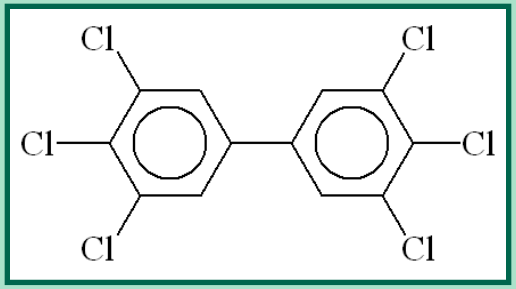
The QuEChERS Extraction Approach and Comprehensive Two-Dimensional Gas Chromatography of Persistent Organic Pollutants in Human Breast Milk

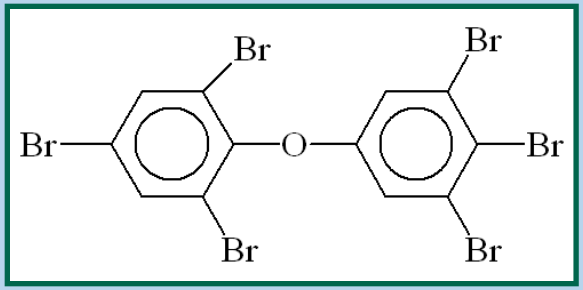
Chris Rattray, Michelle Misselwitz, Jack Cochran and Julie Kowalski

Polychlorinated Biphenyls

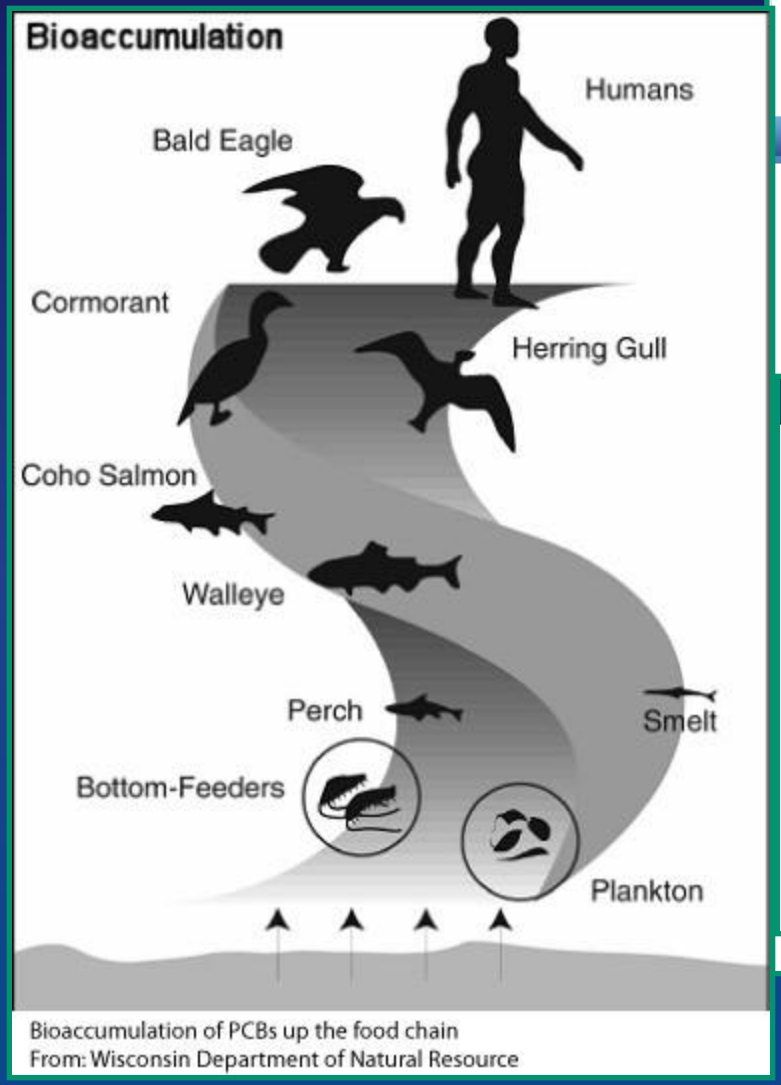


- ✓ Lipophilic
- ✓ Bioaccumulate
- ✓Persistent

Brominated Flame Retardants



Pollutants in Human Milk



Bioaccumulation of PCBs up the food chain
From: Wisconsin Department of Natural Resource

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Chemical Pollution and Mother's Milk
Chemicals in Mother's Milk

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Environmental Contaminants in Breast Milk: Persistent Organic Pollutants (POPs)
Authors and Disclosures
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POPs are a fam

Death Caps
An Ed Rosenberg San Francisco Mystery
TOXIC POLLUTANTS IN BREAST MILK
For nutrition and infant health, authorities agree that the best food is breast milk.(1) Breast milk provides disease-fighting immune factors that infants' immature immune systems cannot provide for themselves. Compared with bottle-fed babies, those who are breast-fed have fewer infections of the intestine, brain, middle ear, respiratory tract, and urinary tract. They gain more benefit from vaccines,(2) and enjoy greater protection against allergies,(3) type 1 diabetes,(4) and ulcerative colitis and Crohn's disease.(5) Breast-feeding also enhances brain development.(6)

Current Methodology

- Liquid-Liquid Extraction, Silica SPE Cleanup
  - Multiple extractions, time consuming and solvent intense
  - Emulsifications, acid washing
- Focused Microwave Extraction, Silica and Alumina SPE Cleanup
  - Capital Equipment
  - Minimized solvent usage
- Semi-Automated Solid Phase Extraction with Multi-Column automated cleanup
  - Large capital equipment
  - Fast sample throughput

QuEChERS

Quick, **E**asy, **Ch**eap, **E**ffective, **R**ugged, and **S**afe

PAHs and PCBs in Seafood

Pesticides in Dietary Supplements

Veterinary Drugs in Milk

BFRs and PCBs in Milk??

Pesticides in Fruits and Vegetables

- Simplified shake extraction for multi- pesticide residue analysis
- Extracted and partitioned using acetonitrile and salt/buffer solution
- Dispersive solid phase extraction cleanup

1. M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, J. AOAC International 86, p. 412-431 (2003).
2. EN 15662, Foods of Plant Origin—Determination of Pesticide Residues Using GC-MS and/or LC-MS/MS Following Acetonitrile Extraction/Partitioning and Clean-up by Dispersive SPE—QuEChERS method.
3. QuEChERS-A Mini-Multiresidue Method for the Analysis of Pesticide Residues in Low-Fat Products. http:// www.quechers.com
4. AOAC Official Method 2007.01, Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate.

QuEChERS for POP Monitoring in Human Milk

- ✓ Low initial start-up costs
  - Centrifuge
  - 50 mL PTFE Tubes
- ✓ Low solvent consumption
  - Approx. 50 mL /sample
- ✓ Fast sample preparation
  - Easily 6 samples/person/day
- ✓ Multi-residue method
  - Simultaneous extraction of multiple-compound classes (ie PCB, BFR, OCP)

QuEChERS Extraction Approach

5 mL Milk + 5 mL LC/MS Water

Add Original Unbuffered QuEChERS Packets 4g $MgSO_4$, 1g NaCl

Shake 1 min

Centrifuge 5 min @ 3000g

2.5 mL aliquot for cleanup

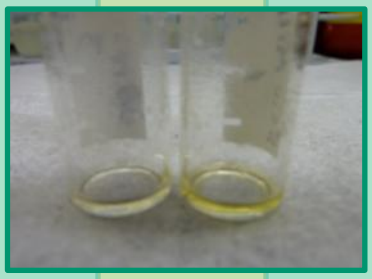
Add 100µL IS (PCB 130)

10 mL Hexane:Acetone (1:1)

Shake 1 min

Cartridge SPE Cleanup

Evaporate to "dry"

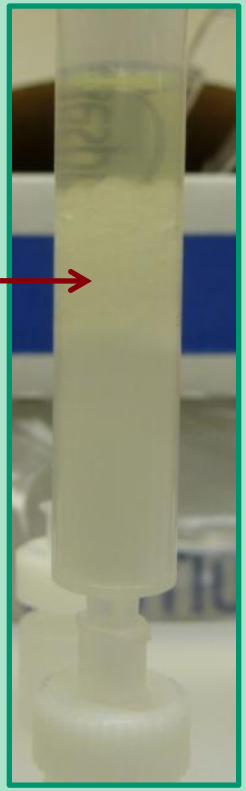


Add 0.5 mL Hexane

Condition Silica SPE Cartridge 3mL/500mg Silica

Add ~ 1cm of Na_2SO_4 to tube

Elute with 5 mL ***Hexane***



Concentrate to 50µL in isooctane

Instrument Conditions

GC X GC Conditions

Injection

Temp: 250°C
Injection: 1 µL fast, splitless (1.0 min)
Liner: Sky Single Taper w/ wool

Column

Rxi-XLB 30m x 0.25mmID x 0.25µm
Rxi-17Sil MS 1.0m x 0.25mm x 0.25µm

Conditions

Oven: 80°C (1 min) to 120°C @ 10°C/min to 300°C @ 3°C/min
Secondary Oven Offset: 5°C
Carrier: He, constant flow 1.4 mL/min
Modulator Temperature Offset: 20°C
Modulation Time: 3 sec

LECO Pegasus® TOFMS Conditions

Transfer Line Temperature: 300°C

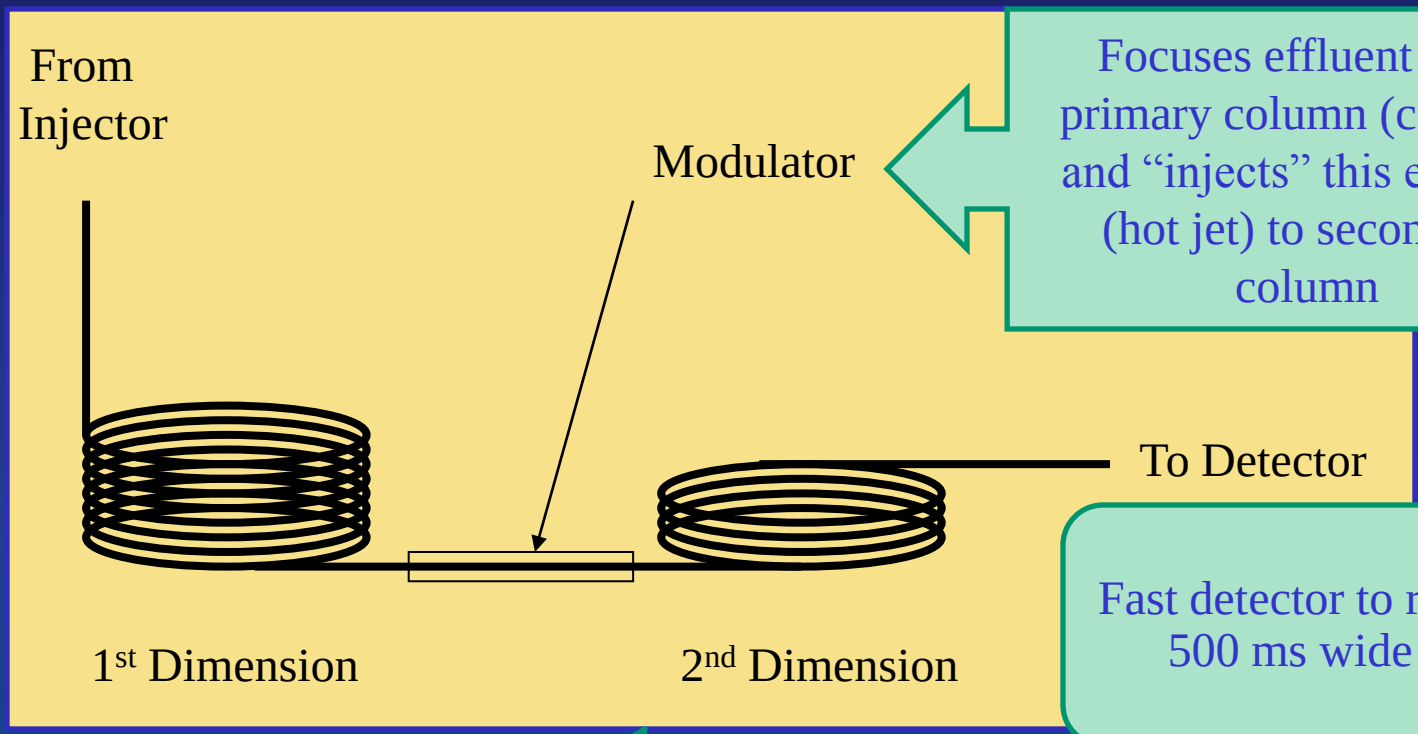
Source Temperature: 225°C

Electron Ionization: 70 eV

Stored Mass Range: 45 to 750 u

Acquisition Rate: 50 spectra/sec

Basics of GCxGC

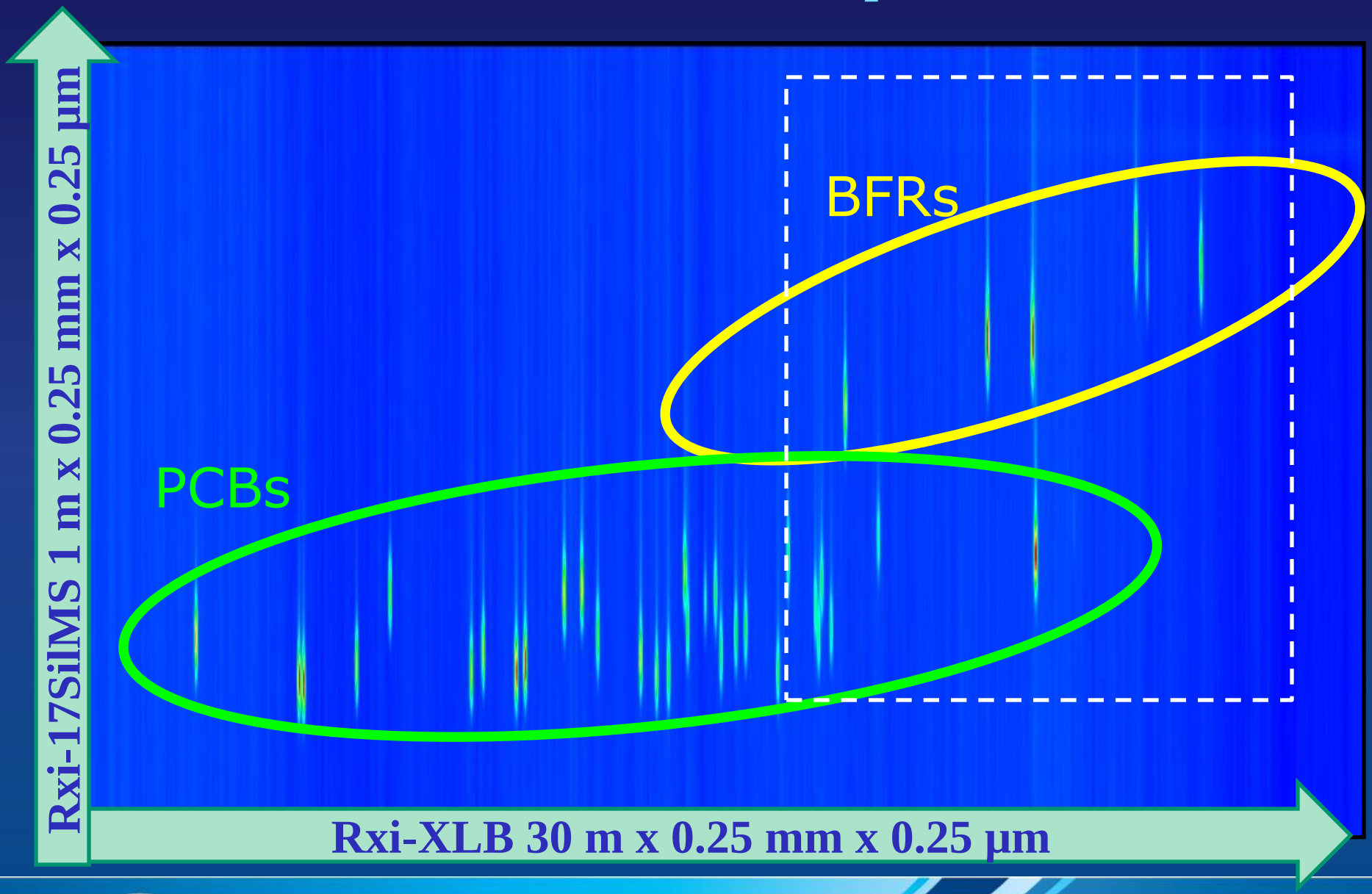


Focuses effluent from primary column (cold jet) and "injects" this effluent (hot jet) to secondary column

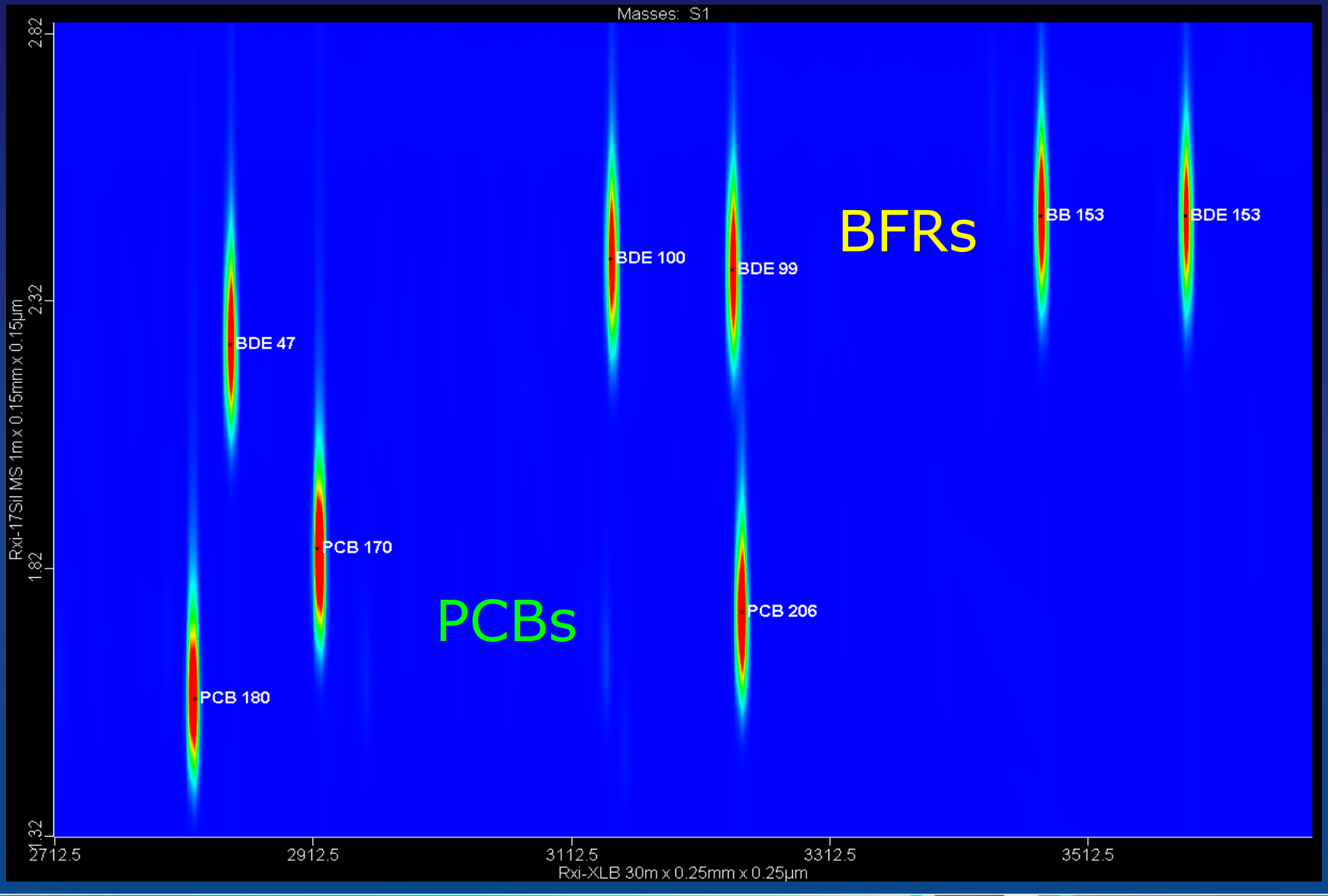
Fast detector to record 50-500 ms wide peaks

Orthogonal phases

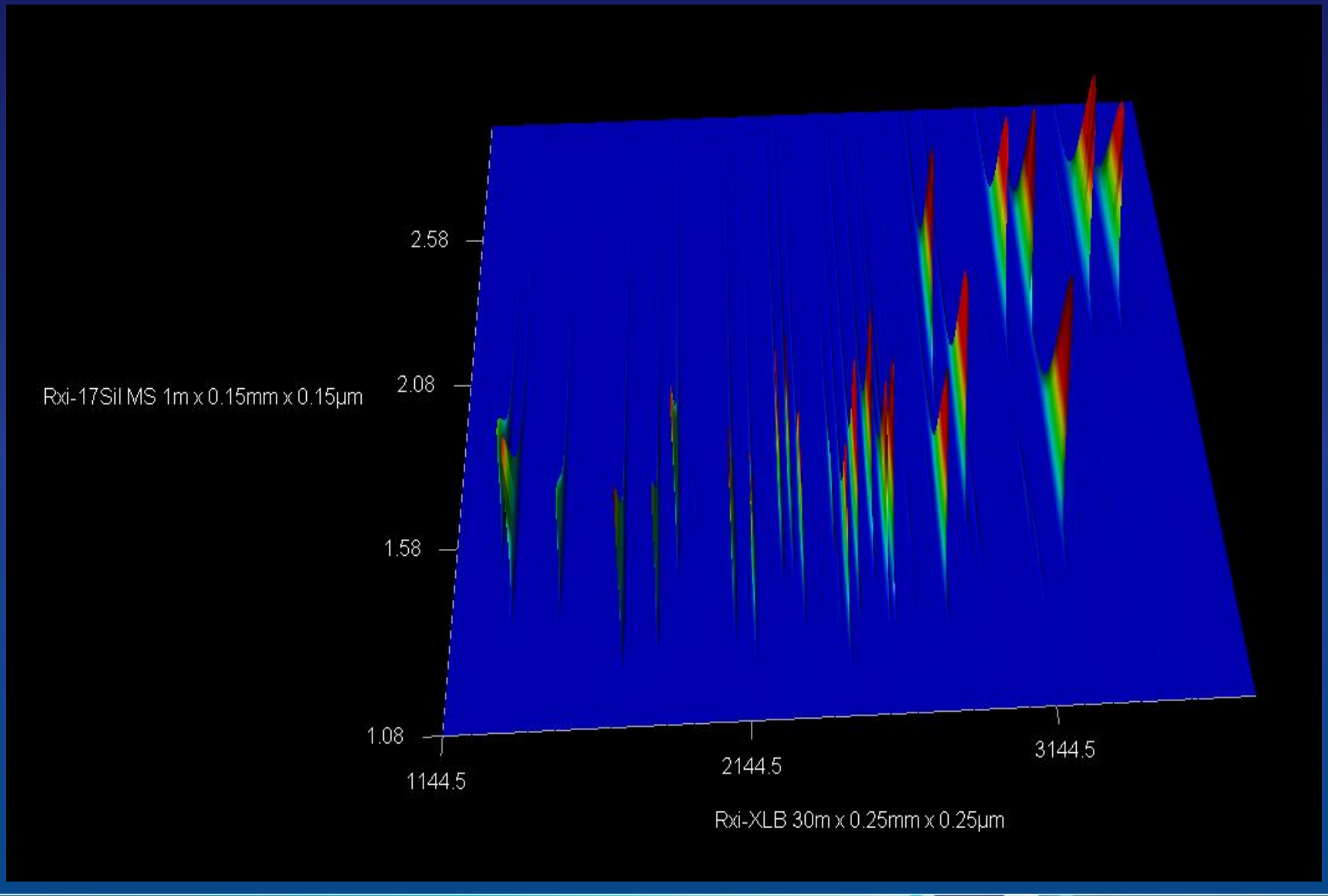
GC x GC Analysis



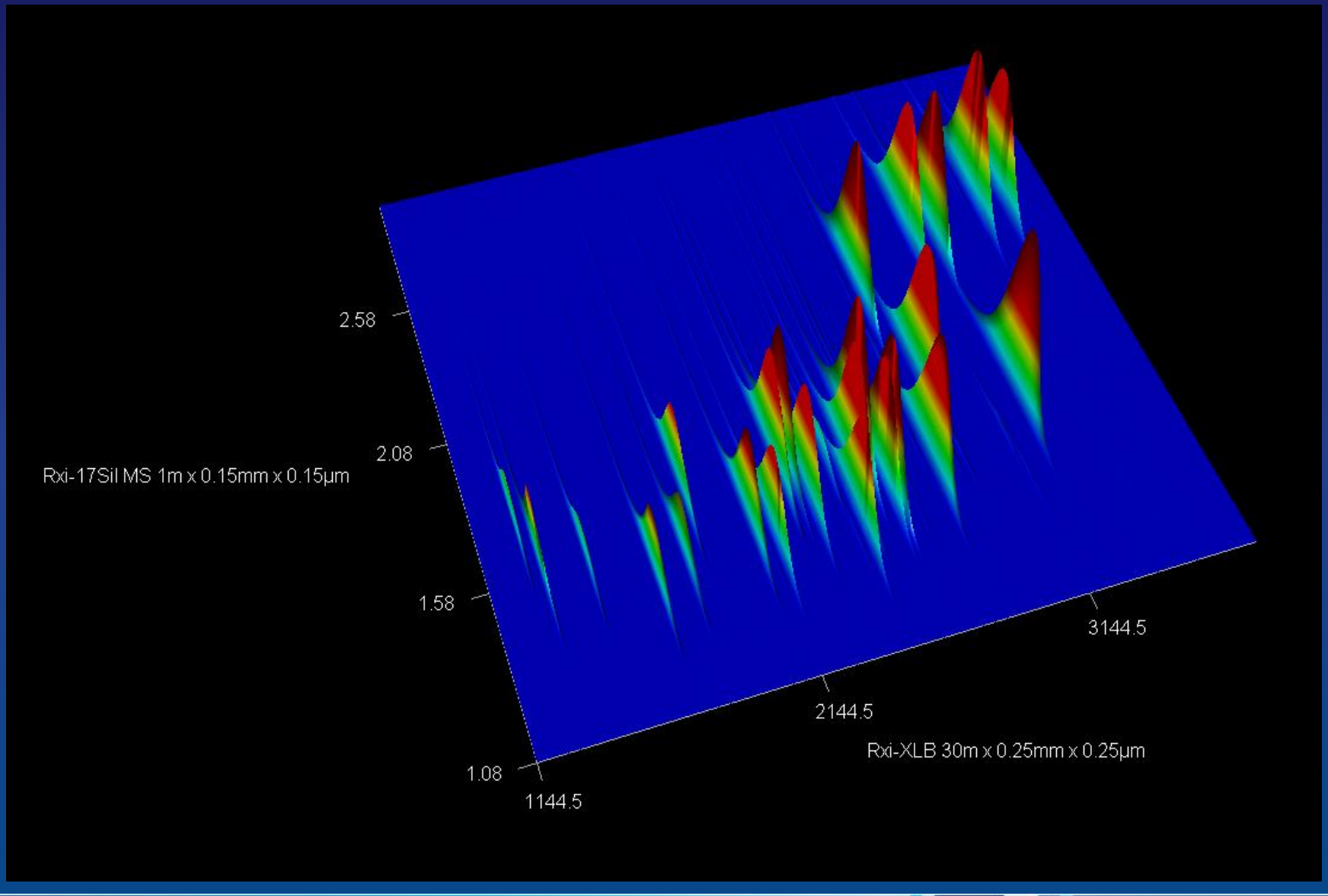
GC x GC Analysis



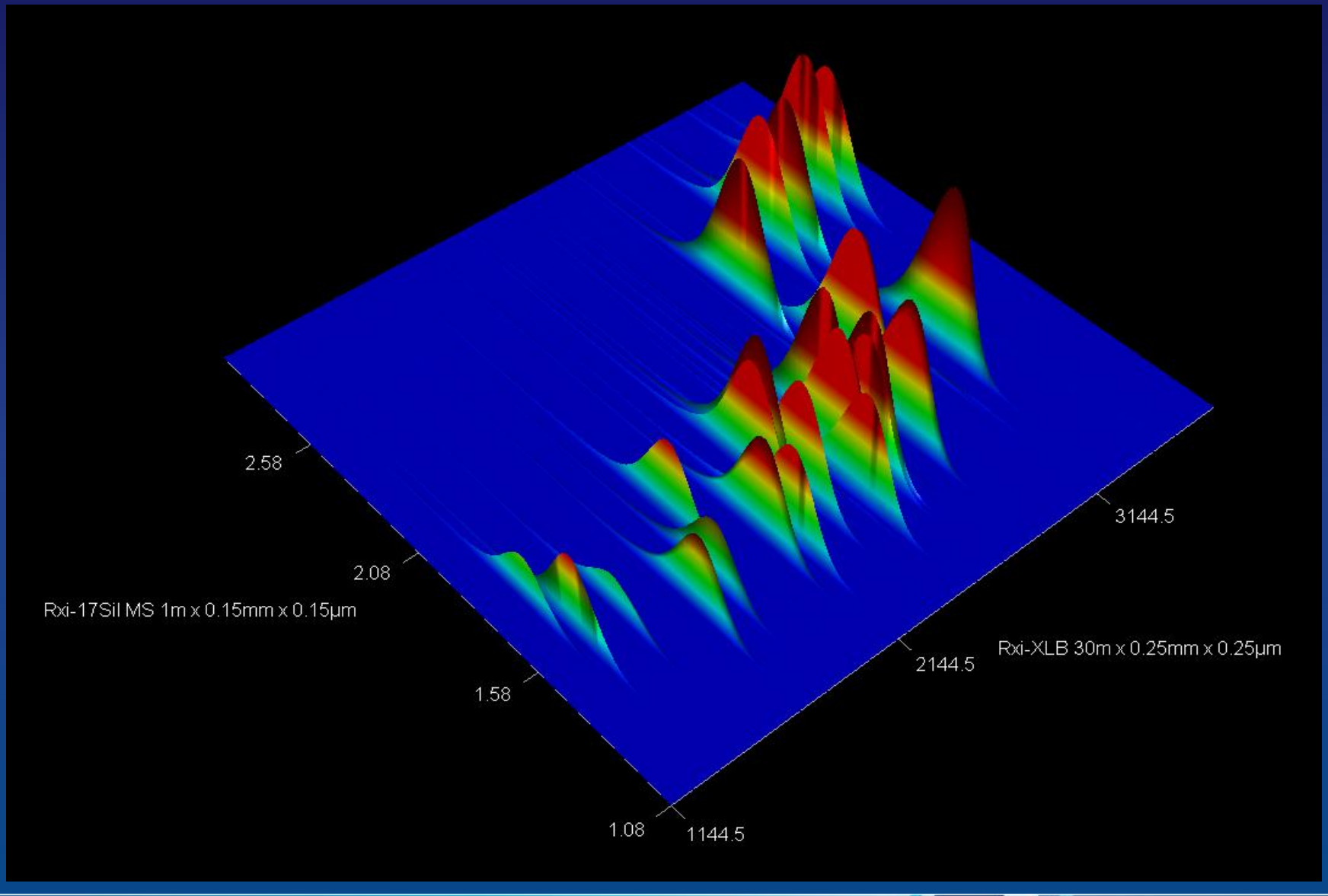
GC x GC Analysis



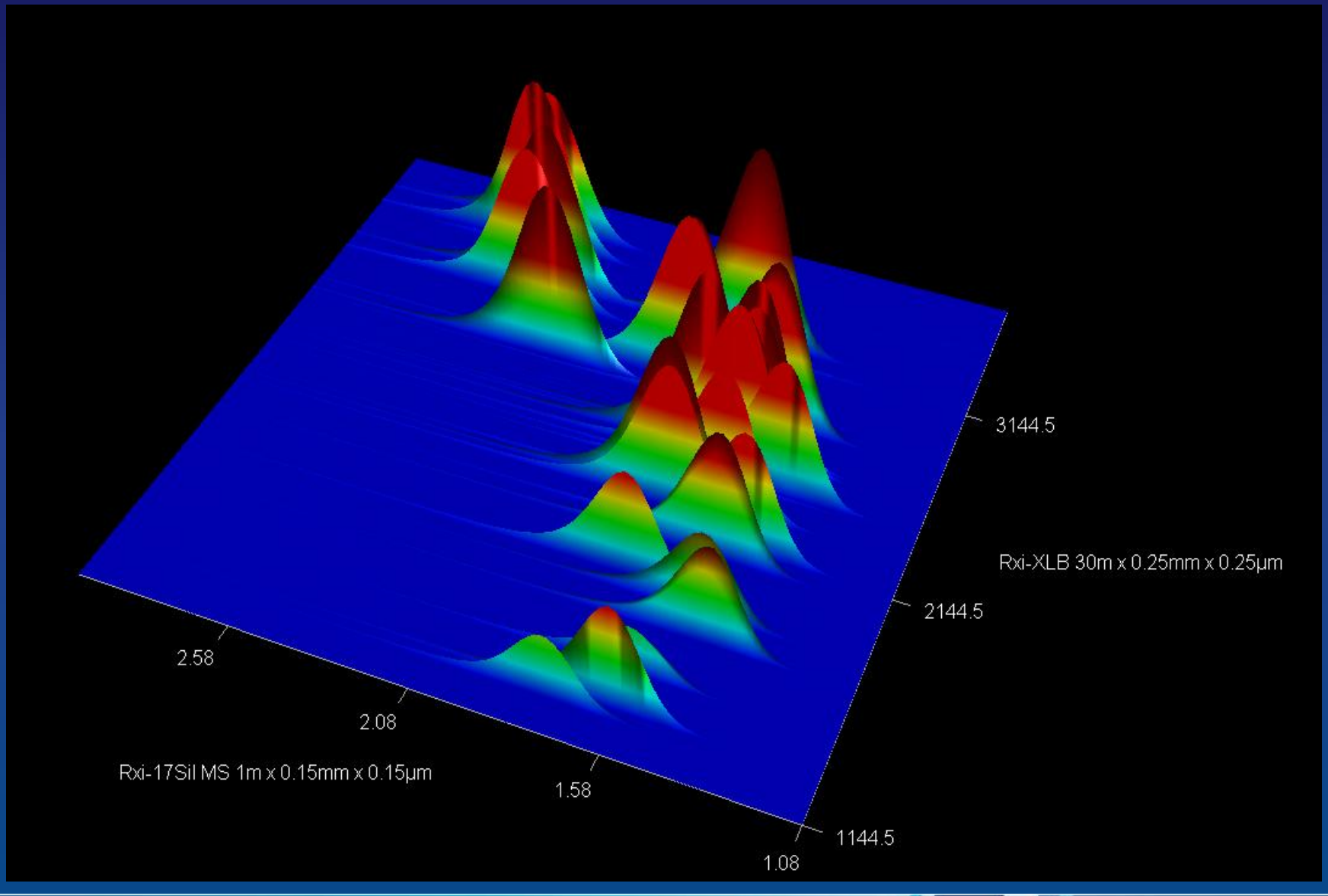
GC x GC Analysis



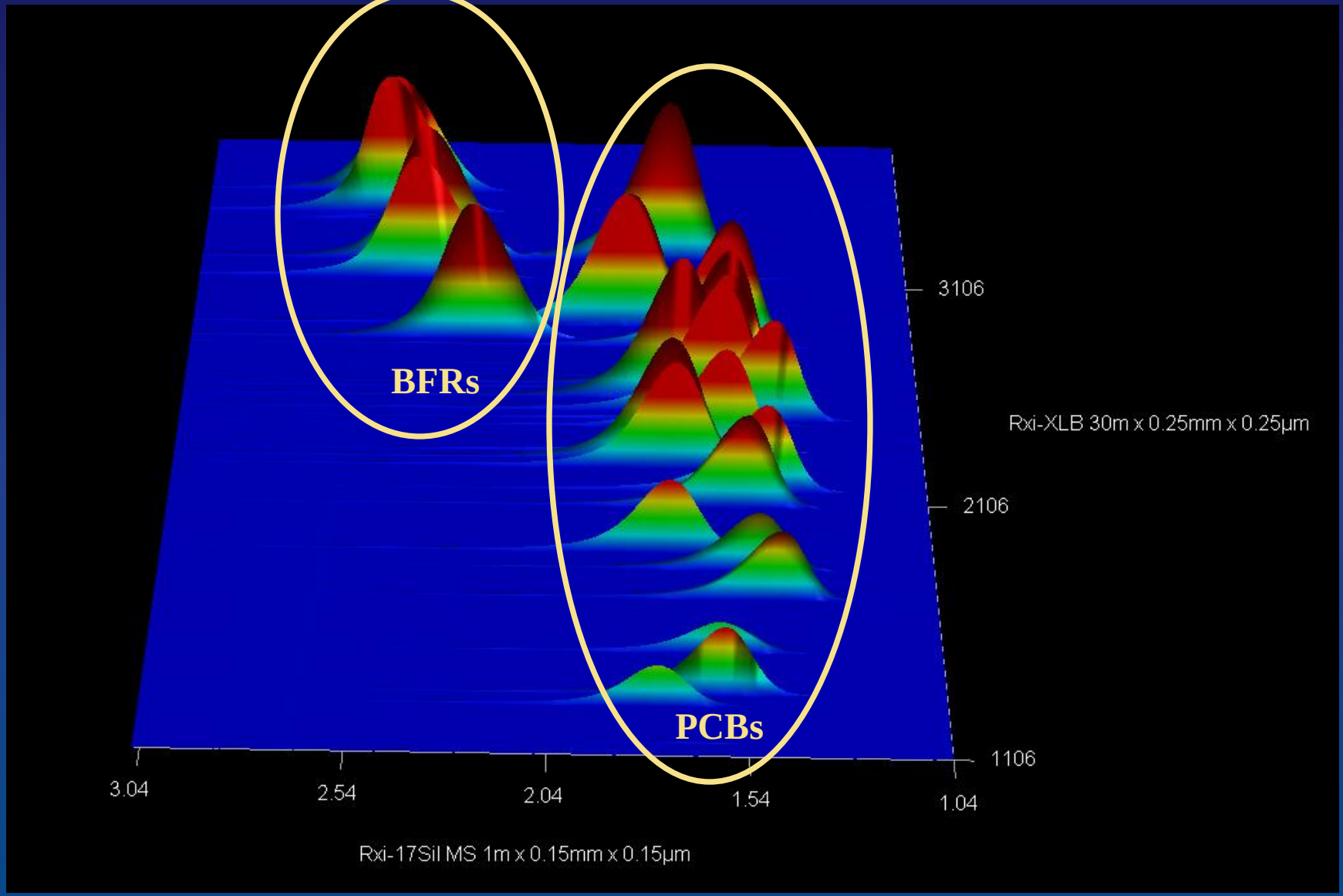
GC x GC Analysis



GC x GC Analysis



GC x GC Analysis



Cow Milk vs Human Milk

Contents	Human	Cow
Protein	1.2	3.3
Lactose	7.0	4.8
Fat	3.8	3.8
Ash (minerals)	0.21	0.71
Total solids	12.4	12.8

Table Written by Harold Eddleman, Ph. D., President, Indiana Biolab, 14045 Huff St., Palmyra IN 47164

Gravimetric Analysis
Non Volatile Extractables

NIST (Human Milk): 3.21%
QuEChERS (Cow Milk): 3.39%

Contents	Human	Cow
fatty acids 8C (%)	trace	6
polyunsaturated fatty acids (%)	14	3

Constituents of Human Milk United Nations University Centre http://unu.edu

Cow Milk - Percent Recoveries (n=3)

	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
PBB 153	107	175	38	86
BDE 153	119	180	32	82
Average	**122**	**129**	**126**	**99**
%RSD	**16**	**18**	**38**	**7**

Blanks

	LC/MS Water	
	ng/kg	pg on-column
PCB 52	3.88	0.97
PCB 99	3.28	0.82
PCB 110	5.2	1.3
PCB 118	4.52	1.13
PCB 153	2.32	0.58
PCB 105	1.48	0.37
BDE 47	67.4	16.9
BDE 100	4.48	1.12
BDE 99	29.4	7.34

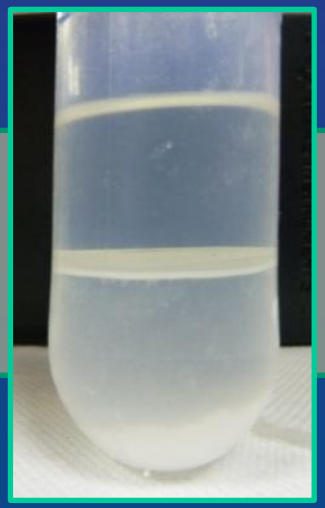
Fortified Human Milk – SRM 1954

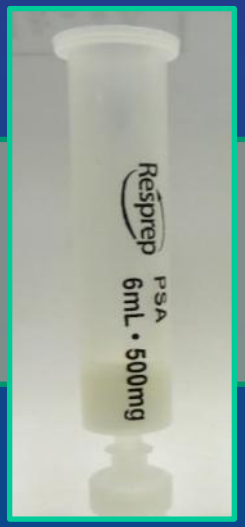
National Institute of Standards & Technology

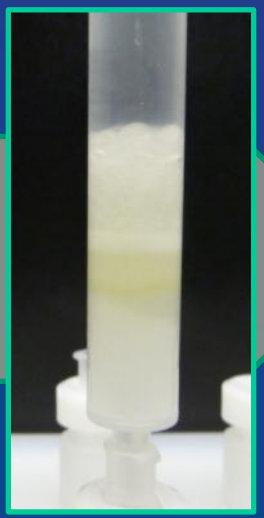
Certificate of Analysis

Standard Reference Material® 1954

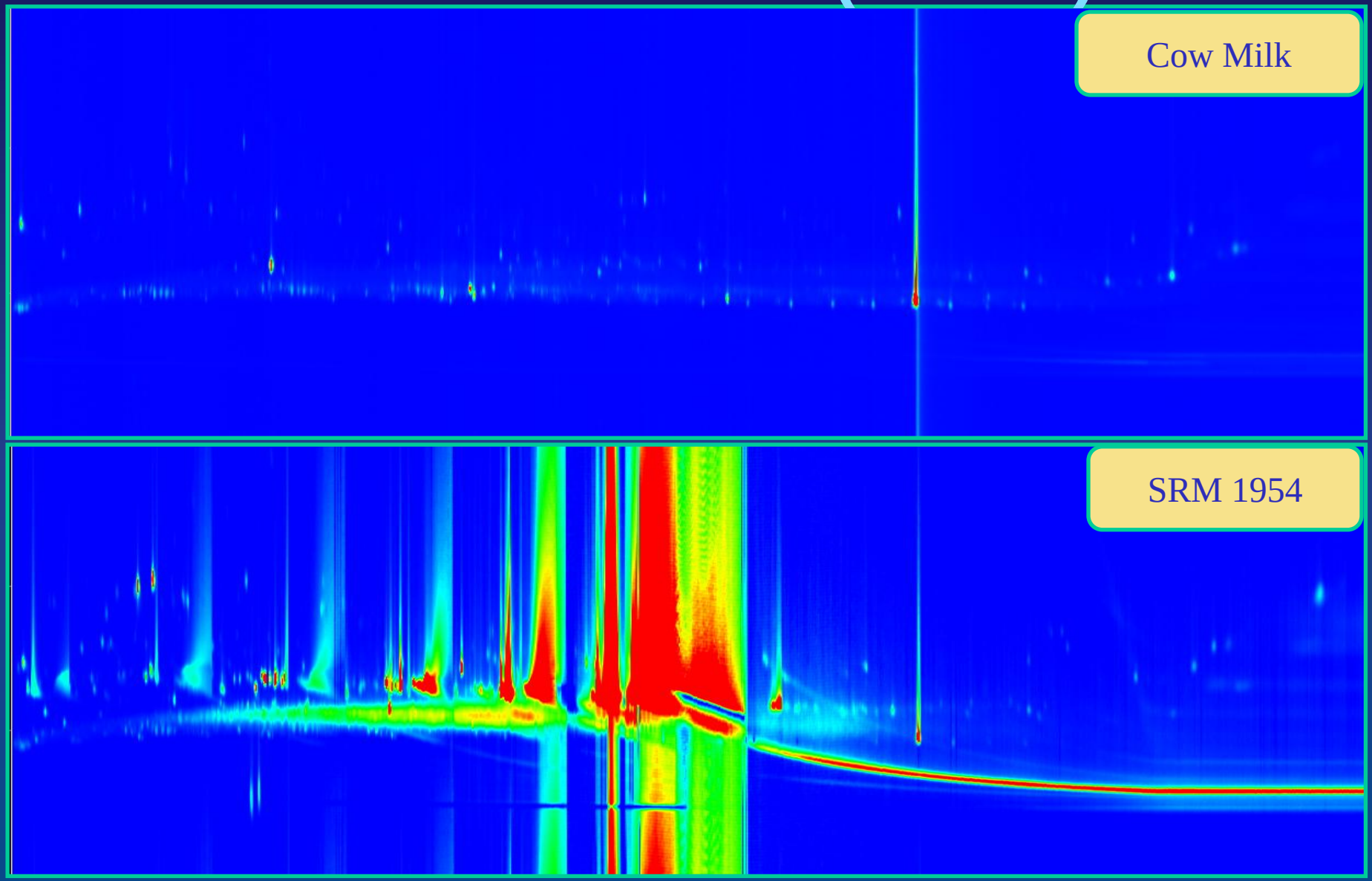
Organic Contaminants in Fortified Human Milk





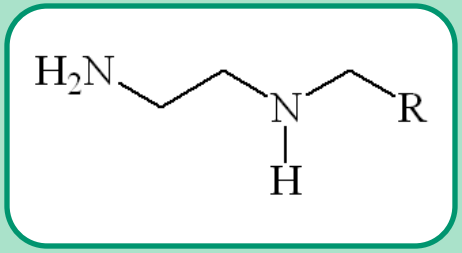


GC x GC TOFMS (m/z 67)



PSA Cartridge SPE Cleanup

PSA = Primary, Secondary Amine

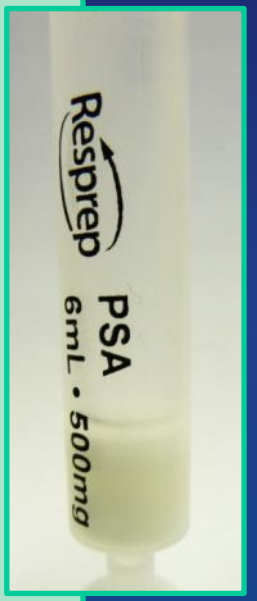


- ✓Fatty Acids
- ✓ Sugars
- ✓ Weak Anion Exchange

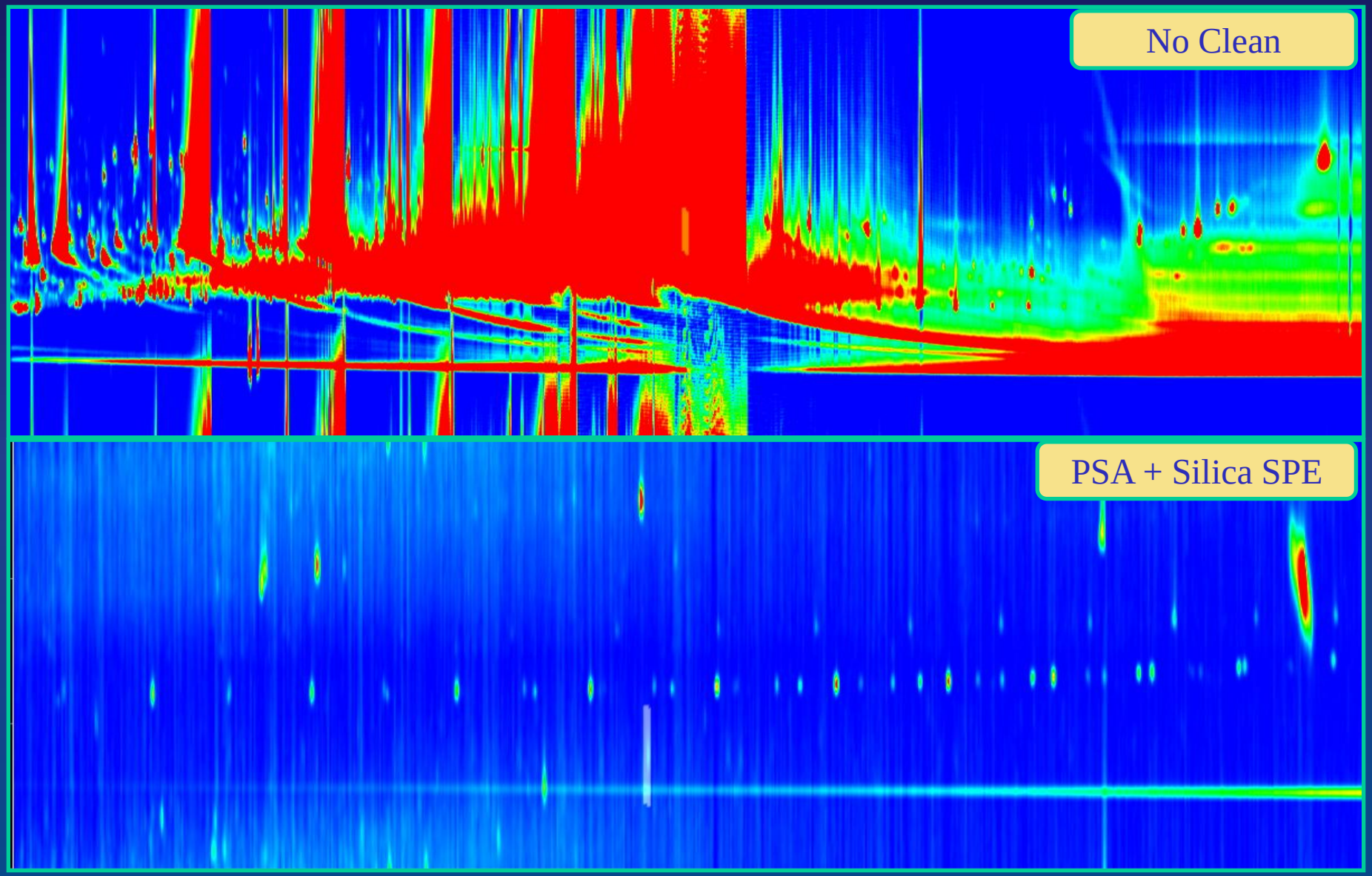
Condition PSA Cartridge
6mL/ 500mg PSA
with Acetone

Load 2.5 mL sample extract
Pass through cartridge

Elute with 5mL Hexane



GC x GC TOFMS of SRM 1954



	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**

National Institute of Standards & Technology

Certificate of Analysis

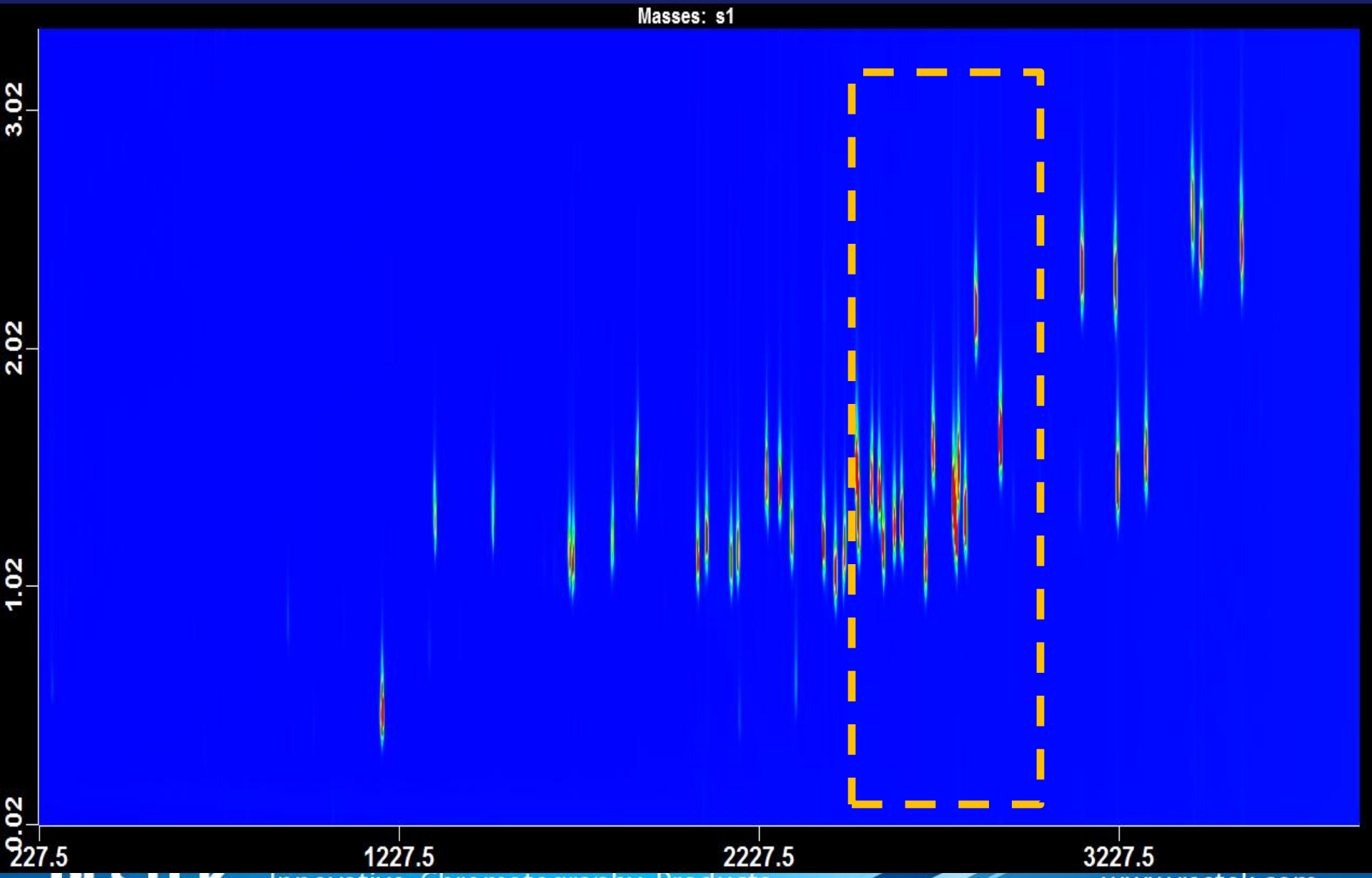
Standard Reference Material® 1953

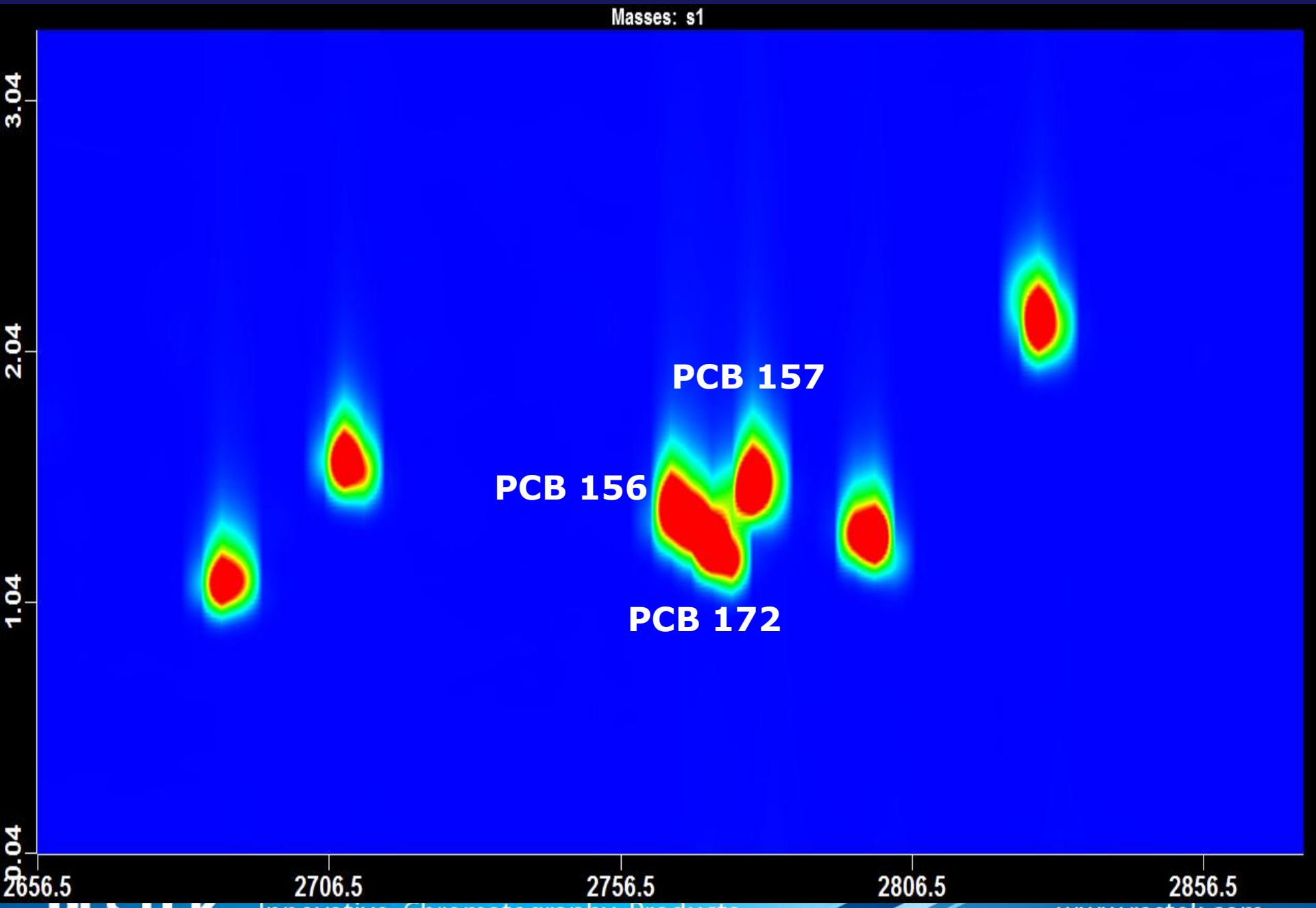
Organic Contaminants in Non-Fortified Human Milk

SRM 1953

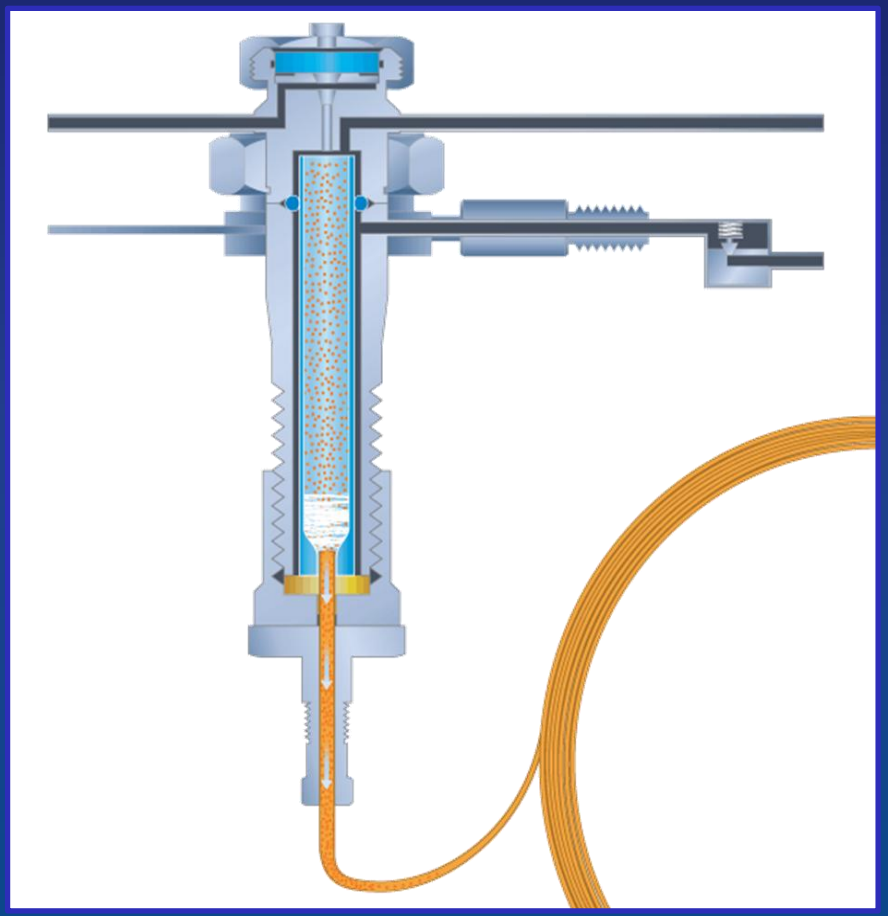
- ✓Non-fortified milk
- ✓Target concentration 10x less than fortified sample
  - Final concentration in low pg/µL amt

GC x GC ECD




Masses: s1
3.04
2.04
1.04
0.04
PCB 157
PCB 156
PCB 172
2656.5
2706.5
2756.5
2806.5
2856.5

Concurrent Solvent Recondensation Large Volume Splitless Injection

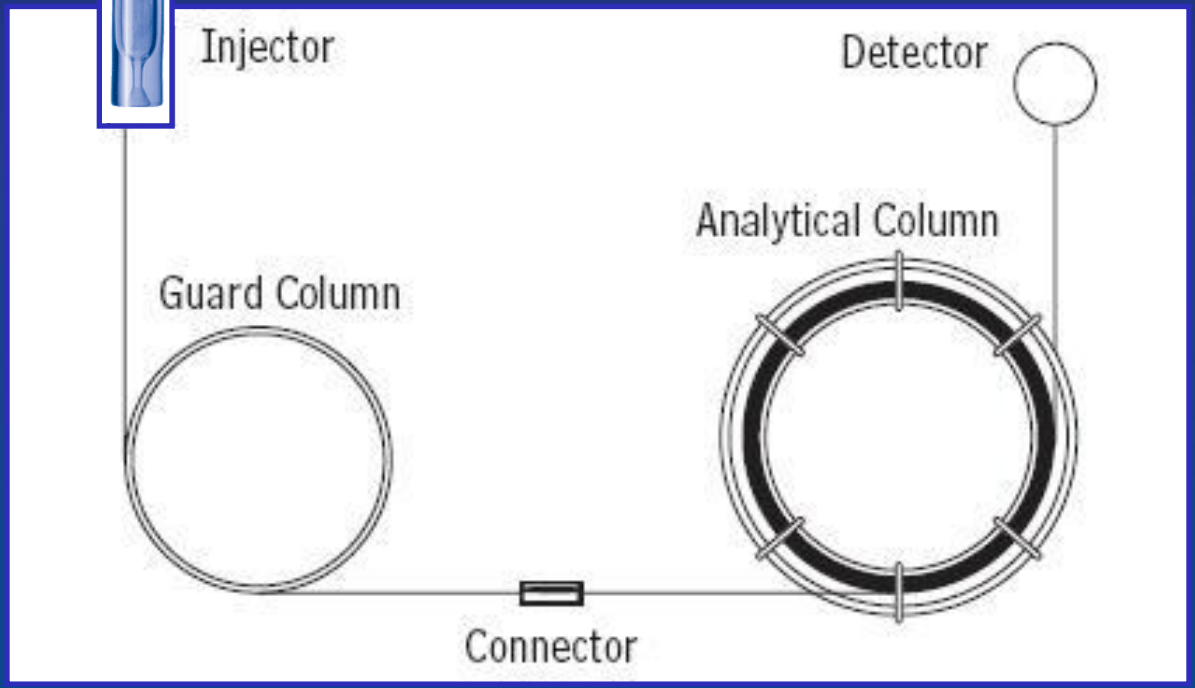


No modifications necessary to inject up to 30 µL on Agilent split/splitless injection port

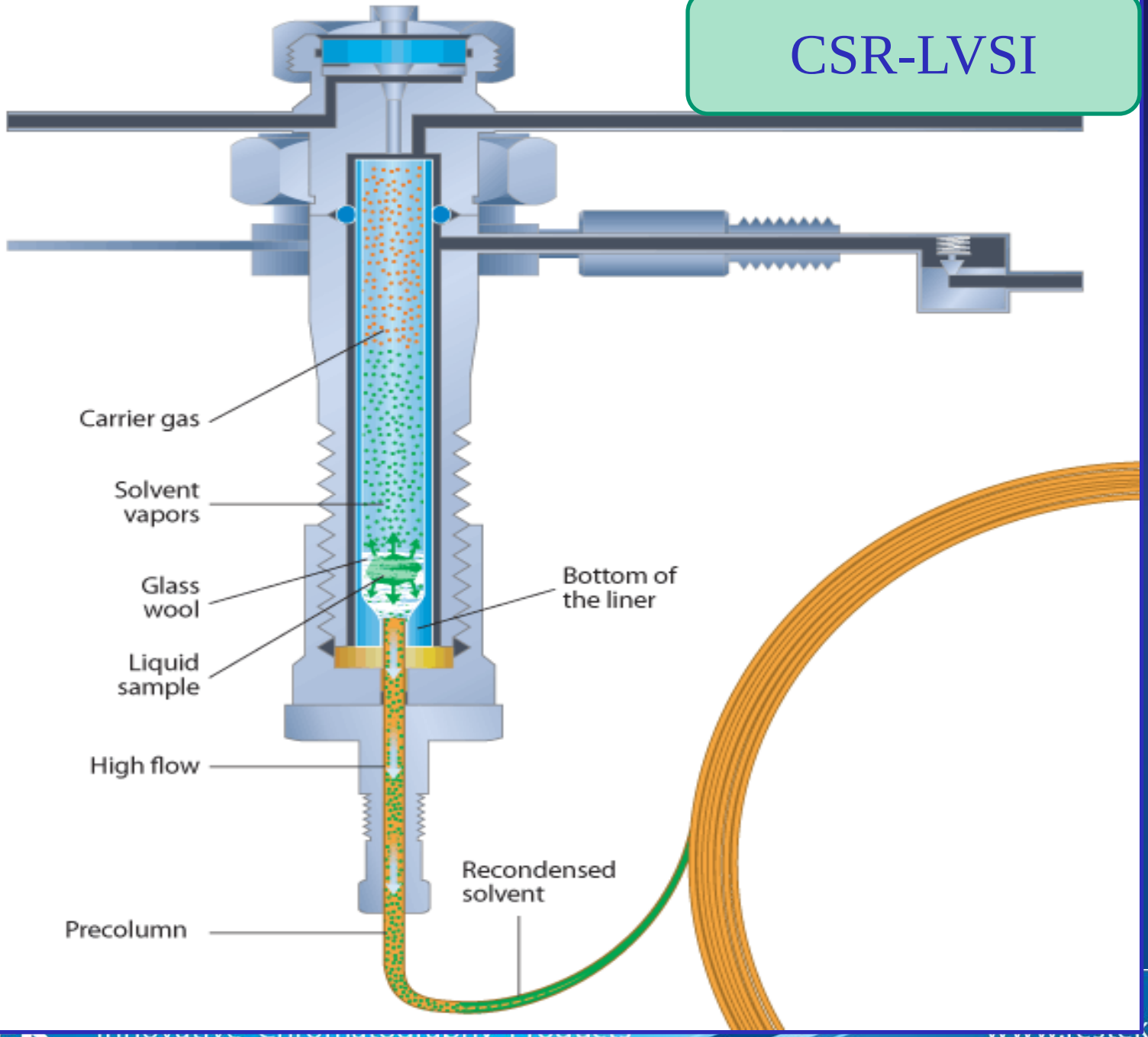
P. Magni, T. Porzano, Concurrent Solvent Recondensation Large Sample Volume Splitless Injection, J. Sep. Sci. 26 (2003) 1491.

Patent No: US 6,955,709 B2.

- ✓ Fast autosampler injection
- ✓ Single taper/gooseneck inlet liner with wool
- ✓ 5 meter guard column / retention gap
- ✓ Initial oven temperature below boiling point of solvent



CSR-LVSI


Carrier gas
Solvent vapors
Glass wool
Liquid sample
High flow
Precolumn
Bottom of the liner
Recondensed solvent

Instrument Conditions

GC X GC Conditions

Injection

Temp: 250°C
Injection: 10 µL fast, splitless (1.0 min)
Liner: Sky Single Taper w/ wool

Column

IP Deact Guard 5m x 0.53mmID
Rxi-XLB 30m x 0.25mmID x 0.25µm
Rxi-17Sil MS 1.0m x 0.25mm x 0.25µm

Conditions

Oven: 80°C (1 min) to 120°C @ 10°C/min to 300°C @ 3°C/min
Secondary Oven Offset: 5°C
Carrier: He, constant flow 1.4 mL/min
Modulator Temperature Offset: 20°C
Modulation Time: 3 sec

LECO Pegasus® TOFMS Conditions

Transfer Line Temperature: 300°C

Source Temperature: 225°C

Electron Ionization: 70 eV

Stored Mass Range: 45 to 750 u

Acquisition Rate: 50 spectra/sec

	QuEChERS (n=3) (ng/kg)	NIST Certified (ng/kg)	Percent Error
PCB 28	46.7	63.1	26
PCB 74	117	149	21
PCB 66	31.6	32.8	4
PCB 99	109	138	21
PCB 110	19.9	12.4	61
PCB 118	**198**	**213**	**7**
PCB 146	42.4	55.6	24
PCB 153	459	480	4
PCB 105	**47.8**	**45.7**	**5**
PCB 138	412	317	30
PCB 178	18.5	22.2	16
PCB 167	**15.4**	**15.8**	**3**
PCB 177	18.1	24.5	26
PCB 156	**51.9**	**59.7**	**13**
PCB 172	11.3	14.8	24
PCB 157	**14.5**	**14.6**	**0**
PCB 180	251	236	7
BDE 47	2700	2200	23
BDE 100	985	839	17
BDE 99	392	342	15
BDE 154	40.6	42.8	5
PBB 153	30.9	36.6	16
BDE 153	968	984	2

QuEChERS Extraction with PSA and Silica SPE Cleanup

- **Overview**

- 5 mL Milk + 5 mL Water
- 10 mL Hexane:Acetone (1:1)
- Unbuffered QuEChERS Extraction
- PSA cSPE pass through
- Silica cSPE cleanup
- GC x GC TOFMS analysis

Summary

- ✓ Good agreement with SRM certified values
  - Avg Error = 15%
- ✓ Low solvent usage
  - ~ 50 mL total
- ✓ Cost Effective

Conclusion

- The QuEChERS extraction approach using Hexane:Acetone is a viable approach for multi-analyte screening in human milk
- Using a combination of PSA then Silica cSPE provides the needed removal of fatty acid interferences in human milk
- Using GC x GC allows simultaneous determination of PCBs and BFRs
- Concurrent solvent recondensation – large volume splitless injection can greatly increase detectability without the need for a specialized injection port. However extract cleanup efficiency may need to be revisited to increase system robustness.

Thank You!