Application Note

Instrument: Pegasus® HRT+ 4D

Enhanced Quantitative Analysis of Polychlorinated Paraffins by Comprehensive GCxGC-HRTOFMS with Negative Chemical Ionization

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Key Words: Quantitative Analysis, Persistent Organic Pollutants, Chlorinated Paraffins, Comprehensive Two-Dimensional Gas Chromatography, High-Resolution Time-of-Flight Mass Spectrometry, Electron Capture Negative Ionization, GCxGC, HRTOFMS, ECNI

Introduction

Household dust is a repository of hazardous compound accumulation and therefore an important indicator of chemical exposure.[1] Humans are regularly exposed to a wide variety of harmful substances in dust such as pesticides, flame-retardants, and persistent organic pollutants (POPs). The occurrence of dangerous chemicals in homes is potentially high due to inadvertent transfer from outside sources, as well as their presence in household goods such as carpets, furniture, paint, textiles, and electronic devices. One group of persistent organic pollutants (POPs) that can be found in dust are polychlorinated paraffins (PCPs). PCPs are synthetic compounds produced by the chlorination of linear alkanes. PCPs have a general formula of $C_nH_{2n+2-z}Cl_z$ and their extent of chlorination by weight ranges from 30 to 70 percent. PCPs are produced in enormous quantities (greater than 1 million tons/year) and are used commercially as flame-retardants, plasticizers, and metalworking lubricants. They are classified according to their carbon length: Short-chain chlorinated paraffins (SCCP, C_{10}-C_{13}), medium-chain chlorinated paraffins (MCCP, C_{14}-C_{17}), and long-chain chlorinated paraffins (LCCP, $\geq C_{18}$). PCPs are difficult to analyze using conventional GC-MS and LC-MS approaches due to their complexity. The number of PCPs is very large not only because of the many different homologs with varying formulas, but also structural and stereoisomers for PCPs with identical formulas. Their high level of chlorination results in significant molecular fragmentation using conventional electron ionization energy, thus reducing sensitivity. Matters are complicated further by their low sample concentrations relative to other dust components such as saturated hydrocarbons, polyaromatic hydrocarbons, phthalates, and other POPs. These compounds can coelute in one-dimensional separations and result in isobaric interferences that can skew quantitative values.

In this study, an improved analytical approach using comprehensive two-dimensional gas chromatography (GCxGC) with high-resolution time-of-flight mass spectrometry (HRTOFMS) and a multi-mode ionization source operating in Electron Capture Negative Ionization (ECNI) mode was developed to quantify PCPs. ECNI-HRTOFMS significantly increased sensitivity, improved selectivity, and minimized isobaric interferences between PCP congeners and other POPs. The implementation of GCxGC resulted in satisfactory separation and quantification of SCCPs and MCCPs in sequential chromatographic groups (Figure 1). In this application note, the terms groups and clouds of PCPs will be used interchangeably.

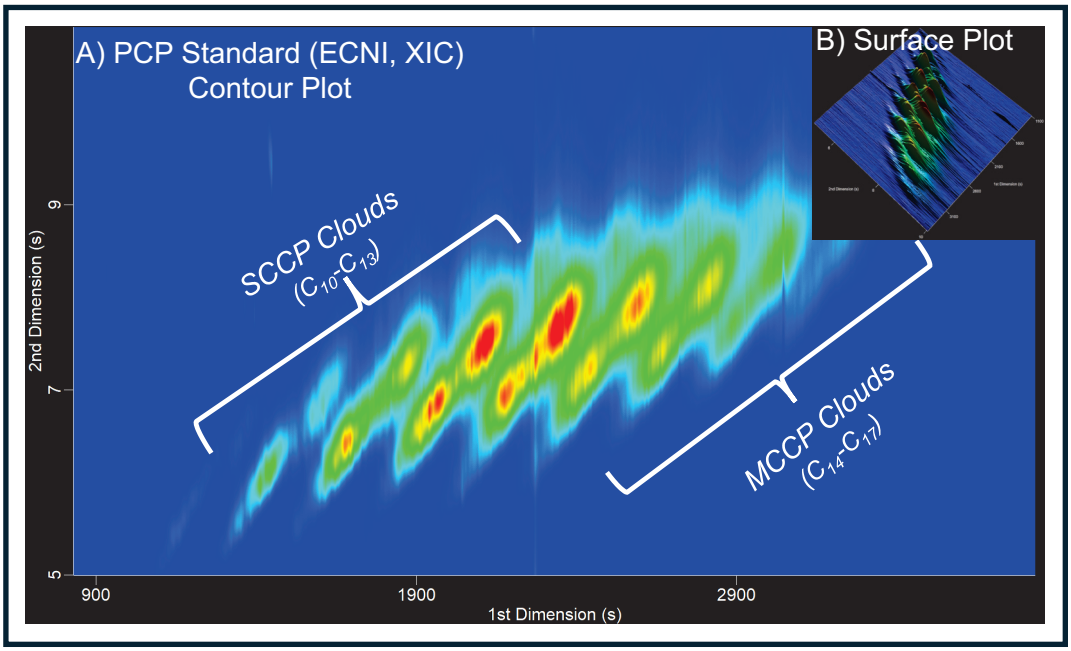

Figure 1. A) ECNI contour and B) surface plot showing short- and medium-chain polychlorinated paraffin clouds in a standard mixture (10 µg/mL).

Experimental

Sample and Standard Preparation

Dust samples were obtained from vacuum cleaner bags used over multiple weeks in local household and business premises. Standard reference material (SRM) 2585 was purchased directly from the National Institute of Standards and Technology (NIST) website and was used for method development. Dust samples were weighed (0.14 ±0.01 g) and transferred to 10 mL glass centrifuge tubes. Three mL of extraction solvent (3:1 dichloromethane/hexane) were added, and the tubes were capped. The heterogeneous mixture was vortexed for 10 seconds and then extracted for 20 minutes via sonication. The supernatant was removed, and the dust was extracted again with an additional three mL of solvent. The combined extracts were filtered through a syringe filter and placed under N_2 gas to evaporate the solvents. The resulting residue was reconstituted in 350 µL of dichloromethane, and 50 µL of ß-hexachlorocyclohexane ($^{13}C_6$, 5 µg/mL) was added to obtain a total volume of 400 µL. The mixture was vortexed for 30s and 100 µL aliquots were transferred to 2 mL vials for analysis.

Calibration standards were prepared by combining SCCP (100 µg/mL in cyclohexane; 51.5, 55.5, and 63 wt. percent), and MCCP standards (100 µg/mL in cyclohexane; 42, 52, and 57 wt. percent), adding 100 µL of ß-hexachlorocyclohexane ($^{13}C_6$, 5 µg/mL), and diluting with the appropriate volume of cyclohexane to produce eight standards at a volume of 1 mL (0.10, 0.30, 0.50, 1.0, 2.5, 5.0, 10.0, and 15.0 µg/mL). The samples were analyzed using the instrument parameters listed below.

Table 1. GC×GC-TOFMS Conditions

Gas Chromatograph	**Agilent 7890B with LECO Dual Stage QuadJet™ Modulator**
Injection	3µL liquid injection, Splitless, 280 °C
Carrier Gas	He @ 1.0 mL/min, Corrected Constant Flow
Primary Column	HP-5MS UI, 30 m x 0.25 mm i.d. x 0.25 µm
Secondary Column	Rxi-17Sil MS, 1.2 m x 0.25 mm x 0.10 µm
Temperature Program	60 °C (1 min) ramp 40 °C/min to 140 °C, then ramp to 320 °C at 2.5 °C/min (Hold 1 min) Secondary oven maintained +15 °C relative to primary oven
Modulation Period	8.0 s; modulator maintained +15 °C relative to secondary oven
Transfer Line	300 °C
Mass Spectrometer	**LECO *Pegasus* HRT+ 4D**
Source Temperature	135 °C
Acquisition Mode	High Resolution, R = 25,000 for m/z 219, Mass Accuracy = 1 ppm
Ionization	ECNI (Reagent Gas = CH_4)
Mass Range (m/z)	50-1000
Acquisition Rate	50 spectra/s

Data Processing

Data processing consisted of mass calibration (PFTBA), PCP cloud classification, and signal processing. The classification feature in ChromaTOF® brand software was used to define PCP group regions within ECNI contour plots as illustrated for the 10 µg/mL standard PCP mixture in Figure 2. Each cloud region delineates PCPs with formulas for which the sum of carbon and chlorine atoms is constant. For example, the sum of the number of carbons and chlorine atoms for group 4 compound formulas equals eighteen (#C + #Cl = 18). Signal processing within the classification regions was restricted to PCPs with 10-17 carbon atoms and 5-10 chlorine atoms (Figure 3).

The two most abundant high-resolution accurate mass (HRAM) ions in the $[M\text{-}Cl]^-$ or $[M\text{-}HCl]^-$ ion clusters for targeted

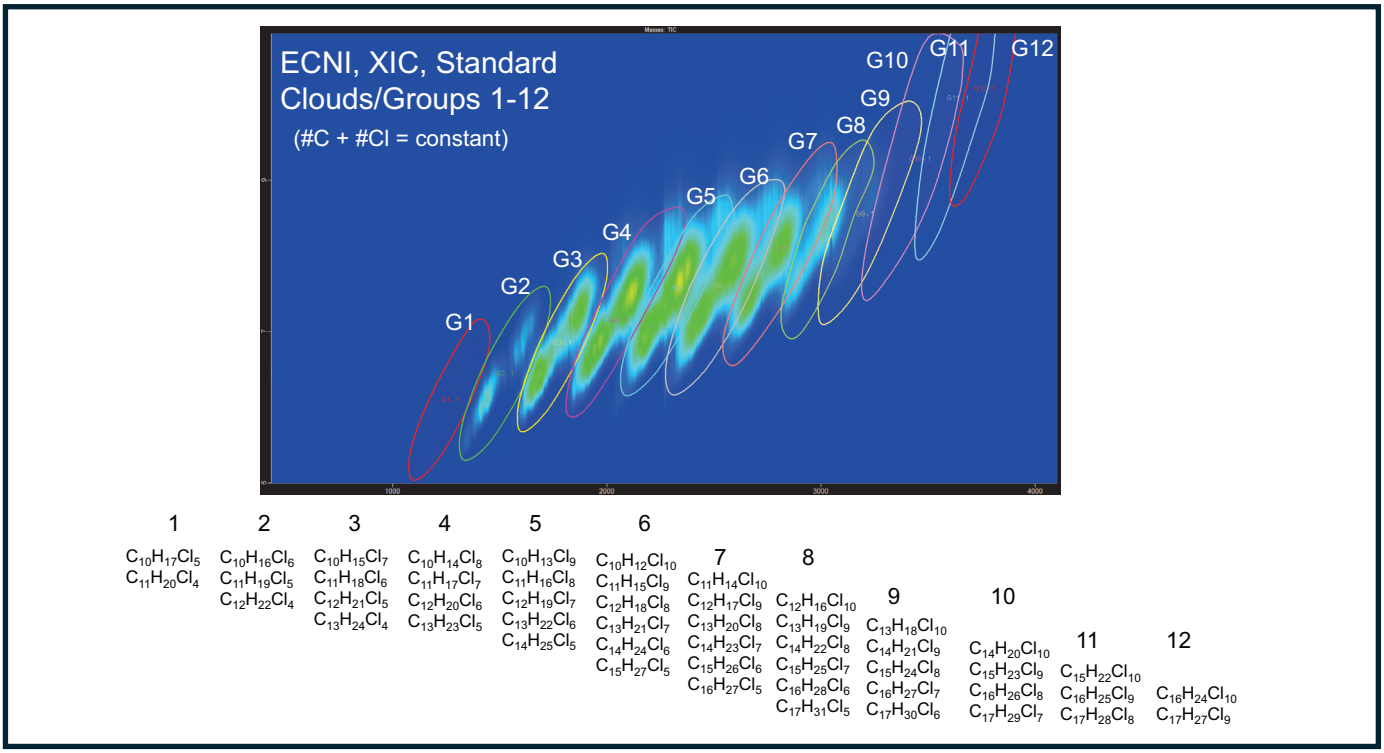

Figure 2. ECNI Contour plot showing polychlorinated paraffins clouds (groups) in a standard mixture at 10 µg/mL. Each cloud represents PCPs where the sum of chlorines and carbons equals a constant number.

A) Method: Mass Calibration, Classification, Signal Processing

Classification - "CS7 3uL sL"

Auto Select

#	Show	Class	Comp	Region	Color	Formula 1	Data Domain	Mass Toleran	Formula 2	Data Domain	Mass Toleran	Formula 3	Data Domain	Mass Toleran	Formula 4	Data Domain	Mass Toleran	Formula 5	Data Domain	Mass Tolera
1	☒	G1		+		277.9982+279.9952	Signal	5 PPM		Signal			Signal			Signal			Signal	
2*	☒	G2		+		312.9671+314.9664	Signal	5 PPM	292.0132+294.0199	Signal	5 PPM		Signal			Signal			Signal	
3	☒	G3		+		346.9285+348.9257	Signal	5 PPM	325.9752+327.9730	Signal	5 PPM	304.0340+306.0301	Signal	5 PPM		Signal			Signal	
4	☒	G4		+		360.9438+362.9407	Signal	5 PPM	339.9909+341.9888	Signal	5 PPM		Signal			Signal			Signal	
5	☒	G5		+		380.8888+382.8862	Signal	5 PPM	394.9048+396.9006	Signal	5 PPM	374.9594+376.9556	Signal	5 PPM	354.0056+356.0036	Signal	5 PPM		Signal	
6	☒	G6		+		430.8621+432.8593	Signal	5 PPM	408.9207+410.9168	Signal	5 PPM	388.9745+390.9722	Signal	5 PPM	368.0223+370.0195	Signal	5 PPM		Signal	
7	☒	G7		+		464.8241+466.8210	Signal	5 PPM	444.8788+446.8760	Signal	5 PPM	422.9361+424.9334	Signal	5 PPM	402.9906+404.9877	Signal	5 PPM	382.0370+38	Signal	5 PPM
8	☒	G8		+		478.8385+480.8350	Signal	5 PPM	458.8927+460.8896	Signal	5 PPM	436.9519+438.9486	Signal	5 PPM	417.0063+419.0030	Signal	5 PPM	396.0527+39	Signal	5 PPM
9	☒	G9		+		492.8536+494.8505	Signal	5 PPM	472.9098+474.9066	Signal	5 PPM	450.9686+452.9657	Signal	5 PPM	431.0151+433.0197	Signal	5 PPM	411.0767+41	Signal	5 PPM
10	☒	G10		+		506.8710+508.8684	Signal	5 PPM	486.9258+488.9231	Signal	5 PPM	464.9828+466.9802	Signal	5 PPM	445.0368+447.0342	Signal	5 PPM		Signal	
11	☐	G11		+		520.8865+522.8838	Signal	5 PPM	500.9415+502.9389	Signal	5 PPM	480.9955+482.9932	Signal	5 PPM		Signal			Signal	
12	☐	G12		+		534.9021+536.9006	Signal	5 PPM	514.9581+516.9546	Signal	5 PPM		Signal			Signal			Signal	
13	☐	ISTD		+		323.0000+324.9980	Signal	5 PPM		Signal			Signal			Signal			Signal	
14		Uncla:					Signal	5 PPM		Signal	5 PPM		Signal			Signal			Signal	

B) Mass Calibration Results

Ion	Formula	m/z calc	m/z obs	PPM
PFTBA200	C_4F_8	199.9878	199.9878	0.11
PFTBA283	$C_5F_{11}N$	282.9861	282.9860	-0.15
PFTBA395	$C_8F_{15}N$	394.9797	394.9796	-0.28
PFTBA414	$C_8F_{16}N$	413.9781	413.9780	-0.27
PFTBA433	$C_8F_{17}N$	432.9765	432.9765	0.04
PFTBA452	$C_8F_{18}N$	451.9749	451.9749	0.03
PFTBA514	$C_{10}F_{20}N$	513.9717	513.9716	-0.24
PFTBA595	$C_{12}F_{23}N$	594.9669	594.9668	-0.14
PFTBA633	$C_{12}F_{25}N$	632.9637	632.9637	0.03

C) Processing Results (Std,10ppm)

#	Class	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
1*	G1	569904.7					
2	G2	447816.9	341180.3				
3	G3	692731.7	4587156.7	546280.9			
4	G4	6898242.2	2827441.8				
5	G5	30949.8	3965294.4	3598223.0	1096070.0		
6	G6	874540.1	3870031.2	1517833.1	1834069.4		
7	G7	218577.3	2486595.5	2072041.4	2434092.4	201832.2	141739.6
8	G8	160579.8	125399.6	3098547.9	272045.1	2756.6	
9	G9	51326.0	1229454.9	70663.6	2168.7	0.0	
10	G10	134557.0	222801.5	27096.9	0.0		
	Total	9519944.2	18759470.7	10365553.9	5413864.3	204570.6	141739.6

Figure 3. A) Cloud quant processing method: Mass calibration, classification, signal processing with HRAM (G1-G10). B) Mass calibration results. C) Processing results for the 10 µg/mL calibration standard mix.

chlorinated paraffins within a cloud were used for signal processing as shown for two group 4 formulas in Figure 4. The mass accuracy values for the most intense HRAM ions for $C_{11}H_{17}Cl_7$ $[M+2-Cl]^-$ and $C_{12}H_{20}Cl_6$ $[M+2-HCl]^-$ in the specified group 4 region were -1.14 and 0.08 ppm respectively.

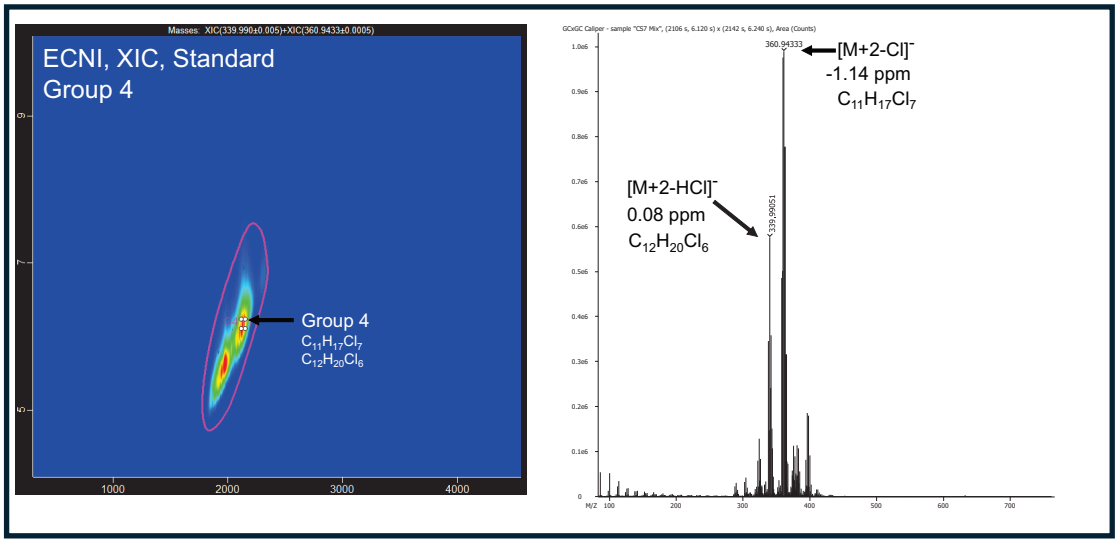

Figure 4. ECNI Contour plot showing the group 4 classification region in a dust sample (left). The contour plot data is based on HRAM eXtracted Ion Chromatograms (XIC) for PCPs with formulas $C_{11}H_{17}Cl_7$ and $C_{12}H_{20}Cl_6$ (right).

Results and Discussion

GCxGC provided increased peak capacity and improved chromatographic resolution via two different mechanisms, allowing separation of sequential groups of PCPs. The combination of GCxGC and HRTOFMS resulted in excellent quantitative PCP data. Instrument response for PCPs intensified as the number of chlorine atoms per PCP increased. For example, the relative ECNI response for octachlorinated PCPs is greater than for tetrachlorinated PCPs under identical instrumental conditions. "Matched PCP standards" have been used for the non-biased quantitative analysis of PCP samples. Unfortunately, perfectly matched standard-sample mixes are not available, and therefore the results obtained for PCPs using this analytical technique are quantitative estimates. All quantitative analysis results depend greatly on the type of instrumental technology used for analysis. Typical GC and LC-MS analysis approaches do not effectively account for differences in PCP congener response, due to a lack of chromatographic separation, and potential isobaric interferences. In this study, a direct quantitative approach, cloud quant, was implemented for the determination of SCCP and MCCP concentrations in dust extracts. This analytical method utilized a mixed PCP standard to populate cloud regions within contour plots for group selective quantitation with GCxGC-ECNI-HRTOFMS. There was a quadratic relationship between concentration and instrumental response (R^2 > 0.99) as shown for groups 5-8 in the calibration standard set (Figure 5).

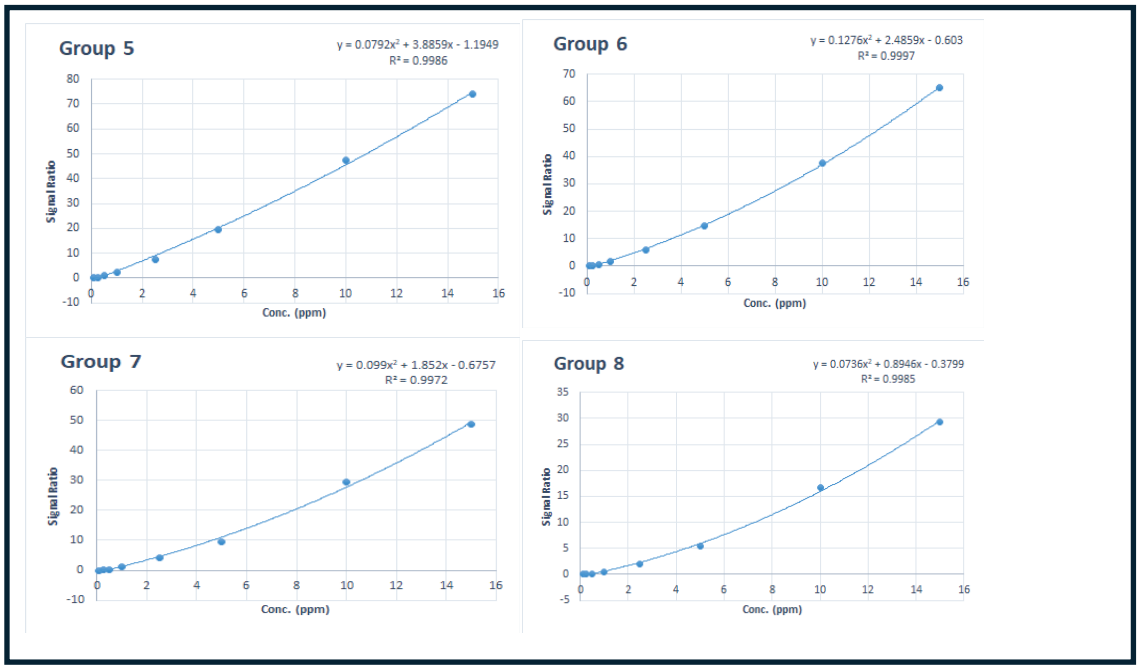

Figure 5. Calibrations curves for groups 5-8. The R^2 values were > 0.99.

NIST 2585 SRM was used to develop and test the methodology since SCCP and MCCP concentrations for this SRM have been previously reported and provided a metric for GCxGC-ECNI-HRTOFMS performance. Representative ECNI (XIC) plots for the NIST SRM and household dust are displayed (Figure 6). The individual group quantitation values (µg/mL) based on the calibration standard set are summarized within each plot. For example, the SRM group 4 value was 0.48 µg/mL which translated into a concentration of 1.4 µg/g of PCPs per sample. The concentration for the complete SCCP and MCCP were 11.1 and 11.2 µg/g respectively. These results were comparable with those reported in the literature for the same SRM (SCCP 7.6 – 8.7 µg/g, MCCP 10 - 16.4 µg/g).[2-4] Analysis of the household dust sample resulted in lower concentration values for both SCCP and MCCPs (8.1 and 8.8 µg/g).

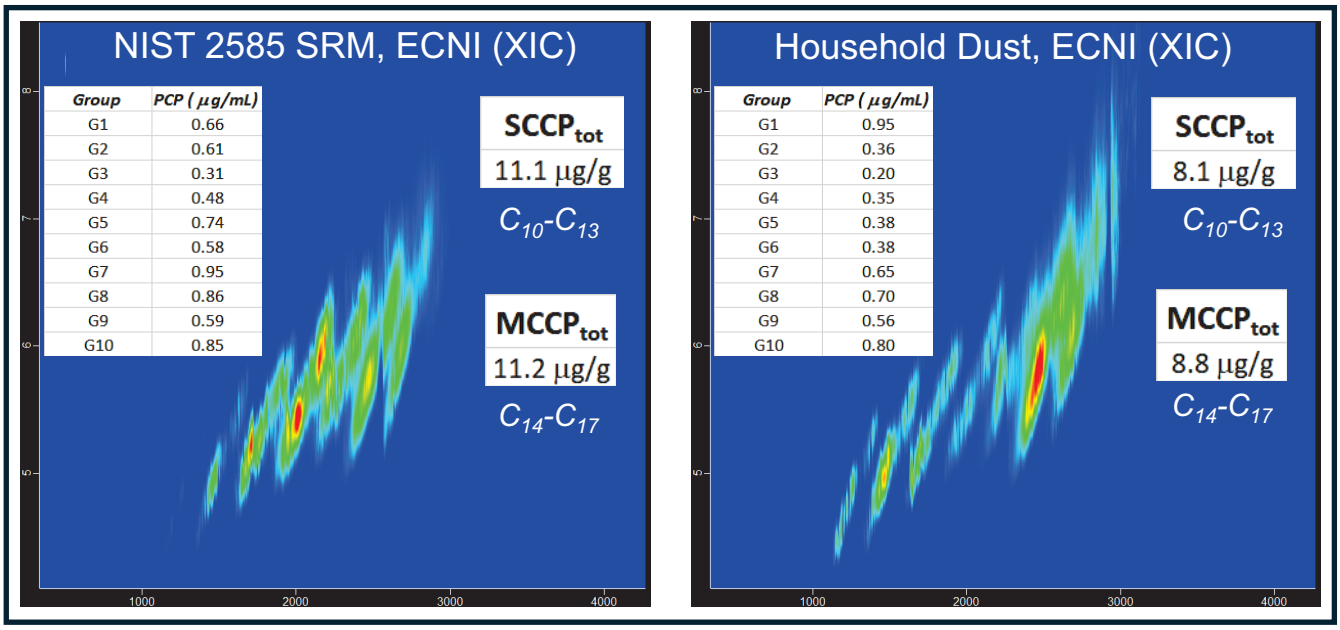

Figure 6. ECNI (XIC) contour plots and quantitative results for NIST 2585 SRM (left) and household dust sample (right).

Conclusion

The LECO *Pegasus* HRT$^+$ 4D is an indispensable tool for the quantification of persistent organic pollutants. Simple extraction and analysis procedures were implemented for the quantitation of PCPs in a standard reference material and household dust sample. The enhanced chromatographic resolution, high resolving power, and electron capture negative ionization mode of the HRTOFMS provided the chromatographic separation and mass spectral selectivity necessary to detect and quantify PCP groups in dust sample extracts. PCP "cloud quant" was accomplished using mass calibration, classification, and signal processing of high-resolution accurate masses. Analysis of the NIST 2585 SRM provided results that were comparable to those reported by researchers using different analytical instrumentation.

References

[1]C. Moschet, T. Anumol, B.M. Lew, D.H. Bennett, T.M. Young, *Environ. Sci. Technol.* **5(52)**, 2878–2887 (2018). DOI: 10.1021/acs.est.7b0576.

[2]H. Shang, X. Fan, C. Kubwabo, P.E. Rasmussen, *Environ. Sci. Pollut. Res.* **26**, 7453-7462 (2019).

[3]M Brits, J. de Boer, E.R. Rohwer, J. De Vos, J.M. Weiss, S.H. Brandsma, *Chemosphere* **238**, 124643.

[4]S.H. Brandsma, M. Brits, Q.R. Groenewoud, M.J.M. van Velzen, P.E.G. Leonards, J. de Boer, *Environ. Sci. Technol.* **53(13)**, 7595-7603 (2019).

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