Analysis of Environmental Contaminants in Surface Water and Wastewater Effluents Using GC/Q-TOF

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Agilent

Trusted Answers

DE6667592593

Growing Interests in Broad Scope Screening of Contaminants

- 1000+ pesticides in use or remain in environment
- Other environmental pollutants are also of concern
- High sensitivity and selectivity needed to meet MRLs in “dirty” matrices
- Growing interests in broadest scope and even non-targeted screening for risk assessment

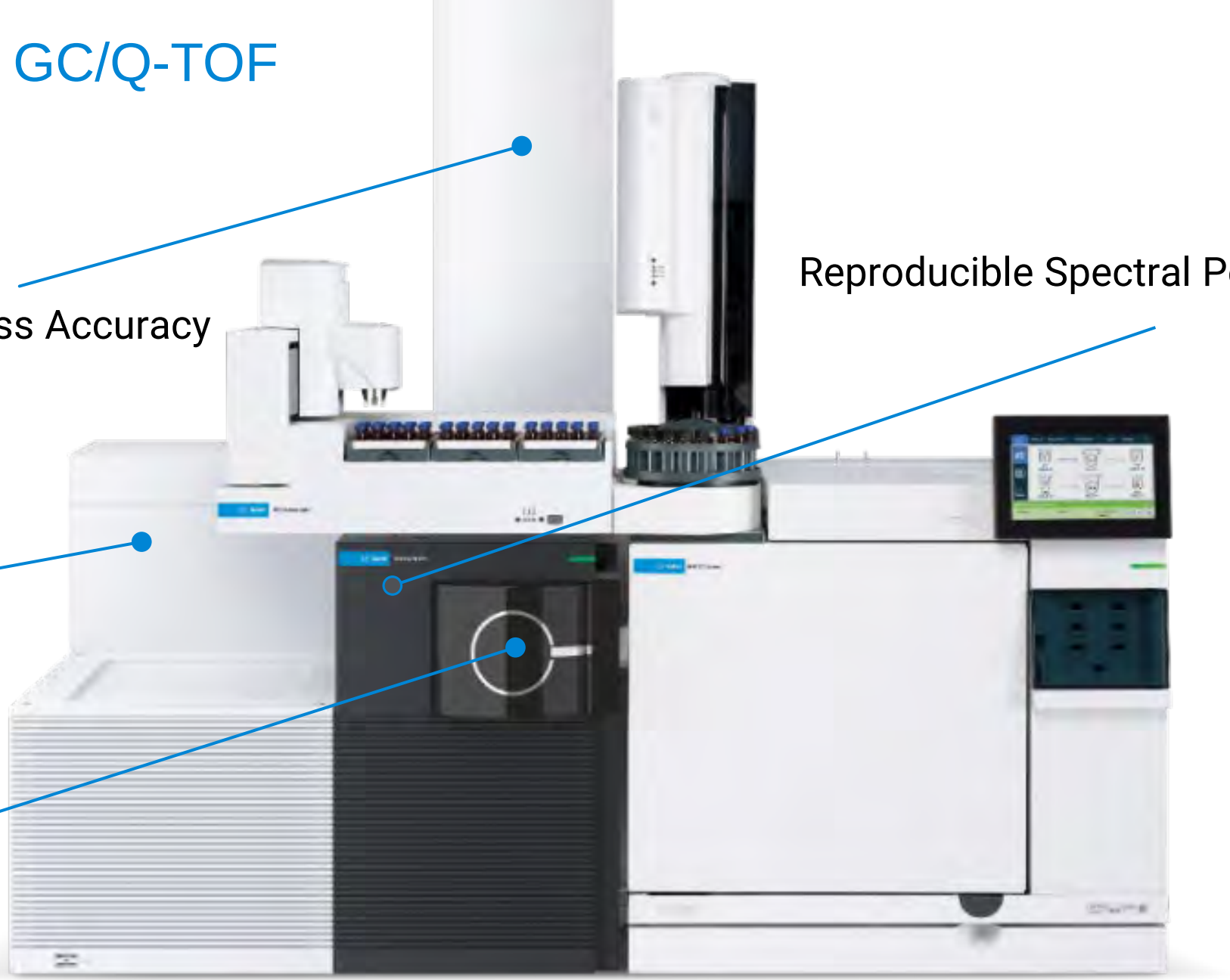
The Agilent 7250 GC/Q-TOF

High Resolution and Mass Accuracy

Reproducible Spectral Performance

Simultaneous
High Resolution and
Wide Dynamic Range

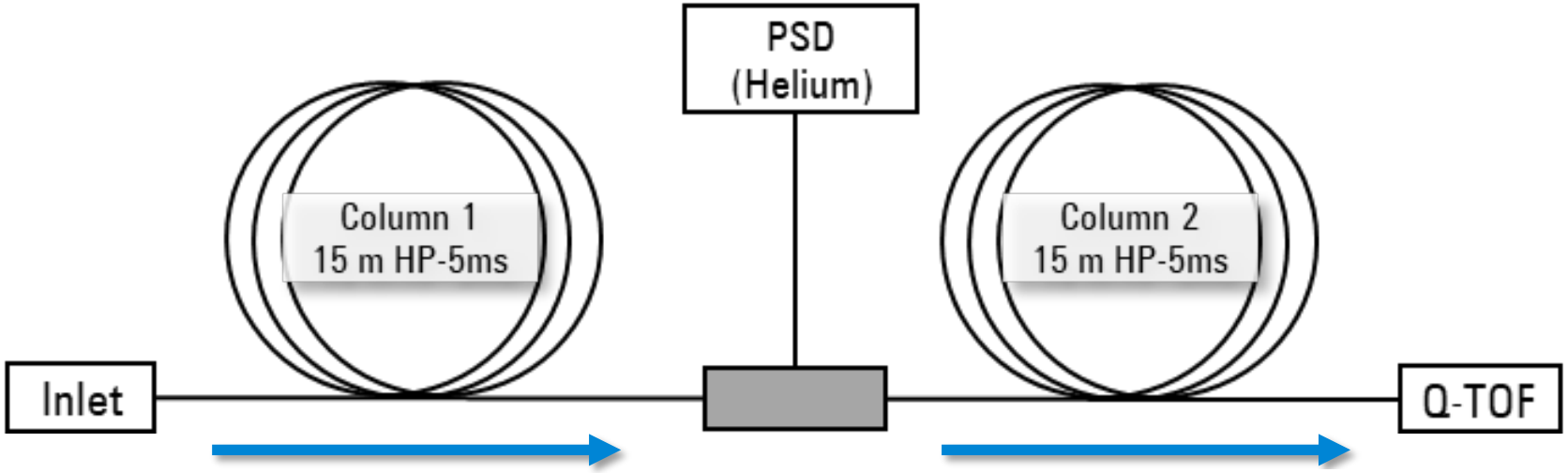
Sensitive Low Energy EI
CI source



GC/Q-TOF Acquisition Modes Used in the Study

- Standard EI (70 eV)
- Negative CI (Methane reagent gas)
- Positive CI (Methane reagent gas)
- Low Energy EI (12 eV)
- MS/MS (Accurate Mass Product Ion Spectra)

GC Configuration with Backflush



- ✓ Reduced run times
- ✓ Enhanced RT stability
- ✓ Longer column lifetime
- ✓ Less ion source contamination

Outline of the Workflow for Screening with GC/Q-TOF

Acquire full-spectrum data

Targeted Method

Calibration standards?

Yes

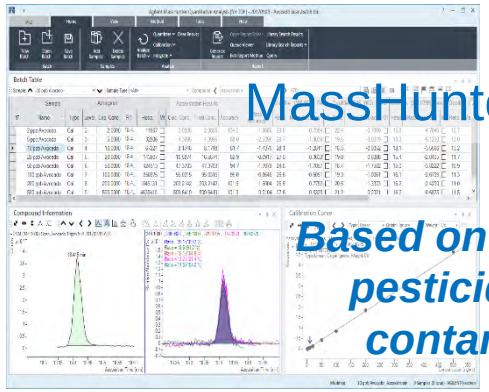
No

Peak Picking

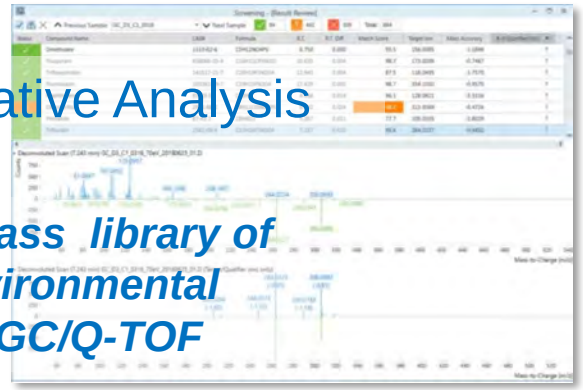
Target Quantitation

Suspect Screening

Non-Target Analysis

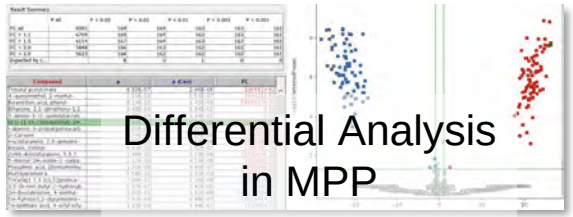


MassHunter Quantitative Analysis



Based on accurate mass library of pesticides and environmental contaminants for GC/Q-TOF

Deconvolution followed by matching public libraries



Differential Analysis in MPP

FDA/09/24/15 Guidelines for Identity Confirmation

Table 1. Summarized Requirements for Confirmation of Identity

MS mode	MS[1]	MS/MS	MS[1] and MS/MS
EIC: signal requirement (absolute)	A criterion to be set by one of the following methods: (1) a S/N threshold ≥ 3; (2) an intensity ratio relative to the comparison standard equal or above a preset threshold		
EIC: retention time, relative to comparison standard	A criterion to be set by one of the following methods: (1) ≤ 0.2 min, or (2) within ±2.5%, not to exceed 0.5 min, or (3) within an established error range, not to exceed 0.5 min		
MS: number of structurally significant ions	Minimum 2	Minimum 2	Minimum 2 combined
MS: mass accuracy	≤ 5 ppm	≤ 10 ppm	MS[1]: ≤ 5 ppm; MS/MS: ≤ 10 ppm

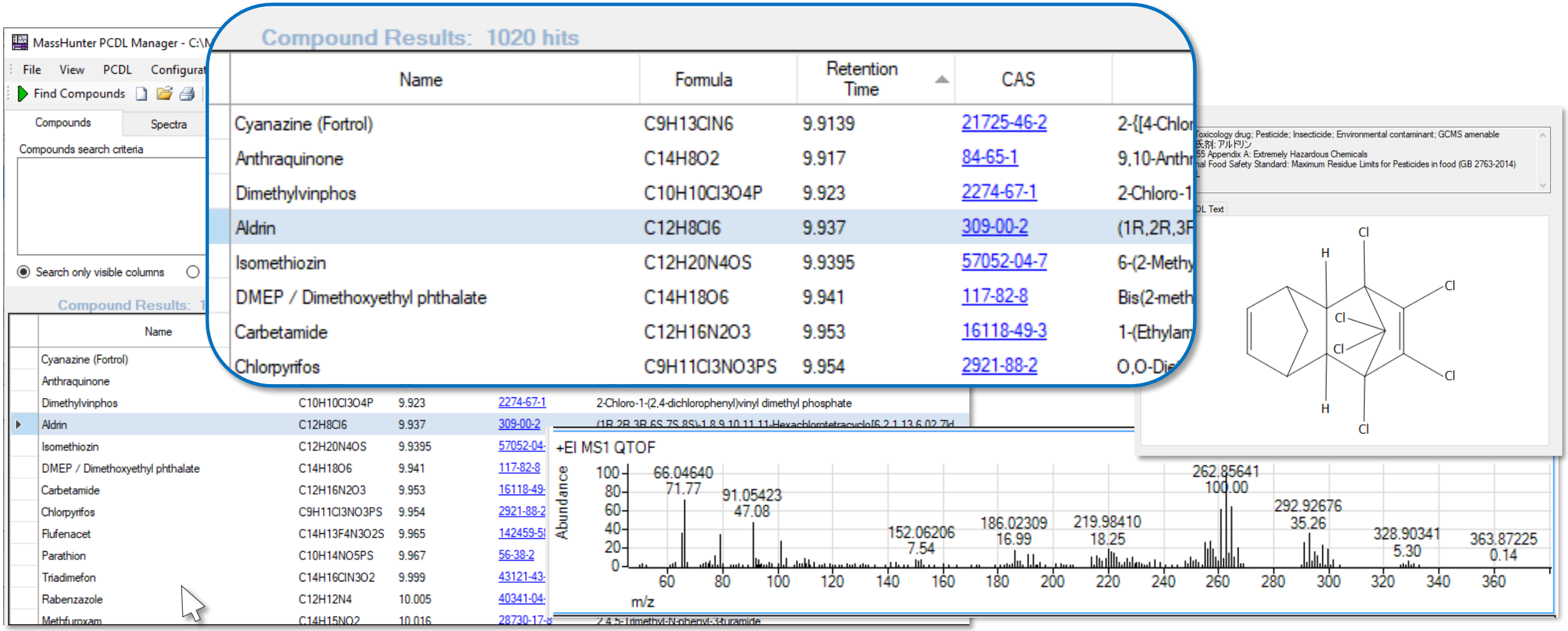
SANTE/12682/2019 Guidelines for Identity Confirmation

MS detector/Characteristics		Acquisition	Requirements for identification	
Resolution	Typical systems (examples)		minimum number of ions	other
Accurate mass measurement	High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS	full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof	2 ions with mass accuracy ≤ 5 ppm[a, b, c]	S/N ≥ 3[d] Analyte peaks from precursor and/or product ion(s) in the extracted ion chromatograms must fully overlap. Ion ratio: see D12

[a] preferably including the molecular ion, (de)protonated molecule or adduct ion
[b] including at least one fragment ion
[c] < 1 mDa for m/z < 200
[d] in case noise is absent, a signal should be present in at least 5 subsequent scans

Accurate Mass Library of Pesticides and Environmental Contaminants (EI)

1,000+ compounds

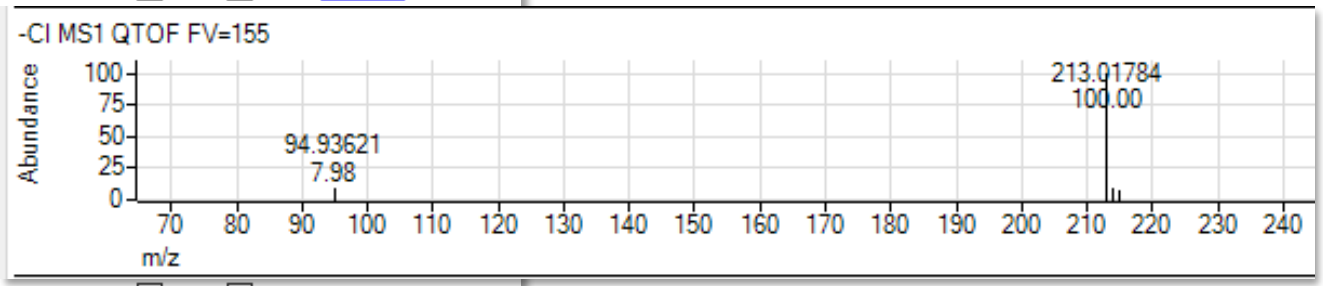


Accurate Mass Library of Environmental Contaminants for Negative CI

100+ compounds

Compound Results: 118 hits

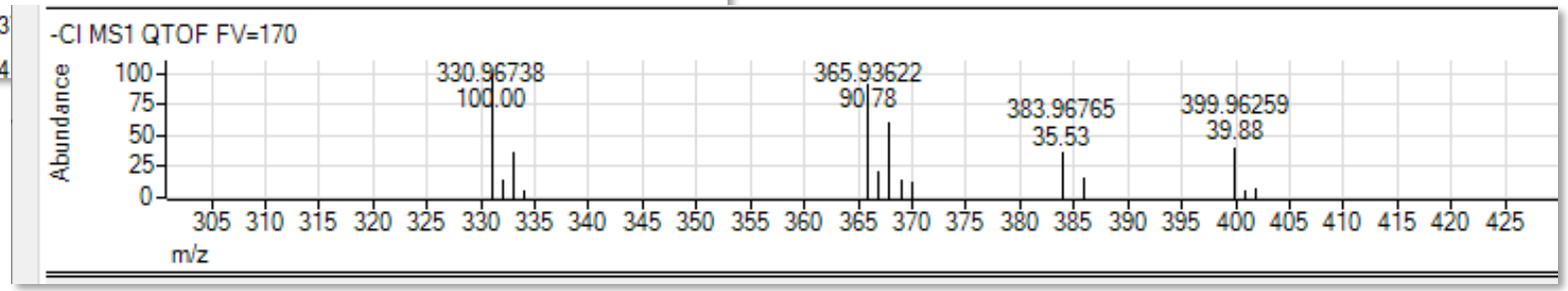
	Name	Formula	Mass	Retention Time	Cation	Anion	CAS
	Trifluralin NCI	C13H16F3N3O4	335.10929	7.27	☐	☐	1582-09-8
	Benfluralin NCI	C13H16F3N3O4	335.10929	7.303	☐	☐	1861-40-1
▶	Cadusafos NCI	C10H23O2PS2	270.08771	7.449	☐	☐	95465-99-9
	Phorate (Isothioate) NCI	C7H17O2PS3	260.01283	7.529	☐	☐	298-02-2
	BHC-alpha NCI	C6H6Cl6	287.86007	7.663	☐	☐	319-84-6
	Hexachlorobenzene (HCB) NCI	C6Cl6	281.81312	7.795	☐	☐	118-74-1
	Dicloran (Dichloran) NCI	C6H4Cl2N2O2	205.96498	7.83	☐	☐	99-30-9
	Dimethoate NCI	C5H12NO3PS2	228.99962	7.837	☐	☐	60-51-5
	BHC-beta NCI	C6H6Cl6	287.86007	8.055	☐	☐	319-85-7
	BHC-gamma (Lindane) NCI	C6H6Cl6	287.86007	8.173	☐	☐	58-89-9
	Terbufos NCI	C9H21O2PS3	288.04413	8.185	☐	☐	13071-79-9
	Fonofos NCI	C10H15OPS2	246.03019	8.276	☐	☐	944-22-9
	Diazinon (Dimpylate) NCI	C12H21N2O3PS	304.10105	8.318	☐	☐	333-41-5
	Tefluthrin NCI	C17H14ClF7O2	418.05705	8.451	☐	☐	79538-32-2



Accurate Mass Library of Environmental Contaminants for Negative CI

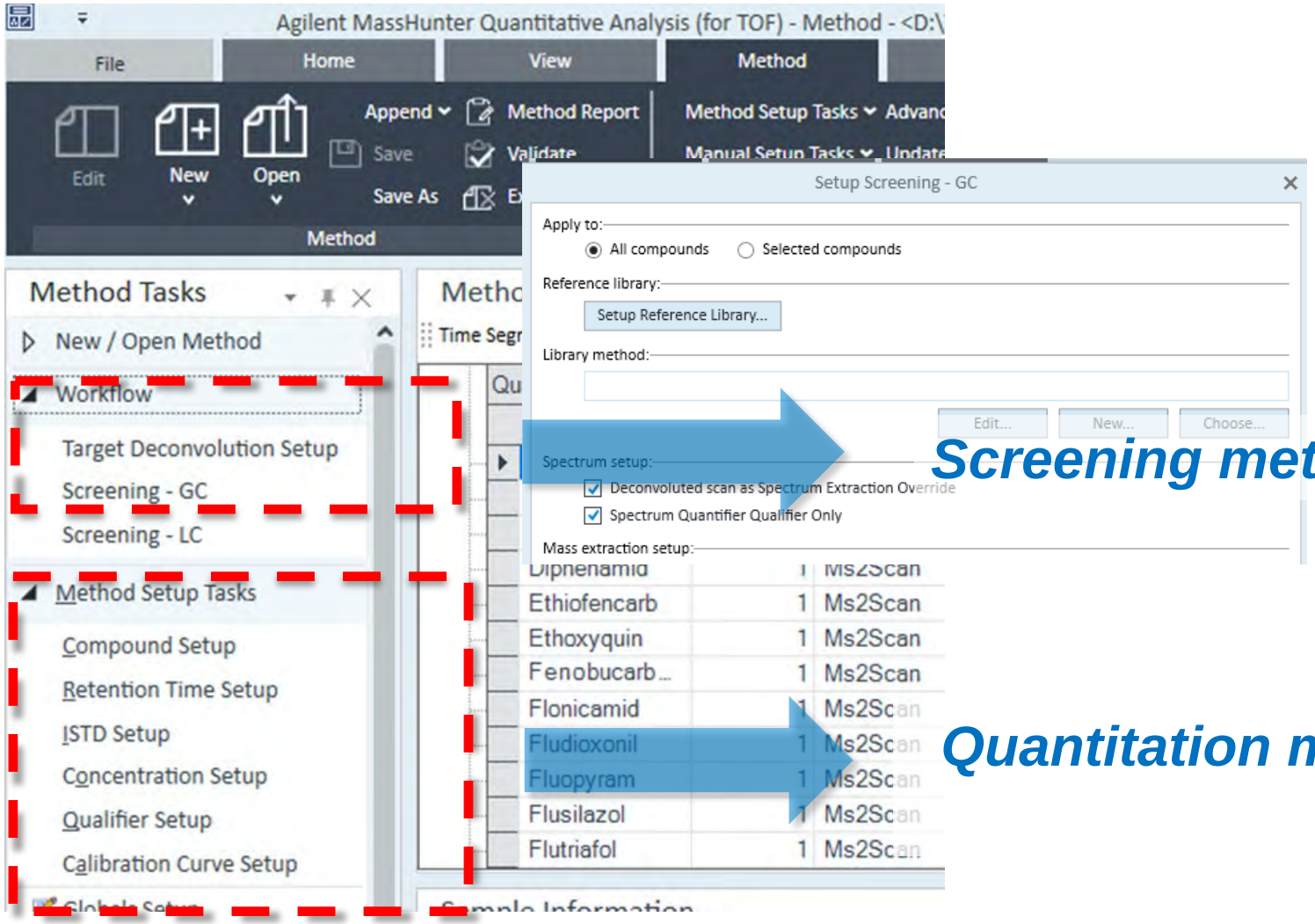
100+ compounds

	Name	Formula	Mass	Retention Time	Cation	Anion	CAS
	Fipronil sulfone NCI	C12H4Cl2F6N4O...	451.93362	11.634	☐	☐	120068-36-2
▶	Fipronil NCI	C12H4Cl2F6N4OS	435.93871	10.537	☐	☐	120068-37-3
	Chlorothalonil NCI	C8Cl4N2	263.88156	8.291	☐	☐	1897-45-6
	Chlorpyrifos NCI	C9H11Cl3NO3PS	348.92628	9.743	☐	☐	2921-88-2
	Bioallethrin (Esbiothrin) NCI	C19H26O3	302.18819	10.545	☐	☐	28434-00-6
	Prallethrin NCI	C19H24O3	300.17254	10.707	☐	☐	23031-36-9
	Tetramethrin NCI	C19H25NO4	331.17836	13.8657	☐	☐	7696-12-0
	Bifenthrin NCI	C23H22ClF3O2	422.12604	13.8189	☐	☐	82657-04-3
	Cyphenothrin (I) NCI	C24H25NO3	375.18344	15.271	☐	☐	39515-40-7
	Cyphenothrin (II) NCI	C24H25NO3	3				
	Esfenvalerate NCI	C25H22ClNO3	4				



Simultaneous Targeted Quantification and Suspect Screening Workflow

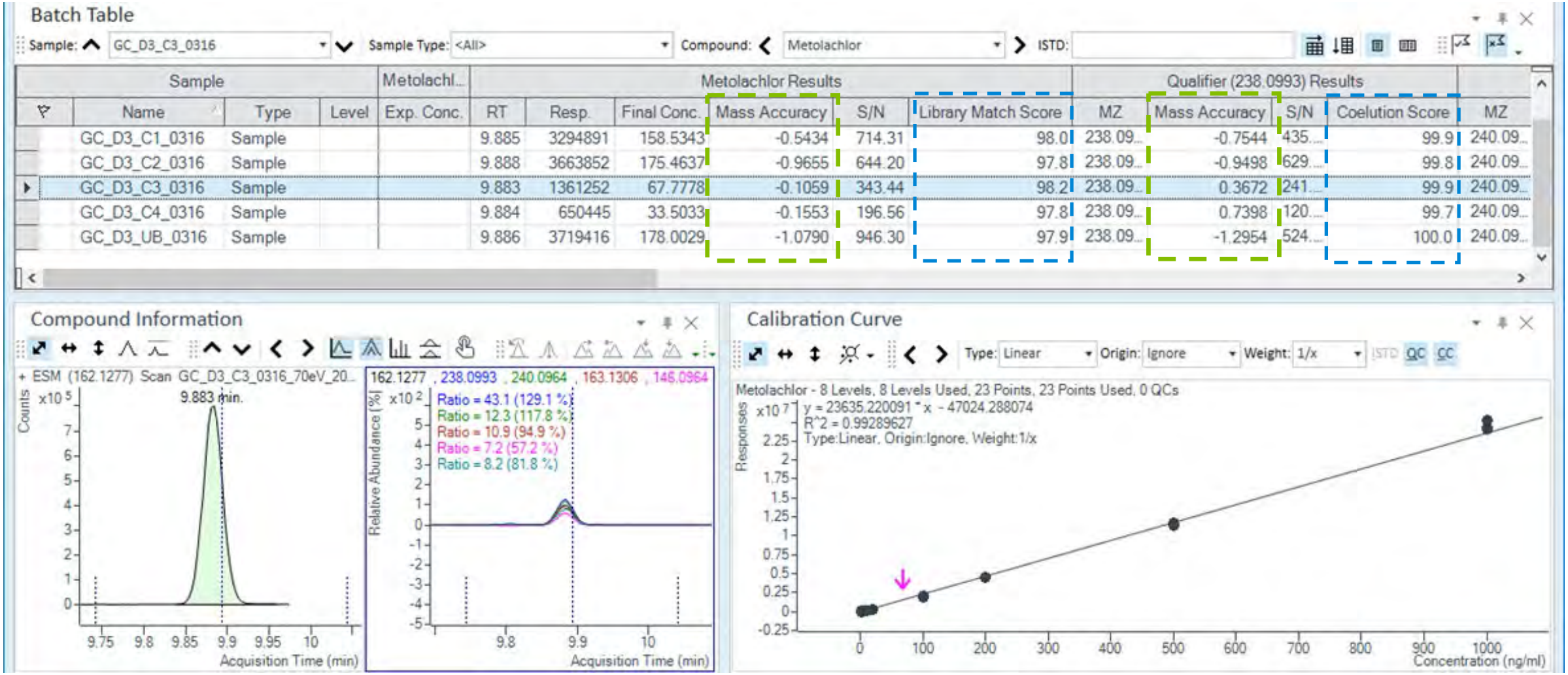
GC/Q-TOF Screener



Screening method setup

Quantitation method setup

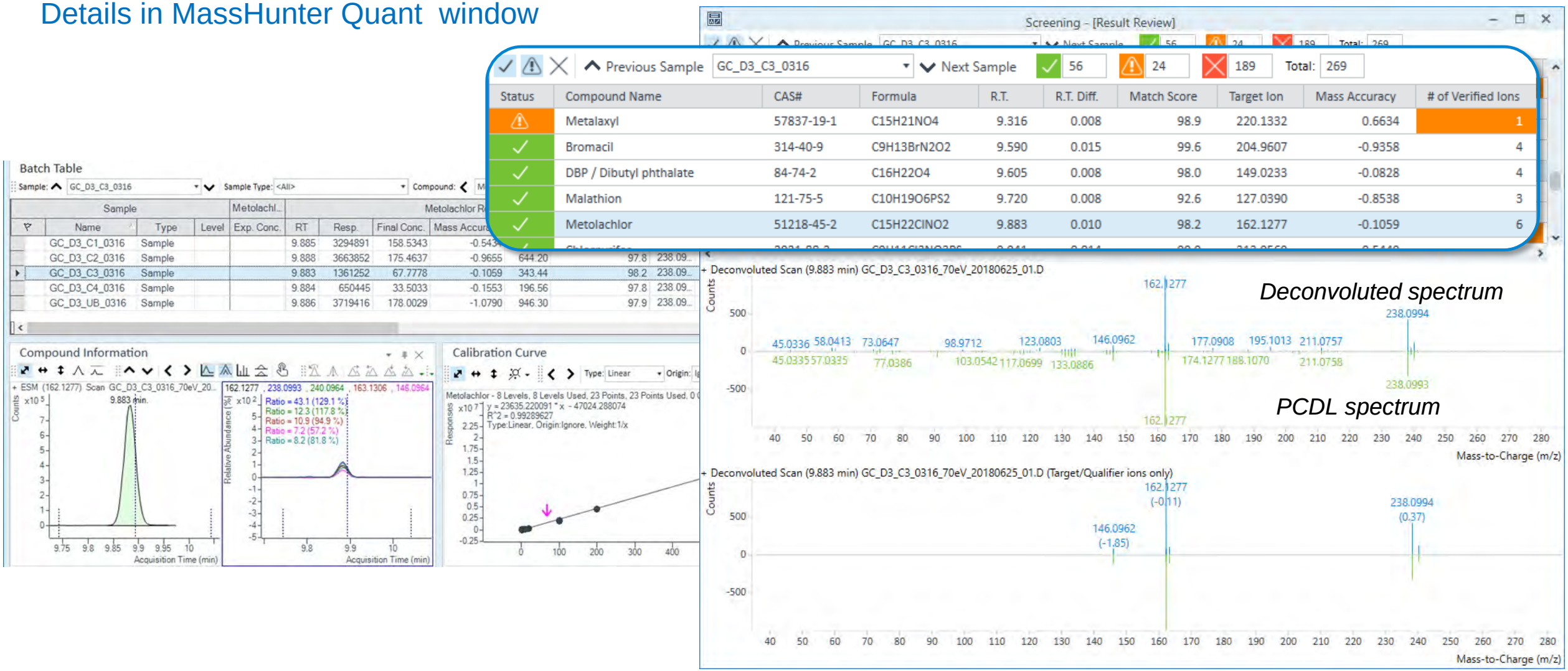
Target Quantitation Window



Screening Window: Results Review

Summary in Screening window

Details in MassHunter Quant window



Screening and Target Quantitation Report

Sample name:	GC_D3_C4_0316		Good	56	Warning	24	Error
Status	Pesticide Screening Report	CAS#	Formula	R.T.	R.T. Diff.	Match Score	Target Ion	Mass Accuracy	# of Qualified Ions
+	Benzaldehyde	100-52-7	C7H6O	3.381	0.013	99.9	105.0335	-2.10 PPM	5
+	Phenol	108-95-2	C6H6O	3.457	0.049	97.4	94.0413	-1.50 PPM	6
+	1,3-Dichlorobenzene (M-Dichlorobenzene)	541-73-1	C6H4Cl2	3.625	0.024	9			
+	1,4-Dichlorobenzene (P-Dichlorobenzene)	106-46-7	C6H4Cl2	3.625	0.008	9			
+	Benzylalcohol	100-51-6	C7H8O	3.717	0.017	9			
+	2-Methylphenol	95-48-7	C7H8O	3.794	0.032	8			
+	Acetophenone	98-86-2	C8H8O	3.868	0.002	9			
+	o-Toluidine	95-53-4	C7H9N	3.899	0.000	8			
+	Hexachloroethane	67-72-1	C2Cl6	3.922	0.016	9			
+	2,4-Dimethylphenol (2,4-Xylenol)	105-67-9	C8H10O	4.204	0.015	8			
+	2,4-Dichlorophenol	120-83-2	C6H4Cl2O	4.354	0.003	9			
+	Naphthalene	91-20-3	C10H8	4.457	0.017	9			
+	4-Chloroaniline	106-47-8	C6H6ClN	4.499	0.011	9			
+	Hexachlorobutadiene	87-68-3	C4Cl6	4.573	0.016	9			
+	Caprolactam	105-60-2	C6H11NO	4.724	0.000	9			
+	4-Chloro-3-methylphenol	59-50-7	C7H7ClO	4.848	0.011	9			
+	2-Methylnaphthalene	91-57-6	C11H10	4.995	0.018	9			
+	1-Methylnaphthalene	90-12-0	C11H10	5.092	0.020	9			
+	Biphenyl	92-52-4	C12H10	5.423	0.019	9			
+	Diphenylether	101-84-8	C12H10O	5.534	0.014	8			
+	1,4-Naphthalenedione	130-15-4	C10H6O2	5.626	0.020	9			
+	Dimethylphthalate	131-11-3	C10H10O4	5.780	0.019	9			
+	Phthalimide	85-41-6	C8H5NO2	5.843	0.018	9			
+	Acenaphthene	83-32-9	C12H10	6.116	0.022	9			
+	Dibenzofuran	132-64-9	C12H8O	6.313	0.020	9			
+	Pentachlorobenzene	608-93-5	C6HCl5	6.344	0.019	8			
+	DiethylPhthalate	84-66-2	C12H14O4	6.679	0.015	9			
+	Fluorene	86-73-7	C13H10	6.780	0.019	9			

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Status	Pesticide Screening Report	CAS#	Formula	R.T.	R.T. Diff.	Match Score	Target Ion	Mass Accuracy	# of Qualified Ions
+	Trifluralin	1582-09-8	C13H16F3N3O4	7.236	0.011	96.1	264.0227	-1.38 PPM	4
+	HCB / Hexachlorobenzene	118-74-1	C6Cl6	7.761	0.022	95.2	283.8096	-2.64 PPM	5
+	Dimethoate	60-51-5	C5H12NO3PS2	7.779	0.012	98.2	124.9821	-0.25 PPM	5
+	Clomazone	81777-89-1	C12H14ClNO2	7.972	0.013	99.8	204.1019	-0.78 PPM	3
+	Diazinon (Dimpylate)	333-41-5	C12H21N2O3PS	8.277	0.009	98.6	179.1179	-4.08 PPM	5
+	Phenanthrene	85-01-8	C14H10	8.326	0.021	99.6	178.0777	-0.58 PPM	5
+	Chlorothalonil	1897-45-6	C8Cl4N2	8.574	0.018	94.1	265.8781	-0.96 PPM	3
+	Bromacil	314-40-9	C9H13BrN2O2	9.592	0.014	99.8	204.9607	-0.87 PPM	4
+	DBP / Dibutyl phthalate	84-74-2	C16H22O4	9.604	0.008	97.8	149.0233	-0.23 PPM	4
+	Malathion	121-75-5	C10H19O6PS2	9.722	0.007	93.0	127.0390	-0.89 PPM	3
+	Metolachlor	51218-45-2	C15H22ClNO2	9.884	0.010	97.8	162.1277	-0.16 PPM	6
+	Chlorpyrifos	2921-88-2	C9H11Cl3NO3PS	9.941	0.013	99.9	313.9569	0.78 PPM	5
+	DCPA / Chlorthal-dimethyl	1861-32-1	C10H6Cl4O4	10.041	0.014	99.9	300.8802	0.73 PPM	6
+	Pendimethalin (Penoxalin)	40487-42-1	C13H19N3O4	10.507	0.013	99.9	252.0979	1.23 PPM	6
+	Fluoranthene	206-44-0	C16H10	10.701	0.025	99.8	202.0777	-0.91 PPM	3
+	Tetrachlorvinphos (Dietreen T)	22248-79-9	C10H9Cl4O4P	11.139	0.007	89.6	328.9298	-0.69 PPM	2
+	Pyrene	129-00-0	C16H10	11.171	0.023	98.5	202.0777	-0.65 PPM	4
+	p,p'-DDE	72-55-9	C14H8Cl4	11.603	0.009	90.3	245.9998	-0.95 PPM	4
+	Myclobutanil	88671-89-0	C15H17ClN4	11.719	0.002	87.2	179.0245	-0.41 PPM	3
+	p,p'-DDD	72-54-8	C14H10Cl4	12.355	0.003	76.5	235.0076	-0.19 PPM	2
+	BBP / Benzyl butyl phthalate (Butylbenzylphthalate)	85-68-7	C19H20O4	12.919	0.000	97.2	149.0233	-0.66 PPM	6
+	Bis(2-ethylhexyl)adipate	103-23-1	C22H42O4	13.190	0.035	90.7	129.0546	-0.94 PPM	2
+	TPPA / Triphenyl phosphate	115-86-6	C18H15O4P	13.357	0.003	100.0	325.0624	-0.42 PPM	6
+	Piperonyl butoxide	51-03-6	C19H30O5	13.368	0.004	94.9	176.0832	-2.06 PPM	3
+	Chlorantraniliprole	500008-45-7	C18H14BrCl2N5O2	14.135	0.001	90.7	278.0008	-0.25 PPM	3
+	Bis(2-ethylhexyl)phthalate	117-81-7	C24H38O4	14.397	0.006	98.7	149.0233	-0.50 PPM	6

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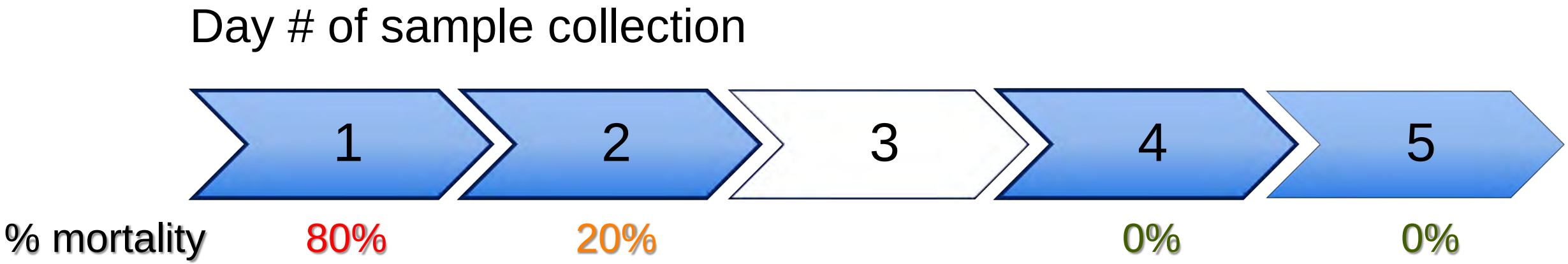
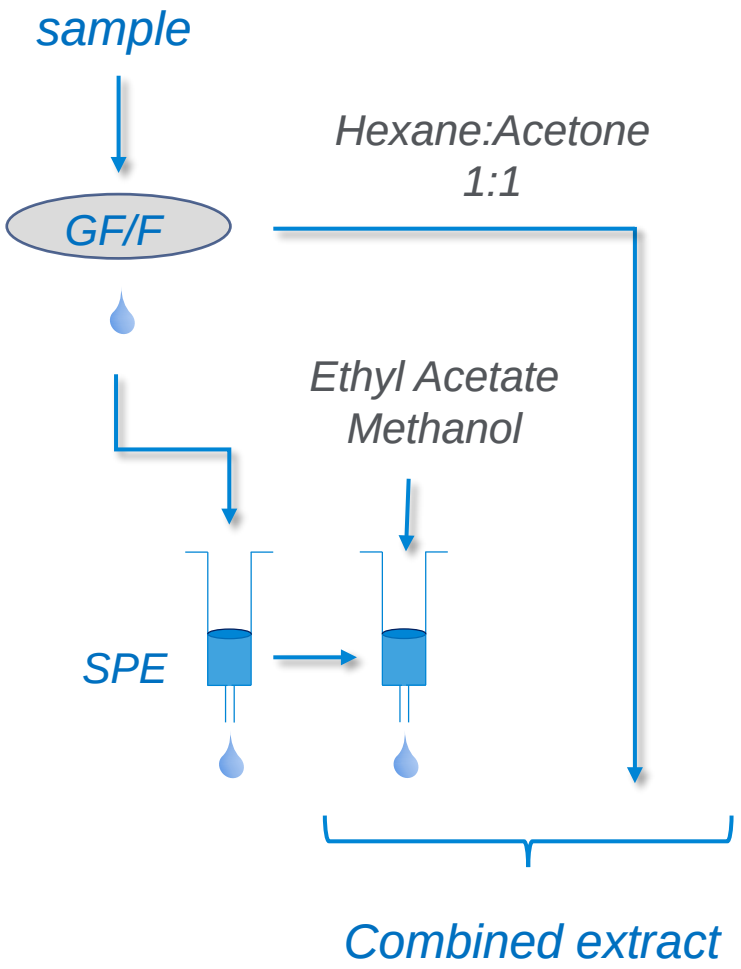
Identification of Toxic Contaminants in the Wastewater Effluent Samples

Sampling



- The wastewater effluent samples were collected on days 1, 2, 4, and 5 of a five-day series
- The samples from days 1 and 2 displayed acute toxicity towards *Ceriodaphnia dubia* (shown by whole effluent toxicity testing)

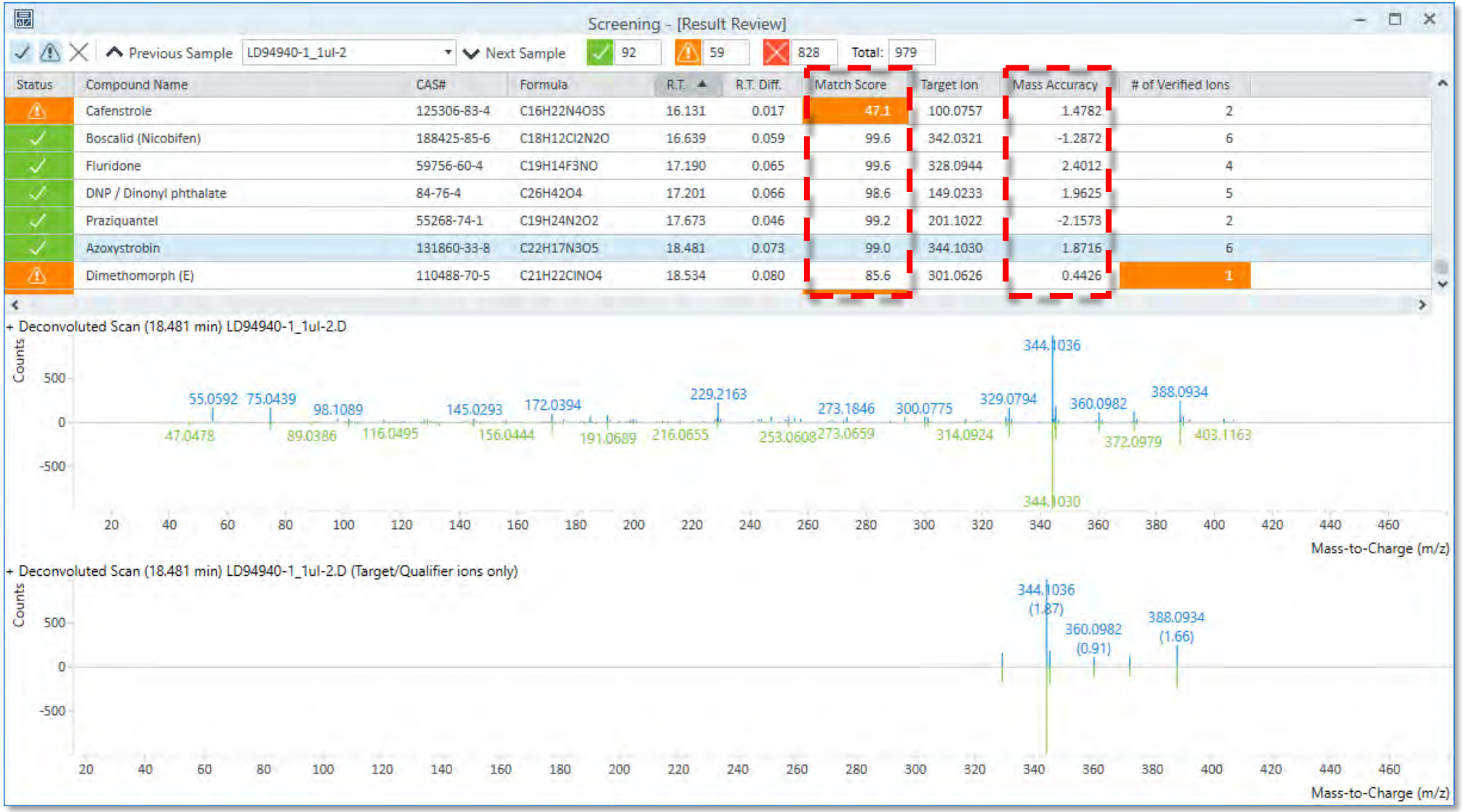
Extractions



- Samples were filtered through a 0.45 µm GF/F filter and passed over a hydrophilic reversed-phase SPE cartridge.
- Dried cartridges were eluted with ethyl acetate and methanol.
- Dried filters were extracted in a sonicating bath with hexane/acetone 1:1.
- Both extracts were combined and spiked with dibromooctafluorobisphenol (DBOFB) as an internal standard.

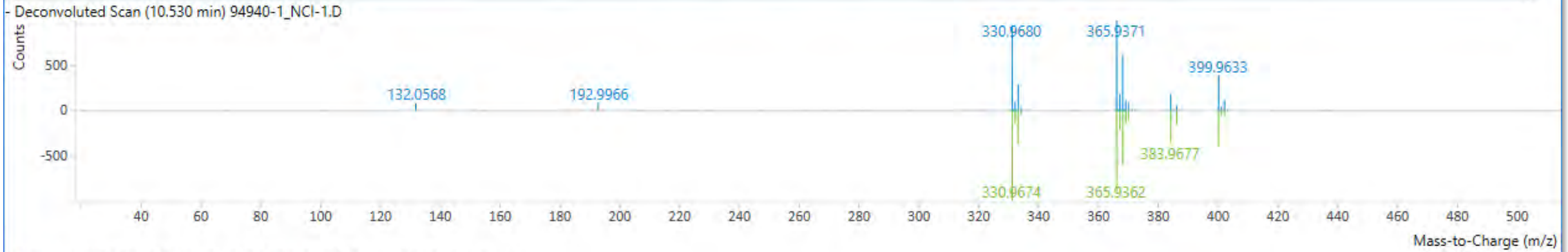
EI Screening Results

Over 90 contaminants were identified in each sample



NCI Screening Results

✓	Triadimefon NCI	43121-43-3	C14H16ClN3O2	9.882	0.144	93.1	126.9956	-1.8779	3
✓	Chlorthal-dimethyl (Dacthal or DCPA) NCI	1861-32-1	C10H6Cl4O4	9.924	0.161	91.6	331.8997	-1.9129	4
✓	Fipronil-sulfide NCI	120067-83-6	C12H4Cl2F6N4S	10.391	0.001	99.9	383.9677	1.6695	2
✓	Fipronil NCI	120068-37-3	C12H4Cl2F6N4OS	10.531	0.005	99.4	365.9362	2.5318	6
✓	Prallethrin NCI	23031-36-9	C19H24O3	10.694	0.013	85.5	167.1078	-2.3838	2



Summary of Suspect Screening Results
Compounds correlated with effluent toxicity

Sample	80 % Mortality						20 % Mortality						0 % Mortality					
	LD94940-1			LD94940-2			LD94941-1			LD94941-2			LD94943-1			LD94943-2		
Compound Name	Response	Mass Error	Library Match score	Response	Mass Error	Library Match score	Response	Mass Error	Library Match score	Response	Mass Error	Library Match score	Response	Mass Error	Library Match score	Response	Mass Error	Library Match score
TBEP/*Tris*(2-butoxyethyl) Phosphate	2013504	2.8	99.9	1502528	3.9	99.9	1289372	2.5	99.9	1559301	3.8	99.9	787113	3.1	99.9	784473	3.8	99.9
tert-Butylphenyldiphenylphosphate	16799	2.1	92.9	4948	3.2	74.6	2828	1.1	82.5	10468	0.8	91.9	2950	1.3	70.6	2766	0.8	91.9
Chlorantraniliprole	6298	0.2	76.8	5330	2.0	79.4	3572	1.7	63.2	3494	1.8	66.4	3458	1.1	52.4	2710	1.8	66.4
Flurprimidol	16518	1.3	80.4	15240	0.5	76.4	10698	2.6	73.7	12065	2.1	80.2	6038	2.0	74.2	4976	2.1	80.2
Paclobutrazol	16985	0.9	96.8	15763	1.6	98.7	10725	0.9	92.4	12090	2.1	94.9	9106	1.8	79.1	8448	2.1	94.9
TBZ/Thiabendazole	1570235	1.4	99.7	1536170	2.4	99.7	1282402	0.6	99.7	1368732	2.2	99.8	774093	0.6	99.7	675439	2.2	99.8
Azoxystrobin	134463	1.8	99.1	139960	3.0	98.9	109579	1.4	98.9	119004	1.7	98.8	104804	1.7	89.9	94511	1.7	98.8

Non-Targeted Analysis

Acquire full-spectrum data

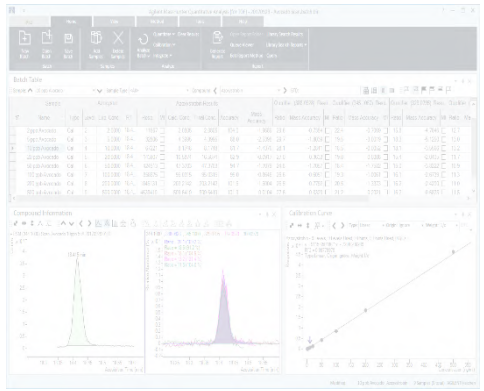
Targeted Method

Yes

Calibrate?

No

Targeted Quantitation



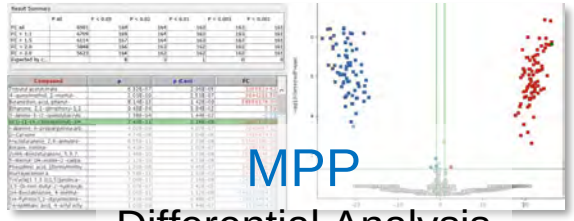
Suspect Screening

Peak Picking

Non-Target Analysis

Unknowns Analysis

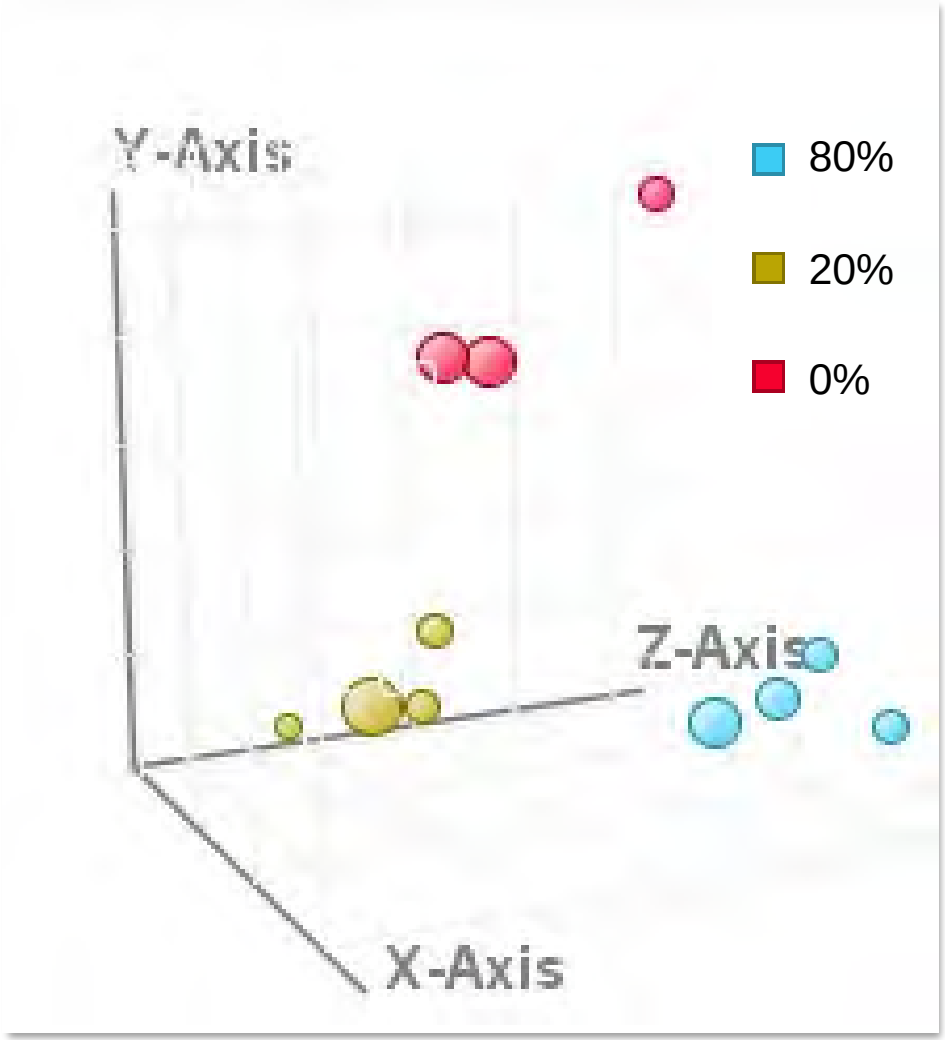
Deconvolution &
NIST search



MPP

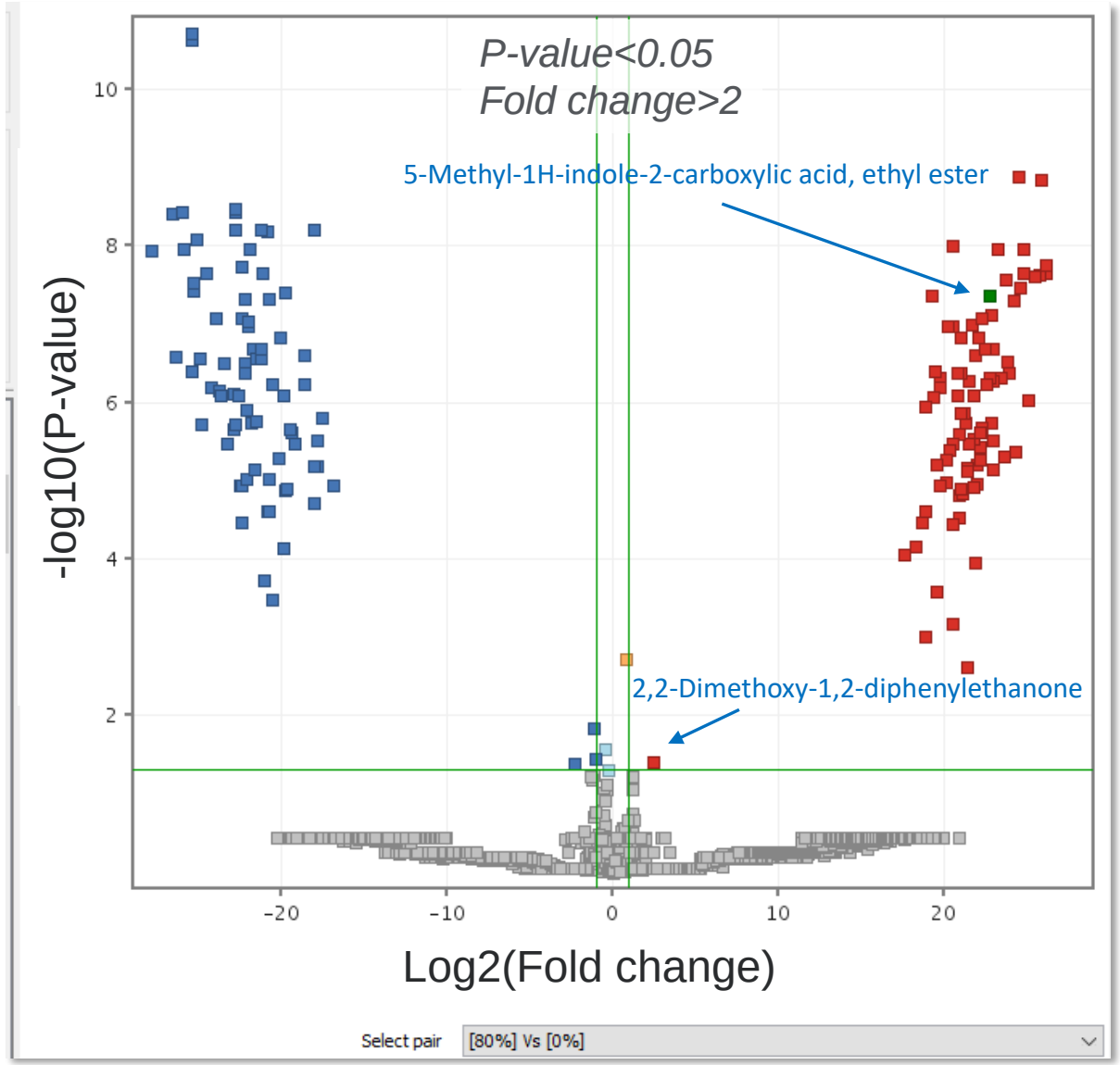
Differential Analysis

Principle Component Analysis Confirmed Separation Between the Groups



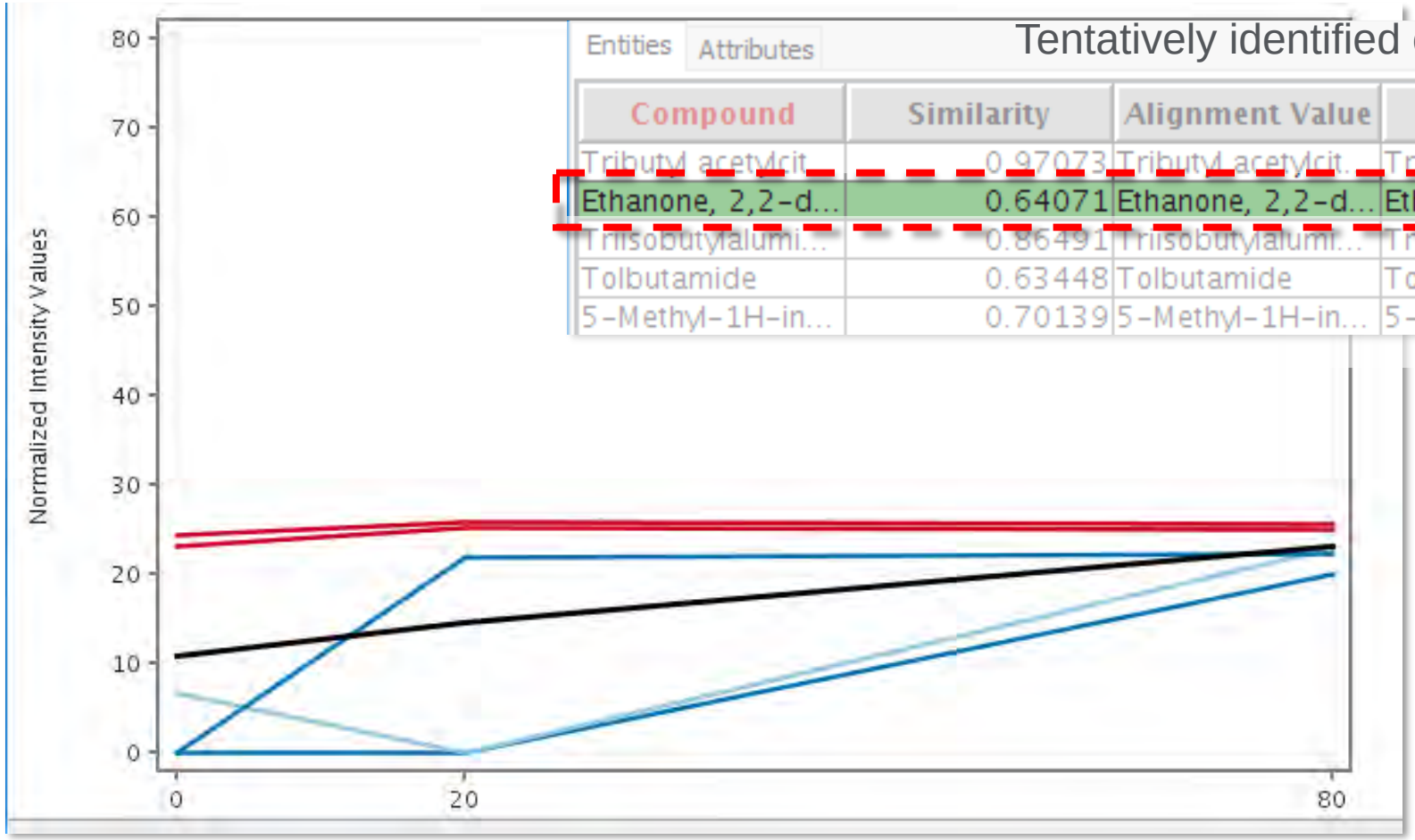
- Compounds were imported to Mass Profiler Professional (MPP)
- Principle Component Analysis (PCA) was performed to visualize the separation of the three groups of the samples

Volcano Plot: Comparison of 80% Mortality vs 0% Mortality



- Compounds found at higher levels in the 80% mortality group
- Compounds found at higher levels in the 0% mortality group

Correlation Analysis



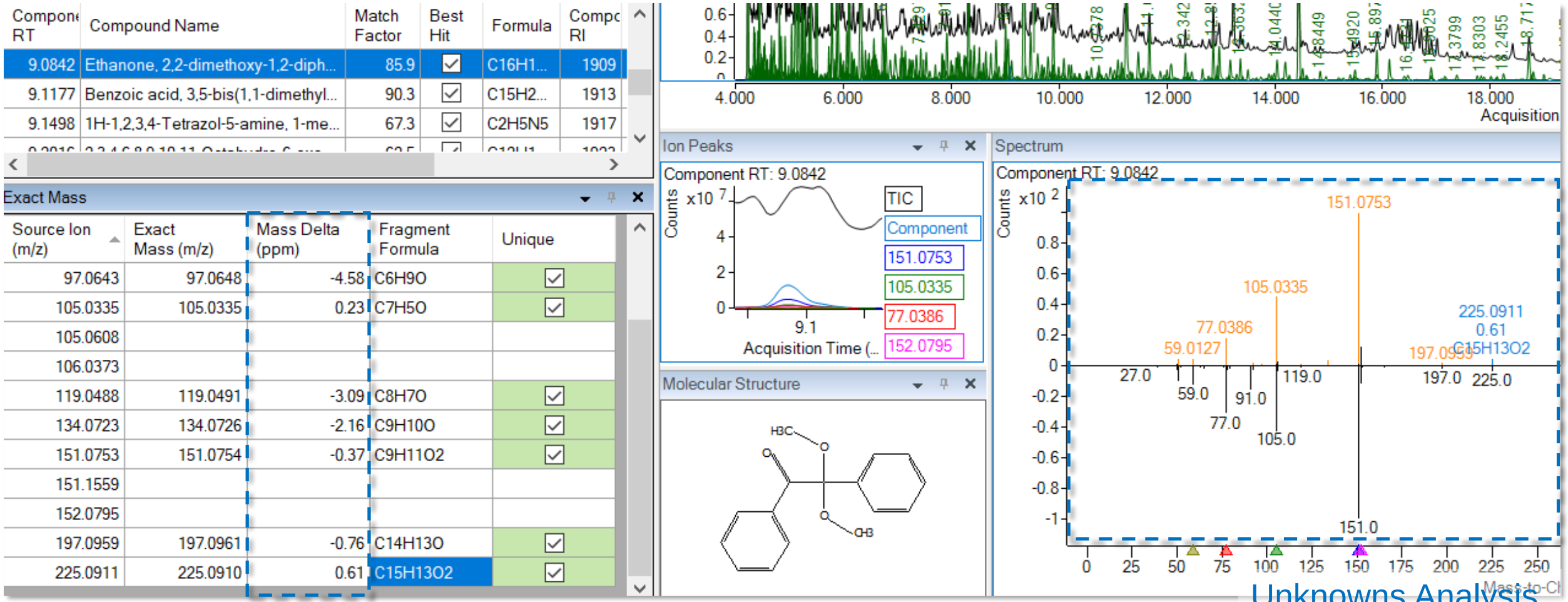
Tentatively identified compounds with NIST

Entities | Attributes

Compound	Similarity	Alignment Value	Annotations	CAS Number
Tributyl acetylcit...	0.97073	Tributyl acetylcit...	Tributyl acetylcit...	77-90-7
Ethanone, 2,2-d...	0.64071	Ethanone, 2,2-d...	Ethanone, 2,2-d...	24650-42-8
Triisobutylalumi...	0.86491	Triisobutylalumi...	Triisobutylalumi...	100-99-2
Tolbutamide	0.63448	Tolbutamide	Tolbutamide [C...	64-77-7
5-Methyl-1H-in...	0.70139	5-Methyl-1H-in...	5-Methyl-1H-in...	1000318-48-3

- Percent mortality was used as a filtering parameter
- Pearson similarity cutoff: 0.6

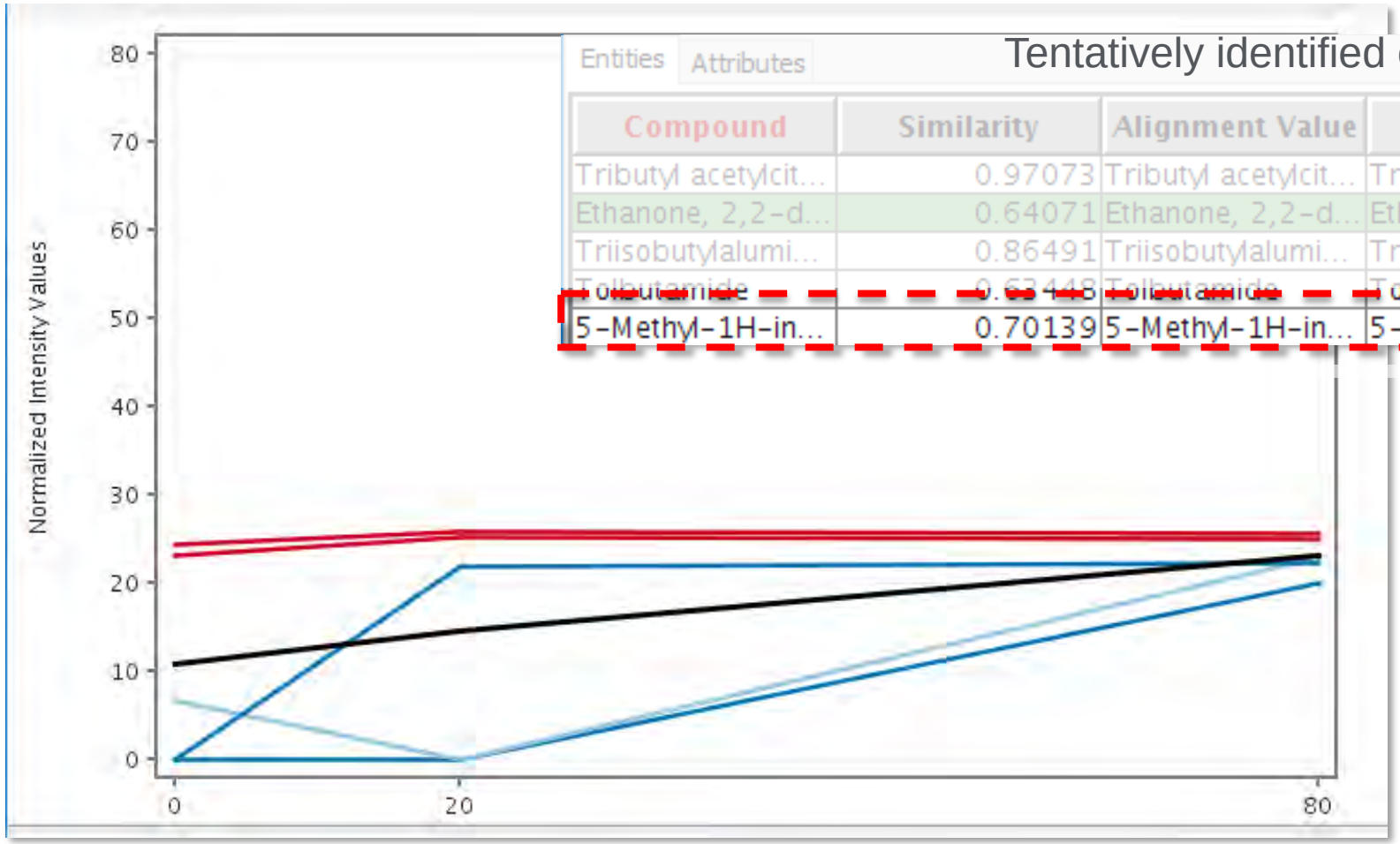
Tentative Hits Confirmation Using Accurate Mass



Unknowns Analysis, NIST

Confirmed by accurate mass

Another Tentative Hit From Correlation Analysis



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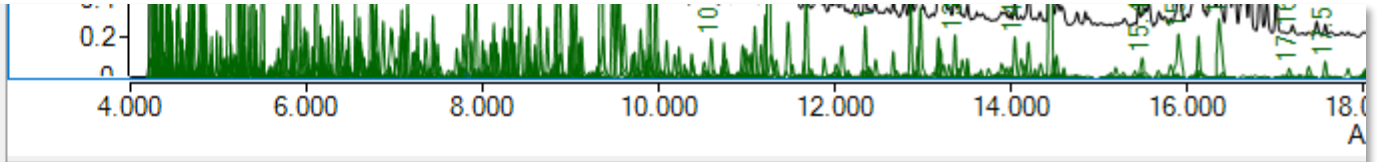
5-Methyl-1H-in...	0.70139	5-Methyl-1H-in...	5-Methyl-1H-in...	1000318-48-3

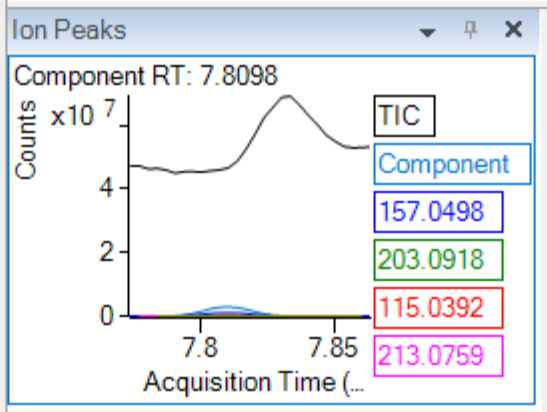
Tentative Hits Confirmation Using Accurate Mass

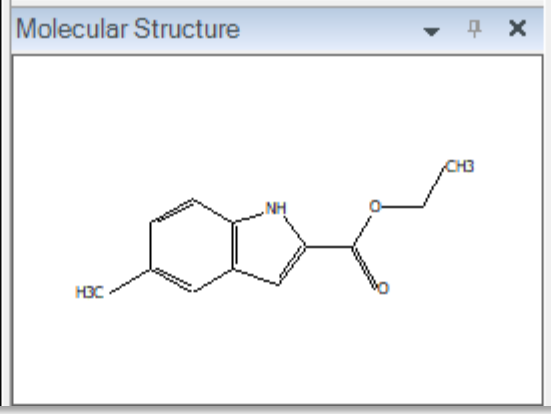
7.8098	5-Methyl-1H-indole-2-carboxylic a...	64.8	☑	C12H13NO2	1754
7.8315	Tetradecanoic acid	87.4	☑	C14H28O2	1757
7.8675	Butanedioic acid, phenyl-	57.3	☑	C10H10O4	1762
7.8994	Naphthalene, 1-isocyano-	59.8	☑	C11H7N	1766
7.9169	Bicyclo[2.2.1]heptane, 2-[9-borabi...	69.2	☑	C18H31BO	1768

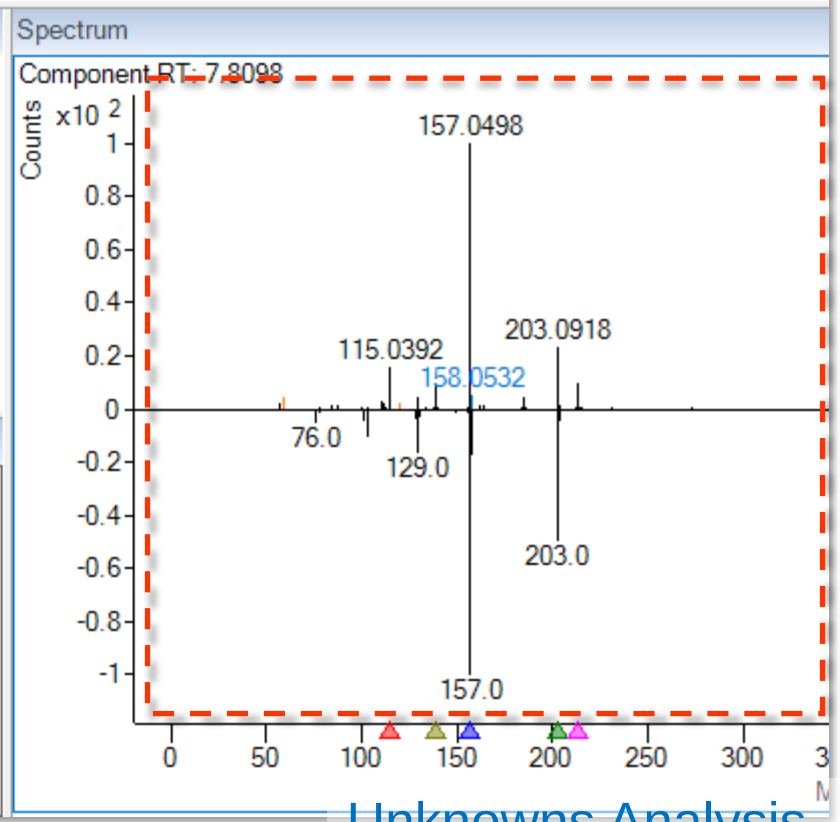
xact Mass

Source Ion (m/z)	Exact Mass (m/z)	Mass Delta (ppm)	Fragment Formula	Unique
57.0808				
59.0492	59.0491	1.79	C3H7O	☑
111.0076				
112.0153				
115.0392				
120.0929	120.0934	-3.50	C9H12	☑
129.0184				
139.0026				
157.0128				
157.0498				
158.0532				





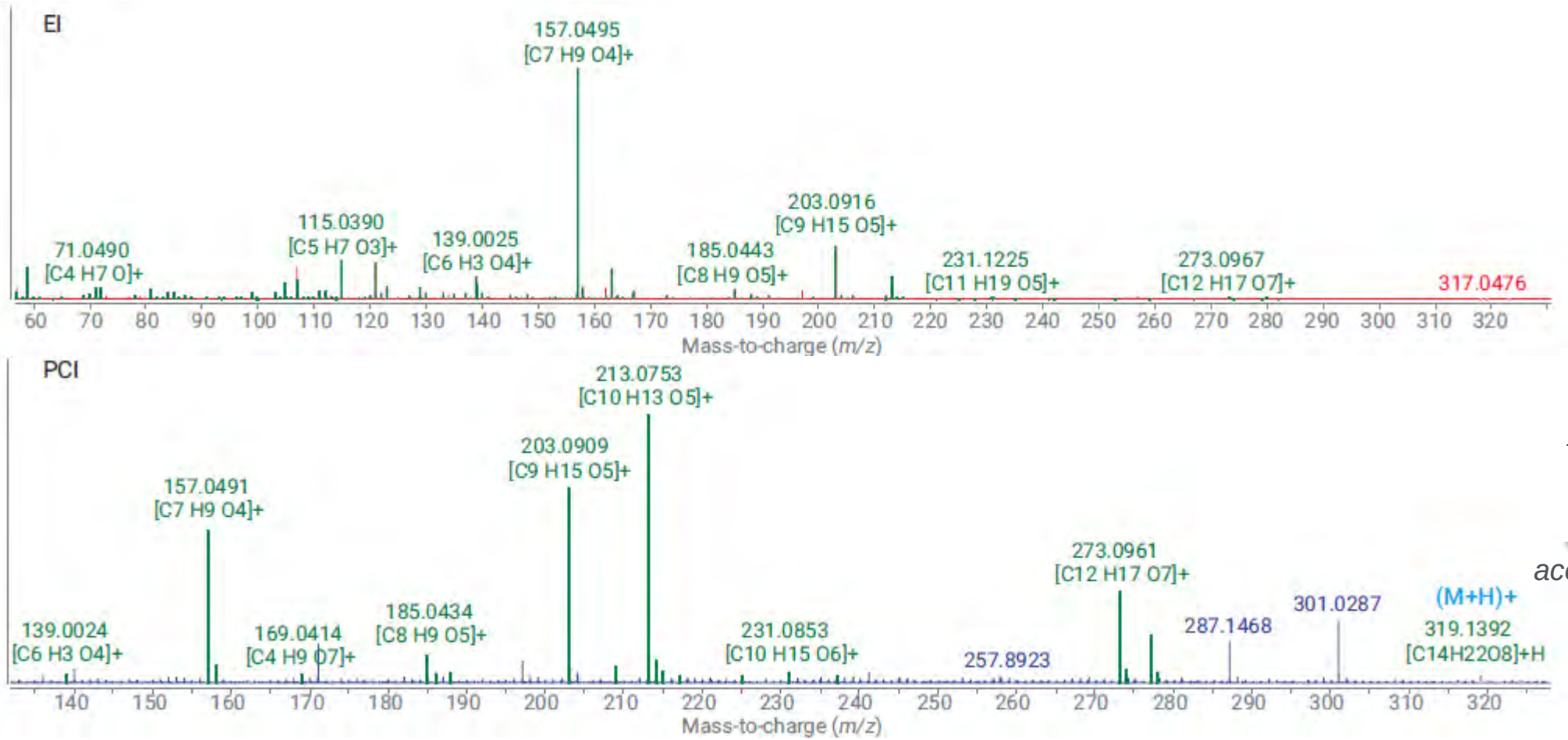


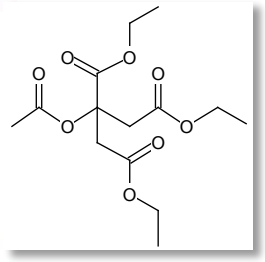


Unknowns Analysis, NIST

Rejected by accurate mass

Identification of an Unknown Compound

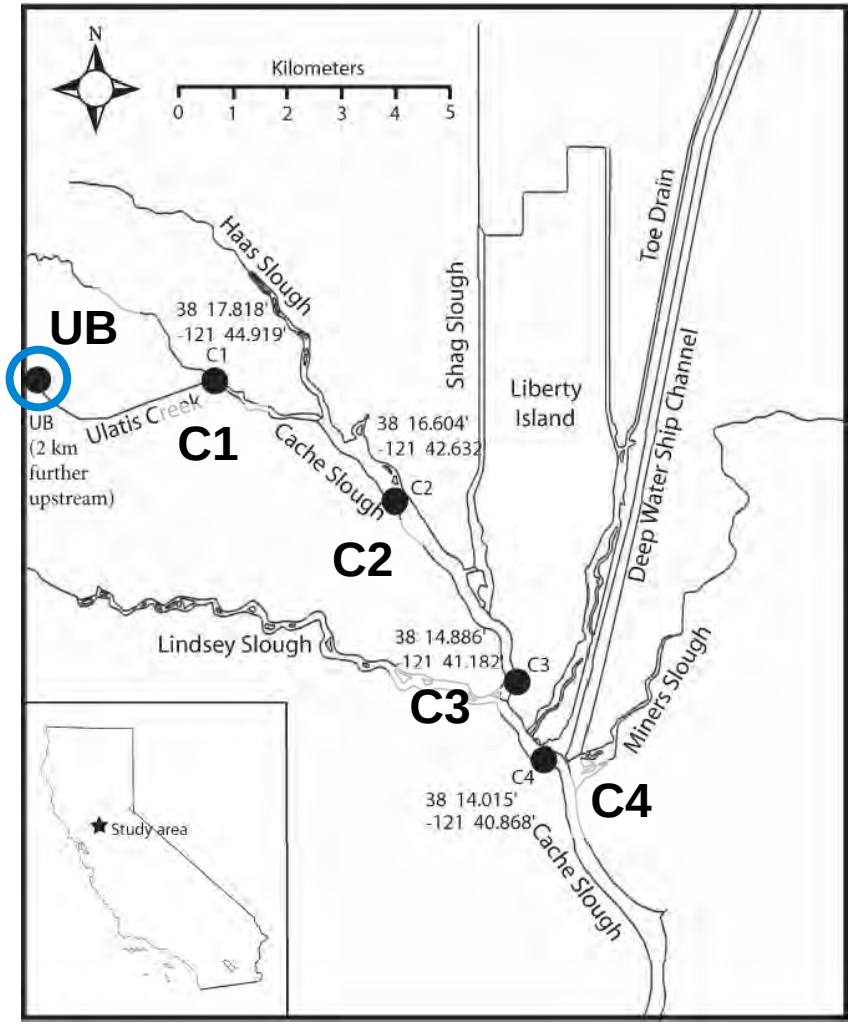




acetyl triethyl citrate

Profiling of Environmental Contaminants in Surface Water

Surface Water Study Site and Sampling



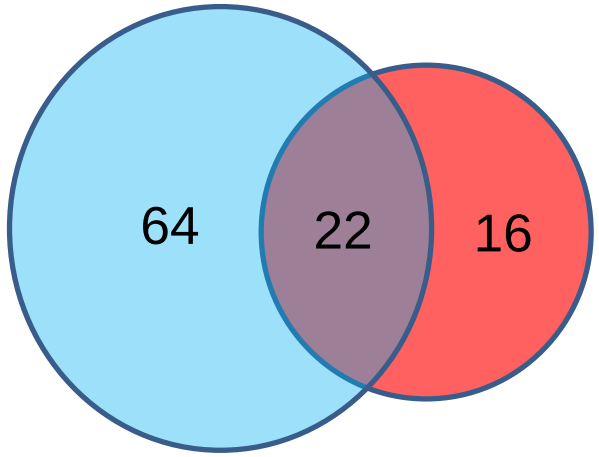
Sampling

- Sampling was carried out at locations throughout the Cache Slough Complex, located in the Sacramento-San Joaquin River Delta in Northern California
- The main input of point-source micropollutants as well as diffuse pollutants is expected to be via Ulatis Creek.
- All samples were cooled during transport and stored in the dark at 4 °C until extraction

Extraction for GC/Q-TOF Analysis

- Surface waters (1L) were passed through a GF/F filter
- The filtrate were passed through a polymeric solid phase extraction (SPE) cartridge
- After drying for one hour, the cartridges were eluted with 10 mL of ethyl acetate.

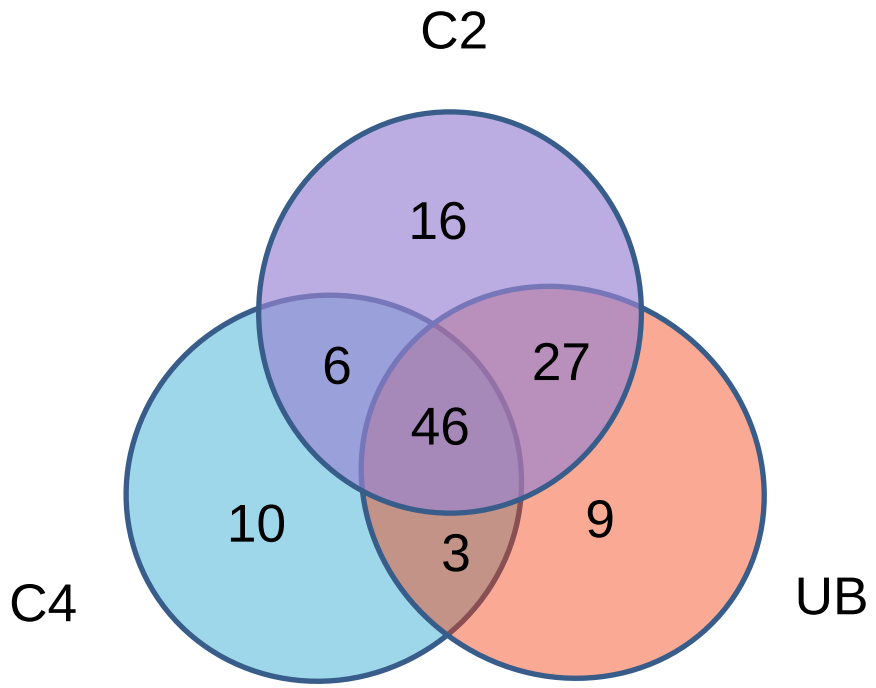
Distribution of the Contaminants between Water and Filter Extracts from UB Site



Compounds uniquely identified in the UB filter extract:

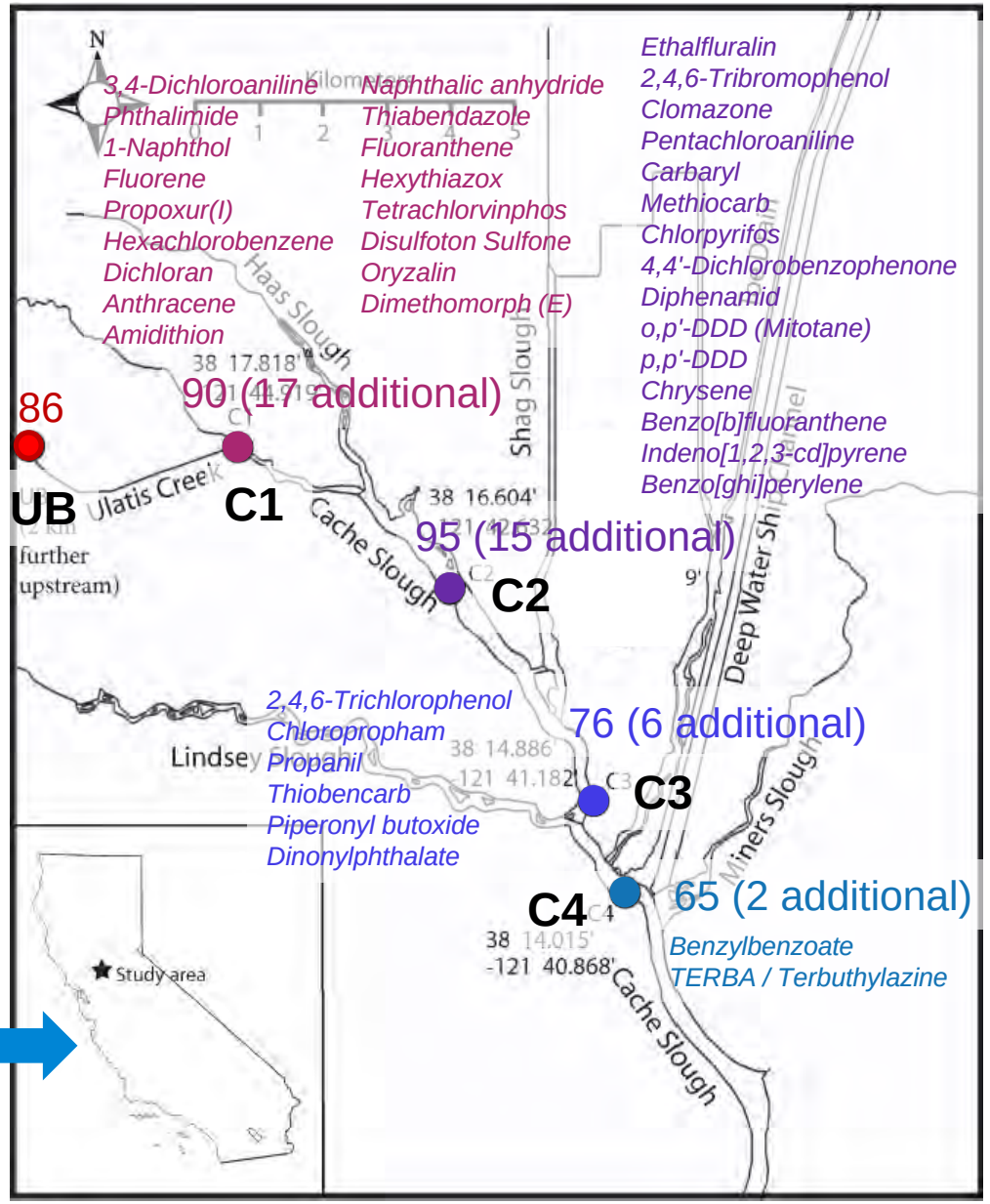
Diphenylamine (DFA)
Hexachlorobenzene
Pentachloroaniline
Fluoranthene
Pyrene
Nonachlor-trans
p,p'-DDD
Dihexylphthalate
Bifenthrin
Chrysene
cis-Permethrin
trans-Permethrin
Benzo[b]fluoranthene
Benzo[a]pyrene
Dinonylphthalate
Indeno[1,2,3-cd]pyrene

Geographic Distribution of the Pollutants

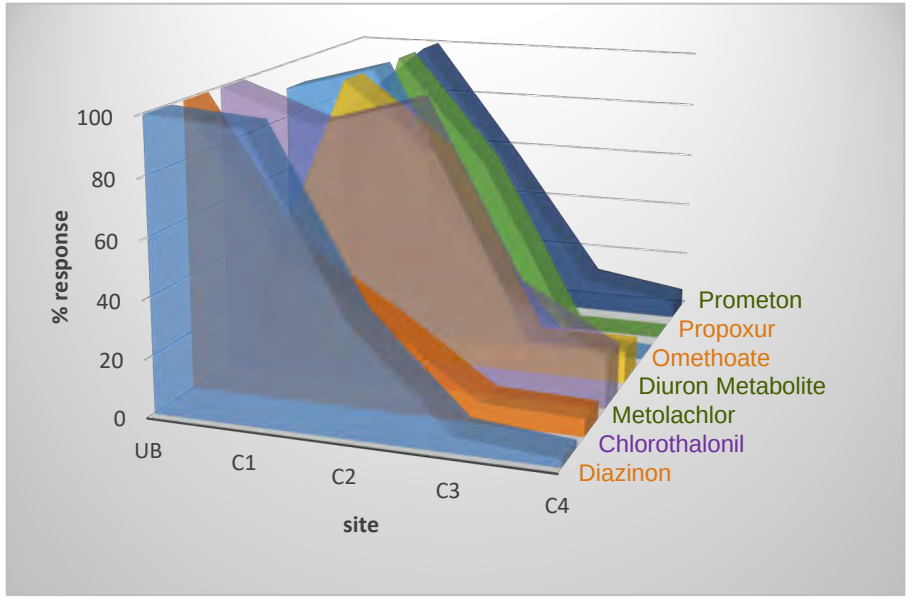


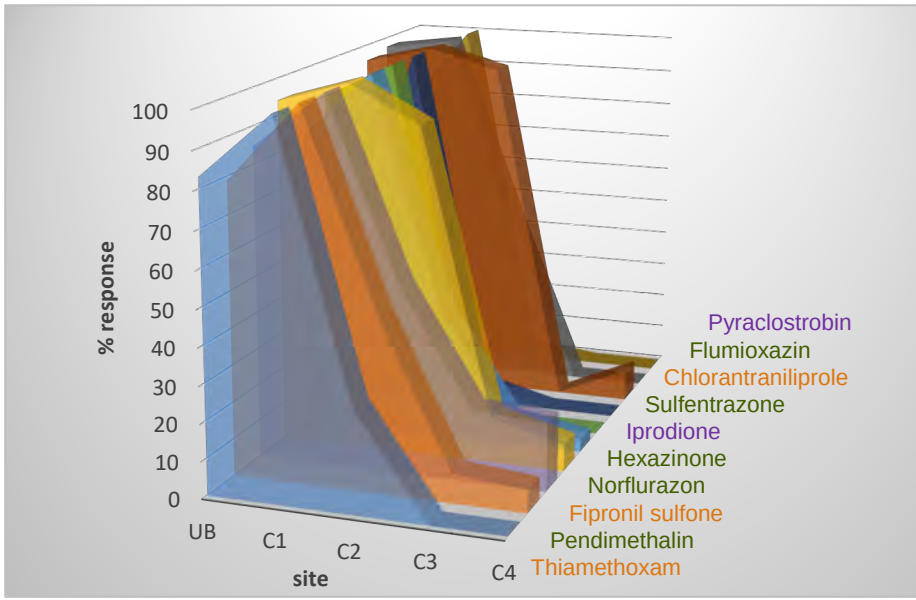
Comparison of the identified contaminants between UB, C2 and C4 sites

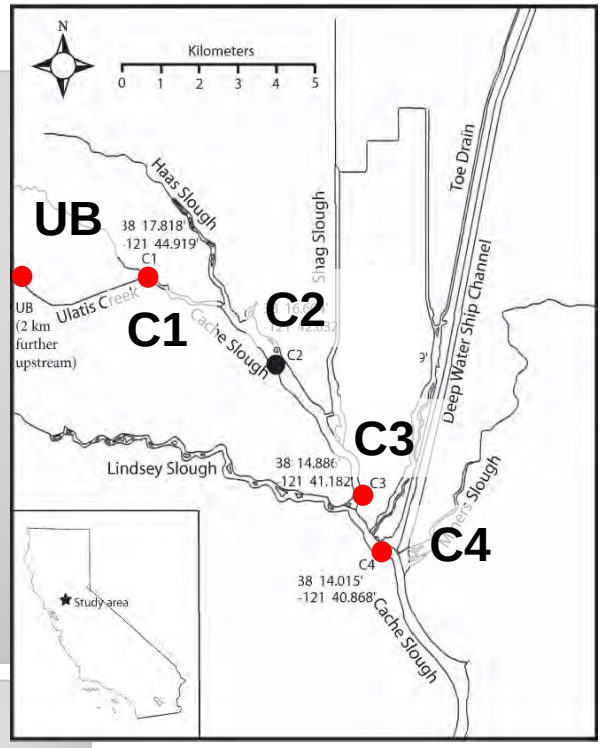
Sampling map showing the number of identified pollutants as well as the new contaminants added to the flow stream from each site

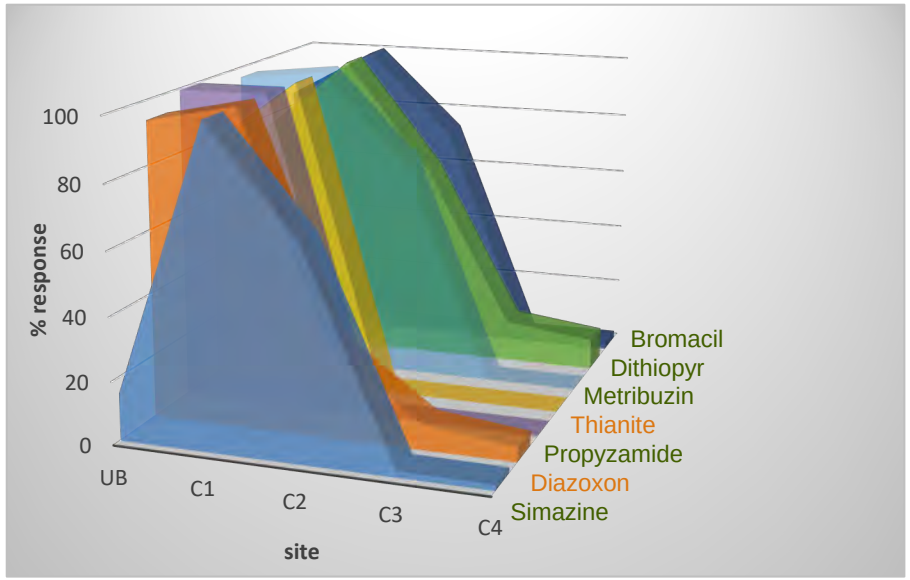


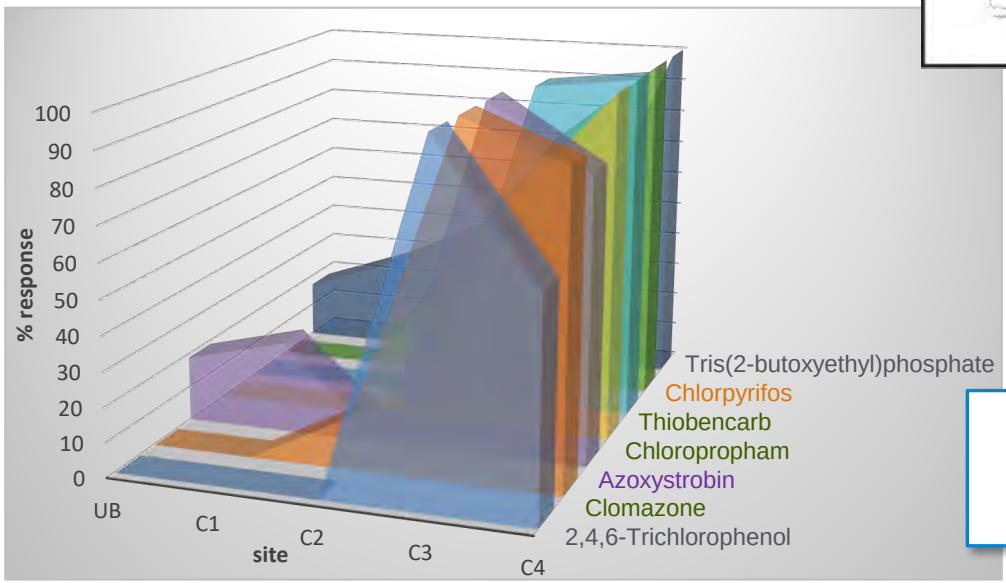
Geographic Distribution of the Pollutants





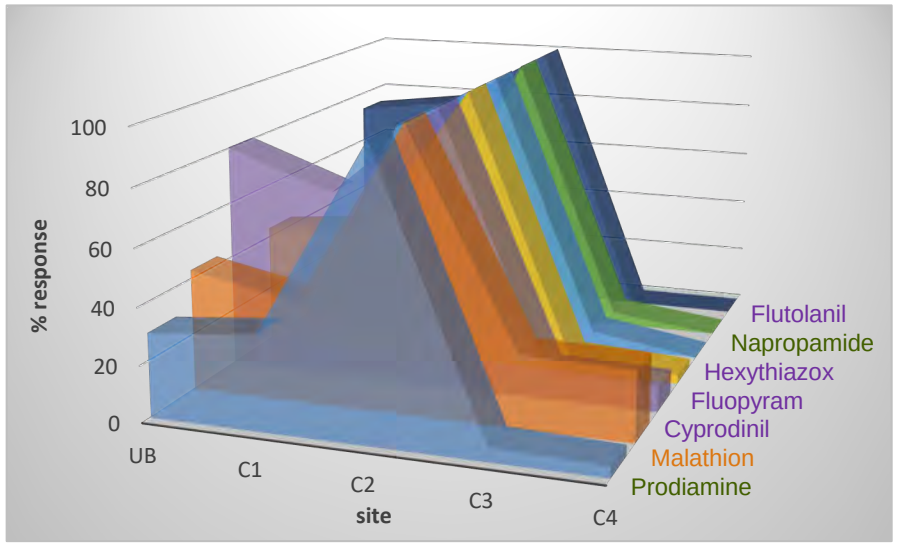


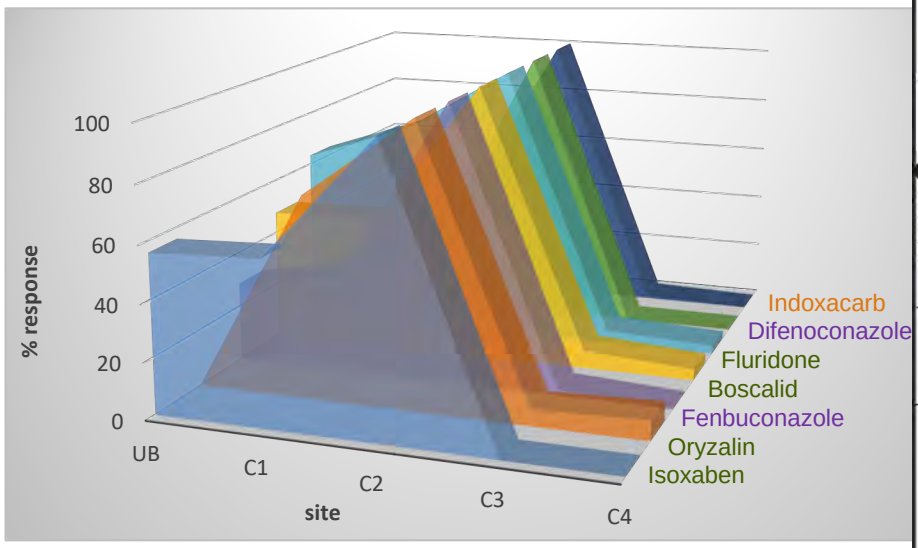


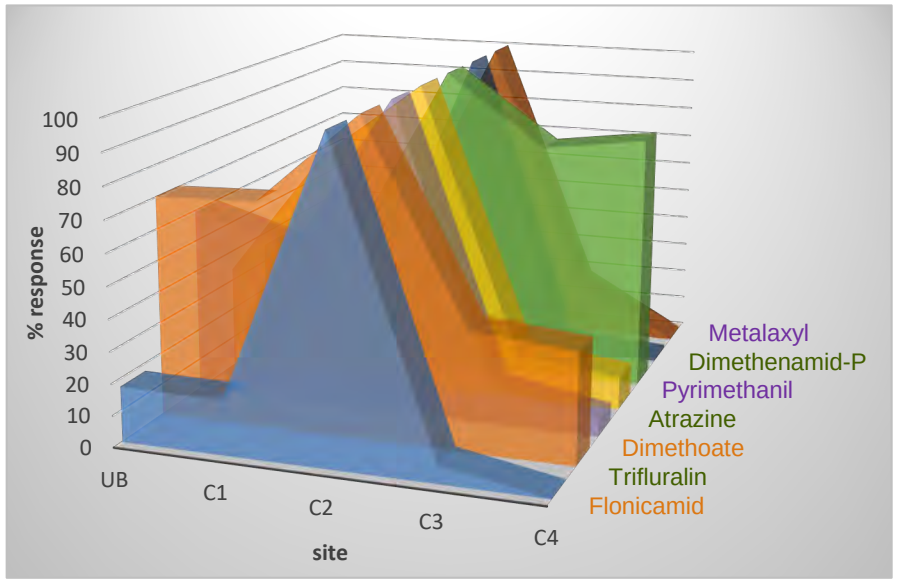


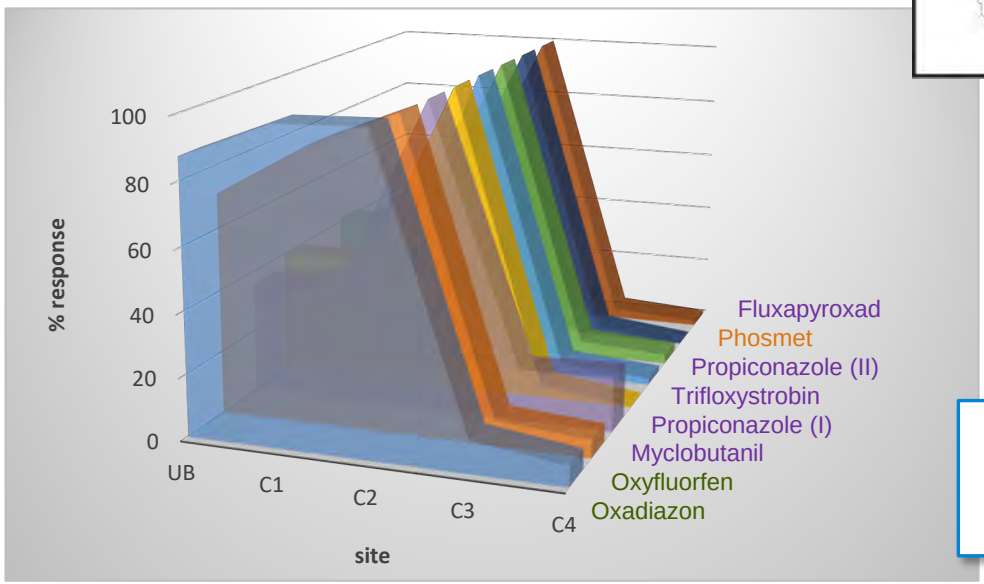
Herbicide
Insecticide
Fungicide

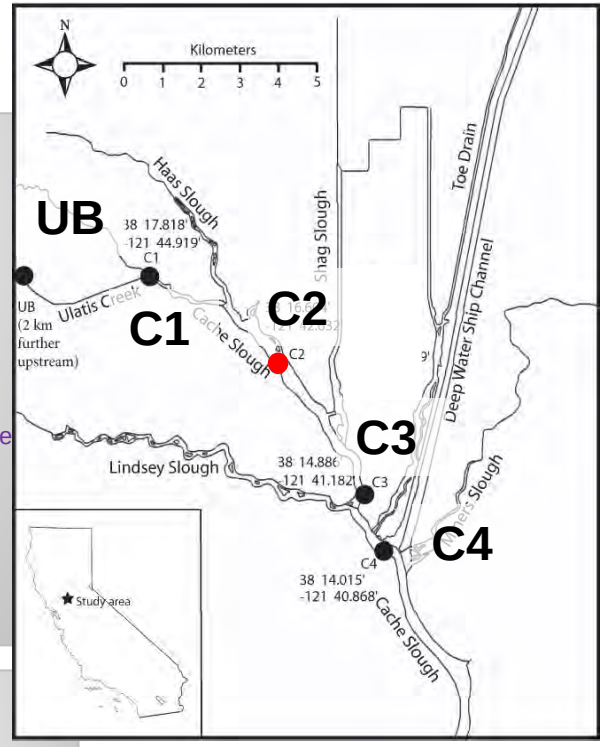
Geographic Distribution of the Pollutants







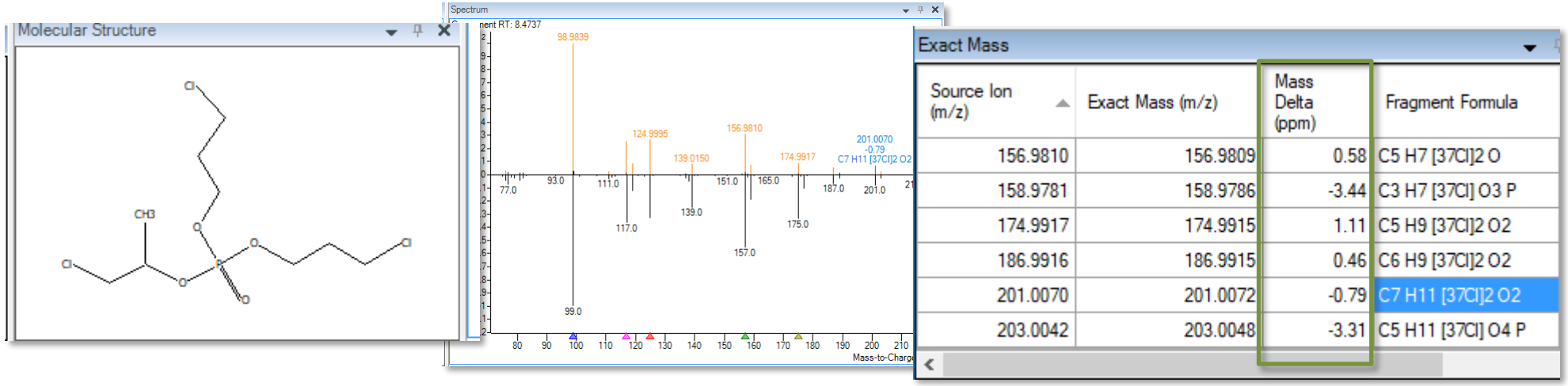




Herbicide
Insecticide
Fungicide

Examples of Contaminants Identified in Non-Targeted Screening

Tentative hit: ***Bis(3-chloro-1-propyl)(1-chloro-2-propyl)phosphate (C9H18Cl3O4P)***

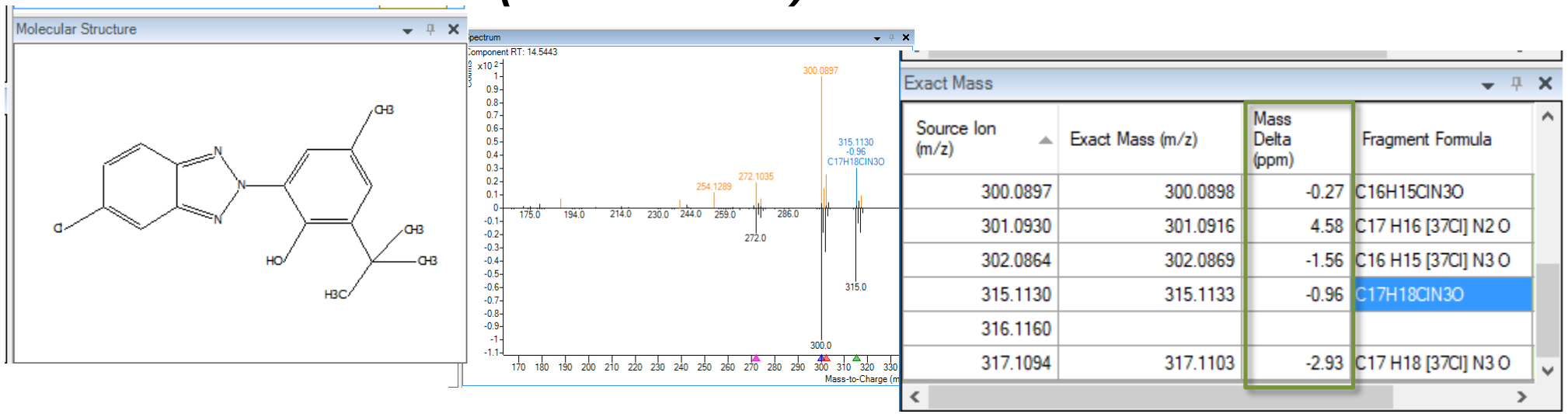


- Example of tentatively identified contaminants from UB site, using Unknowns Analysis and NIST17.L library.

- Low mass error for the fragments in the deconvoluted spectrum provides additional point for confirmation of the molecular formula of the hit.

Examples of Contaminants Identified in Non-Targeted Screening

Tentative hit: ***Bumetrizole (C27H18ClN3O)***

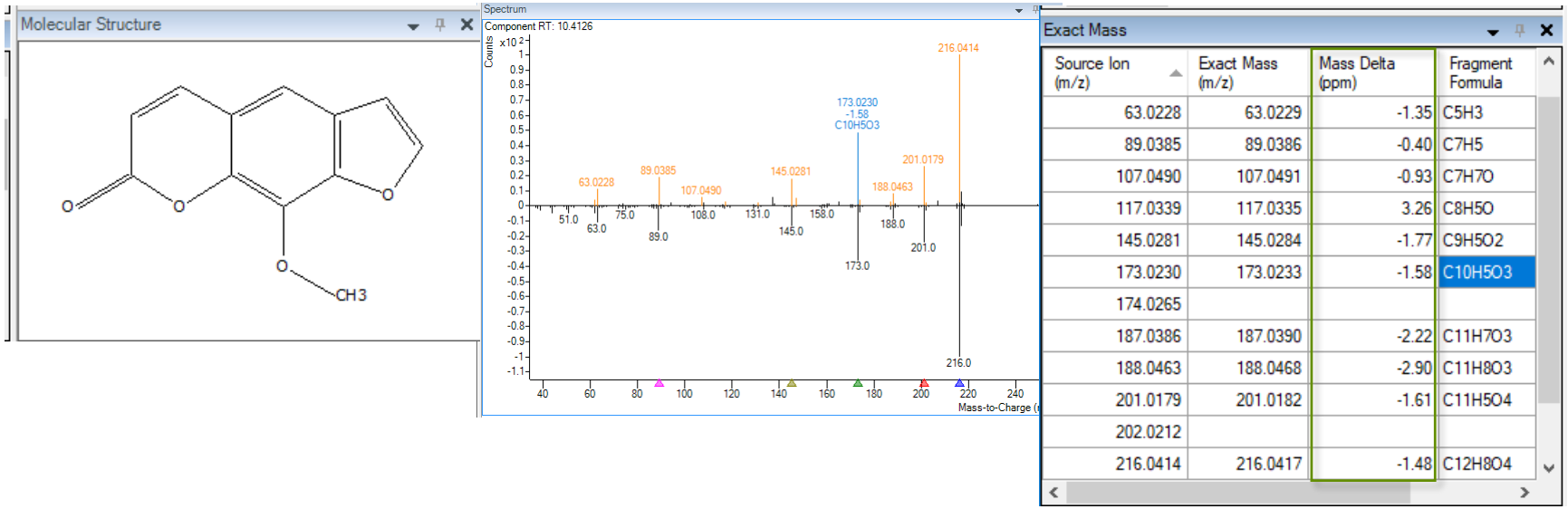


- Example of tentatively identified contaminants from UB site, using Unknowns Analysis and NIST17.L library.

- Low mass error for the fragments in the deconvoluted spectrum provides additional point for confirmation of the molecular formula of the hit.

Examples of Contaminants Identified in Non-Targeted Screening

Tentative hit: ***Methoxsalen (C12H8O4)***

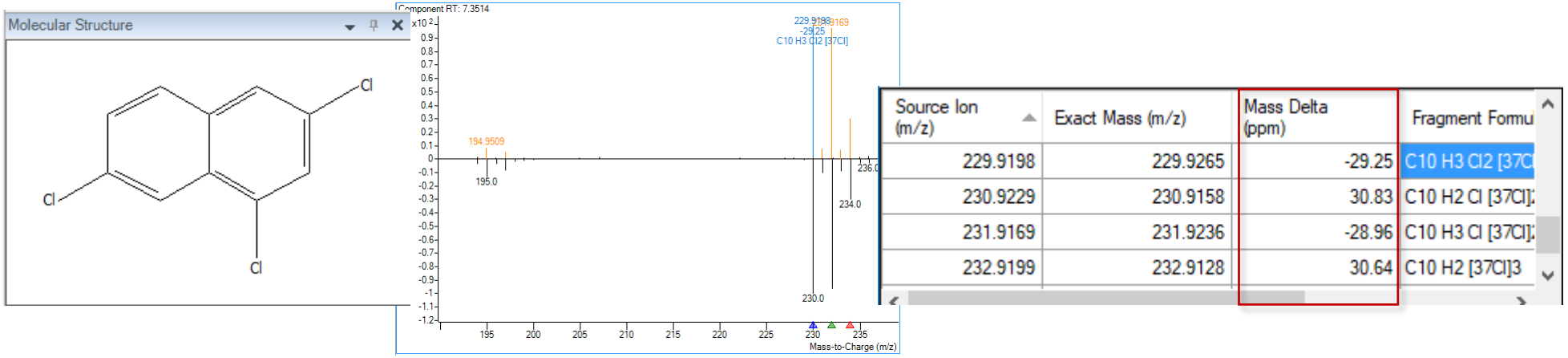


- Example of tentatively identified contaminants from UB site, using Unknowns Analysis and NIST17.L library.

- Low mass error for the fragments in the deconvoluted spectrum provides additional point for confirmation of the molecular formula of the hit.

Unknowns Structure Elucidation

Tentative NIST17 hit: ***1,3,7-trichloronaphthalene (C10H5Cl3)***



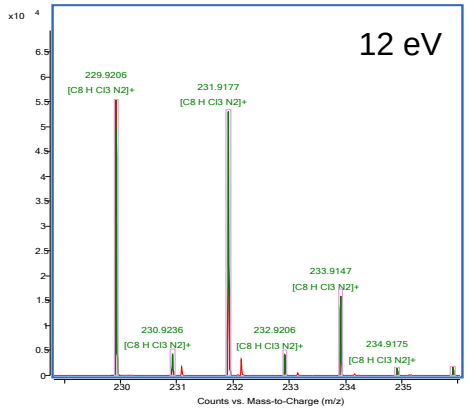
Source Ion (m/z)	Exact Mass (m/z)	Mass Delta (ppm)	Fragment Formu
229.9198	229.9265	-29.25	C10 H3 Cl2 [37C
230.9229	230.9158	30.83	C10 H2 Cl [37Cl]
231.9169	231.9236	-28.96	C10 H3 Cl [37Cl]
232.9199	232.9128	30.64	C10 H2 [37Cl]3

- Identity confirmation and structure elucidation of one of the tentative hits
- Significant mass error suggested incorrect identity of the compound

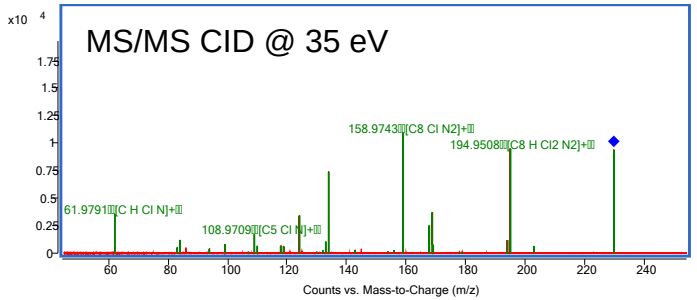
UB site

Unknowns Structure Elucidation

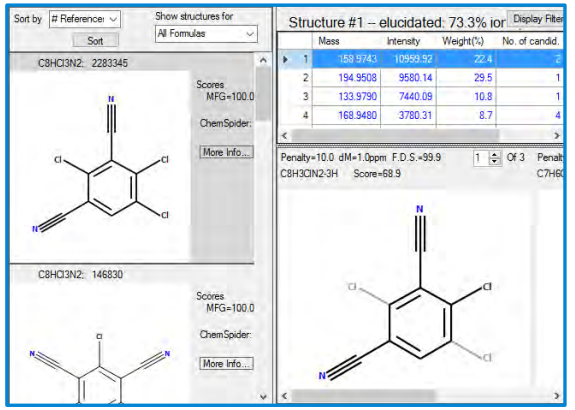
Step 1: Confirm M^+



Step 2: Confirm fragment ions



Step 3: Structure elucidation on candidate

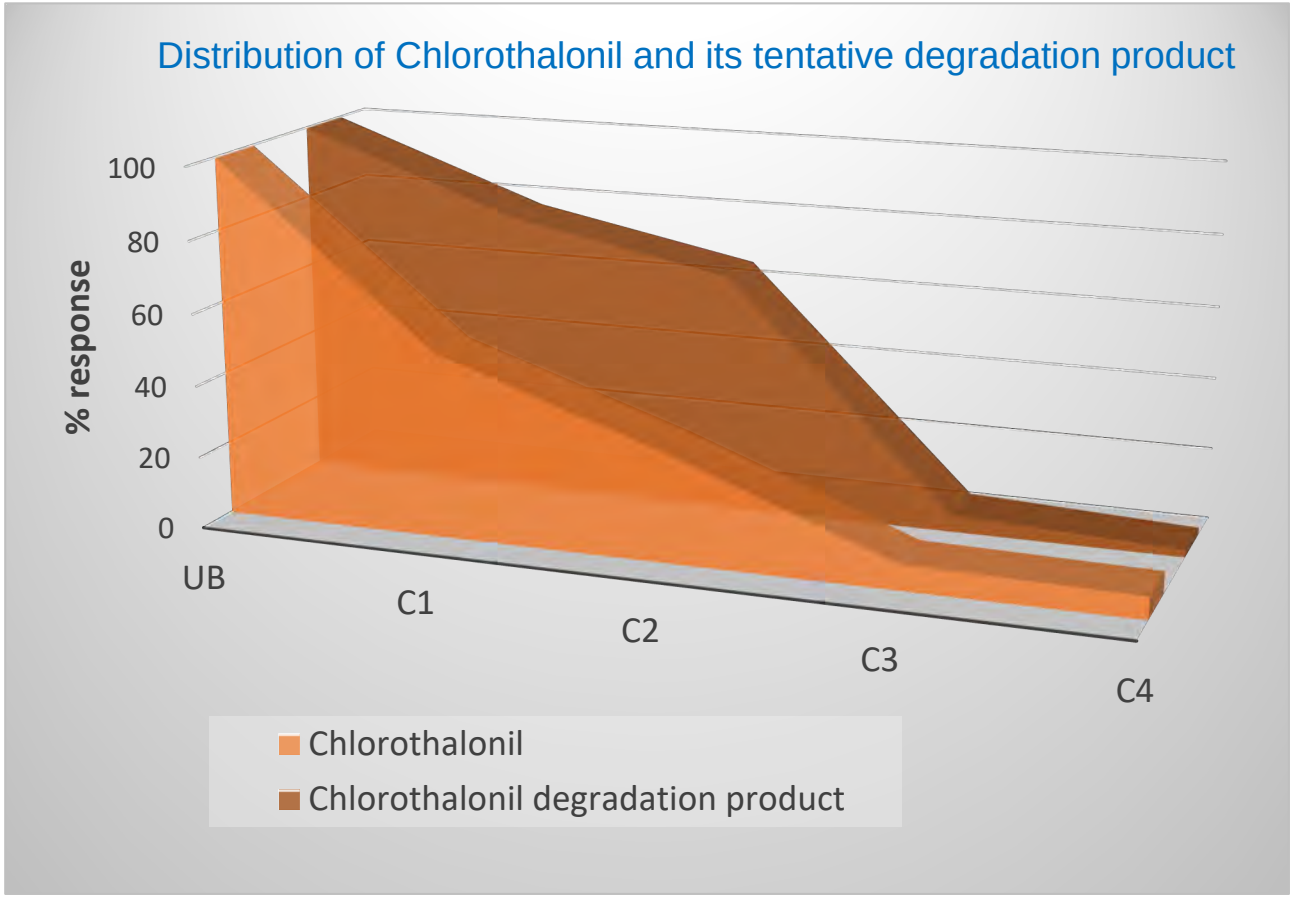


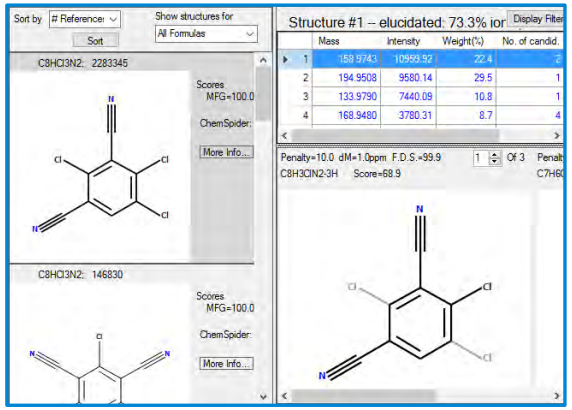
Most likely: ***2,4,5-Trichloroisophthalonitrile***.
A degradation product of Chlorothalonil

The compound was identified using Molecular Structure Correlator tool with accurate mass product ion spectrum as an input

UB site

Unknowns Structure Elucidation





Most likely: ***2,4,5-Trichloroisophthalonitrile***.
A degradation product of Chlorothalonil

UB site

Summary

- The EI and NCI suspect screening approach combined with nontargeted screening were used to identify environmental contaminants in surface water and wastewater effluents using a high-resolution GC/Q-TOF.
- A few compounds, including pesticides such as flurprimidol, paclobutrazol, azoxystrobin, and chlorantraniliprole, were identified predominantly in the wastewater effluent samples associated with some degree of toxicity.
- When using the nontargeted approach, that is unlikely to detect minor differences in the levels of trace compounds, it was able to identify additional potential contaminants outside of the accurate mass library.
- Low energy EI and accurate mass MS/MS facilitated structure elucidation of unknowns

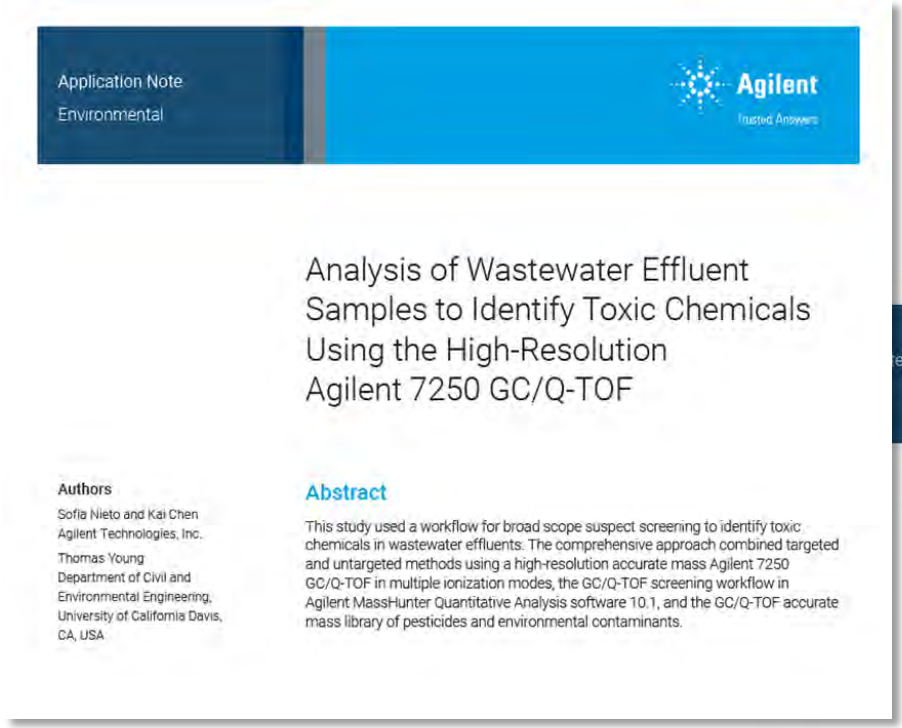
Acknowledgements & References

Thomas Young

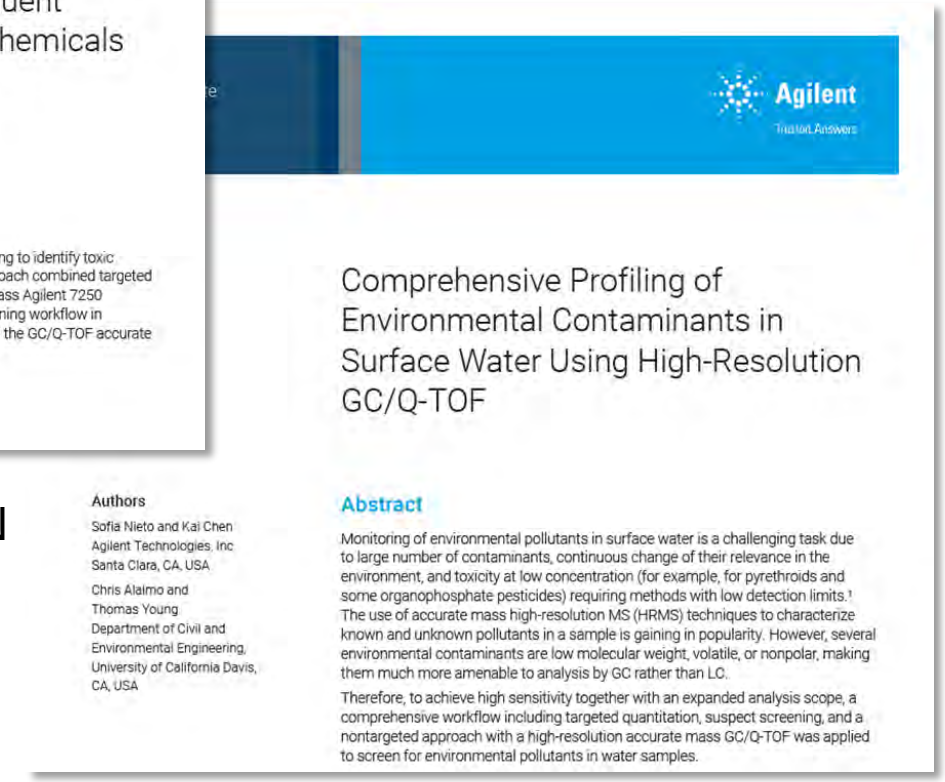
Department of Civil and Environmental Engineering
University of California, Davis

Kai Chen

Agilent Technologies, Inc.



Application Note: 5994-1345EN



Application Note: 5994-1371EN

High Resolution GC/Q-TOF for Routine Analysis of Dioxins

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competenza italiana nel settore analisi

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The BioChemie Project

Dioxin, PCB Dioxin Like and PCB Markers analysis by different high-resolution technology in food and environmental matrices

The magnetic sector is used for the analysis of classes of compounds, mainly dioxins, furans and PCBs, only to specific congeners. And not in multi-residual and multi-class areas such as Pesticides, micropollutants in food and Persistent Organic Pollutants, chemicals that are very resistant to decomposition and that have some toxic properties. Due to their persistence and toxicity characteristics, they are particularly harmful to human health and the environment.

Prerequisite in the identification and quantification in high resolution is that each ion extracted / acquired has a maximum deviation of 5 ppm vs. the exact mass and that the Mass Spectrometer Resolution is ≥ 10,000 at 10% valley (Resolving Power).

The BioChemie Project

Dioxin, PCB Dioxin Like and PCB Markers analysis by different high-resolution technology in food and environmental matrices

The analysis of Poly Chlorine Dibenzo Dioxins (PCDD), Poly Chlorine Dibenzo Furans (PCDF) and Poly Chlorine Biphenyls (PCB) in food and environmental matrices are usually performed with magnetic sector mass spectrometers.

The magnetic sector mass spectrometer normally is not used for untarget and target analysis (Pesticides) where the identification and quantification scope is required on a large number of compounds at the same time in different classes; in fact, its main focus is the analysis of compounds that have the same characteristics to be monitored (Dioxins, Furans and PCBs).

Using a technology like the Q-TOF (Time of Flight) allows to respect these conditions not only in the narrow intervals of the SICP (Selected Ion Current Profile of the Magnetic Sector; the line described by a signal at its exact value of mass charge ratio [m/z]), but in the whole scan interval in high resolution from 20 m/z to 1200 m/z. Its high speed allows it to acquire the entire spectral range in Profile without signal loss with a "Mass Drift" within 5ppm.

EPA Method Requirement

Method 1613

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

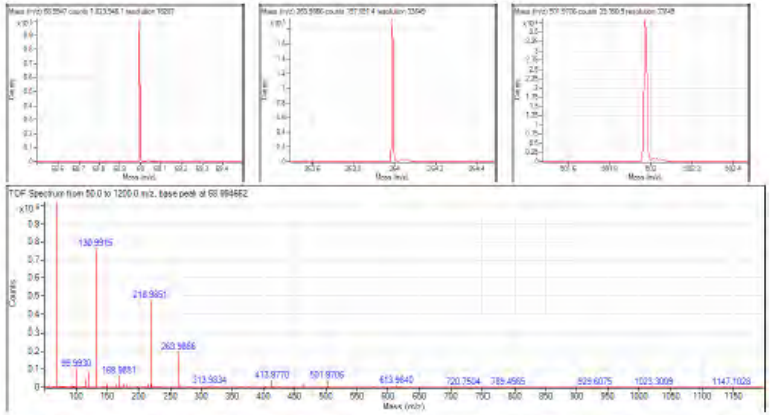
15.2 MS Resolution—A static resolving power of at least 10,000 (10% valley definition) must be demonstrated at the appropriate m/z before any analysis is performed. Static resolving power checks must be performed at the beginning and at the end of each 12-hour shift according to procedures in Section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

Enabled	Target Mass	Actual Mass	Accuracy (ppm)	Abundance	Resolution	Time of Flight (ns)
selected	68.9947	68.9947	-0.14	1,023,948.1	18267	25249.99
selected	130.9915	130.9914	-0.28	780,087.0	26184	34324.14
selected	218.9851	218.9851	-0.05	486,046.9	32399	44017.34
selected	263.9866	263.9866	0.00	197,091.4	33649	48207.76
selected	413.9770	413.9770	0.12	40,158.1	35983	60056.87
selected	463.9738	463.9736	-0.38	18,996.9	36395	63507.51
selected	501.9706	501.9706	0.01	39,350.9	37849	66007.14
selected	613.9642	613.9643	0.26	7,524.6	37665	72868.98

GC/Q-TOF System Verification - Tune

Instrument Name	GCQTOF 7250 / US1919E302	MS Model	7250
Tune Date & Time	11/30/2019 07:28:13 PM	Source	LE-EI
Software Version	10.0.368	Firmware Version	G.7250.02.02R
Tune File	D:\MassHunter\GCMS\1\7250\atunes.eihs.tune.xml Modified		

TOF



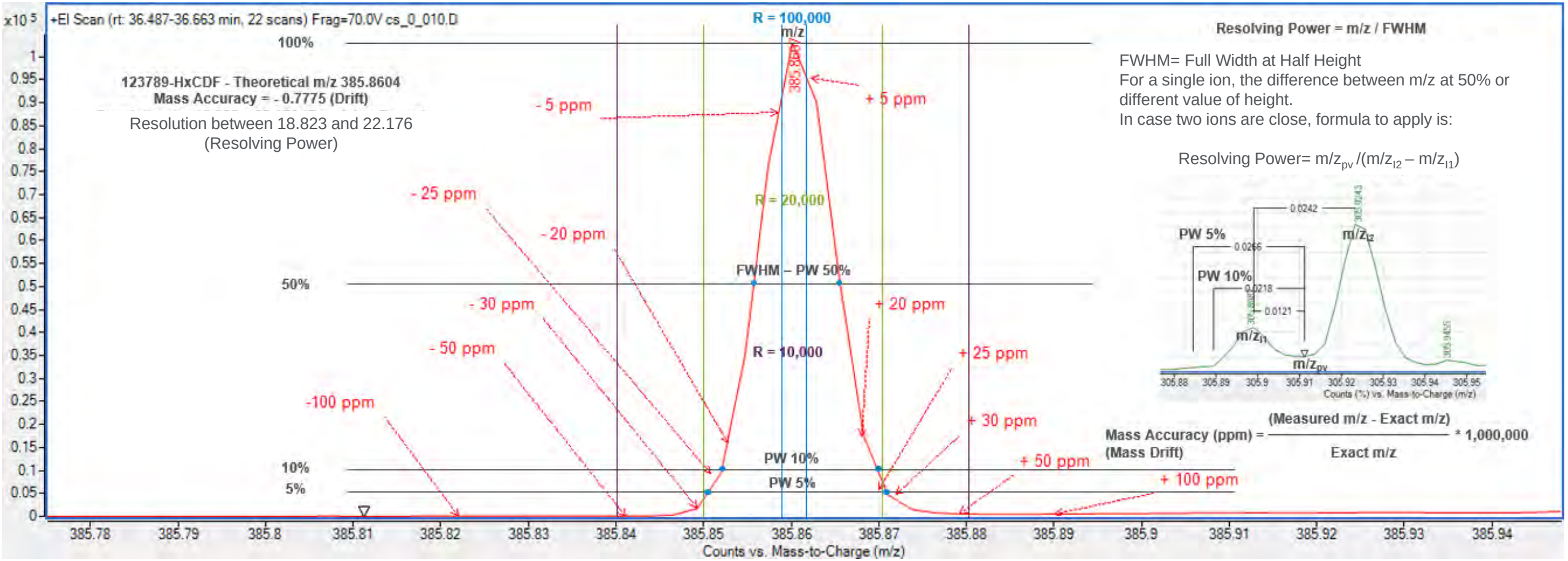
Enabled	Target Mass	Actual Mass	Accuracy (ppm)	Abundance	Resolution	Time of Flight (ns)
selected	68.9947	68.9947	-0.14	1,023,948.1	18267	25249.99
selected	130.9915	130.9914	-0.28	780,087.0	26184	34324.14
selected	218.9851	218.9851	-0.05	486,046.9	32399	44017.34
selected	263.9866	263.9866	0.00	197,091.4	33649	48207.76
selected	413.9770	413.9770	0.12	40,158.1	35983	60056.87
selected	463.9738	463.9736	-0.38	18,996.9	36395	63507.51
selected	501.9706	501.9706	0.01	39,350.9	37849	66007.14
selected	613.9642	613.9643	0.26	7,524.6	37665	72868.98

Mass accuracy < 1.0 ppm	
Maximum mass accuracy error is -0.4 ppm	OK
Base peak should be 69.00 or 131.00	
Base peak is 68.9947	OK
Base peak abundance should be > 100000	
Base peak abundance = 1023948.1	OK
Resolution should be > 25000 for peaks > 100 amu	
Lowest resolution for peaks > 100 amu = 26184	OK

Agilent | Page 1 of 3 | 11/30/2019 07:55:18 PM

Verify Mass Accuracy (Drift) and Resolving Power

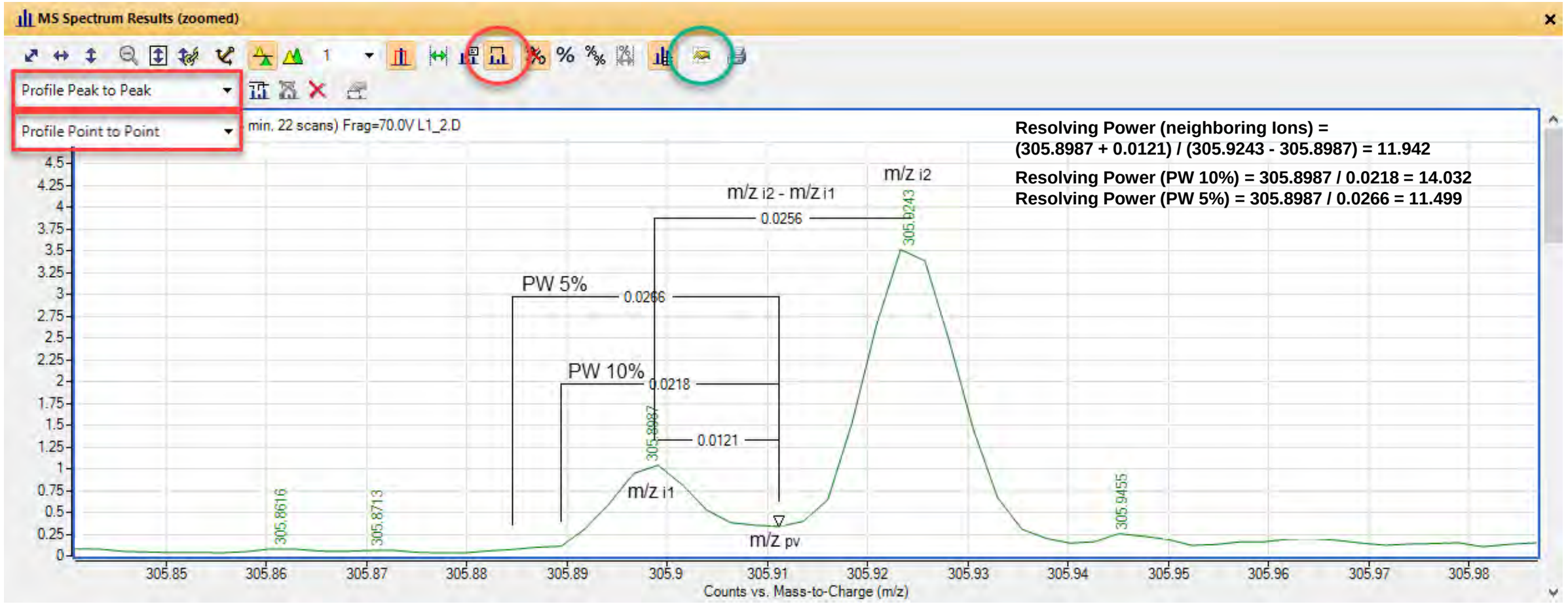
10.1.2.3 Using a PFK molecular leak, tune the instrument to meet the minimum required resolving power of 10,000 (10% valley) at m/z 304.9824 (PFK) or any other reference signal close to m/z 304 (from TCDF). For each descriptor (Table 8), monitor and record the resolution and exact m/z's of three to five reference peaks covering the mass range of the descriptor. The resolution must be greater than or equal to 10,000, and the deviation between the exact m/z and the theoretical m/z (Table 8) for each exact m/z monitored must be less than 5 ppm.



Verified Mass Accuracy (Drift) and Resolving Power

using MassHunter Qual by Caliper Measurement Tool

In the spectrum window after appropriate zoom of the ion 305.3987, use the "Delta Mass Caliper“ in "Profile Point to Point" mode for the intervals where at least one of the points is not a maximum of an ion peak (valley point), and in "Profile Peak to Peak" to select a maximum of an ions peak. Tools are marked in red in the Spectrum Figure.

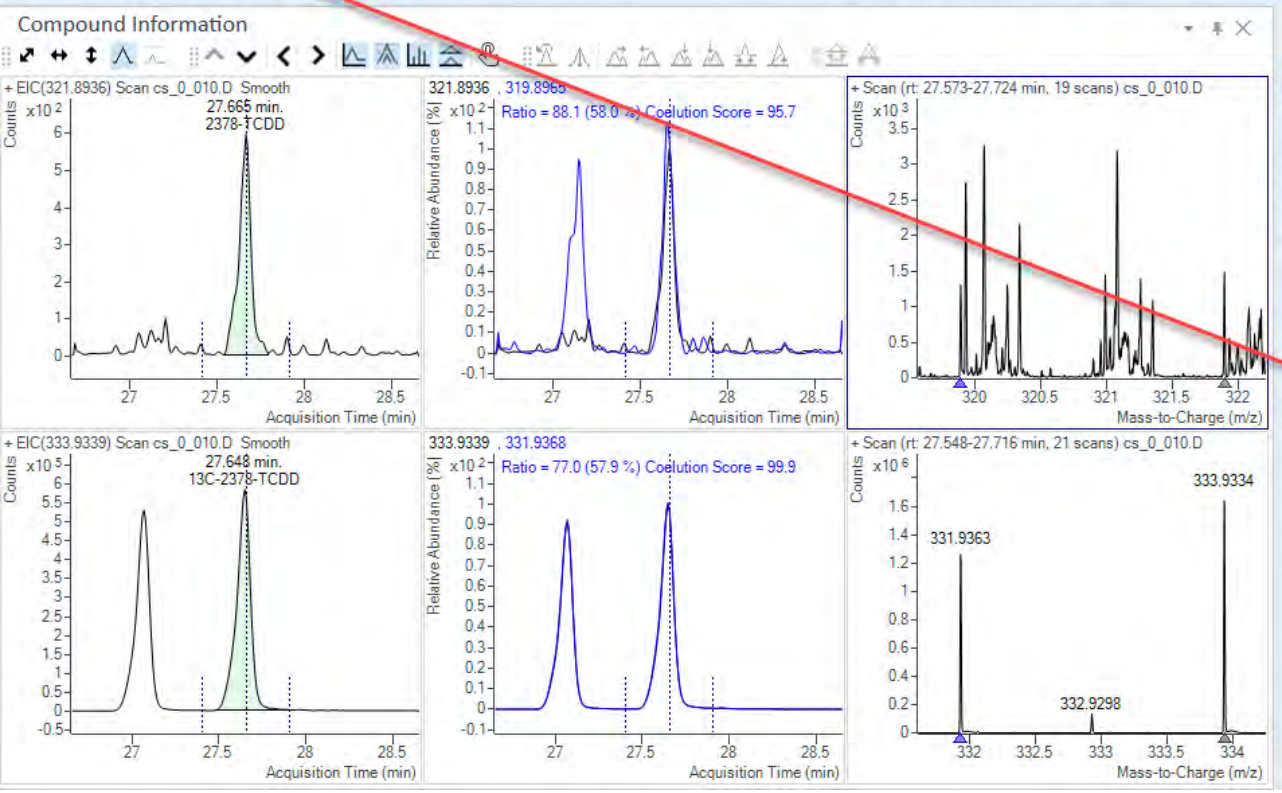


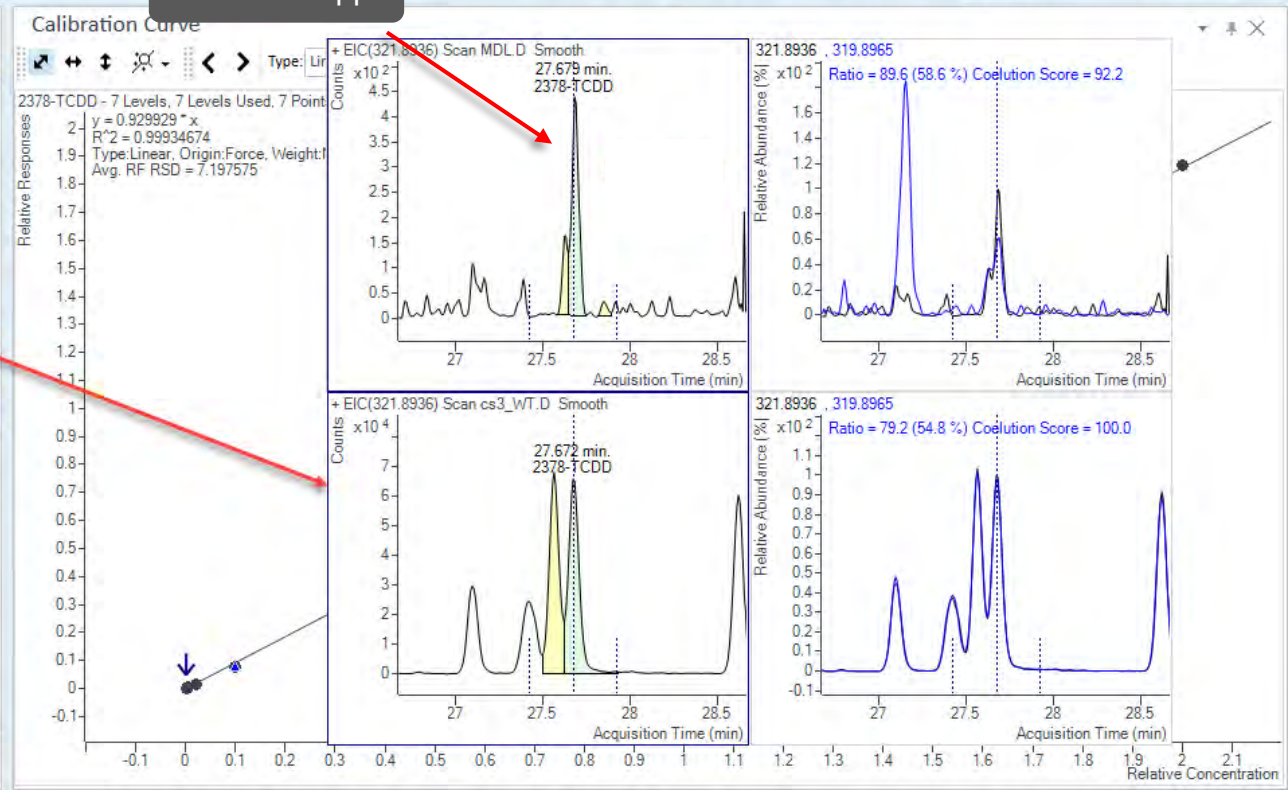
2,3,7,8-TCDD at 10ppt and 2ppt

New column and filament

Sample							2378-TCD..	2378-TCDD Results								Qualifier (319.8965) Results				13C-2378-TCDD (ISTD) Results				Qualifier (331.9368			
		Name	Data File	Type	Level	Acq. Date-Time	Dil.	Exp. Conc.	RT	MI	Resp.	Final Conc.	Accuracy	Mass Accuracy	S/N	Resolution F.	Ratio	MI	Area	Mass Accuracy	RT	MI	Resp.	Mass Accuracy	Ratio	MI	Area	M
▶		cs_0_010	cs_0_010.D	Cal	CSL	11/02/2019 09:33 AM	1.0	10.0000	27.665	☐	5394	9.9564	99.6	-1.8239	288.66	0.2	88.1	☐	2526	-1.0645	27.648	☐	5825914	-1.3997	77.0	☐	2534820	
	⚑	cs_0_025	cs_0_025.D	Cal	CS0.5	11/02/2019 10:29 AM	1.0	25.0000	27.686	☐	12428	21.9754	87.9	-2.0615	169.06	0.0	67.6	☐	5011	-4.2123	27.653	☐	6081394	-2.1884	75.6	☐	2618665	
		cs_0_050	cs_0_050.D	Cal	CS1	11/02/2019 11:25 AM	1.0	50.0000	27.667	☐	23231	42.5849	85.2	-1.1871	379.62	0.0	70.8	☐	9632	-4.0700	27.650	☐	5866400	-1.9859	75.5	☐	2523032	
		cs_0_2	cs_0_2.D	Cal	CS2	11/02/2019 12:21 PM	1.0	200.0000	27.672	☐	92111	174.4189	87.2	-2.2779	2188.23	0.0	78.1	☐	40401	-2.3115	27.655	☐	5678930	-1.0051	76.3	☐	2458229	
		cs_1	cs_1.D	Cal	CS3	11/02/2019 01:16 PM	1.0	1000.0000	27.670	☐	462834	853.0784	85.3	-3.3888	473.66	0.0	75.7	☐	199442	-3.0392	27.653	☐	5834273	-2.3737	76.5	☐	2528027	
		cs_4	cs_4.D	Cal	CS4	11/02/2019 02:12 PM	1.0	4000.0000	27.671	☐	1950419	3570.7063	89.3	-2.4982	2094.11	0.0	75.2	☐	837249	-2.6045	27.654	☐	5873866	-2.1610	76.8	☐	2551149	
		cs_20	cs_20.D	Cal	CS5	11/02/2019 03:08 PM	1.0	20000.0000	27.672	☐	11679828	20093.4830	100.5	-1.1718	3412.53	0.0	78.3	☐	127517	-1.3513	27.655	☐	6250742	-1.6898	76.3	☐	2705766	
	⚑	cs3_WT	cs3_WT.D	QC	CS3	11/04/2019 08:21 PM	1.0	1000.0000	27.672	☐	515631	902.5551	90.3	-2.9006	1857				27924	-3.2997	27.655	☐	6143495	-2.2320	76.8	☐	2669394	

MDL test 2 ppt





The Soil Ring Test, on the good way

Parametro	N° Dati Totali	N° Action	N° Warning	Dati Adeguati %	N° Blunder	Unità di misura	Valore assegnato x_{pt}	Media Robusta (Tutti i dati) x*	Scarto tipo assegnato relativo σ_{pt}%	Scarto Tipo Robusto relativo (Tutti i dati) s*%
2378TCDD	48	2	3	90	0	ng/kg	3,8	3,9	35	27,3
2378TCDF	54	2	2	93	0	ng/kg	483,3	463,4	30	19,5
12378PeCDD	55	0	2	96	0	ng/kg	110,5	105,3	30	22,4
12378PeCDF	53	1	2	94	0	ng/kg	314,5	310,9	30	18,1
23478PeCDF	56	1	3	93	0	ng/kg	492,0	462,4	30	26,6
123478HxCDD	56	3	2	91	1	ng/kg	275,8	272,8	30	23,5
123478HxCDF	56	2	4	89	0	ng/kg	1012,3	1007,8	25	18,0
123678HxCDD	58	0	5	91	0	ng/kg	2416,4	2251,3	30	29,3
123678HxCDF	55	2	4	89	1	ng/kg	222,4	219,6	30	22,1
123789HxCDD	56	0	6	89	0	ng/kg	1115,5	1071,9	25	23,8
234678HxCDF	55	2	3	91	0	ng/kg	132,3	133,6	30	26,5
1234678HpCDD	59	2	4	90	0	ng/kg	11849,6	11173,9	25	19,4
1234678HpCDF	58	2	5	88	0	ng/kg	598,3	566,9	25	21,0
1234789HpCDF	54	3	0	94	0	ng/kg	149,0	143,4	25	31,0
OCDD	58	3	7	83	0	ng/kg	11959,6	11127,5	25	23,5
OCDF	57	4	4	86	2	ng/kg	354,5	353,4	25	24,1
PCDD/DF - TEQ	53	1	3	92	0	ng/kg	1044,3	1017,9	20	16,2

Parametro	Materiale	Unità di misura	x_i	z-score	z/z'	Segnale
2378TCDD	SU/2-2-19	ng/kg	3,6	-0,12	z	-
2378TCDF	SU/2-2-19	ng/kg	573,4	0,62	z	-
12378PeCDD	SU/2-2-19	ng/kg	86,6	-0,72	z	-
12378PeCDF	SU/2-2-19	ng/kg	337	0,24	z	-
23478PeCDF	SU/2-2-19	ng/kg	312	-1,22	z	-
123478HxCDD	SU/2-2-19	ng/kg	240,3	-0,43	z	-
123478HxCDF	SU/2-2-19	ng/kg	865	-0,58	z	-
123678HxCDD	SU/2-2-19	ng/kg	2146,8	-0,37	z	-
123678HxCDF	SU/2-2-19	ng/kg	247,9	0,38	z	-
123789HxCDD	SU/2-2-19	ng/kg	1212,5	0,35	z	-
234678HxCDF	SU/2-2-19	ng/kg	75,4	-1,43	z	-
1234678HpCDD	SU/2-2-19	ng/kg	11116,8	-0,25	z	-
1234678HpCDF	SU/2-2-19	ng/kg	510,9	-0,58	z	-
1234789HpCDF	SU/2-2-19	ng/kg	168,6	0,53	z	-
OCDD	SU/2-2-19	ng/kg	12106,5	0,05	z	-
OCDF	SU/2-2-19	ng/kg	325,9	-0,32	z	-
PCDD/DF - TEQ	SU/2-2-19	ng/kg	849,3	-0,93	z	-

Metodi di prova utilizzati

- HRMS: EPA 1613, (26 laboratori); UNI EN 16190 (3 laboratori) altro metodo (1 laboratorio)
- LRMS: EPA 8280 (19 laboratori); UNI 11199 (7 laboratori) altro metodo (4 laboratori).

Accreditation Body: Water and Soil

ACCREDIA

BIOCHEMIE LAB S.r.l. Via di Limite 27/G 50013 Campi Bisenzio FI	Numero di accreditamento: **0195 L** Sede **A**
	Revisione: **56** Data: **19/03/2020**
	pag. **12** di **48** UNI CEI EN ISO/IEC 17025:2018

Acque, Suoli

Denominazione della prova / Campi di prova	*Metodo di prova*	*Tecnica di prova*	*O&I*
PCDD e PCDF: Policlorodibenzodiossine (PCDD) sostituite in 2,3,7,8: 2,3,7,8-Tetraclorodibenzodiossina (TCDD); 1,2,3,7,8-Pentaclorodibenzodiossina (PeCDD); 1,2,3,4,7,8-Esaclorodibenzodiossina (HxCDD); 1,2,3,6,7,8-Esaclorodibenzodiossina (HxCDD); 1,2,3,7,8,9-Esaclorodibenzodiossina (HxCDD); 1,2,3,4,6,7,8-Eptaclorodibenzodiossina (HpCDD); Octaclorodibenzodiossina (OCDD); Policlorodibenzofurani (PCDF) sostituiti in 2,3,7,8: 2,3,7,8-Tetraclorodibenzofurano (TCDF); 1,2,3,7,8-Pentaclorodibenzofurano (PeCDF); 2,3,4,7,8-Pentaclorodibenzofurano (PeCDF); 1,2,3,4,7,8-Esaclorodibenzofurano (HxCDF); 1,2,3,6,7,8-Esaclorodibenzofurano (HxCDF); 1,2,3,7,8,9-Esaclorodibenzofurano (HxCDF); 2,3,4,6,7,8-Esaclorodibenzofurano (HxCDF); 1,2,3,4,6,7,8-Eptaclorodibenzofurano (HpCDF); 1,2,3,4,7,8,9-Eptaclorodibenzofurano (HpCDF); Octaclorodibenzofurano (OCDF)	EPA 1613B 1994	GC-HRMS	
Somma PCDD/PCDF I-TEQ (somma dei prodotti tra le concentrazioni dei 17 congeneri PCDD/PCDF cloro sostituiti nelle posizioni 2,3,7,8 ed i NATO CCMS TEF 1988)	EPA 1613B 1994 + NATO CCMS Report n°176 1988	calcolo	
Somma PCDD/PCDF WHO-TEQ (somma	EPA 1613B 1994 + UNEP/POPS/COP.3/INF/27 11/04/2007 (somma dei prodotti tra le concentrazioni dei 17 congeneri PCDD/PCDF cloro sostituiti nelle posizioni 2.3,7.8 ed i WHO-TEF - Rif. UNEP/POPS/COP.3/INF/27 del 11/04/2007)	calcolo	

The best Choice

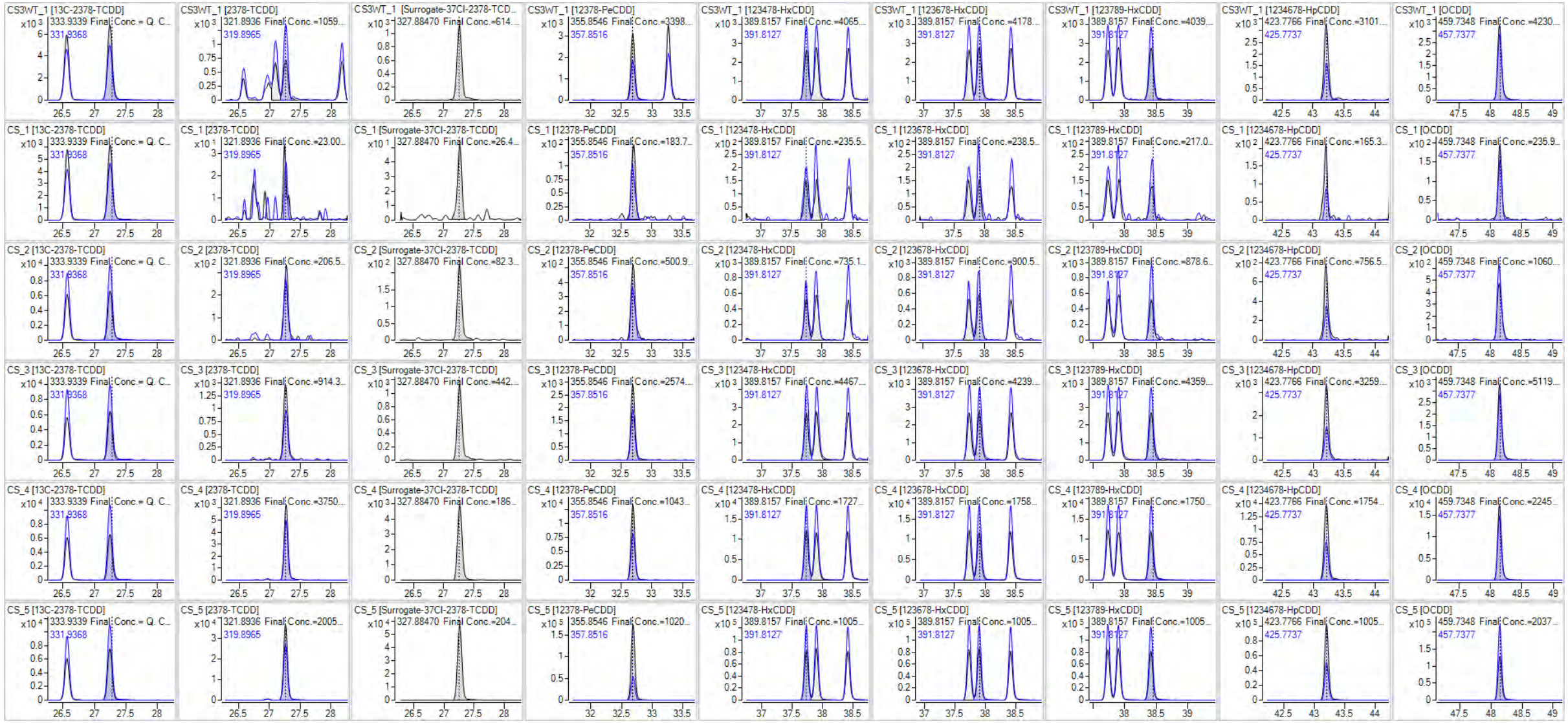
In MassHunter Quant are available several Datafiles format to analyze Accurate Mass Data and possibility to choose different Mass Extraction windows for the acquired signal. Also Different Acquisition Rate are settable to balance ion statistic and peaks datapoints.

The final choices are:

- Profile: method requirement to calculate the Resolution at 10% valley
- Mass Extraction: 5 ppm should be the most common way. 25 ppm can be used too. It is possible to work on a double approach, with a first screening batch at 5ppm to check the mass drift and a second quantitation batch at 25 ppm to quantitate the samples.
- Acquisition Rate: 2 spectra/s

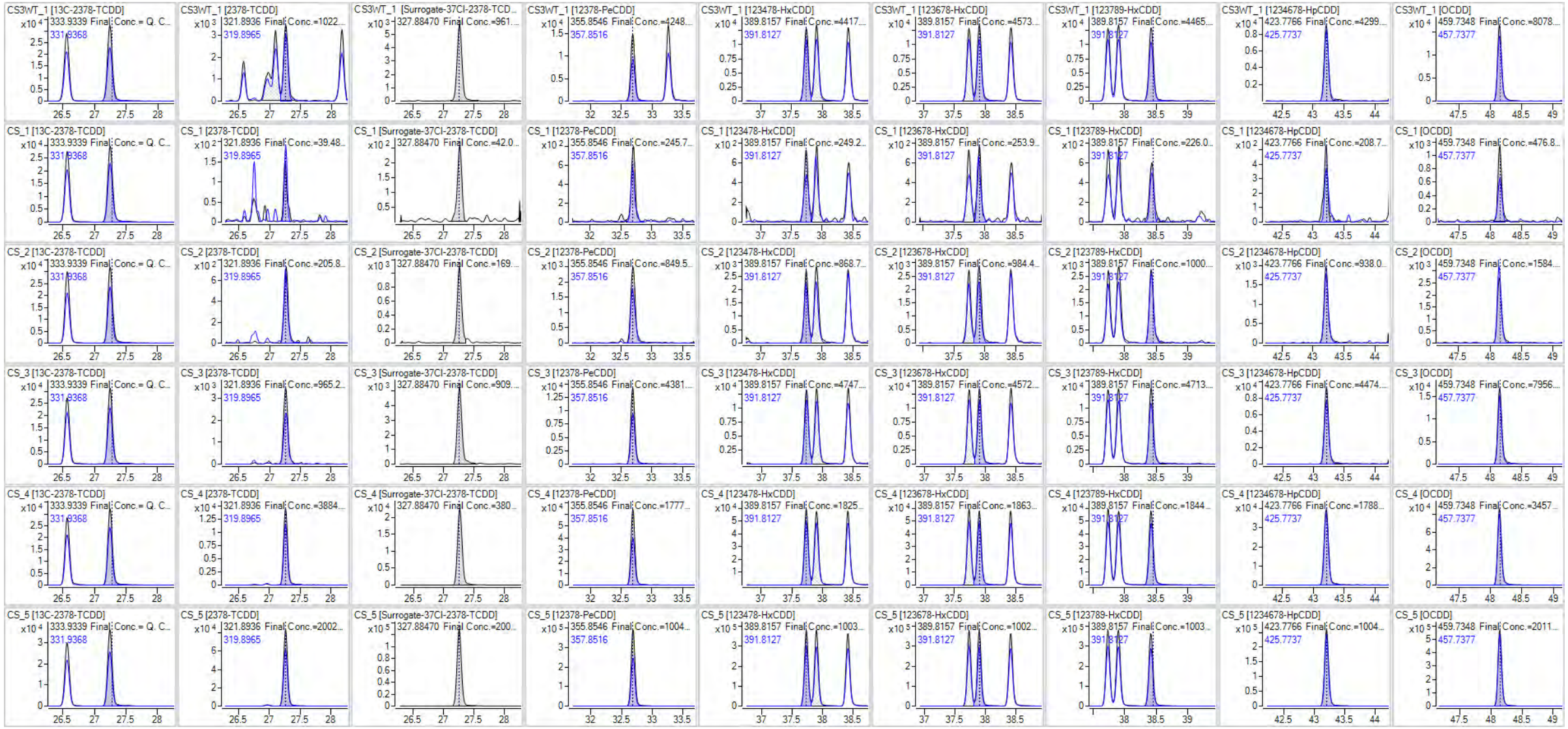
Dioxins in Profile

2 scans/s Acq Rate and 5 ppm Mass Extraction - Calibration CS1-CS5



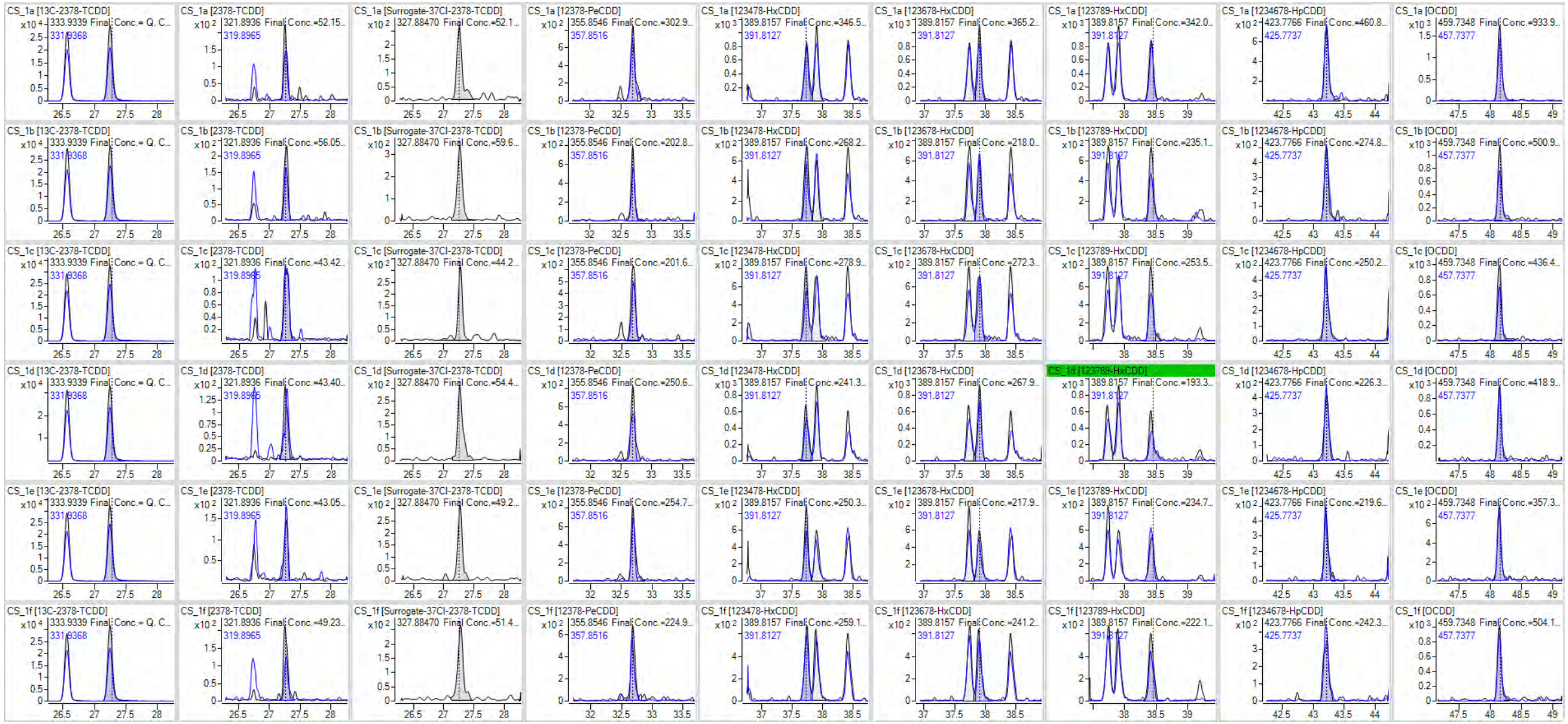
Dioxins in Profile

2 scans/s Acq Rate and 25 ppm Mass Extraction - Calibration CS1-CS5



Dioxin in Profile

2 scans/s Acq Rate and 25 ppm Mass Extraction - Reproducibility CS1



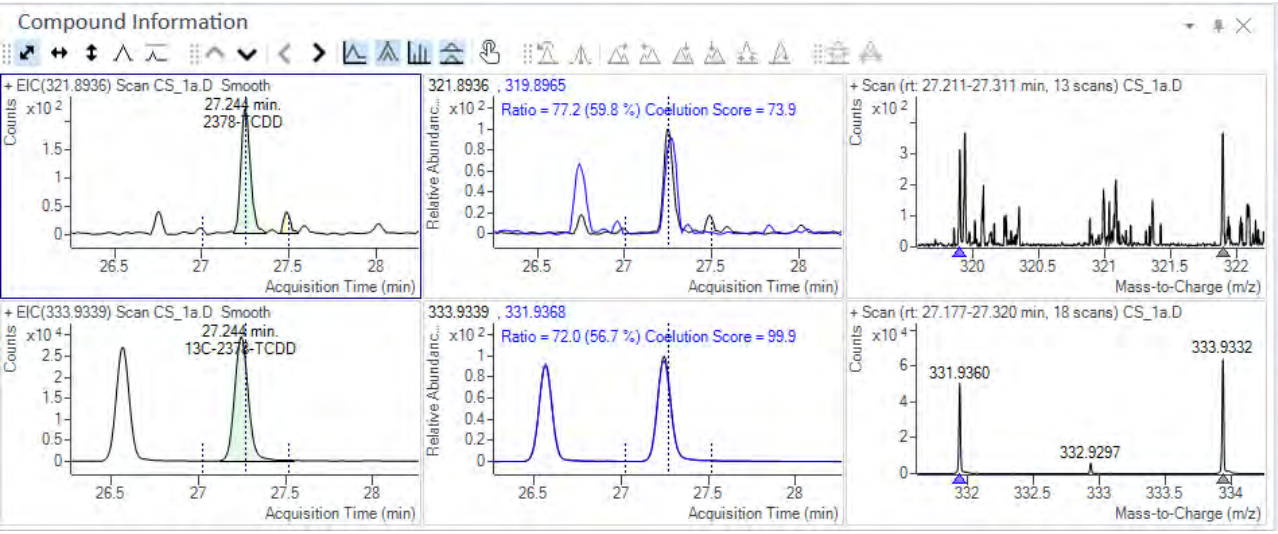
Dioxin in Profile

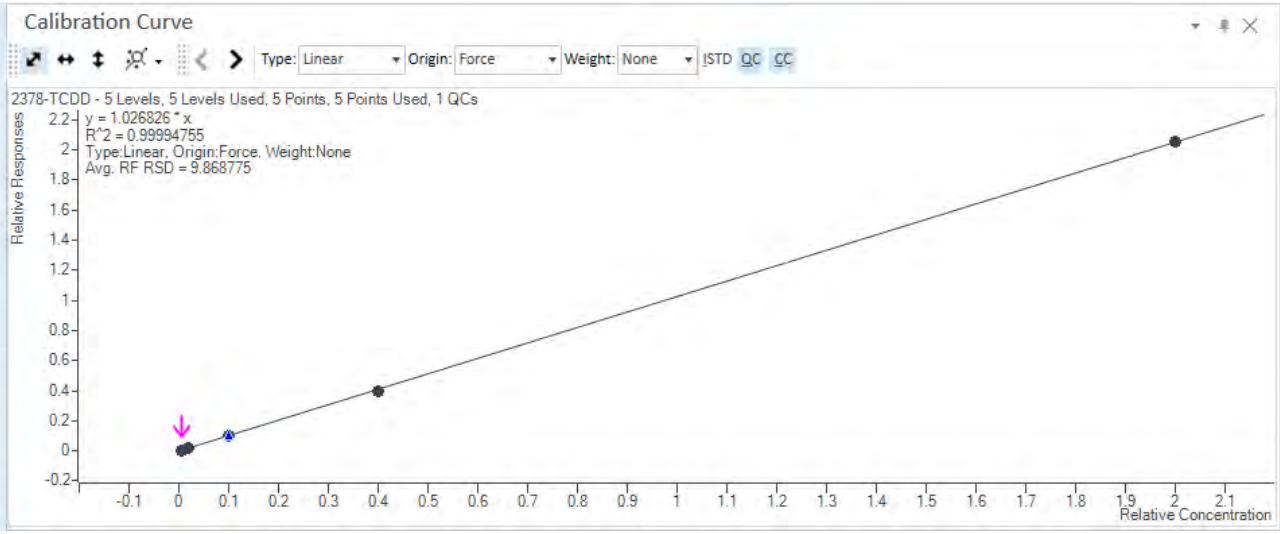
2 scans/s Acq Rate and 25 ppm Mass Extraction - Reproducibility CS1

(ppt) CS1 Tetra 50 - Penta Hexa Hepta 250 - Octa 500

Compound Method			CS_1a	Qualifi...	ISTD R...	ISTD...	CS_1b	Qualifi...	ISTD R...	ISTD...	CS_1c	Qualifi...	ISTD R...	ISTD...	CS_1d	Qualifi...	ISTD R...	ISTD...	CS_1e	Qualifi...	ISTD R...	ISTD...	CS_1f	Qualifi...	ISTD R...	ISTD...
Name	Transition	RT	Final Conc.	Area	Area	Area	Final Conc.	Area	Area	Area	Final Conc.	Area	Area	Area	Final Conc.	Area	Area	Area	Final Conc.	Area	Area	Area	Final Conc.	Area	Area	Area
2378-TCDD	321.8936	27.279	52.1543	663	165202	118924	56.0576	634	169520	124865	43.4254	653	179666	134474	43.4039	693	180876	127314	43.0536	696	175571	132947	49.2302	532	175135	121083
12378-PeCDD	355.8546	32.708	302.9143	3381	140137	86800	202.8719	2235	146923	96312	201.6435	2213	152777	98012	250.6058	2891	149925	100458	254.7759	2752	145873	95914	224.9952	2436	144496	85735
123478-HxCDD	389.8157	37.760	346.5083	3752	126203	93712	268.2883	2591	129305	96885	278.9580	3020	136659	99780	241.3882	2701	128142	103883	250.3892	2597	133559	103519	259.1539	3068	128267	95336
123678-HxCDD	389.8157	37.936	365.2158	4385	137178	100143	218.0835	3077	136419	111544	272.3659	3407	143314	119958	267.9914	3268	143430	115647	217.9321	2563	140002	107200	241.2093	2749	134612	104280
123789-HxCDD	389.8157	38.447	342.0160	3807	131691		235.1310	2472	132862		253.5199	2806	139986		193.3342	2278	142089	115277	234.7888	3241	136781		222.1199	2190	131439	
1234678-HpCDD	423.7766	43.234	460.8025	3930	78991	73158	274.8537	2790	87148	80398	250.2685	2535	92629	86724	226.3505	2207	90774	86534	219.6219	2252	86656	86468	242.3600	2384	84306	86360
OCDD	459.7348	48.156	933.9701	7068	151211	124801	500.9108	3935	160898	148349	436.4790	3311	172539	149627	418.9746	3721	172723	151693	357.3865	3145	162961	150642	504.1717	4232	167429	140046

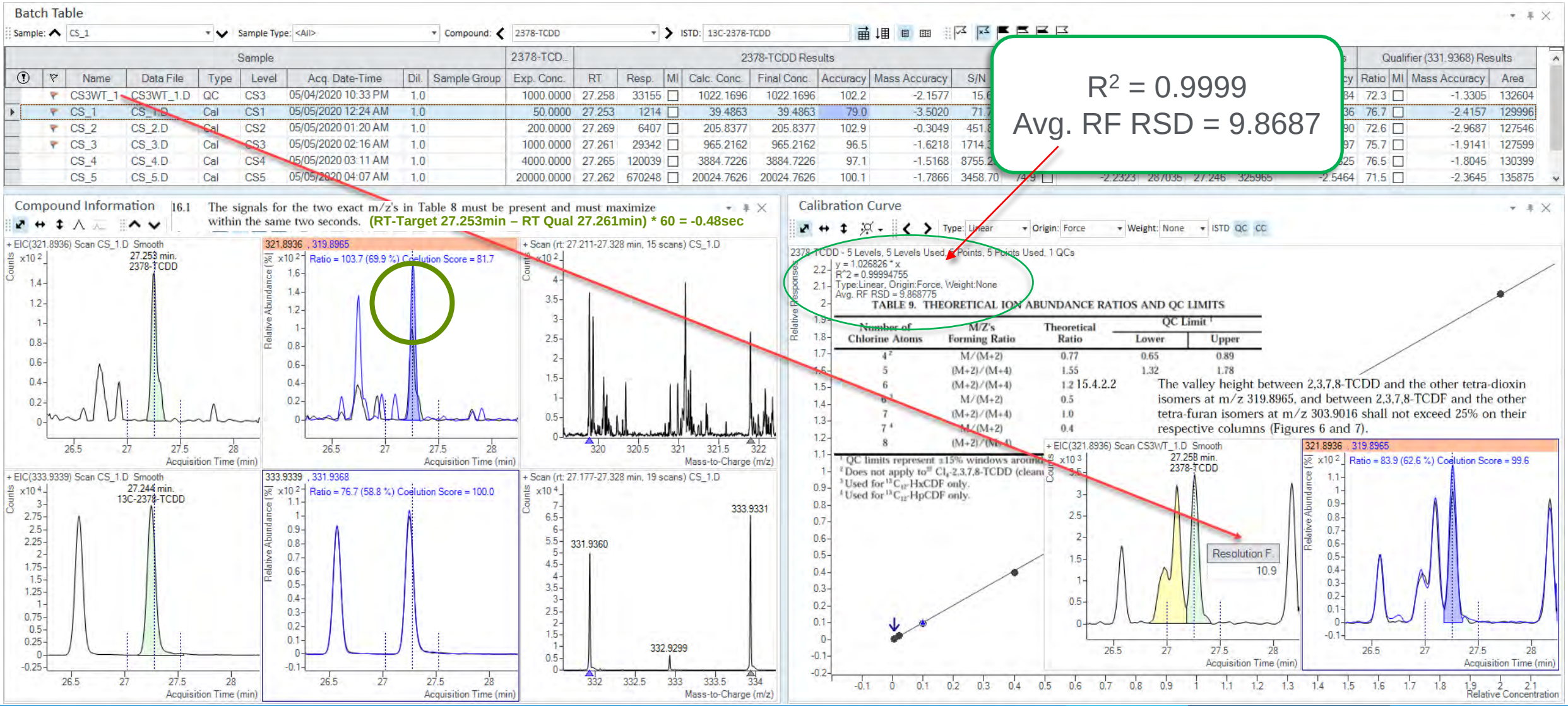
CS1b-CS_1f	2378-TCDD	12378-PECDD	123478-HxCDD	123678-HxCDD	123789-HxCDD	1234678-HpCDD	OCDD
RSD%	10.4	12.2	8.3	11.7	16.6	9.7	12.1





Ion Ratio results in Profile

2 scans/s Acq Rate and 25 ppm Mass Extraction - Native Compound Dioxin



Calibration Range

The Calibration Range is associated not only with the instrument sensitivity, but also with the capacity to detect a low concentration and at the same time satisfy the identification and quality requirement of the Method, but most important the Law Limits.

16.0 Qualitative Determination

A CDD, CDF, or labeled compound is identified in a standard, blank, or sample when all of the criteria in Sections 16.1 through 16.4 are met.

16.1 The signals for the two exact m/z's in Table 8 must be present and must maximize within the same two seconds.

16.2 The signal-to-noise ratio (S/N) for the GC peak at each exact m/z must be greater than or equal to 2.5 for each CDD or CDF detected in a sample extract, and greater than or equal to 10 for all CDDs/CDFs in the calibration standard (Sections 10.2.3 and 15.3.3).

±15% windows around the theoretical ion abundance ratios.

16.3 The ratio of the integrated areas of the two exact m/z's specified in Table 8 must be within the limit in Table 9, or within ±10% of the ratio in the midpoint (CS3) calibration or calibration verification (VER), whichever is most recent.

16.4 The relative retention time of the peak for a 2,3,7,8-substituted CDD or CDF must be within the limit in Table 2. The retention time of peaks representing non-2,3,7,8-substituted CDDs/CDFs must be within the retention time windows established in Section 10.3.

Calibration Range: Standard

NATIVE PCDDs & PCDFs	1613CSL (ng/ml)	1613CS0.5 (ng/ml)	1613CS1 (ng/ml)	1613CS2 (ng/ml)	1613CS3 (ng/ml)	1613CS4 (ng/ml)	1613CS5 (ng/ml)
2,3,7,8-Tetrachlorodibenzo-p-dioxin	0.1	0.25	0.5	2	10	40	200
1,2,3,7,8-Pentachlorodibenzo-p-dioxin	0.5	1.25	2.5	10	50	200	1000
1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	0.5	1.25	2.5	10	50	200	1000
1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	0.5	1.25	2.5	10	50	200	1000
1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	0.5	1.25	2.5	10	50	200	1000
1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	0.5	1.25	2.5	10	50	200	1000
Octachlorodibenzo-p-dioxin	1.0	2.5	5.0	20	100	400	2000
2,3,7,8-Tetrachlorodibenzofuran	0.1	0.25	0.5	2	10	40	200
1,2,3,7,8-Pentachlorodibenzofuran	0.5	1.25	2.5	10	50	200	1000
2,3,4,7,8-Pentachlorodibenzofuran	0.5	1.25	2.5	10	50	200	1000
1,2,3,4,7,8-Hexachlorodibenzofuran	0.5	1.25	2.5	10	50	200	1000
1,2,3,6,7,8-Hexachlorodibenzofuran	0.5	1.25	2.5	10	50	200	1000
1,2,3,7,8,9-Hexachlorodibenzofuran	0.5	1.25	2.5	10	50	200	1000
2,3,4,6,7,8-Hexachlorodibenzofuran	0.5	1.25	2.5	10	50	200	1000
1,2,3,4,6,7,8-Heptachlorodibenzofuran	0.5	1.25	2.5	10	50	200	1000
1,2,3,4,7,8,9-Heptachlorodibenzofuran	0.5	1.25	2.5	10	50	200	1000
Octachlorodibenzofuran	1.0	2.5	5.0	20	100	400	2000
LABELLED PCDDs & PCDFs							
2,3,7,8-Tetrachloro[$^{13}C_{12}$]dibenzo-p-dioxin	100	100	100	100	100	100	100
1,2,3,7,8-Pentachloro[$^{13}C_{12}$]dibenzo-p-dioxin	100	100	100	100	100	100	100
1,2,3,4,7,8-Hexachloro[$^{13}C_{12}$]dibenzo-p-dioxin	100	100	100	100	100	100	100
1,2,3,6,7,8-Hexachloro[$^{13}C_{12}$]dibenzo-p-dioxin	100	100	100	100	100	100	100
1,2,3,4,6,7,8-Heptachloro[$^{13}C_{12}$]dibenzo-p-dioxin	100	100	100	100	100	100	100
Octachloro[$^{13}C_{12}$]dibenzo-p-dioxin	200	200	200	200	200	200	200
2,3,7,8-Tetrachloro[$^{13}C_{12}$]dibenzofuran	100	100	100	100	100	100	100
1,2,3,7,8-Pentachloro[$^{13}C_{12}$]dibenzofuran	100	100	100	100	100	100	100
2,3,4,7,8-Pentachloro[$^{13}C_{12}$]dibenzofuran	100	100	100	100	100	100	100
1,2,3,4,7,8-Hexachloro[$^{13}C_{12}$]dibenzofuran	100	100	100	100	100	100	100
1,2,3,6,7,8-Hexachloro[$^{13}C_{12}$]dibenzofuran	100	100	100	100	100	100	100
1,2,3,7,8,9-Hexachloro[$^{13}C_{12}$]dibenzofuran	100	100	100	100	100	100	100
2,3,4,6,7,8-Hexachloro[$^{13}C_{12}$]dibenzofuran	100	100	100	100	100	100	100
1,2,3,4,6,7,8-Heptachloro[$^{13}C_{12}$]dibenzofuran	100	100	100	100	100	100	100
1,2,3,4,7,8,9-Heptachloro[$^{13}C_{12}$]dibenzofuran	100	100	100	100	100	100	100
CLEANUP STANDARD							
2,3,7,8-[$^{37}Cl_4$]-Tetrachlorodibenzo-p-dioxin	0.1	0.25	0.5	2	10	40	200
INTERNAL STANDARDS							
1,2,3,4-Tetrachloro[$^{13}C_{12}$]dibenzo-p-dioxin	100	100	100	100	100	100	100
1,2,3,7,8,9-Hexachloro[$^{13}C_{12}$]dibenzo-p-dioxin	100	100	100	100	100	100	100

For the Calibration Curve the Standard Solution for EPA Method 1613 CLS, CS0.5, CS1, CS2, CS3, CS4, CS5 and CS3WT, from Wellington Laboratories - Guelph, Ontario - Canada, are ready to use. We perform an additional dilution 1:10 with a solvent mix of Nonane/Toluene 95:5.

CS3WT — EPA Method 1613; Calibration and Verification Solution (CS3) combined with Window Defining and 2378-TCDD Resolution Testing Congeners

QUANTITATIVE ANALYTES		(ng/ml)	SEMI-QUANTITATIVE ANALYTES	(ng/ml)
NATIVE PCDDs & PCDFs:			**WINDOW DEFINERS:***	
2,3,7,8-Tetrachlorodibenzo-p-dioxin		10	1,3,6,8-Tetrachlorodibenzo-p-dioxin	10
1,2,3,7,8-Pentachlorodibenzo-p-dioxin		50	1,2,8,9-Tetrachlorodibenzo-p-dioxin	10
1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin		50	1,2,4,7,9-Pentachlorodibenzo-p-dioxin	50
1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin		50	1,2,3,8,9-Pentachlorodibenzo-p-dioxin	50
1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin		50	1,2,4,6,7,9-Hexachlorodibenzo-p-dioxin	50
1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	(WD)	50	1,2,3,4,6,7,9-Heptachlorodibenzo-p-dioxin	50
Octachlorodibenzo-p-dioxin		100		
QUANTITATIVE ANALYTES		(ng/ml)	**SEMI-QUANTITATIVE ANALYTES**	(ng/ml)
NATIVE PCDDs & PCDFs:			**WINDOW DEFINERS:***	
			1,3,6,8-Tetrachlorodibenzofuran	10
2,3,7,8-Tetrachlorodibenzofuran		10	1,2,8,9-Tetrachlorodibenzofuran	10
1,2,3,7,8-Pentachlorodibenzofuran		50	1,3,4,6,8-Pentachlorodibenzofuran	50
2,3,4,7,8-Pentachlorodibenzofuran		50	1,2,3,8,9-Pentachlorodibenzofuran	50
1,2,3,4,7,8-Hexachlorodibenzofuran		50	1,2,3,4,6,8-Hexachlorodibenzofuran	50
1,2,3,6,7,8-Hexachlorodibenzofuran		50		
1,2,3,7,8,9-Hexachlorodibenzofuran		50	**2,3,7,8-TCDD RESOLUTION TESTING ISOMERS:**	
2,3,4,6,7,8-Hexachlorodibenzofuran		50	1,2,3,4-Tetrachlorodibenzo-p-dioxin	5
1,2,3,4,6,7,8-Heptachlorodibenzofuran	(WD)	50	1,2,3,7/1,2,3,8-Tetrachlorodibenzo-p-dioxin mix	5
1,2,3,4,7,8,9-Heptachlorodibenzofuran	(WD)	50	1,2,3,9-Tetrachlorodibenzo-p-dioxin	10
Octachlorodibenzofuran		100		
LABELLED PCDDs & PCDFs:				
2,3,7,8-Tetrachloro[$^{13}C_{12}$]dibenzo-p-dioxin		100	2,3,7,8-Tetrachloro[$^{13}C_{12}$]dibenzofuran	100
1,2,3,7,8-Pentachloro[$^{13}C_{12}$]dibenzo-p-dioxin		100	1,2,3,7,8-Pentachloro[$^{13}C_{12}$]dibenzofuran	100
1,2,3,4,7,8-Hexachloro[$^{13}C_{12}$]dibenzo-p-dioxin		100	2,3,4,7,8-Pentachloro[$^{13}C_{12}$]dibenzofuran	100
1,2,3,6,7,8-Hexachloro[$^{13}C_{12}$]dibenzo-p-dioxin		100	1,2,3,4,7,8-Hexachloro[$^{13}C_{12}$]dibenzofuran	100
1,2,3,4,6,7,8-Heptachloro[$^{13}C_{12}$]dibenzo-p-dioxin		100	1,2,3,6,7,8-Hexachloro[$^{13}C_{12}$]dibenzofuran	100
Octachloro[$^{13}C_{12}$]dibenzo-p-dioxin		200	1,2,3,7,8,9-Hexachloro[$^{13}C_{12}$]dibenzofuran	100
CLEANUP STANDARD:			2,3,4,6,7,8-Hexachloro[$^{13}C_{12}$]dibenzofuran	100
2,3,7,8-[$^{37}Cl_4$]-Tetrachlorodibenzo-p-dioxin		10	1,2,3,4,6,7,8-Heptachloro[$^{13}C_{12}$]dibenzofuran	100
			1,2,3,4,7,8,9-Heptachloro[$^{13}C_{12}$]dibenzofuran	100
INTERNAL STANDARDS:				
1,2,3,4-Tetrachloro[$^{13}C_{12}$]dibenzo-p-dioxin		100		
1,2,3,7,8,9-Hexachloro[$^{13}C_{12}$]dibenzo-p-dioxin		100		

Calibration Range: The Law

The Italian Law for the matrices investigate Water and Soil is Legislative Decree 3 April 2006 N° 152 and modification at December 2019, it is transposing the Environmental Directive of the European Parliament.

For Groundwater, the threshold limits are reported in the Table 2 Annex 5 Part IV Title V, equal to $4*10^{-6}$ µg-TEQ/L for the sum of Dioxins and Furans.

For Soil, the threshold limits are reported in the Table 1 Annex 5 Part IV Title V, in column A for Public Garden and column B for Commercial and Industrial Sites equal to $1*10^{-5}$ and $1*10^{-4}$ mg-TEQ/Kg dry matter for the sum of Dioxins and Furans.

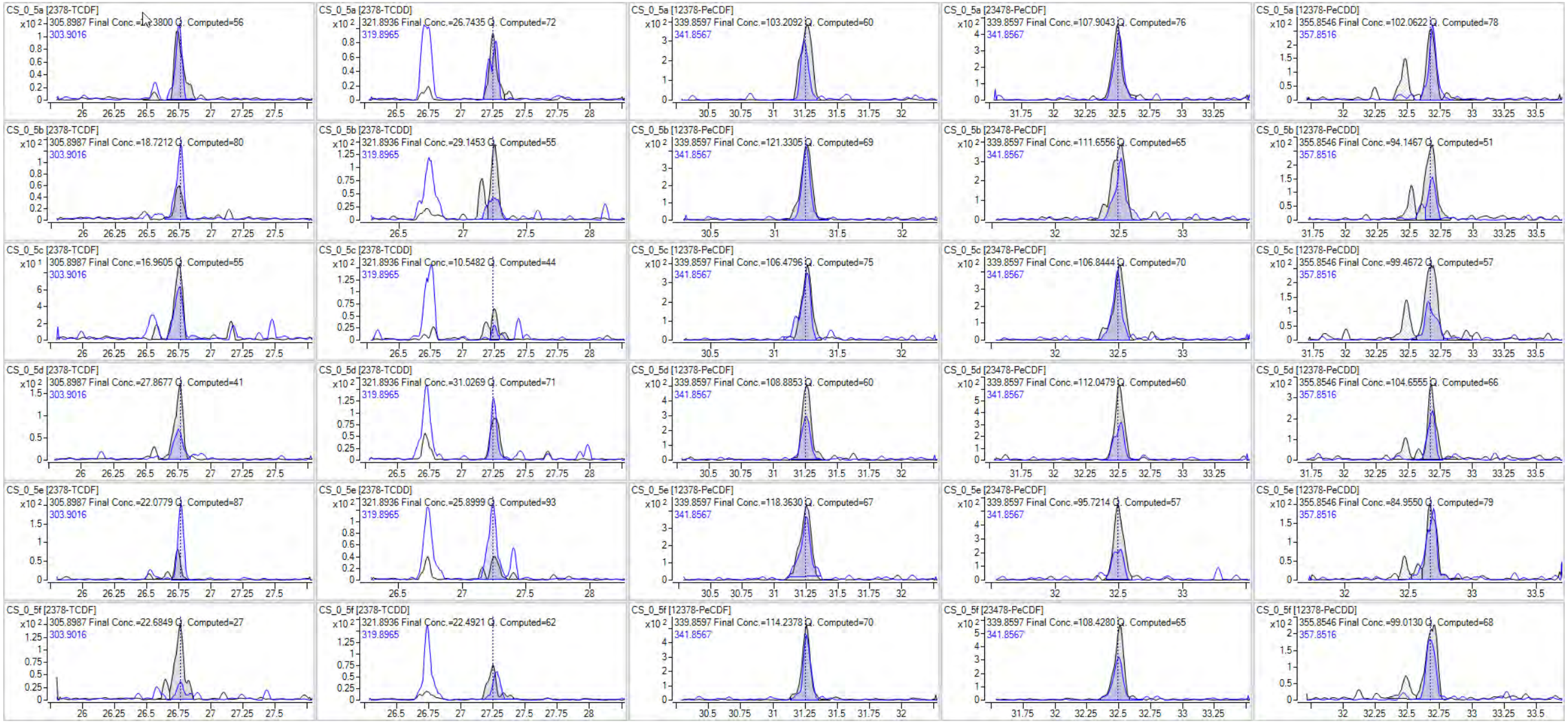
The same Decree, where possible, ask to guarantee a LOQ less than 1/10 of the threshold limits.

Usually for Environmental Samples is recommended the Medium Bound approach for TEQ.

- **NATO/CCMS: North Atlantic Treaty Organization/Committee on the Challenges of Modern Society. International Toxicity Equivalency Factor (I-TEF) method of risk assessment for complex mixtures of dioxin and related compounds, 186, 1988**
- **WHO: World Health Organization - The 2005 World Health Organization Re-evaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-like Compounds. Van den Berg, M. et al. ToxSci Advance Access published July 7, 2006.**

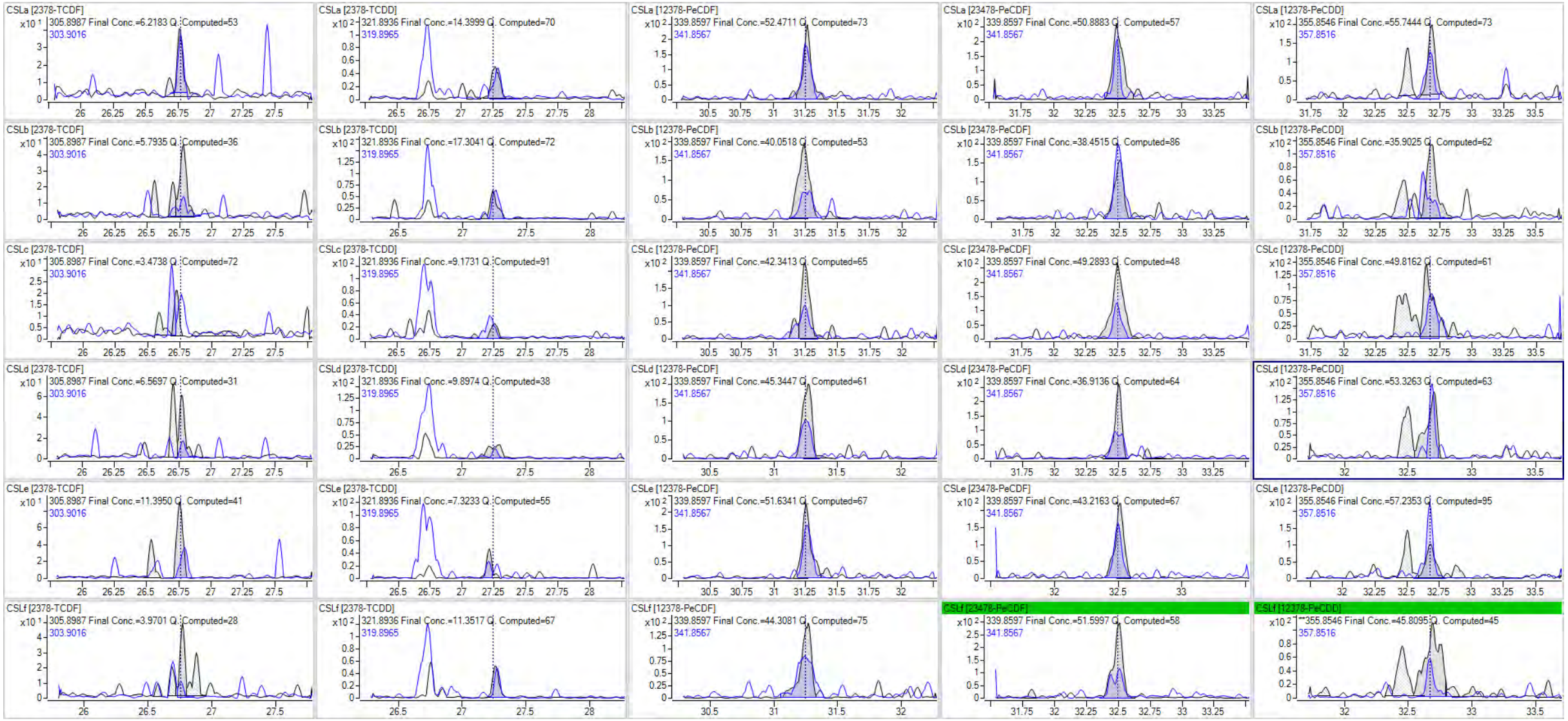
CS0.5 - (ppt) Tetra 25 and Penta 125

Hexa and Heptha 125; Octa 250 (after 500 runs)



CSL - (ppt) Tetra 10 and Penta 50

Hexa and Heptha 50; Octa 100 (after 500 runs)



Sample Preparation – Water samples
Calibration starting from CS1

1800 mL sample (900mL in 1L bottle, volumes are gravimetryly determined by use a balance at 0.01g) plus 100µL of Labeled Compounds LCS solution at 1µg/L (50µL per bottle) and 20mL of Hexane (10mL per bottle) are mechanical extracted by use an Agitator (Collomix by DTO Servizi Srl - Spinea (VE) Italy) with a program of 3-4 minutes.

After the extraction, with the help of a Pasteur, the top Organic phases in the bottles are collected inside a separation funnel together with other Hexane added in the bottles after a short manual agitation, then in order to not lose solvent also a minimum part of water is collected.

The whole Organic phase in the separation funnel is collected in a 40mL Vial through a paper filter with sodium sulfate, in order to retain the residual water; both the separator funnel and the filter need to be washed with Hexane, also this solvent must be collected.

The Organic phase is concentrated under nitrogen flow (Techne Dri-Block DB100/3) at 50°C, and transferred in a 12mL test tube, with a Pasteur, together with other little Hexane aliquots added in the 40mL Vial after a short manual agitation to wash the Vial walls.

The Organic phase in the 12mL test tube is reduced in volume, close to 100µL, and transferred in an autosampler Vial at micro volume, together with other 100µL Hexane aliquots added in 12ml test tube with a soft manual agitation to wash the walls.

The Organic phase in the autosampler Vial is dried and finally recovered with 10 µL of Internal Standard ISS solution at 10ng/mL.

The sample is concentrated 180,000 times. Less factors if calibration starts from CS0.5 or CSL.

Sample Preparation – Soil samples
Calibration starting from CS1

The soil sample is dried for 24h in an oven at 40°C, then pestled in a mortar, homogenized and sifted at 2mm. 1g is then directly weighed in a technical balance (0.01g) inside a 10mL cell for ASE Extractor (100°C, 1500psi, Hexane as solvent, single cycle and two series of washes, collection in 40mL Vial), after addition of 160µL Labeled Compounds LCS solution at 1µg/L.

To the extract is added 200µL of Cleanup Standard CSS solution at 0.08µg/L, in order to verify the success of the next purification process.

The Organic phase is concentrated under nitrogen flow (Techne Dri-Block DB100/3) at 50°C, at about 5-7mL, a quantity of acid is added directly into the 40mL Vial (pure Sulfuric Acid equal to approximately the quantity of extract). With the necessary precautions, a first step is performed only by slightly shaking the Vial, in order to avoid the appearance of emulsions. By using a Pasteur, by tilting the Vial, the Sulfuric Acid, which forms the underlying part, is withdrawn at rest and discharged, taking care not to take small parts of Hexane, in the form of drops. Proceed with a second step by adding again the same amount of Sulfuric Acid, this time, always with caution, shake the Vial more vigorously to ensure that the sample is sufficiently purified. After having discharged the Sulfuric Acid again, carry out an evaluation of the success of the purification process by observing the limpidity of the extract. If it is not clear enough, proceed with further steps (up to a maximum of three cycles), if the clarity of the solution is not appreciable, the sample is treated with an equivalent aliquot of 5% NaCl in water, with caution and shaking more vigorously, in order to eliminate all the acid present; also in this case discharge the underlying part with a Pasteur.

After the purification process, transfer the extract in a 12mL test tube, with a Pasteur, through a paper filter with sodium sulfate, in order to retain the residual water; both the 40mL Vial and the filter need to be washed with Hexane, perform a short manual agitation to wash the Vial walls, also this solvent must be collected. The Organic phase in the 12mL test tube is reduced in volume, close to 100µL, and transferred in an autosampler Vial at micro volume, together with other 100µL Hexane aliquots added in 12mL test tube with a soft manual agitation to wash the walls.

The Organic phase in the autosampler Vial is dried and finally recovered with 16µL of Internal Standard ISS solution at 10ng/mL.

The sample is concentrated 62.5 times. Less factors if calibration starts from CS0.5 or CSL.

Sample Preparation – Optional Purification step

An optional step, depending on the dirt present in the sample, is possible after the extraction step.

For the Water samples, proceed by adding 125µl of CSS at 0.08µg/L, while for those of Soil with what has already been provided in the previous slide.

For both matrices, the purification phase involves the use of an automatic system, schematized alongside.

Loading the solution into the system, which has three different columns: Silica (acidic, neutral and basic), Alumina and Carbon. All columns are disposable. The elution from the same occurs using different solvents such as: Hexane; Dichloromethane/Hexane 20:80; Dichloromethane/Hexane 50:50; Toluene. These allow to trap unwanted materials in the column phase and divide the extract into two distinctly collected fractions, PCB and Dioxins/Furans.

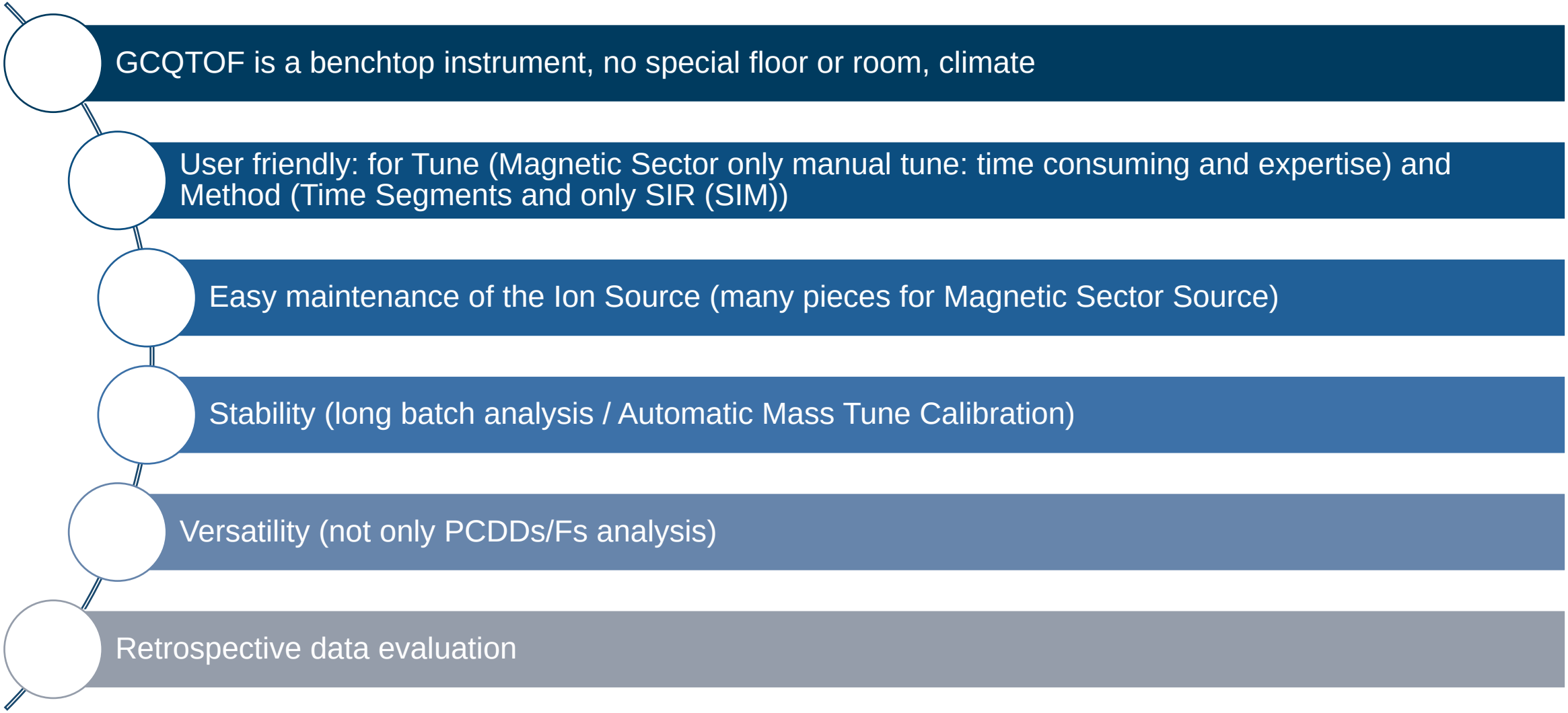
LCTech GmbH - DEXTech Heat
Automated Sample Clean-up in PCB and Dioxin analysis

1 - Sample Vial

2 - Tubings

3 - Sample Loop

GCQTOF (advantages) vs Magnetic Sector



Thank You!
Now Q&A session

An awesome Instrument, fine tuned on Hardware and Software for this application

Special Thanks to Agilent: Anna, Fabrizio and Marica. BioChemie: Alessio, Cristian, Erika, Mattia and Davide.