Application Note

Converting Target Analysis of Organochlorine Pesticides 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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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Organochlorine pesticides (OCPs) are among the high production volume chemicals produced in the past that had wide application and exhibit significant persistence in the environment. DDT, for example, has a half-life in sediment of up to 15 years meaning that it may not be 90% degraded for 45 to 60 years.[1] Furthermore, they are associated with a number of negative health effects when bioaccumulated in humans and animals. These are among the reasons that nine of the initial 12 compounds addressed by the 2004 Stockholm Convention on Persistent Organic Pollutants (POPs) belong to this compound class.[2]

Due to the complexity of matrices, such as sediment and biota, 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 instrumentation. 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]

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 and biota extracts for the presence of 39 OCPs. Performance characteristics and sample quantification results for APGC-MS/MS were compared with results for the same aliquots run using GC-EI-HRMS.

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

Slow rates of degradation in the environment and the semivolatile properties of OCPs combined with the sheer volume of these chemicals deployed across the years has resulted in their global distribution even to areas where primary urban, industrial, and agricultural inputs are absent.[4] This widespread distribution leads to OCPs being found not only in water and sediment samples but also incorporated into the food web making biota samples important to study as well. Environmental processes, such as thawing of the cryosphere, that may be exacerbated by anthropogenic climate change also contribute to transport of these persistent organic pollutants (POPs) to remote regions in the Arctic. These factors combine to keep the need for monitoring for the presence of OCPs in environmental matrices current and relevant despite the fact that many of them have been banned from production and their original intended use for many years.

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.[5] Many environmental analyses have the combination of needs that make EI-GC-HRMS a required analytical technique. Therefore, it became widely adopted for this work. However, the technique presents challenges in many labs where the facilities requirements for installation and operator expertise required for these systems are beyond the means of many organizations. To add to that, 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.[6] A current trend in recent years then 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.[7]

In an effort to further expand on the applications being successfully transitioned to GC-APCI MS/MS, this study used a well characterized EI-GC-HRMS method for the quantification of OCPs in environmental matrices.[7] Extracted samples and standard reference materials (SRMs) were analyzed first using He carrier gas for method development and direct comparison to the prior method and then using N_2 to compare performance with a more sustainable, less costly alternative carrier gas.[8]

Experimental

Sample Preparation

Sample preparation was performed at Environment and Climate Change Canada's (ECCC) Quebec Laboratory for Environmental Testing (QLET). Samples and standards included a five point calibration curve, blanks, standard reference materials (SRMs) and four samples of biota and sediment. Samples were placed in a cellulose thimble containing Na_2SO_4 and extracted using soxhlet extraction (16-hour reflux in hexane:acetone 1:1 for biota, toluene for sediment). Extracts were passed through GPC followed by silica deactivated with 3% H_2O column clean-up.

GC Conditions

System: Xevo TQ XS Tandem Quadrupole Mass Spectrometer

Ionization: APGC+, dry source charge exchange

Column (He): Restek Rxi 5 Sil MS 60 m x 0.25 mm ID, 0.25 µm film at 1.50 mL/min

Column (N2): Restek Rxi 5 Sil MS 40 m x 0.18 mm ID, 0.18 µm film at 0.45 mL/min

Injection: 1 µL, Split/Splitless injector in splitless mode

MS acquisition: MRM

MS Conditions

System: Autospec™ Premier Magnetic Sector Mass Spectrometer

Ionization: EI+ at 35 eV

Column (He): Agilent DB5-MS 60 m x 0.25 mm x 0.25 µm film at 1.0 mL/min

Injection: 1 µL, Cool-on-Column injector

MS acquisition: HR SIR

In order to reduce variables, the same 39-minute temperature program was used for all carrier gas and column configurations.

Gradient Table

Ramp (°C/min)	Temp (°C)	Hold (min)
Initial	90	0
60	190	0
6.5	200	0
3	260	8
25	325	5

Results and Discussion

The first stage of method development was performed using the APGC source and the 60 m column with He carrier gas to reproduce the reference EI-GC-HRMS 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 1.5 mL/min in order to match retention times and separating efficiency between the two methods. This resulted in equivalent separation between all critical pairs plus RTs closely matched across all analytes, surrogates and internal standards (data not shown).

The method was next adapted for N_2 carrier gas using a 40 m column scaled to maintain the same phase ratio as the reference method column. This allowed the N_2 carrier gas APGC method to achieve RTs within an average of 1.1 seconds of the He method and separations equivalent to the APGC He carrier gas method, Figure 1, and reference method. An example of the separation between hexachlorobenzene and pentachloroanisole using APGC and each carrier gas is shown in Figure 2. Because of the close match in chromatographic performance between the two carrier gases, the exact same MRM acquisition file and quantification processing method are able to be used on both data sets. Furthermore, comparable sensitivity, +/- a factor of 2X, was achieved between the two carrier gas methods, Figure 3. Linear response was observed for all OCPs in the He and N_2 data across the range of 1 to 200 ppb with an average R^2 of 0.9960 and 0.9991, respectively. Because the lowest calibration point was 1 ppb, which is well above the LOD for the analytes in this method, the top two MRM transitions are both sufficiently sensitive to be summed together for use as the primary quantification trace.

Upon completion of APGC MS/MS method development, extracted samples and SRMs of biota and sediment were analyzed using the N_2 carrier gas method. All aliquots had been previously analyzed using the EI HRMS reference method. Results for the SRMs have been previously published[8]. Two extracts of sediment named Sample A and Sample B plus two extracts of biota named Sample 5 and Sample 6 were analyzed. For analytes with reported concentrations within the calibrated range of 1 to 200 ppb, the average agreement between the two methods was within 20.2% for all analytes and all samples. See Figure 6 for results from Samples 5 and 6 which are within an average of 5.95% across all detected analytes in the 15 to 65 ppb range. Although chloronthalonilchlorothalonil is included in the analyte list, it was excluded from the comparison because it was showed high variability across all samples and SRMs and is known to present problems with recovery from complex matrices.[9] These samples were also stored for over three years between the original analysis by EI HRMS and subsequent APGC-MS/MS analysis which may have contributed to its variability. Higher variability was also observed for calculated concentrations in the 1 to 2 ppb range. Average agreement between the two methods was, therefore, recalculated for the range of 2 to 200 ppb resulting in all being within an average of 10.6%. Examples of low levellow-level analyte detections in extracted samples are shown in Figures 7 and 8. The calculated concentration for trans-nonachlor in sediment Sample A is within 6.6% of the reference method value and the dieldrin in biota Sample 6 value is within 4.2% of the reference method. Because the criteria for agreement between the two methods was to be within 30%, all of the above values indicate initial success in method translation with further method evaluation and refinement planned.

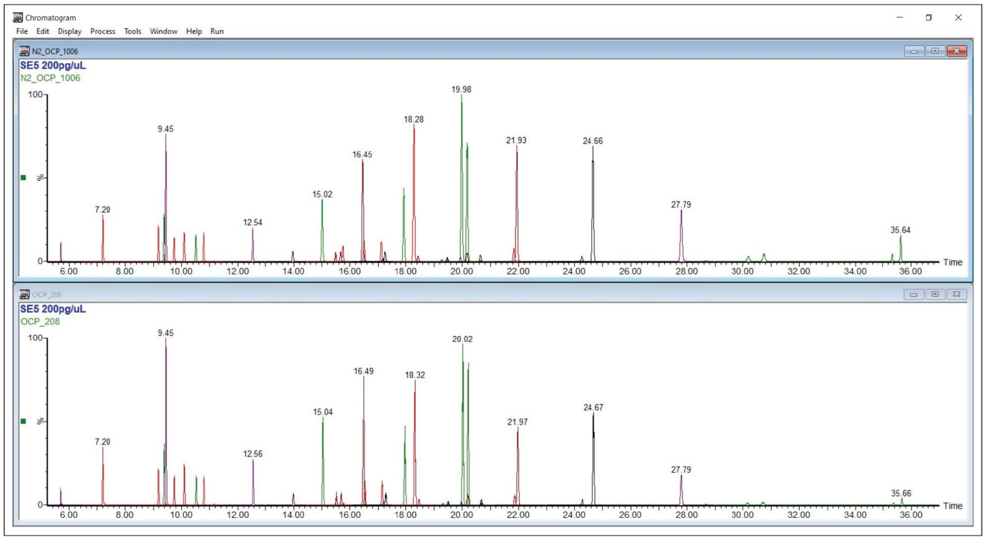


Figure 1. Overlaid APGC MRM TICs for N_2 (upper) and He (lower) carrier gas.

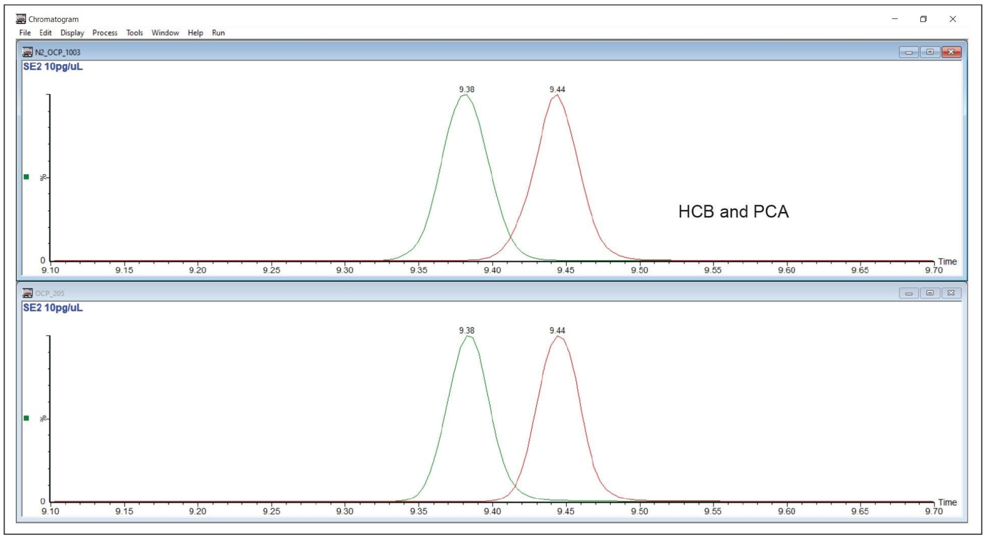


Figure 2. APGC data showing chromatographic resolution of hexachlorobenzene and pentachloroanisole using nitrogen (upper) and He (lower) carrier gas.

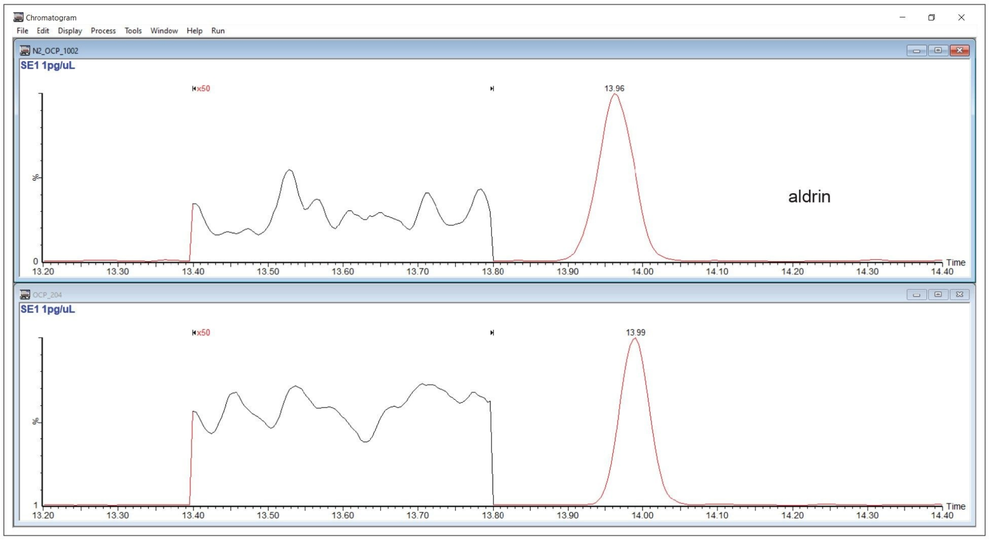
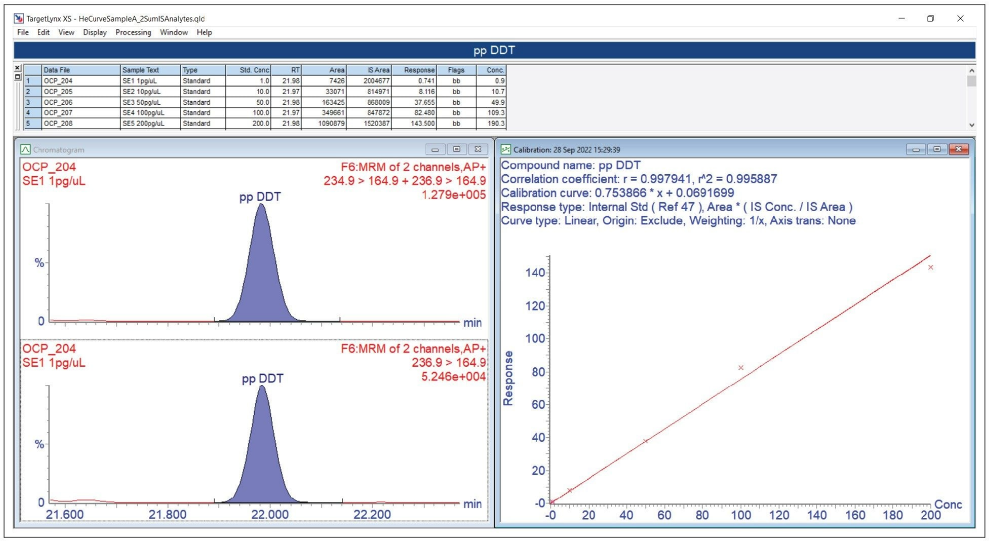


Figure 3. Aldrin APGC sensitivity comparison N_2 (upper) and He (lower) carrier gas for 1 ppb (1 µL at 1 pg/µL = 1 pg mass-on-column).

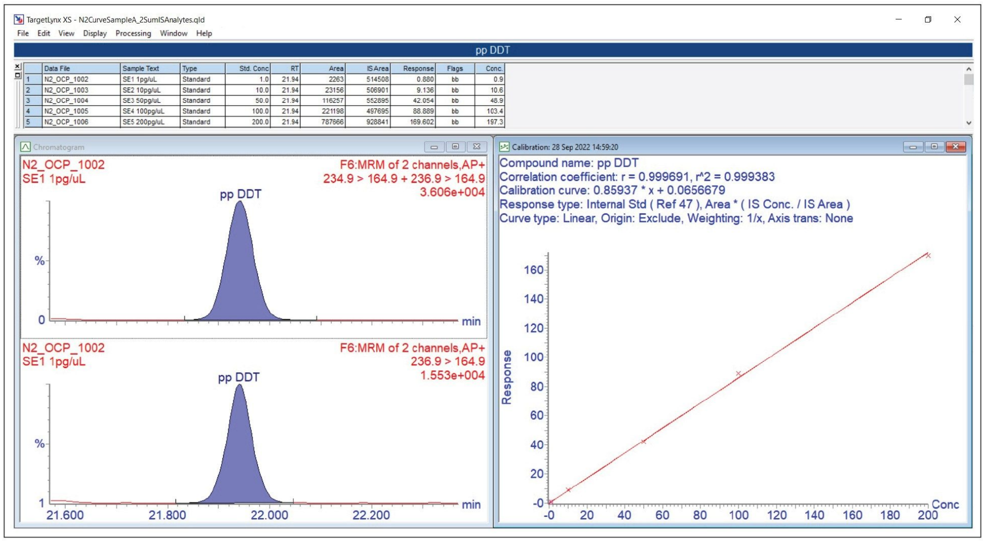


	Data File	Sample Text	Type	Std. Conc	RT	Area	IS Area	Response	Flags	Conc.
1	OCP_204	SE1 1pg/uL	Standard	1.0	21.98	7426	2004677	0.741	bb	0.9
2	OCP_205	SE2 10pg/uL	Standard	10.0	21.97	33071	814971	8.116	bb	10.7
3	OCP_206	SE3 50pg/uL	Standard	50.0	21.98	163425	868009	37.655	bb	49.9
4	OCP_207	SE4 100pg/uL	Standard	100.0	21.97	349661	847872	82.480	bb	109.3
5	OCP_208	SE5 200pg/uL	Standard	200.0	21.98	1090879	1520387	143.500	bb	190.3

Calibration: 28 Sep 2022 15:29:39

Compound name: pp DDT
Correlation coefficient: r = 0.997941, r^2 = 0.995887
Calibration curve: 0.753866 * x + 0.0691699
Response type: Internal Std (Ref 47), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

Figure 4. He carrier gas calibration curve example for p,p DDT with 1 ppb chromatogram.



	Data File	Sample Text	Type	Std. Conc	RT	Area	IS Area	Response	Flags	Conc.
1	N2_OCP_1002	SE1 1pg/uL	Standard	1.0	21.94	2263	514508	0.880	bb	0.9
2	N2_OCP_1003	SE2 10pg/uL	Standard	10.0	21.94	23156	506901	9.136	bb	10.6
3	N2_OCP_1004	SE3 50pg/uL	Standard	50.0	21.94	116257	552895	42.054	bb	48.9
4	N2_OCP_1005	SE4 100pg/uL	Standard	100.0	21.94	221198	497695	88.889	bb	103.4
5	N2_OCP_1006	SE5 200pg/uL	Standard	200.0	21.94	787666	928841	169.602	bb	197.3

Compound name: pp DDT
Correlation coefficient: r = 0.999691, r^2 = 0.999383
Calibration curve: 0.85937 * x + 0.0656679
Response type: Internal Std (Ref 47), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

Figure 5. N_2 carrier gas calibration curve example for p,p DDT with 1 ppb chromatogram.

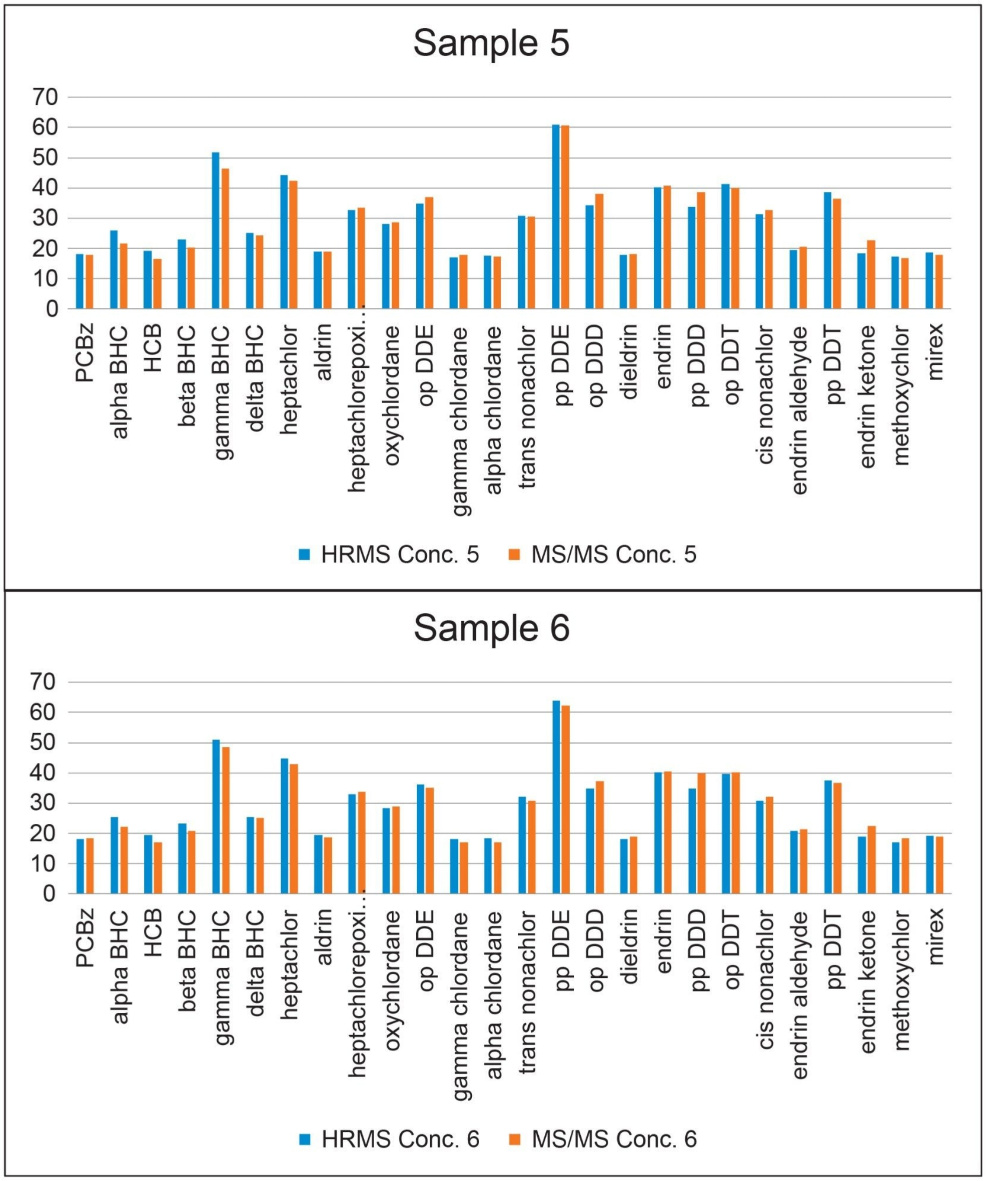


Figure 6. Concentration comparison (ppb on y-axis) between the reference method and APGC-MS/MS method using N_2 carrier gas.

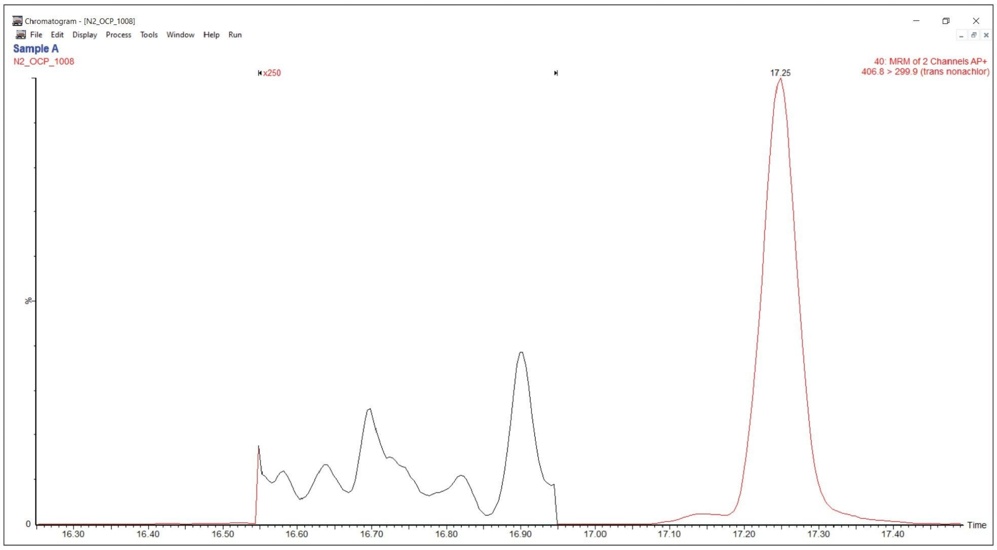


Figure 7. Trans-nonachlor at 2.52 ppb in sediment Sample A. Note baseline magnification of 250 times.

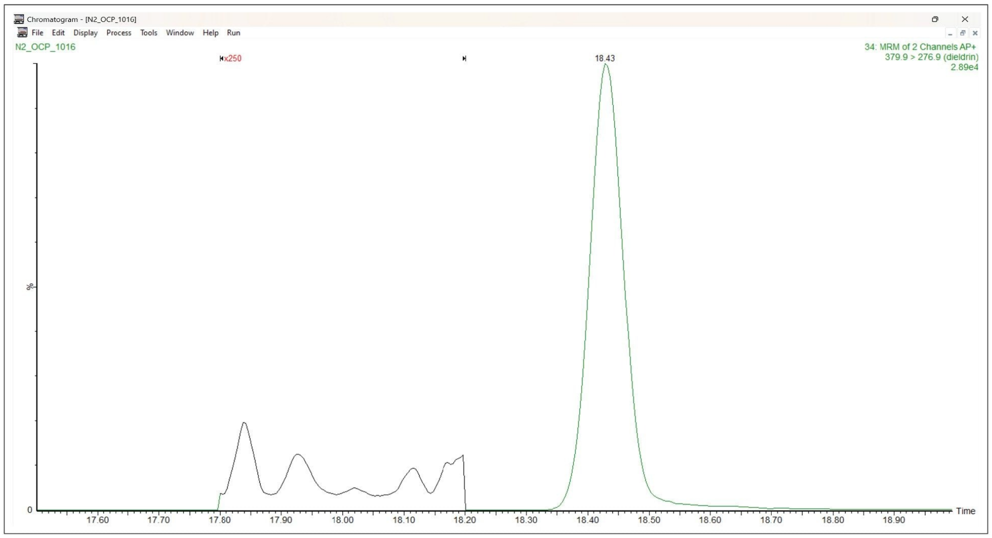


Figure 8. Dieldrin at 18.9 ppb in biota Sample 6. Note baseline magnification of 250 times.

Conclusion

The GC-EI-HRMS reference method separations and runtime were matched on the APGC-MS/MS system with only minor modification to the He carrier gas flowrate. Scaling of the column dimensions for use with N_2 carrier gas effectively reproduced the reference method separations and total runtime which allows the use of the same acquisition and processing files across both configurations. 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. Analysis of extracted samples of biota and sediment indicate 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.

References

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