

## Target Analysis of Semivolatile Compounds Using Atmospheric Pressure GC-MS/MS

---

Douglas M. Stevens, Frank Dorman, Kenneth J. Rosnack, Peter Hancock

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

---

### Abstract

Across the past decade, the use of gas chromatography atmospheric pressure ionization mass spectrometry (GC-APCI-MS) has steadily increased.<sup>1</sup> Many publications using this technique have focused on analytes of a specific chemical class or specific applied-use.<sup>2,3</sup> Optimization for these different analyses has typically involved operation of the source to favor either charge exchange ionization or a protonation ionization mechanism. While the design of the source used in this study does allow for precise control of either charge exchange or protonation, the nature of some multi-class analyses leads to the need for concurrent ionization of analytes amenable to either or both ionization mechanisms. Analysis of multi-class semivolatile organic compounds is one such method.

This work will evaluate the sensitivity, reproducibility, and dynamic range of the Waters Atmospheric Pressure Gas Chromatography (APGC™) ionization source while operating in a configuration that provides simultaneous charge exchange and protonation ionization.

### Benefits

---

- The sensitivity and specificity of APGC-MS/MS allow the use of split injection which increases instrument utilization and column life
- Broader compound coverage through dual chemical ionization allows APGC to be used for an expanded range of targeted semivolatile analytes relevant in environmental analysis

---

## Introduction

Interest in semivolatile compounds (SVOCs) stretches back to the early days of modern environmental analysis when GC/MS was among the primary instrumental techniques employed and, in fact, helped define this category of analysis.<sup>4</sup> At this time it was much more common for contaminated sites to have multiple types of compounds from multiple sources all present at a single location.<sup>5,6,7</sup> Early studies of the occurrence, fate, and transport of these compounds in the environment combined with studies on adverse health effects on humans, animals, and organisms as well as the components and compartments that make up the overall environment, contributed to the recognition of the need for deliberate and thorough monitoring of SVOCs as part of comprehensive and data-driven environmental protection plan.<sup>8</sup>

While studying the fate and effects of various industrial chemicals in and on the environment, it has often been necessary to focus on a single analyte, compound class, or applied use which leads to increased time and effort when attempting to thoroughly characterize samples. The need for focus has previously been caused by factors related to the analytical technique such as excessive fragmentation during ionization, low specificity, and relatively poor sensitivity. Together these performance challenges created the need for extensive sample clean up and enrichment as well as longer analysis times. While current generation electron ionization (EI) GC/MS systems have better performance characteristics than early models, they do still have inherent limitations based on the fundamental traits of EI and single stage MS. To help overcome these specific challenges, it is possible to combine the use of low energy CI with Tandem Mass Spectrometry (MS/MS) using APGC on a tandem quadrupole MS operating in multiple reaction monitoring (MRM) mode. The low energy ionization of atmospheric CI contributes to both the specificity and sensitivity advantages over EI by generating molecular ions of high relative and absolute abundance for the vast majority of analytes. The specificity of MRM significantly reduces chemical noise as compared with single stage MS scanning and selected ion monitoring/recording (SIM/SIR) modes of acquisition. It has recently been found that it is possible to create stable conditions in which an

individual analyte ionizes through one of two different mechanisms based on its characteristic proton affinity or ionization energy.

The overall category of volatile organic compounds is divided into subcategories including very volatile (VVOC), volatile (VOC), and SVOCs. VVOCs, often referred to as gases, and VOCs have previously been studied using APGC.<sup>9</sup> SVOCs are characterized by higher boiling points and molecular weights as compared with gases and VOCs. Investigation of SVOCs using this approach is needed to ensure that it meets performance requirements for quantitative target analysis.

---

## Experimental

A standard SVOC calibration mixture (Restek, #31850) was diluted to allow evaluation of the sensitivity, reproducibility, and linearity of the APGC source across the range of 5 pg/ $\mu$ L to 50 ng/ $\mu$ L. The mix contains 76 analytes that elute in 75 peaks (one isomeric pair co-elutes). A mix of six commonly used internal standards was also added (Restek, #31006) at a concentration of 8 ng/ $\mu$ L to each injection aliquot.

Separation was performed on an Rxi®-5Sil MS, 30 m x 0.25 mm id x 0.25  $\mu$ m film column with a flow of 2 mL/minutes helium and a 100:1 split injection at 300 °C with a straight 4 mm split injection port liner with wool. The GC was held at 40 °C for one minute, ramped to 120 at 10 °C/minute, then to 320 at 25 °C and held for four minutes.

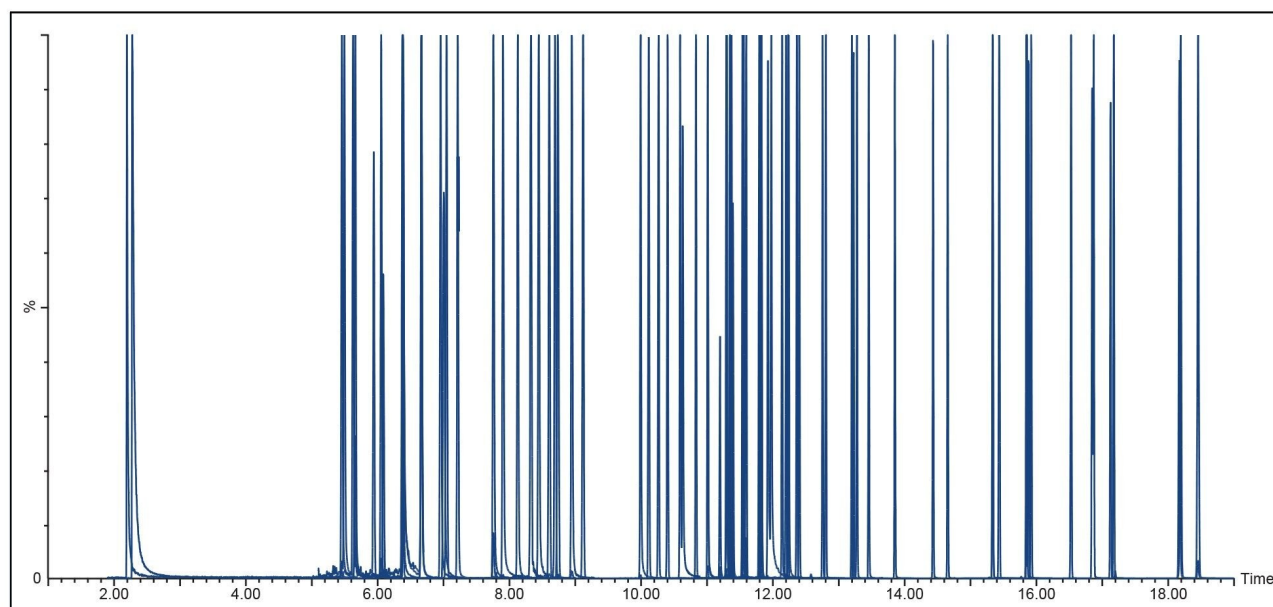
GC-MS/MS analysis was performed on a Xevo TQ-XS™ Tandem Quadrupole Mass Spectrometer System equipped with the APGC source option. In order to create concurrent conditions for protonation and charge exchange ionization, water was added to the source and Cone Gas was run at 240 L/hour.

---

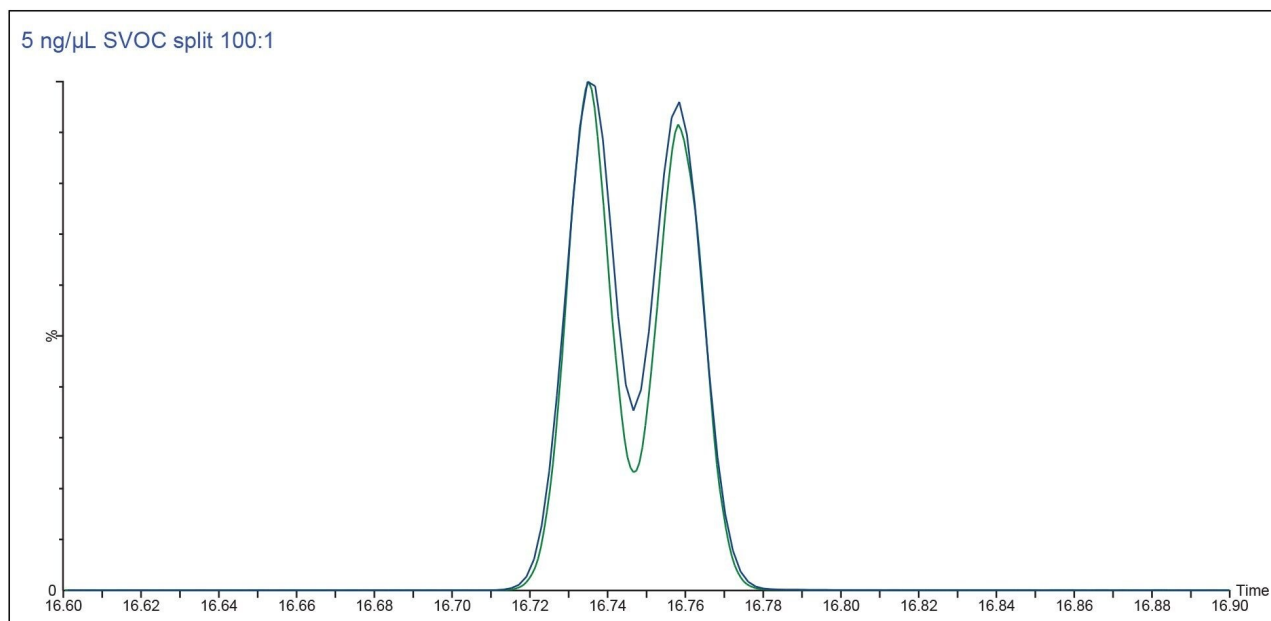
## Results and Discussion

A high-level standard containing multiple classes of SVOCs was used to develop a chromatographic separation that results in all analytes eluting under twenty minutes (Figure 1) using a 100:1 split injection. The requirement to separate specific isomeric pairs with a minimum of a 50% valley between chromatographic peaks was among

the criteria considered during this method development. Four steps of carrier gas flowrate from 1.8 to 2.5 mL/minute of helium were evaluated with a 20–30% valley achieved between the isomeric compounds benzo[b]fluoranthene and benzo[k]fluoranthene (Figure 2). Subsequent method development focused on creation of optimized MRM transitions for all analytes and internal standards. Multiple MRM transitions were obtained for each analyte and entered into a Quanpedia™ Database. This allows sharing of the optimized MRM transitions for labs implementing this method.



*Figure 1. MRM TIC Chromatograms of 76 SVOCs. 1 ng/μL aliquot split 100:1 (10 pg on-column).*



*Figure 2. Separation of benzo[b]fluoranthene and benzo[k]fluoranthene at 1.8 mL/min (green) and 2.3 mL/min (blue) helium flow. Chromatograms aligned by 0.213 min for overlay purposes.*

Overall, 87% of the analytes achieved an  $r^2$  value of greater than 0.99 across three orders of magnitude in the range of 10 pg to 10 ng/μL (100 fg to 100 pg on-column) based on three injections of each level. Example calibration curves and chromatograms at 1 pg on-column are shown in Figures 3 and 4 to demonstrate sensitivity and linearity. The compounds, hexachlorocyclopentadiene and benzo[a]pyrene, were chosen as they represent analytes that ionize through charge exchange and protonation, respectively. One of the internal standards, perylene-d12, was found to ionize well in both modes. This allowed its use as a traditional response reference correction standard as well as to monitor for stable dual mode operation through the evaluation of the ion ratio between the protonated and charge exchange forms. The ion ratio between the two forms had a 0.49% RSD across 30 hours of continuous operation indicating stable ionization in both modes across time periods compatible with typical SVOC sample batches.

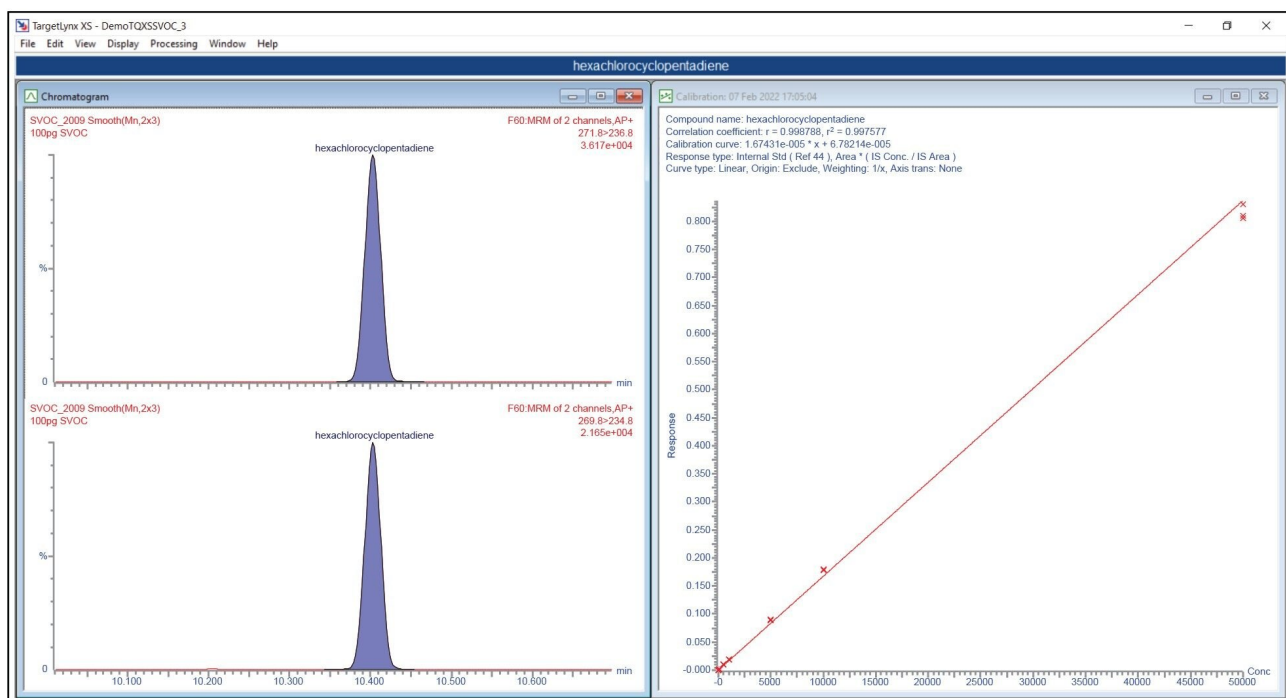


Figure 3. Calibration curve for hexachlorocyclopentadiene (10 pg to 50 ng,  $n = 3$ ).

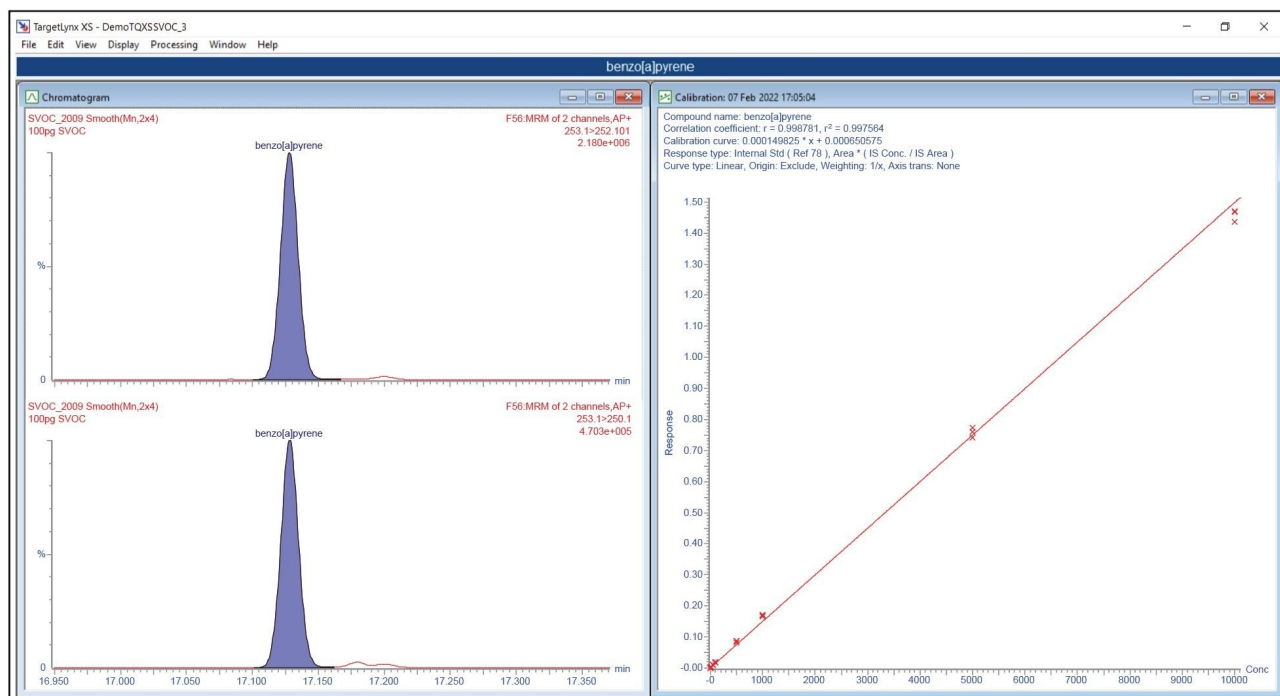


Figure 4. Calibration curve for benzo[a]pyrene. 10 pg to 10 ng,  $n=3$ ).

Finally, during the course of this work not only did the price of helium rise rapidly but the possibility of a complete interruption of the helium supply was encountered. This led to the evaluation of nitrogen as a carrier gas. The conversion from helium to nitrogen carrier was facilitated by the fact that high purity, dry nitrogen is supplied to the system for use as the APGC make-up and reagent gases. An example of the separation of benz[a]anthracene and chrysene with each carrier gas is shown in Figure 6. The column and most analytical conditions were kept constant between the two examples except for nitrogen carrier gas flowrate was 0.7 mL/minute versus 2.0 mL/minute for helium and the temperature program was 40 °C for one minute to 320 °C at 20 °C/minute with a hold of five minutes. The peak widths achieved for helium carrier gas were 2.4 s versus 3.0 s for nitrogen carrier gas. Sensitivity for hexachlorobenzene was also evaluated using nitrogen carrier gas with greater than 1000:1 signal-to-noise being achieved for 500 fg mass-on-column (Figure 7). In future work, further adaptation of the method for optimum nitrogen carrier gas performance will include scaling the column dimensions in order to achieve peak widths and resolution from nitrogen carrier gas that are comparable to helium.

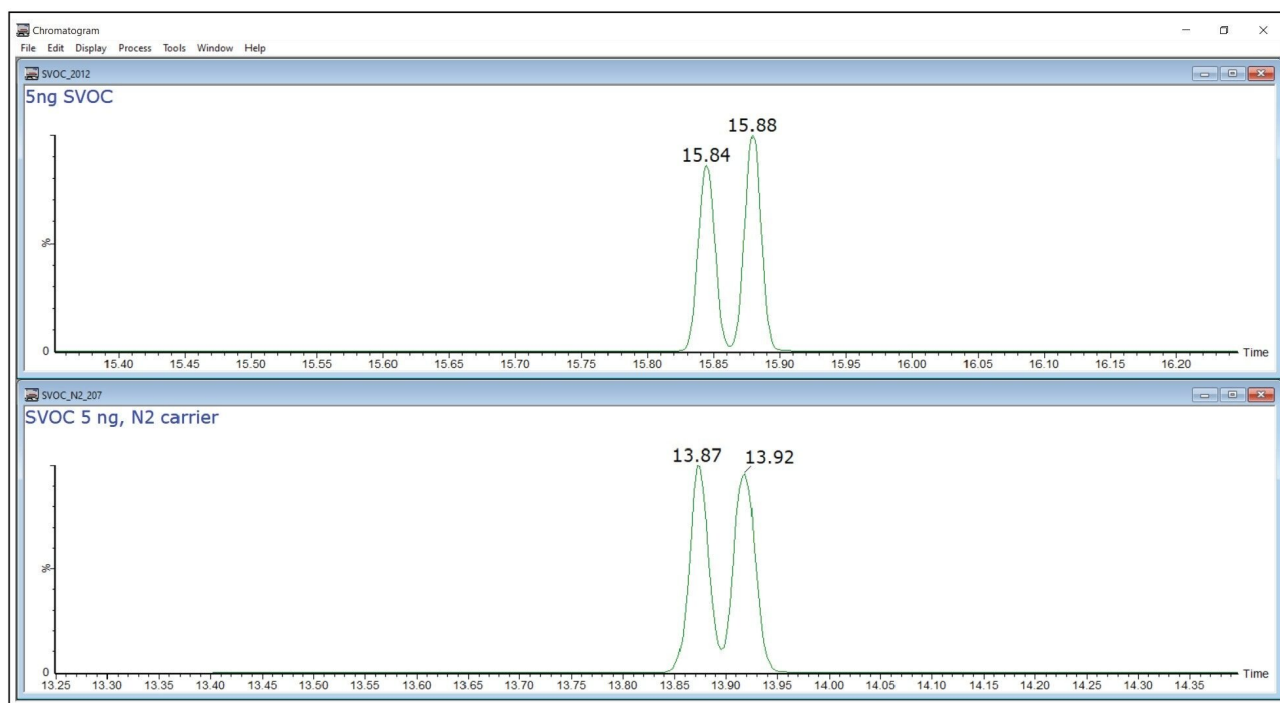


Figure 6. Separation of benz[a]anthracene and chrysene using same column with helium carrier gas (upper chromatogram) and nitrogen carrier gas (lower chromatogram).



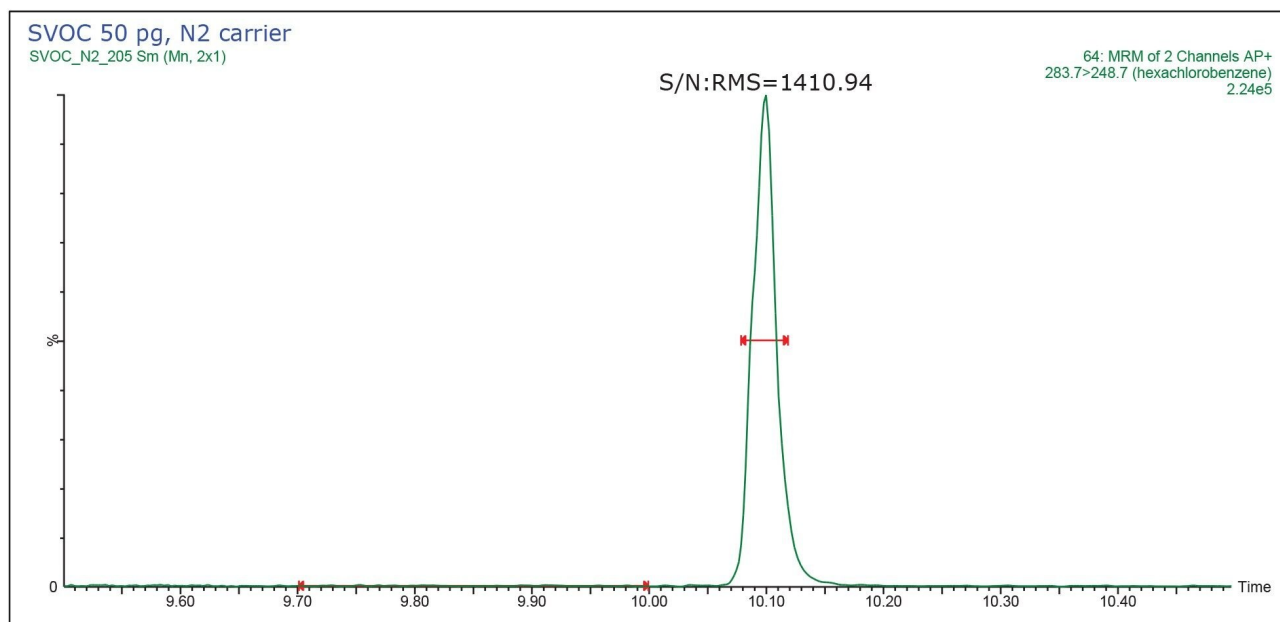


Figure 7. Sensitivity of hexachlorobenzene, 500 fg mass-on-column, with nitrogen carrier gas.

---

## Conclusion

The use of the APGC source in a dual chemical ionization mode demonstrated linearity and stability suitable for the routine quantitative analysis of semivolatile organic compounds. The sensitivity achieved allowed the use of a high split ratio for injections which decreases the amount of matrix introduced to the column as well as the MS. Split injection also reduces the residence time in the GC inlet, generally resulting in improved inertness and robustness. This helps extend column life and the time between routine maintenance procedures. Future work will investigate the performance of this concurrent ionization scheme and the use of nitrogen as a carrier gas for target analysis of SVOCs in extracted samples of environmental relevance.

---

## References

---

1. Li, Du-Xin, *et al.* "Gas Chromatography Coupled to Atmospheric Pressure Ionization Mass Spectrometry (GC-API-MS): Review." *Analytica chimica acta* 891 (2015): 43–61.
2. Portolés, Tania, *et al.* "Potential of Atmospheric Pressure Chemical Ionization Source in GC-QToF MS MS for Pesticide Residue Analysis" *Journal of Mass Spectrometry* 45.8 (2010): 926–936.
3. Xu, Shanshan, *et al.* "Monitoring Temporal Trends of Dioxins, Organochlorine Pesticides and Chlorinated Paraffins in Pooled Serum Samples Collected From Northern Norwegian Women: The Misa Cohort Study." *Environmental Research* 204 (2022): 111980.
4. Heller, Stephen R., John M. McGuire, and William L. Budde. "Trace Organics by GC/MS [Gas Chromatography/Mass Spectrometry]." *Environmental Science & Technology* 9.3 (1975): 210–213.
5. US Environmental Protection Agency, Superfund History, 2021, [Superfund Site Link < https://www.epa.gov/superfund/superfund-history-printable-version%20>](https://www.epa.gov/superfund/superfund-history-printable-version%20) , Accessed 25 March 2022.
6. Campbell, J. H., *et al.* "Groundwater Quality Near an Underground Coal Gasification Experiment." *Journal of Hydrology* 44.3–4 (1979): 241–266.
7. Wallin, Bruce K. Fate of Toxic and Nonconventional Pollutants in Wastewater Treatment Systems Within the Pulp, Paper, and Paperboard Industry. US *Environmental Protection Agency*, Industrial Environmental Research Laboratory, 1981.
8. Campisano, R., K. Hall, J. Griggs, S. Willison, S. Reimer, H. Mash, M. Magnuson, L. Boczek, and E. Rhodes. Selected Analytical Methods for Environmental Remediation and Recovery (SAM) 2017. U.S. *Environmental Protection Agency*, Washington, DC, EPA/600/R-17/356, 2017.
9. Douglas M. Stevens, Analysis of Residual Solvents in Hemp Oil Using Headspace Sampling and Atmospheric Pressure GC-MS/MS, Waters Application Note: [720007150](#), 2021.
10. Raro, M., *et al.* "Potential of Atmospheric Pressure Chemical Ionization Source in Gas Chromatography Tandem Mass Spectrometry for the Screening of Urinary Exogenous Androgenic Anabolic Steroids" *Analytica Chimica Acta* 906 (2016): 128–138.

---

## Featured Products

Xevo TQ-XS Triple Quadrupole Mass Spectrometry <<https://www.waters.com/134889751>>

Waters Atmospheric Pressure Gas Chromatography (APGC) <<https://www.waters.com/10100362>>

MassLynx MS Software <<https://www.waters.com/513662>>

720007595, April 2022

© 2022 Waters Corporation. All Rights Reserved.

[Terms of Use](#)

[Privacy](#)

[Trademarks](#)

[Sitemap](#)

[Careers](#)

[Cookies](#)

[Cookie-Einstellungen](#)