

Tandem GC/MS Analysis of Sub-pg/ μ L Quantities of PBDEs, Including BDE-209 Using the Novel High Efficiency EI Source

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Introduction

Polybrominated diphenyl ethers (PBDE) are stable and bioaccumulate in fatty tissues. They may act as endocrine disruptors and BDE-209 has even shown evidence of carcinogenic potential¹. That makes them an important class of compounds to monitor.

The analysis of PBDE has been the domain of sector field instruments using gas chromatography isotope dilution high resolution mass spectrometry. In the past only accurate mass instruments have been able to reach the required detection limits. The introduction of a High Efficiency Source (HES) has increased the ion flux over traditional tandem GC/MS instruments by a factor of 10 to 20-fold with a multi-fold improvement in EI detection limits. The HES allows the analysis of BDE-209 samples to be run on a tandem GC/MS at trace levels that have to this point only been run on sector field instruments.

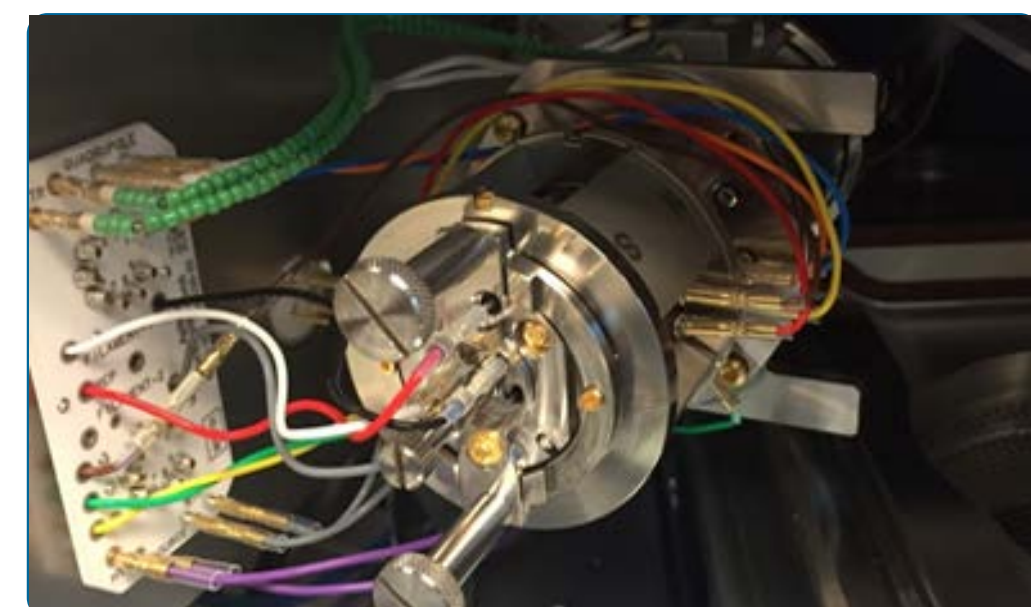


Figure 1. High Efficiency EI Source.

With the increase in EI sensitivity we are able to:

- Easily quantitate BDE-209 at 0.2 pg/ μ L.
- Use one concentration range (0.2 – 100 pg/ μ L) for all PBDEs.
- Have excellent linearity for all PBDEs from tribromo to decabromo BDEs.

Experimental

Most of the traditional GC/MS based PBDE methods developed out of EPA 8270 culminating in 1614A. These methods often use a relatively short, thin-film column to minimize BDE-209 breakdown. In this case a longer 25 m x 200 μ m x 0.11 μ m column was used as a compromise between resolution and minimal BDE-209 breakdown. In this case we rely on a tandem quad GC/MS using the new High Efficiency Source (HES) to reach sub-pg/ μ L concentrations of all the PBDEs, including BDE-209.

Experimental

The sample preparative procedure is straightforward and highly automated^{2,3}. This procedure also works for other flame retardants such as polybrominated biphenyl (BB-153) and PCBs such as CB-153. There are two Gilson 215 liquid handlers used for standard addition and liquid-liquid extraction. There is also a RapidTrace SPE workstation used to remove lipids. The general steps involved with PBDE sample preparation were:

- Two grams of serum were weighed into 16x100 mm culture tubes.
- The aliquots were placed on the Gilson 215 configured for internal standard addition and mixing by rotation.
- HCl was added by hand and the sample tubes were vortexed.
- A second Gilson 215 liquid handler was used for automated liquid-liquid extraction. The organic phase (top layer) was transferred to a 16 x 125 mm culture tube. This extraction step was repeated and the combined extracts were evaporated to dryness.
- Lipids were removed using a two layered cleanup cartridge, automated using the RapidTrace SPE workstation. The top layer of the cleanup cartridge was 0.2g of activated silica gel and the lower layer was 1.0g of 33% sulfuric acid in silica gel (w/w). 10mL of 5% dichloromethane in hexane was used for elution.
- The samples were evaporated and transferred to a GC vial containing an external recovery standard for analysis on the Agilent 7010 Triple Quadrupole GC/MS.

GC and MS Conditions:		Agilent 7890B/7010
Column		HP-Ultra 1, 17 meter, 0.2 mm ID, 0.11 μ m film
Injection volume		2 μ L Pulse Splitless
Pressure pulse		20 psi until 0.6 min
Split/Splitless inlet temperature		260 °C
Purge flow to split vent		50 mL/min at 0.75 min
Oven temperature program		140 °C for 1min
Carrier gas		Helium at 1 mL/min constant flow
Transfer line temperature		290 °C
Ionization mode		EI
Source temperature		290 °C
Quadrupole temperature		150 °C
Quench gas		Helium, 4 mL/min
Collision gas		Nitrogen, 1.5 mL/min
Acquisition mode		MRM

Table 1. GCMS Parameters. The helium quench gas flow was increased from 2.25 mL/min to 4 mL/min. This reduces neutral noise by lowering the abundance of helium metastables so that they are less likely to contribute to neutral noise.

Experimental

Compound	RT (min)	Target MRM	CE	Qualifier MRM	CE	Qualifier MRM	CE
¹³ C ₁₂ -Tri	6.761	419.9→260	25				
Tri	6.763	405.7→246	25	407.9→248.0	25	405.7→167.0	55
1234-TCDD	7.527	327.6→199.9	45	327.6→263.9	15		
¹³ C ₁₂ -Tetra	8.756	497.9→337.9	30				
Tetra	8.761	485.9→325.9	30	483.6→326.0	30	483.6→324.0	30
¹³ C ₁₂ -Penta	10.216	575.9→415.9	35				
Penta	10.223	405.8→296.9	40	563.9→403.9	35	405.8→298.9	40
¹³ C ₁₂ -Hexa	11.781	655.6→495.9	20				
BB-153	11.760	308.0→148.0	35	468.0→308.0	40		
Hexa	15.717	483.6→374.9	30	483.6→376.9	30		
Hexa	11.786	643.6→483.9	20				
¹³ C ₁₂ -Hepta	13.920	733.6→574.0	50				
Hepta	13.931	721.6→561.8	50	561.7→454.9	50		
¹³ C ₁₂ -PBDE-209	20.795	971.6→812.2	60				
PBDE-209	20.804	799.4→639.5	55				

Table 2. MRM Transitions for PBDEs.

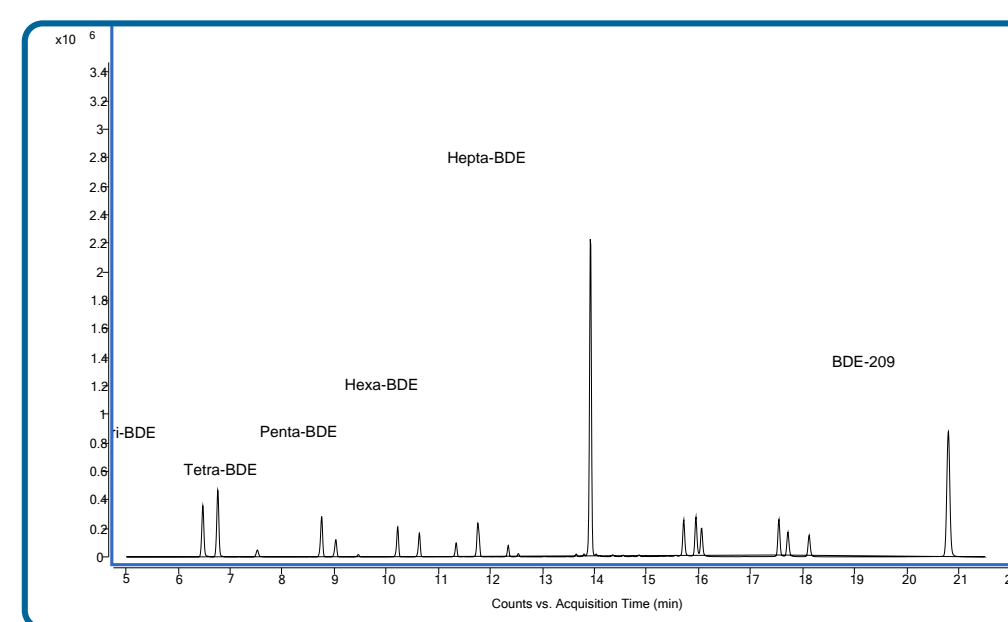


Figure 2. Relative Response of BDE-209 to other brominated flame retardants at identical concentration. The enhanced response of decabromodiphenyl ether is due to the high ion flux and low noise characteristic of the novel High Efficiency Source (HES).

Results and Discussion

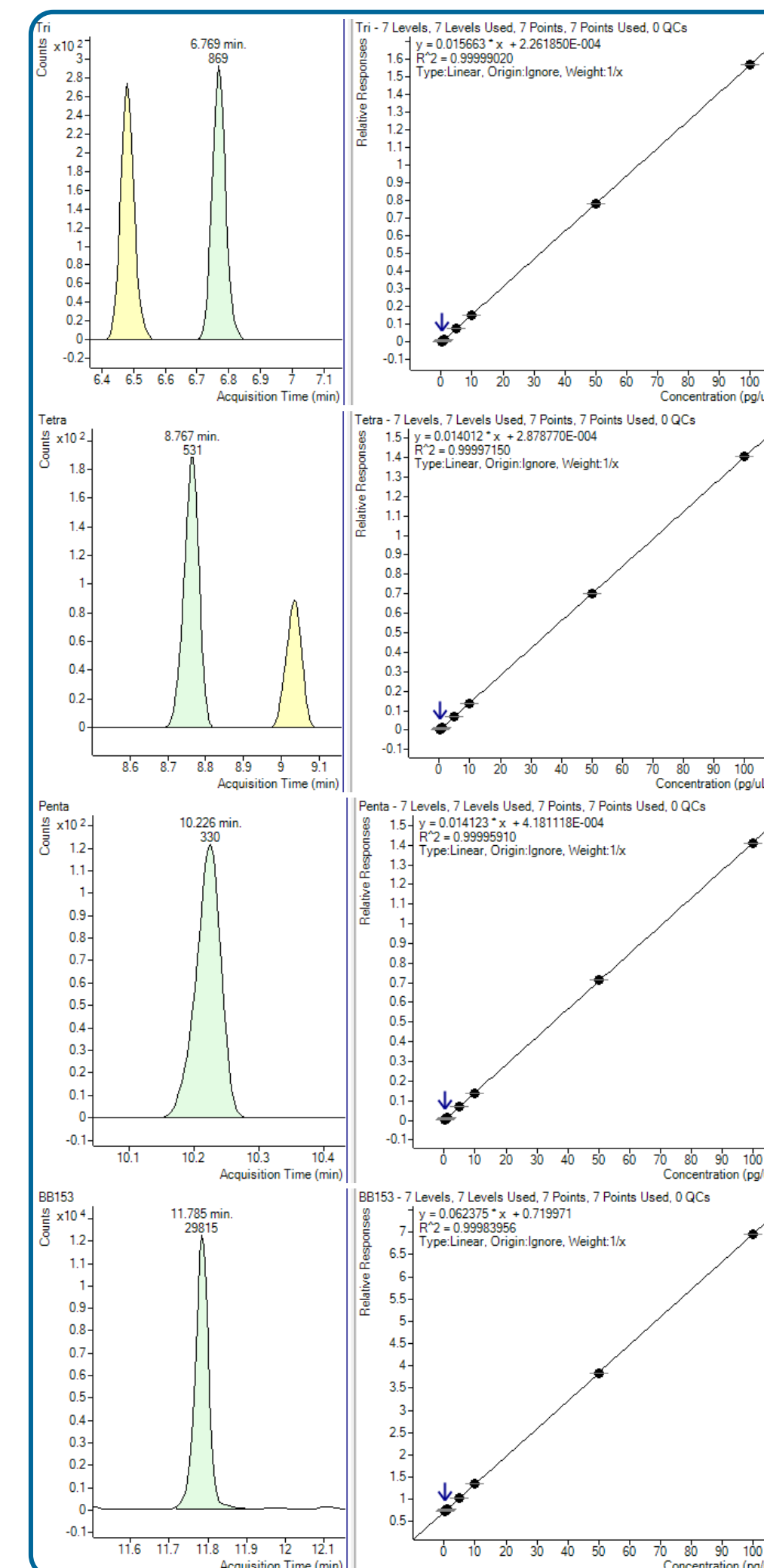


Figure 3. Tri-BDE through BB-153.

Results and Discussion

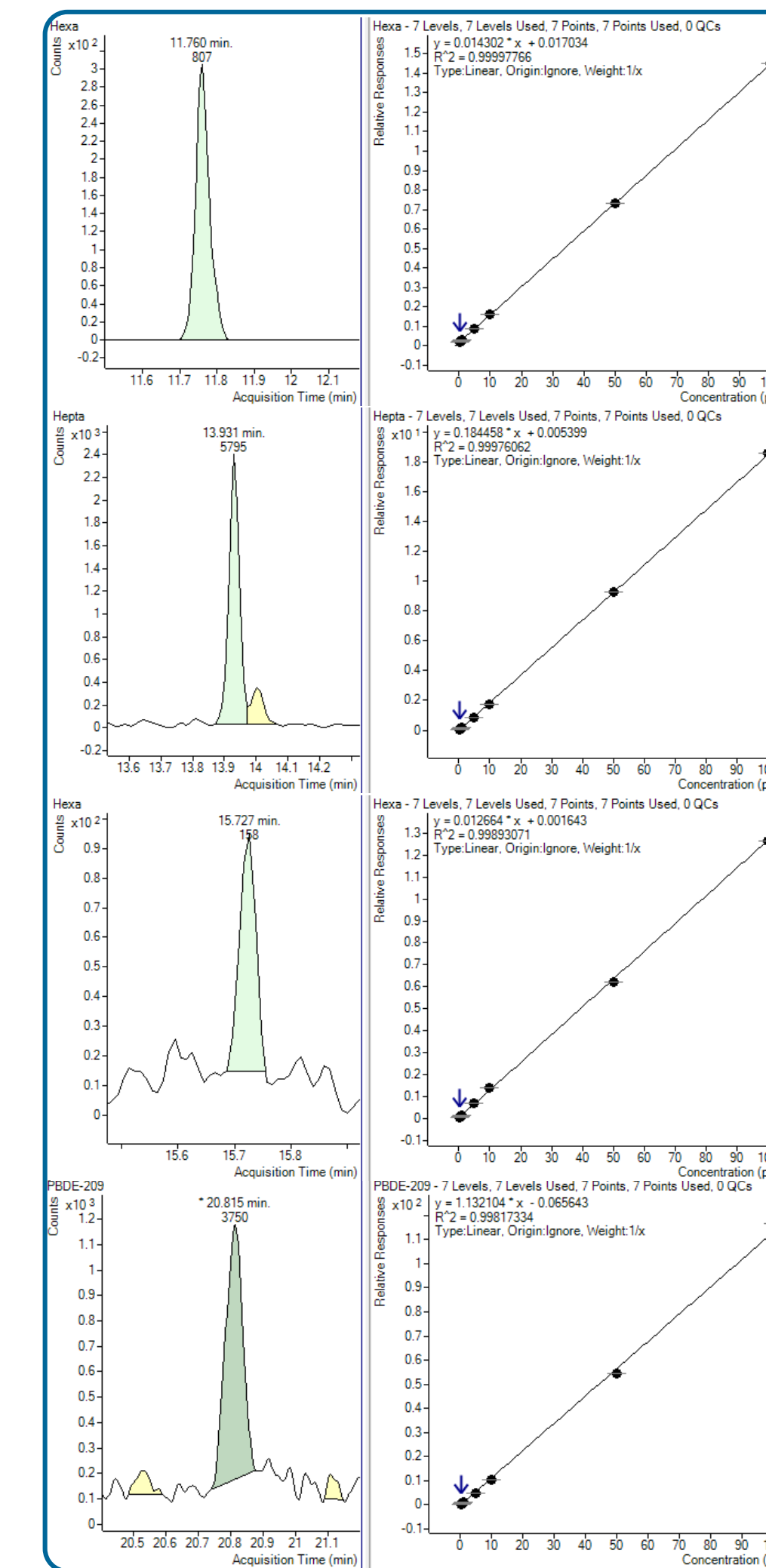


Figure 4. Hexa-BDE through BDE-209.

The HES installed on a tandem GC/MS is more sensitive and selective than the ubiquitous accurate mass GC/MS traditionally used for EPA method 1614A. **Too much sensitivity becomes an unforeseen concern** as we want to keep all the target compounds in their linear range. Of the brominated flame retardants, only decabromo-diphenyl ether benefited from a gain of 100. Electron multiplier based detectors saturate at ion abundances in the 10⁷ range. We have several options to keep the signal from saturating in the cases of the other brominated flame retardants:

- Lower the gain. The range goes from 0.1 to 100.
- Select less abundant MRM transitions.
- Lower the dwell time.
- Narrow the peak widths.

The standard helium quench gas flow is 2.25 mL/min. It can be increased to reduce neutral noise. This is only effective in cases where there is very little chemical noise. Note that the collision energy might need a slight adjustment if the helium flow rate is increased significantly. In the case of brominated flame retardants we noticed a reduction in noise when we increased the quench gas flow.

Conclusions

The High Efficiency Source (HES) introduces an inexpensive and yet highly-sensitive approach to selective PBDE analysis in blood serum at biologically relevant concentrations down to sub-pg/mL (0.2-0.4 pg on-column). Figure 2 shows the TIC of BDE-209 where it has a significantly higher response relative to other brominated flame retardants. The strong response for PBDE-209 is in contrast to the weak response that has been typically shown on either earlier GC/MS/MS or GC/HRMS instruments. This is an indication of the applicability of the HES for the analysis of compounds like PBDEs, PBB, and PCBs at concentrations that have been traditionally difficult to obtain.

References

- ¹EPA Technical Fact Sheet – Polybrominated Diphenyl Ethers (PBDEs) and Polybrominated Biphenyls (PBBs), Jan 2014
- ²Andreas Sjödin et al, Anal. Chem. 2004, 76, 1921-1927
- ³Jones, R.; Edenfield, E.; Anderson, S.; Zhang, Y.; Sjödin, A. Semiautomated extraction and cleanup method for measuring persistent organic pollutants in human serum. Organohalogen Compd. 2012, 74, 97-98

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