ANALYSIS OF HALOGENATED PERSISTENT ORGANIC POLLUTANTS IN HUMAN BREAST MILK USING THE QUECHERS EXTRACTION APPROACH AND COMPREHENSIVE TWO - DIMENSIONAL GAS CHROMATOGRAPHY

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Introduction

Persistent organic pollutants (POPs) are a group of chemicals that include brominated flame retardants (BFRs) and polychlorinated biphenyls (PCBs). These chemicals can be distributed throughout the environment through soil, water, and air contamination and once in the environment do not readily break down. Due to the lipophilic nature of these components they accumulate in the fatty tissue of animals and bioaccumulate up the food chain[1]. According to the World Health Organization human breast milk is an ideal matrix to monitor the levels of POPs in not only the mother and infant, but also as a key indicator of the levels of these chemicals in the local environment.

Current sample preparation for the analysis of PCBs and BFRs typically involves liquid-liquid or pressurized fluid extraction with extensive clean-up[2,3]. These methods often include expensive equipment and/or large amounts of solvent usage. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) methodology was developed for the determination of multi-residue pesticides in fruits and vegetables[4]. The low solvent consumption and multi-residue approach of QuEChERS may be an attractive sample preparation alternative for biomonitoring efforts for halogenated POPs in human breast milk.

Previous work presented at Dioxin 2011 focused on the initial method development of POPs in cow milk using the QuEChERS extraction, silica cartridge cleanup, and determination by comprehensive two-dimensional gas chromatography (GCxGC) with an electron capture detector (ECD)[5]. Continuing from this work, further method optimization has been completed for the analysis of POPs in human milk using a NIST certified standard reference material (SRM). Using GCxGC with a time-of-flight mass spectrometer (TOFMS) provided full mass acquisition for both target and non-target analysis.

Materials and Methods

Initial method development was performed on whole cow milk samples fortified at 0.1, 0.5, 1 and 10 µg/kg with a mixed standard containing select polybrominated diphenyl ethers (BDEs), a brominated biphenyl (PBB) and polychlorinated biphenyls (PCBs). Using a modification of the original unbuffered QuEChERS sample preparation approach, 5 mL of milk diluted with 5 mL water was extracted with 10 mL of hexane:acetone (1:1) and partitioned with 4 g magnesium sulfate and 1 g sodium chloride. The extract was centrifuged and a 2.5 mL aliquot was taken through cleanup prior to analysis. Silica cartridge SPE and primary secondary amine (PSA) cartridge SPE were evaluated for extract cleanup. Method performance was evaluated by analyzing a NIST standard reference material of fortified human milk (NIST SRM 1954).

An Rxi-XLB 30m x 0.25mm x 0.25µm column coupled to a 1m x 0.15mm x 0.15µm Rxi-17Sil MS in a LECO GCxGC-ECD was used to determine recoveries for the extraction experiments. In addition, extracts were evaluated on a LECO Pegasus 4D GCxGC-TOFMS system.

Results and Discussion

Cleanup Evaluation

The QuEChERS methodology uses dispersive solid phase extraction (dSPE) cleanup with PSA, C18 and/or graphitized carbon black (GCB). During initial method development it was determined that dSPE did not have the capacity to provide an effective cleanup of the co-extracted fat in the milk extracts and cartridge cleanup (cSPE) was necessary. A 500 mg silica cartridge SPE cleanup with 5 mL hexane:dichloromethane (3:1 v/v) elution was from a determination of PCBs and PBDEs in fish[6]. This solvent scheme did not provide a clean enough extract, and therefore a hexane-only elution was used. A 3x5 mL hexane fractionation study using spiked cow milk found that almost 100% of PCBs and BDEs were present in the first fraction; therefore only 5 mL hexane was used as the elution solvent for subsequent experiments (Figure 1).

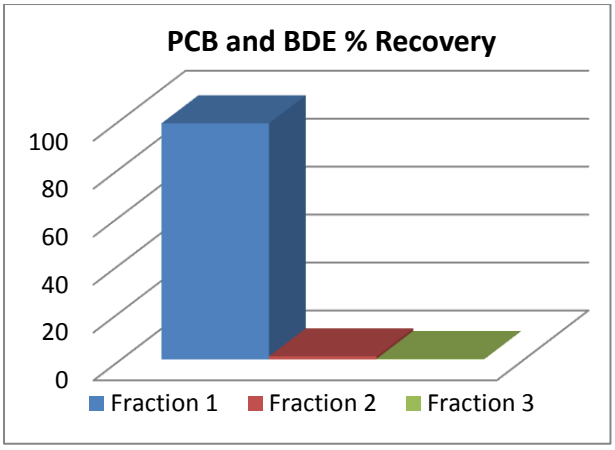

Figure 1: 3x5 mL fractions of hexane evaluated for PCB and BDE recoveries from 500 mg silica cartridge.

After the method had been optimized, a NIST SRM of fortified human milk (SRM 1954) was taken through the process. Using GCxGC with an ECD proved to be very difficult for the human milk analysis. The retention times of target analytes were shifting, making quantitation almost impossible. The resultant extract was then analyzed using GCxGC-TOFMS. Looking at the total ion chromatogram (TIC) of the human milk extract highlighted that large amounts of fatty acids were still present in the extract (Figure 2).

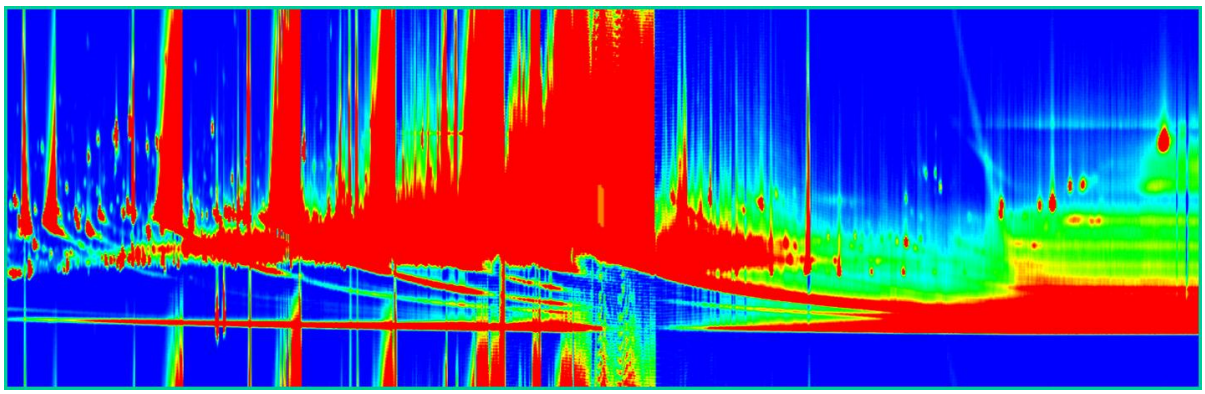

Figure 2: GCxGC-TOFMS analysis of NIST Standard Reference Material 1954 (fortified human milk) extract with silica cSPE cleanup revealed that many interferences still remained in the extract.

Subsequent method development focused on the removal of fatty acid interferences in the human milk that were not present in the cow milk. While cow milk and human milk have similar fat content, they differ in fatty acid content, especially polyunsaturated fatty acids that are essential for human brain development.

Primary secondary amine (PSA) sorbent is commonly used to clean up sugars and fatty acids found in QuEChERS extracts. A 500 mg PSA cartridge was evaluated as a standalone cleanup step and in conjunction with the silica cartridge cleanup. It was determined that the PSA cartridge in addition to the silica cartridge provided a clean enough extract to properly quantify targeted analytes in human milk (Figure 3).

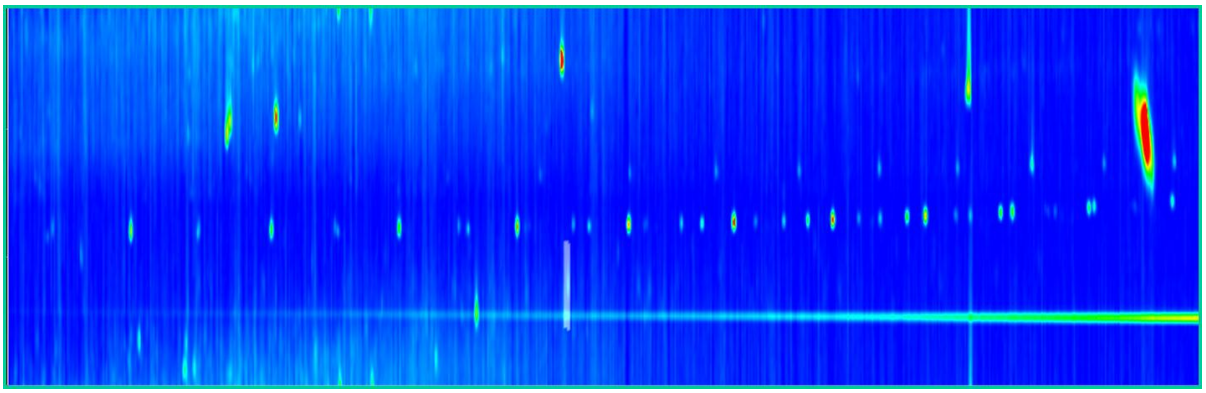

Figure 3: GCxGC-TOFMS analysis of NIST Standard Reference Material 1954 (fortified human milk) extract with PSA and silica cSPE cleanup removed large interferences.

Percent Recoveries

Triplicate spike experiments in whole cow milk at 0.1, 0.5, 1 and 10 µg/kg yielded average recoveries of 122±16, 129±18, 126±38 and 99±7 percent respectively using silica cSPE cleanup only. Evaluation of the NIST SRM fortified human milk using a PSA cSPE cleanup followed by a silica cSPE cleanup with GCxGC-TOFMS provided results with an average of 15% error of the certified value (Table I). In addition to quantitation data (shown in bold font in the table below), several other analytes that were present in the SRM but did not have a reference standard were evaluated on a semi-quantitative basis (shown in non-bold font).

	QuEChERS (n=3) (ng/kg)	NIST Certified (ng/kg)	Percent Error
PCB 28	562	519	8
PCB 74	643	563	14
PCB 66	**384**	**428**	**10**
PCB 99	655	558	17
PCB 110	**399**	**458**	**13**
PCB 118	530	597	11
PCB 146	503	492	2
PCB 153	**1080**	**977**	**11**
PCB 105	360	482	25
PCB 138	**982**	**639**	**54**
PCB 178	514	449	14
PCB 167	478	467	2
PCB 177	470	447	5
PCB 156	516	511	1
PCB 172	578	443	31
PCB 157	450	467	4
PCB 180	**896**	**696**	**29**
BDE 47	**2970**	**2570**	**16**
BDE 100	**1220**	**1280**	**5**
BDE 99	**637**	**739**	**14**
BDE 154	414	464	11
PBB 153	**297**	**474**	**37**
BDE 153	**1340**	**1440**	**7**

Table I: Comparison of NIST certified values and QuEChERS approach methodology values obtained for standard reference material of fortified human milk.

Comprehensive Two-Dimensional Gas Chromatography

Using the selective and sensitive ECD allows detection of multiply halogenated analytes in fg amounts. Chromatographic resolution is very important for a single channel detector and GCxGC allows two independent separations in one analytical run. In a one-dimension GC analysis some of the PCBs and BFRs will co-elute. However, the second dimension Rxi-17Sil MS helps resolve BFRs from PCBs (Figure 3).

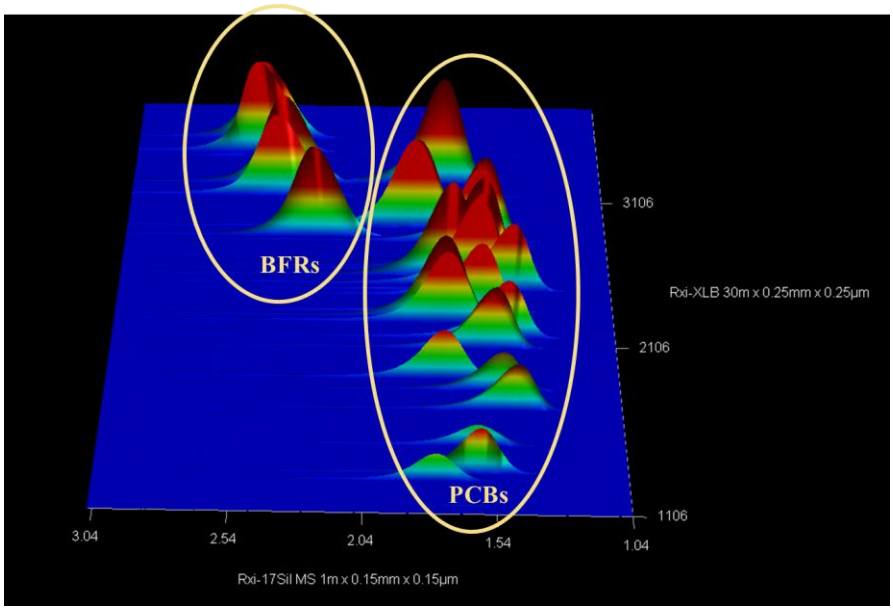

Figure 4: GCxGC-ECD contour plot of select BFR and PCB standard using an Rxi-XLB (30 m x 0.25 mm x 0.25 µm) and Rxi-17SilMS (1 m x 0.15 mm x 0.15 µm) allows the separation of BFRs and PCBs in the second dimension.

References

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