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Modernizing Persistent Organic Pollutants (POPs) Analysis

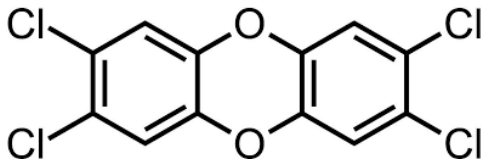
Agenda

- Introduction to POPs
- Dioxin analysis with the DFS
- Recent regulatory changes:
  - Dioxin analysis in food with the Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system
- Analysis of PBDEs with the Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system
- Analysis of PBDEs with the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS
- Analysis of SCCPs wit the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS
- Conclusions

What are Persistent Organic Pollutants (POPs)

- POPs are toxic chemicals that adversely affect humans
- Bioaccumulate and transferred via the environment and the food chain
- Long half lives and difficult to break down
- POPs are regulated by the Stockholm convention

- 2,3,7,8-TCCD is know the most toxic compound in the world



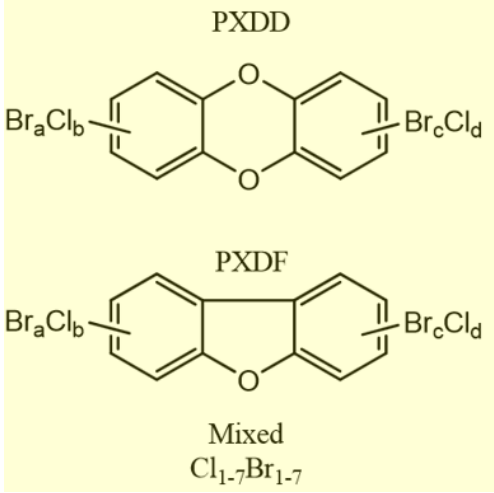
Viktor Yushchenko exposure to dioxins causing chloracne

Aldrin	*A*
Chlordane	*A*
DDT	*B*
Dieldrin	*A*
Endrin	*A*
Heptachlor	*A*
Hexachlorobenzene	*A and C*
Mirex	*A*
Toxaphane	*A*
Polychlorinated biphenyls (PCB)	*A and C*
Polychlorinated dibenzo-p-dioxins (PCDD)	*C*
Polychlorinated dibenzofurans (PCDF)	*C*

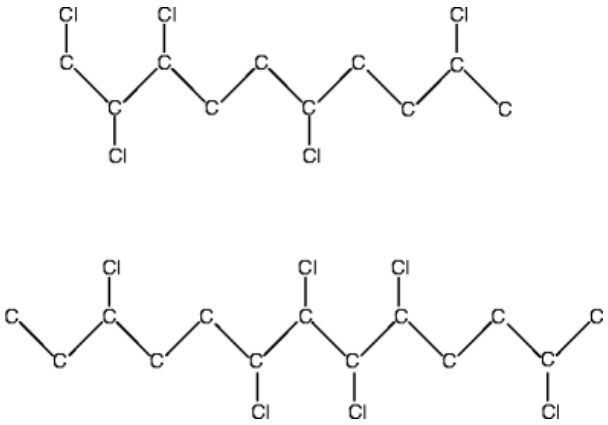
Alpha hexachlorocyclohexane	*A*
Beta hexachlorocyclohexane	*A*
Chlordecone	*A*
Hexabromobiphenyl	*A*
Hexabromocyclododecane	*A*
Hexabromodiphenyl ether and heptabromodiphenyl ether (commercial "Octa")	*A*
Hexachlorobutadiene	*A*
Lindane	*A*
Pentachlorobenzene	*A and C*
Pentachlorophenol and its salts and esters	*A*
Perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride	*A*
Polychlorinated naphthalenes	*A and C*
Technical Endosulfan and related isomers	*A*
Tetrabromodiphenyl ether and pentabromodiphenyl ether (commercial "Penta")	*A*

Emerging POPs and Analysis Challenges

- Emerging POPs of interest:
  - Mixed halogenated dioxins and furans
  - Short-chain chlorinated paraffins (SCCPs)
- Challenges with the analysis of POPs:
  - Low regulatory limits
  - Varied matrices
  - Covering expanding lists of target analytes
  - Producing consistent results
- Need instrumentation suitable for the analysis and the regulation



4000+ congeners of mixed halogenated dioxins and furans



Short chained chlorinated paraffins (SCCPs)

Thermo Scientific DFS Magnetic Sector GC-HRMS – *worldwide compliance*

- **Global compliance** with any official Dioxin, PCB, or PBDE method (e.g. EPA 1613, 1668..)
- **Proven leadership** with *robust sensitivity* for routine applications thanks to large-volume ion source
- **Exceptional productivity and flexibility** with *New DualData XL* option, for up to doubled sample throughput
- **Future committed** for Dioxins and POPs *regulations compliance* today and tomorrow

thermoscientific.com/GoldStandard

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Worldwide Compliance - Official Methods Recognizing Magnetic Sector Technology

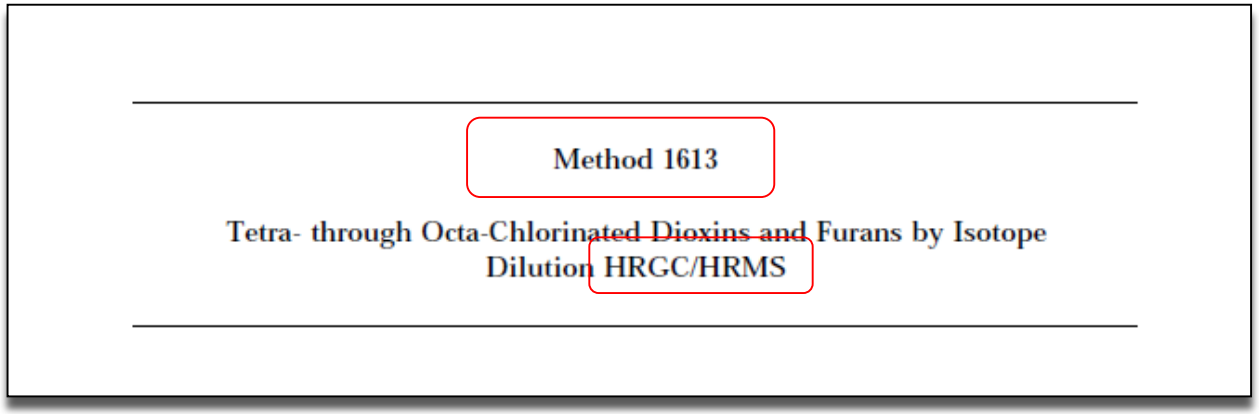
Application	Regulation/Norm	DFS Magnetic Sector GC-HRMS
Food safety	EU Regulatory Feed Control (at ML)	Approved
Food safety	EN 16215	Approved
Food safety	Background food studies (<1/5th EU ML)	Recommended by EURL
Clinical research	Human studies at trace levels	Recommended by EURL
Environmental	EN 1948	Approved
Environmental: Dioxins and Furans	US EPA 1613 B for strict EPA compliance	Approved
Environmental: PBDEs	US EPA 1614	Approved
Environmental	US EPA Method 23	Approved
Environmental	US EPA Method 8290	Approved
Environmental: PCBs	US EPA Method 1668	Approved
Environmental: Pesticides	US EPA Method 1699	Approved
Environmental: Hormones and steroids	US EPA 1698	Approved
Environmental	JIS K0311	Approved
Environmental	JIS K0312	Approved

Do you have international customers?

Are you working globally? Already now or in the future…

Official Method Compliance – EPA 1613

Here: EPA 1613 compliance



Method 1613

Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS

DFS & EPA 1613:

- 10.000 resolution
- Resolution checks
- Use PFK
- mass-drift correction
- lock mass monitoring

15.2 MS Resolution - A static **resolving power** of at least **10,000** (10% valley definition) must be demonstrated at the appropriate m/z before any analysis is performed. Static **resolving power checks** must be performed at the beginning and at the end of each 12 hour shift according to procedures in Section 10.1.2. Corrective actions must be implemented whenever the resolving power does not meet the requirement.

→ DFS

→ DFS

10.2.1.2 The mass spectrometer shall be operated in a **mass-drift correction mode**, using **perfluorokerosene (PFK)** to provide lock m/z's. The lock-mass for each group of m/z's is shown in Table 8. Each **lock mass shall be monitored** and shall not vary by more than ±20% throughout its respective retention time window. Variations of the lock mass by more than 20% indicate the presence of coeluting interferences that may significantly reduce the sensitivity of the mass spectrometer.

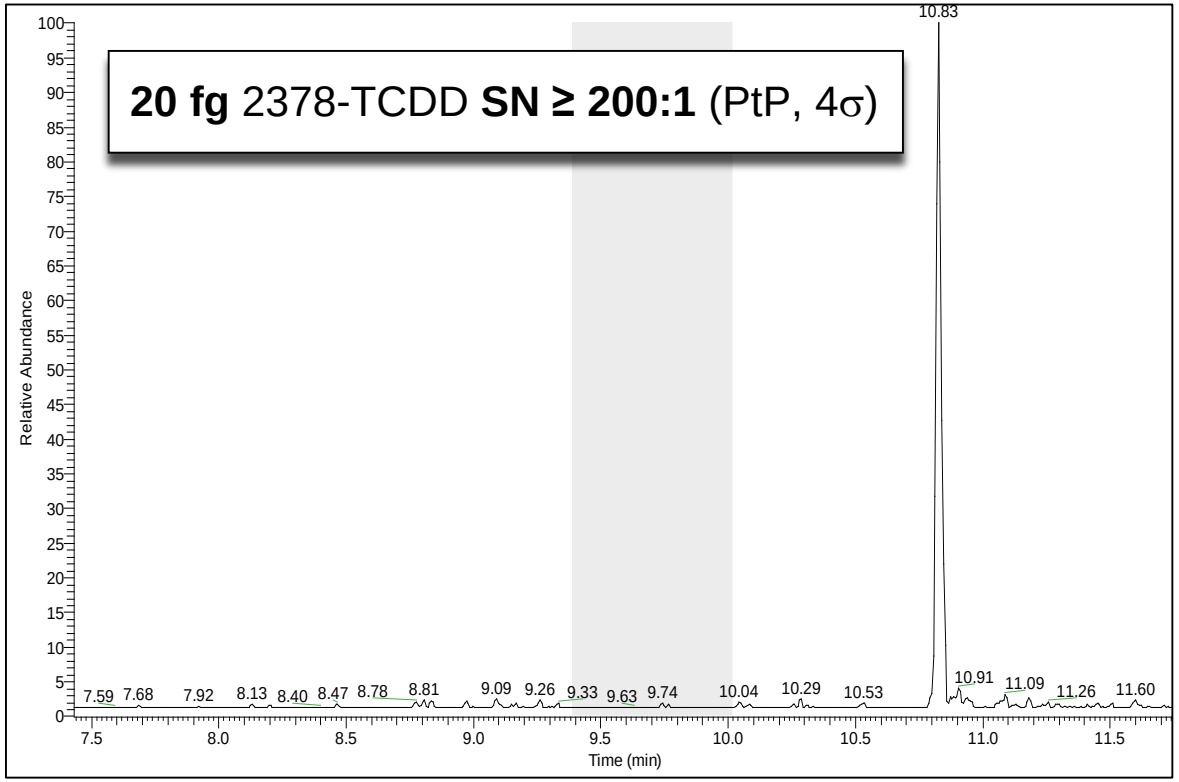
→ DFS

→ DFS

→ DFS

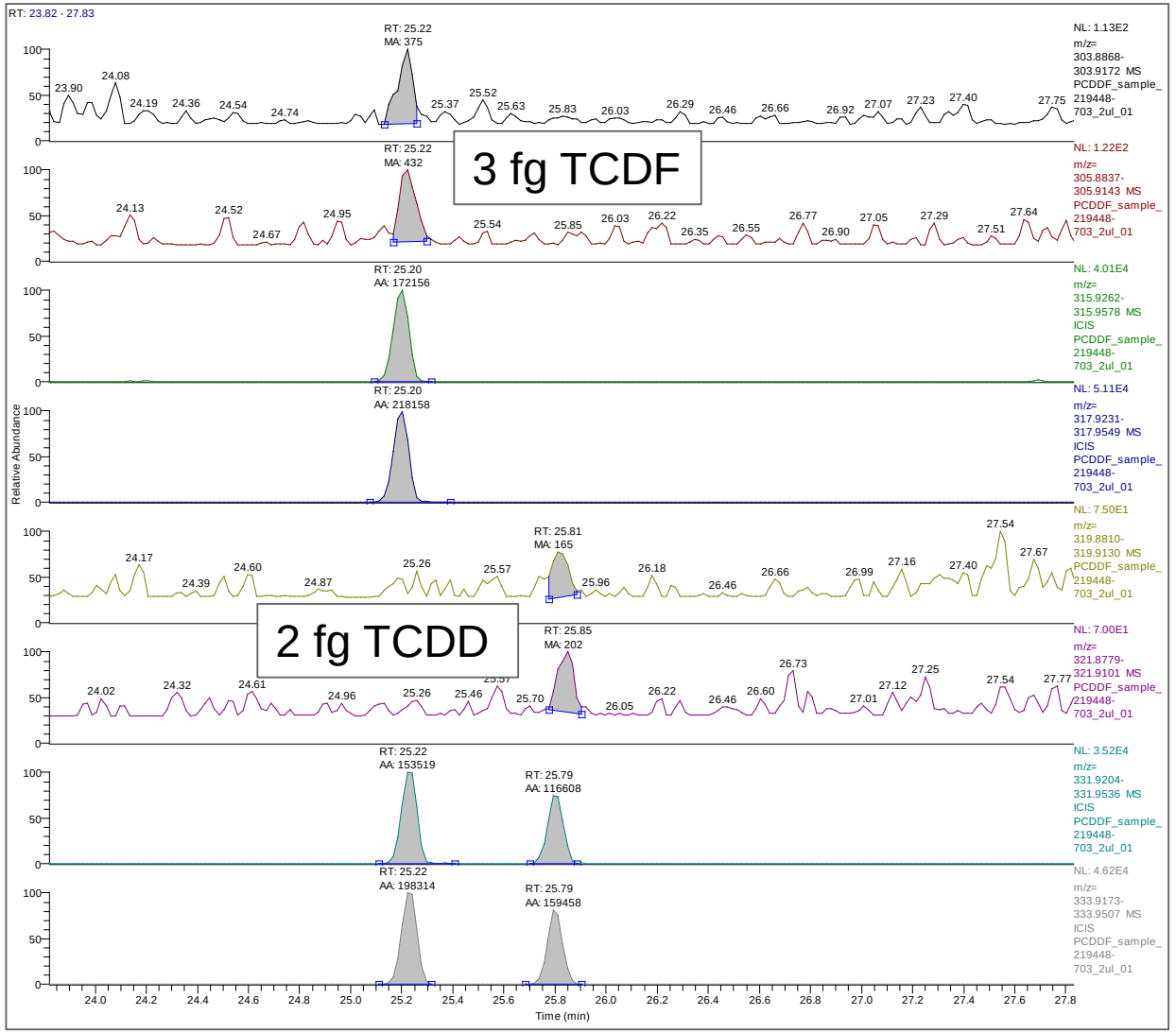
Ultimate Sensitivity Combined with Robustness

Best Dioxin specification on the market



Proof spec demonstrated to customers during DFS GC-HRMS installation

Real sample: serum extract (col.: 60m x 0.25 (0.25))



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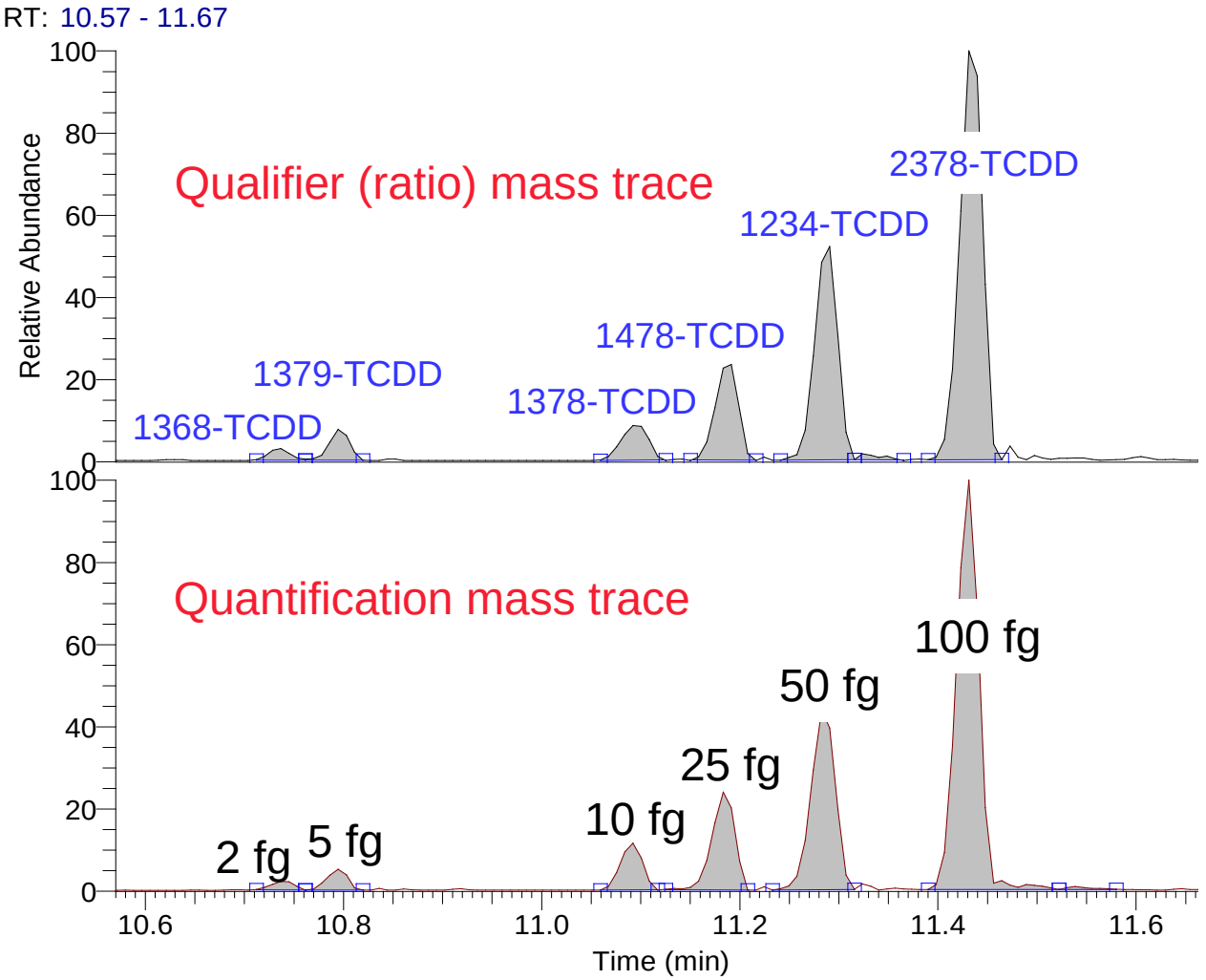
Ultimate Sensitivity for Confidence

➔ 6 different native tcdd isomers at **different** concentrations (2 – 100 fg/ul)



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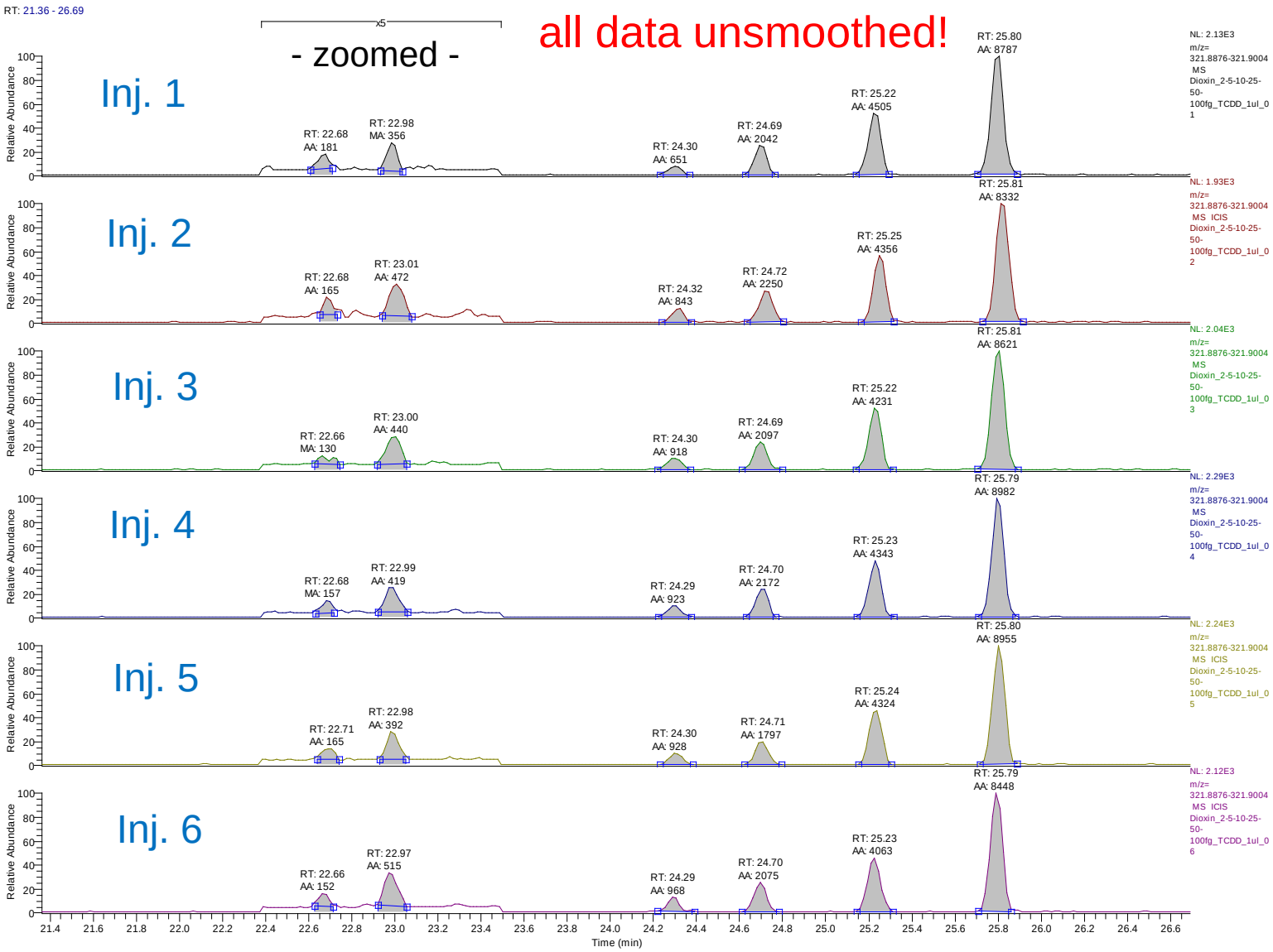
2 – 100 fg TCDD on 60 m Column



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Customer Lab in Japan (2010)

10 times repeated TCDD spec

#	tcdd	S/N (4σ)
1	20 fg	212 : 1
2	20 fg	217 : 1
3	20 fg	205 : 1
4	20 fg	237 : 1
5	20 fg	242 : 1
6	20 fg	236 : 1
7	20 fg	212 : 1
8	20 fg	235 : 1
9	20 fg	222 : 1
10	20 fg	227 : 1

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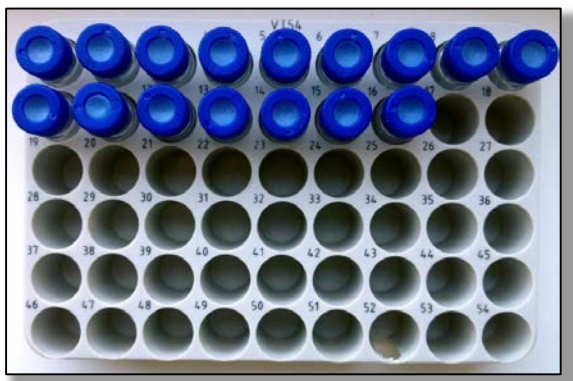
Dioxins & POPs Productivity – Thermo Scientific DFS DualData XL Option

Technology based on Magnetic Sector MS provides a solution for maximum productivity in terms of number of samples analyzed per time.

With DualData XL Option you can **double your throughput**, even with mixed Applications such as Dioxins, PCBs, PBDEs, OCPs and other.

- same hardware
- same software
- same lab space

= same cost of ownership
as standard magnetic sector MS
...but with up to double productivity



DualDataXL

16 samples in 12 hour shift

32 samples in 12 hour shift

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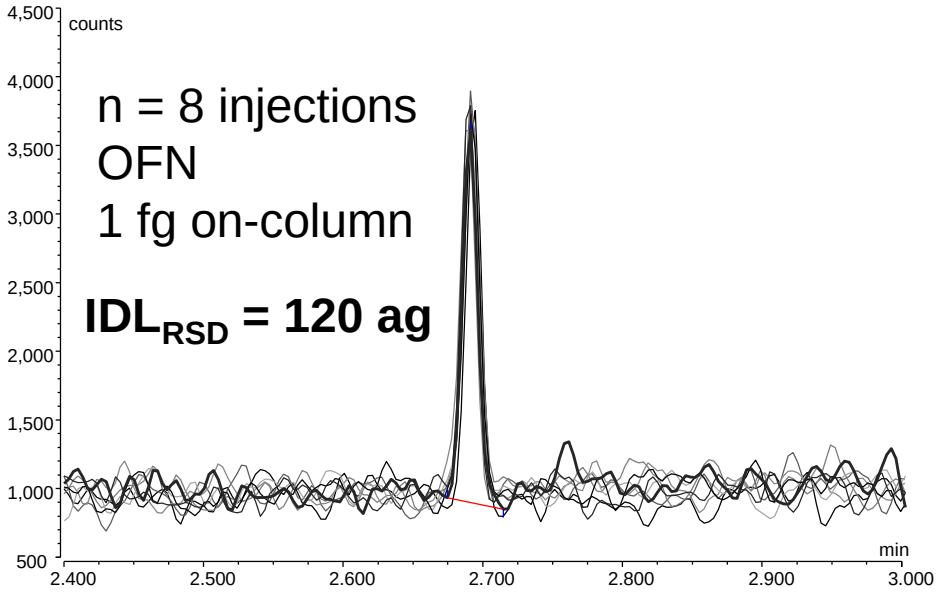
Thermo Scientific TSQ 9000 Triple Quadrupole GC-MS/MS System

Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system

Feature	Benefit
Highly efficient ionization	A greater ion flux reaching the detector
A more tightly focused ion beam	Less ion burn and a higher degree of robustness

Thermo Scientific™ Advanced Electron Ionization (AEI) source



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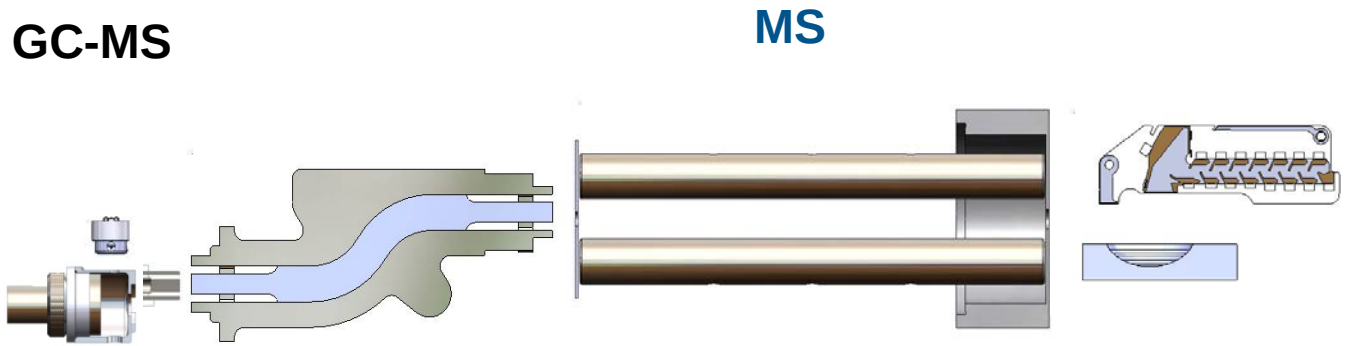
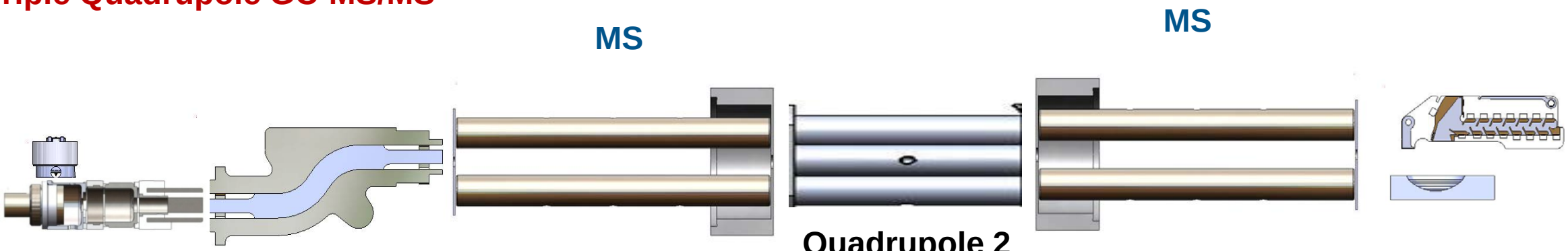
Why Triple Quadrupole GC-MS/MS?

Single Quadrupole GC-MS



Ion Source | Ion Transfer Optics | Quadrupole | EM Detector

Triple Quadrupole GC-MS/MS



Ion Source | Ion Transfer Optics | Quadrupole 1 (Q1) | **Quadrupole 2 (Q2) Collision Cell** | **Quadrupole 3 (Q3)** | EM Detector

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GC-MS/MS – What's So Special?

- **Low detection limits**
- **Optimized sample preparation**
- **Consolidated analytical methods**
- **Faster, automated data processing**

...it's a high selectivity technique...

EU Compliance – Thermo Scientific TSQ 9000 triple quadrupole GC-MS/MS system

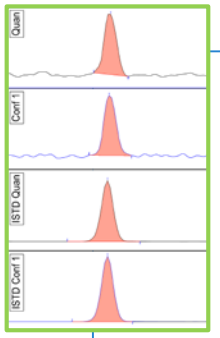
EU regulations amended in 2014 (589/2014 & 709/2014)
Allowing the use of GC-MS/MS for confirmatory methods providing the following criteria are met:

Chromatographic separation of Heptafurans better than 25% peak to peak.

2 specific SRM transitions for all labelled and native congeners.

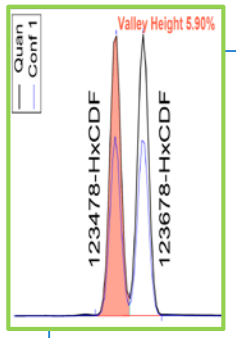
Ion ratio ± 15% max deviation from theoretical value for product ions of the two transitions

Resolution ≤1 (separation of two peaks one mass unit apart) for each quadrupole



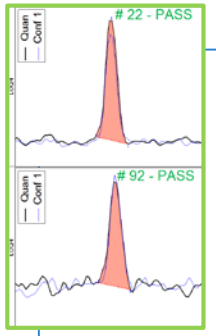
Regulatory compliant sensitivity

- 10fg on-column TCDD with ion ratios (**2 specific SRM transitions)** within **±15% tolerance**.
- Routine method (all 17 toxic PCDDFs + 4 non-ortho dl-PCBs).



Chromatographic and mass spectral resolution

- Peak to peak separation of HxCDF isomers <25% (5.9%)
- Q1 and Q3 both set to 0.7Da @FWHM – giving EU compliant separation between masses

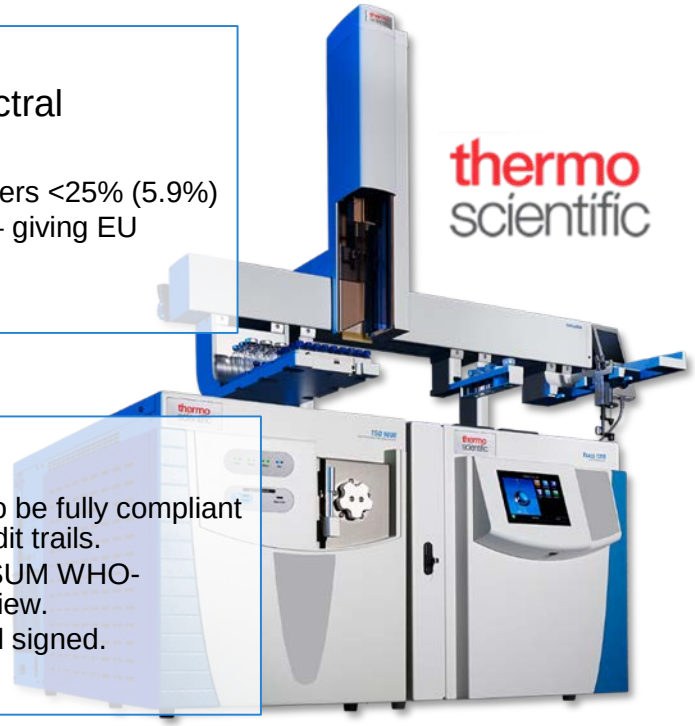


LOQ compliance

- For GC-MS/MS European guidance is to demonstrate LOQ throughout sequence (as opposed to using S:N ratios.
  - **±15% ion ratios.**
  - **≤30% deviation from average RF.**
- Demonstrated over multi-site validation study on three separate systems and ~2 weeks continuous runtime.

21 CFR, part 11 compliance

- Chromeleon software has the tools to be fully compliant with FDA requirements, including audit trails.
- Real time results updates including SUM WHO-PCDD/F-TEQ values during data review.
- All data can be digitally reviewed and signed.



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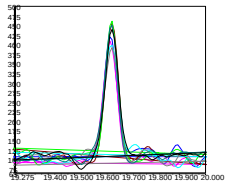
Method Development Resource: The AppsLab Library of Analytical Applications

- … An online search engine for Thermo Fisher Scientific applications
- … Provides comprehensive application information and ready-to-run analytical methods
- … A central repository for Thermo Scientific chromatography and MS application information

Visit: www.thermofisher.com/appslab

LOQ Consistency for Dioxin Analysis in Matrix

- Consistent results at the LOQ
- Reproducibility within the regulatory requirements



IDL
0.58 fg

Injection #			
22	88.33	71.76	94.59
	93.48	67.18	96.42
23	101.79	62.34	92.28
	104.45	69.42	97.60
53	107.36	60.28	91.57
	97.07	73.03	100.00
66	90.36	67.25	89.31
79			
92			
101			

n = 8 injections 2,3,7,8-TCDD

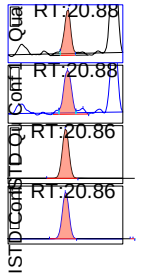
5fg on-column

10 fg on-column 40 fg on-column 160 fg on-column

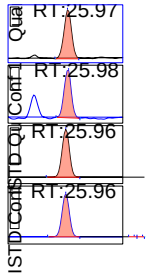
Reporting to Regulations Made Easy with Chromeleon

Peak Name	Ion Ratio	ISTD Rec	<LOQ?	UB WHO TEQ	MB WHO TEQ	LB WHO TEQ
	%	%		pg/g	pg/g	pg/g
2378-TCDF	96.37	79.2		0.0493	0.0493	0.0493
2378-TCDD	*105.26*	*89.2*		*0.0683*	*0.0683*	*0.0683*
12378-PeCDF	88.79	94.2		0.0041	0.0041	0.0041
23478-PeCDF	78.57	94.8		0.0686	0.0686	0.0686
12378-PeCDD	75.45	99.7		0.3410	0.3410	0.3410
123478-HxCDF	55.86	81.2		0.0113	0.0113	0.0113
123678-HxCDF	59.95	87.3		0.0113	0.0113	0.0113
234678-HxCDF	68.85	76.8		0.0112	0.0112	0.0112
123478-HxCDD	65.58	88.4		0.0477	0.0477	0.0477
123678-HxCDD	65.59	96.0		0.1020	0.1020	0.1020
123789-HxCDD	65.90	96.0		0.0557	0.0557	0.0557
123789-HxCDF	IR High	71.2	<LOQ	0.0071	0.0035	0.0000
1234678-HpCDF	85.61	60.7	<LOQ	0.0017	0.0008	0.0000
1234678-HpCDD	80.13	94.2		0.0688	0.0688	0.0688
1234789-HpCDF	78.94	81.8	<LOQ	0.0012	0.0006	0.0000
OCDD	94.97	91.3		0.0023	0.0023	0.0023
OCDF	102.53	83.0	<LOQ	0.0001	0.0001	0.0000
		SUM PCDD/Fs		0.852	0.847	0.842

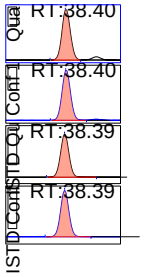
Automatic flagging of results outside tolerance



2,3,7,8-TCDD



12378-PeCDD



OCDD

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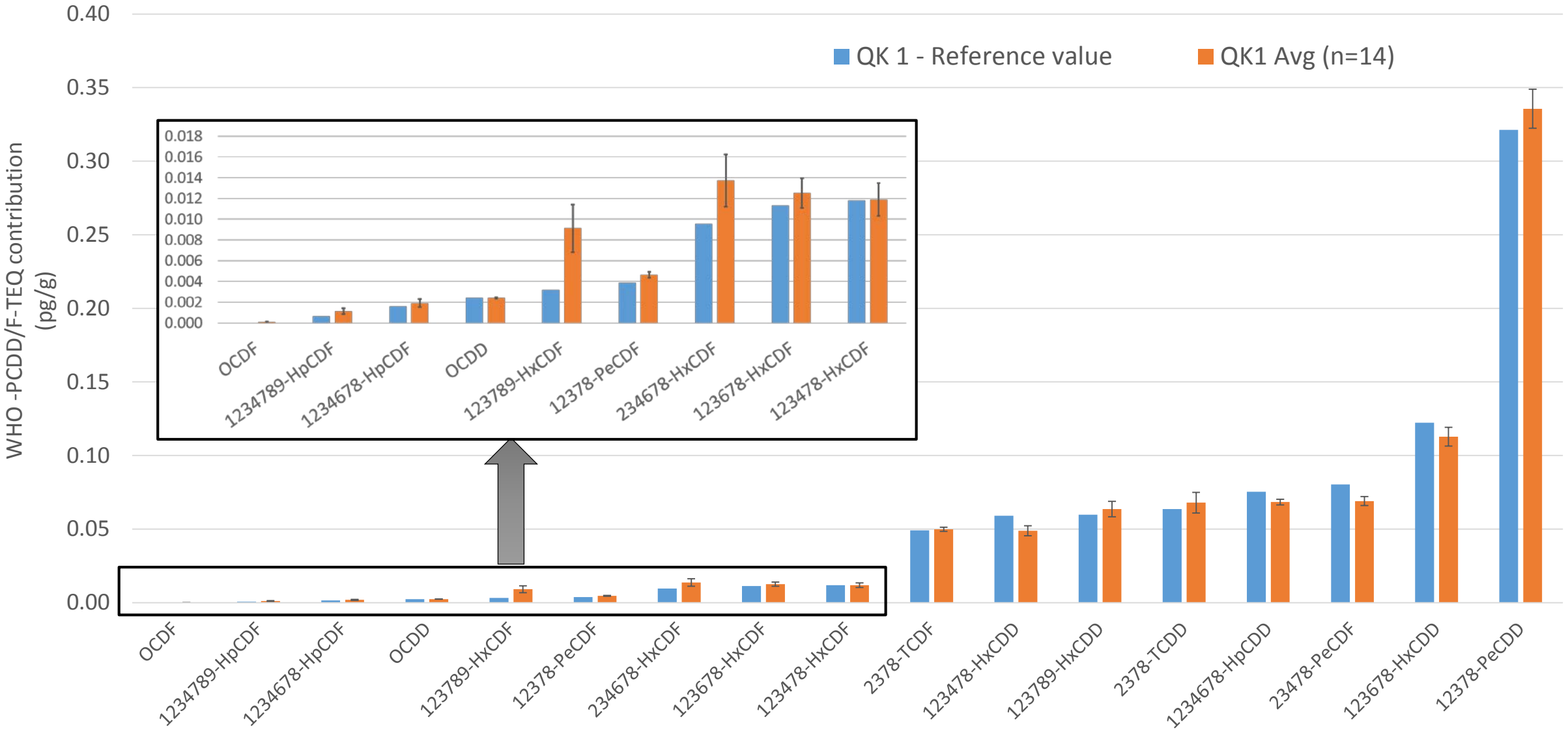
Agreement of Total TEQ with Reference Animal Fat Sample



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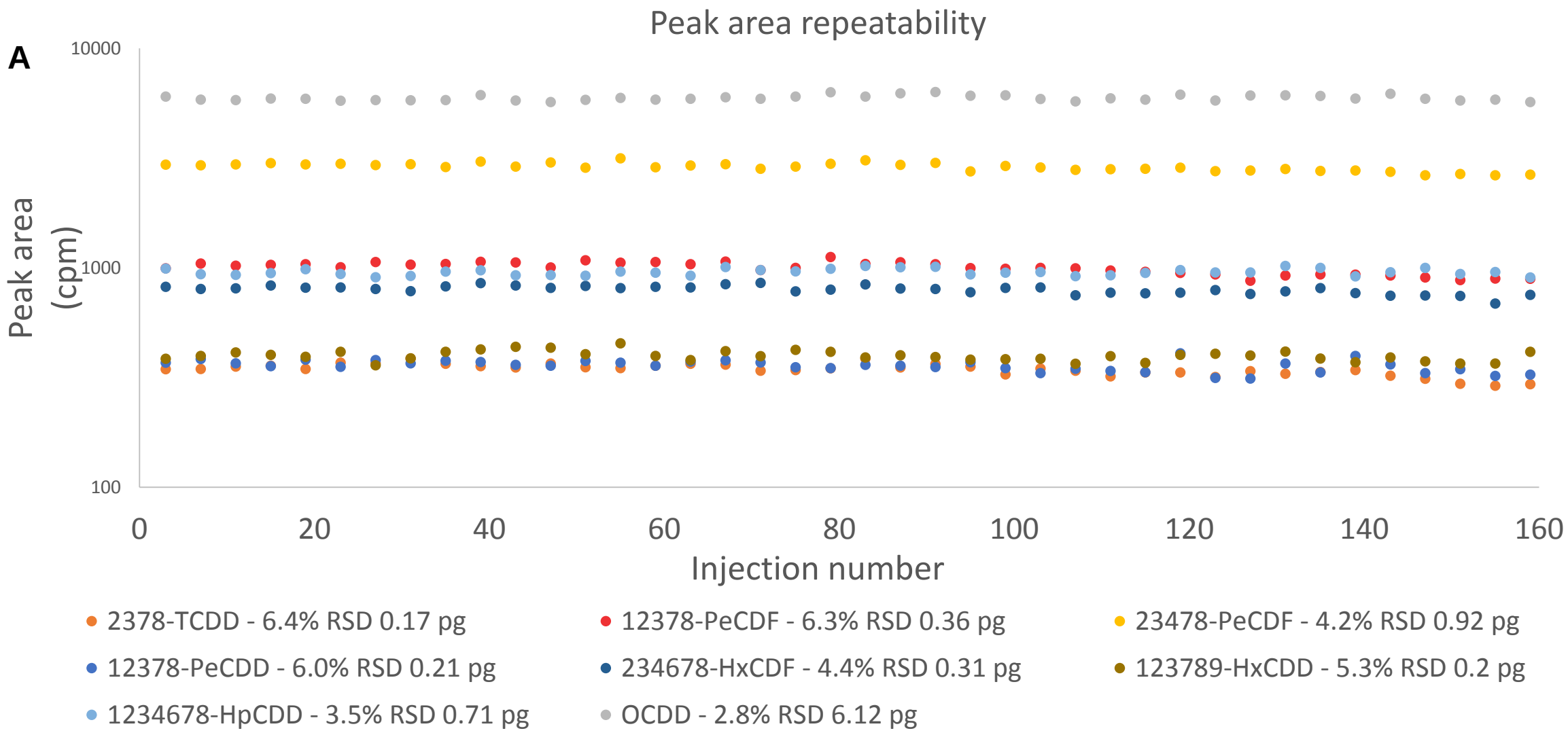
Long Term Robustness for Dioxin Analysis



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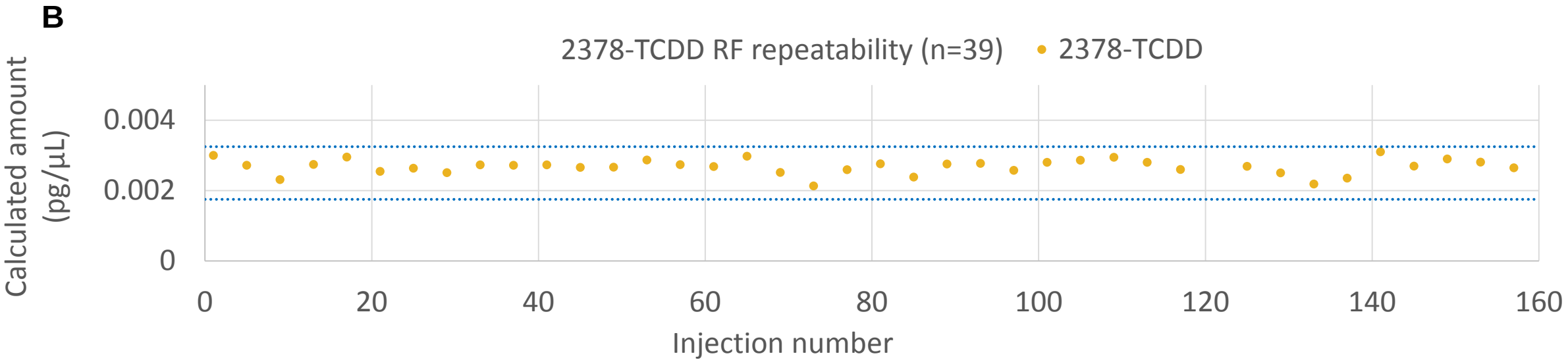
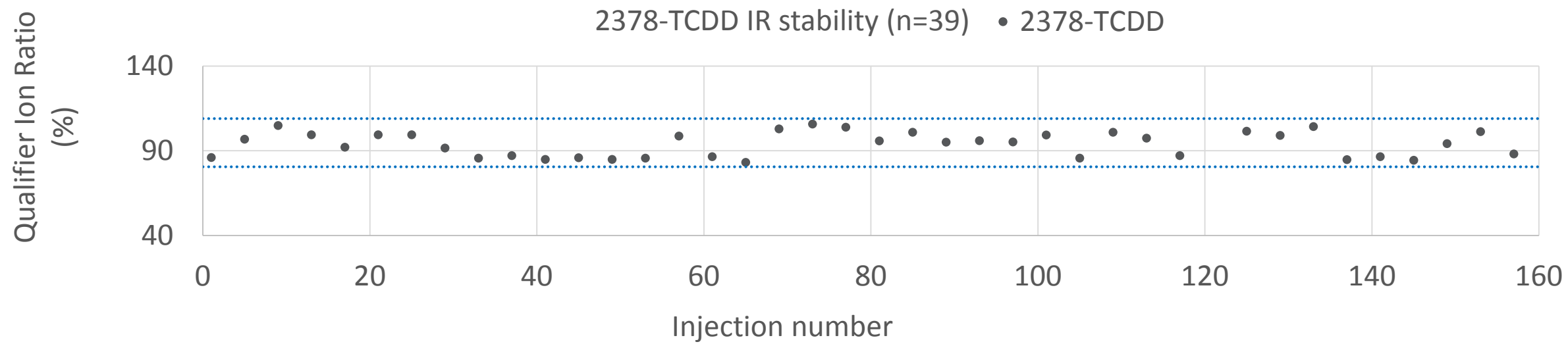
LOQ Calculation Reproducibility

B





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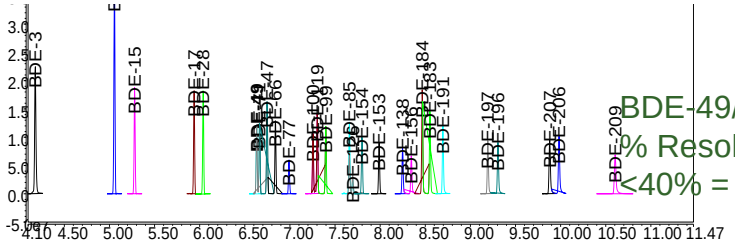
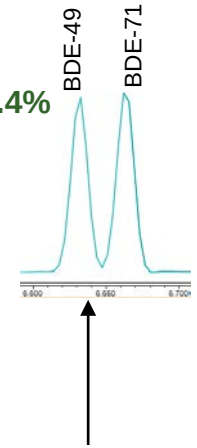
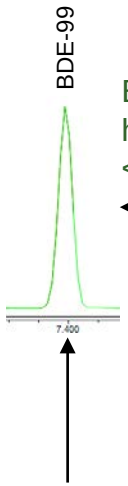
PBDE Analysis - Highest Calibration Standard



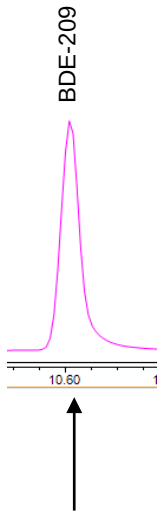
BDE-49/71
% Resolution = **0.4%**
<40% = PASS





BDE-99 USP Tf @10% height = **1.1**
<3 = PASS

BDE-209 USP Tf @10% height = **1.4**
<3 = PASS



Counts

Mono **Di** **Tri** **Tetra** **Penta** **Hexa** **Hepta** **Octa** **Nona** **Deca**

RT (minutes)

800 pg oc*	1600 pg OC	4000 pg OC

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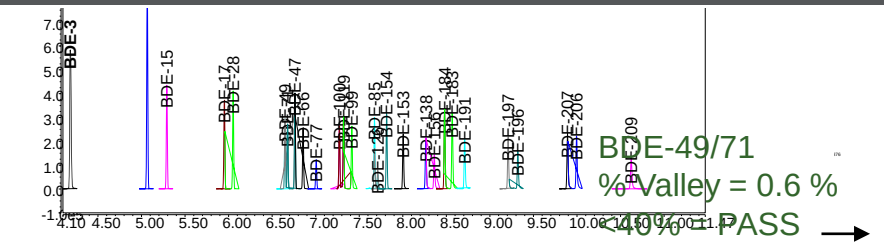
PBDE Analysis - Lowest Calibration Standard



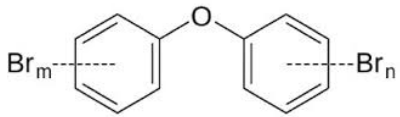
BDE-49/71
% Valley = 0.6 %
<40% = PASS
(EPA-1614 criteria)

BDE-49

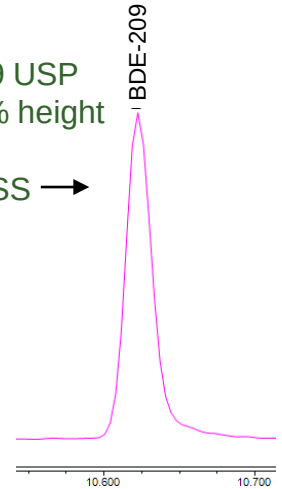
BDE-71

Counts

BDE-99 USP
Tf @10% height
= 0.8
<3 = PASS



BDE-209 USP
Tf @10% height
= 1.3
<3 = PASS



Mono **Di** **Tri** **Tetra** **Penta** **Hexa** **Hepta** **Octa** **Nona** **Deca**

RT (minutes)

2 pg oc*	4 pg OC	10 pg OC

- *oc = on column, solvent standard, overlay of quantification SRM transitions. (No signal normalization)

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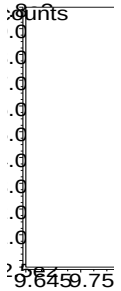
Sensitivity



BDE-209, 500 fg OC, n=15 injections
% RSD peak area = 7.7 %
Calculated IDL = 100 fg OC (0.1 ng/Kg in sample)

Counts

RT (minutes)

- Solvent standard 0.25 pg/uL
- t-score = 2.624
- n=15 injections
- n=14 degrees of freedom
- 99% confidence level
- Peak area % RSD < 15%

Excellent sensitivity for PBDEs using the AEI source

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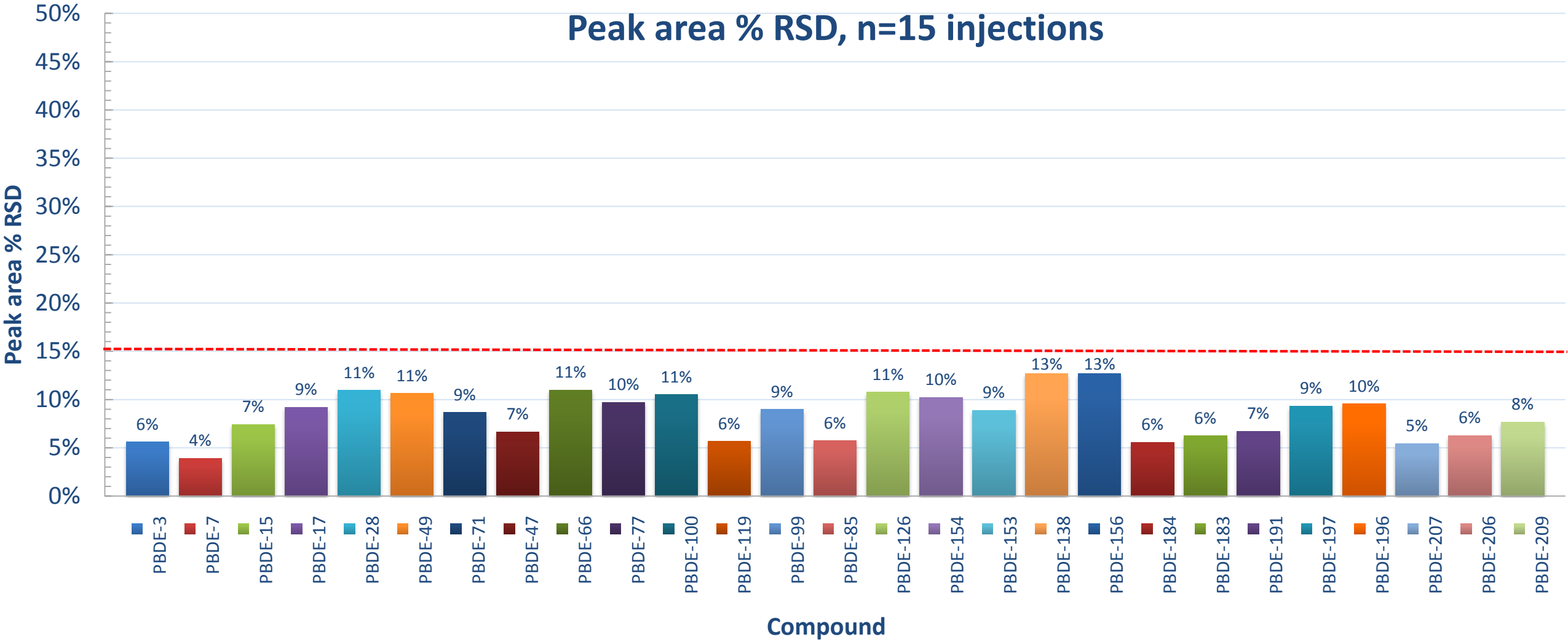
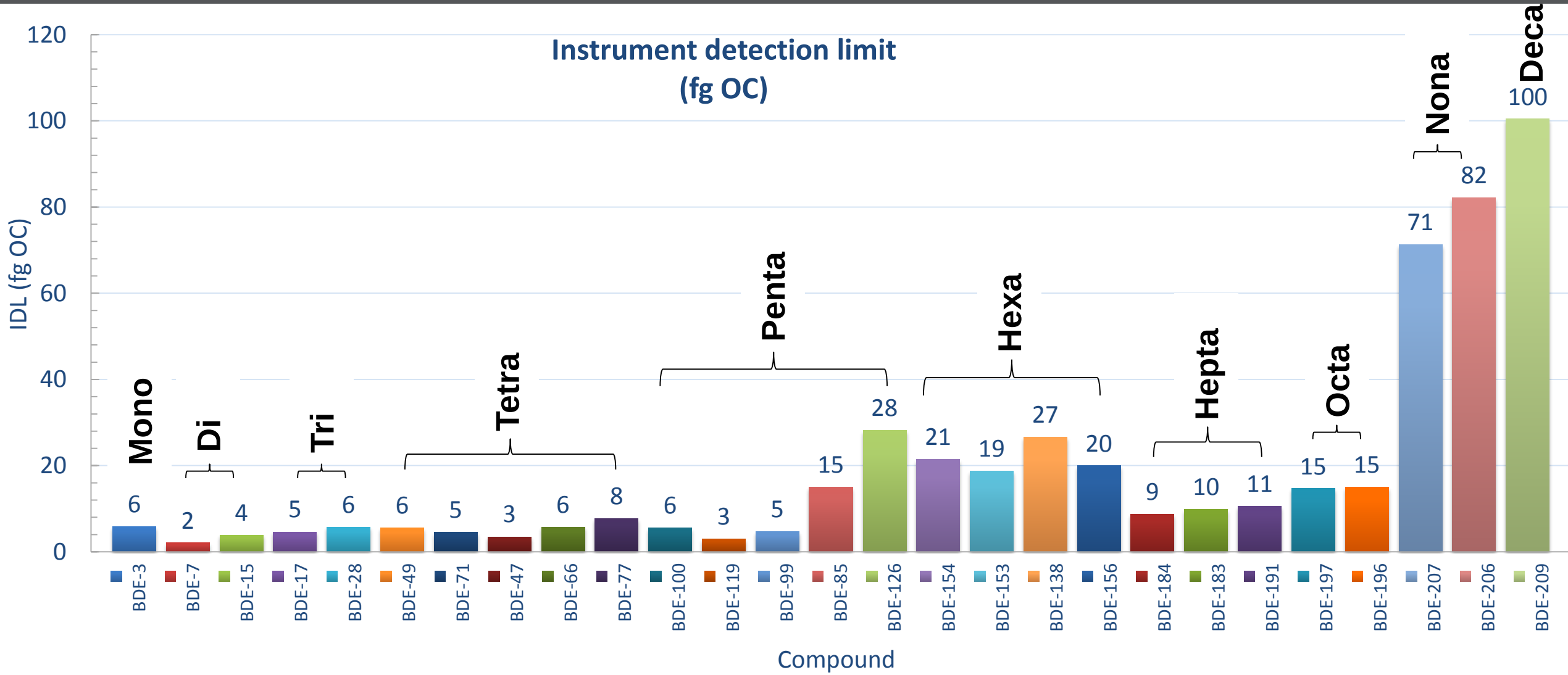
Peak Area Repeatability (IDL concentration)



- Solvent standards 0.02-0.25 pg/uL, t-score = 2.624, n=15 injections, n=14 degrees of freedom, 99% confidence level and peak area % RSD < 15%.

Instrument Detection Limit (IDL)



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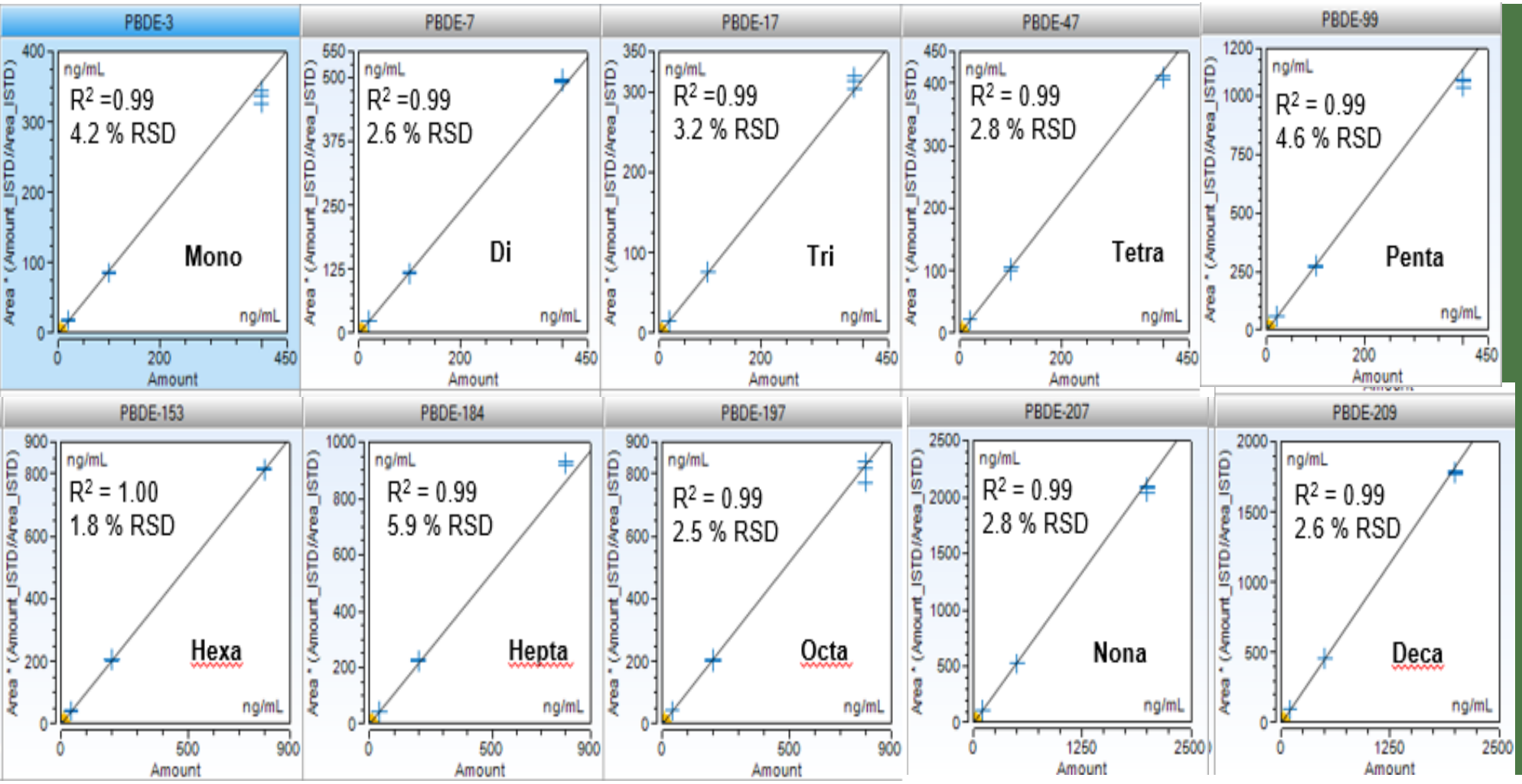
Example Linearity of Response

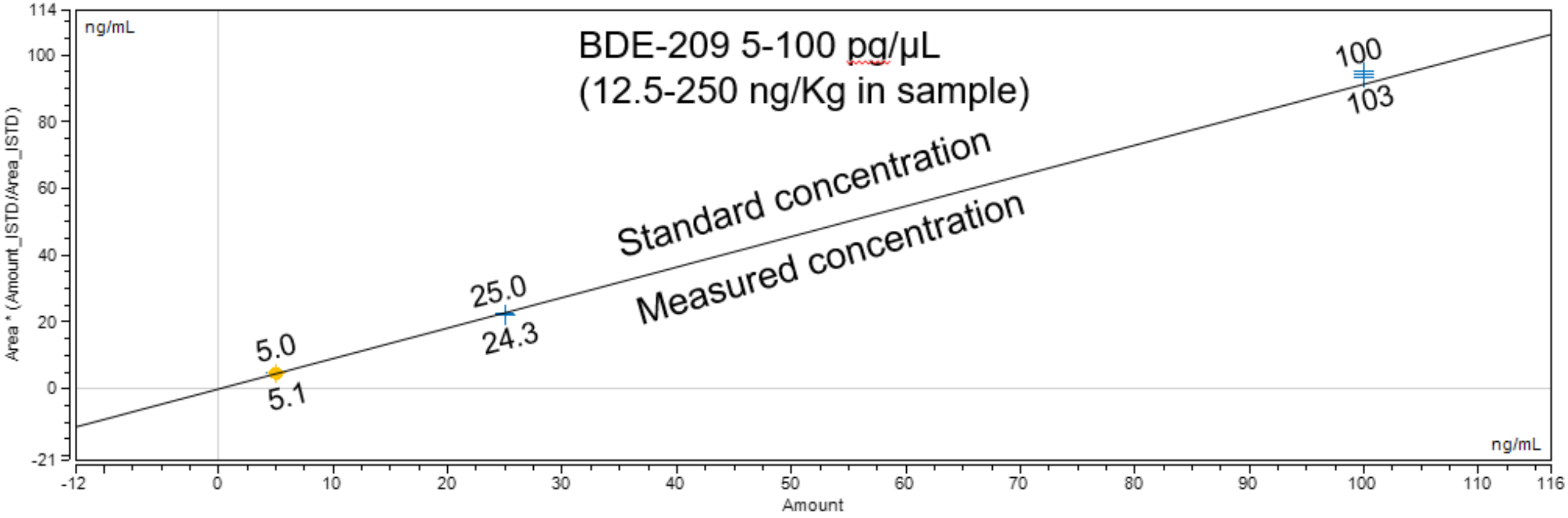


- Solvent standards
- 1.0-2000 pg/uL
- Internal standard adjusted with ^{13}C BDE stds
- No weighting applied as RRF was used
- Triplicate injection per level

Excellent linearity, R^2 > 0.98 and RRF % RSD < 10%

ThermoFisher SCIENTIFIC

Linearity of Response



Excellent low end precision and accuracy for triplicate standard injections

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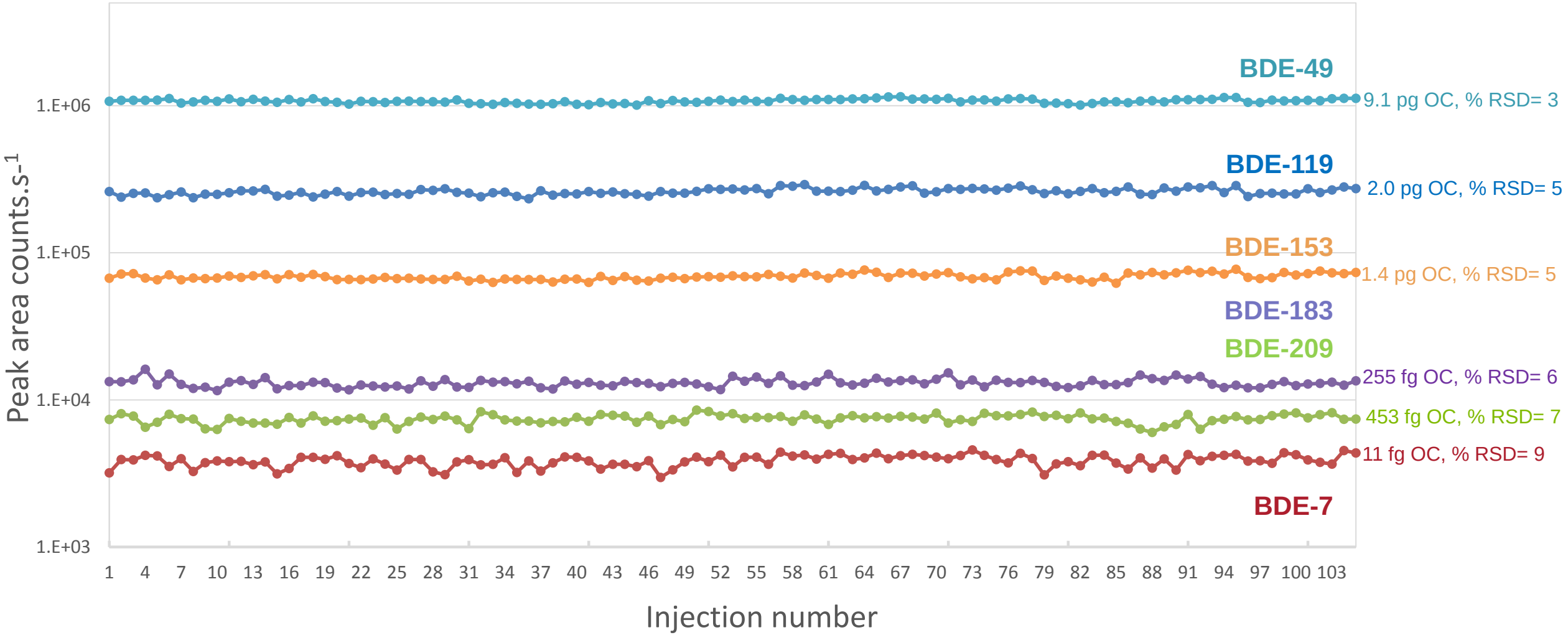
Robustness

Peak area repeatability: n=105 unspiked extracted fish matrix injections



Peak area stable (no inlet, column, MS maintenance or tuning)

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Orbitrap GC-MS Family

Redefining Routine GC-MS

RP 60,000 (FWHM @ *m/z* 200)

EI (VeV) / CI; Full-scan; Timed-SIM

NACRW 2016: Thermo Scientific™ Exactive™ GC Orbitrap™ GC-MS system

ASMS 2015: Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system

Unprecedented Depth in Analysis

RP 120,000 (FWHM @ *m/z* 200)

EI (VeV) / CI; Full-scan, Timed-SIM

MS/MS capability

Instrument.com 2015
China Outstanding
New Instrument

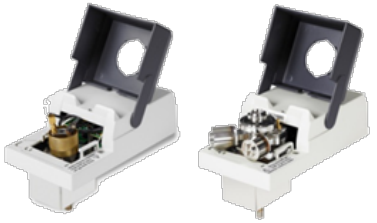
ThermoFisher SCIENTIFIC

Well Established and Proven Technologies

Orbitrap mass analyzer

Incredible HR/AM performance

Highly regarded Q Exactive platform



TRACE 1310 GC

Unique modular injector and detector design

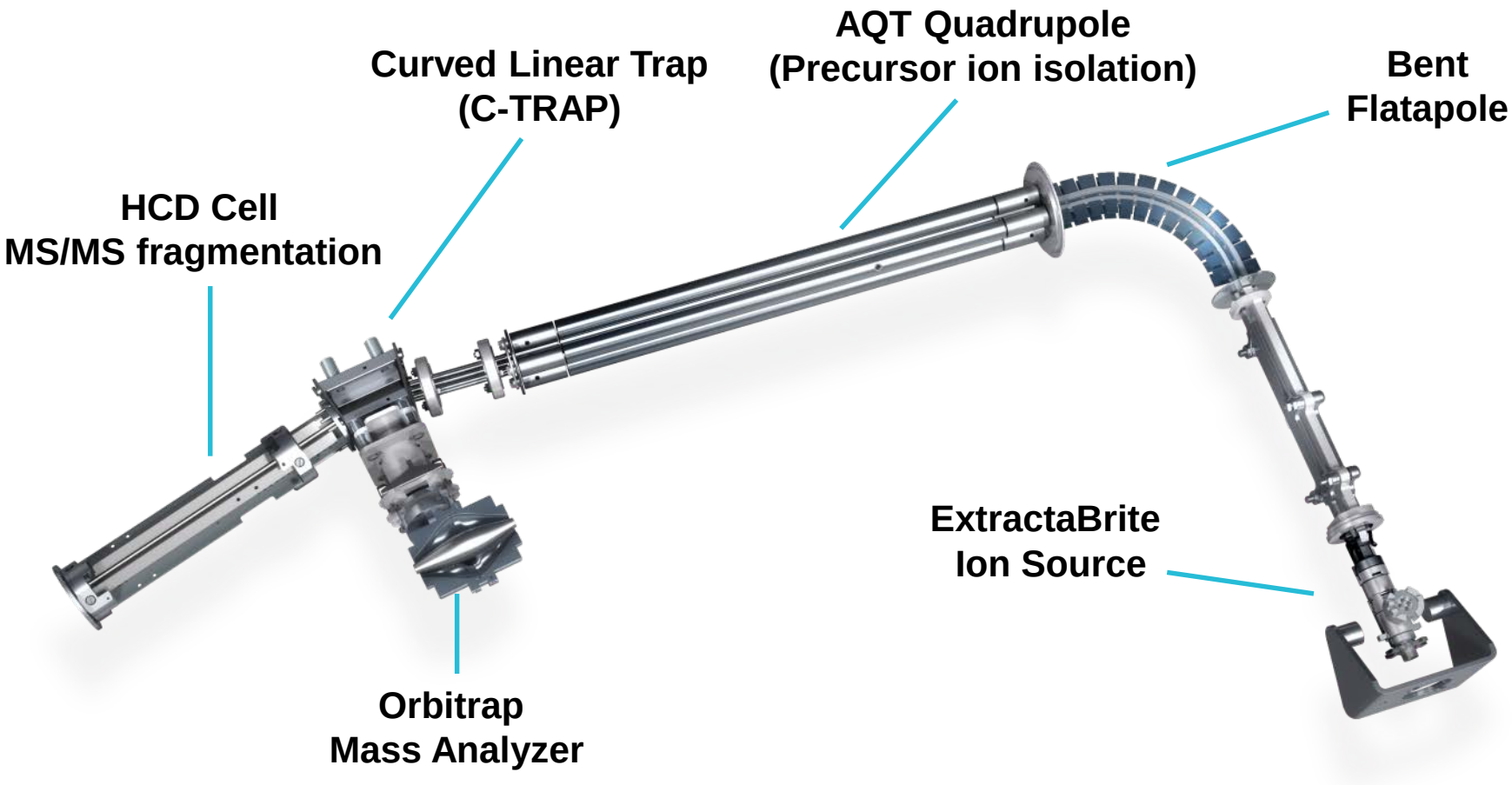
Rapid heat cycling

NeverVent™ technology

ExtractaBrite™ ion source

V-Lock source plug

Thermo Scientific Q Exactive GC Orbitrap GC-MS/MS System : The Technology Inside



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Orbitrap GC System Highlights

Resolving Power

Up to 120,000 at *m/z* 200

Mass Accuracy

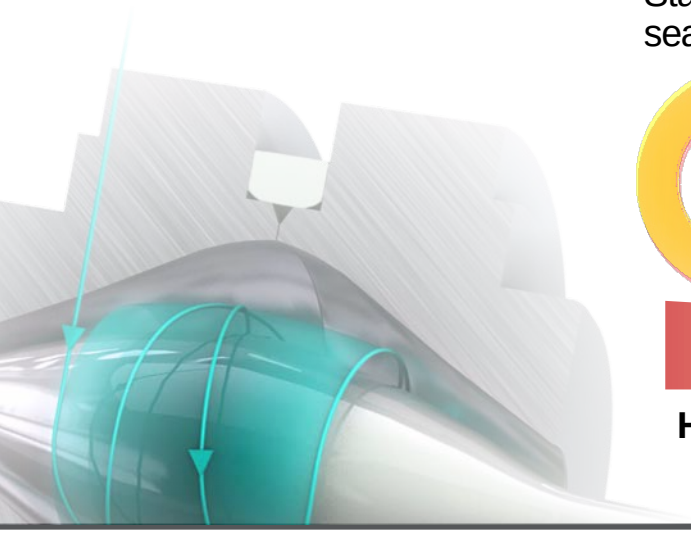
< 1ppm

Sensitivity

ppt

Dynamic Range

>6 orders



- Standard library searchable spectra

EI 70eV

- Molecular ion confirmation (incl. adducts)

CI

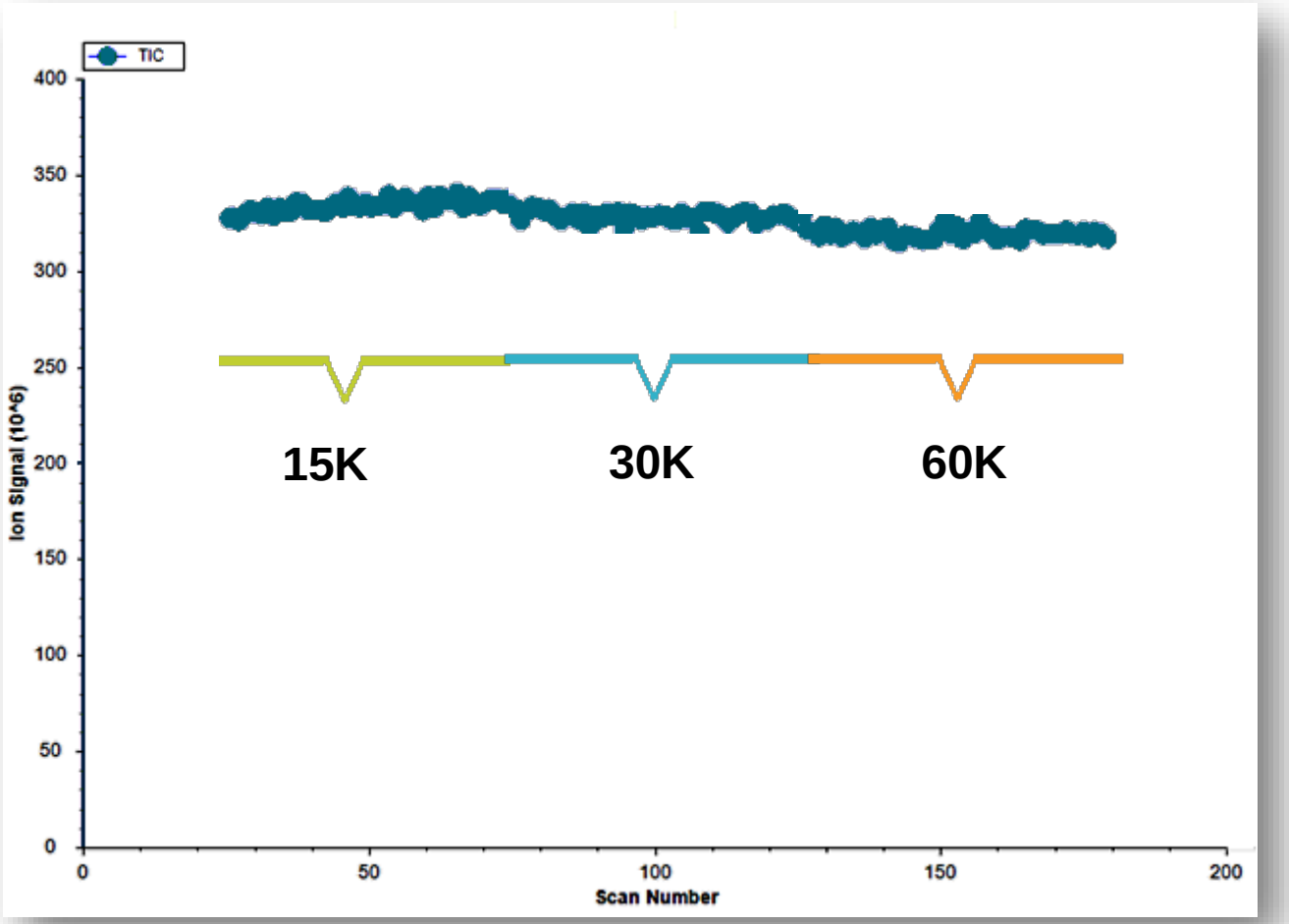
Harder

Softer

Ionization Range

ThermoFisher SCIENTIFIC

Achieve Maximum Resolution and Maintain Sensitivity



- TIC signal intensity vs. scan number
- Increase the resolving power during acquisition
- Negligible drop in sensitivity

High selectivity analysis all of the time

EURL European Union Reference Laboratory Dioxins and PCBs

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Advantages for POPs analysis

Key Benefits

- Fast instrument and method set-up
- Method consolidation
- High efficiency data processing
- Quantitative and qualitative information in a single injection
- Adjustable scope of analysis
- Retrospective data analysis

Polybrominated Diphenyl Ethers

C S B Consorci Sanitari de Barcelona

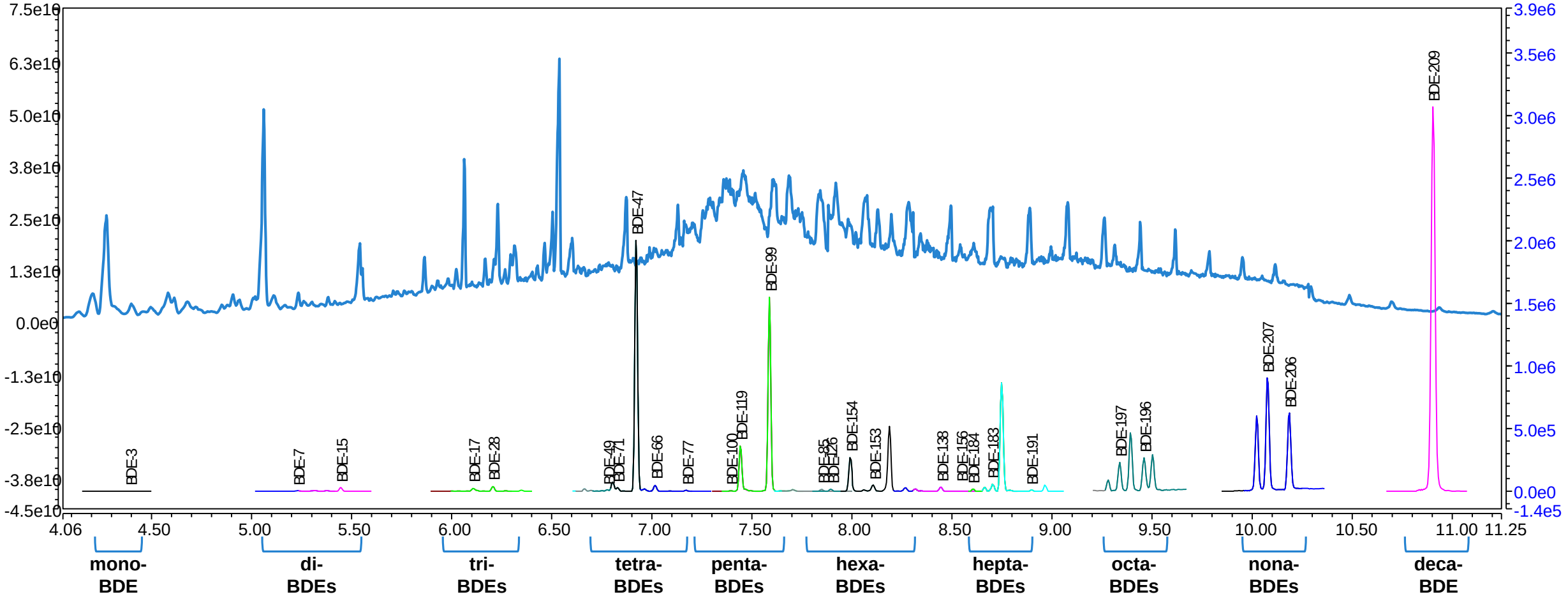
Agència de Salut Pública

Persistent Organic Pollutants

"GC-Orbitrap is a useful tool for control labs, **outstanding sensitivity**…**high selectivity** when analyzing complex matrices…**high robustness** …helps to fulfill new regulations with extremely low limits (e.g. PCBs, PBDEs)"

Practical Experiences of Implementing POPs Methods using Orbitrap™ GC-MS. Nuria Cortés-Francisco, BFR 2017

PBDE Analysis of Environmental Samples



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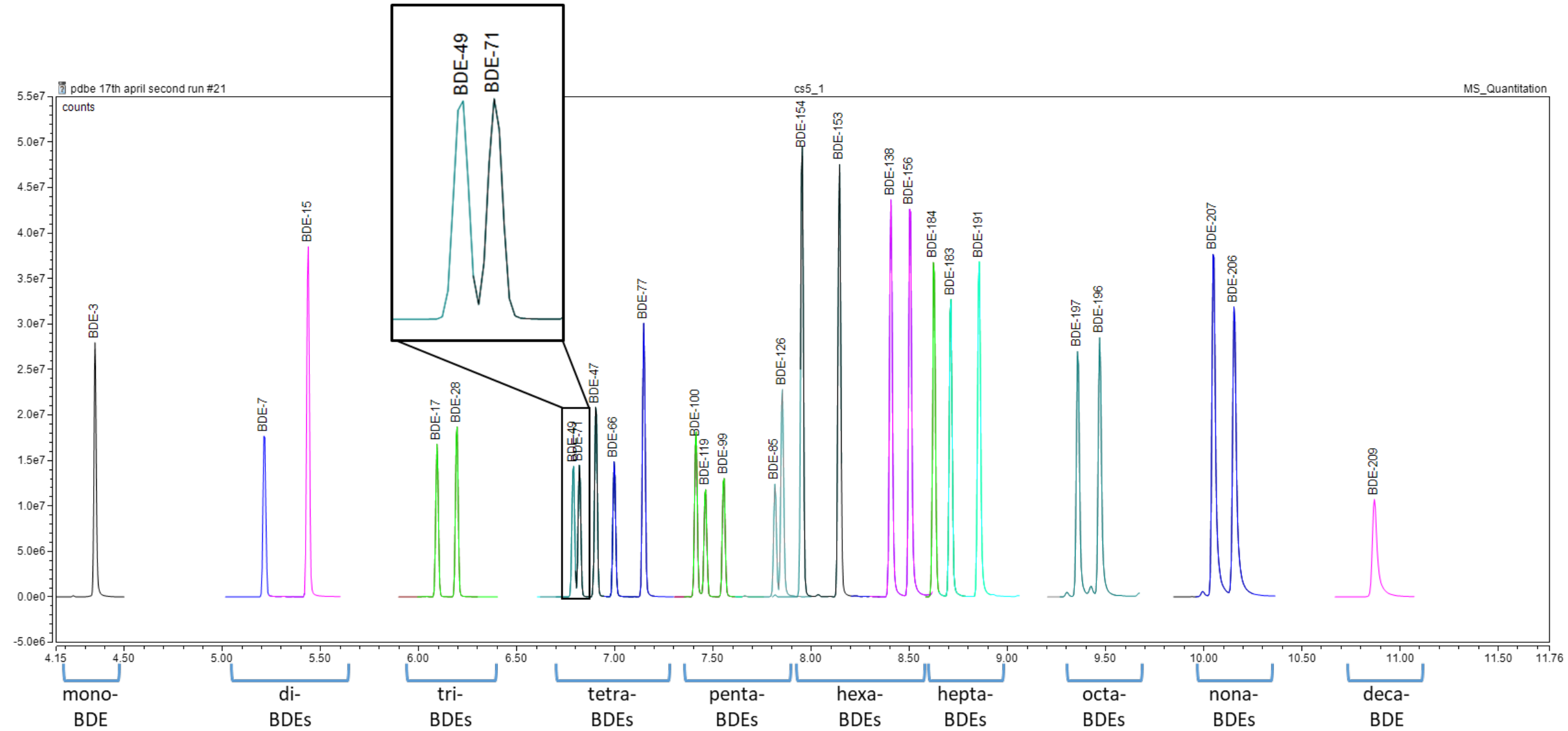
Chromatography – Extracted Ion Chromatogram



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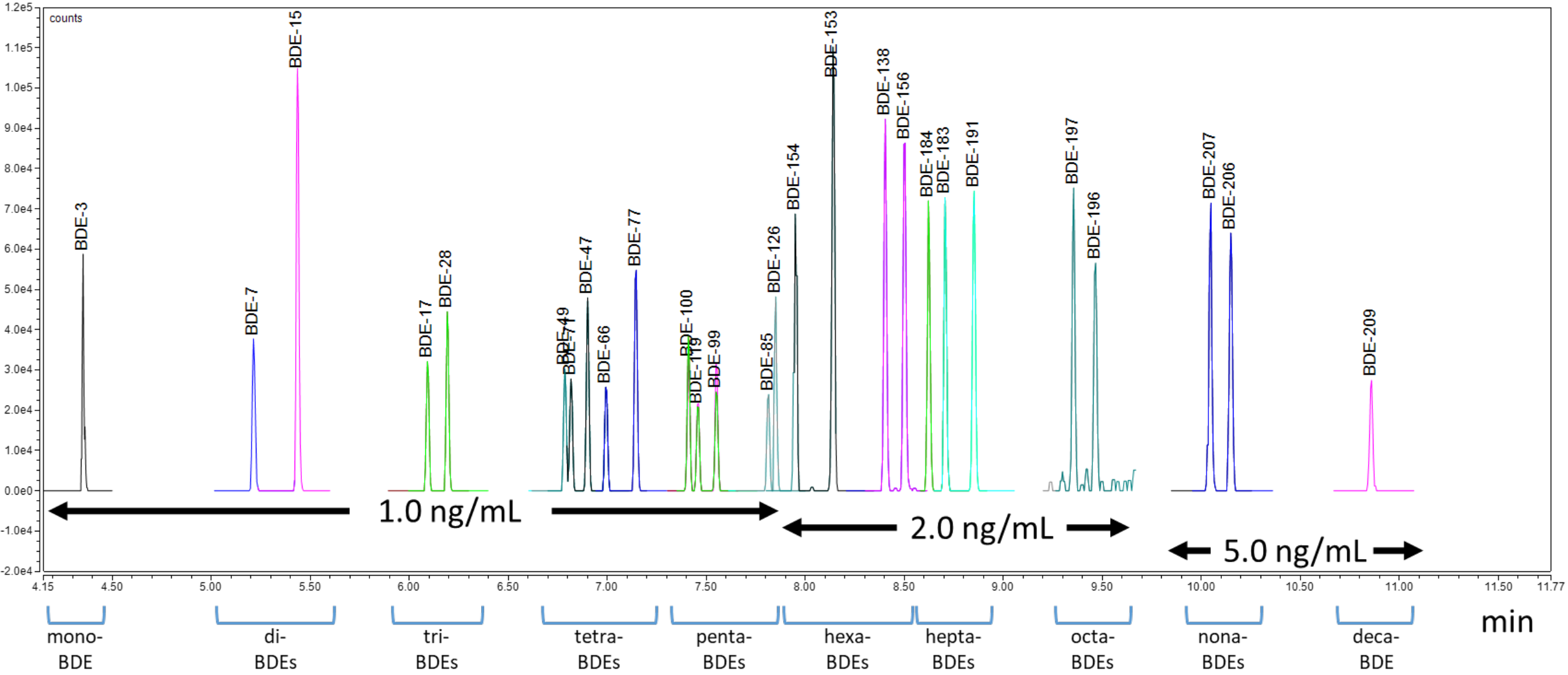
Sensitivity



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Tuna Sample Spiked 0.01 ng/g PBDEs

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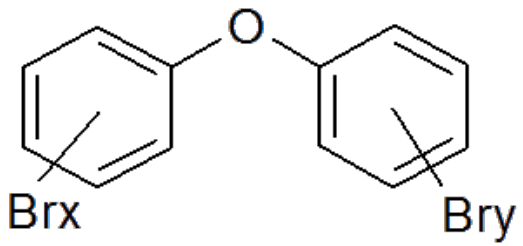
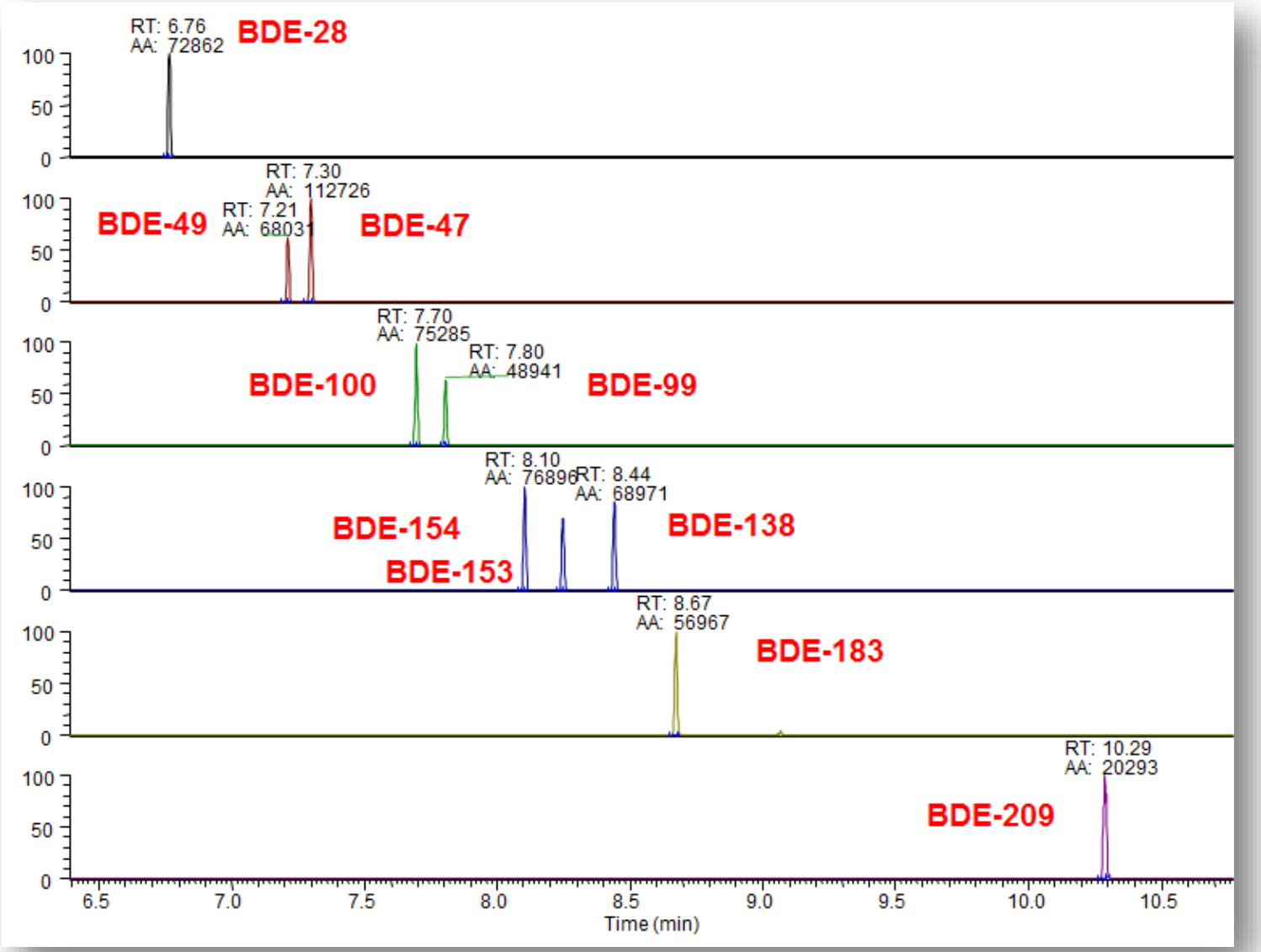
Agència de Salut Pública



x=1-5, y=0-5



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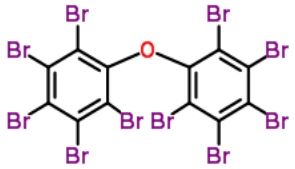
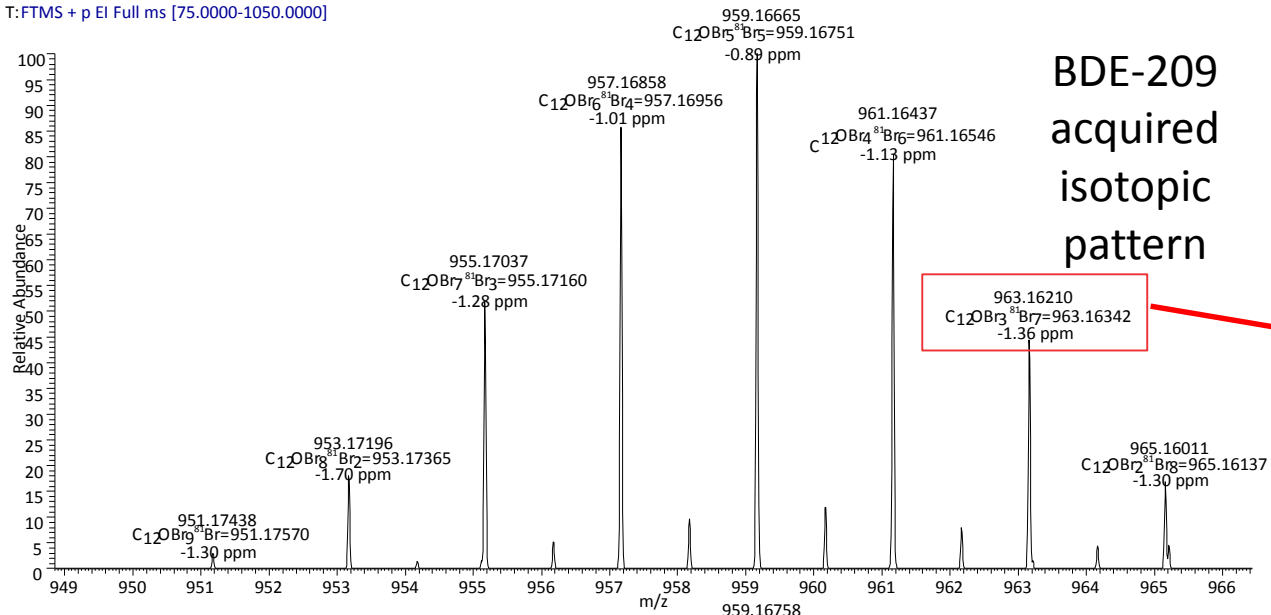
Mass Accuracy and Isotope Pattern

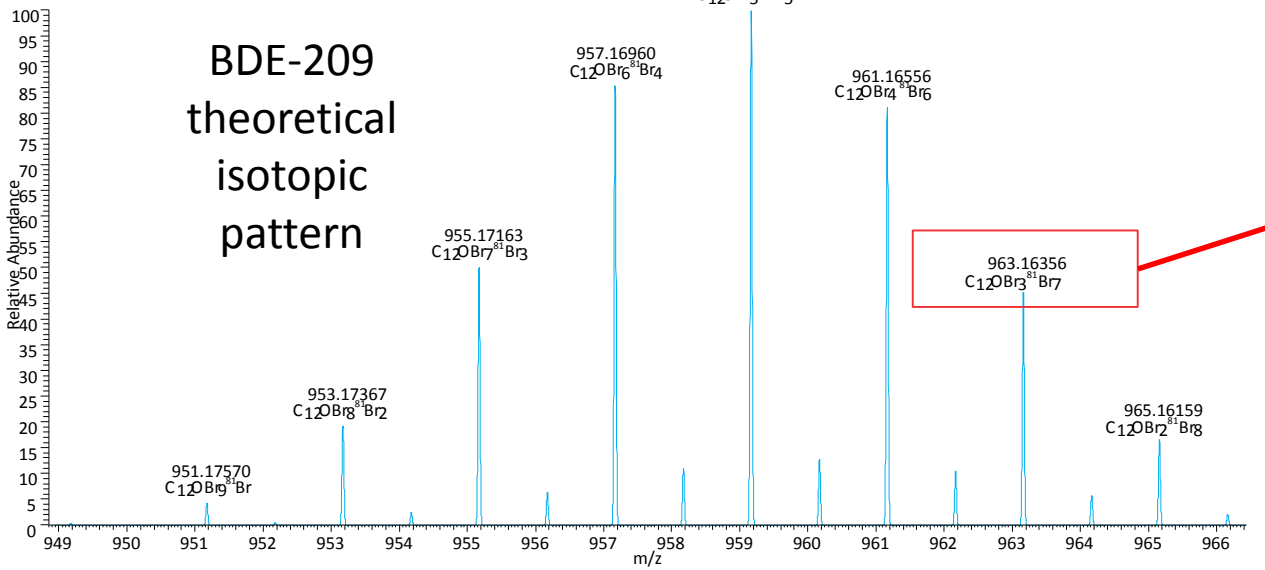


BDE-209



BDE-209 acquired isotopic pattern

963.16210
$C_{12}OBr_3{}^{81}Br_7 = 963.16342$
-1.36 ppm



BDE-209 theoretical isotopic pattern

963.16356
$C_{12}OBr_3{}^{81}Br_7$

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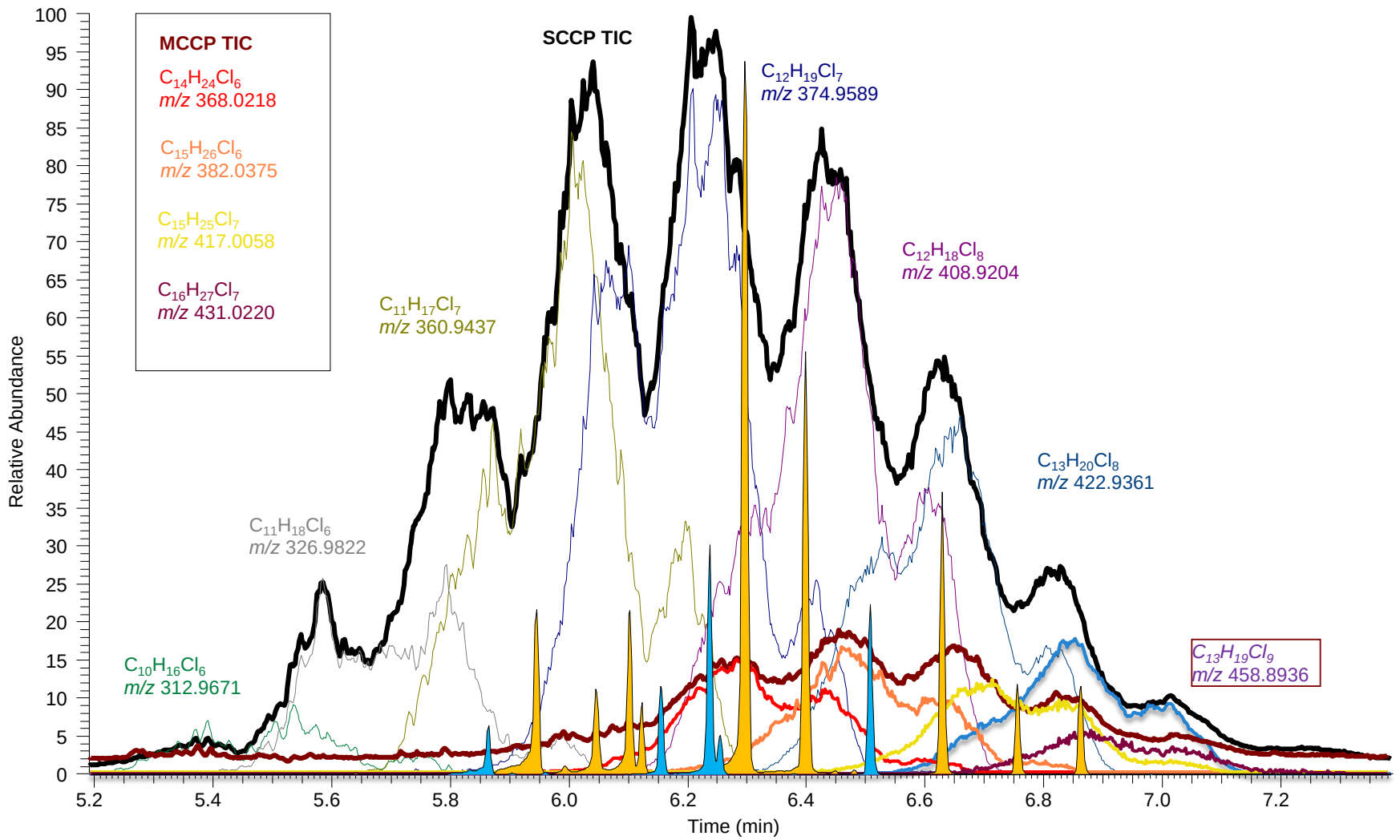
POPs in Salmon: A New Approach using High Resolution Full Scan

SCCPs
MCCPs
PCBs,
Pesticides
Toxaphenes



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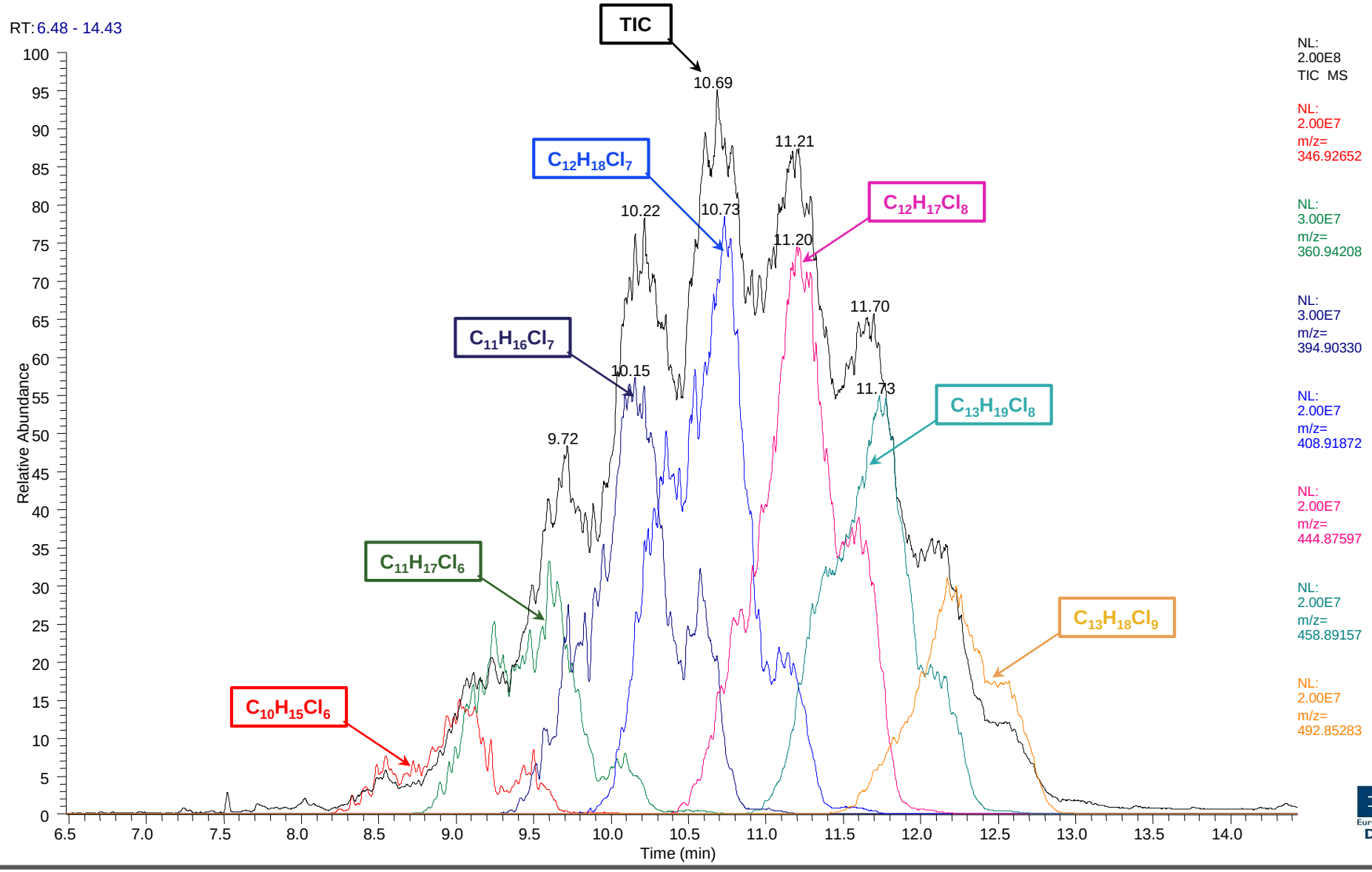
HRAM Selectivity 63% C_{10}-C_{13} Technical Mix, NCI, 60k Resolution



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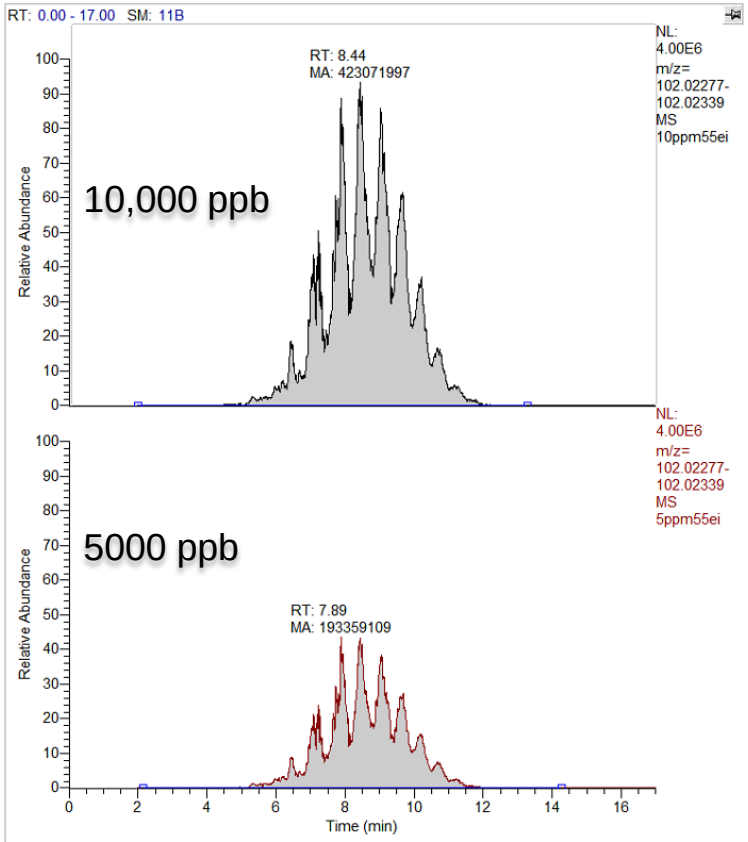
SCCP Linearity

- Linearity was assessed using the following dilution series for each SCCP chlorination degree (63% and 55.5%):
  - **25**
  - **50**
  - **100**
  - **250**
  - **500**
  - **1,000**
  - **5,000**
  - **10,000**



- Concentrations as **pg/µL**.
- In each standard, ~400 pg/µL p-Terphenyl-d14 was added and used as an IS

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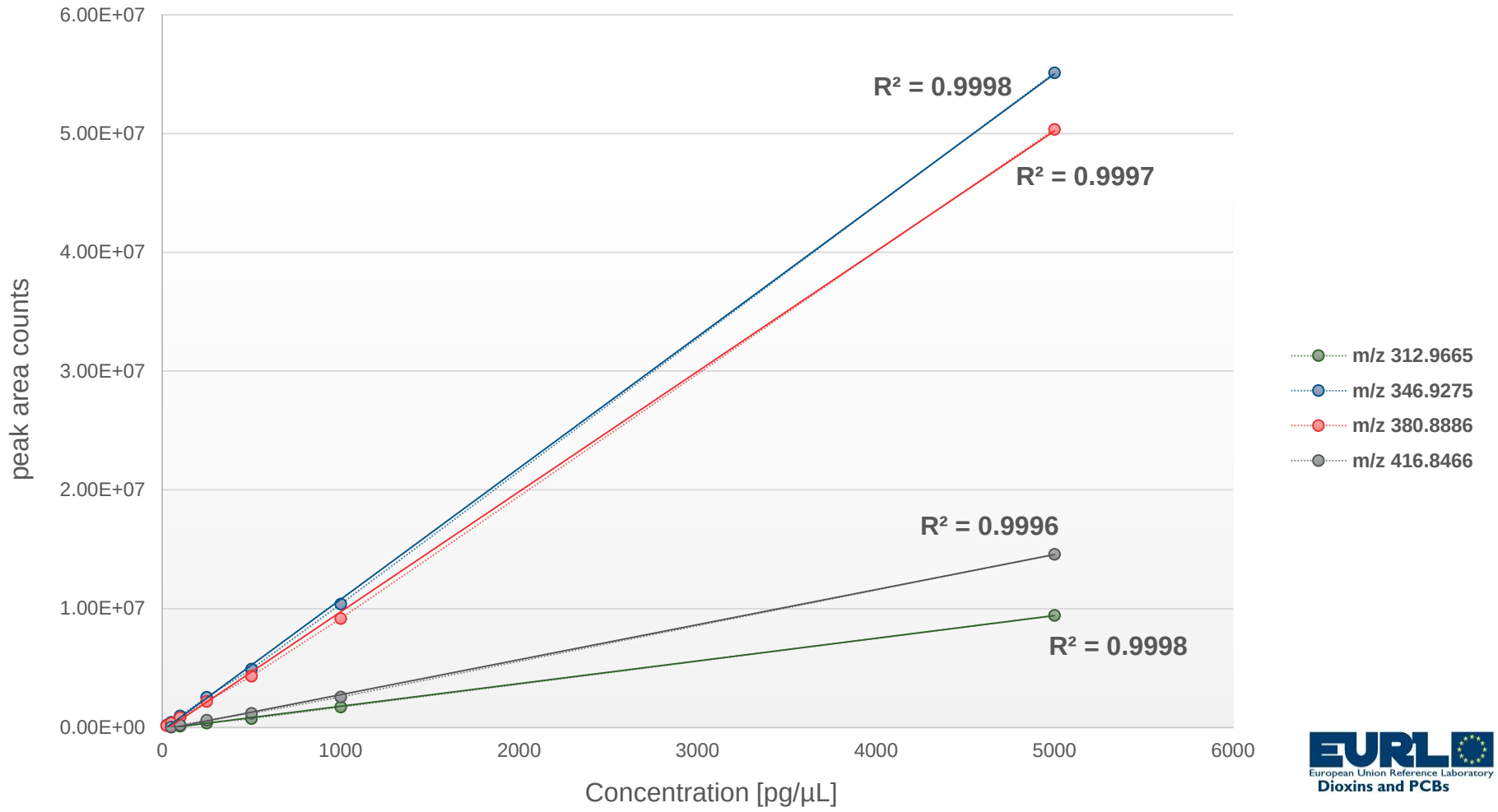
Negative Chemical Ionization: Linearity for SCCP 63% C10

Excellent compound linearity (25-5,000 ppb) was obtained for these C10 chlorinated homologues present in the SCCP 63% technical mixture.



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Peak Area Repeatability

- Peak area repeatability using full-scan NCI across n=10 repeat injections of 25 ppb solvent standard (63% C_{10}-C_{13}).

inj. no	*m/z* 492.8546 ($C_{13}H_{18}Cl_9$)	*m/z* 458.8936 ($C_{13}H_{19}Cl_8$)
1	765881	1308232
2	822551	1428540
3	795041	1361253
4	781911	1363928
5	776597	1321808
6	731874	1250508
7	761201	1305483
8	749797	1284342
9	737987	1257718
10	757772	1286412
mean	768061	1316822
StDev	27217.2	54540.3
%RSD	**3.5**	**4.1**

Chlorinated Paraffins, PCBs, toxaphenes, pesticides

EURL
European Union Reference Laboratory
Dioxins and PCBs

"using high resolution, accurate mass Orbitrap-MS **enables much deeper insights** into the pattern and content of CPs **without having to fear mass interferences** from other CPs or halogenated compounds such as PCBs. Preliminary results suggest that determination of both CPs and PCBs in the same sample in one run is possible, representing **a potential for shorter sample preparation and quicker analyses** of these types of POPs in food"

HIGH RESOLUTION ACCURATE MASS SCREENING FOR CHLORINATED PARAFFINS IN FOOD SAMPLES USING GC-ORBITRAP MASS SPECTROMETRY (Kerstin Krätschmer et al. Dioxin 2017)

ThermoFisher SCIENTIFIC

Summary - POPs Portfolio

- Analysis of POPs is a challenging and varied application
- Legislation changes are allowing methods to be run on different instrumentation
- Thermo offers instrumentation to meet all the requirements for current and future analysis

Magnetic Sector GC-HRMS

Thermo Scientific™ DFS™ Magnetic Sector GC-HRMS

- Target compound analysis in routine
- World-Wide compliance (e.g. EPA 1613)
- Sensitivity combined with robustness for routine analysis
- Robust-by-size with large volume ion source

GC-MS Triple Quadrupole

Thermo Scientific™ TSQ™ 9000 GC-MS/MS

- Target compound analysis in routine
- Ease-of-use
- Compliant with EU regulations for Dioxin food & feed analysis
- Great price/performance ratio

Orbitrap GC-MS

Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS

- Untargeted compound analysis
- Method consolidation
- Highest resolution and mass accuracy
- Unique unknown identification capabilities

Thank You!

Q & A

ThermoFisher SCIENTIFIC

Thank You

Please return our survey to receive a drink ticket for our daily networking event where you can continue discussions with our experts!