Environmental Chemistry Laboratory

Simultaneous Determination of PCBs, PBDEs, and Organochlorine Pesticides Using GC-Tandem Mass Spectrometry

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April 23, 2014

ECL Seminar

Background

- Three classes of compounds
  - Polychlorinated biphenyls (PCBs)
  - Polybrominated diphenyl ethers (PBDEs)
  - Organochlorine pesticides (OCPs)
- Persistent
  - Lipophilic
  - Bio-accumulative
- Health Effects
  - Endocrine disruptors
  - Reproductive effects

Outline

- POP Serum Analysis Method
- Method Development
  - SGE HT8-PCB
  - Agilent DB-5ms
  - Results from Studies
- Method Updates
- Future Work
- Conclusions

Steps for Serum Extraction

Sample Preparation:
Defrost, add surrogate spike, and denature with formic acid

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Automated Sample Extraction using Waters HLB Column

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Sample Evaporation to ~1mL

↓

Sample Clean-up with manually packed acid silica column

↓

Matrix change and sample concentration to ~80uL

↓

Internal Standard spike and transfer to GC vial for analysis

Analysis Methods

Biomonitoring CA

- Contemporary Samples
- Analysis on GC-HRMS
- Two GC columns
  - PCBs/OCPs (~ 1hr/sample)
  - PBDEs (~ ½ hr/sample)
- Total time:
  ~ 1.5 hrs/sample

Federally Funded Projects

- Archived Samples (1960s) and Contemporary Samples
- Analysis on GC-ECD
- Dual Column Method
  - Run concurrently
  - PCBs/OCPs
- Total time:
  ~ 2.5 hrs/sample
- Upgraded to GC-tandem MS

Compounds of Interest

Measured on GC-ECD (two columns) or GC-HRMS (one column)		Measured on GC-HRMS (one column)
Organochlorine Pesticides (OCPs)	**Polychlorinated Biphenyls (PCBs)**	**Polybrominated Diphenyl Ethers (PBDEs)**
β-BHC	PCB66	BDE17
HCB	PCB74	BDE28
Oxychlordane	PCB99	BDE47
Trans-nonachlor	PCB101	BDE66
p,p'-DDE	PCB105	BDE85
o,p'-DDT	PCB118	BDE99
p,p'-DDT	PCB138	BDE100
	PCB153	BDE153
	PCB156	BDE154
	PCB170	BDE183
	PCB180	BDE196
	PCB183	BDE197
	PCB187	BDE201
	PCB194	BDE202
	PCB203	BDE203
		BDE206
		BDE207
		BDE208
		BDE209

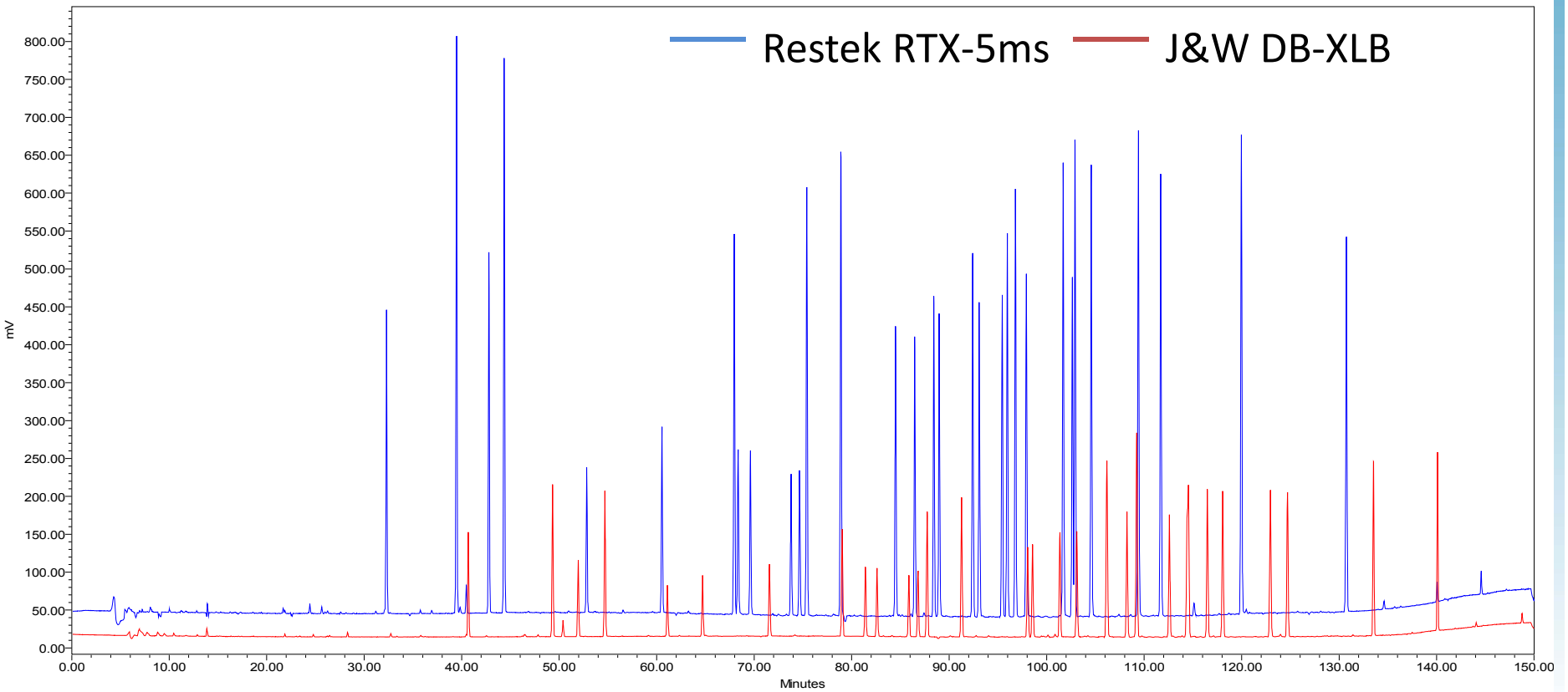
Method Development

- New Method
  - Transfer method from GC-ECD to GC-triple quadrupole mass spectrometer (GC-QqQ)

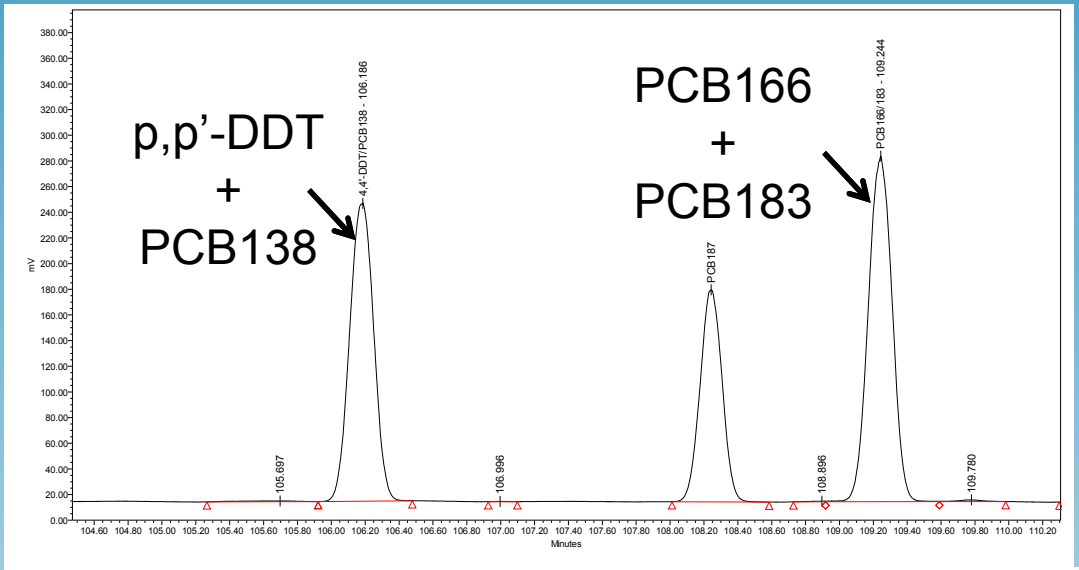
- Goals for Method Transfer:
  - All analytes from GC-ECD (PCBs/OCPs)
  - Shorter analysis time than GC-ECD
  - Additional analytes
    - PCB28, PCB167
    - “major” PBDEs

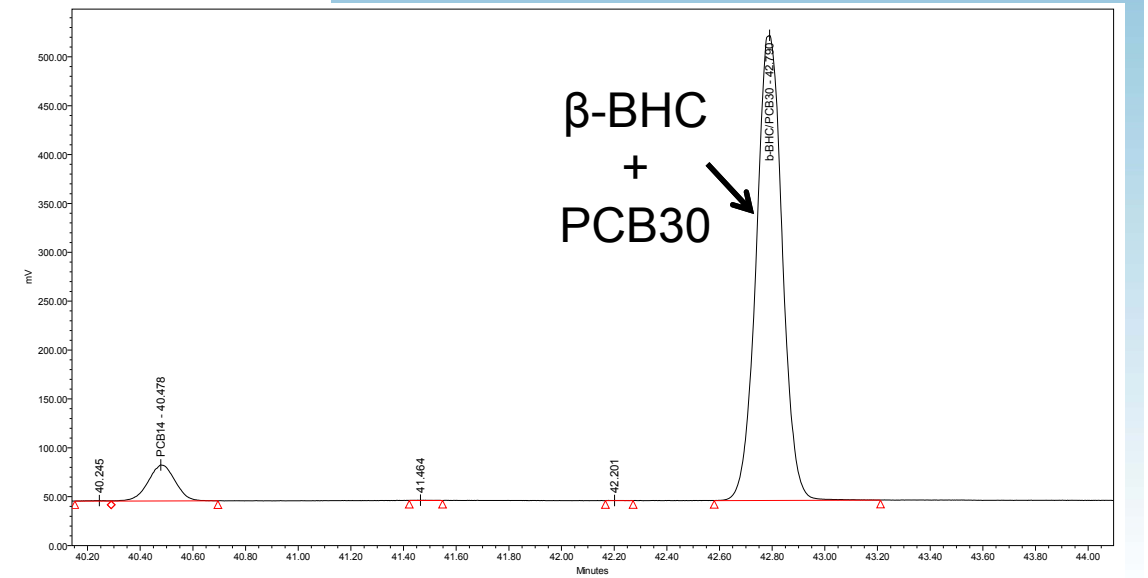
GC-ECD method

- External surrogates
  - TCMX, PCB14, PCB65, PCB166



GC-ECD method





GC-QqQ

Agilent 7890A GC

- Injector
  - Pulsed Splitless
  - 250°C
- Column
  - Flow Rate: 1mL/min
  - Carrier Gas: Helium

Agilent 7000B Series

- Source
  - Electron Impact Extractor (EIEX)
  - 70eV
  - 275°C
- Collision Cell
  - Nitrogen (1.5mL/min)
  - Helium (2.25mL/min)
- Q1 and Q3
  - 150°C
  - MS1 resolution: 1.2amu
  - MS2 resolution: 1.2amu
- Detector:
  - Time filter peak width: 0.7s

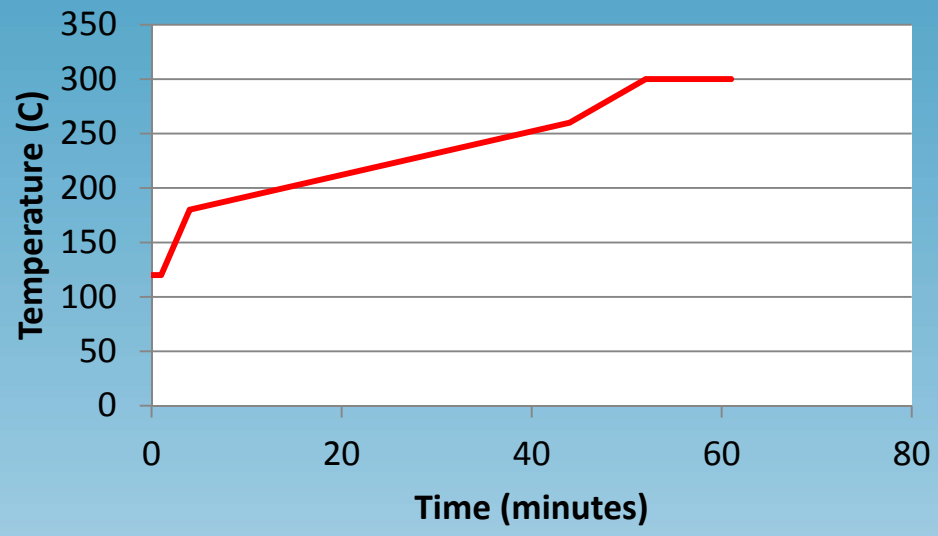
SGE HT8-PCB

Column Length: 60m

Column I.D.: 0.25mm

Transfer Line: 300°C

Run Length: ~ 61 min



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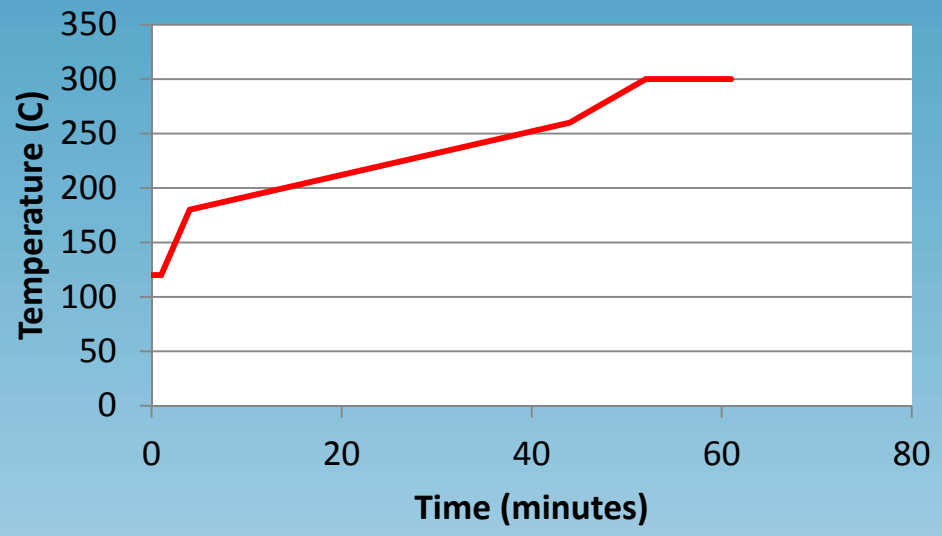

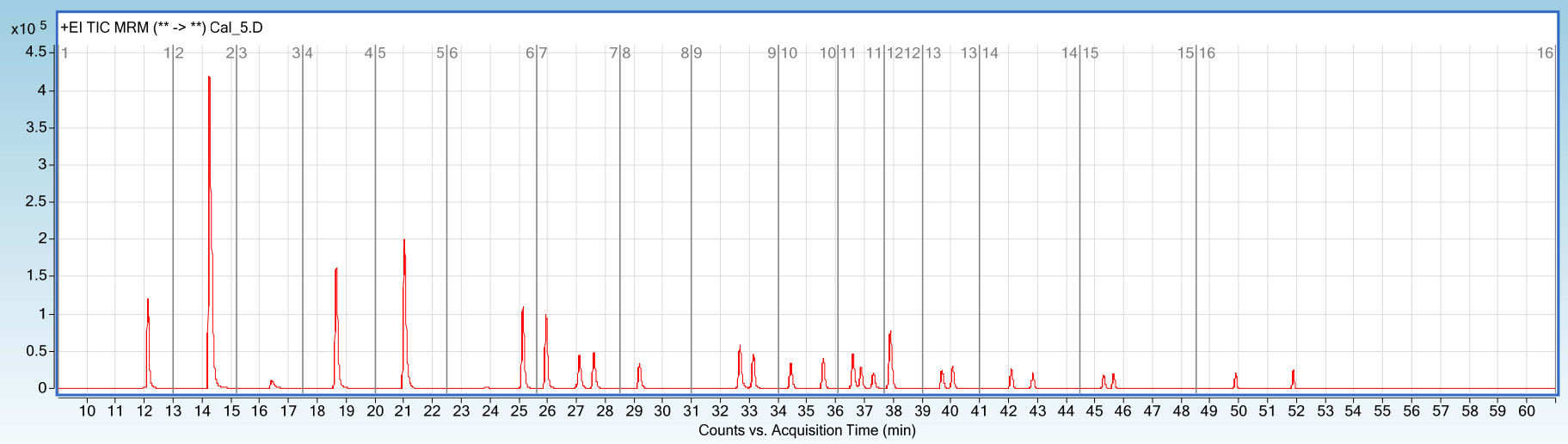

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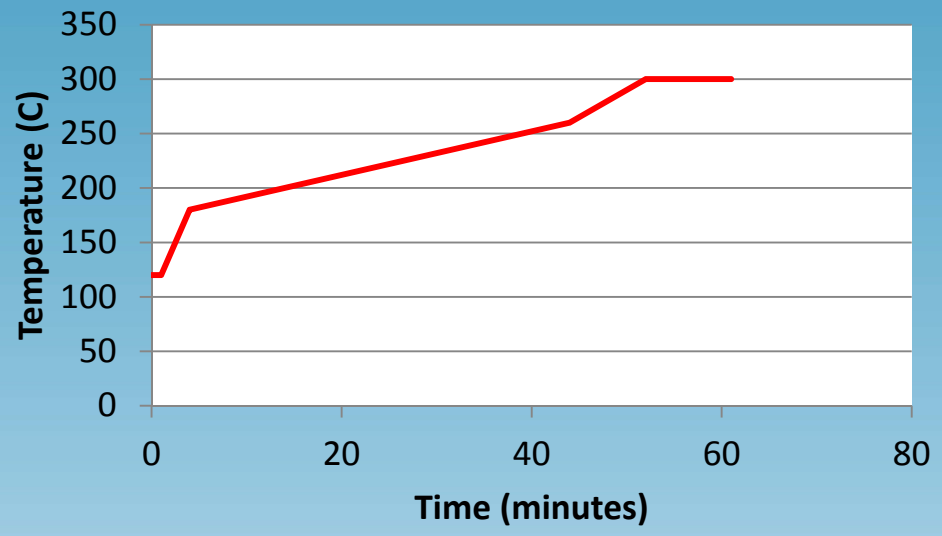
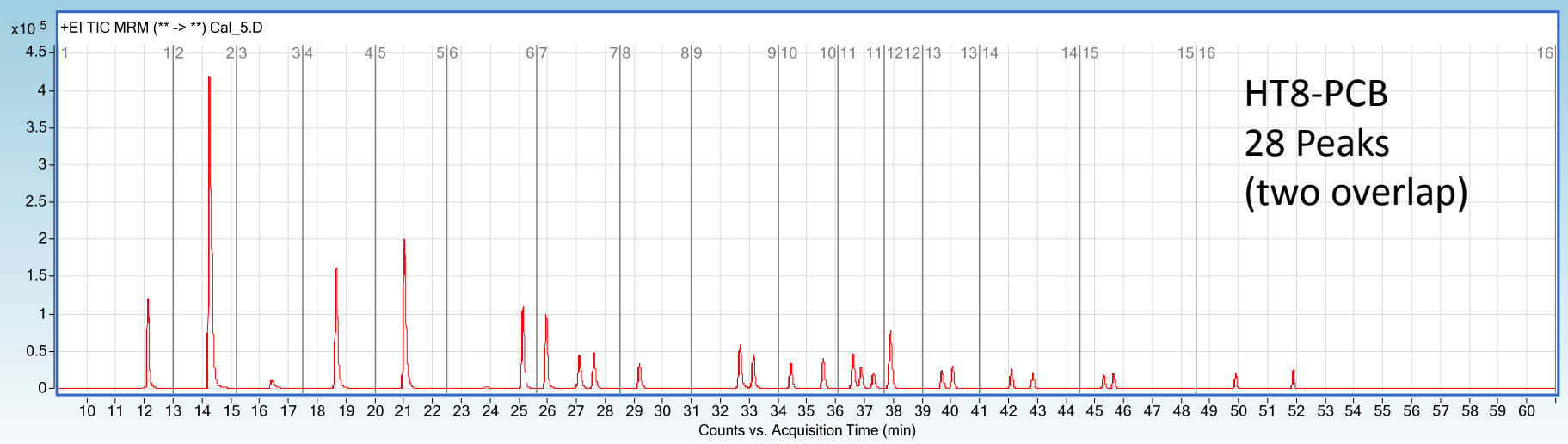




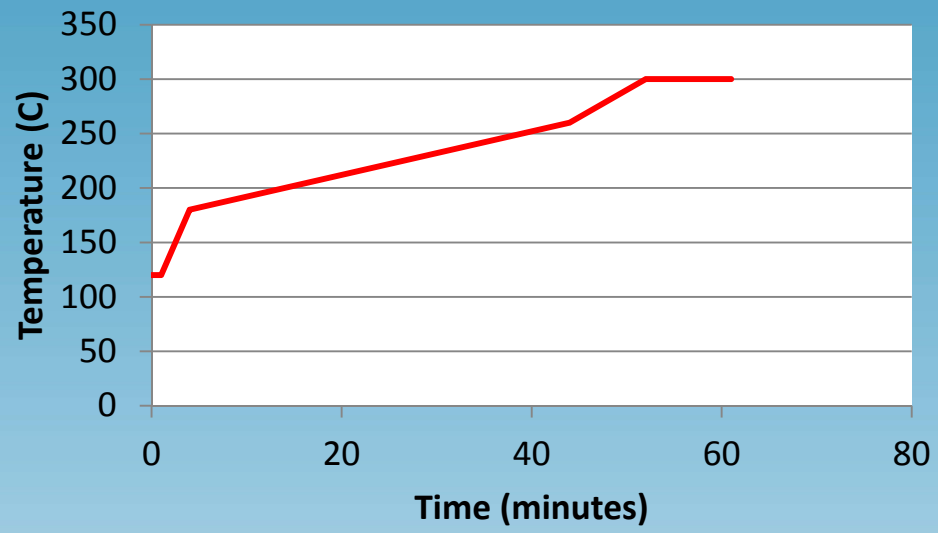
SGE HT8-PCB

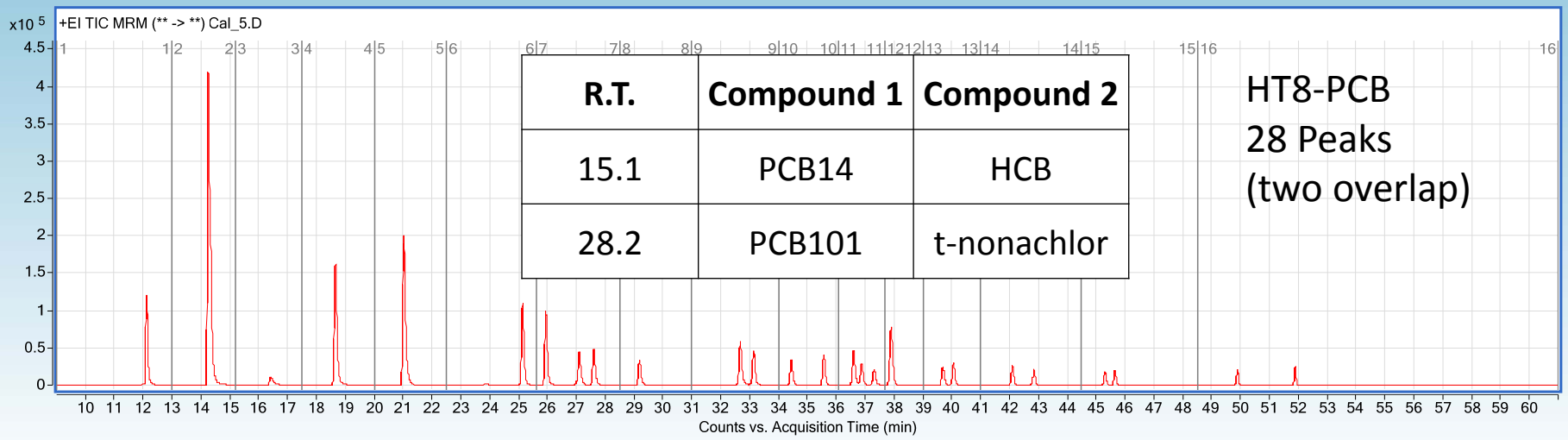
Column Length: 60m

Column I.D.: 0.25mm

Transfer Line: 300°C

Run Length: ~ 61 min



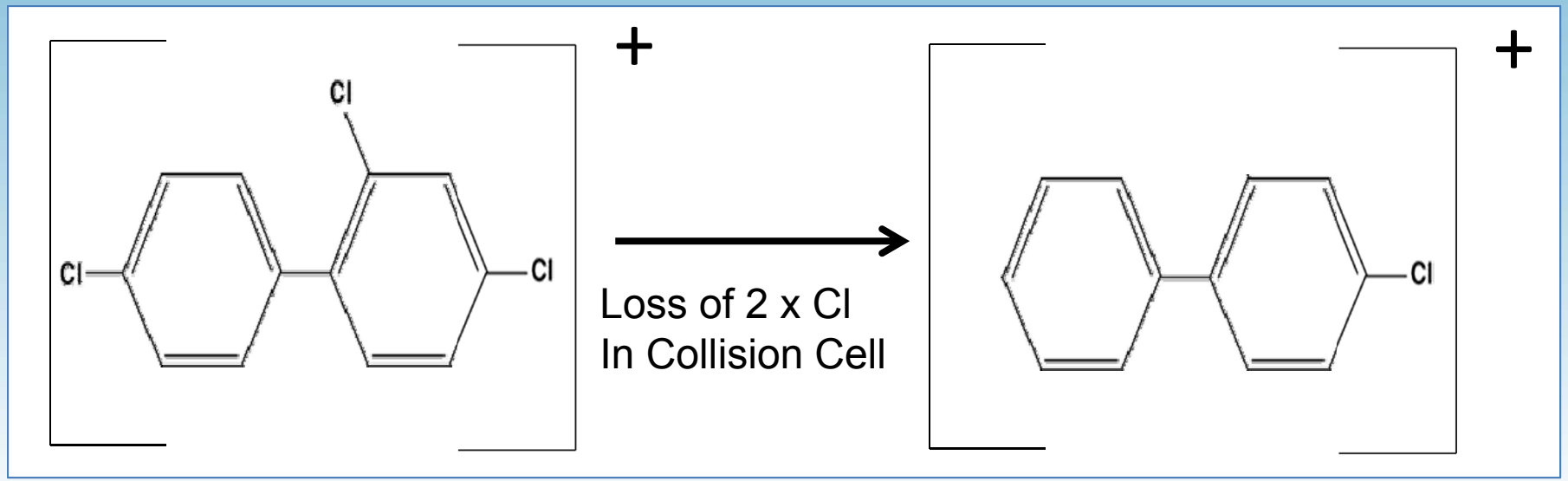


R.T.	Compound 1	Compound 2
15.1	PCB14	HCB
28.2	PCB101	t-nonachlor

HT8-PCB
28 Peaks
(two overlap)

MRM Transitions

- PCBs
  - Precursor Ion: [M+]
  - Product Ion: [M – 2Cl]+
  - Qualifier uses ^{37}Cl loss



MRM Transitions

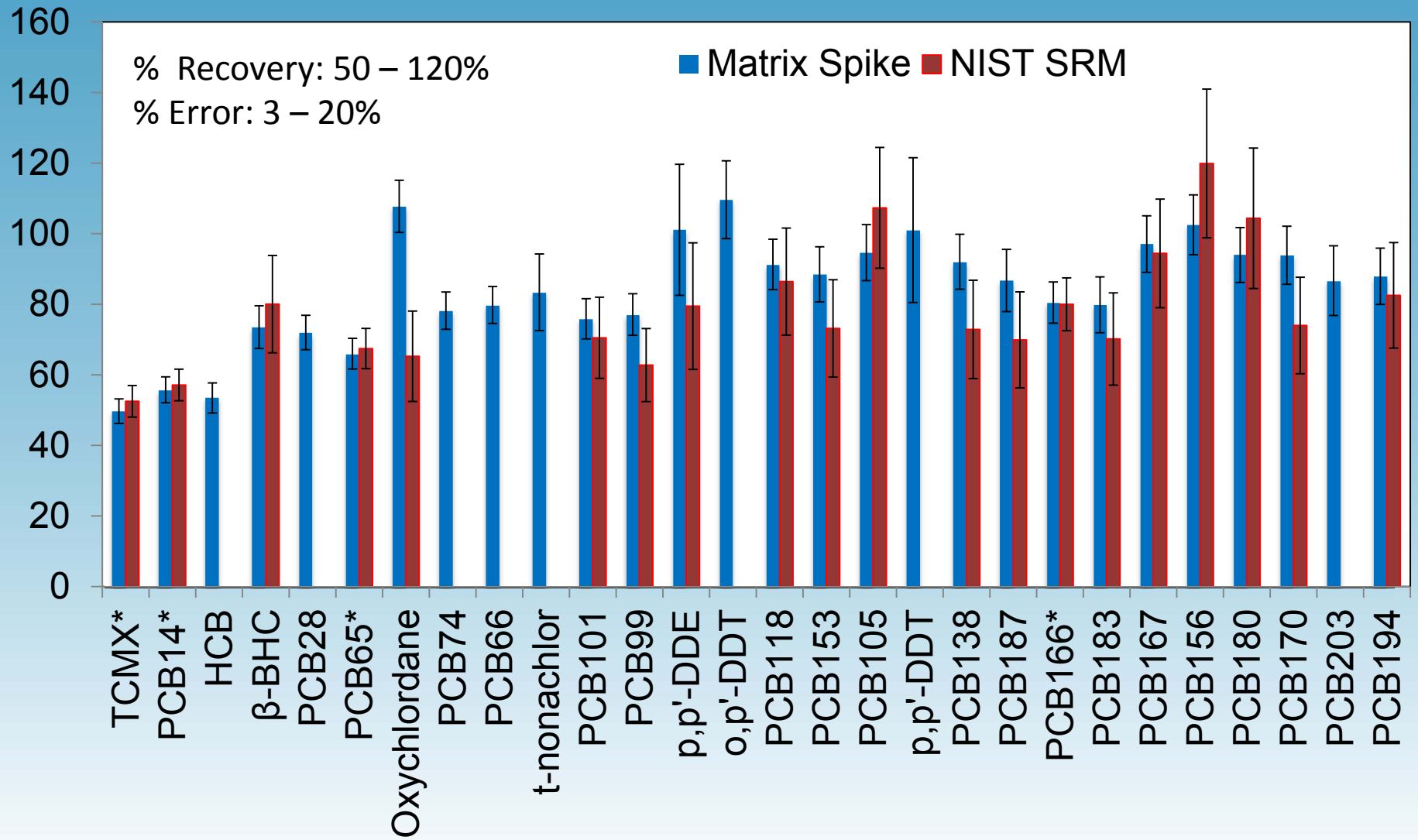
- OCPs
  - Depends on compound

	Precursor	Product	Loss of
TCMX	M+	[M - 1Cl]+	1 x Cl
HCB	M+	[M - 2Cl]+	2 x Cl
p,p'-DDE	M+	[M - 2Cl]+	2 x Cl
t-nonachlor	[M - 1Cl]+	[M - 3Cl]+	2 x Cl
β-BHC	[M - 3Cl]+	[M - 4Cl]+	1 x Cl
DDT (o,p' and p,p')	[M - CCl_3]+	[M - CCl_3 -2Cl]+	2 x Cl
Oxychlordane			

HT8-PCB: Pedigree Study

- Breast Cancer Study
  - Samples from 1960's
  - N = 195
- Limits of Detection
  - 0.005 - 0.02 ng/mL for PCBs
  - 0.05 - 0.15 ng/mL for OCPs
- Linearity Ranges
  - 0.01 – 3 ng/mL for PCBs
  - 0.05 – 80 ng/mL for OCPs

Pedigree Study QC: % Recoveries



* External Surrogate

Detection Frequencies for BDEs

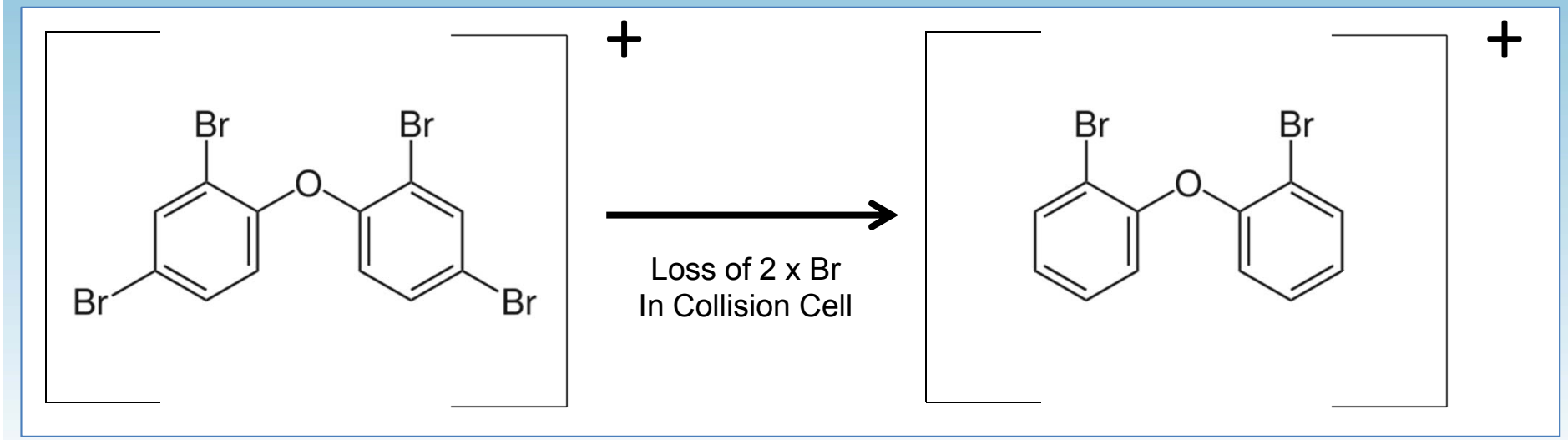
	BEST	MIEEP (M)	MIEEP (CB)	Zota	FA Study	FOX Study
	N = 110	N = 77	N = 63	N = 38	N = 25	N = 101
BDE17	2%	0%	3%	0%	0%	1%
BDE28	92%	36%	13%	32%	84%	97%
BDE47	99%	92%	67%	74%	84%	99%
BDE66	9%	4%	0%	0%	0%	6%
BDE85	20%	6%	3%	5%	16%	35%
BDE99	81%	61%	25%	63%	92%	90%
BDE100	89%	90%	56%	74%	100%	98%
BDE153	99%	90%	37%	68%	100%	100%
BDE154	17%	9%	0%	0%	12%	20%
BDE183	4%	4%	2%	3%	8%	5%
BDE196	3%	3%	0%	0%	0%	2%
BDE197	43%	36%	0%	3%	60%	62%
BDE201	5%	3%	0%	3%	0%	5%
BDE202	1%	0%	0%	0%	0%	1%
BDE203	1%	1%	0%	0%	0%	1%
BDE206	5%	4%	3%	0%	0%	12%
BDE207	18%	23%	3%	0%	0%	50%
BDE208	3%	9%	2%	0%	0%	8%
BDE209	38%	53%	17%	0%	36%	29%

“Major” BDEs

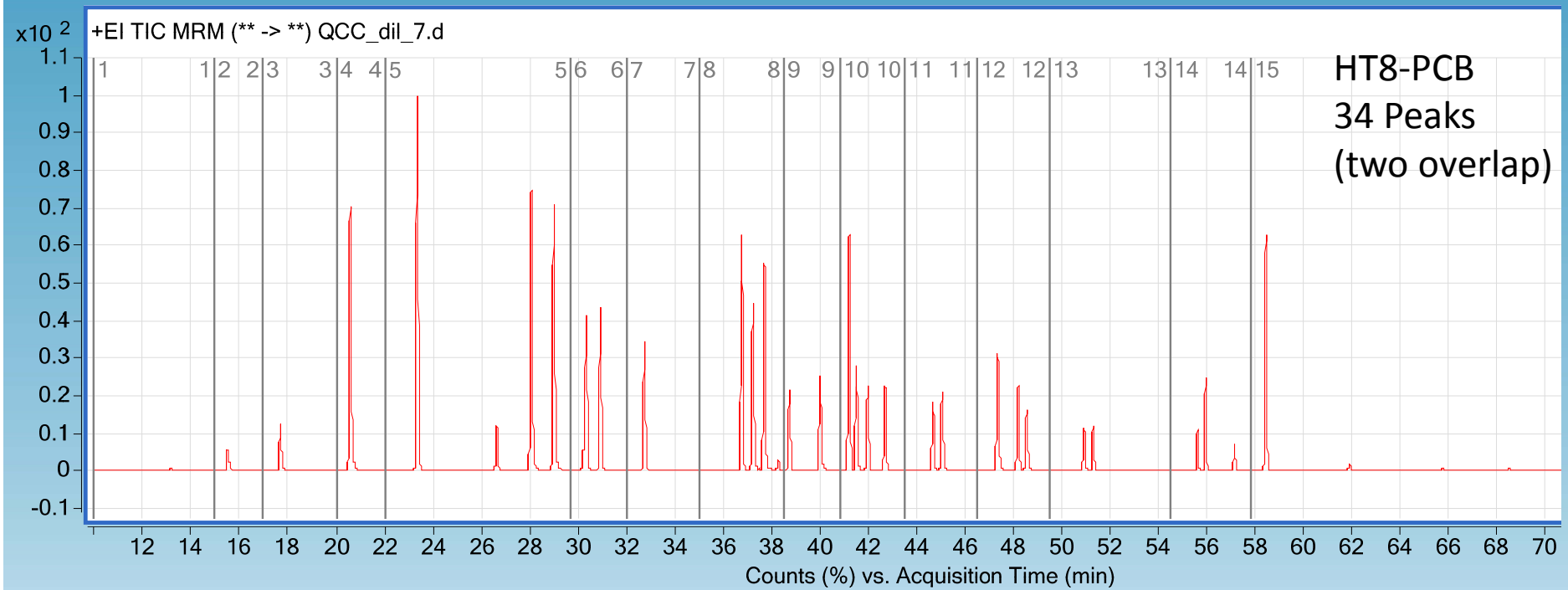
	BEST	MIEEP (M)	MIEEP (CB)	Zota	FA Study	FOX Study
	N = 110	N = 77	N = 63	N = 38	N = 25	N = 101
BDE17	2%	0%	3%	0%	0%	1%
BDE28	92%	36%	13%	32%	84%	97%
BDE47	99%	92%	67%	74%	84%	99%
BDE66	9%	4%	0%	0%	0%	6%
BDE85	20%	6%	3%	5%	16%	35%
BDE99	81%	61%	25%	63%	92%	90%
BDE100	89%	90%	56%	74%	100%	98%
BDE153	99%	90%	37%	68%	100%	100%
BDE154	17%	9%	0%	0%	12%	20%
BDE183	4%	4%	2%	3%	8%	5%
BDE196	3%	3%	0%	0%	0%	2%
BDE197	43%	36%	0%	3%	60%	62%
BDE201	5%	3%	0%	3%	0%	5%
BDE202	1%	0%	0%	0%	0%	1%
BDE203	1%	1%	0%	0%	0%	1%
BDE206	5%	4%	3%	0%	0%	12%
BDE207	18%	23%	3%	0%	0%	50%
BDE208	3%	9%	2%	0%	0%	8%
BDE209	38%	53%	17%	0%	36%	29%

MRM Transitions

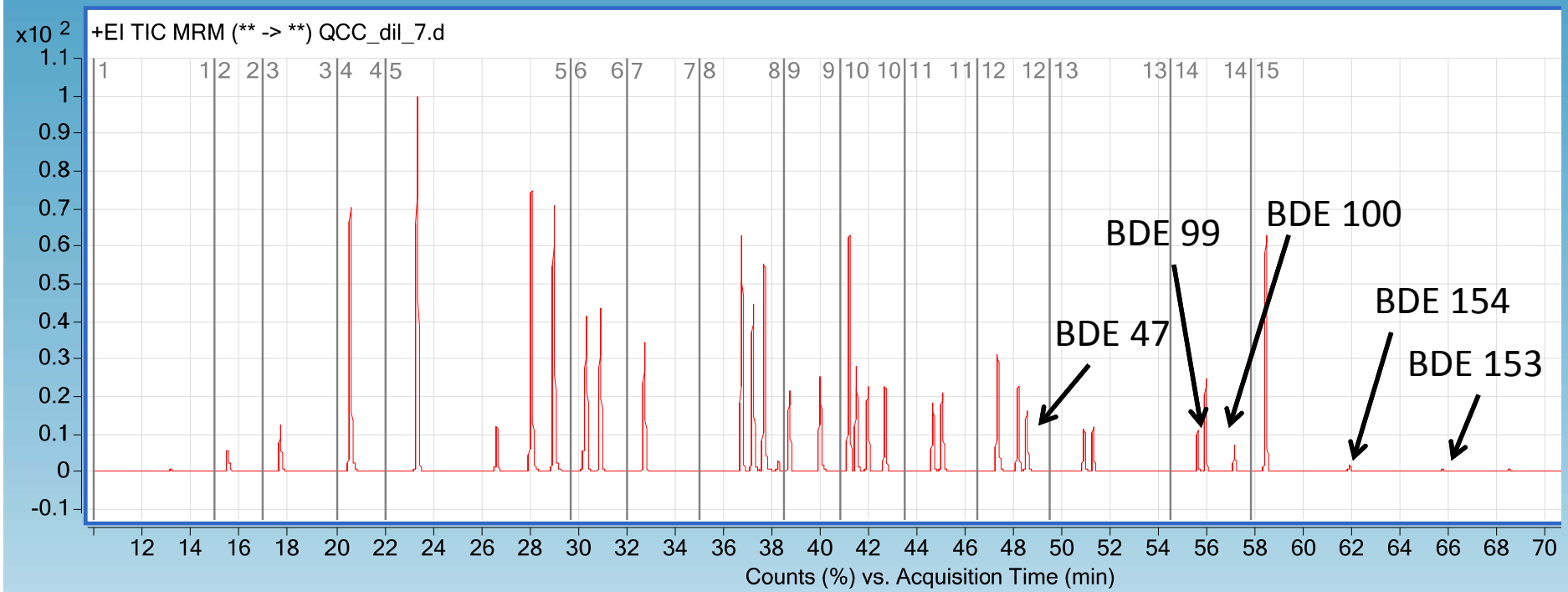
- PBDEs
  - Precursor Ion: [M+]
  - Product Ion: [M+] – 2Br
  - Qualifier uses ^{81}Br loss



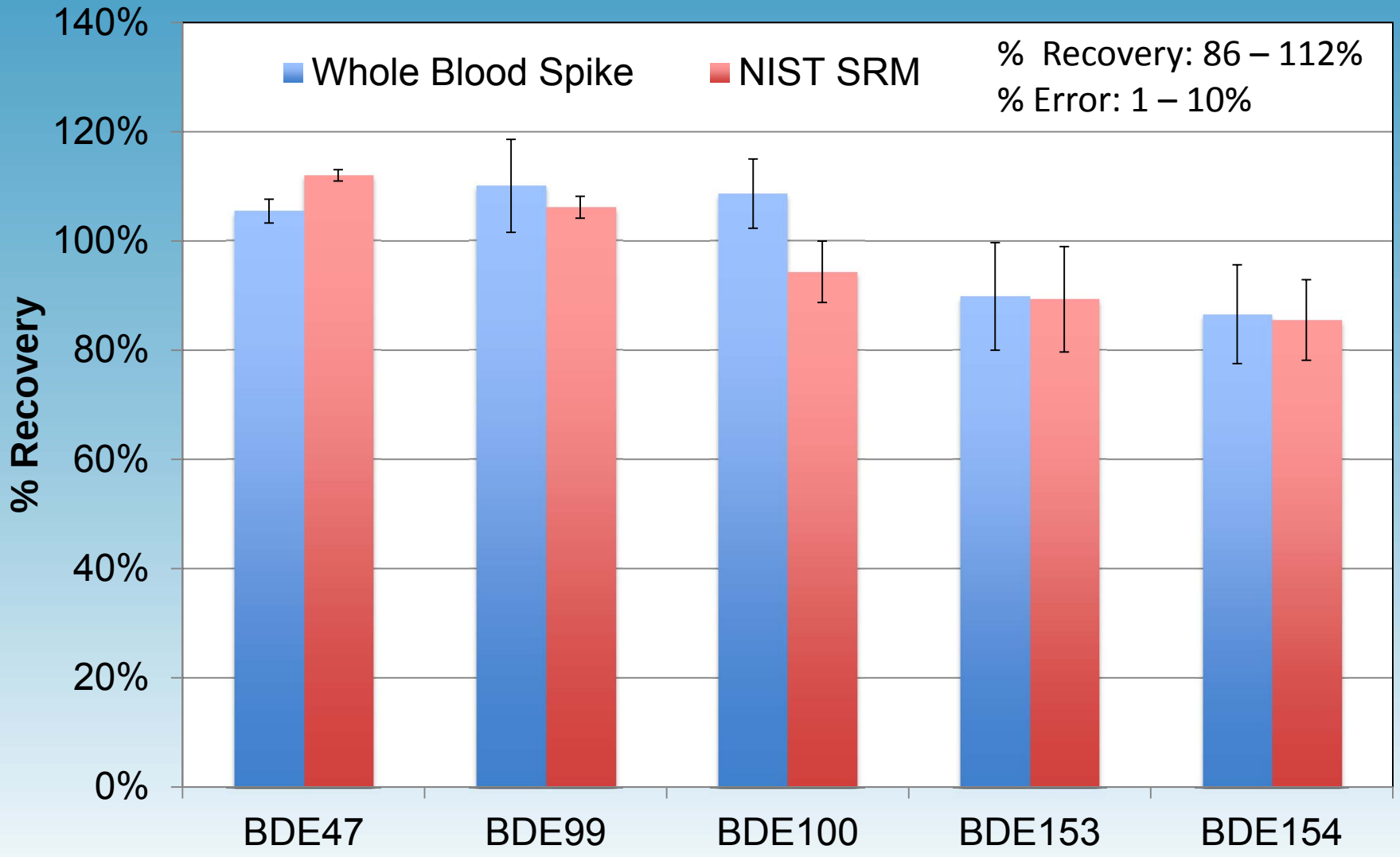
HT8-PCB: PBDE Results



HT8-PCB: PBDE Results

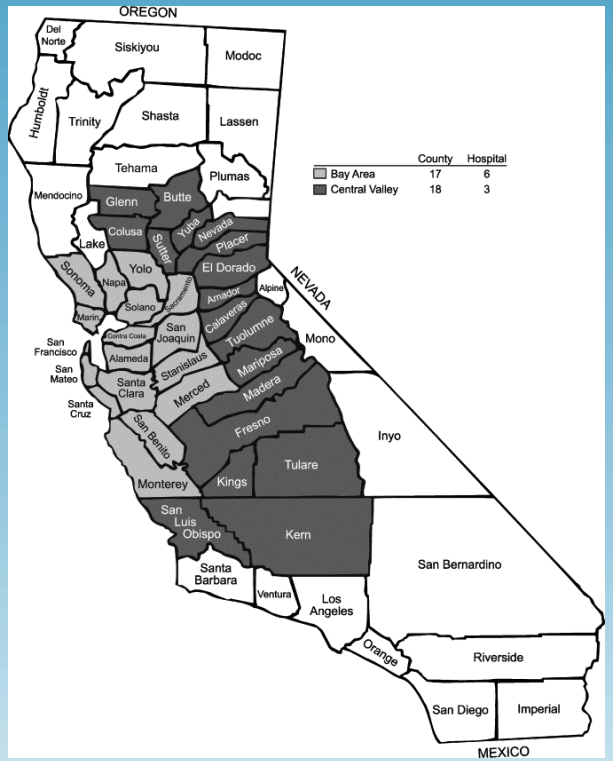


HT8-PCB: PBDE Results



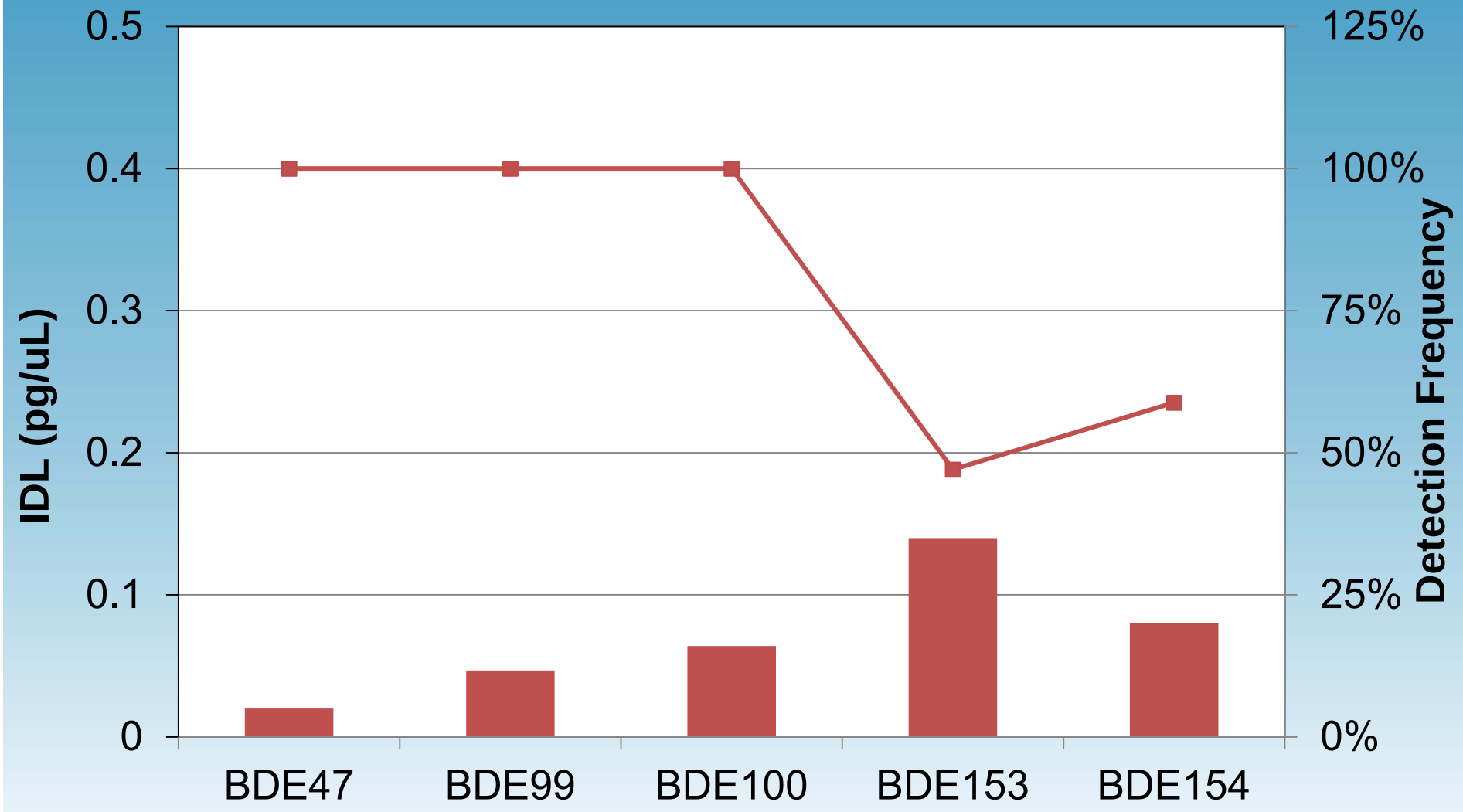
CA Childhood Leukemia Study

- Case-control study started in 1995
- Seeks to identify genetic and environmental risk factors for childhood leukemia
- Children's Whole Blood (Case)



GOAL: To measure PCBs, PBDEs, and OCPs in 100uL of whole blood using a GC-MS/MS with a single injection

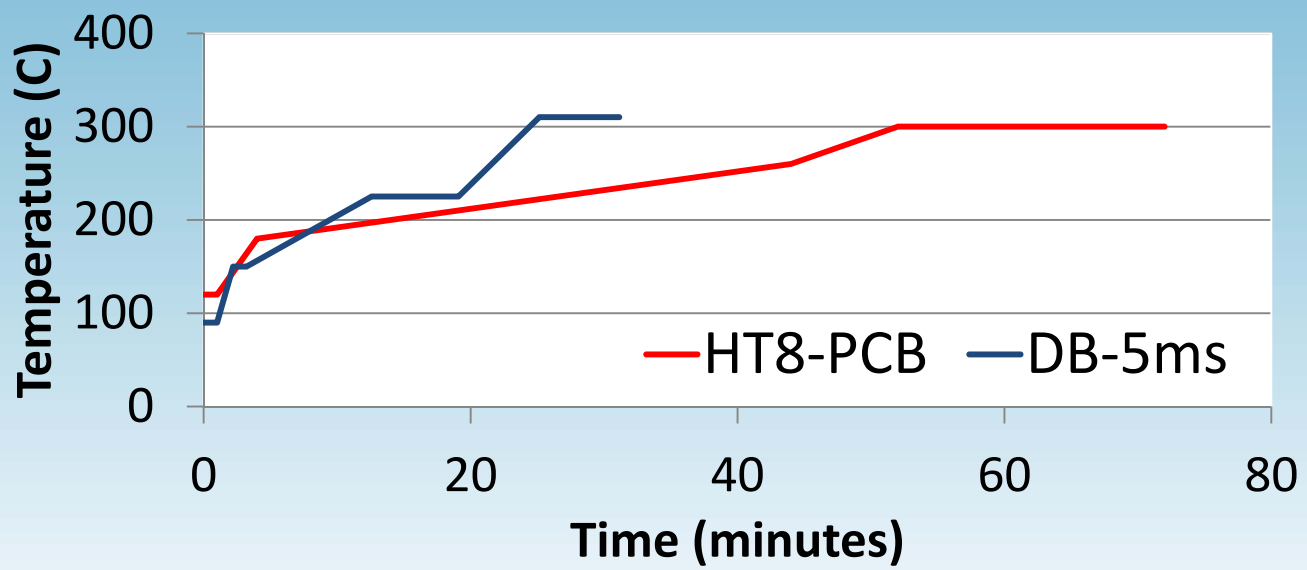
HT8-PCB: PBDE Results



New Column

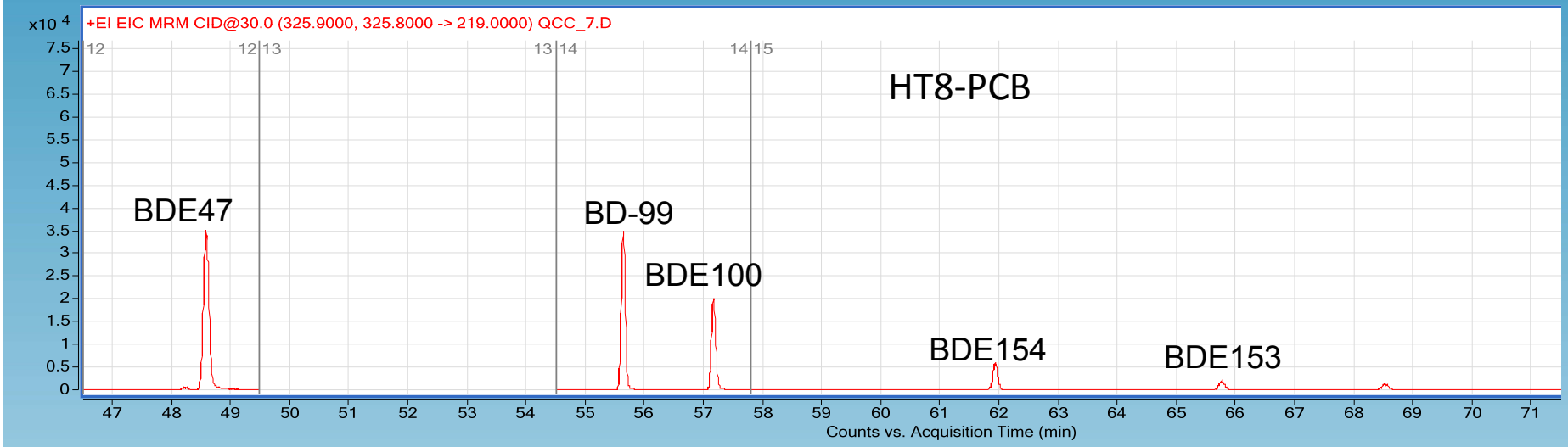
SGE HT8-PCB
(60m, 0.25mm)
Transfer Line: 300°C
Run Length: 75min

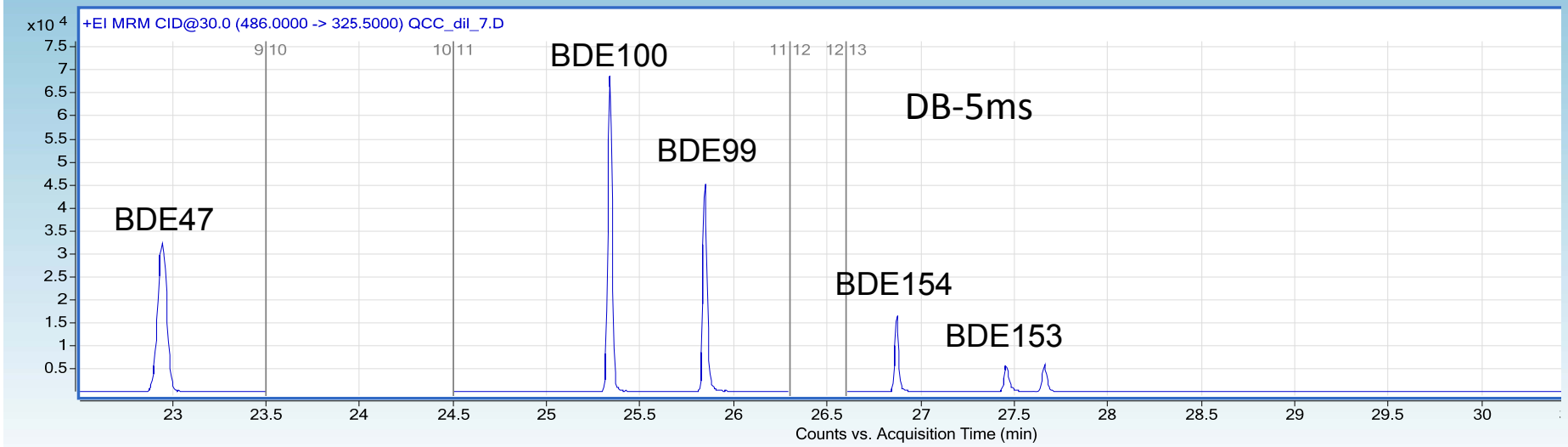
Agilent DB-5ms*
(30m, 0.25mm, 0.25um)
Transfer Line: 280°C
Run Length: 32min



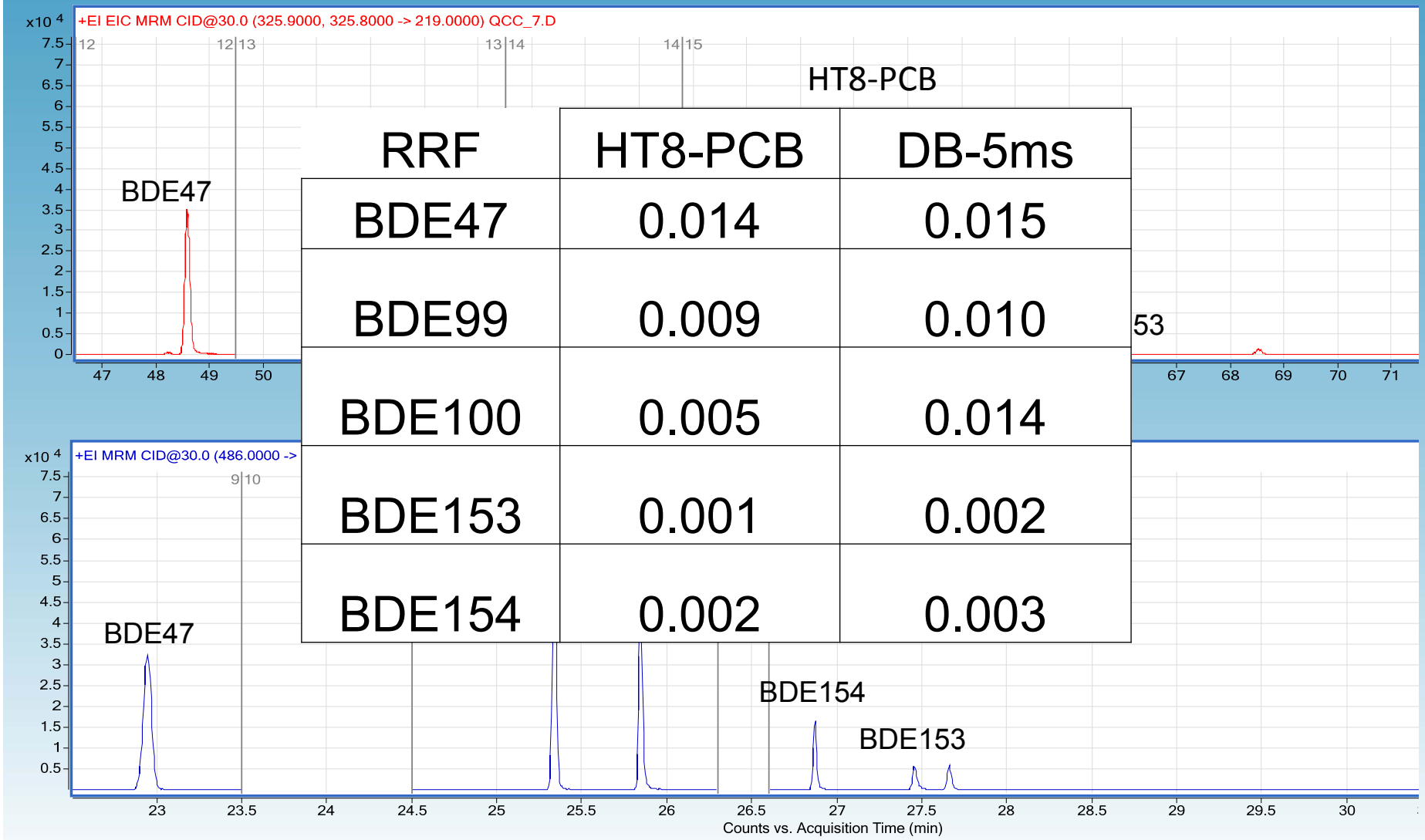
* Based on Lin et al, 2013 Talanta 113: 41–48

HT8-PCB and DB-5ms



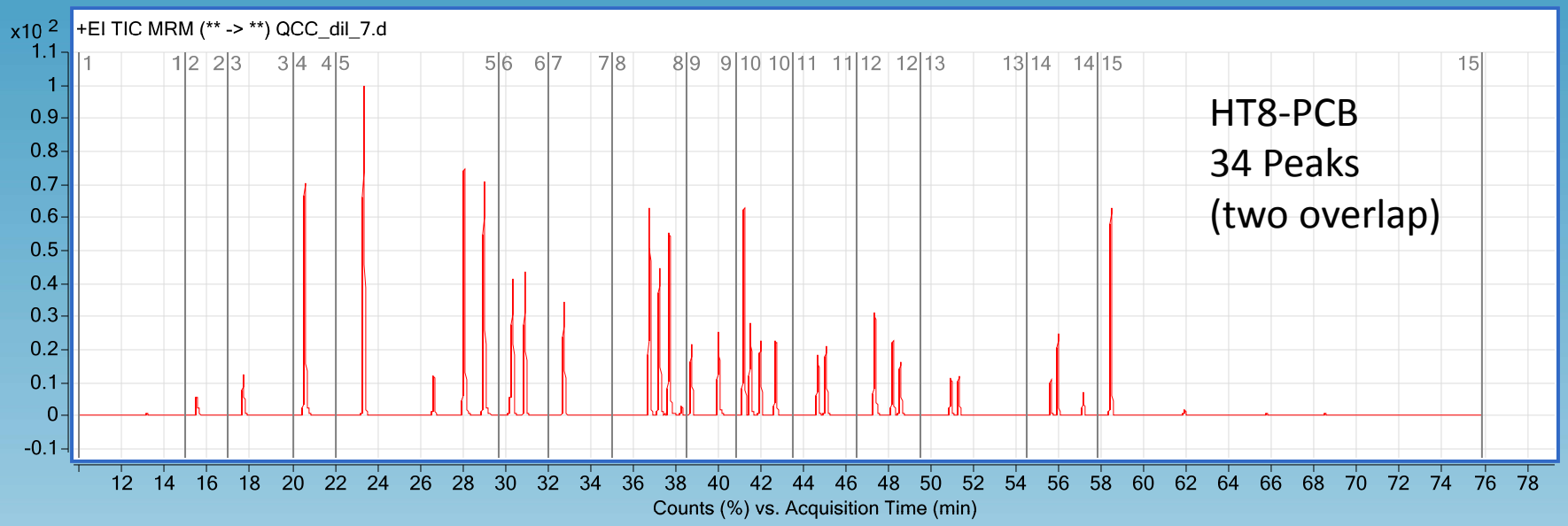


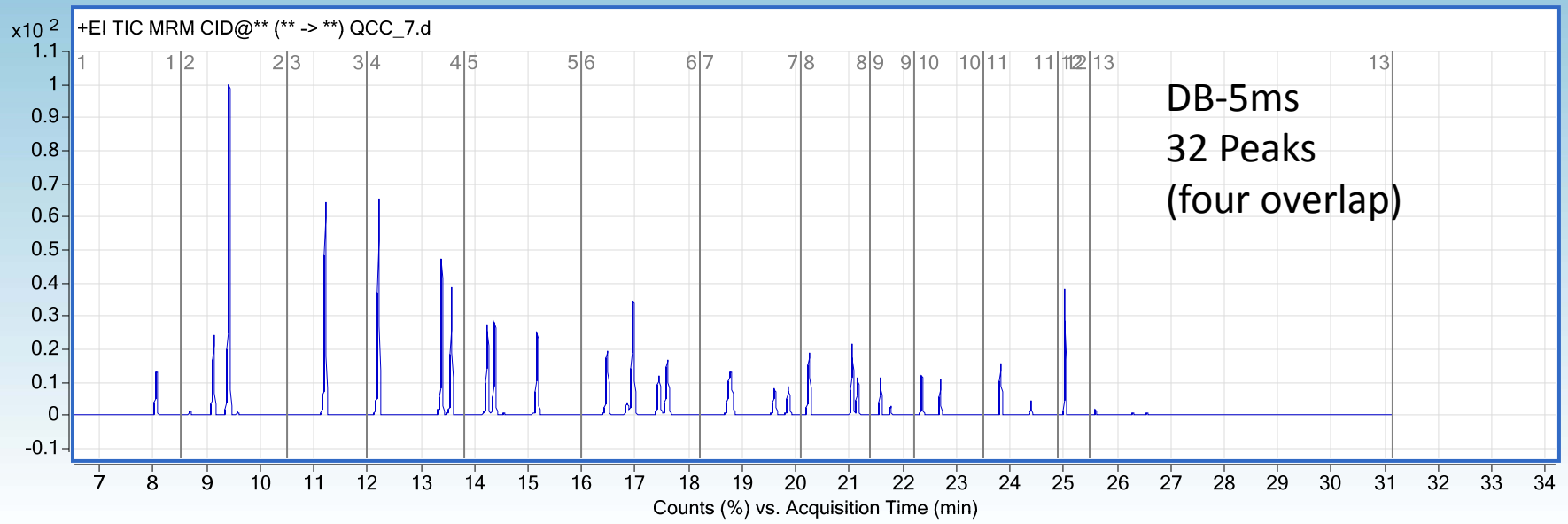
HT8-PCB and DB-5ms



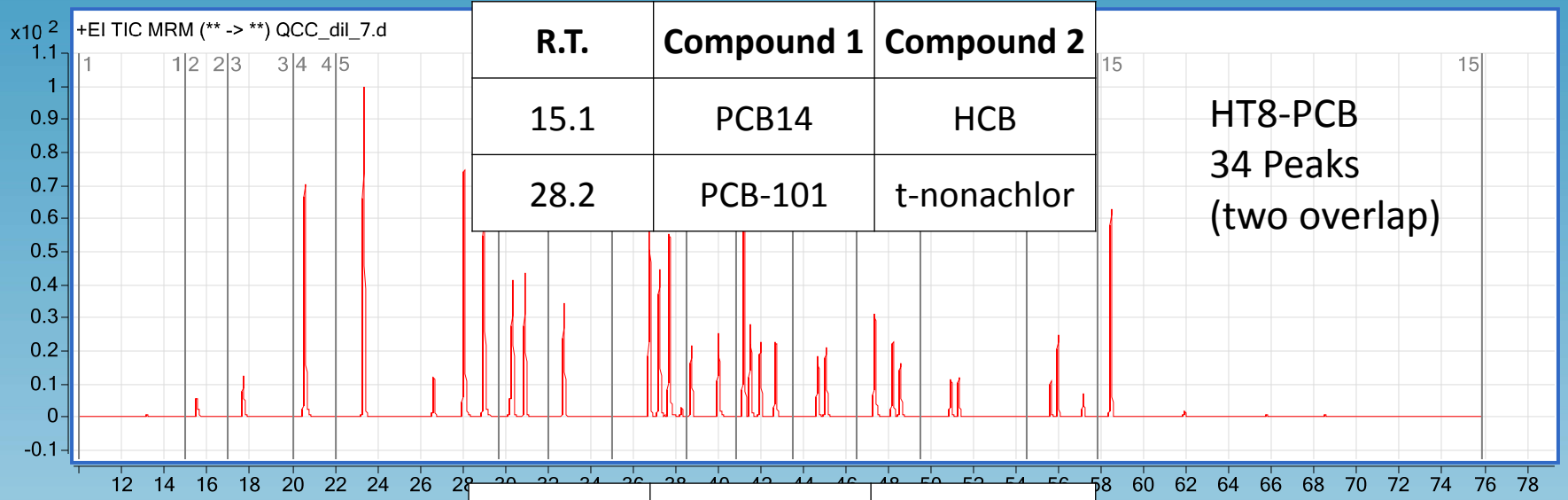
RRF	HT8-PCB	DB-5ms
BDE47	0.014	0.015
BDE99	0.009	0.010
BDE100	0.005	0.014
BDE153	0.001	0.002
BDE154	0.002	0.003

HT8-PCB vs DB-5ms (TIC %)



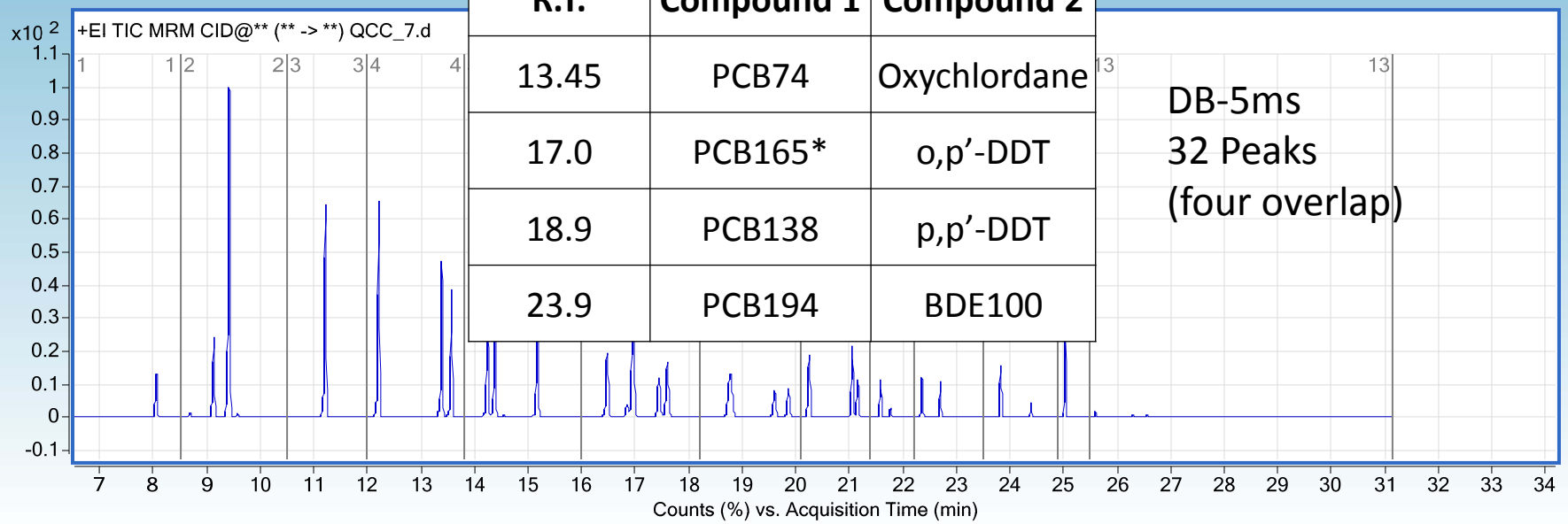


HT8-PCB vs DB-5ms (TIC %)



R.T.	Compound 1	Compound 2
15.1	PCB14	HCB
28.2	PCB-101	t-nonachlor

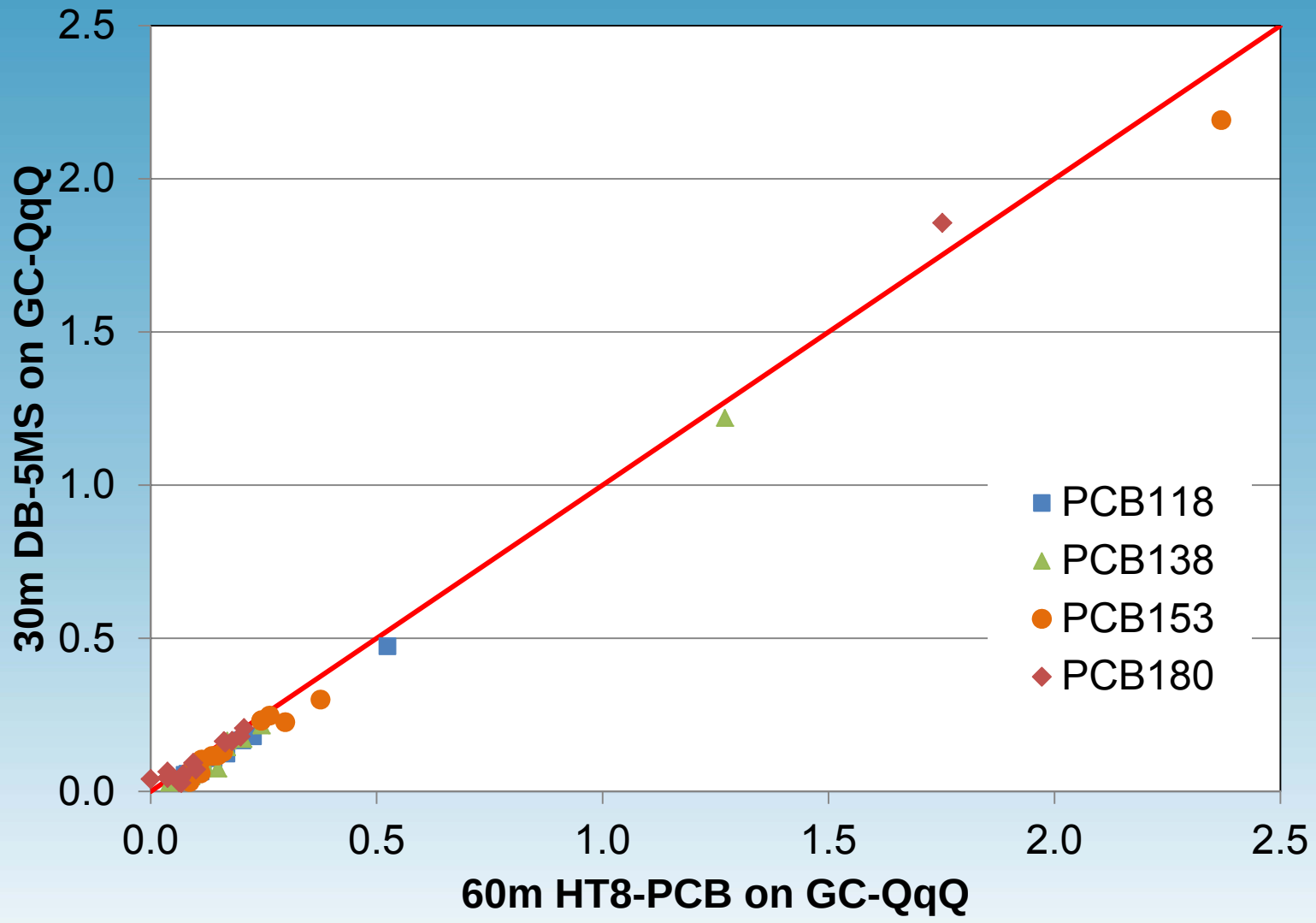
HT8-PCB
34 Peaks
(two overlap)



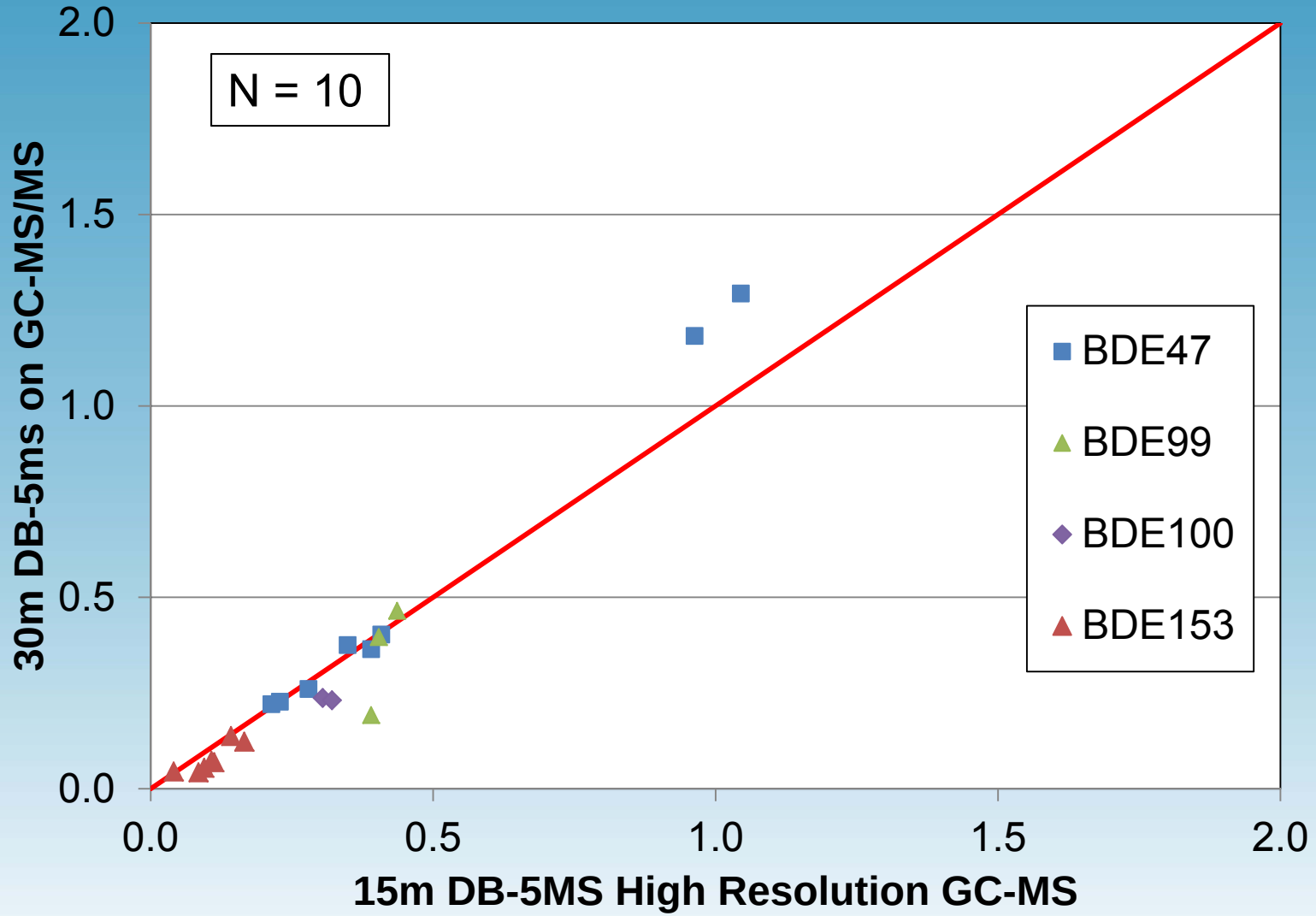
R.T.	Compound 1	Compound 2
13.45	PCB74	Oxychlordane
17.0	PCB165*	o,p'-DDT
18.9	PCB138	p,p'-DDT
23.9	PCB194	BDE100

DB-5ms
32 Peaks
(four overlap)

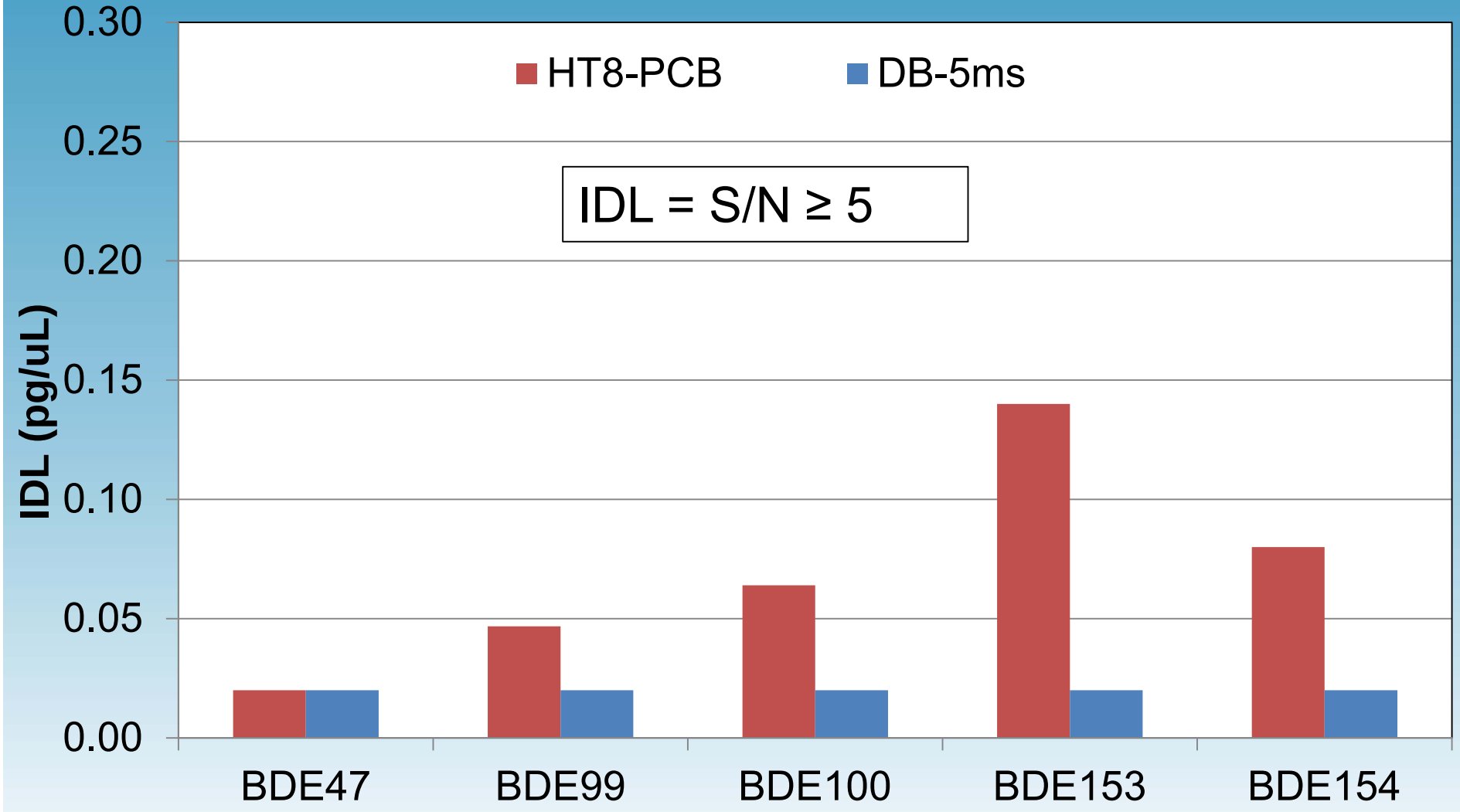
Column Comparison Results



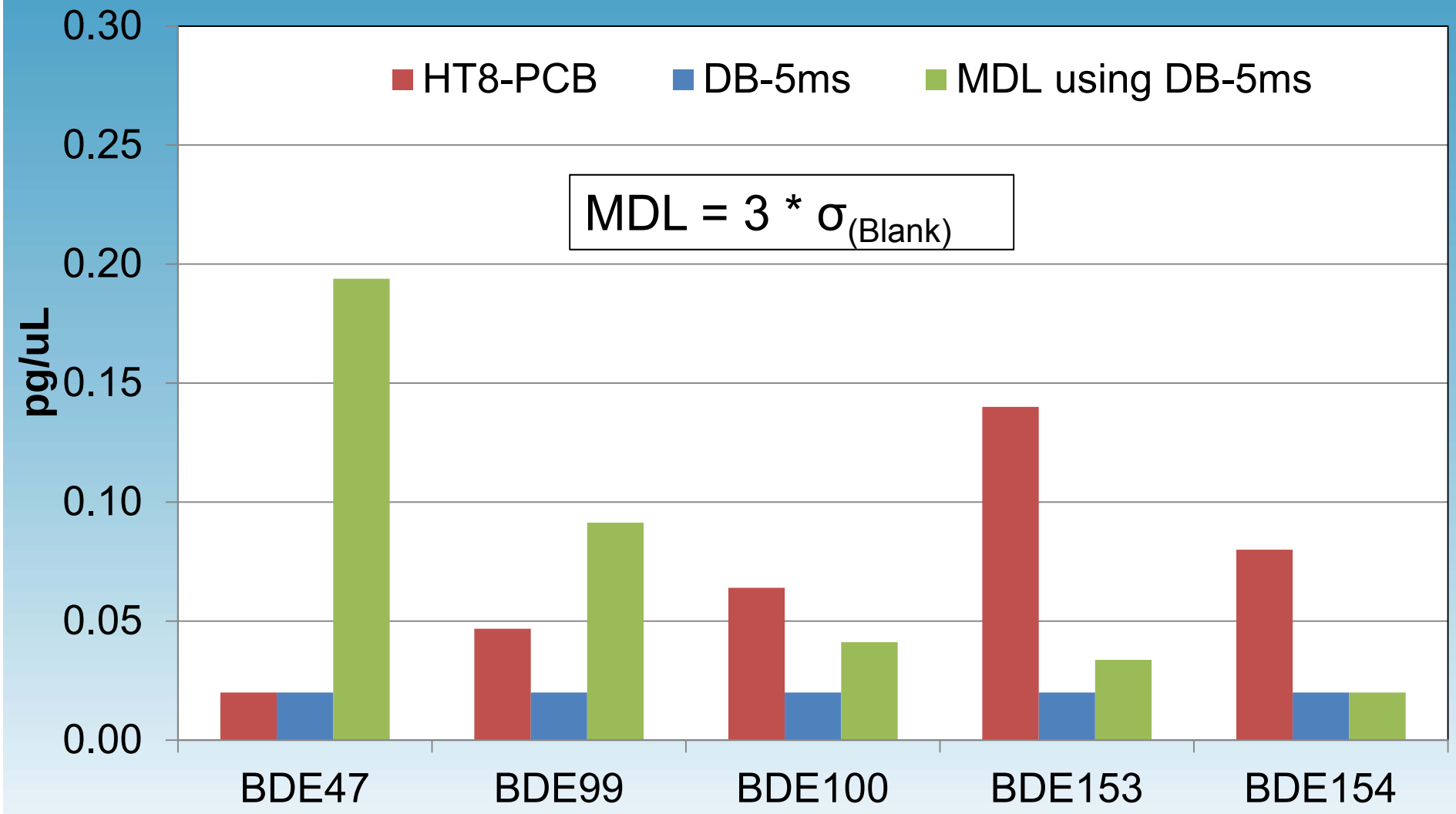
GC-HRMS vs GC-QqQ Results



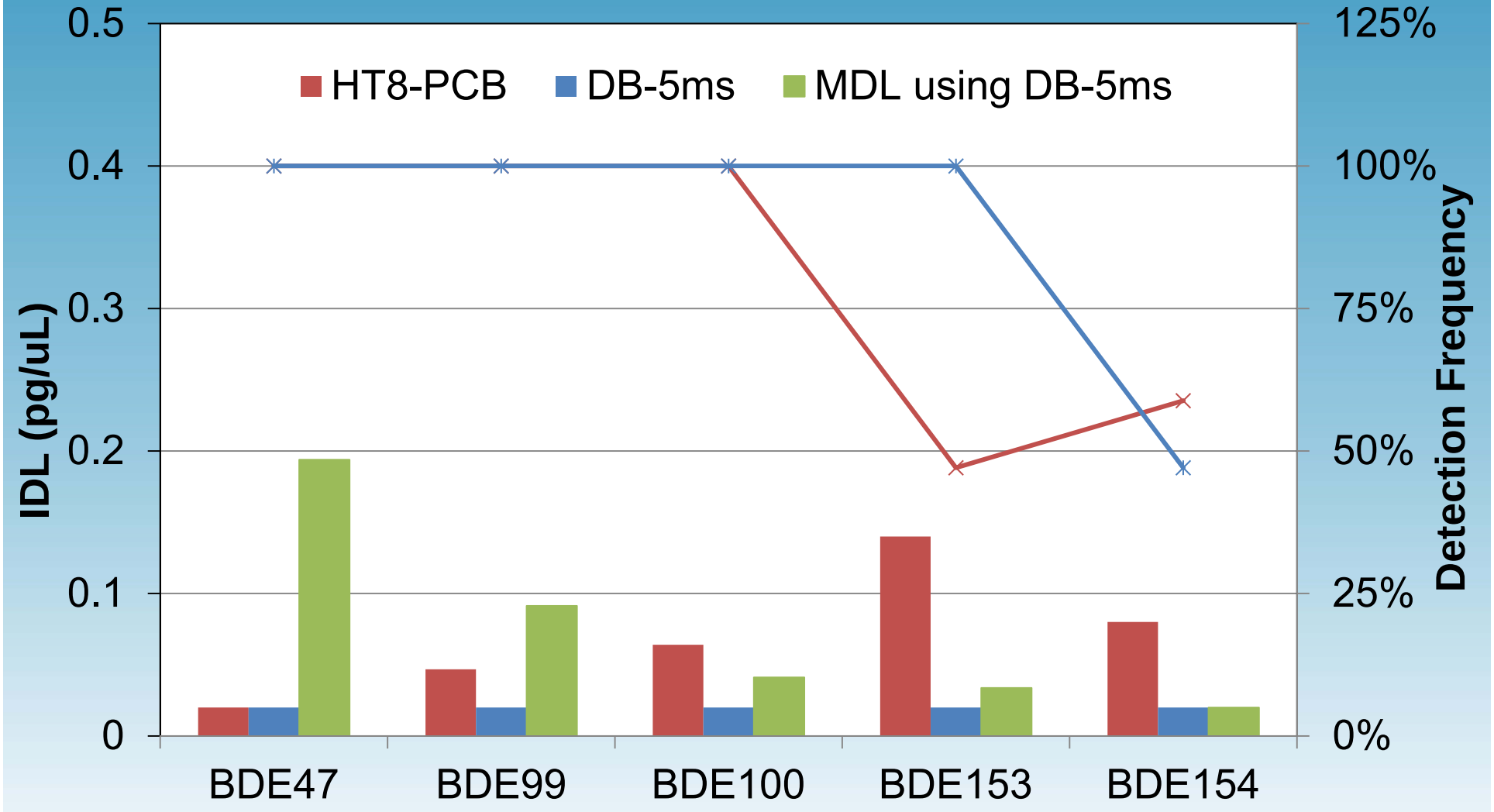
Instrument Detection Limits based on Column Selection



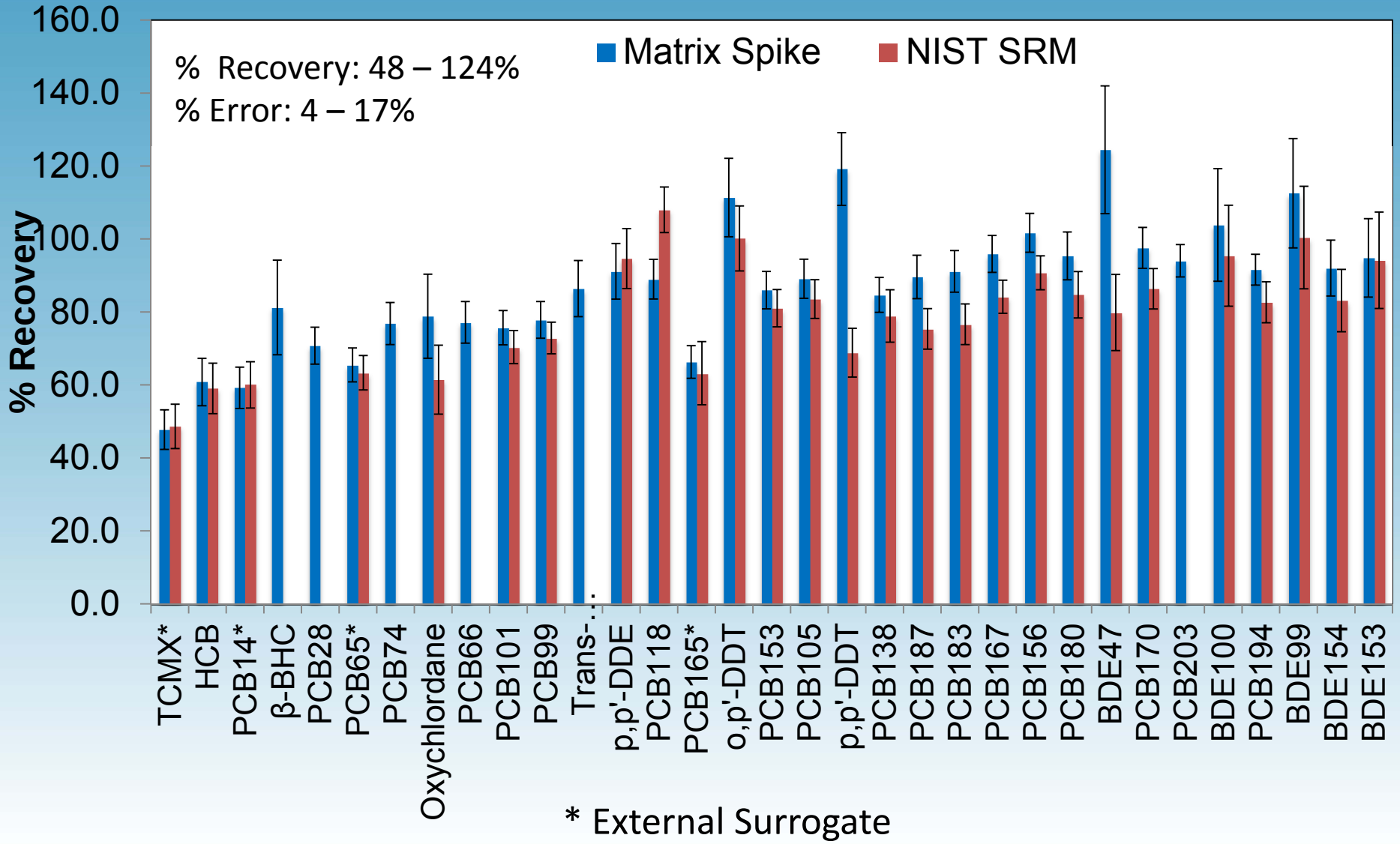
IDL and Method Detection Limits



IDL and MDL and Detection Frequency



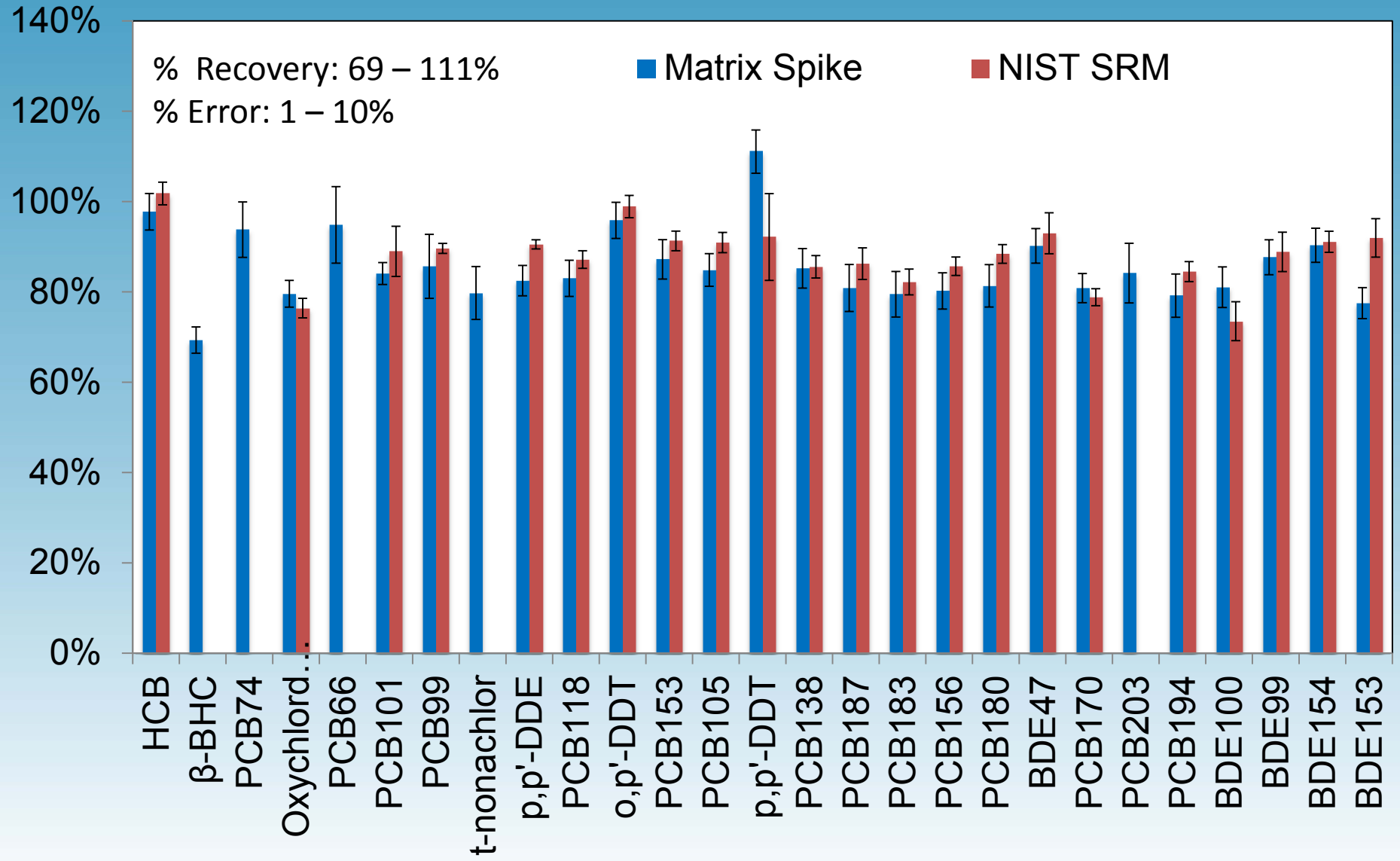
DB-5ms QC Recoveries



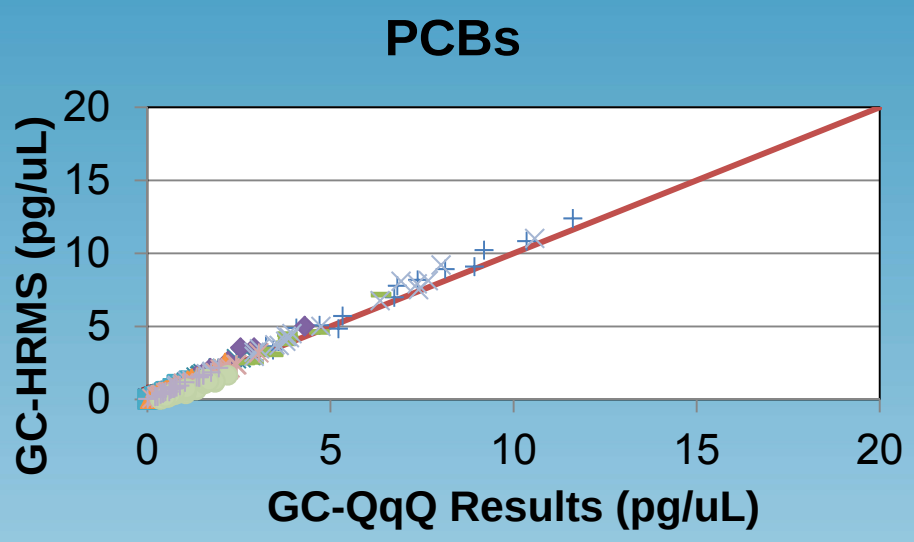
* External Surrogate

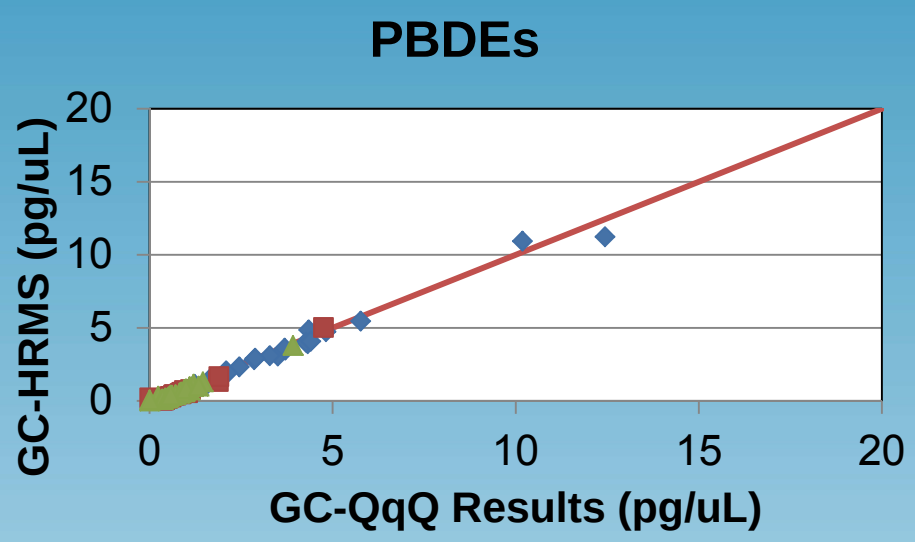
	CCLS		Santa Rosa	Three Generation Study	
	Children	Mothers	BBCC Study	Mothers	Daughters
N	194	50	100	492	300
Vol Serum	100uL	1mL	2mL	1mL	1mL
D.F. > 50%	PCB105	PCB99	PCB28	PCB28	PCB28
	PCB118	PCB105	PCB74	PCB66	PCB66
	PCB138	PCB118	PCB99	PCB74	PCB74
	PCB153	PCB138	PCB101	PCB99	PCB99
	PCB180	PCB153	PCB118	PCB101	PCB101
	p,p'-DDE	PCB170	PCB138	PCB105	PCB118
	p,p'-DDT	PCB180	PCB153	PCB118	PCB138
	Trans-nonachlor	PCB187	PCB156	PCB138	PCB153
	BDE47	PCB194	PCB170	PCB153	PCB156
	BDE99	β-BHC	PCB180	PCB156	PCB167
	BDE100	HCB	PCB187	PCB167	PCB170
	BDE153	Oxychlordane	PCB203	PCB170	PCB180
		o,p'-DDT	β-BHC	PCB183	PCB183
		p,p'-DDE	HCB	PCB187	PCB187
		p,p'-DDT	Oxychlordane	PCB180	PCB194
		Trans-nonachlor	p,p'-DDE	PCB194	PCB203
		BDE47	p,p'-DDT	PCB203	HCB
		BDE99	Trans-nonachlor	β-BHC	Oxychlordane
		BDE100	BDE47	HCB	o,p'-DDT
		BDE153	BDE99	Oxychlordane	p,p'-DDE
			BDE100	o,p'-DDT	p,p'-DDT
			BDE153	p,p'-DDE	Trans-nonachlor
				p,p'-DDT	BDE47
				Trans-nonachlor	BDE99
					BDE100
					BDE153

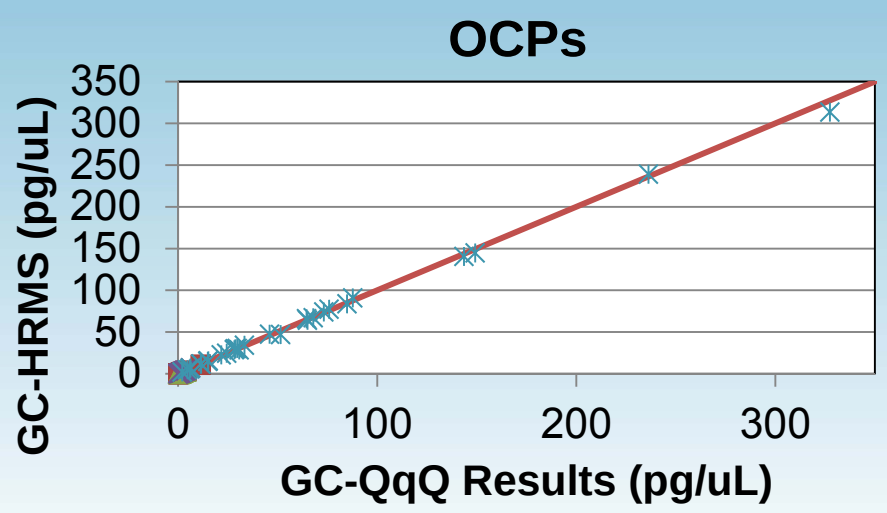
Current Work - Surrogates



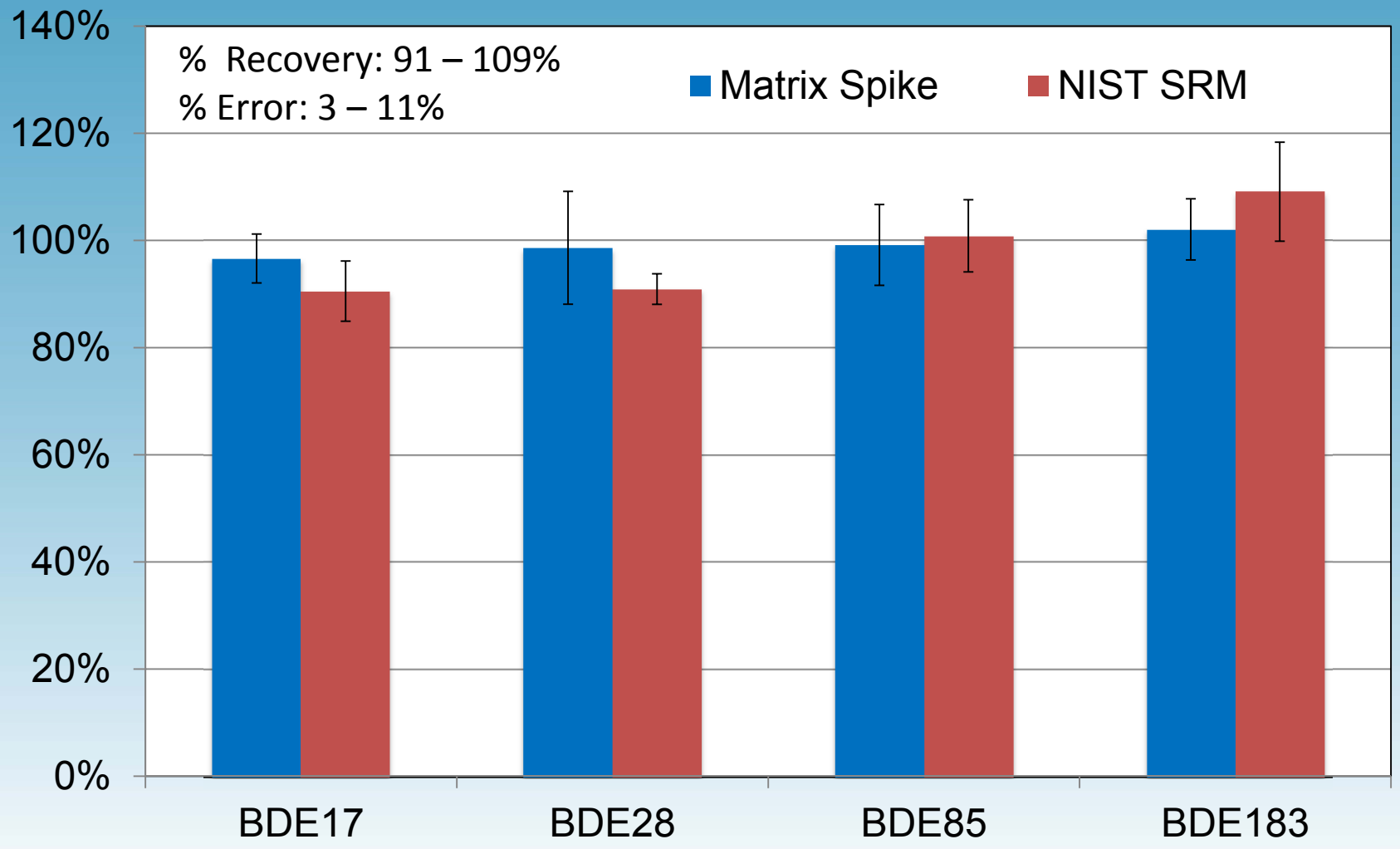
GC-HRMS vs GC-QqQ: Samples





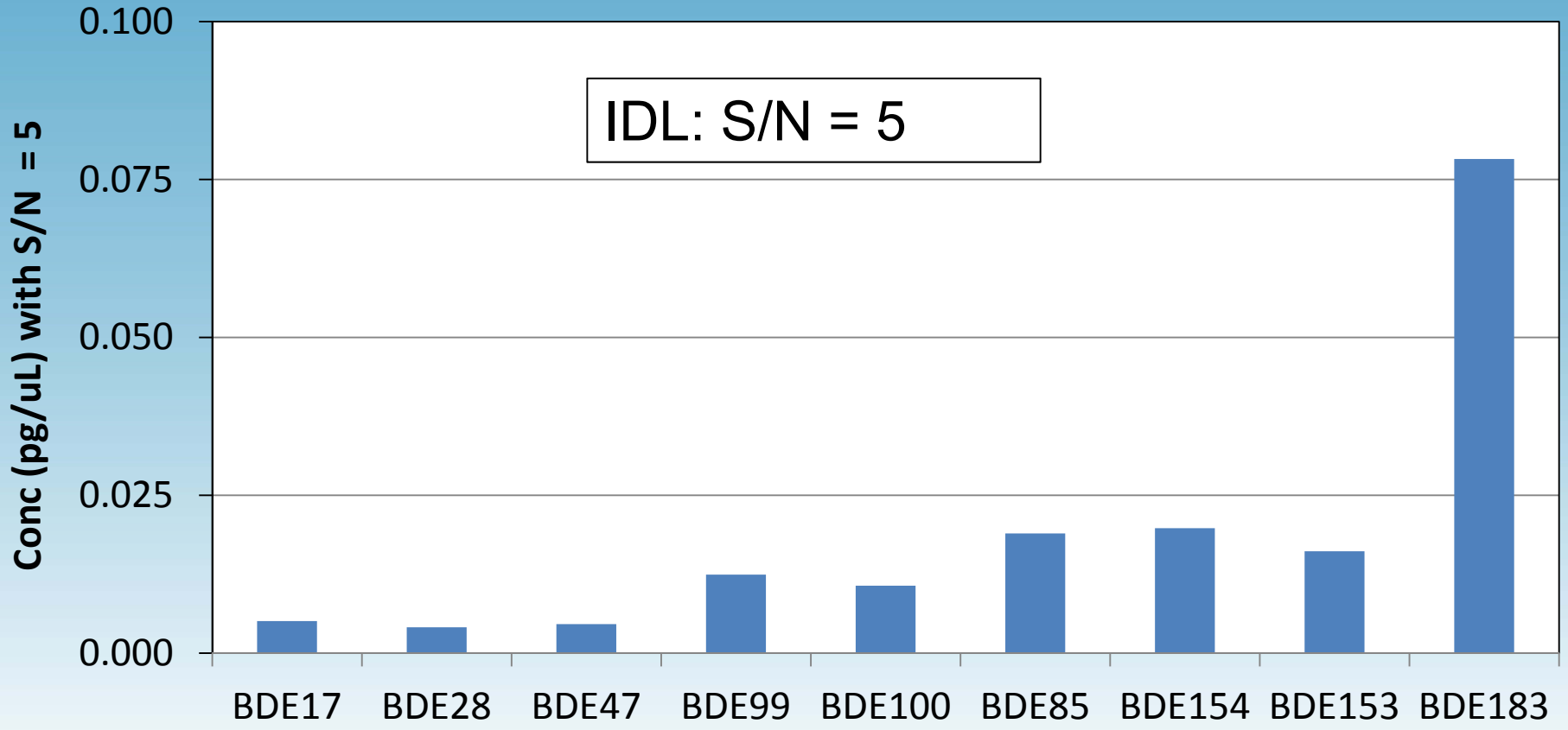


Current Work: Additional PBDEs



Issue To Address

- Instrument detection limits for new PBDEs
  - Especially BDE85 and BDE183



Future work

- Address New PBDE instrument detection limits
- Implement DB-5ms column method on GC-HRMS
  - Will increase sample throughput
  - No high MW BDEs (e.g. BDE209)
- Process Expanded BEST study on GC-HRMS with new method

Conclusions

- GC-MS/MS method allows for the analysis of PCBs, PBDEs, and OCPs in a single 32 min run
  - No detected interferences for serum analysis
- When method is transferred to GC-HRMS should reduce sample analysis time and increase sample throughput by ~ 3-fold.

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