High Performance Gas Chromatography Time of Flight Mass Spectrometry Analysis of Halogenated Organic Compounds in Pet Food

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Introduction

Halogenated organic compounds (HOCs) have been used for decades as pesticides, insecticides and fire retardants. HOCs when mishandled or misused can result in contaminated food and feed supplies. These anthropogenic compounds can leach out of commercial products, enter the environment and cause harm to humans and animals. Their chemical stability and lipid solubility contribute to both their bioaccumulation and biomagnification in the environment. Novel HOCs are continually developed and introduced as replacements for banned or regulated compounds. Figure 1 shows some of the HOCs targeted in this study. They include traditional persistent organic pollutants (POPs) such as polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), dioxins, and emerging HOCs such as dechlorane plus (*syn-* and *anti-*, decabromodiphenylethane (DBDPE), and hexachlorodibromocyclooctane (HBCD).

Analyses of samples containing HOCs can be complicated due to unintentional elimination of targeted compounds (e.g., PBDEs) during sample cleanup, dirty matrices, and mass spectral interferences between coeluting analytes. These problems can be minimized by improving sample preparation and/or enhancing instrument selectivity. LECO Corporation's Pegasus® GC-HRT (Figure 2) with Folded Flight Path (FFP™) technology has the increased resolving power, sensitivity and selectivity necessary to analyze pet food samples.

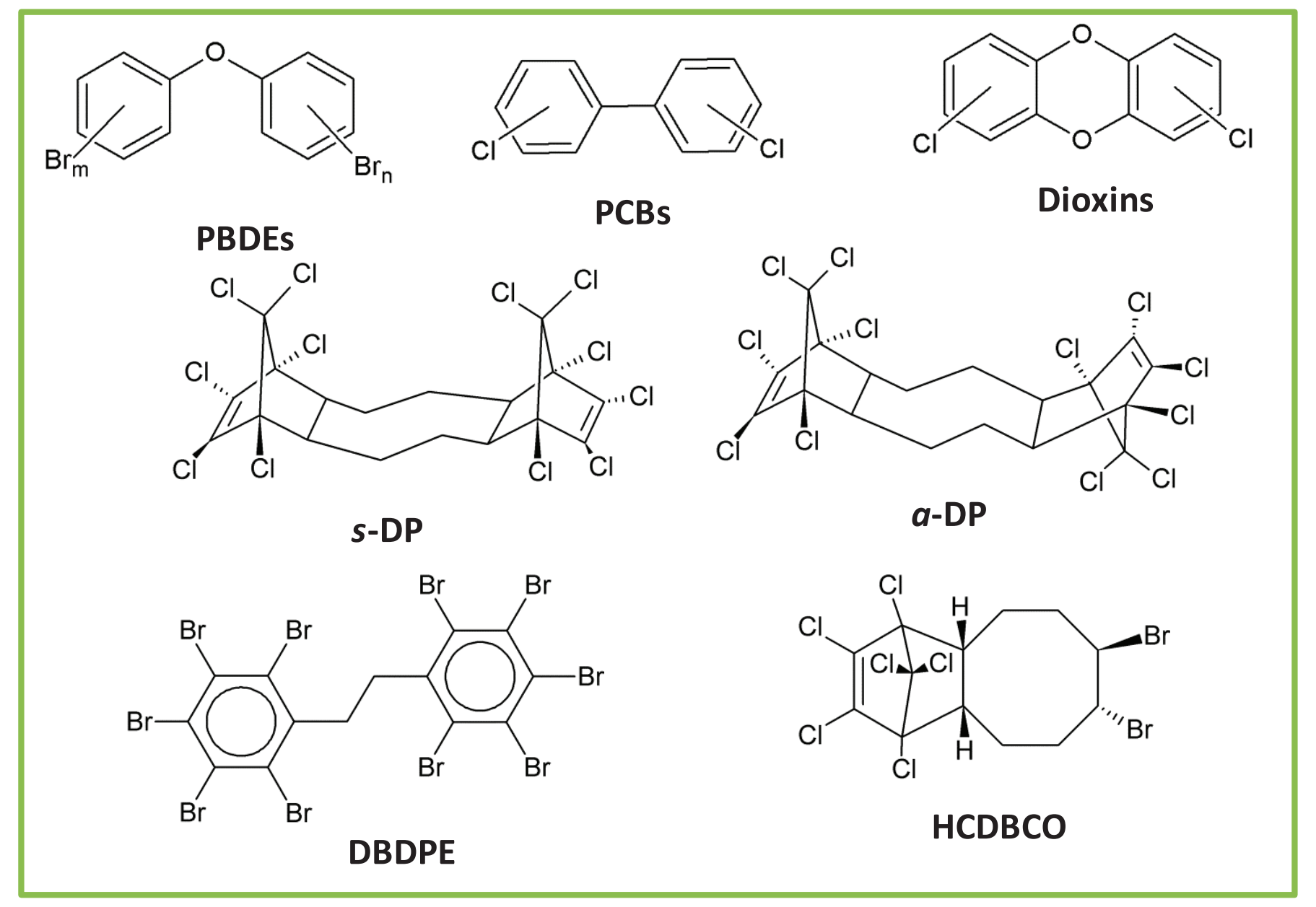

Figure 1. Representative halogenated organic compounds

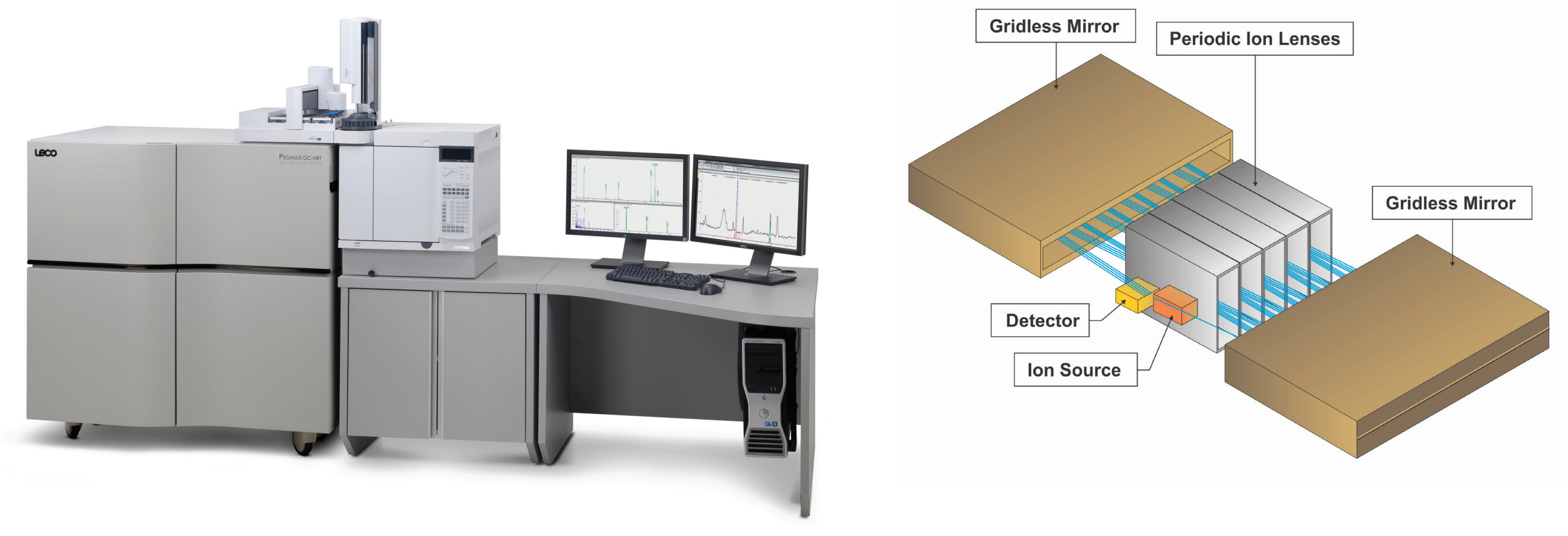

Figure 2. LECO's Pegasus® HRT and FFP™ Mass Analyzer.

Experimental

Dog and cat food samples were spiked with surrogate standards (Accustandard PCBs WHO/NIST/NOAA: 28 PCBs at 10 µg/mL) and extracted with acetonitrile using Qsep Q150 extraction salts (Restek #26213). The samples were analyzed right after extraction or treated with dispersive Solid Phase Extraction (dSPE) sorbents before analysis.

Gas Chromatography high performance time-of-flight mass spectrometry (TOFMS) was used for characterization of HOCs in four different varieties of cat and dog food. Spectral data were collected using two of three possible TOFMS operating modes (Figure 3): high resolution (R = 25,000, FWHM at 218.985080 m/z) and ultra-high resolution mode (R = 50,000, FWHM at 218.985080 m/z).

Sample Preparation

1.0 gram of pet food was placed in a 50 mL centrifuge tube, spiked with congener standard (50 or 100 ng standard/ g pet food) and vortexed at 2200 rpm for 30 seconds. Water (9 g) and acetonitrile (10 mL) were added to the food and the mixture was homogenized by vortexing at 2200 for one min. Extraction salts were added, mixed vigorously for one min., vortexed at 2200 for an additional min., and then centrifuged at 3000 rpm for 5 mins. A 1.0 mL aliquot of the acetonitrile layer was spiked with internal standards (30 µL of BDE-118 and BB-209, conc. = 50 µg/mL) and transferred to an AS vial for analysis. Additional 1.0 mL portions of samples were transferred to dSPE sample clean-up tubes (Restek # 26125 red, 26215 yellow, 26219 orange, 26218 grey), and vortexed for 2 mins., centrifuged at 3000 rpm for 5 mins., and 0.4 mL portions were transferred to AS vials for analysis.

Instrument Parameters

GC Parameters

GC	Agilent 7890
Column Type:	Restek Rtx-1614 (15m, 0.25 mm ID, 0.18 mm df)
Injection	Splitless, 2 µL , 250 °C
Oven	70 °C (1 min.) to 320 °C at 20 °C/min (1.5 min.)
Carrier Gas	He, Constant Flow (1.00 mL/min.)

MS Parameters

Spectrometer:	LECO PEGASUS GC-HRT®
Ion Source:	EI
Polarity:	Positive
Flight Path:	HR, UHR (R = 25K and 50K, FWHM)
Spectral Acquisition:	6 spectra / second
m/z Range:	50 – 1000 (HR Mode); 200 – 700 (UHR Mode)
m/z Calibration:	PFTBA

Results (HR Mode)

Quechers extraction of cat food sample A, followed by dSPE cleanup using either Resprep Q213 (150mg $MgSO_4$, 25mg PSA, 7.5mg GCB) or Resprep Q252 (150mg $MgSO_4$, 50mg PSA, 50mg C18, 50mg GCB) resulted in the most effective removal of potential interfering matrix components (Figure 3). The high resolving power of the Pegasus HRT is very useful for the analysis of extracted food samples with or without dSPE cleanup as shown in Figure 4. High quality spectral data were obtained for targeted HOCs, as well as, major matrix components such as fatty acids and sterols. Mass spectral data for octadecanoic acid and cholesta-3,5-diene were searched against commercially available libraries (e.g., NIST, Wiley 9) and resulted in matches of 814 and 874 out of a possible 1000. In addition, mass accuracy values for the acid and cholestadiene were 0.16 and 0.008 ppm respectively. All surrogate (28 PCBs) and internal standards (BDE-118 and BB-209) were easily extracted from the data as shown in Figure 5.

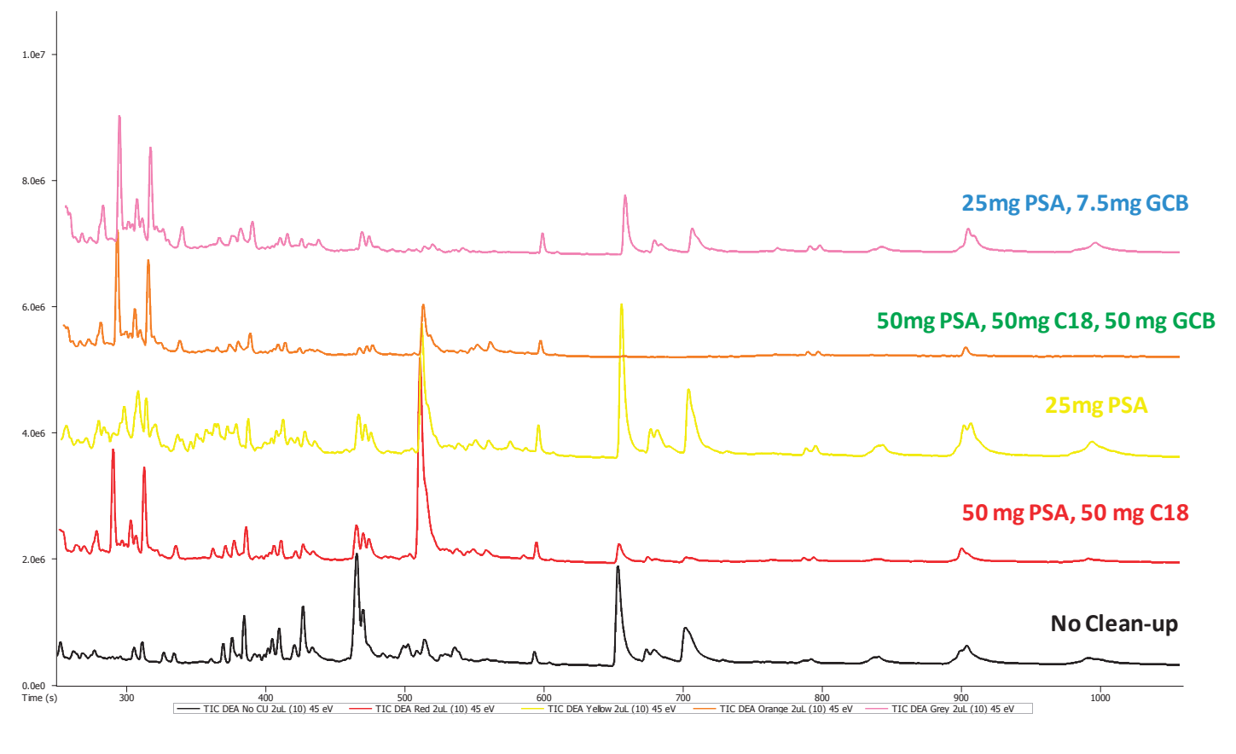

Figure 3. TICs -- Cat Food A

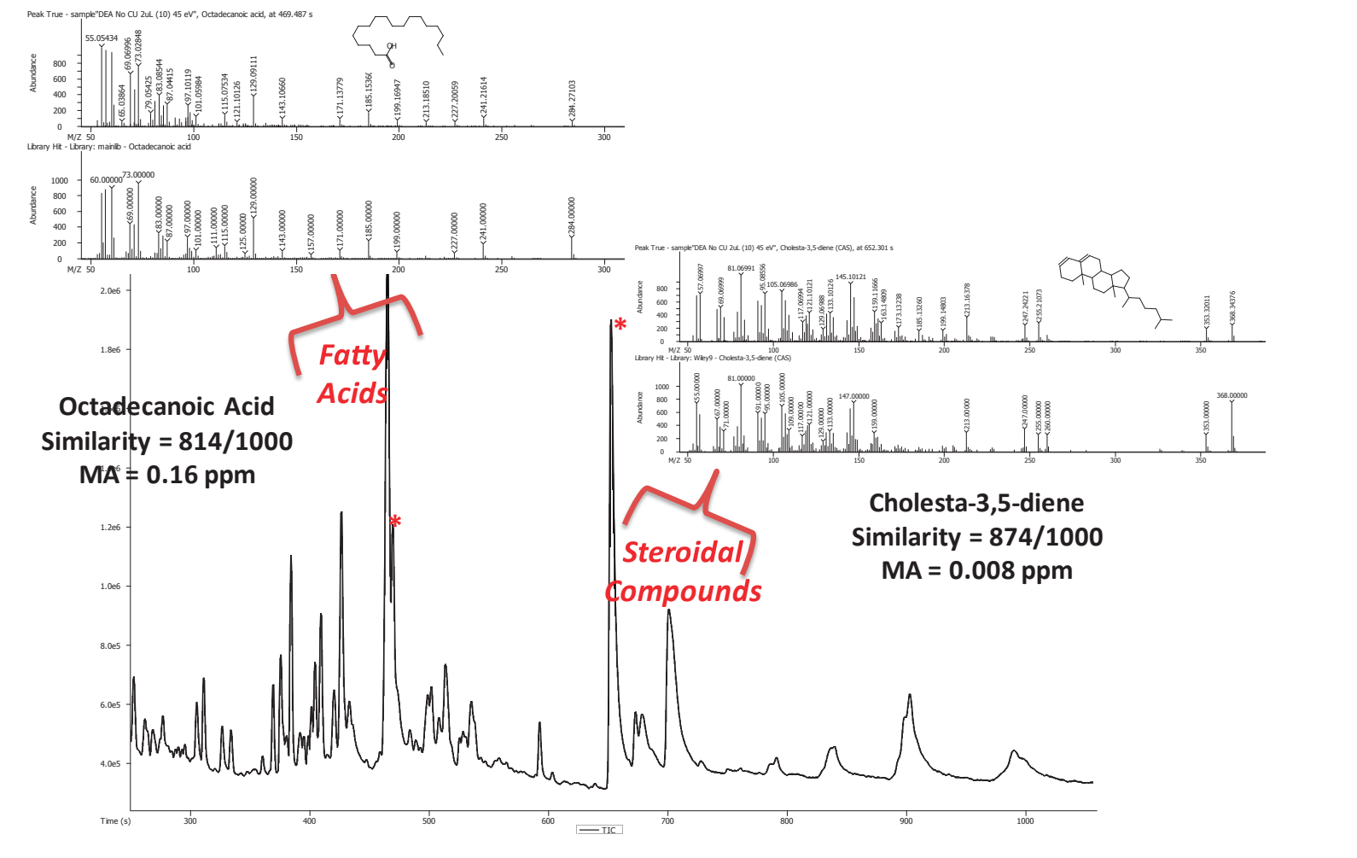

Figure 4. MS Data – Cat Food A

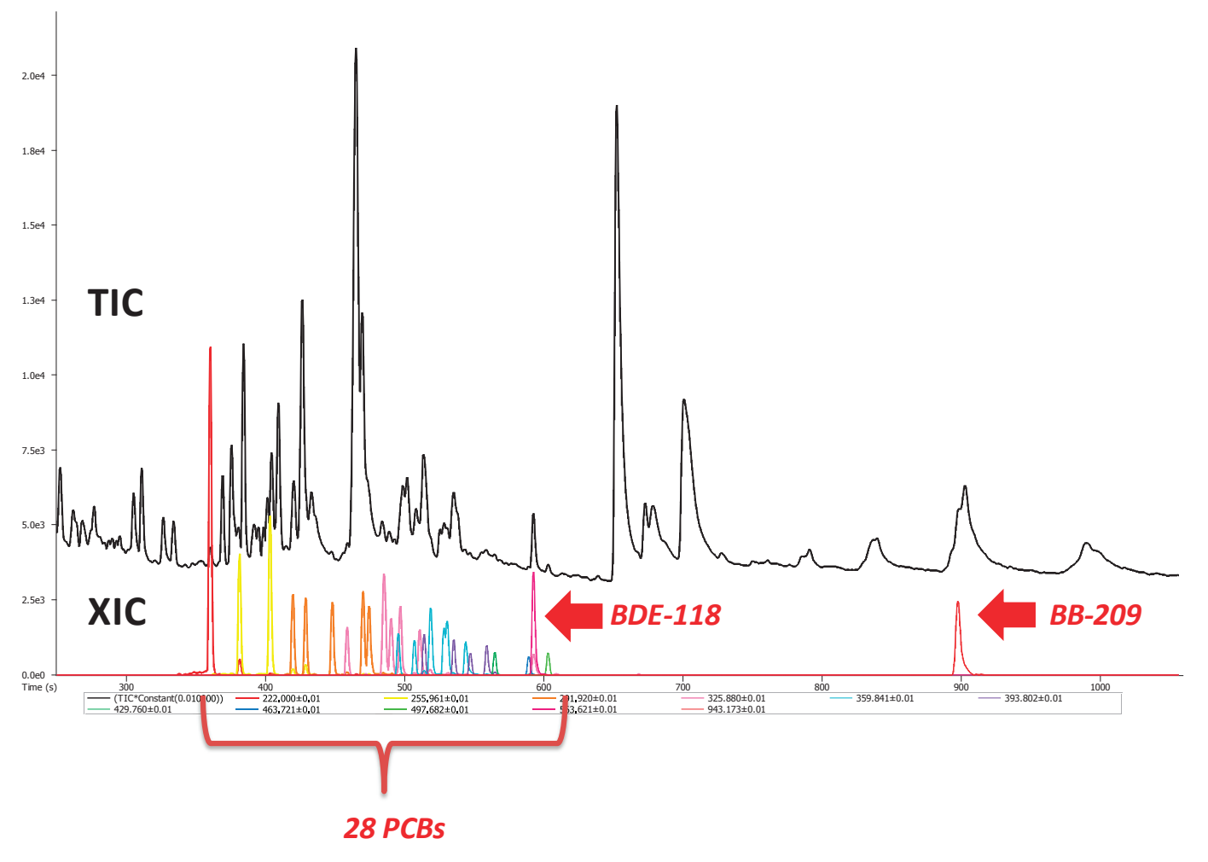

Figure 5. TIC & XIC – Cat Food A

A TIC and extracted ion chromatogram (XIC) of cat food sample B is shown in Figure 6. Further evidence of the high quality data produced by the GC-HRT can be seen in Figure 7 where mass spectra for CB-209, BDE-118 and BB-209 are displayed. Mass accuracy values for these HOCs were -0.36, 0.52 and 0.17 ppm. An average of 0.64 ppm was obtained for all HOCs in this sample (Table 1)

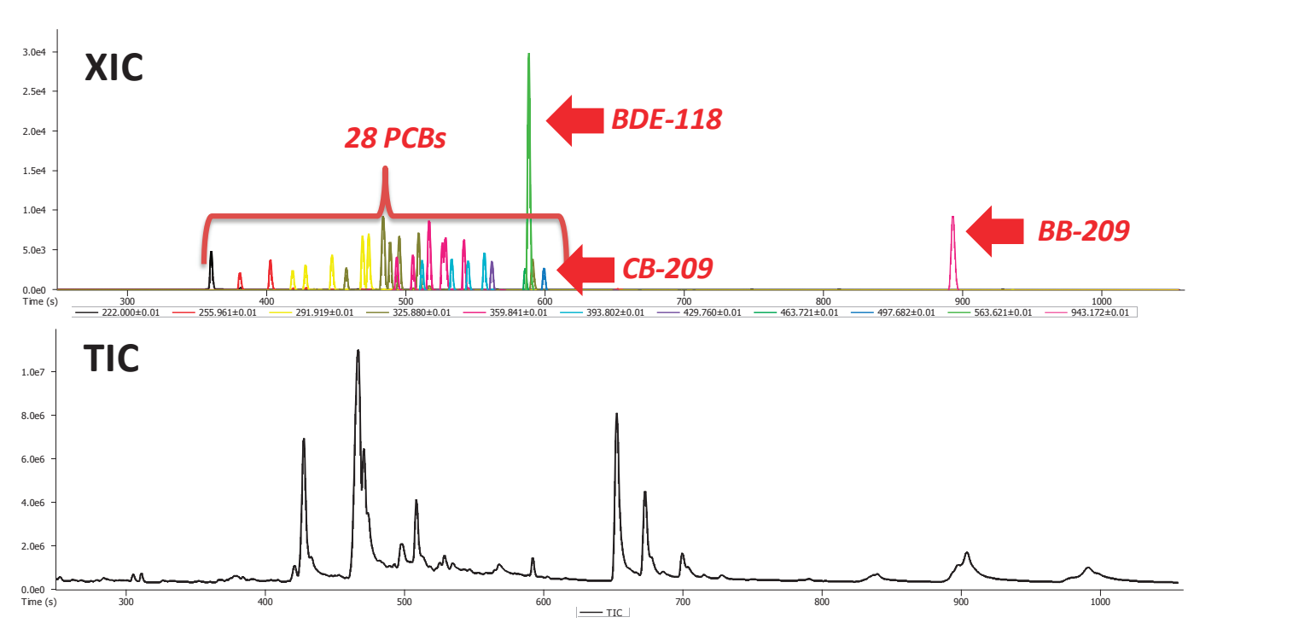

Figure 6. TIC & XIC – Cat Food B

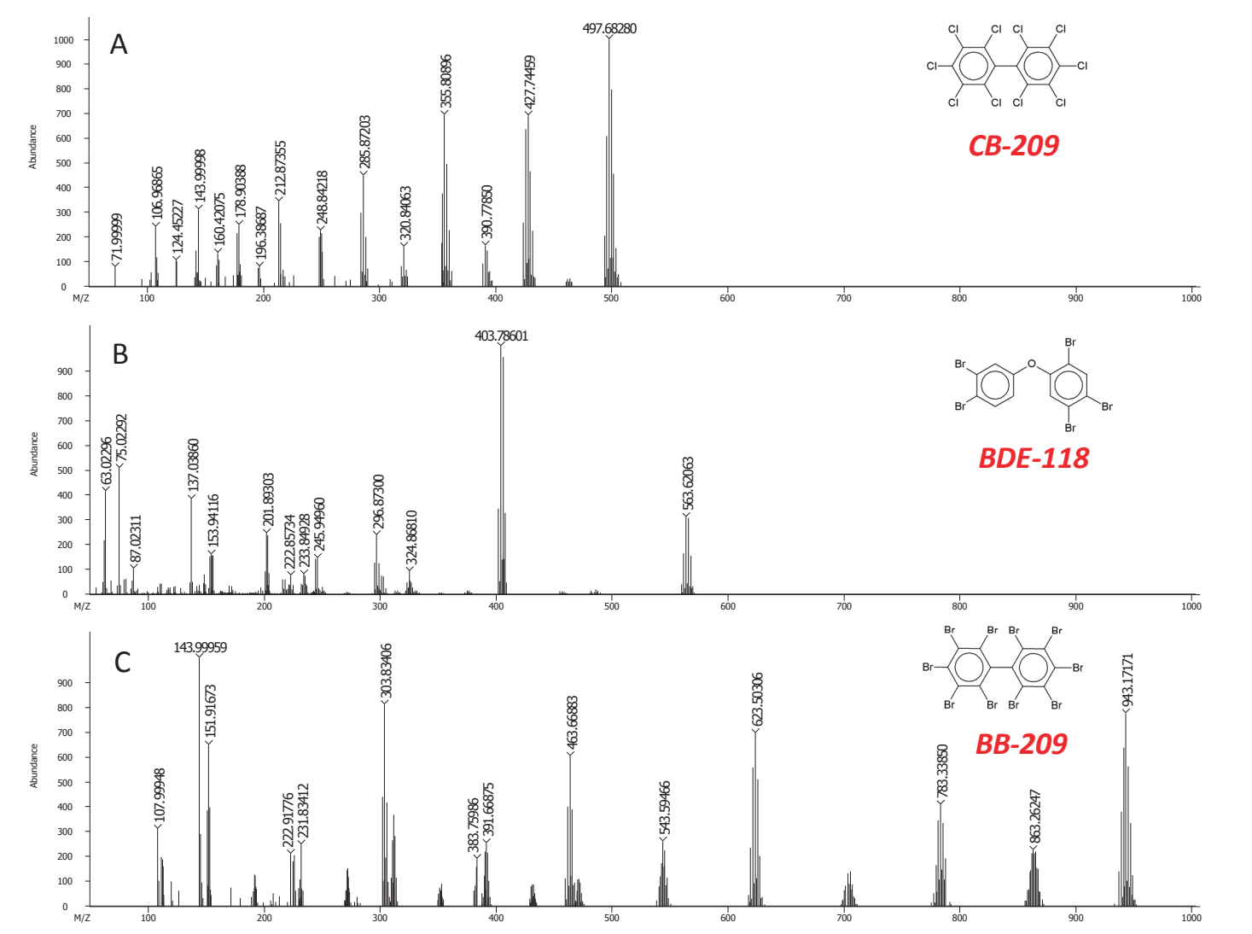

Figure 7. MS Data – Cat Food B

Compound	R.T. (s)	Formula	Calculated Ion m/z	Observed Ion m/z	Mass Accuracy (ppm)
di-PCB	360.31	C12H8Cl2	221.99976	221.99982	0.30
tri-PCB	381.331	C12H7Cl3	255.96078	255.96081	0.10
tri-PCB	403.258	C12H7Cl3	255.96078	255.96076	-0.09
tet-PCB	419.657	C12H6Cl4	289.92181	289.92154	-0.93
tet-PCB	447.892	C12H6Cl4	289.92181	289.92158	-0.79
tet-PCB	469.917	C12H6Cl4	289.92181	289.92233	1.80
tet-PCB	474.448	C12H6Cl4	289.92236	289.92181	-1.18
tet-PCB	428.9	C12H6Cl4	289.92181	289.92178	-0.10
pen-PCB	458.652	C12H5Cl5	323.88284	323.88297	0.39
pen-PCB	485.324	C12H5Cl5	323.88284	323.88336	1.59
pen-PCB	490.211	C12H5Cl5	323.88284	323.88303	0.60
pen-PCB	496.749	C12H5Cl5	323.88284	323.88242	-1.29
pen-PCB	510.495	C12H5Cl5	323.88284	323.88325	1.26
pen-PCB	485.657	C12H5Cl5	323.88339	323.88284	0.77
hex-PCB	495.454	C12H4Cl6	357.84387	357.84418	0.86
hex-PCB	506.995	C12H4Cl6	357.84387	357.84393	0.18
hex-PCB	518.64	C12H4Cl6	357.84387	357.84401	0.39
hex-PCB	528.206	C12H4Cl6	357.84387	357.84394	0.19
hex-PCB	530.535	C12H4Cl6	357.84387	357.84369	-0.48
hex-PCB	543.703	C12H4Cl6	357.84387	357.84337	-1.38
hex-PCB	530.75	C12H4Cl6	357.84442	357.84387	-0.81
hep-PCB	514.067	C12H3Cl7	391.80490	391.80472	-0.45
hep-PCB	535.224	C12H3Cl7	391.80490	391.80503	0.34
hep-PCB	547.109	C12H3Cl7	391.80490	391.80485	-0.12
hep-PCB	558.837	C12H3Cl7	391.80490	391.80498	0.22
oct-PCB	564.643	C12H2Cl8	425.76647	425.76592	-1.14
non-PCB	589.088	C12HCl9	459.72695	459.72716	0.45
dec-PCB	602.924	C12Cl10	493.68798	493.68780	-0.36
BDE-118	592.445	C12H5Br5O	559.62518	559.62547	0.52
BB-209	897.685	C12Br10	933.18337	933.18282	0.17

Table 1. Mass Accuracies – Cat Food B.

Figure 8 shows a TIC and XIC for dog food sample A. Five *penta*-chlorinated biphenyls, one which coelutes with BDE-118 are visible in the XIC of the sample. The mass spectrum in Figure 9 illustrates the GC-HRT's ability for the separation of low abundance isobaric ions with nominal mass 324. BDE-118 mass accuracy values for the molecular ion and $[M-Br_2]^{+\bullet}$ fragment were 0.049 and -0.64 ppm.

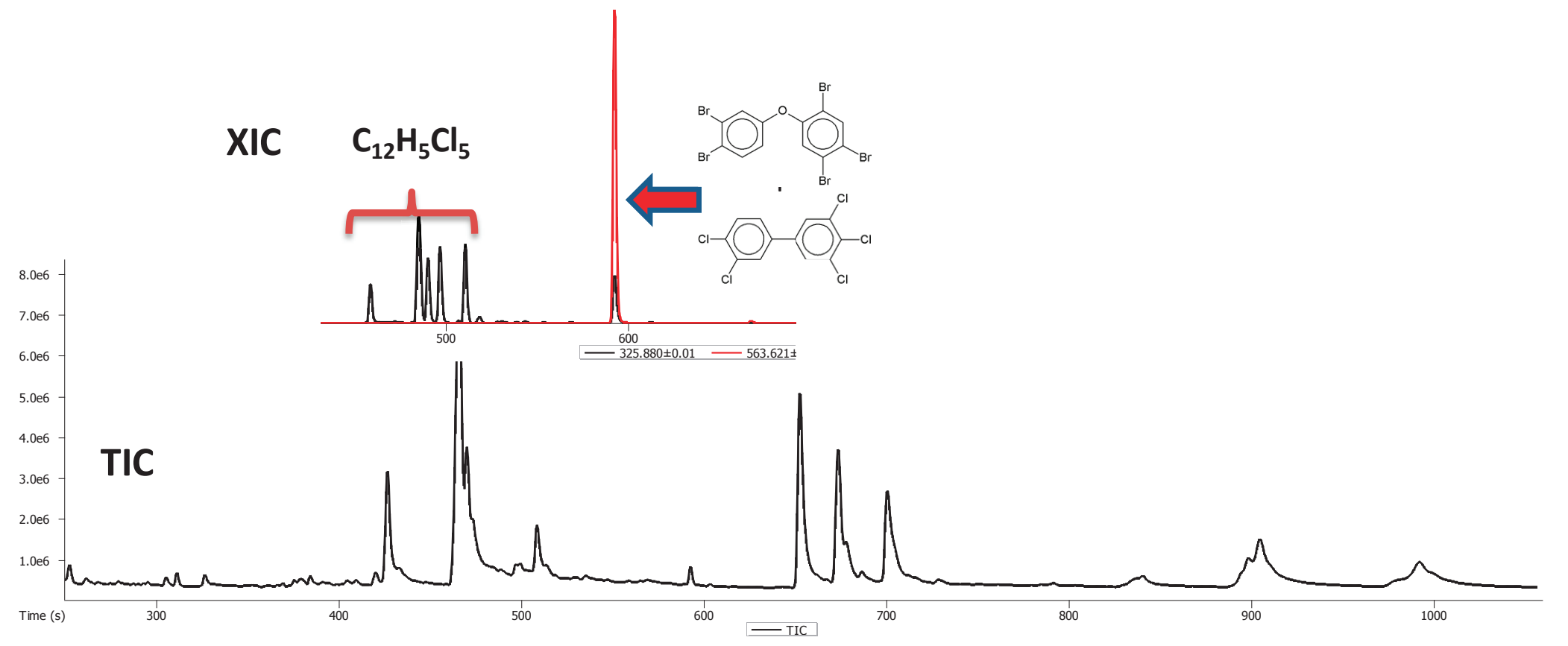

Figure 8. TIC and XIC – Dog Food A

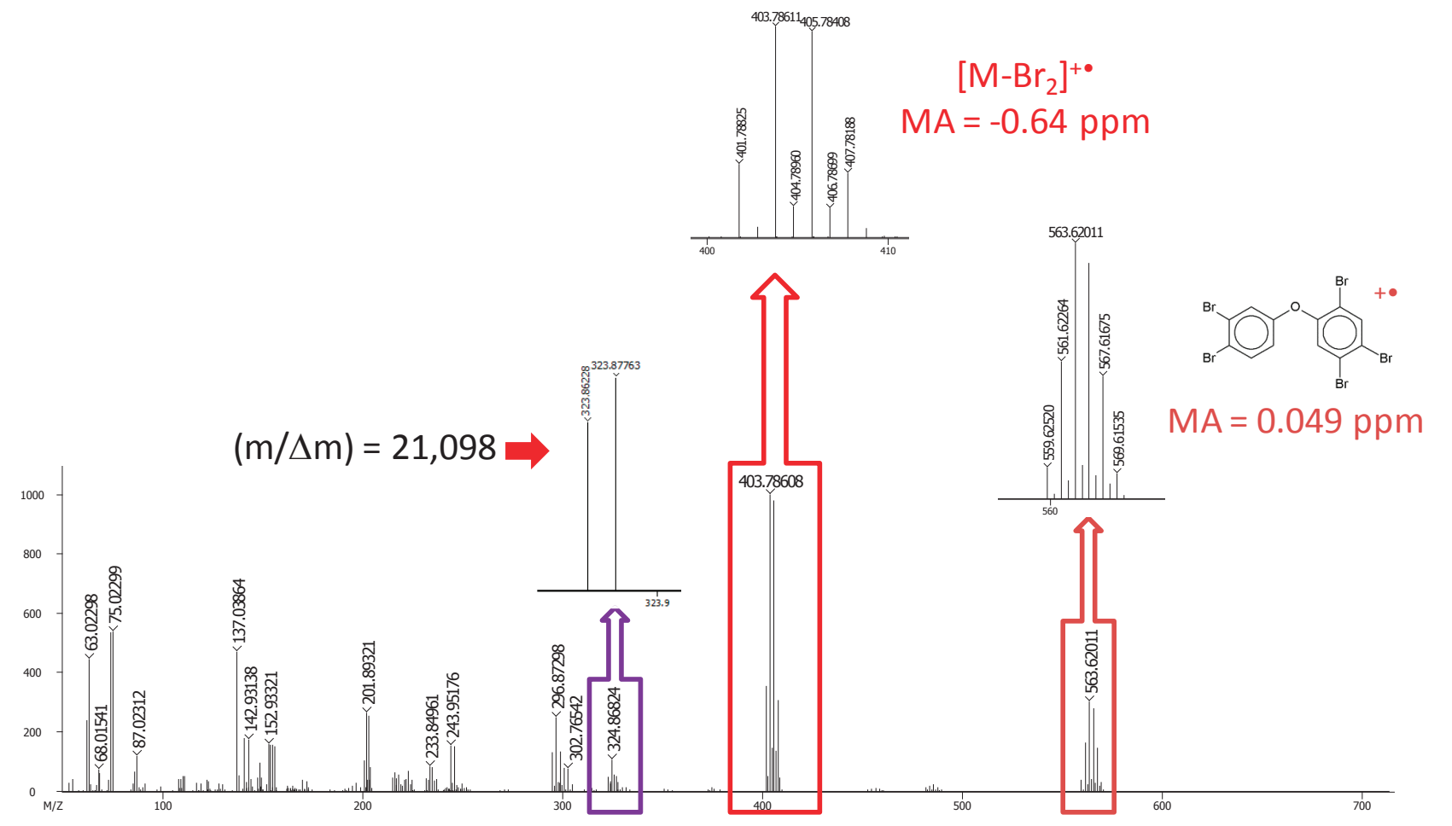

Figure 9. MS data – Dog Food A

System Performance (UHR Mode)

Operation of the GC-HRT in ultra-high resolution mode significantly reduces HOC interferences as shown in Figure 10. Robust analysis of the mass spectrum for CB-180 and BDE-47 would be difficult without a high performance mass spectrometer to isobaric fragment ions such as the ones shown in Figure 11. Operation of the GC-HRT in ultra-high resolution mode resulted in a calculated resolution of 23,937 (m/Δm) for the $[M-Cl_2+2]^{+\bullet}$ and $[M-Br_2]^{+\bullet}$ ions of CB-180 and BDE-47. ChromaTOF-HRT software provided deconvoluted spectra for each of the compounds (Figure 11).

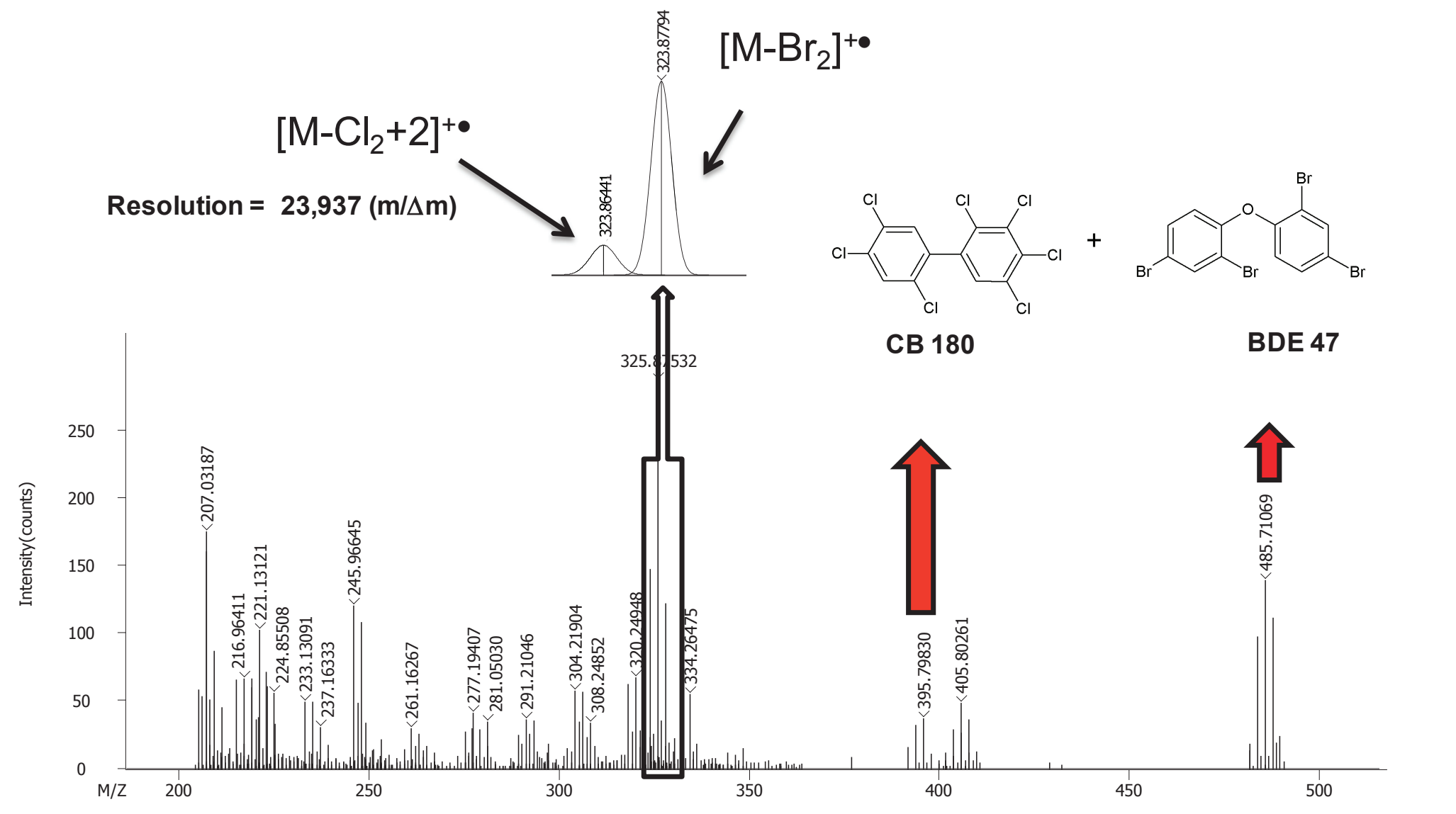

Figure 10. MS data for coeluting HOCs –Environmental Sample

Figure 11. Peak True MS Data for Coeluting HOCs – Environmental Sample

Summary

The data in this poster demonstrates the selectivity and performance of LECO Corporation's Pegasus GC-HRT. The instrument minimizes background interference problems caused by coeluting compounds. The GC-HRT provided the high resolution and enhanced selectivity required for the analysis of complex matrices such as pet food. Dispersive clean-up was not necessary for these samples since targeted and untargeted analysis can be easily accomplished with LECO's Pegasus HRT and ChromaTOF HRT software. The high performance GC-HRT can be successfully employed for analysis of complex matrices.