Screening of Environmental Contaminants using GC/Q-TOF and Accurate Mass Library

Kai Chen and Philip Wylie

Agilent Technologies, Inc.

Thomas Young

Department of Civil and Environmental Engineering
University of California

Agilent
Trusted Answers

UCDAVIS
UNIVERSITY OF CALIFORNIA

Presentation Outline

- **Introduction on Broad Scope Target Screening Using Accurate Mass Library**
- **Method Evaluation**
- **Case Study: Screening of Contaminants in Surface Water Samples**
- **Explore Unknowns**
- **Conclusion**

Broad Scope Target Screening Using Accurate Mass Library

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

Challenges for Environmental Scientists

How much of each calibrated target compound is present?

Dozens of Compounds
e.g., 100~200 Targets

Are other target residues present?

100's of Compounds
e.g., 800 Targets

Is there anything else in my sample?

1000's of Compounds

Workflow Strategy

Acquire full-spectrum data

Targeted Method

Yes

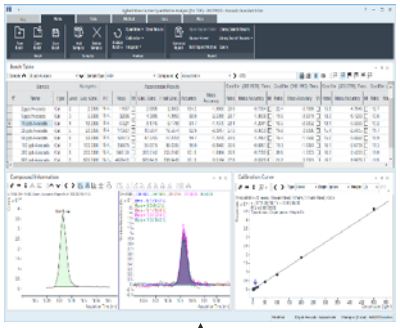
Calibrate?

No

Targeted Quantitation

Suspect Screening

Non-targeted Screening



NIST

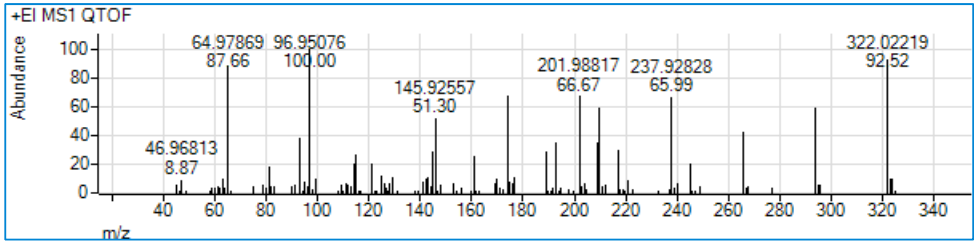
Deconvolution followed by matching public libraries

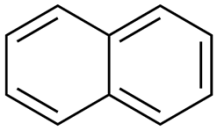
Suspect added to target

GC/Q-TOF Accurate Mass Library

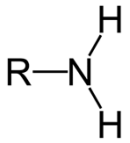
GC/Q-TOF Pesticides & Environmental Pollutants PCDL – now with ***1000+ compounds***:

- High Resolution Spectra
- Expert curation
- Better compound alignment with EPA SVOC targets and Agilent GC/TQ MRM database

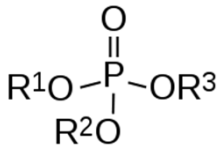




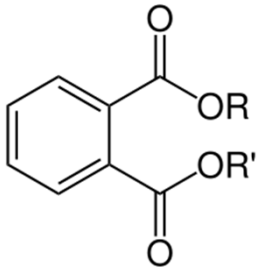
PAHs



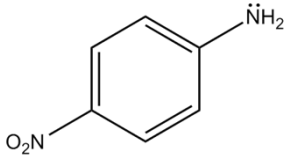
Amines



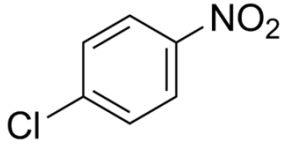
Organophosphates



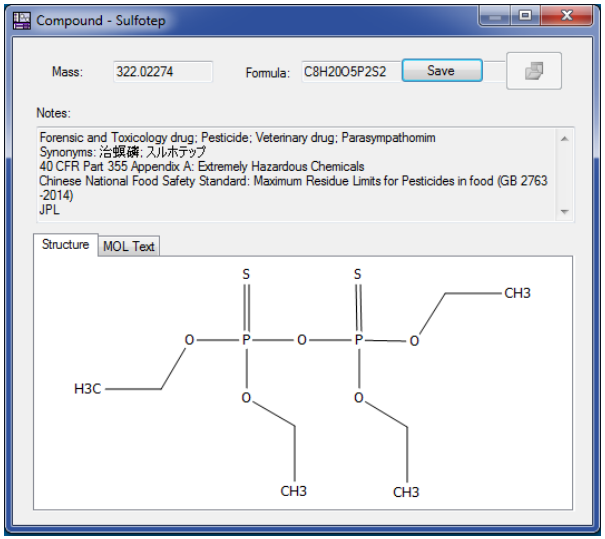
Phthalates



Nitroanilines



Chloronitrobenzenes



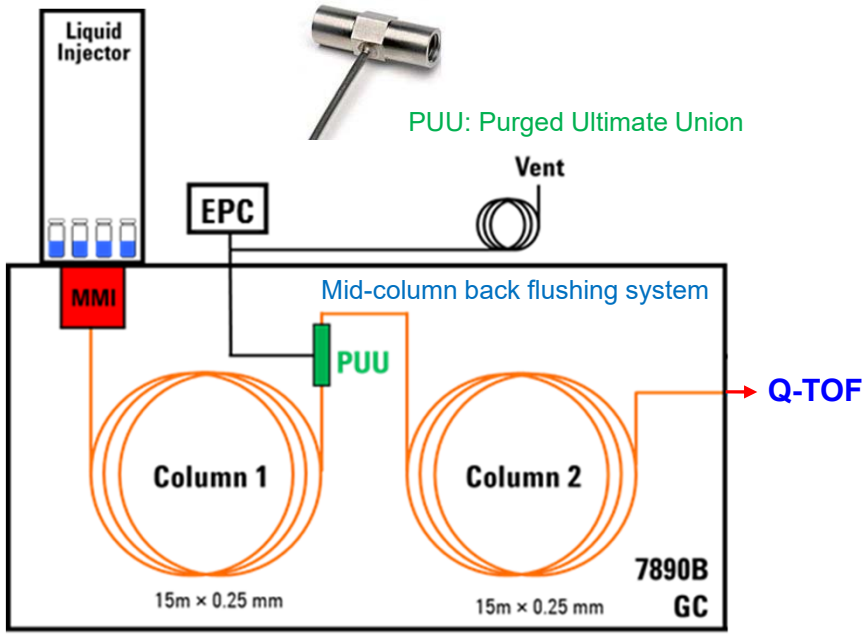
Method Evaluation

Targeted Quantitation

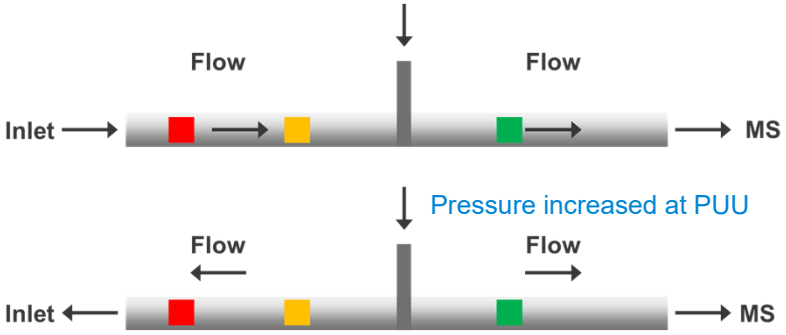
Evaluation Samples

- A standard mixture of 240+ contains representative pesticides and environmental pollutants
- 0.05% RGO serves as a complex background
- Spiking concentration range: 1, 5, 10, 20, 100, 200, 500 and 1000 ng/mL
- Replicates at each level

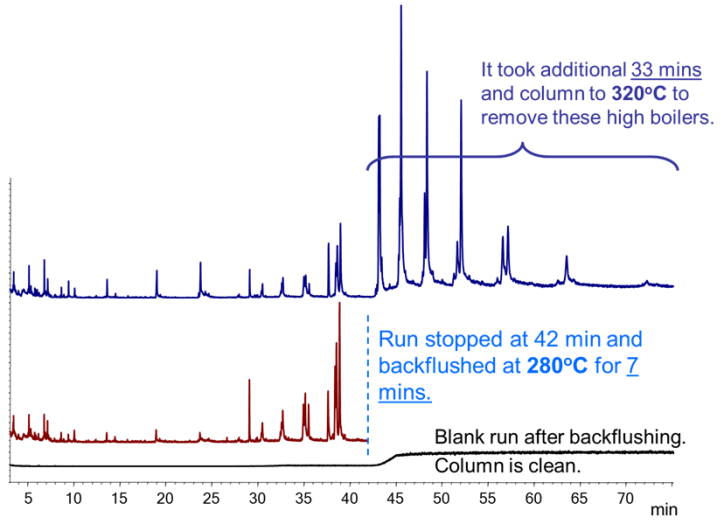
Configuration Optimized with Backflushing



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



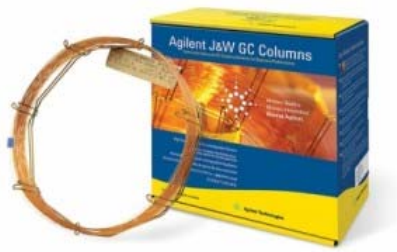
Pressure drops at inlet



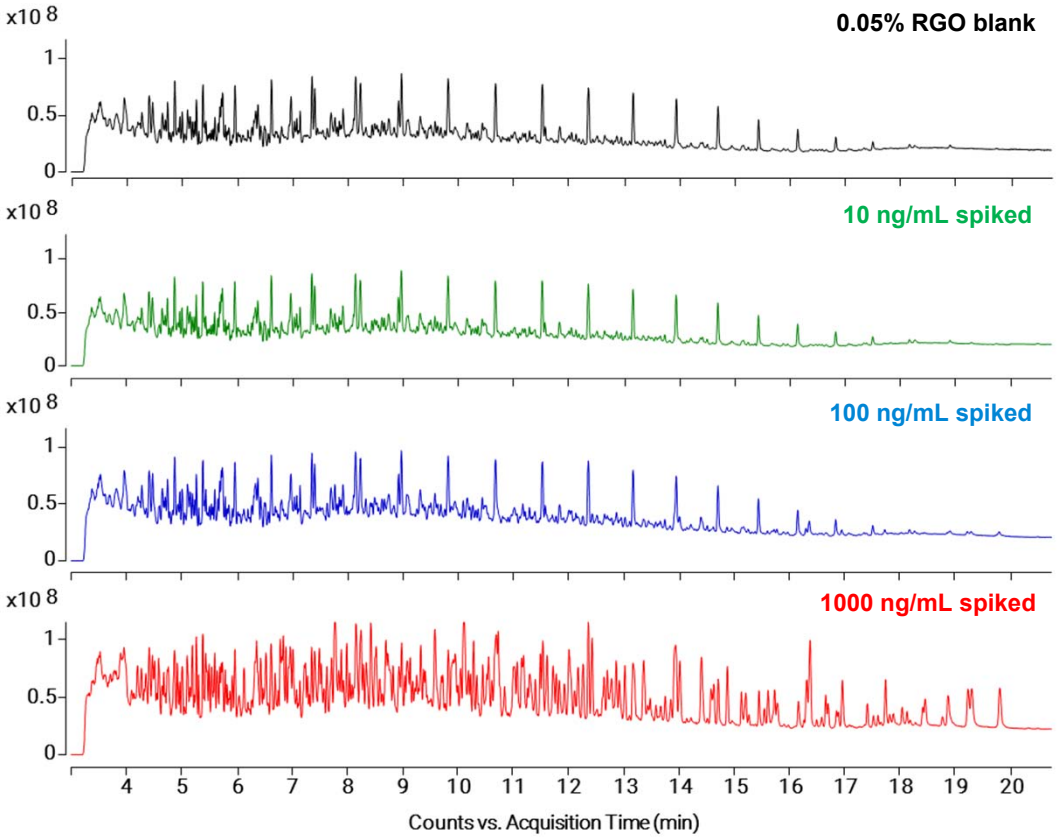
7250 GC/Q-TOF Method Parameters

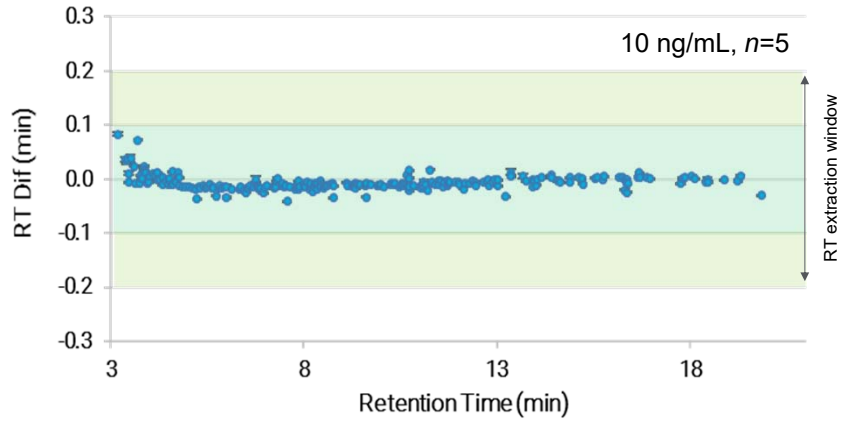
Agilent 7890 GC	
Parameter	**Value**
Inert flow path configuration	Mid-column backflush
Columns	Agilent HP-5ms UI, 15 m, 0.25 mm id, 0.25 µm film (two each)
Inlet	MMI, 4 mm UI liner single taper w wool
Injection volume	1 µL
Injection mode	Cold Splitless 60 °C for 0.2 minutes 600 °C/min to 300 °C, hold 330 °C, post run
Inlet flow (column 1)	1.0 mL/min (**Chlorpyrifos-methyl locked at 9.143 min**)
PUU flow (column 2)	column 1 flow + 0.2 mL/min
Oven temperature program	60 °C (hold 1 min) then 40 °C/min to 170 °C, then 10 °C/min to 310 °C (hold 3 min) **Run time 20.75 min**
Transfer line	280 °C
Midcolumn Backflush	
Timing	5 min duration during post-run
Oven temperature	310 °C
Aux EPC pressure	~50 psi
Inlet pressure	~2 psi

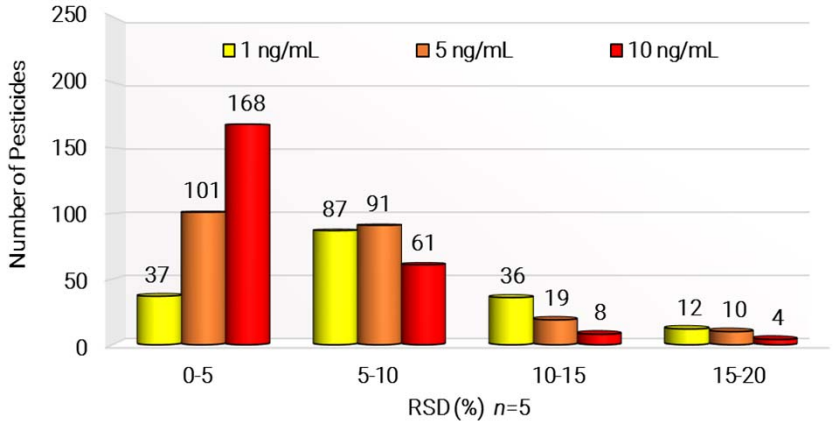
Agilent 7250 Q-TOF	
Parameter	**Value**
Source temperature	280 °C (70 eV), 250 (°C)
Quad temperatures	150 °C
Collison cell gas flows	1 mL/min N_2 4 mL/min He
Electron energy	70 eV (Standard EI), 15 eV (Low energy EI)
Acquisition mass range	45-550 m/z
Spectral acquisition rate	5 spectra/sec



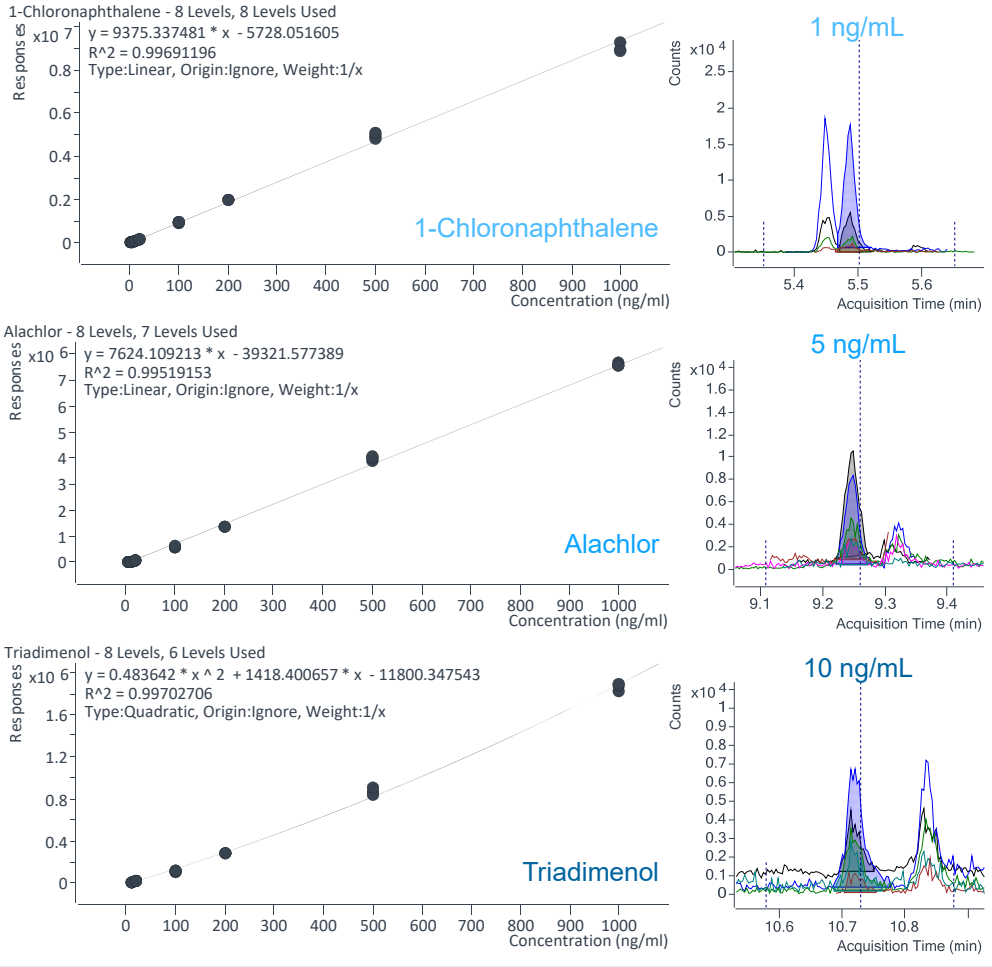
Replicate Precision

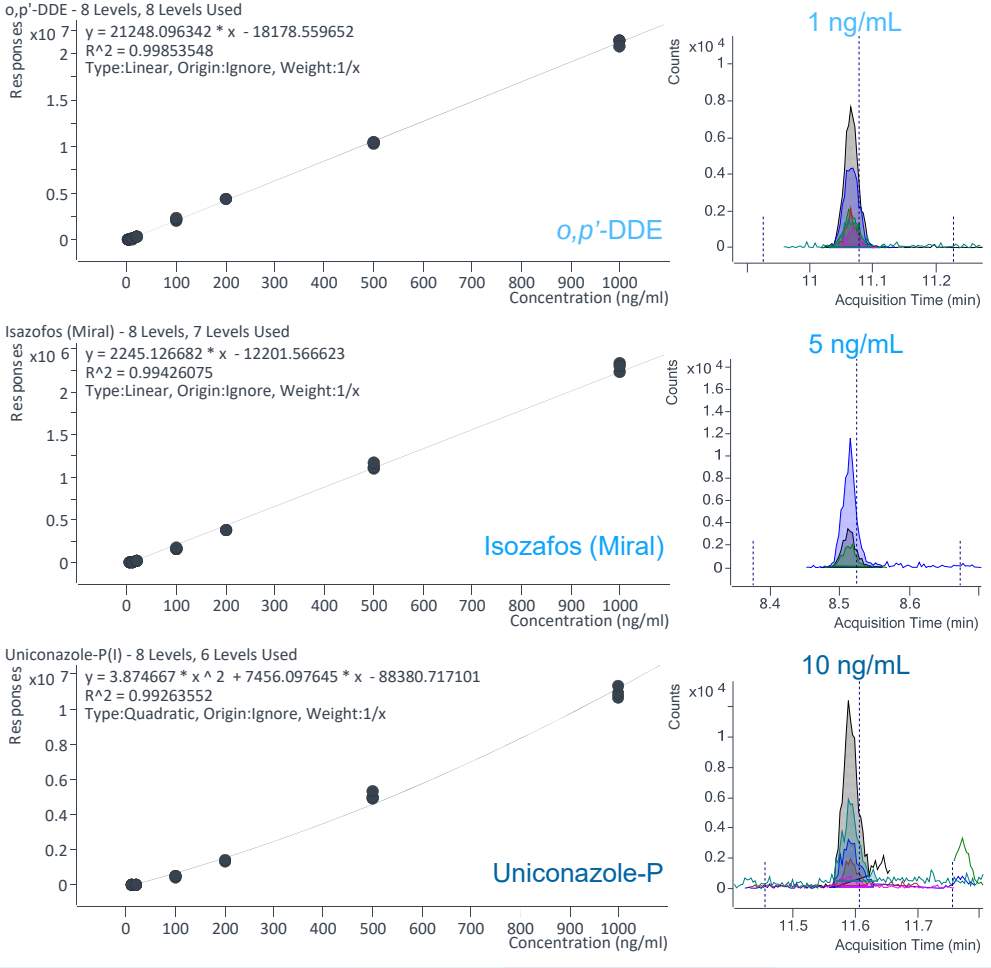




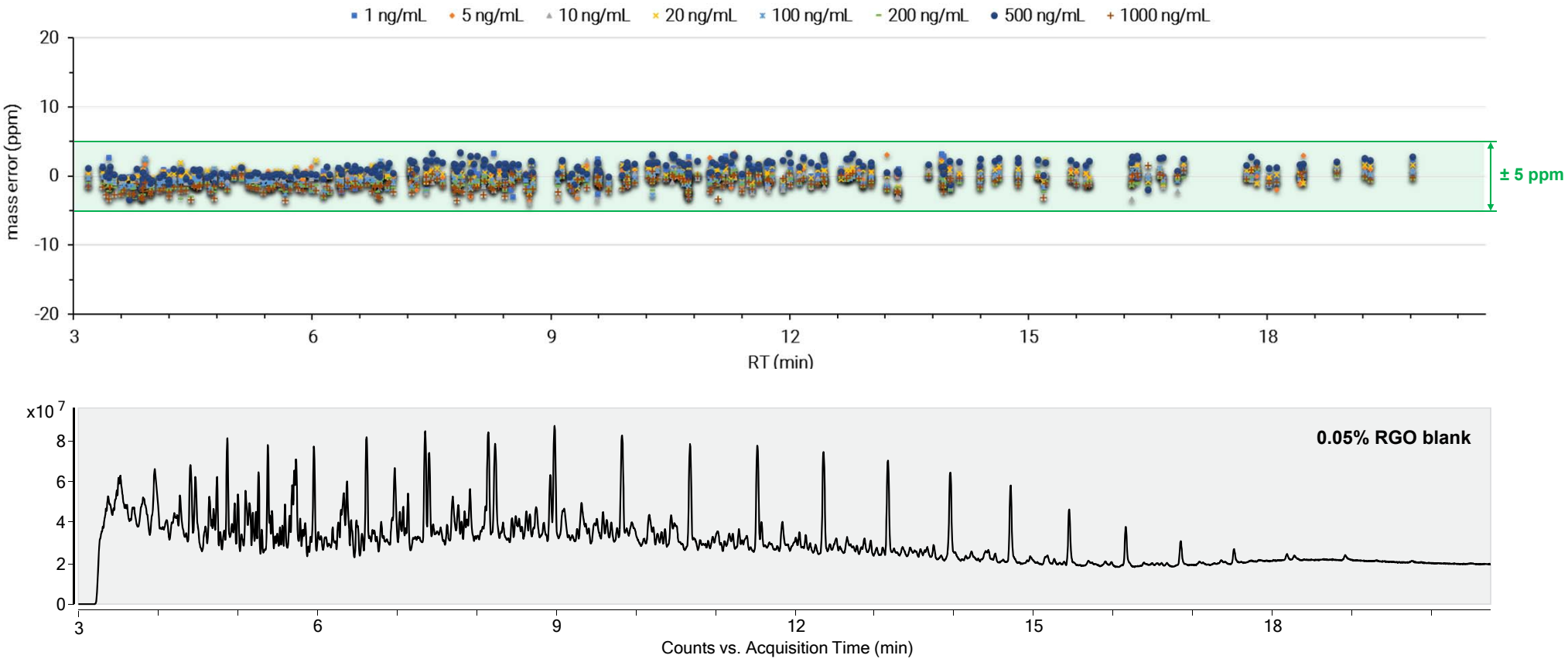


Calibration Range (1-1000 ng/mL)





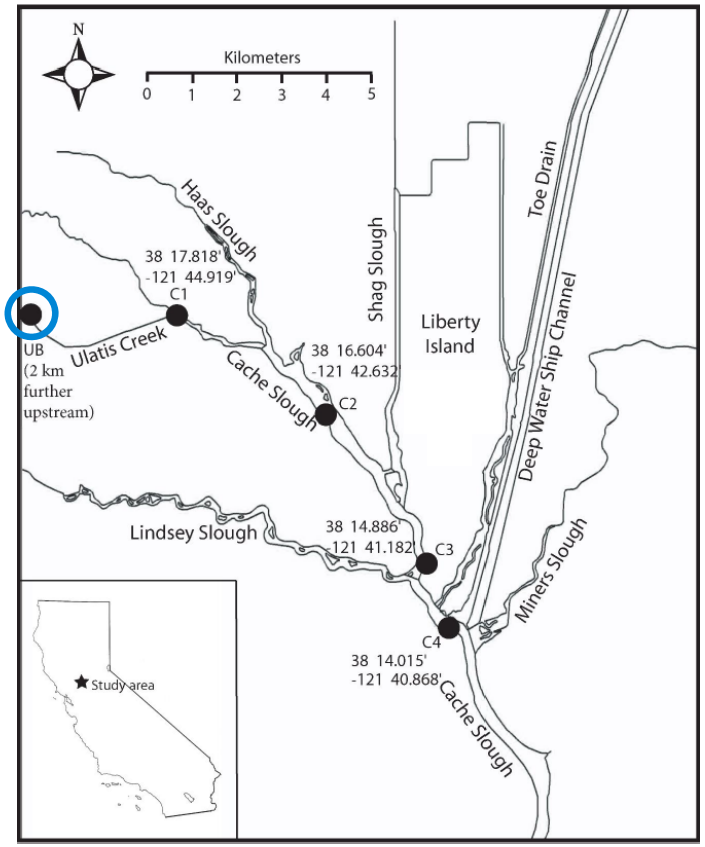
Confidence Enhanced by Mass Accuracy



Case Study: Screening of Contaminants in Surface Water Samples

Targeted Quantitation + **Suspect Screening**

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.

Guideline to Identify Compounds

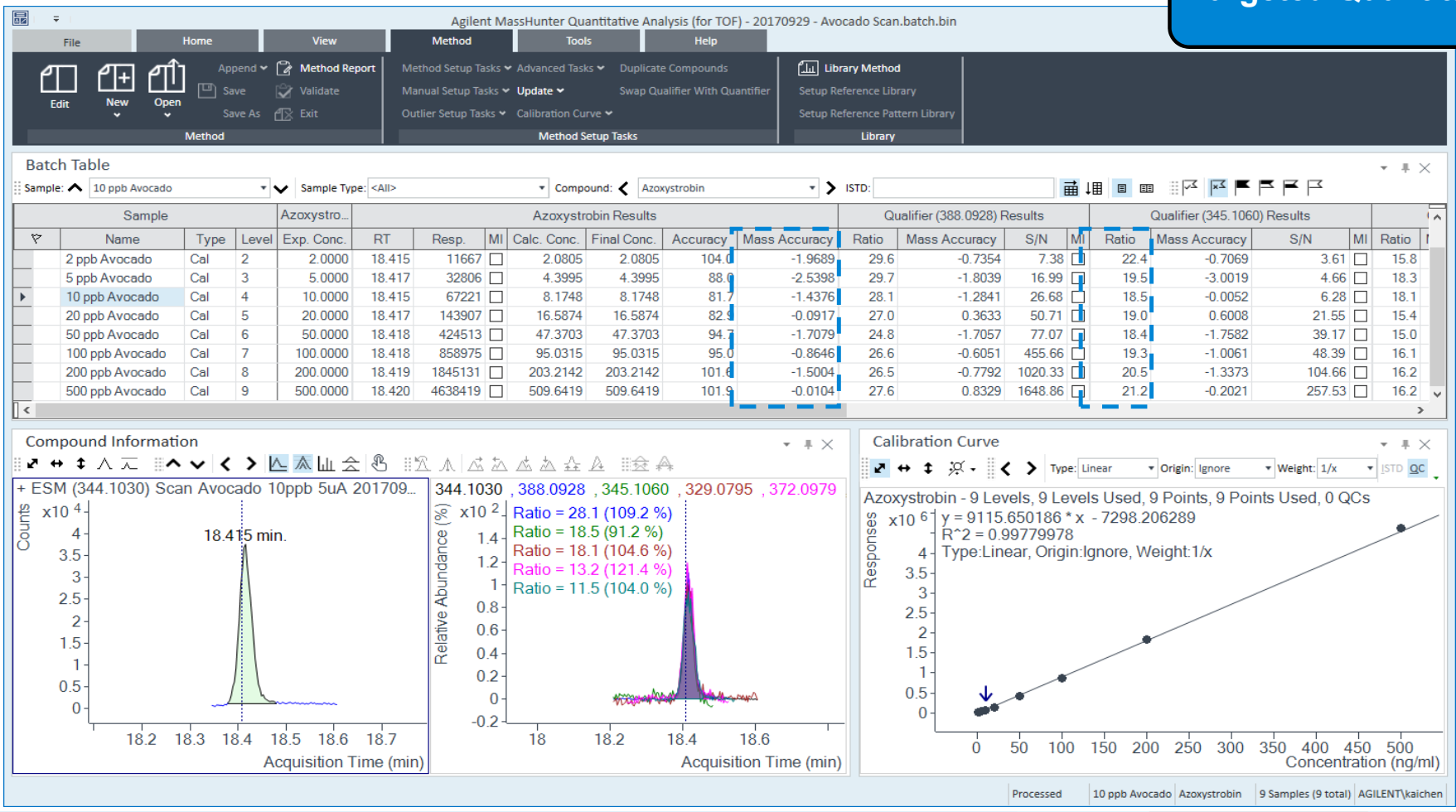
SANTE/11813/2017 Guidelines

MS detector/Characteristics		Acquisition	Requirements for identification	
Resolution	Typical systems (examples)		minimum number of ions	other
Unit mass resolution	Single MS quadrupole, ion trap, TOF	full scan, limited m/z range, SIM	3 ions	S/N ≥ 3[d] Analyte peaks from both product ions in the extracted ion chromatograms must fully overlap. Ion ratio from sample extracts should be within **±30% (relative)** of average of calibration standards from same sequence
	MS/MS triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap	selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 product ions	
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

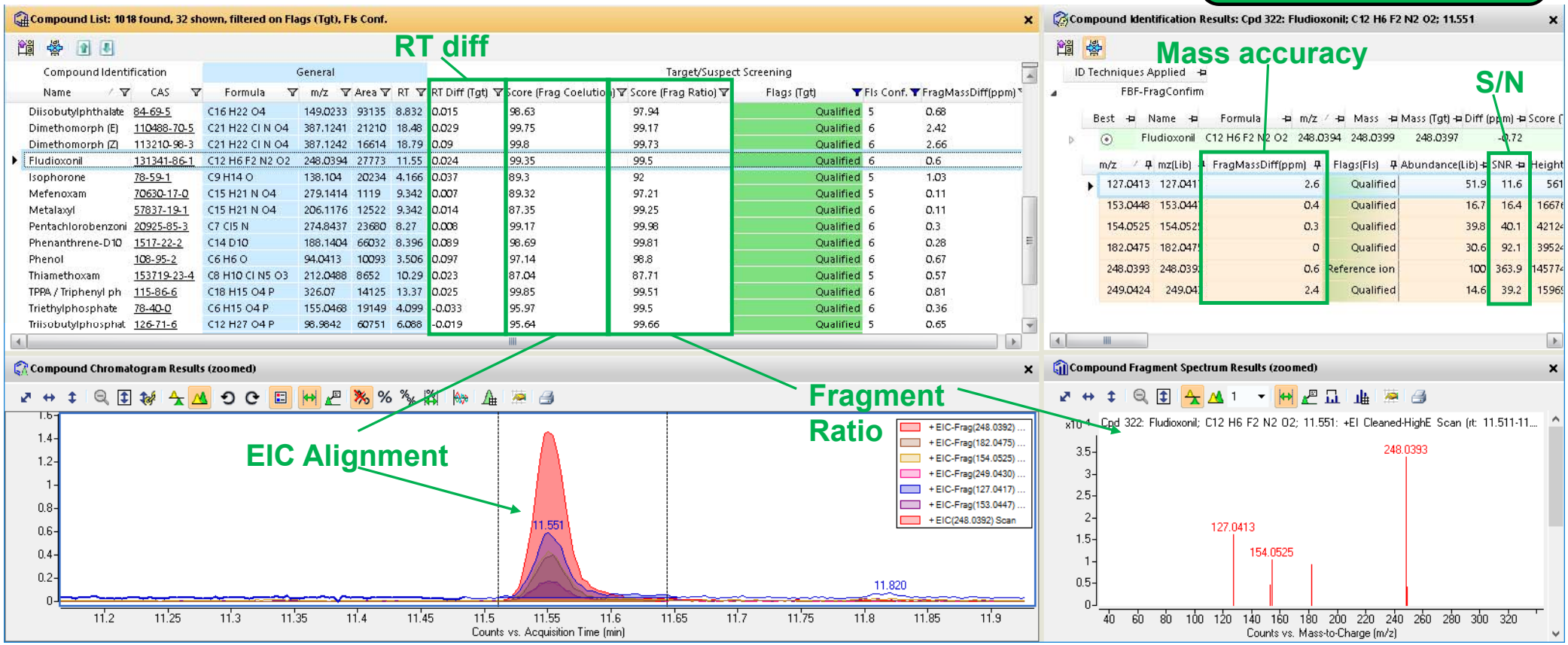
How to Quantify Targets

Targeted Quantitation



How to Confirm Suspect Identification

Suspect Screening



Broad Scope Target Screening Result

Results example from UB sampling site

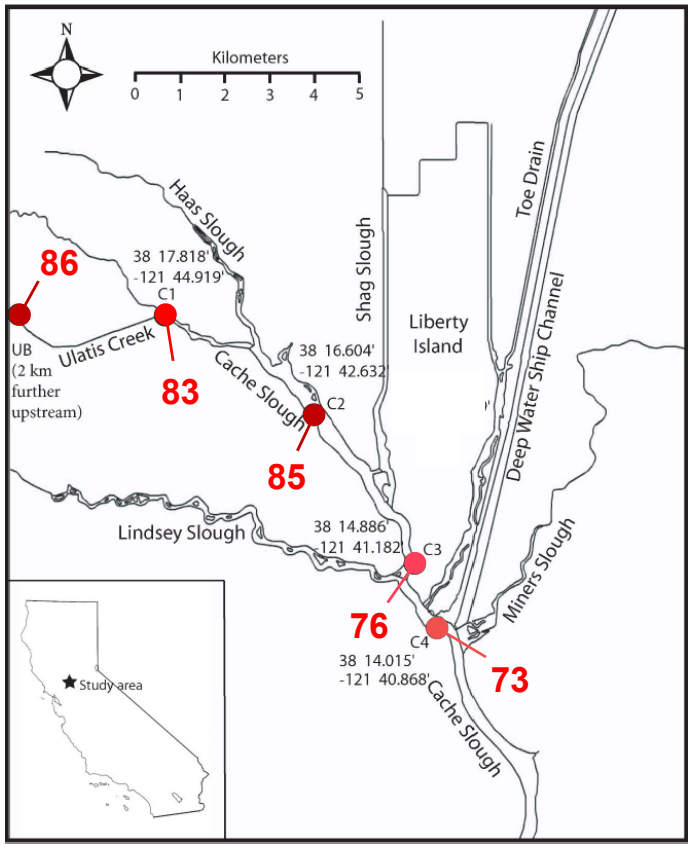
Compound name	RT diff (min)	Score Coelution	Score Frag ratio	Mass diff (ppm)	Amount (ng/mL)
1,4-Naphthalenedione	-0.018	97.1	81.9	0.71	17.9
1-Methylphenanthrene	-0.066	94.7	88.6	3.21	ID only
2-Chlorophenol	0.03	94.9	92.2	1.85	3.8
2-Phenylphenol	-0.02	90.8	86.2	0.59	ID only
4-Chloroaniline	-0.007	83.2	74.0	1.71	1.9
Anthraquinone	-0.015	96.5	93.7	2.35	ID only
Atrazine	-0.006	90.3	98.5	0.77	6.5
Atrazine-desethyl	0.001	85.8	77.1	8.20	ID only
Atrazine-desisopropyl	0.004	97.5	94.4	2.42	ID only
Azoxystrobin	0.002	98.5	99.9	0.89	95.1
BAM / Dichlorbenzamide	-0.002	91.0	84.3	0.57	ID only
Bis(2-chloroethoxy)methane	0.033	85.9	80.2	0.60	3.2
Boscalid (Nicobifen)	0.005	99.8	99.8	0.03	ID only
Bromacil	0.000	99.5	99.4	0.53	116.5
Carbazole	0.034	72.9	87.0	3.70	5.3
Chlorantraniliprole	0.003	96.8	96.1	0.59	304.6
Chloroneb	-0.013	84.3	96.1	0.57	ID only
Chlorothalonil	-0.014	97.6	99.9	0.83	7.3
Coumaphos	0.007	83.8	88.4	0.47	ID only
Cyprodinil	0.006	97.0	99.7	4.00	ID only
DEET / Diethyltoluamide	0.011	87.7	99.7	3.00	ID only
Diazinon (Dimpylate)	-0.009	99.9	86.5	0.86	265.0
Diazoxon	-0.002	96.7	99.5	0.21	ID only
Dibenzothiophene	-0.019	93.1	96.9	0.11	ID only
Dichlobenil	-0.012	97.5	98.1	1.24	ID only
Difenoconazole(I)	0.004	88.3	95.7	1.32	26.1
Difenoconazole(II)	0.013	91.2	96.1	1.00	32.1
Dimethenamid-P	-0.006	99.3	99.0	1.11	ID only
Dimethoate	-0.006	99.2	98.6	2.03	1048.1

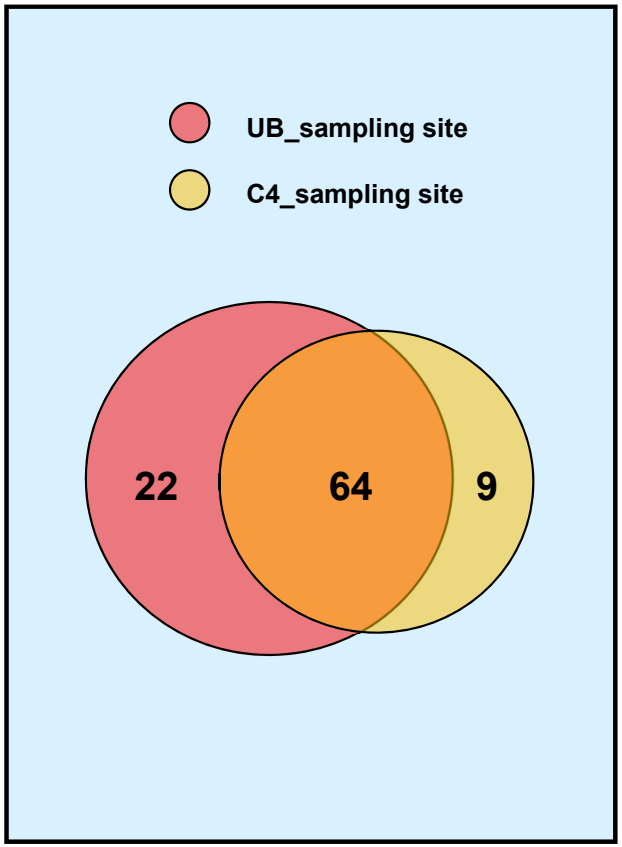
Compound name	RT diff (min)	Score Coelution	Score Frag ratio	Mass diff (ppm)	Amount (ng/mL)
Dimethomorph (E)	0.002	70.5	79.4	4.15	5.7
Disugran	0.076	85.8	67.9	2.44	ID only
Disulfoton Sulfone	0.005	81.4	94.8	1.09	3.6
Dithiopyr	-0.003	99.8	99.8	1.38	ID only
DPA	0.010	90.3	96.3	3	ID only
Ethalfluralin	-0.013	84.1	78.0	1.57	ID only
Fenbuconazole	0.018	95.1	92.8	0.64	ID only
Fipronil	0.021	95.6	91.9	1.26	ID only
Fipronil sulfide	0.016	79.0	99.6	0.27	ID only
Fipronil sulfone	0.051	97.1	99.9	0.06	ID only
Flonicamid	0.049	90.7	89.1	0.73	ID only
Flumioxazin	0.003	93.7	96.6	0.26	ID only
Fluopyram	-0.005	99.7	99.1	1.11	ID only
Fluridone	0.012	97.8	96.1	1.43	ID only
Flurprimidol	0.004	89.1	92.6	2.3	ID only
Flutolanil	0.083	89.9	78.5	0.34	ID only
Fluxapyroxad	0.012	97.6	99.3	0.9	ID only
Fthalide	-0.070	91.7	84.9	1.22	ID only
Hexazinone	0.015	99.6	84.4	1.89	ID only
Indoxacarb	0.009	96.4	71.6	1.5	37.9
Malathion	-0.006	97.0	94.5	0.98	7.9
Metalaxyl	-0.001	79.7	90.4	0.59	11.6
Methiocarb	-0.006	95.5	76.5	1.43	3.5
Metolachlor	-0.007	99.7	99.1	0.21	178.0
Metribuzin	0.010	76.9	97.4	2.98	ID only
Myclobutanil	0.009	88.0	99.5	1.22	10.0
Napropamide	-0.002	91.4	90.7	0.47	11.5
Norflurazon	0.015	96.0	96.3	5	ID only
Norflurazon-desmethyl	0.024	99.6	94.7	0.75	ID only

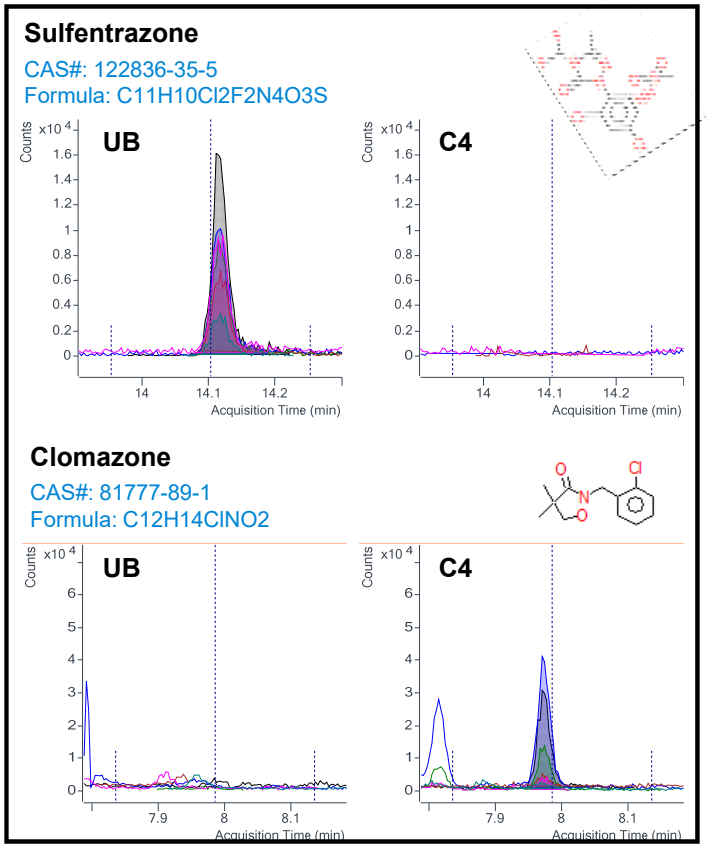
Compound name	RT diff (min)	Score Coelution	Score Frag ratio	Mass diff (ppm)	Amount (ng/mL)
Octhilinone	-0.003	98.9	94.3	1.06	ID only
Omethoate	-0.003	96.1	98.5	0.19	31.8
Oryzalin	0.005	95.9	99.8	0.35	ID only
Oxadiazon	0.004	99.3	99.9	0.78	ID only
Oxyfluorfen	0.005	99.3	99.2	0.27	ID only
p,p'-DDE	-0.004	86.6	99.8	1.41	1.9
PCP / Pentachlorophenol	-0.009	78.3	72.8	1.35	3.1
Pentachloroanisole	-0.018	83.4	89.8	0.09	ID only
Pentachlorobenzonitrile	-0.012	92.2	99.5	0.09	ID only
Phenothiazine	-0.011	94.9	87.5	1.43	ID only
Phosmet (Imidan)	0.043	96.4	80.6	1.79	ID only
Prodiamine	0.001	96.2	99.9	0.31	ID only
Prometon	0.004	97.7	90.1	1.04	ID only
Propiconazole(I)	0.004	99.8	99.3	1.13	ID only
Propiconazole(II)	0.005	99.8	99.4	0.42	ID only
Propyzamide (Pronamide)	-0.003	85.2	80.1	1.07	2.2
Pyraclostrobin	0.001	96.7	93.8	0.71	ID only
Pyrimethanil	0.009	92.3	88.6	2.26	ID only
Quinoline	-0.055	98.3	27.9	1.61	ID only
Simazine	-0.005	99.8	99.8	0.27	ID only
Sulfentrazone	0.015	95.4	99.9	0.32	ID only
Tebuconazole(I)	0.004	94.5	91.4	1.03	ID only
Tebuthiuron	-0.015	95.3	90.4	0.89	ID only
tert-Butylphenyldiphenylphosphate	-0.02	78.3	89.6	1.38	ID only
Tetrachlorvinphos (Dietreen T)	-0.012	71.3	83.5	0.86	2.9
Tetraconazole	0.003	90.2	84.3	1.74	ID only
Thiamethoxam	0.003	90.9	97.1	1.24	34.1
Triclosan	0	84.8	95.7	1.15	ID only

* Amount reported as concentration in the vial for injection

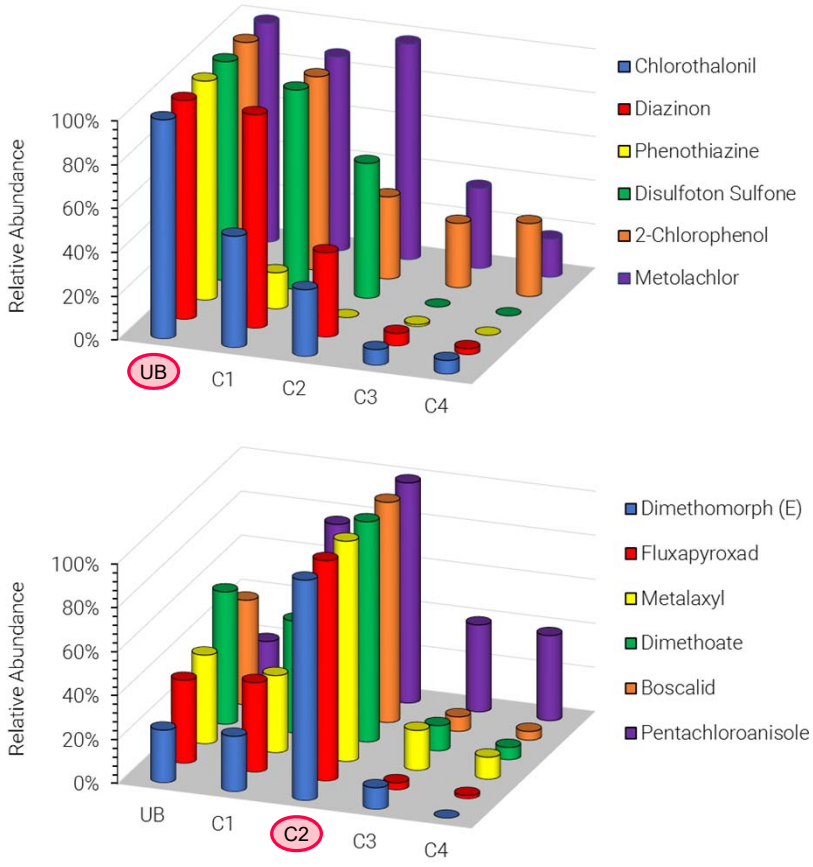
Geographic Distribution of Pollutants

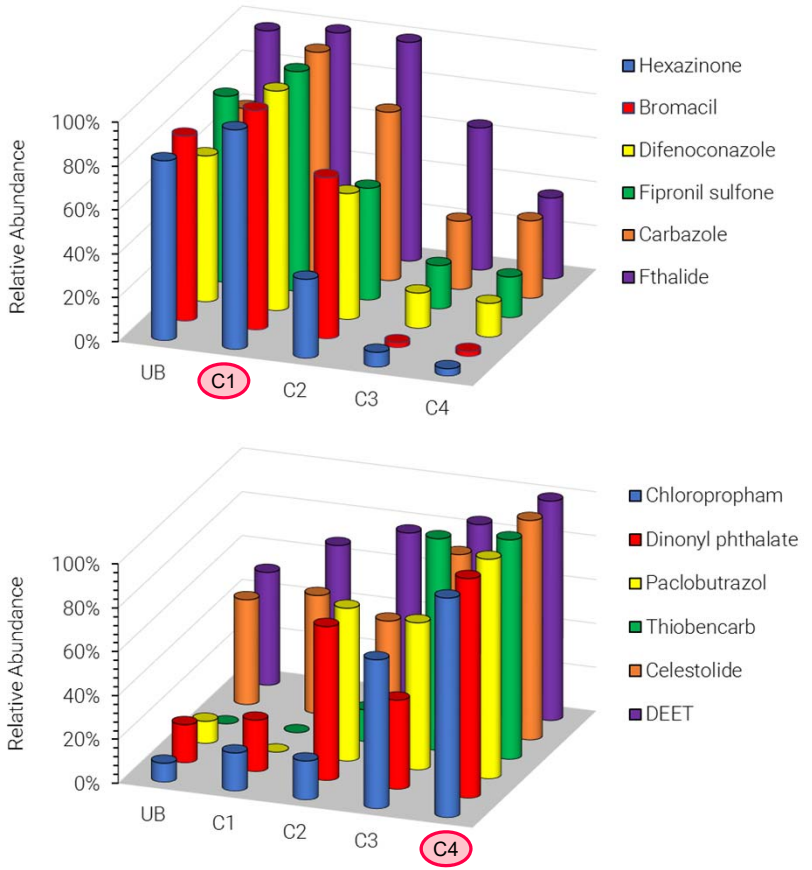






Geographic Distribution of Pollutants

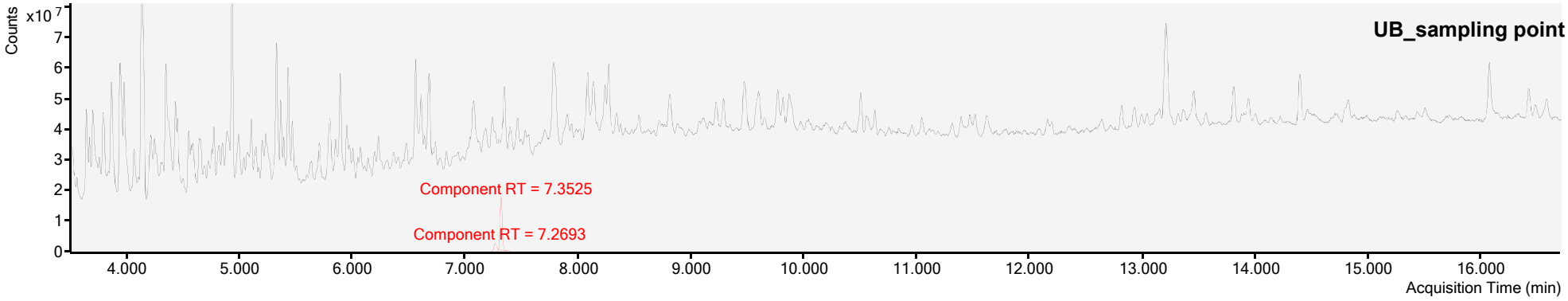


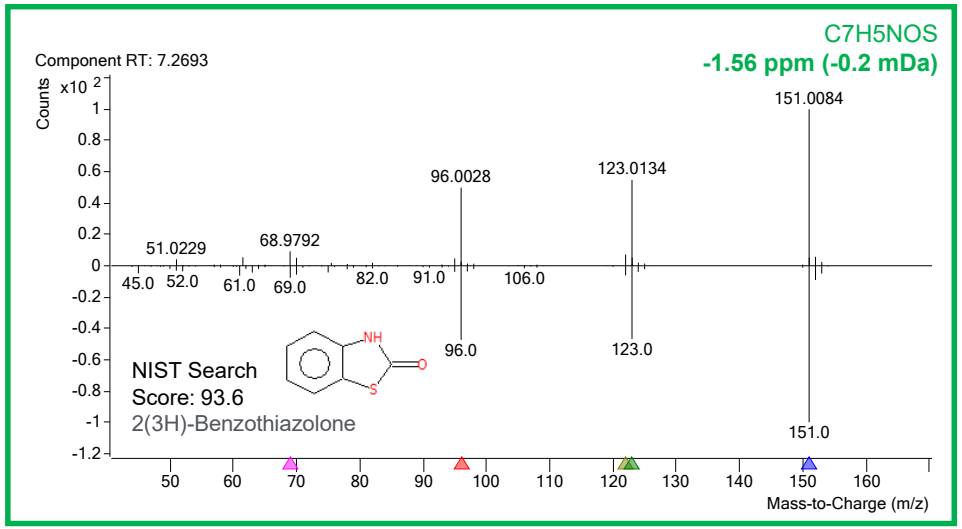


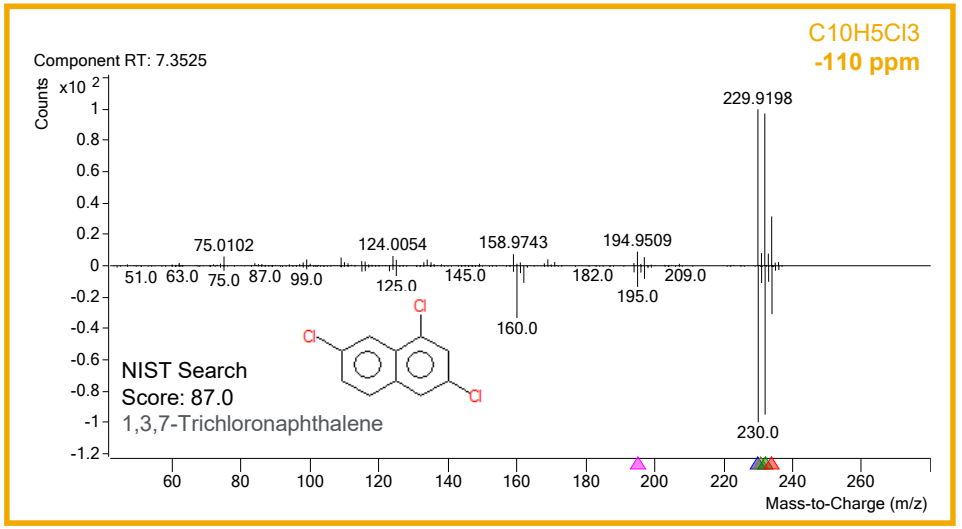
Explore Unknown

Non-targeted Screening

Non-targeted Screening (NIST)



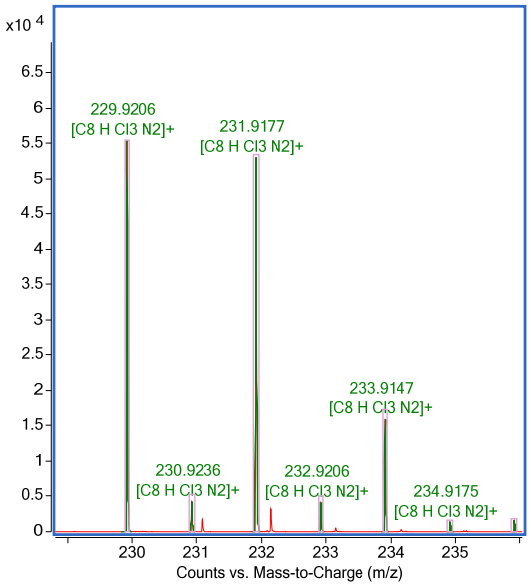




Elucidate structures on C8-H-Cl3-N2

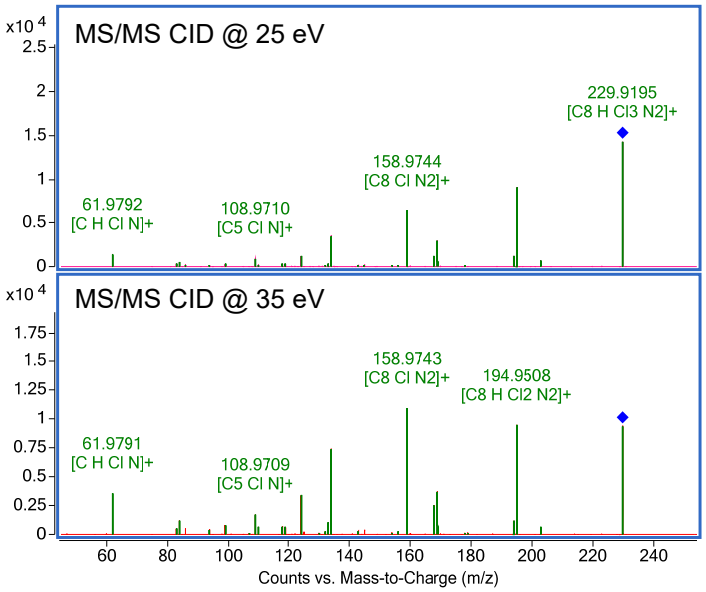
low energy EI, MS/MS and accurate mass

Step 1: Confirm M+

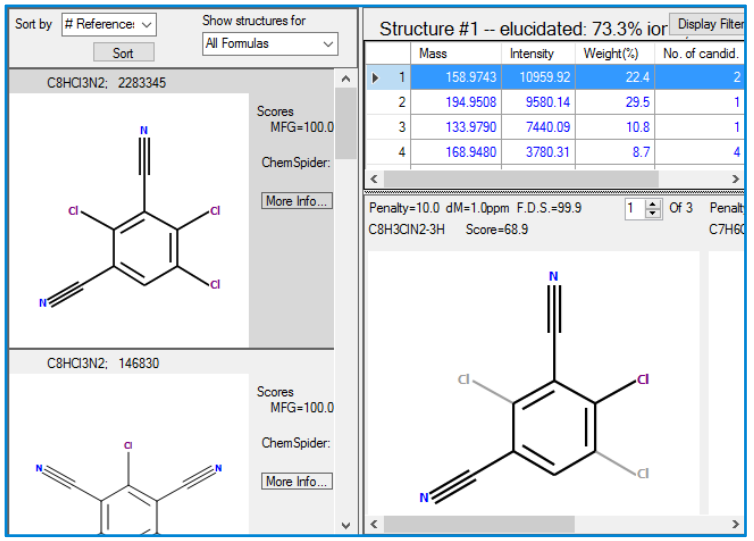


Low energy EI (12 eV)

Step 2: Confirm fragment ions



Step 3: Structure elucidation on candidate



C8-H-Cl3-N2 turns out to be 2,4,5-Trichloroisophthalonitrile.
That is not NIST. It is in fact a degradation product of Chlorothalonil

Conclusion

- An accurate mass library combined with GC/Q-TOF has been used to successfully screen pesticides and environmental pollutants in environmental samples.
- The confidence in results is enhanced by RTL method and excellent mass accuracy.
- Low energy EI and accurate mass MS/MS facilitate untargeted screening and unknowns elucidation.
- Library is scalable to fit the purpose

Acknowledgement

Chris Alaimo

Sofia Nieto
Matthew Curtis

Thanks for your attentions!

Q&A

Appendix

Scalable Library to Fit the Purpose

non-targeted Screening (NIST)

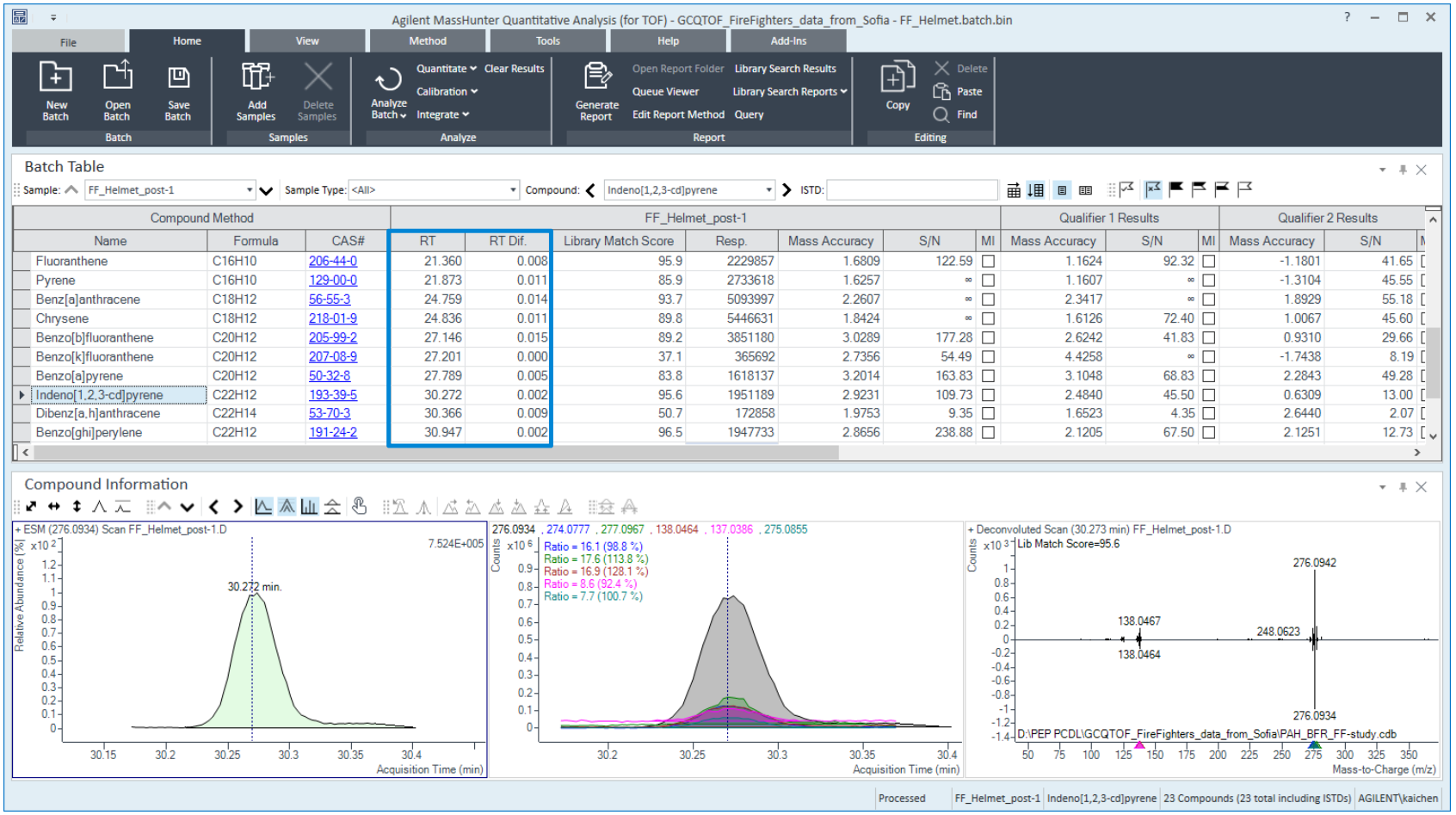
Samples taken from helmet and neck prior to and post fire

4 in^2 area wiped with cotton cloth and IPA

Extraction of cloth with 10mL DCM twice

Evaporation of extract to 1mL

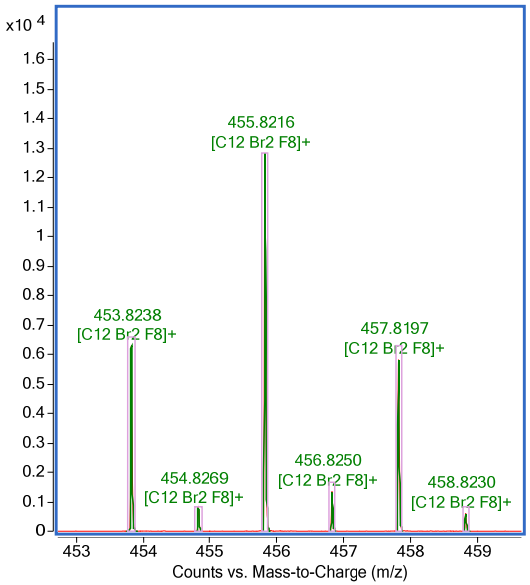
non-targeted Screening (NIST)



Elucidate structures on C12-Br2-F8

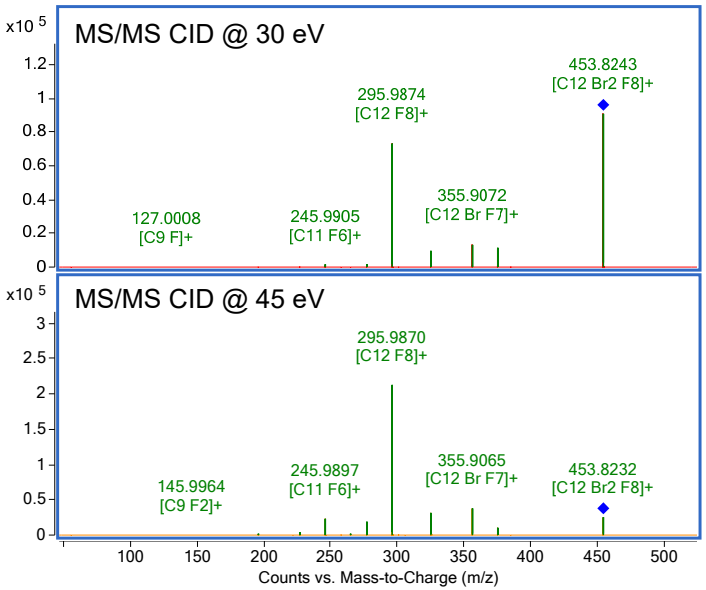
low energy EI, MS/MS and accurate mass

Step 1: Confirm M+



Low energy EI (12 eV)

Step 2: Confirm fragment ions



Step 3: Structure elucidation on candidate