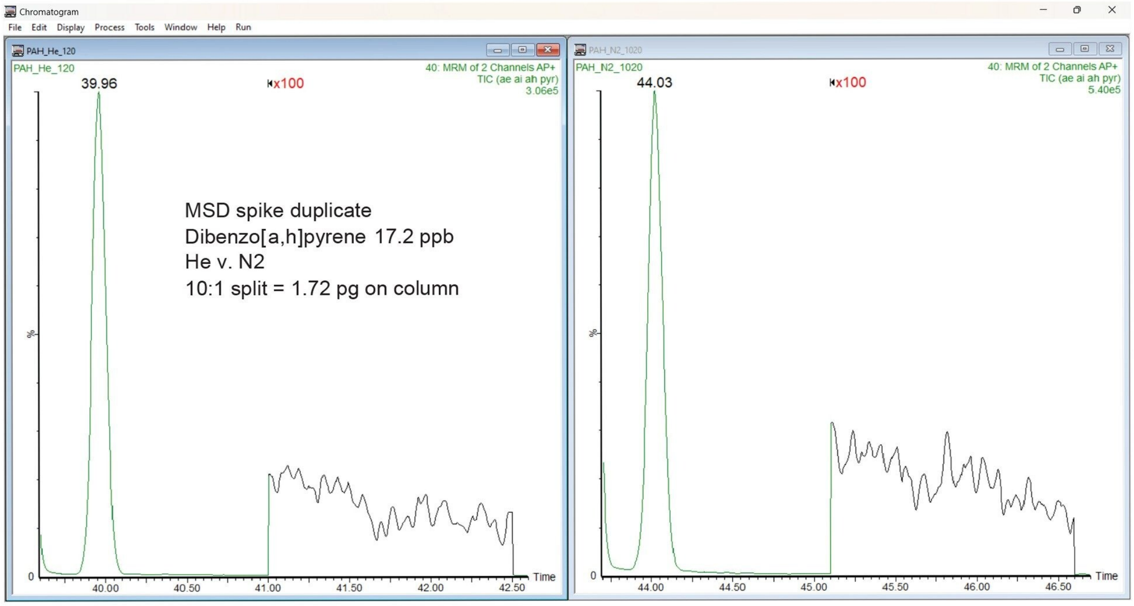
Ramp (°C/min)	Temp (°C)	Hold (min)
Initial	95	0
5	155	0
25	275	0
2.5	335	0
3	**350**	**7**
N_2 50.8 min runtime		

Table 2. N_2 Temperature Program

Results and Discussion

The first stage of method development was to adapt the He carrier gas flow to match APGC MS/MS retention times (RTs) with those of the EI-GC-HRMS reference method. Because the column outlet is near atmospheric pressure when using APGC versus being under vacuum in the reference method, the flowrate of He was increased from 1.0 to 1.7 mL/min which resulted in equivalent separation between all critical pairs plus RTs corresponding well across all analytes, surrogates, and internal standards. To maintain identical chromatographic separations and runtime when switching from He to N_2 carrier gas, it is commonly recommended to scale the column to a narrower inner diameter while keeping the phase ratio between column inner diameter and the stationary phase film thickness constant.[9] However, there was no commercially available column in the optimum dimensions for this scaling to be achieved. Therefore, in this study the identical column was used for both carrier gases.

The method was next adapted for N_2 carrier gas. Due to the lower optimum linear velocity requirement for N_2 carrier gas, the flow was set to 0.80 mL/min. This allowed the N_2 carrier gas APGC method to achieve a total runtime of 2.0 minutes (4.1%) longer than the He carrier gas method with only minor modification to the temperature program as shown above in the Experimental section. Furthermore, the latest eluting analyte, dibenzo(a,h)pyrene a 6-ring PAH at 302 Da, eluted only 4.07 min (10.2%) later, as shown in Figure 1, with comparable sensitivity to the He method. Figures 2 through 4 include examples of critical separations illustrating functionally equivalent sensitivity and chromatographic separations were achieved for all analytes, internal standards, and surrogates with each carrier gas.



MSD spike duplicate
Dibenzo[a,h]pyrene 17.2 ppb
He v. N2
10:1 split = 1.72 pg on column

Figure 1. Last eluting peak, dibenzo(a,h)pyrene. He carrier gas (left) versus N_2 carrier gas (right). Spiked sediment sample at 17.2 ppb in-aliquot concentration equaled 1.72 pg mass-on-column. Note baseline magnification of 100 times.

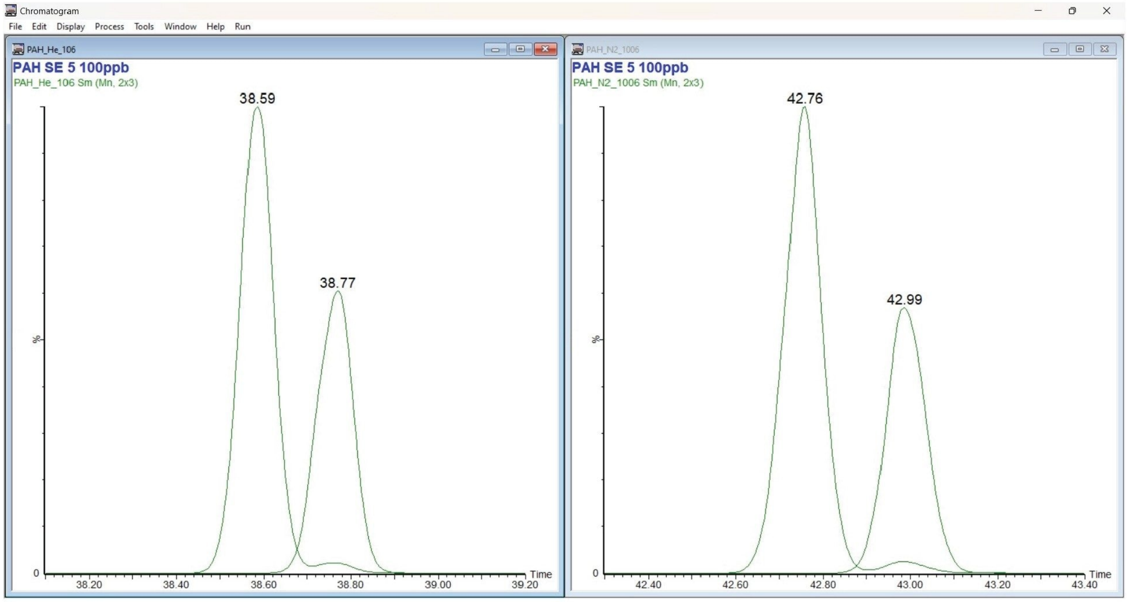


Figure 2. Separation of dibenzo(a,e)pyrene, and coronene using APGC MRM and He carrier gas (left) versus N_2 carrier gas (right).

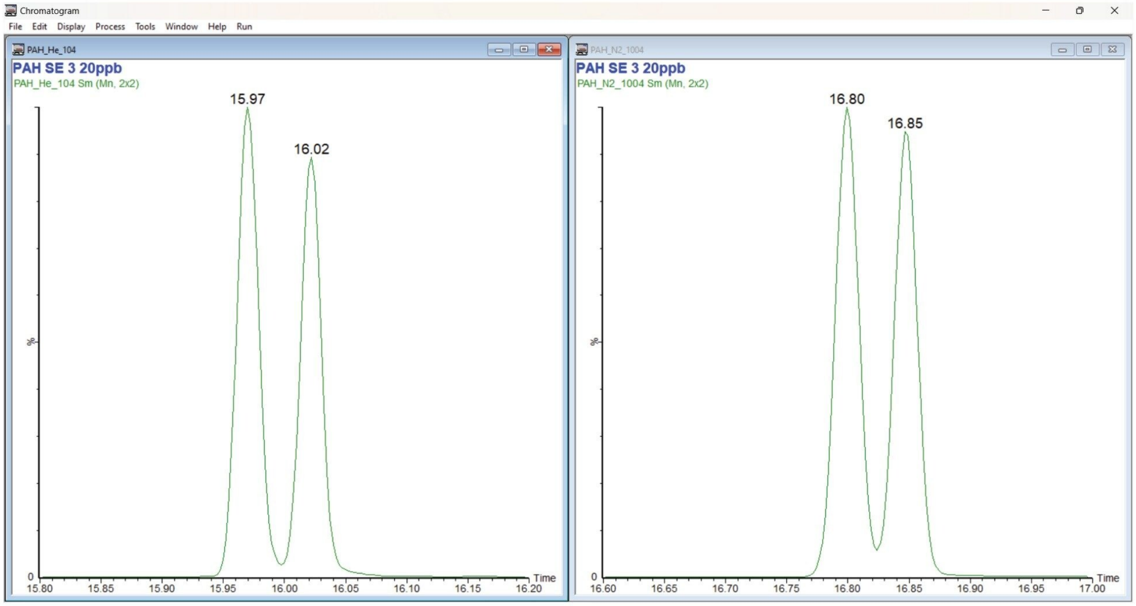


Figure 3. Separation of phenanthrene and anthracene using APGC MRM and He carrier gas (left) versus N_2 carrier gas (right).

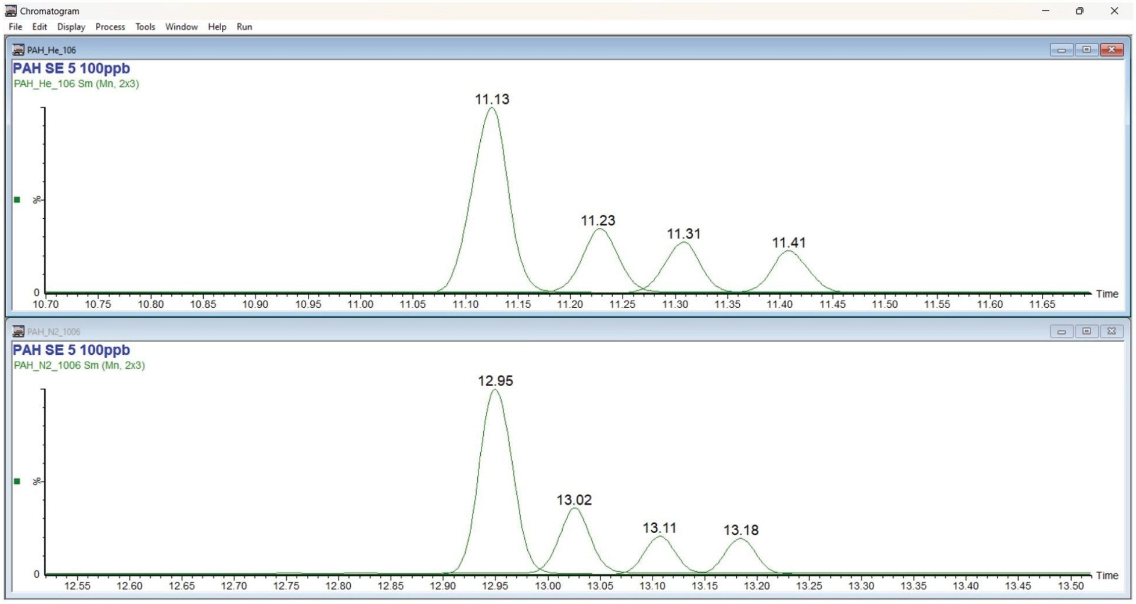
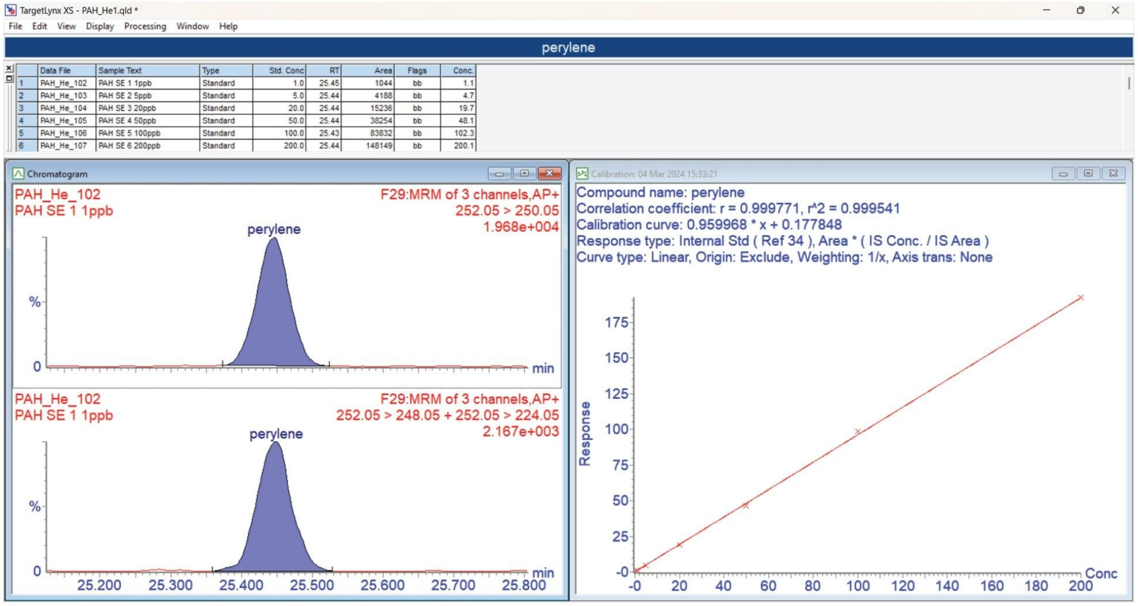


Figure 4. Separation of biphenyl-d10, biphenyl, 2-chloronaphthalene and 1-chloronaphthalene using APGC MRM and He carrier gas (upper) versus N_2 carrier gas (lower).

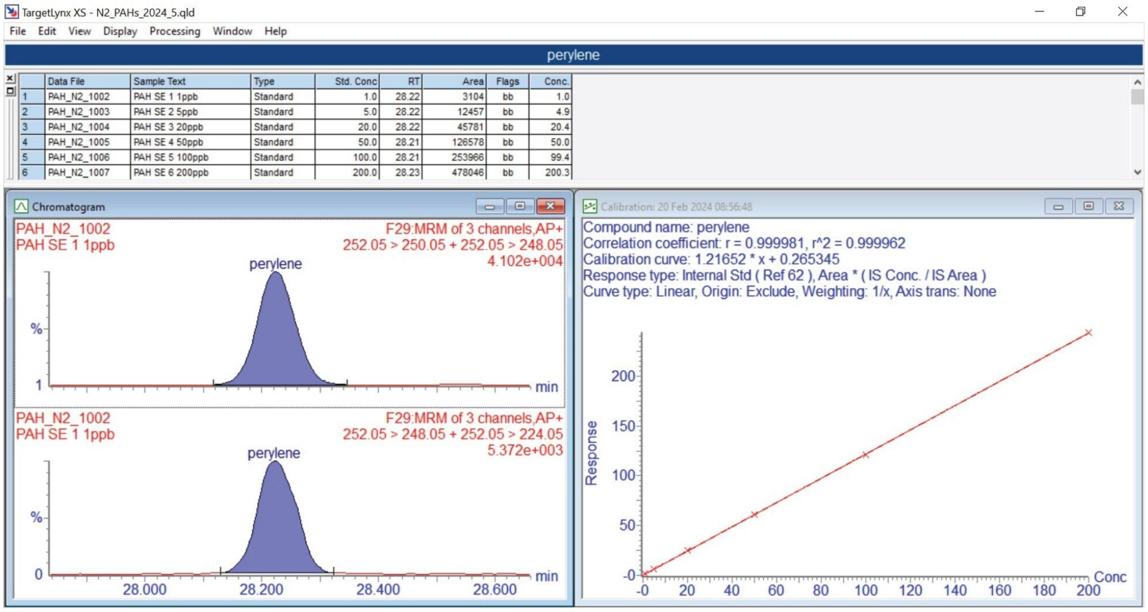
Analysis of the standards provided, demonstrated linear response for all PAHs in both the He and N_2 data across the range of 1 to 200 ppb with an average R^2 of 0.9978 and 0.9984, respectively. Figures 5 and 6 show linearity for perylene along with calculated concentration deviation for each point of the curve and the chromatogram for the 1 ppb level standard.



	Data File	Sample Text	Type	Std. Conc	RT	Area	Flags	Conc.
1	PAH_He_102	PAH SE 1 1ppb	Standard	1.0	25.45	1044	bb	1.1
2	PAH_He_103	PAH SE 2 5ppb	Standard	5.0	25.44	4188	bb	4.7
3	PAH_He_104	PAH SE 3 20ppb	Standard	20.0	25.44	15236	bb	19.7
4	PAH_He_105	PAH SE 4 50ppb	Standard	50.0	25.44	38254	bb	48.1
5	PAH_He_106	PAH SE 5 100ppb	Standard	100.0	25.43	83832	bb	102.3
6	PAH_He_107	PAH SE 6 200ppb	Standard	200.0	25.44	148149	bb	200.1

Compound name: perylene
Correlation coefficient: r = 0.999771, r^2 = 0.999541
Calibration curve: 0.959968 * x + 0.177848
Response type: Internal Std (Ref 34), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

Figure 5. He carrier gas calibration curve example for perylene with 1 ppb chromatogram.



	Data File	Sample Text	Type	Std. Conc	RT	Area	Flags	Conc.
1	PAH_N2_1002	PAH SE 1 1ppb	Standard	1.0	28.22	3104	bb	1.0
2	PAH_N2_1003	PAH SE 2 5ppb	Standard	5.0	28.22	12457	bb	4.9
3	PAH_N2_1004	PAH SE 3 20ppb	Standard	20.0	28.22	45781	bb	20.4
4	PAH_N2_1005	PAH SE 4 50ppb	Standard	50.0	28.21	126578	bb	50.0
5	PAH_N2_1006	PAH SE 5 100ppb	Standard	100.0	28.21	253966	bb	99.4
6	PAH_N2_1007	PAH SE 6 200ppb	Standard	200.0	28.23	478046	bb	200.3

Compound name: perylene
Correlation coefficient: r = 0.999981, r^2 = 0.999962
Calibration curve: 1.21652 * x + 0.265345
Response type: Internal Std (Ref 62), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

Figure 6. N_2 carrier gas calibration curve example for perylene with 1 ppb chromatogram.

Upon completion of APGC MS/MS method development, spiked sediment extracts and SRMs were analyzed using the N_2 carrier gas method. All aliquots had been previously analyzed using the EI HRMS reference method and have been published.[10] For analytes with reported concentrations within the range of 1 to 300 ppb the average agreement between the two methods was within 14.1% for all analytes in the matrix spike and duplicate spike samples (Figure 7). A single analyte, 1-nitropyrene, gave reproducibly high (62.4%) average error in all measurements. Future efforts to investigate this phenomenon will include evaluation of multiple replicate spiked extracts and alternative MRM transitions.

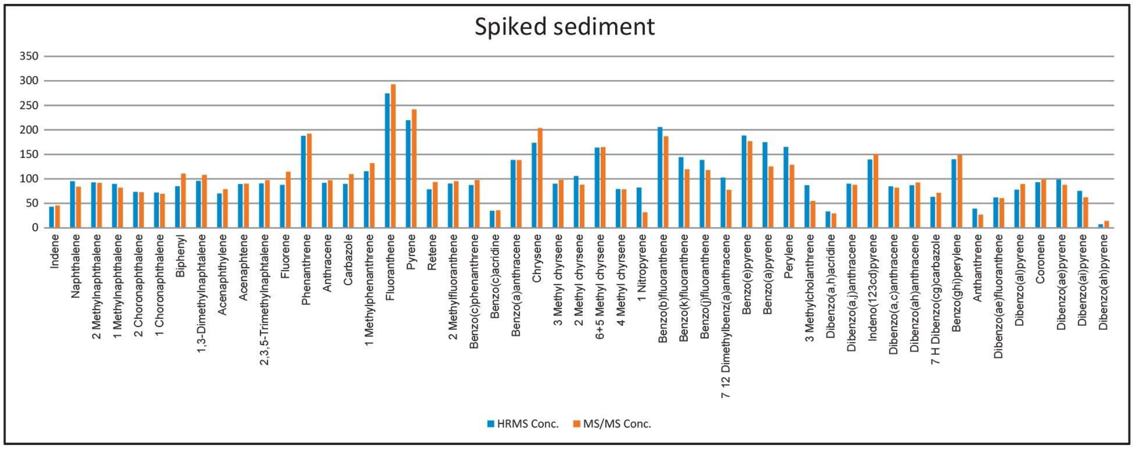


Figure 7. Spiked sediment sample calculated concentration comparison (ppb on y-axis) between the reference method and APGC-MS/MS method using N_2 carrier gas.

Conclusion

The reference method separations and runtime were successfully reproduced using He carrier gas with APGC-MS/MS. Adapting the method for N_2 carrier gas was achieved with a minimal increase in total runtime and comparable separation efficiency. The comparison between the APGC He and N_2 carrier gas data shows that sensitivity is also maintained within a factor of two times between the two carrier gases across all analytes. Analysis of spiked sediment sample extracts indicates good agreement between the APGC N_2 carrier gas method and the EI HRMS reference method values. These results demonstrate the feasibility of modernizing the traditional reference method with a technique that is more easily integrated into most labs and more accessible for most operators.

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