

The QuEChERS Extraction Approach and Comprehensive Two - Dimensional Gas Chromatography of Halogenated Persistent Organic Pollutants in Cow Milk and Human Breast Milk

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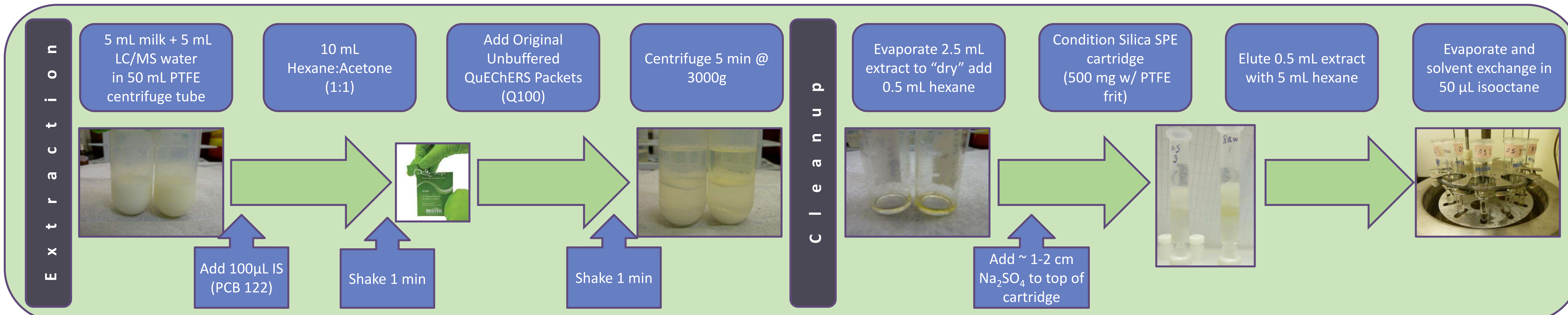
Abstract

- Persistent organic pollutants (POPs) are a group of chemicals that include halogenated pesticides, brominated diphenyl ethers (BDEs) and polychlorinated biphenyls (PCBs).
- Due to the lipophilic nature of these components they accumulate in the fatty tissue of animals and bioaccumulate up the food chain.
- 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 methodology for the analysis of halogenated pesticides, PCBs and BDEs can be expensive, solvent intense and time consuming ^{1,2}.
- The QuEChERS extraction approach coupled to a silica cartridge SPE cleanup may be an attractive sample preparation alternative for biomonitoring efforts for halogenated POPs in milk.
- Comprehensive two-dimensional gas chromatography (GCxGC) with an electron capture detector (ECD) may also offer a more cost-effective alternative.
- Method development was done using whole cow milk and later compared to a NIST Standard Reference Material of Human Breast Milk.

Materials and Methods

- Using the original unbuffered QuEChERS extraction approach³, 5 mL of milk plus 5 mL of LC/MS water 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 including acetonitrile, hexane, ethyl acetate and hexane:acetone (1:1 v/v) were evaluated for extraction efficiency of target analytes while also monitoring the amount of co-extracted fat.
- After extract concentration, a 500 mg/3mL silica cartridge solid phase extraction tube (SPE), with 5 mL hexane elution was used to remove fat from the extract.
- 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.
- Triplicate recovery experiments were performed on whole cow milk samples fortified at 0.1, 0.5, 1.0 and 10 µg/kg with a mixed standard containing select BDEs and PCBs.
- This methodology (Figure 1) was used on a NIST reference material of fortified human breast milk (SRM 1954) to determine method accuracy.

Figure 1: QuEChERS Extraction Approach and Silica SPE Cleanup used for Cow Milk and Human Breast Milk Analysis



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. Ethyl acetate and hexane:acetone (1:1 v/v) emerged as viable extraction solvents.
- Initial experiments followed a similar procedure to remove co-extracted fat on a silica SPE cartridge in fish samples with hexane:dichloromethane (3:1 v/v) elution⁴. However, it was determined a hexane only elution provided better removal of fat while still allowing target BDEs and PCBs to elute in a single 5 mL fraction.
- Triplicate recoveries of whole cow milk fortified at 0.1, 0.5, 1.0 and 10 µg/kg produced average percent recoveries of 122%, 129%, 126% and 99% respectively. (Table I)
- The percent error of the QuEChERS extraction approach with silica SPE cleanup and GCxGC-ECD determination to the NIST certified values of fortified human breast milk averaged 23% removing the outliers PCB 18 and 138, which were biased high due to coelutions. (Table II)

Comprehensive Two-Dimensional Gas Chromatography Electron Capture Detection

- 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.
- The Rxi-17sil MS secondary column provided the needed selectivity to resolve the BDEs and PCBs in the second dimension, which coelute in a one-dimension GC analysis. (Figure 2)
- Two-dimensional chromatography also facilitates the separation of target analytes from matrix interferences seen by GCxGC TOFMS analysis. (Figure 3)
- Using the selective and sensitive ECD allows quantification of multiply halogenated analytes in the low pg range and detection in fg amounts.

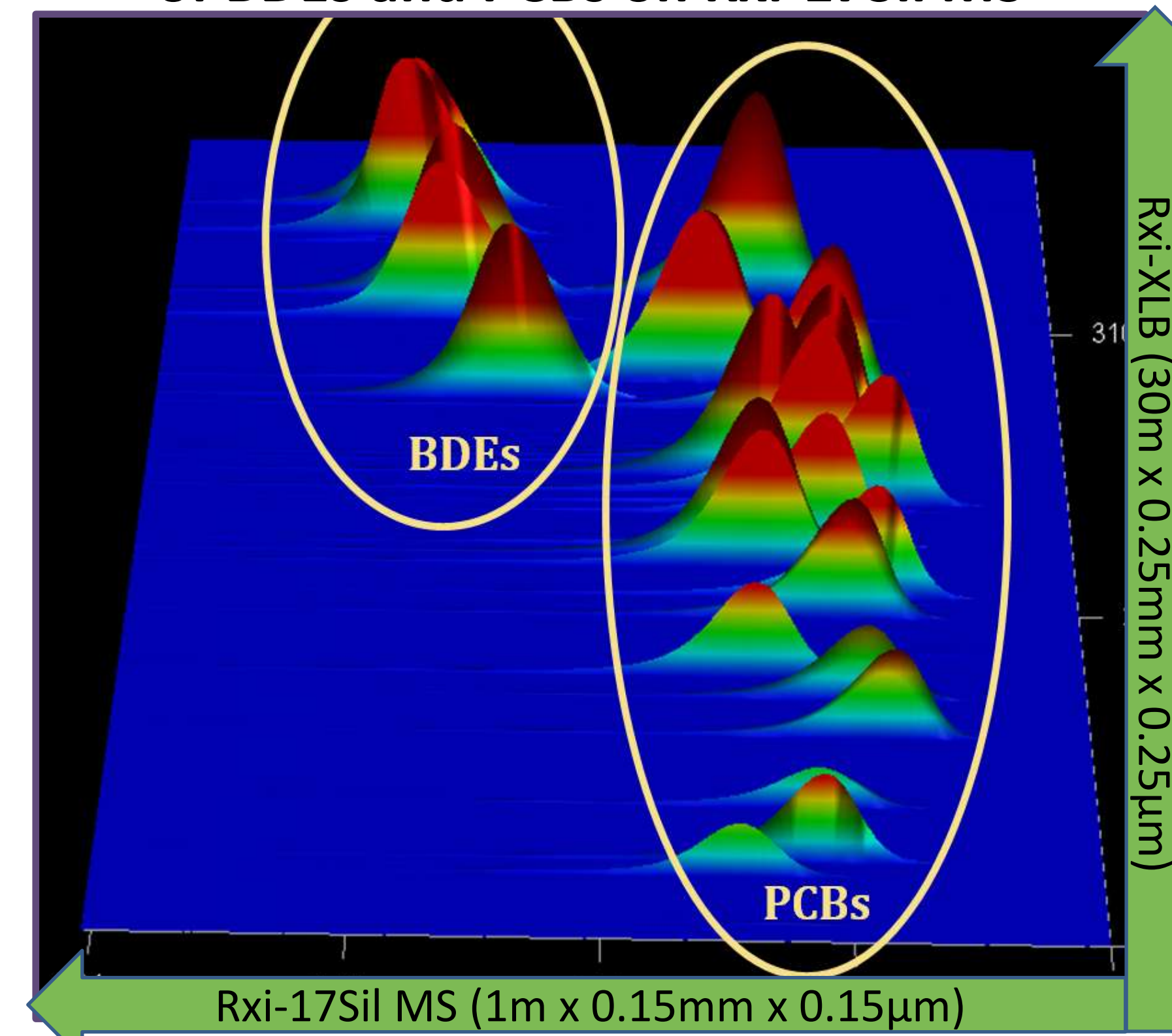
Table I: Triplicate Spike Average Percent Recoveries in Whole Cow Milk

	0.1 ppb	0.5 ppb	1 ppb	10 ppb
PCB 18	138	103	152	90
PCB 52	131	122	163	97
PCB 44	111	107	157	98
PCB 66	108	112	165	99
PCB 101	114	111	162	101
PCB 110	110	112	167	102
PCB 151	109	111	161	103
PCB 153	114	116	154	104
PCB 138	149	118	155	106
PCB 187	118	116	155	106
PCB 183	110	118	154	106
PCB 180	111	124	144	107
BDE 47	186	140	83	98
PCB 170	114	125	138	107
BDE 100	131	167	63	96
BDE 99	130	154	53	94
PCB 206	114	136	101	104
BDE 153	107	175	38	86
BDE 153	119	180	32	82

Table II: Comparison of NIST Certified Values of Fortified Human Breast Milk (SRM 1954)

	NIST Certified Value (SRM 1954) pg/µL	QuEChERS Approach w/ SPE cleanup (n=2) pg/µL	% Error
PCB 18	9	169	1804
PCB 52	11	19	78
PCB 44	10	19	84
PCB 66	11	17	62
PCB 101	11	14	29
PCB 110	11	10	11
PCB 151	10	13	21
PCB 153	24	25	2
PCB 138	16	35	116
PCB 187	13	12	3
PCB 183	11	11	3
PCB 180	17	17	2
BDE 47	64	62	3
PCB 170	13	12	1
BDE 100	32	32	1
BDE 99	18	22	16
PCB 206	12	11	7
BB 153	12	16	33
BDE 153	36	72	99

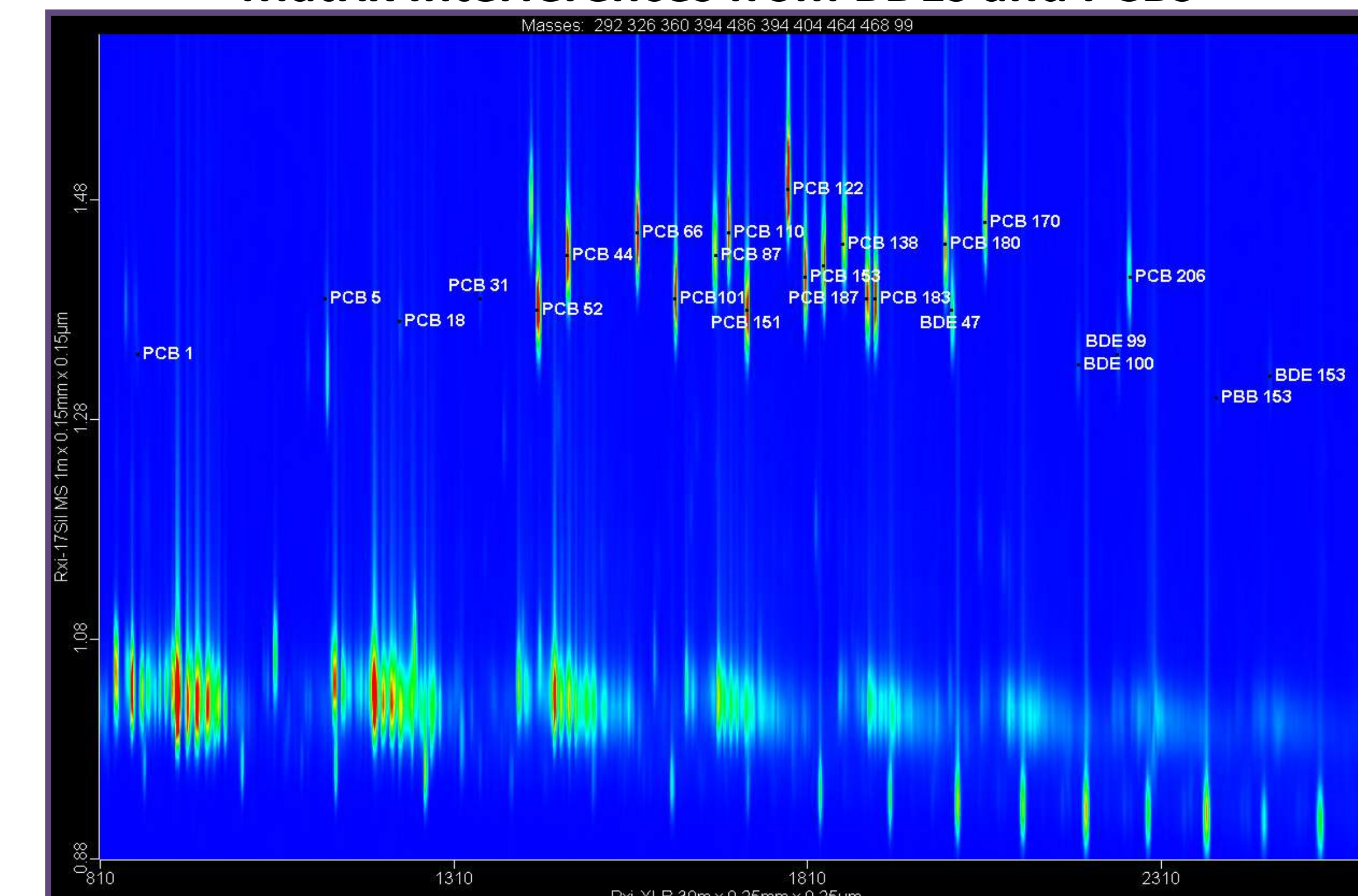
Figure 2: Second Dimension Separation of BDEs and PCBs on Rxi-17Sil MS



GC x GC - ECD Conditions

Column 1: Rxi-XLB 30m x 0.25mm x 0.25µm
 Column 2: Rxi-17Sil MS 1m x 0.15mm x 0.15µm
 Inlet: 250°C, splitless (1 min), Sky Single Taper Liner with Wool
 GC Oven: 80°C (1 min) to 120°C at 10°C/min to 300°C at 3°C/min (primary oven); 85°C (1 min) to 125°C at 10°C/min to 305°C at 3°C/min (secondary oven)
 Modulator: 3.5 sec, 20°C offset
 Detector: µ-ECD @ 325°C

Figure 3: Comprehensive Two-Dimensional GC Separates Matrix Interferences from BDEs and PCBs



References

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