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

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Introduction:

Persistent organic pollutants (POPs) are a group of chemicals that include halogenated pesticides, brominated diphenyl ethers (BDEs) 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 halogenated pesticides, PCBs and BDEs 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.

An expensive high resolution mass spectrometer is often used for the determination of halogenated POPs in breast milk extracts. Comprehensive two-dimensional gas chromatography (GCxGC) with an electron capture detector (ECD) may offer a more cost-effective alternative, due to the sensitivity of the ECD, coupled with the selectivity afforded by a multidimensional separation.

Materials and methods:

Initial method development was performed on whole cow milk samples fortified at 100 µg/kg with a mixed standard containing select organochlorine pesticides (OCPs), BDEs and PCBs. Using the original unbuffered QuEChERS extraction approach, 10 mL of milk was extracted with 10 mL of an organic solvent and partitioned with 4 g magnesium sulfate and 1 g sodium chloride, prior to centrifugation and withdrawal of extract for cleanup prior to analysis. Several organic solvents were evaluated for extraction efficiency of target analytes while also monitoring the amount of co-extracted fat. These solvents included acetonitrile, which is typically used with QuEChERS extractions, hexane, ethyl acetate and hexane:acetone (1:1 v/v). Silica cartridge SPE and primary secondary amine (PSA) cartridge SPE were evaluated for extract cleanup.

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..

Results and discussion:

Extraction Efficiency and Percent Recoveries

Closely following the original unbuffered QuEChERS methodology 10 mL of acetonitrile was added to 10 mL of 100% milk, 50% milk, 25% milk and 0% milk with de-ionized water making up the remaining percentage. Recoveries of target compounds increased as the percentage of milk decreased. Lipophilic compounds such as PCBs and BDEs were not efficiently extracted by the polar solvent. Other extraction solvents were first

evaluated by gravimetrically determining the amount of fat extracted by evaporating 4 mL of milk extract (Table I). Ethyl acetate and hexane:acetone (1:1 v/v) emerged as viable extraction solvents. Removal of co-extracted fat was achieved on a silica SPE cartridge eluted with hexane:dichloromethane (3:1 v/v). Recoveries were compared prior to SPE clean-up and after silica SPE clean-up (Table II). Some analytes such as endrin aldehyde and methoxychlor were not completely recovered from the silica cleanup, while almost all other target analytes benefited from the extract cleanup. This is especially evident for the late eluting BDEs that may have been poorly transferred from the GC inlet because of high molecular weight fats.

Table I: Gravimetric analysis of total fat content with different extraction solvents

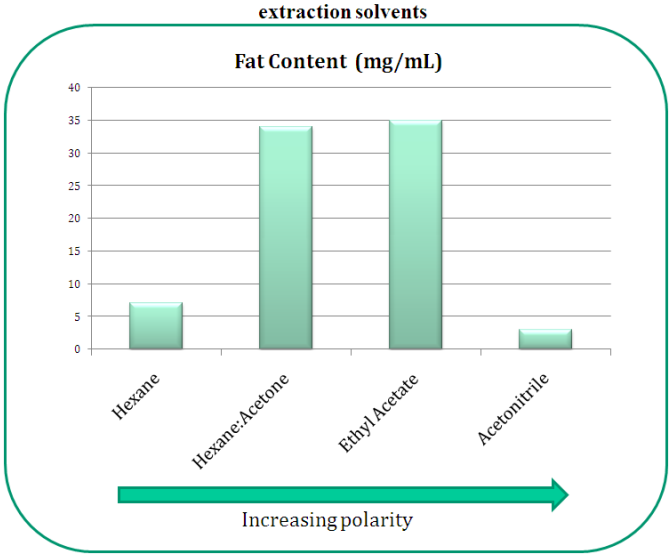


Table II: Percent Recoveries of OCPs, PCBs, and BDEs with and without Silica SPE clean-up

Analyte	Hexane:Acetone (1:1)		Ethyl Acetate	
	Silica SPE	No SPE	Silica SPE	No SPE
alpha-BHC	63	90	53	93
beta-BHC	80	91	69	91
Heptachlor	77	94	68	84
Aldrin	66	82	61	92
Heptachlor epoxide	79	93	70	90
gamma-chlordane	81	87	59	95
PCB 66	79	97	74	91
PCB 99	63	85	80	91
4,4'-DDE	116	101	17	89
PCB 110	81	98	73	77
4,4'-DDD	81	88	91	25
PCB 153	88	95	78	76
Endrin Aldehyde	1	27	1	96
PCB 146	86	99	79	77
PCB 187	92	98	84	73
PCB 183	89	97	79	55
Methoxychlor	2	89	1	95
Endrin Ketone	33	90	24	104
PCB 180	89	91	77	44
BDE-47	79	87	73	92
PCB 170	84	92	74	49
BDE-100	80	66	76	88
BDE-99	78	53	71	85
BB-153	81	39	72	88
BDE-153	90	36	93	93

Comprehensive Two-Dimensional Gas Chromatography Electron Capture Detection

Using the selective and sensitive ECD allows detection of multiply halogenated analytes in fg amounts. Chromatographic resolution is very important without the ability to spectrally resolve analytes through mass spectrometry and GCxGC-ECD allows two independent separations in one analytical run (Figure 1). In a one-dimension GC analysis some of the PCBs and BDEs will co-elute. However, the second dimension Rxi-17Sil MS helps resolve BDEs from PCBs (Figure 2).

Figure 1: GCxGC-ECD surface plot of a mixed standard containing OCPs, PCBs and BDEs

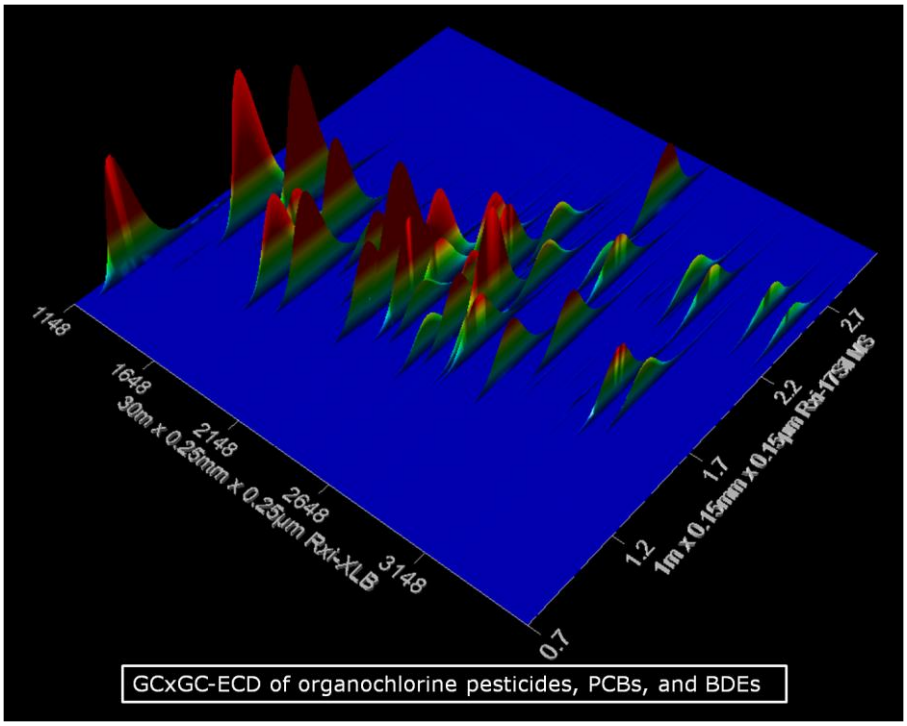
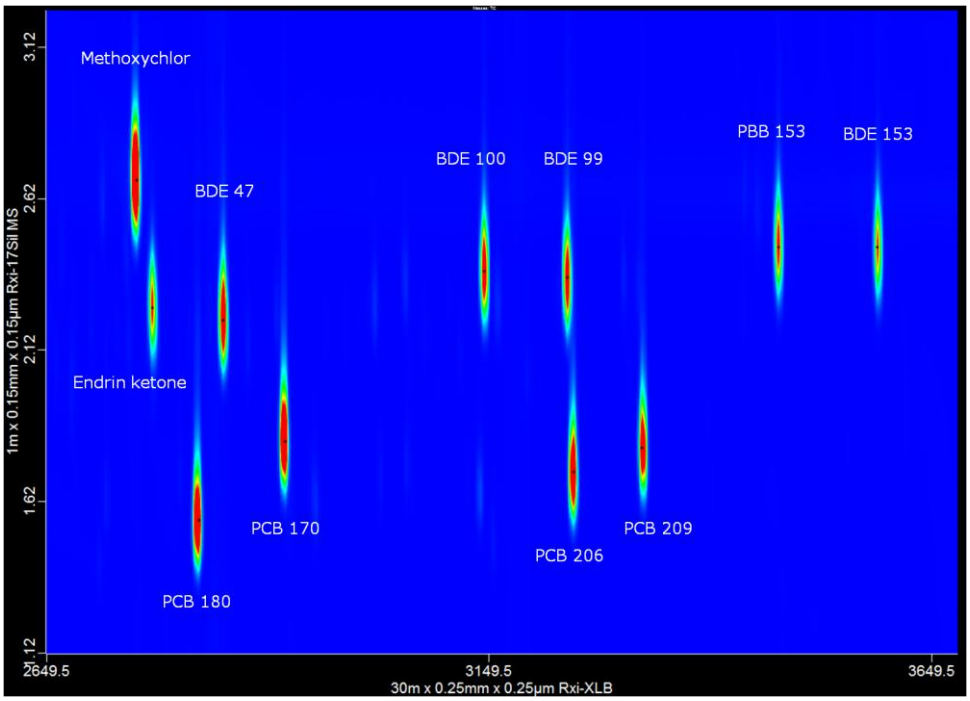


Figure 2: GCxGC-ECD contour plot showing BDEs eluting above PCBs. Especially note resolution of BDE 99 and PCB 206 in the second dimension



References:

1. Stockholm Convention on Persistent Organic Pollutants, http://chm.pops.int
2. Burke E, Holden A, Shaw I. (2003); *Chemosphere* 50: 529-535
3. Sjödin A, McGahee E, Focant J, Jones R, Lapeza C, Zhang Y, Patterson D. (2004); *Anal. Chem*. 76: 4508-4517
4. Anastassiades M, Lehotay S, Stajnbaher D, Schenck F. (2003); *J. AOAC International* 86: 412-431