ChromChem – Chromatography & Chemometrics
Dept. Analytical Chemistry, IIAA – Institute of Food Analysis and Research, University of Santiago de Compostela, Spain

Chromatography-mass spectrometry analysis of polar chemicals in water and transformation products elucidation

José Benito Quintana
University of Santiago de Compostela

Who we/I are/am

- University of Santiago de Compostela, Spain

Who we/I are/am

- ChromChem: Research Group of Chromatography and Chemometrics
- IIAA: Institute of Food Analysis and Research

www.usc.es/gcqprega

www.usc.es/persoais/jb.quintana

Who we/I are/am

- Sample preparation
- Chromatography (-MS)
- LC-QTOF, GC-QTOF (and soon SFC-QTOF)
- Emerging pollutants in the water cycle
- Wastewater-based epidemiology
- Transformation products
- Marine pollutants
- Food and drinks pesticides and other pollutants (wine)
- Chemometrics

www.usc.es/gcqprega

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Index

Polar pollutants: analytical perspective:

- The PROMOTE project
- Improving the determination of PMOCs

Transformation products:

- What we do
- Example

4 Take-home messages

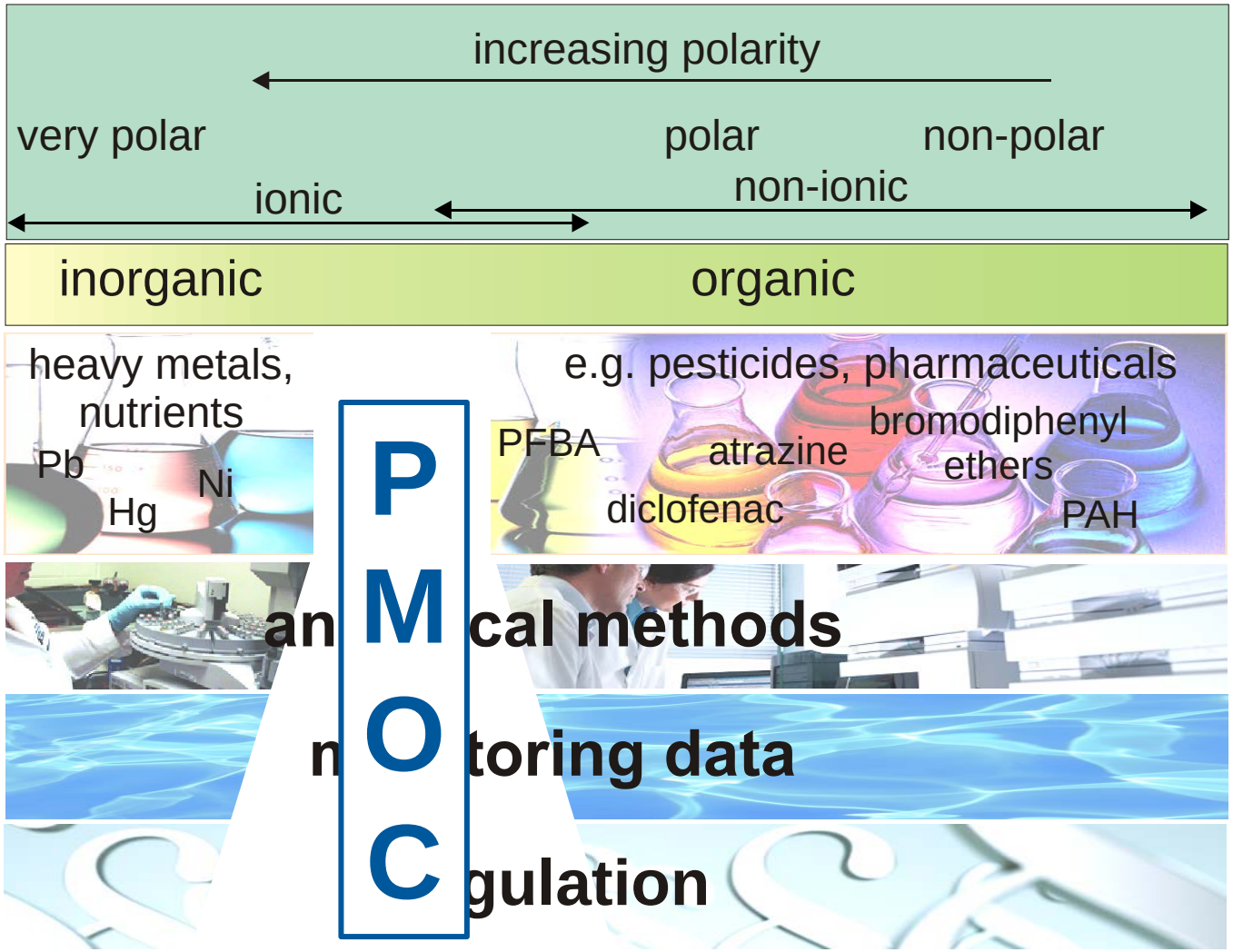

Help! Help! I'm dissolving!
But bears are insoluble...
That's easy for you to say...
You're not Polar!

The PROMOTE project

www.promote-water.eu

- “PROtecting water resources from MObile TracE chemicals”
- Water JPI Pilot Call, runs 2015-2017

The PROMOTE project



The 'polarity gap' representing a knowledge gap with respect to **P**ersistent and **M**obile **O**rganic **C**hemicals (PMOCs)

PMOCs

Environmental Science
Processes & Impacts

PAPER

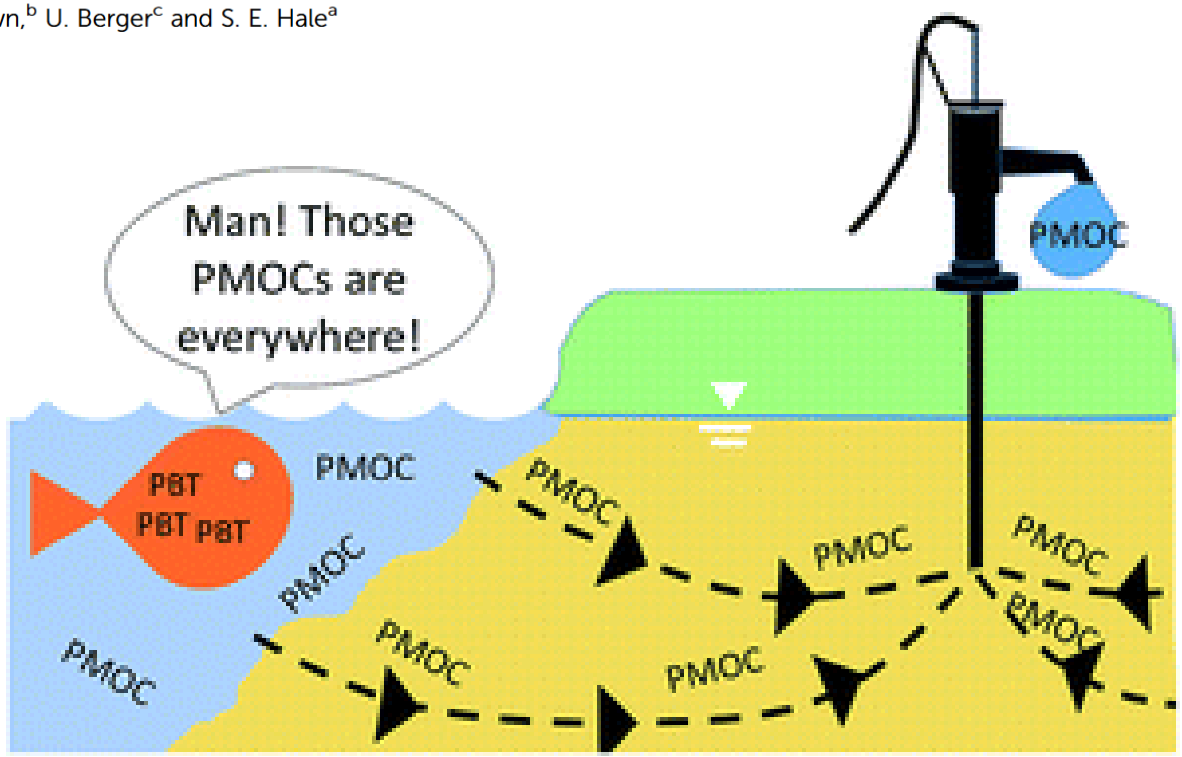
View Article Online
View Journal

Check for updates

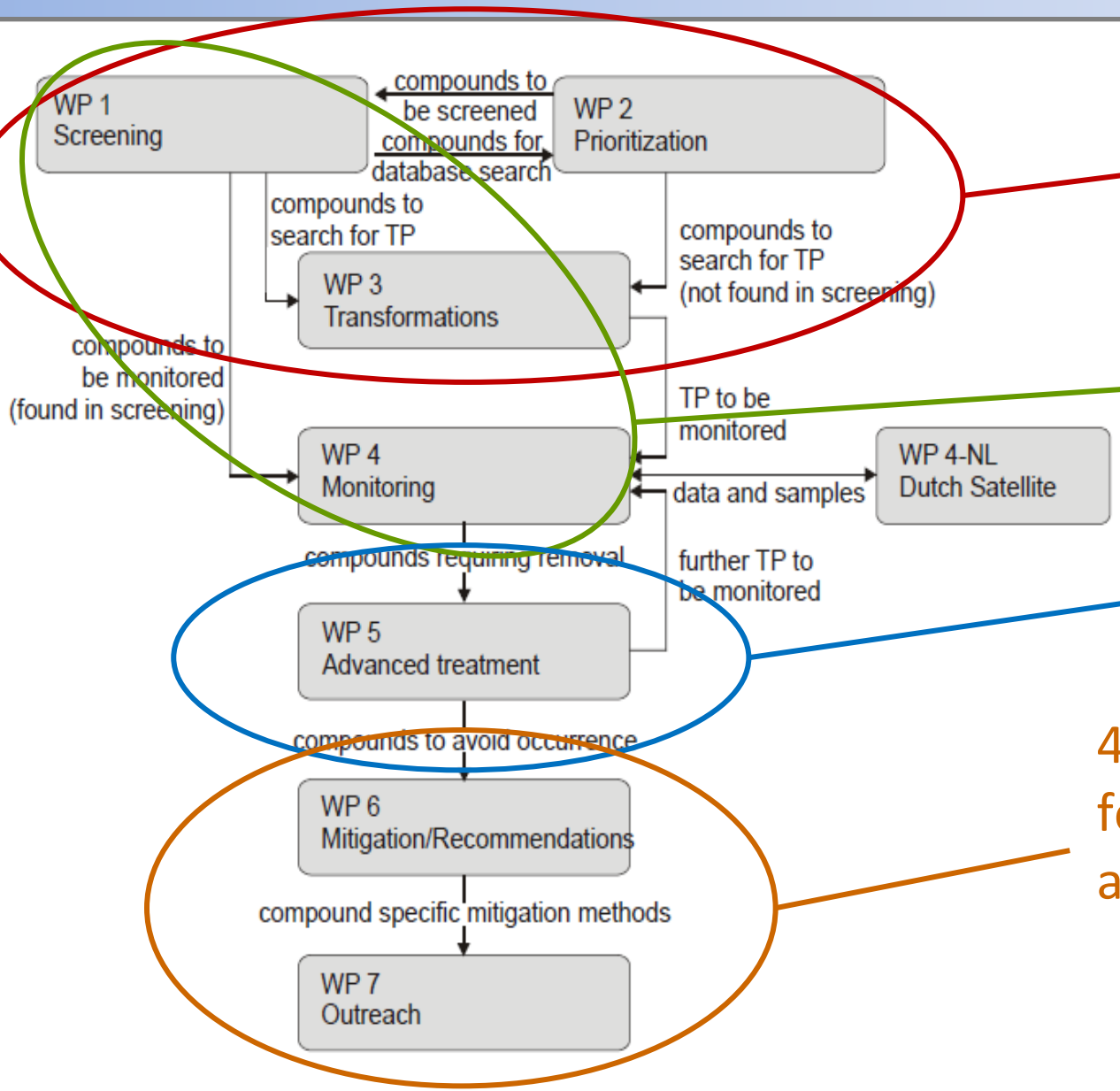
Cite this: DOI: 10.1039/c7em00158d

Ranking REACH registered neutral, ionizable and ionic organic chemicals based on their aquatic persistency and mobility†

H. P. H. Arp, *[a] T. N. Brown,[b] U. Berger[c] and S. E. Hale[a]



The PROMOTE project



1. Identification and prioritization of PMOCs

2. Analytical methods and monitoring

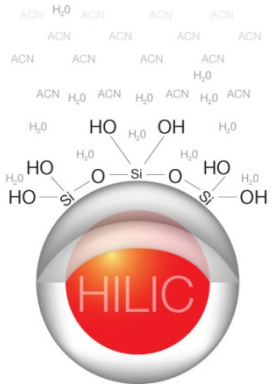
3. Advanced clean-up strategies

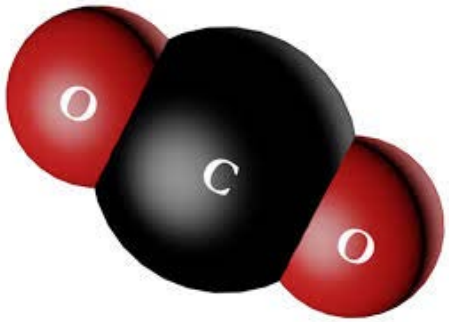
4. Recommendations for mitigation strategies and regulation

Improving PMOCs determination

- **Reversed-Phase LC (RPLC) is the standard (in water analysis)**
- **Alternatives?**

- Ion-pair RPLC
- Ion-exchange
- HILIC (hydrophilic interaction chromatography)
- SFC (supercritical fluid chromatography)
- MMLC: mixed-mode LC





Mixed-mode LC for HRMS screening

DOI: 10.1021/acs.est.6b05135
Environ. Sci. Technol. 2017, 51, 6250−6259

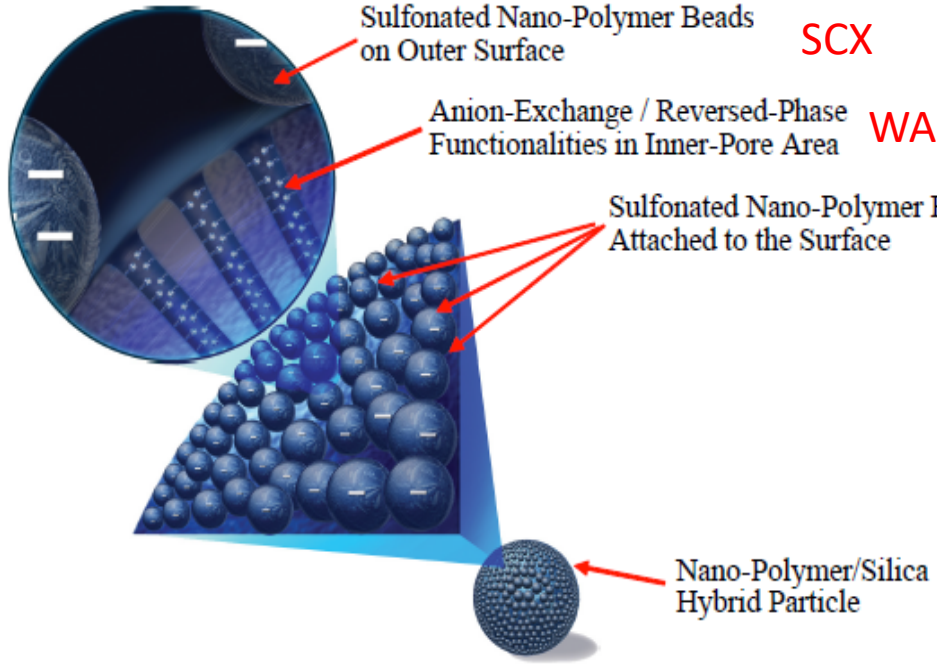
ENVIRONMENTAL Science & Technology

Article

pubs.acs.org/est

Screening for Polar Chemicals in Water by Trifunctional Mixed-Mode Liquid Chromatography−High Resolution Mass Spectrometry

Rosa Montes,* Josu Aguirre, Xandro Vidal, Rosario Rodil, Rafael Cela, and José Benito Quintana*

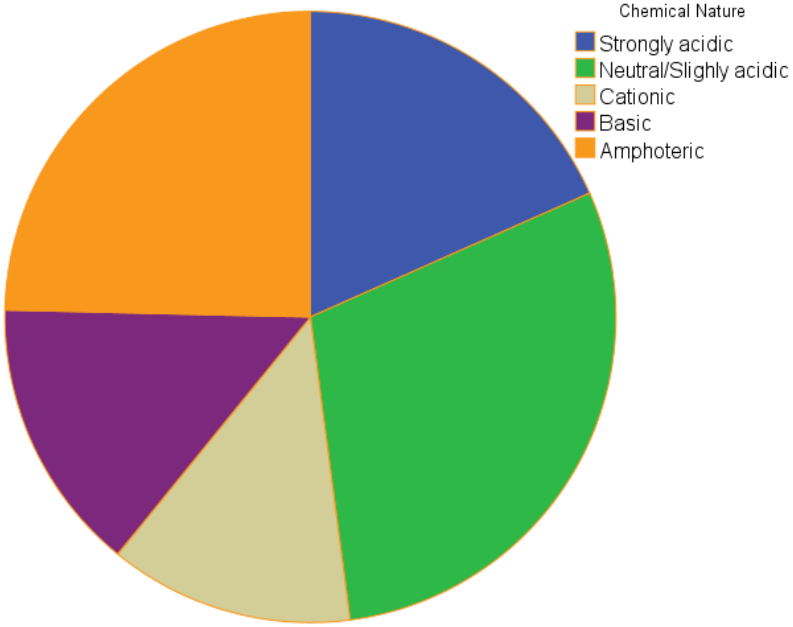


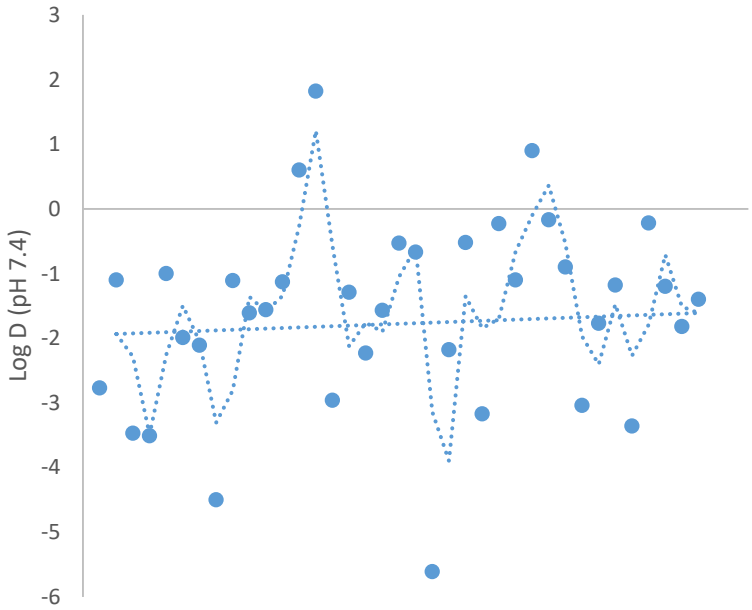
DIONEX

Acclaim® Trinity™ P1

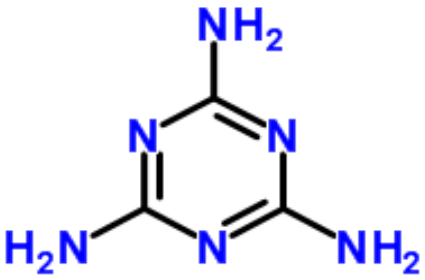
Mixed-mode LC for HRMS screening

- **45 model compounds** including pesticides, sweeteners, flame retardants, UV filters, drugs, etc.
- Acidic, basic, cationic, anionic, amphoteric and neutral chemicals
- Low Log D (pH 7.4)

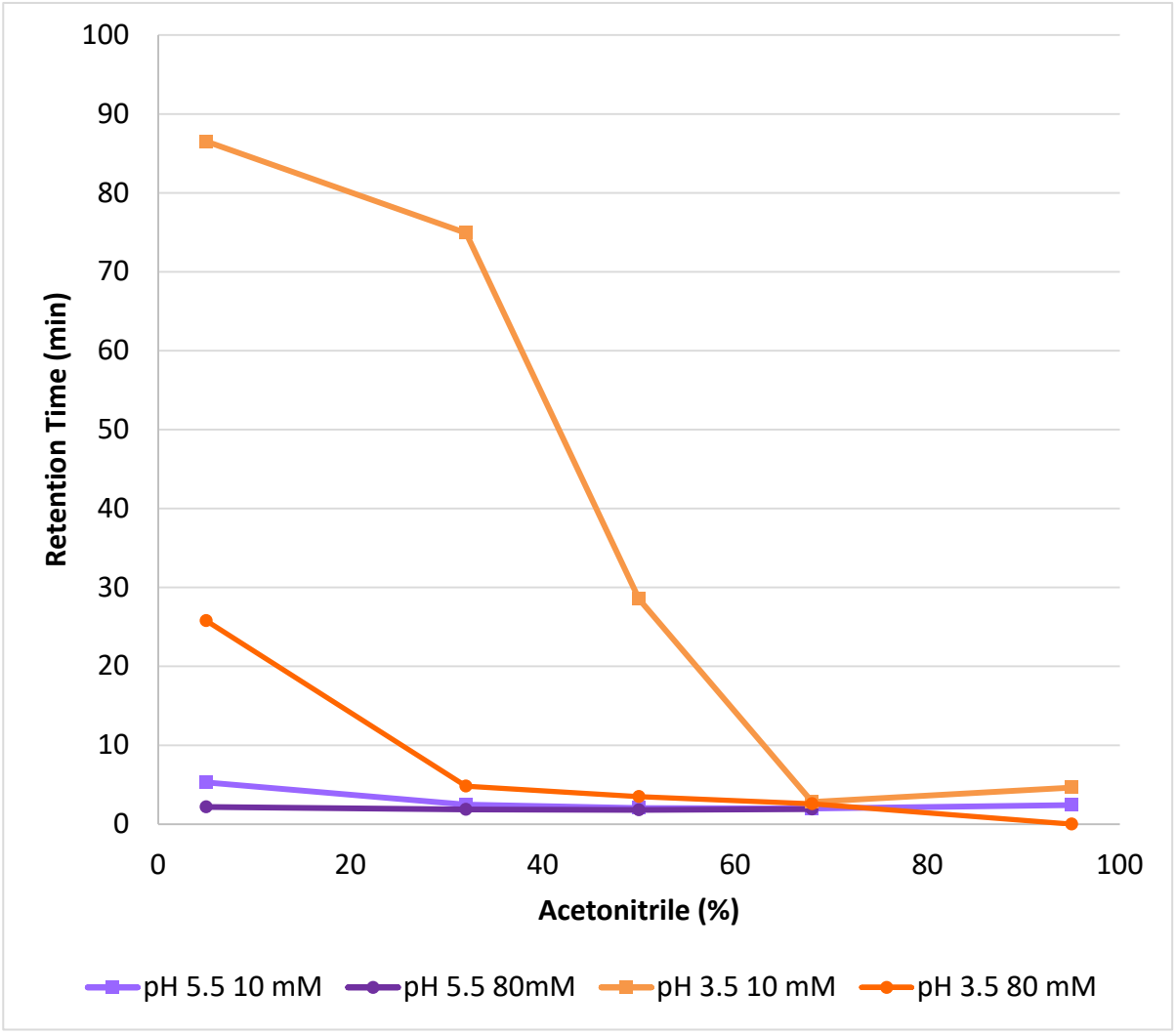




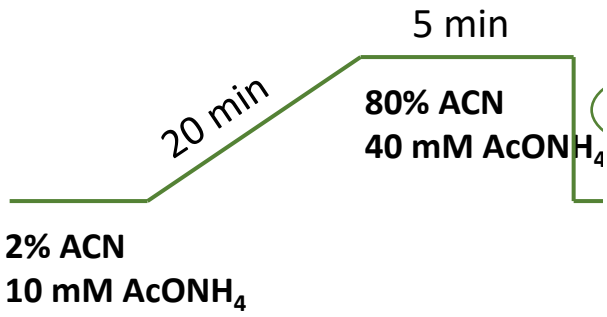
Mixed-mode LC for HRMS screening

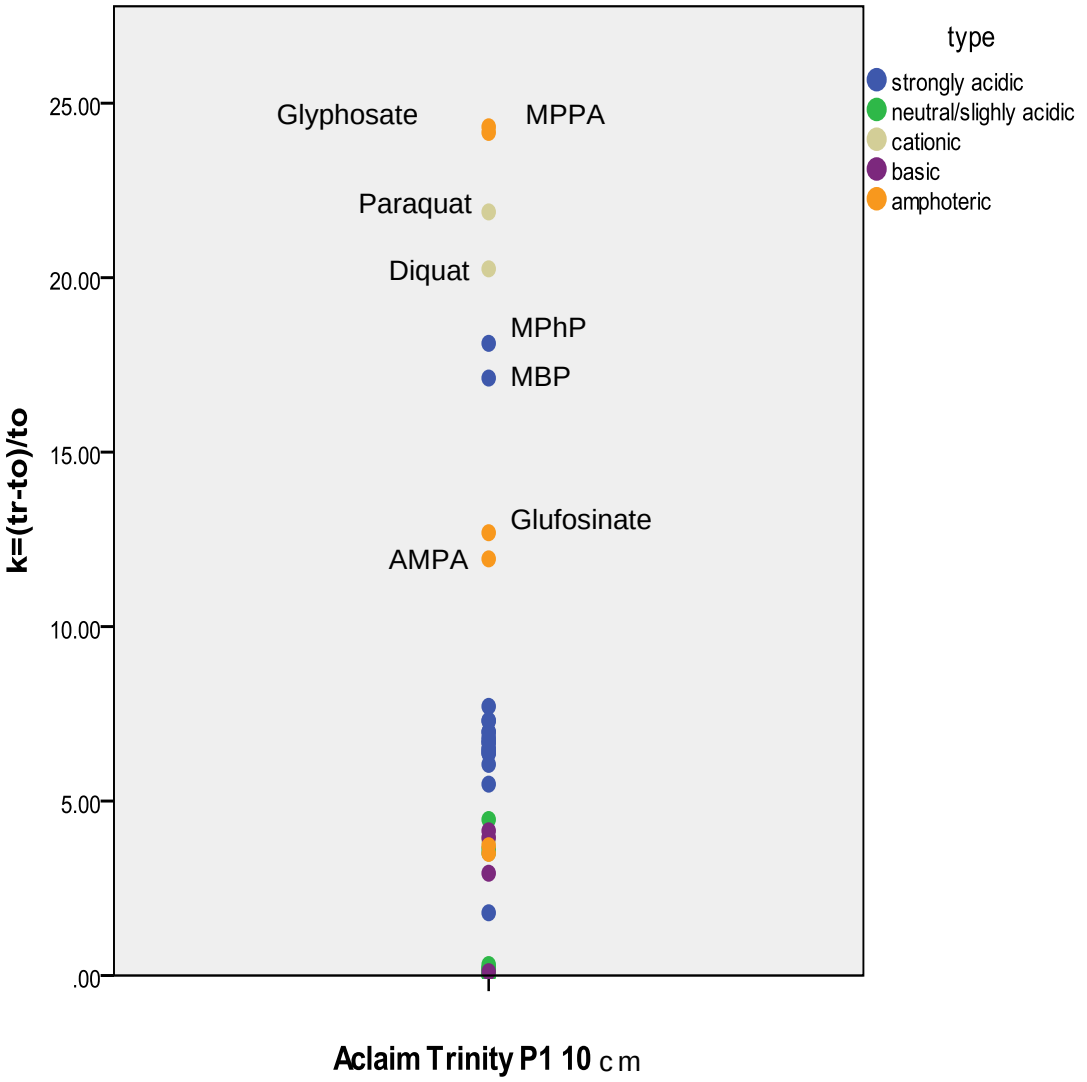


MELAMINE
logD (pH7.4) -1.18

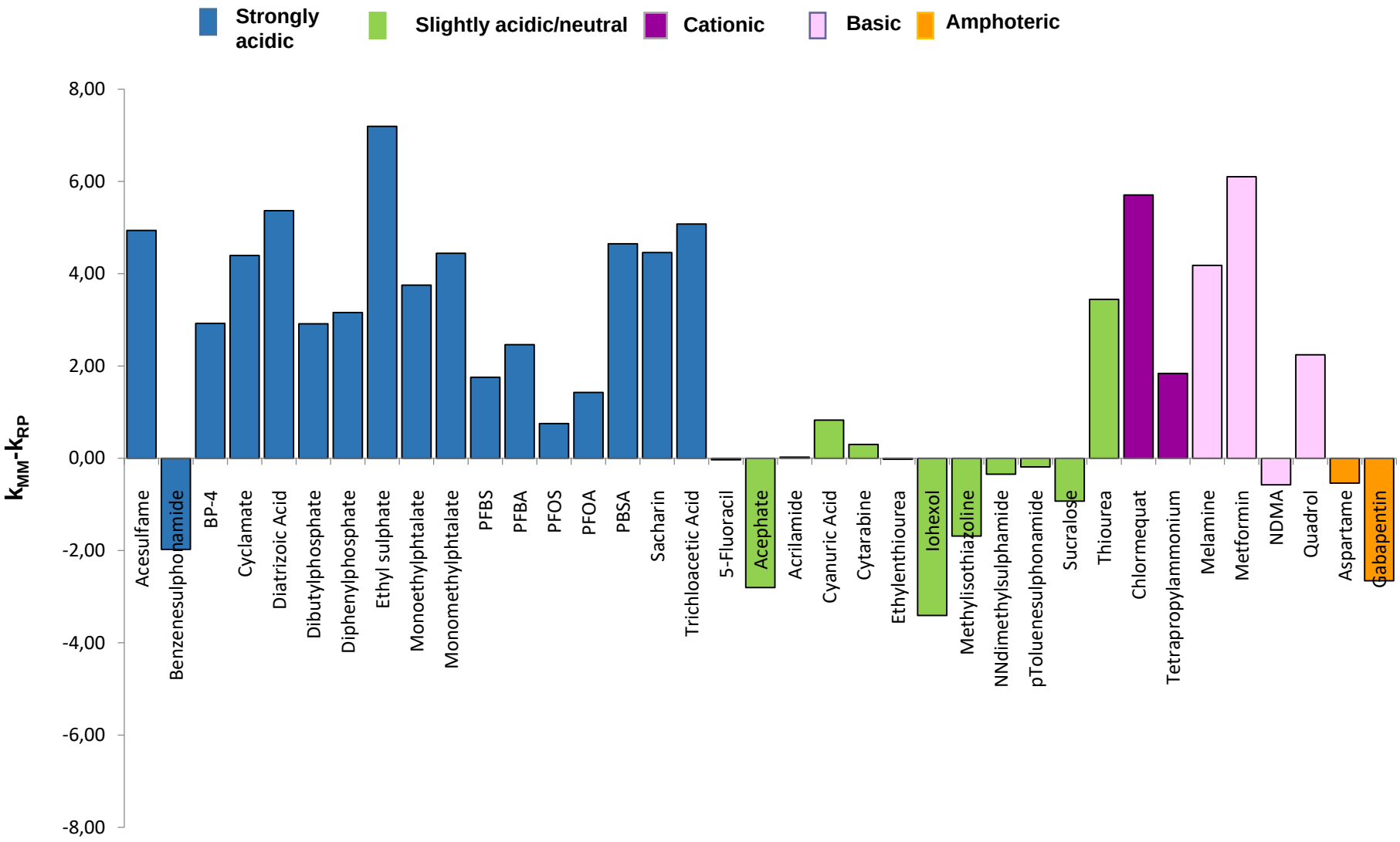


Mixed-mode LC for HRMS screening





Mixed-mode LC for HRMS screening



Mixed-mode LC for HRMS screening

Filter sample

100 mL sample

Freeze

Freeze-dry

3 x 5mL MeOH

Filter
0.22 µm

Evaporate to dryness
(Syncore® Polyvap)

Recontitute in
200 µL ACN:MQ
(1:9)

MMLC—QTOF

Mixed-mode LC for HRMS screening

SUSPECT SCREENING WORKFLOW

Data analysis MS
Suspect screening MassHunter software

Find by Formula Algorithm

Database 1: Water Res. 2015 Feb 1;69:274-83
≈ 2000 common water pollutants

Database 2: PROMOTE consortium
≈ 1000 PMOCs from REACH

- Maximum mass error (± 5ppm)
- Isotopic profile
- Minimum peak heigh (100 counts)

Accurate MS identified candidates

Data analysis MS/MS
Confirmation of positives

Injection in MS/MS mode C.E. 15 and 30 V

Standard not available

MS/MS spectrum interpretation

MS/MS spectrum comparison with databases (e.g. Metlin/Massbank)

Standard available

Comparison of:
- Retention time
- At least 2 product ions

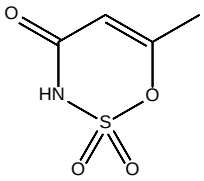
Mixed-mode LC for HRMS screening

CONFIRMED: ACESULFAME

(a) Compound identified by FBF algorithm Database 1

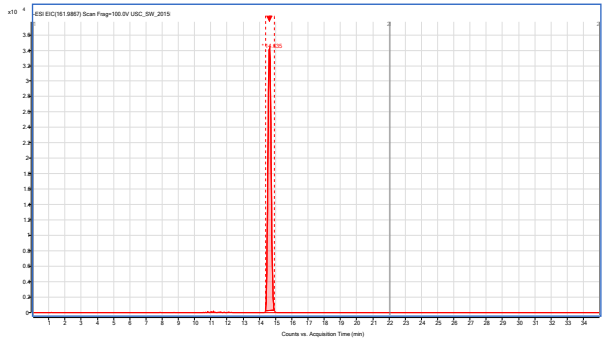
ID Techniques Applied									
FBF									
Best	Name	Formula	Species	m/z	Mass	Mass (Tgt)	Diff (ppm)	Score (Tgt)	RT	RT (
	Acesulfame	C4 H5 N O4 S	(M-H)-	161.9867	162.9937	162.9939	1.58	86.57	14.496	

m/z	Species	Height	Score (MS)	Score (mass)	Score (iso. abund)	Score (iso. spacing)
161.9867	(M-H)-	4557.8	86.57	99.22	78.51	70.95

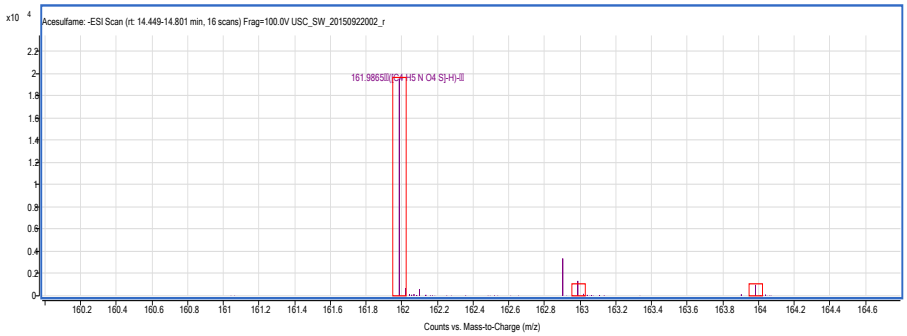


Detected 90% samples

(b) Chromatographic peak



(c) MS spectrum



Mixed-mode LC for HRMS screening

CONFIRMED: 1,3-DI-O-TOLYLGUANIDINE

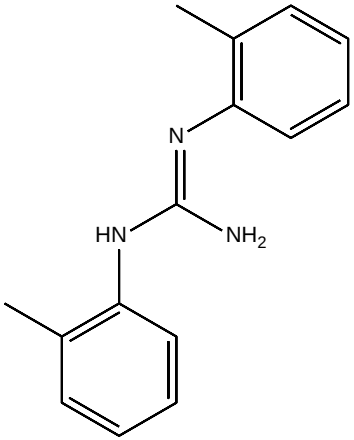
(a) Compound identified by FBF algorithm Database 2

ID Techniques Applied

FBF

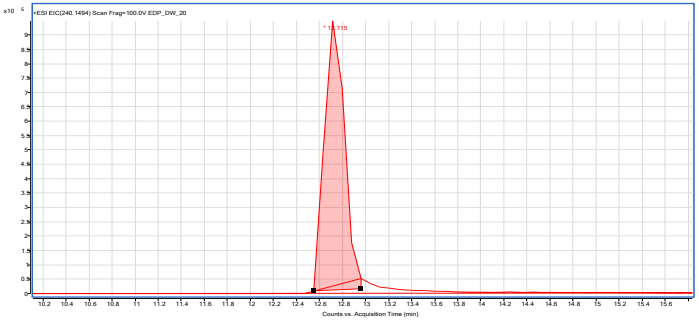
Best	Name	Formula	m/z	Mass	Mass (Tgt)	Diff (ppm)	Score (Tgt)	RT	RT (Tgt
◉	Promote 509	C15 H17 N3	240.1487	239.1414	239.1422	3.42	96.21	12.589	

m/z	Species	Height	Score (MS)	Score (mass)	Score (iso. abund)	Score (iso. spacing)
240.1487	(M+H)+	118502.7	96.21	94.2	96.47	99.91

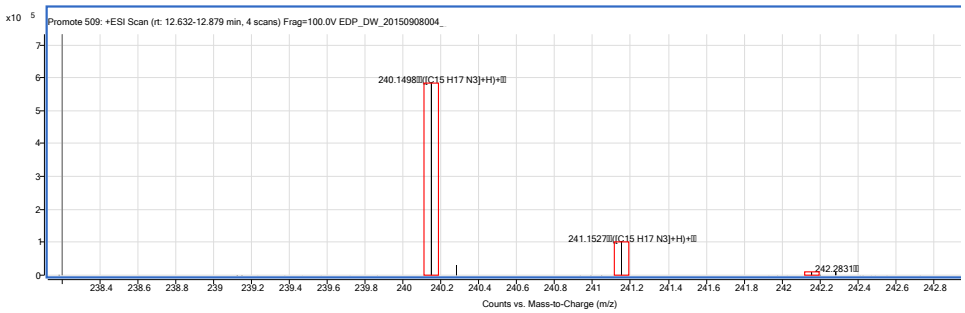


Detected 20% samples

(b) Chromatographic peak



(c) MS spectrum



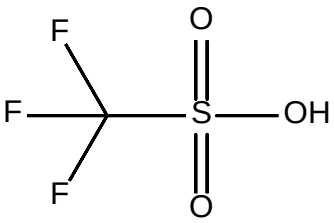
Mixed-mode LC for HRMS screening

CONFIRMED: TRIFLUOROMETHANE SULFONIC ACID

(a) Compound identified by FBF algorithm Database 2

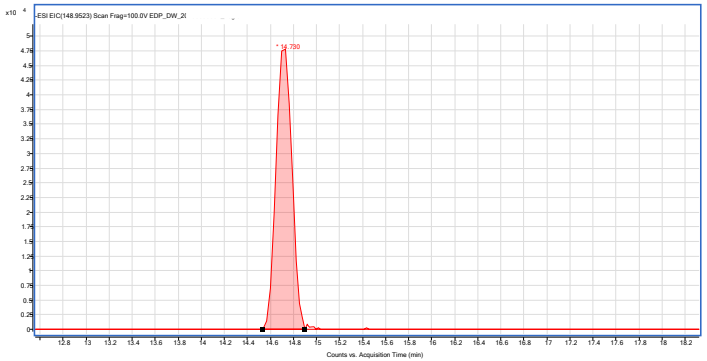
ID Techniques Applied									
FBF									
Best	Name	Formula	m/z	Mass	Mass (Tgt)	Diff (ppm)	Score (Tgt)	RT	RT (Tg
	Promote 515	C H F3 O3 S	148.9524	149.9597	149.9598	1.06	93.49	14.501	

m/z	Species	Height	Score (MS)	Score (mass)	Score (iso. abund)	Score (iso. spacing)
148.9524	(M-H)-	38306.2	93.49	99.69	77.8	99.94

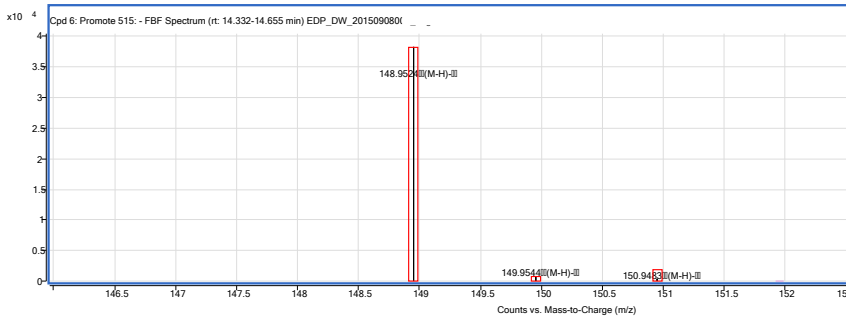


Detected 100% samples

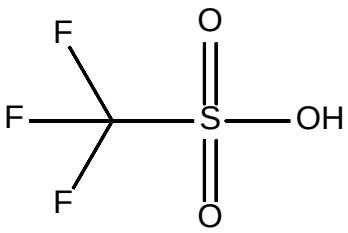
(b) Chromatographic peak

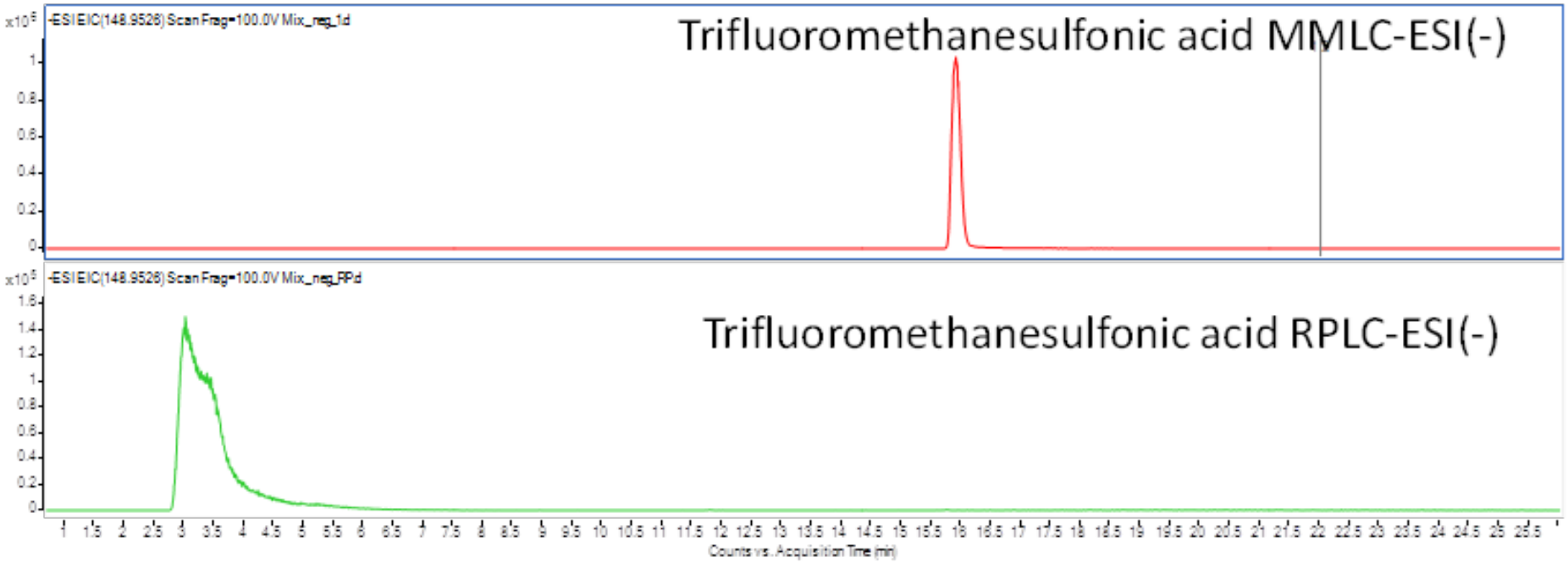


(c) MS spectrum



Mixed-mode LC for HRMS screening





Improving PMOCs determination

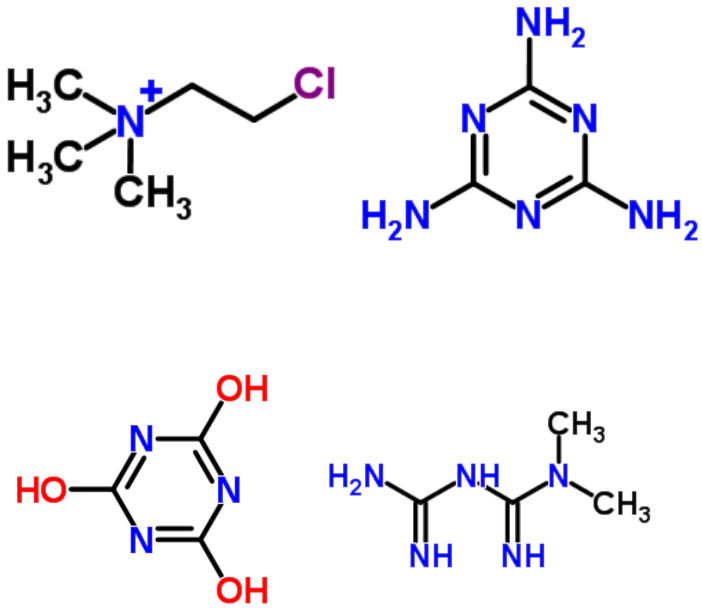
Environmental Science & Technology

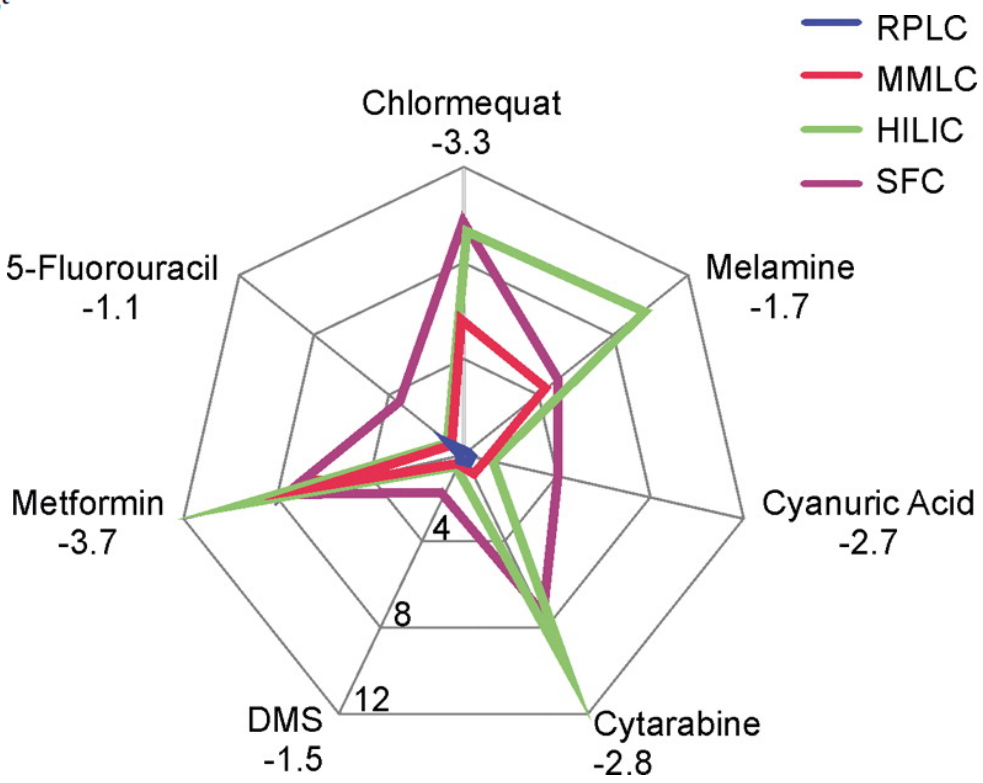
Feature

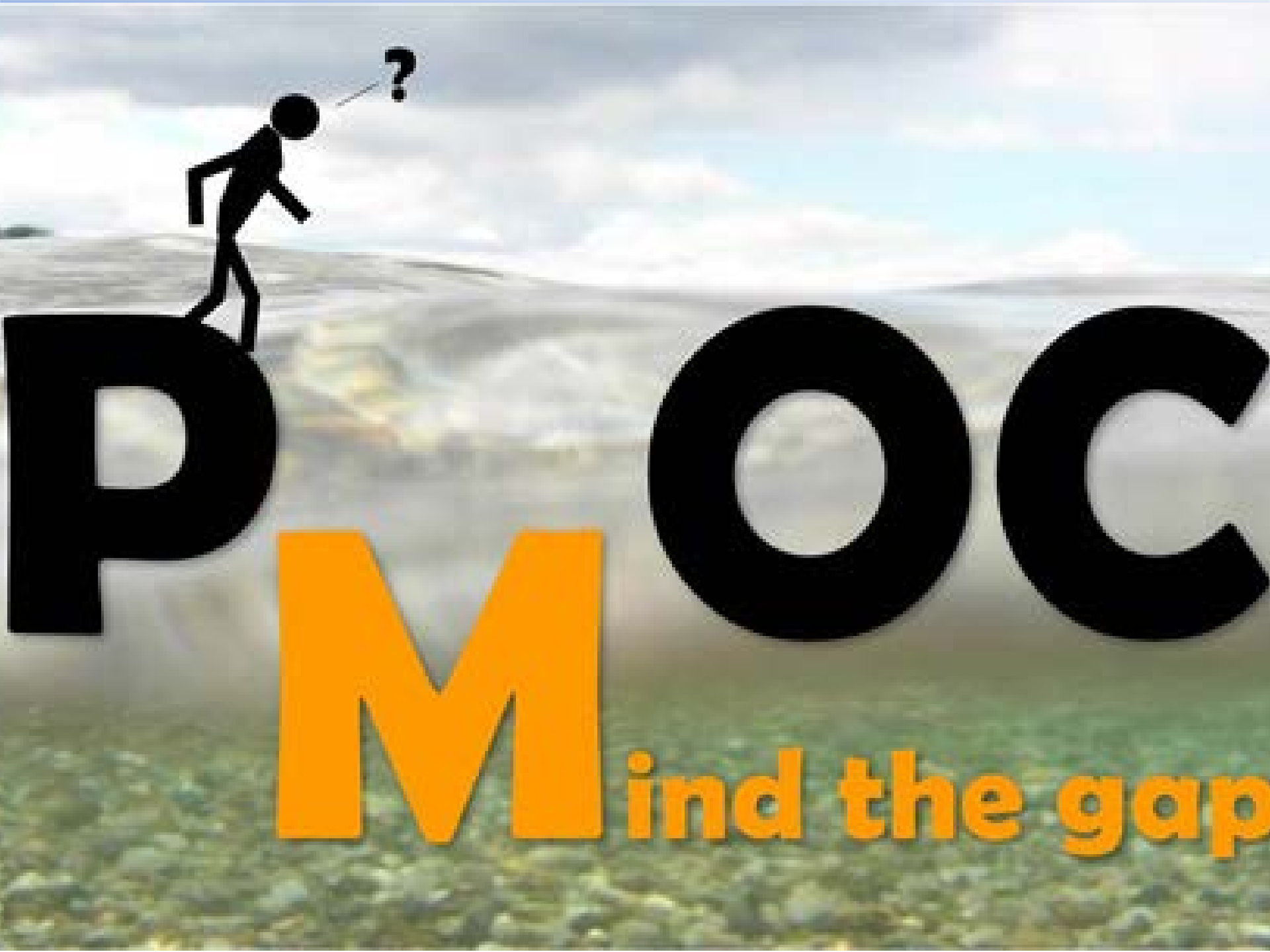
DOI: 10.1021/acs.est.6b03338
Environ. Sci. Technol. 2016, 50, 10308−10315

Mind the Gap: Persistent and Mobile Organic Compounds—Water Contaminants That Slip Through

Thorsten Reemtsma,[*,†] Urs Berger,[†] Hans Peter H. Arp,[‡] Hervé Gallard,[§] Thomas P. Knepper,[∥] Michael Neumann,[⊥] José Benito Quintana,[#] and Pim de Voogt[∇,○]

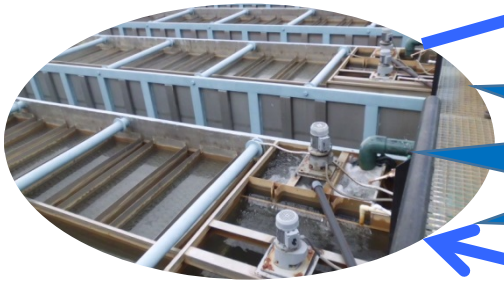





?
POC
Mind the gap

The water cycle

WWTP



DWTP

Transformation products (TPs)

Surface water

Transformation products (TPs)

At ChromChem we have experience with:

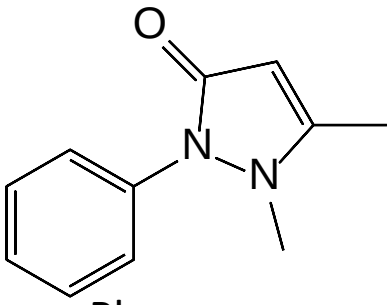
- Chlorination, permanganate, UV/Solar photolysis, biotransformation, AOPs…

Our workflow

1. Preliminary experiments
2. Kinetic study: pH, dose, natural ions / catalizers
3. TPs identification:
    - HRMS + statistical tools
    - HRMS/MS elucidation
4. QSAR toxicity assessment
5. Real samples

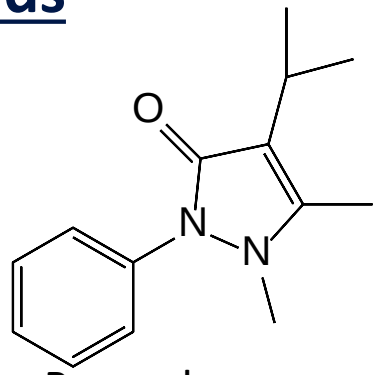
Example: phenazone-type analgesics

Considered compounds



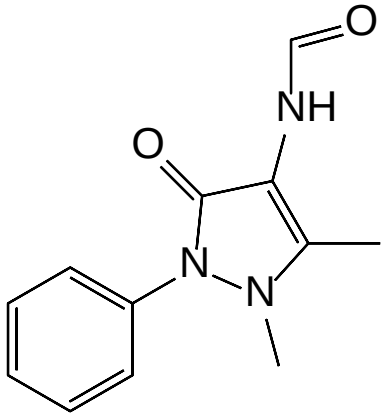
Phenazone

Phe



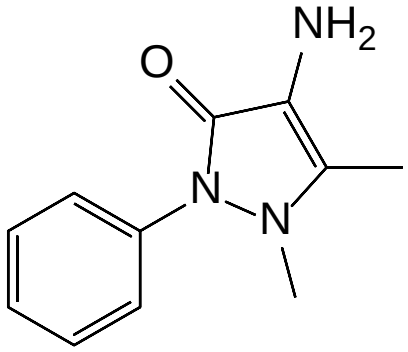
Propyphenazone

PrPhe



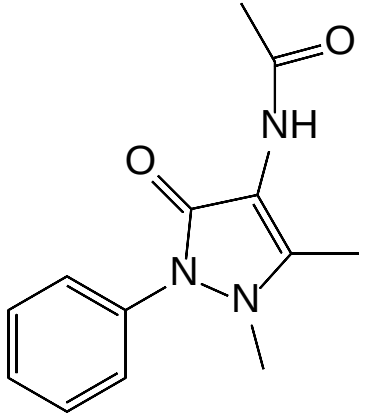
4-formylaminoantipyrine

FAA



aminoantipyrine

AA



4-acetoamidoantipyrine

AAA

AIMS

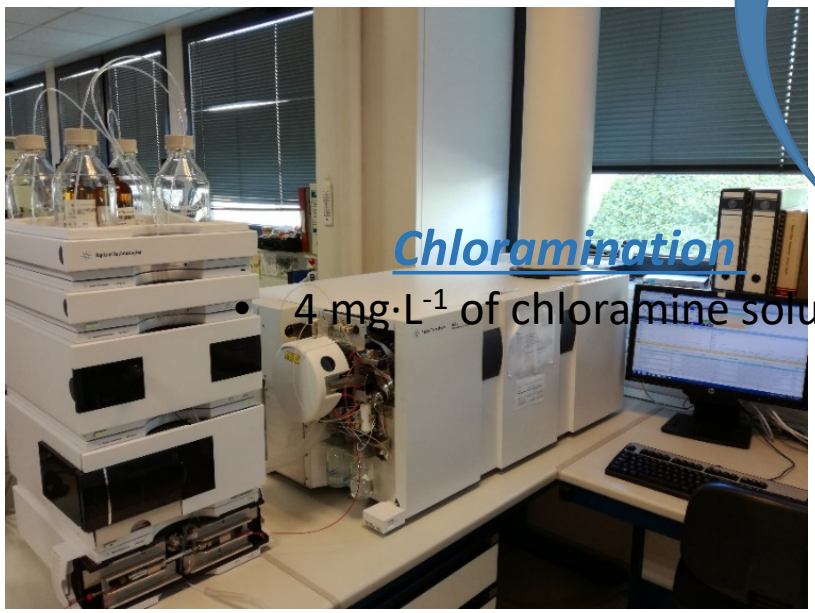
- Study the reaction kinetics of **Phe**, **PrPhe**, **AA**, **FAA** and **AAA** with:
  - Free chlorine
  - Chlorine + bromide
  - Chloramine
- Identification of transformation products (TPs)

Methodology

- 10 mL ultrapure water
- 30 mM pH buffer (pH 5.7 – pH 8.3)
- 50 µg·L^{-1} or 1 mg ·L^{-1} of compound

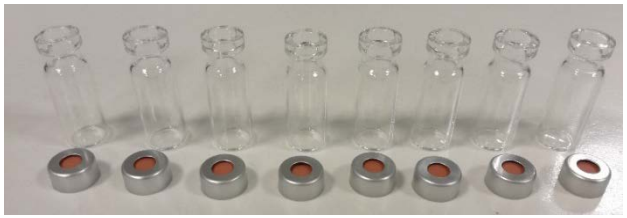
Chlorination (+ bromide)

- 1-10 mg·L^{-1} of chlorine
- 0-100 µg·L^{-1} bromide



Chloramination

- 4 mg·L^{-1} of chloramine solution



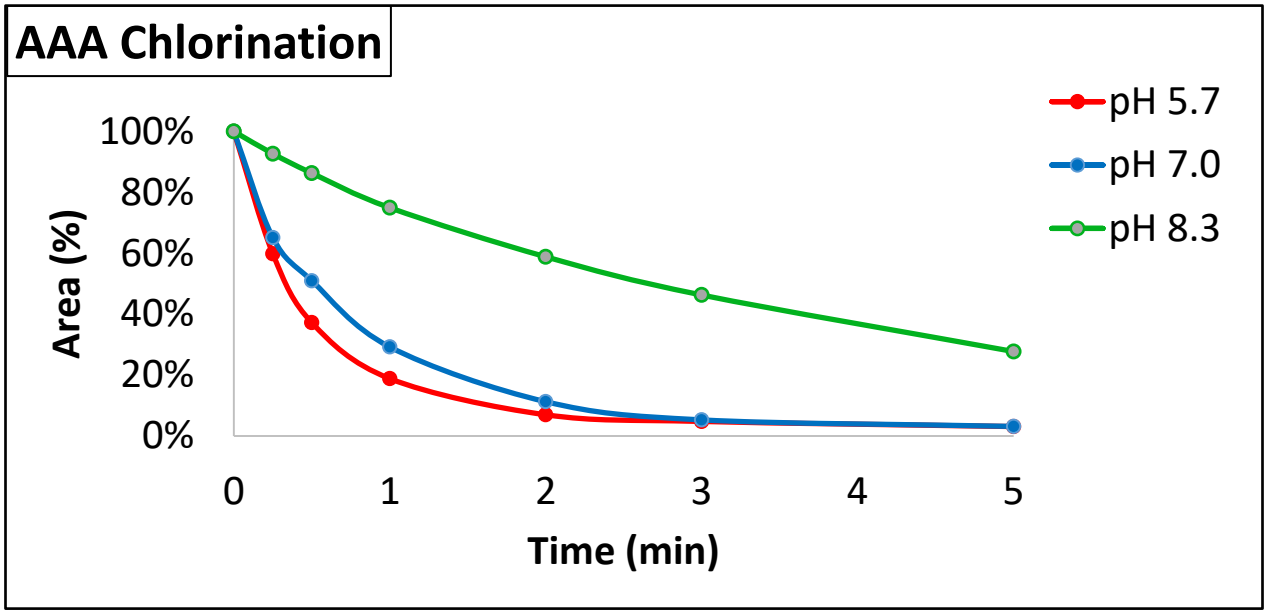
- 1 mL aliquots at different times
- Stopped with ascorbic acid

Kinetics

Chlorine

Half-lives at 10 mgL^{-1} Cl_2

Milli-Q pH	Phe (s)	PrPhe (s)	AA (s)	AAA (s)	FAA (s)
5.7	0.9	0.4	3.7	25	50
7.0	1.8	0.5	3.6	43	52
8.3	4.1	0.9	3.5	163	59

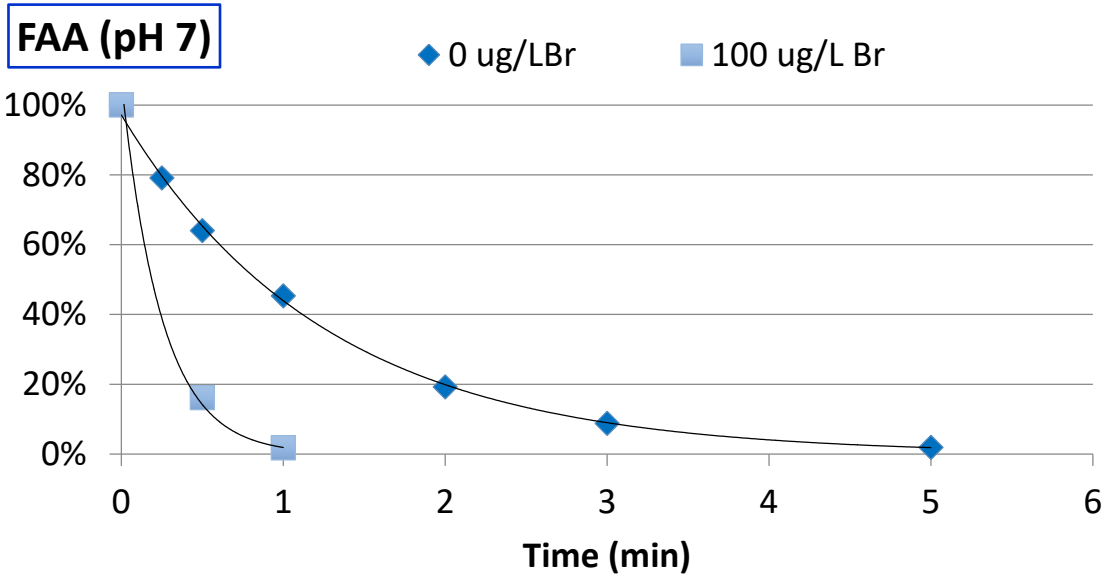


Kinetics

Chlorine + bromide

Half-lives at 10 mgL^{-1} Cl_2

Milli-Q pH 7	Phe (s)	PrPhe (s)	AA (s)	AAA (s)	FAA (s)
0 μgL^{-1} Br-	1.8	0.5	3.6	43	52
100 μgL^{-1} Br-	1	1.1	5	10	12

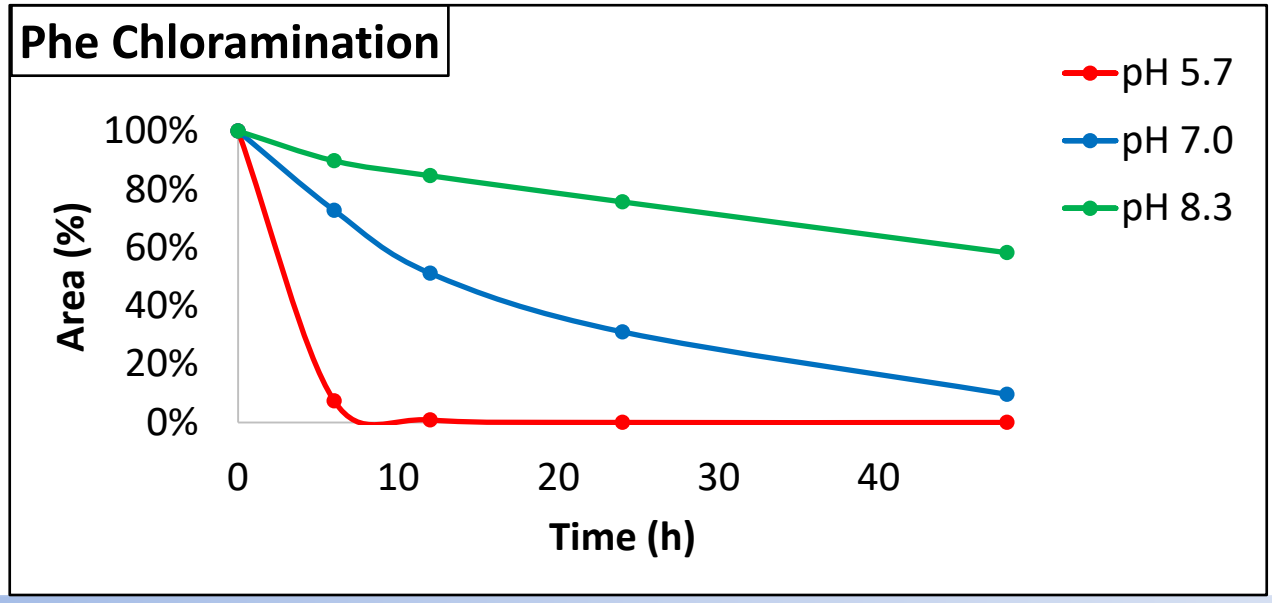


Kinetics

Chloramine

Half-lives at 4 mgL^{-1} NH_2Cl

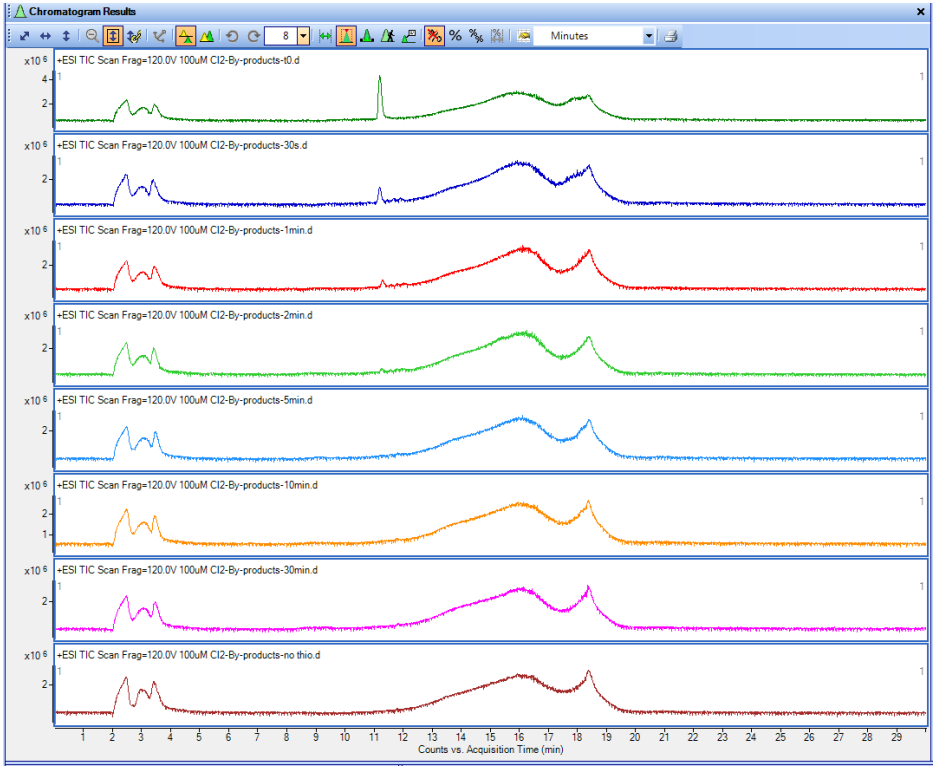
Milli-Q pH	Phe (h)	PrPhe (h)	AA (h)	AAA	FAA
5.7	1.93	0.62	0.23	-	-
7.0	14.75	5.92	50	-	-
8.3	69.31	30.14	4.07	-	-

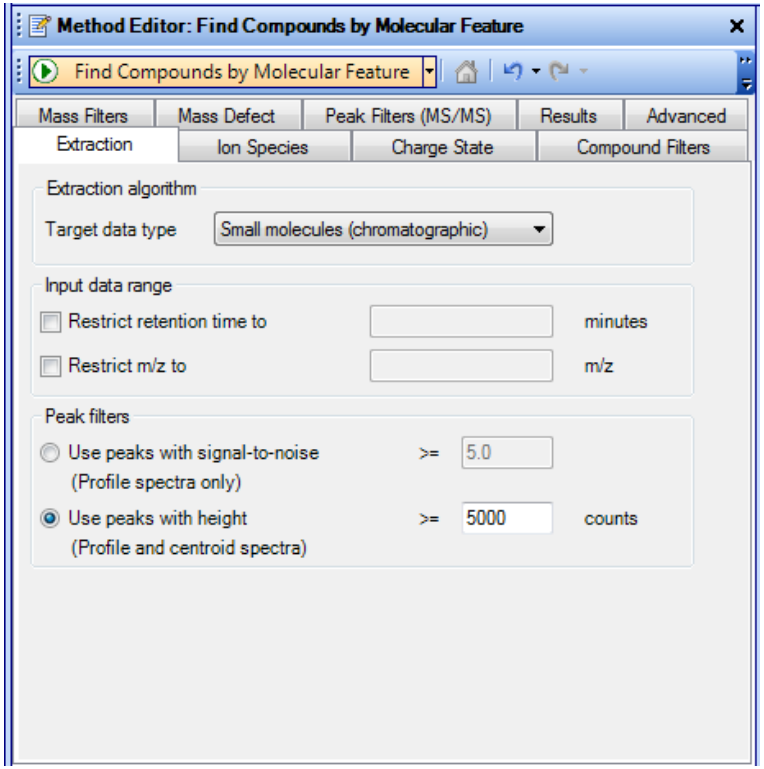


Chasing the TPs

Data treatment: finding suspects

1. MassHunter Qualitative (software by Agilent Technologies)
   - Open data files
   - Algorithm *Find Compounds by Molecular Feature*
   - Export data results as .cef

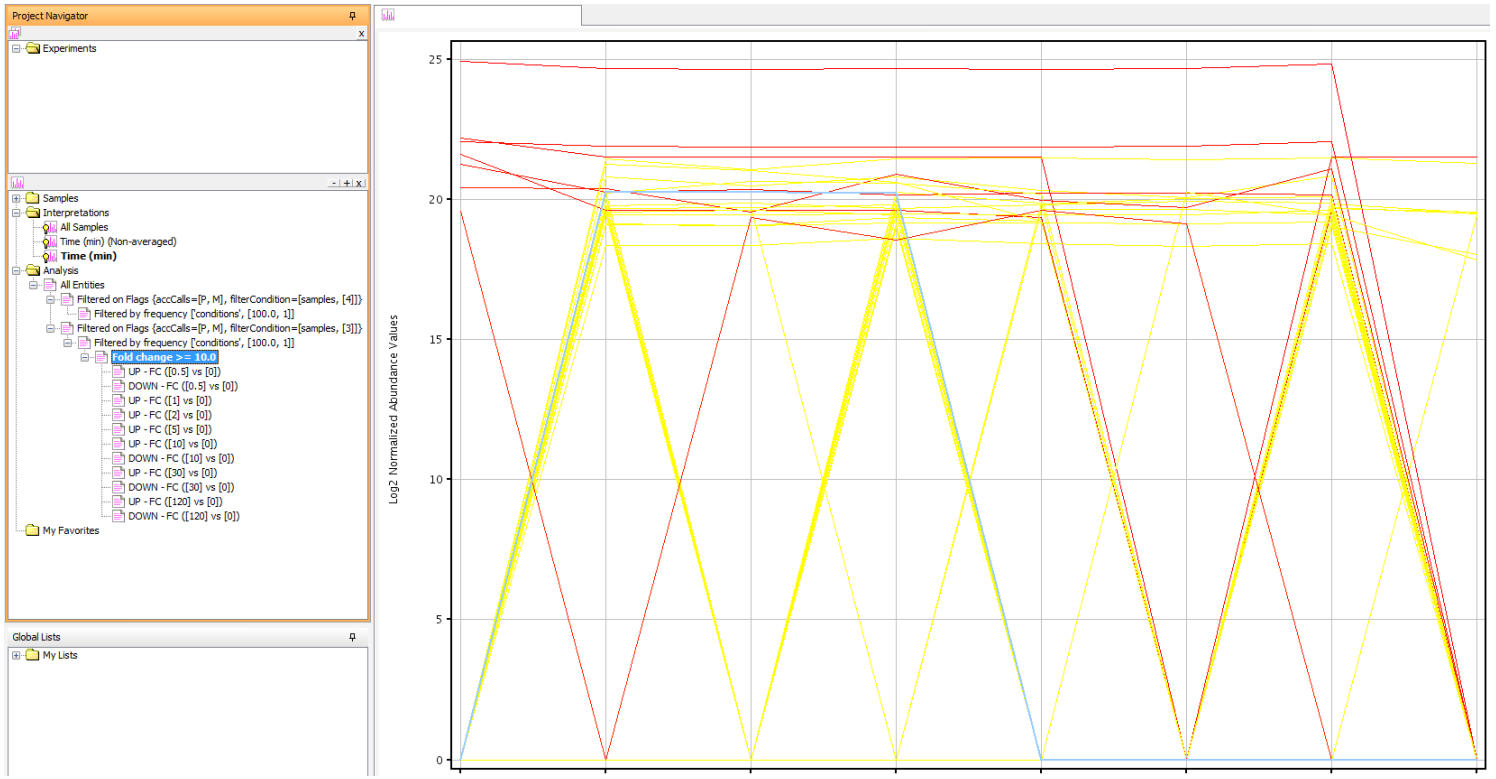




Chasing the TPs

Data treatment: finding suspects

2. Mass Profiler Professional (Agilent Technologies)
   - Open .cef files
   - Set up data analysis (alignment, ANOVA, fold changes, etc.)
   - Seek “overexpressed features”



Chasing the TPs

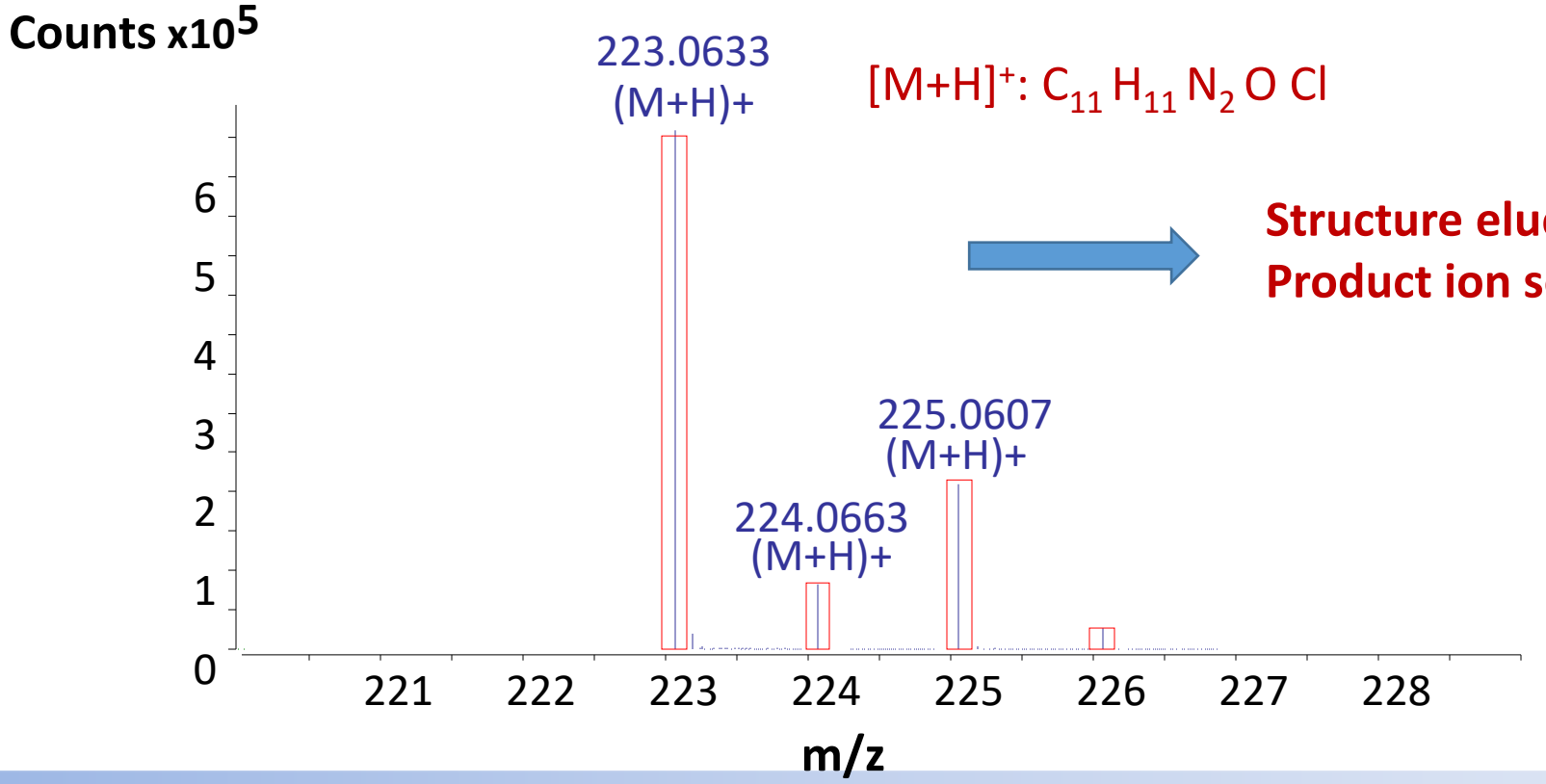
Data treatment: structure elucidation

Formula generation:

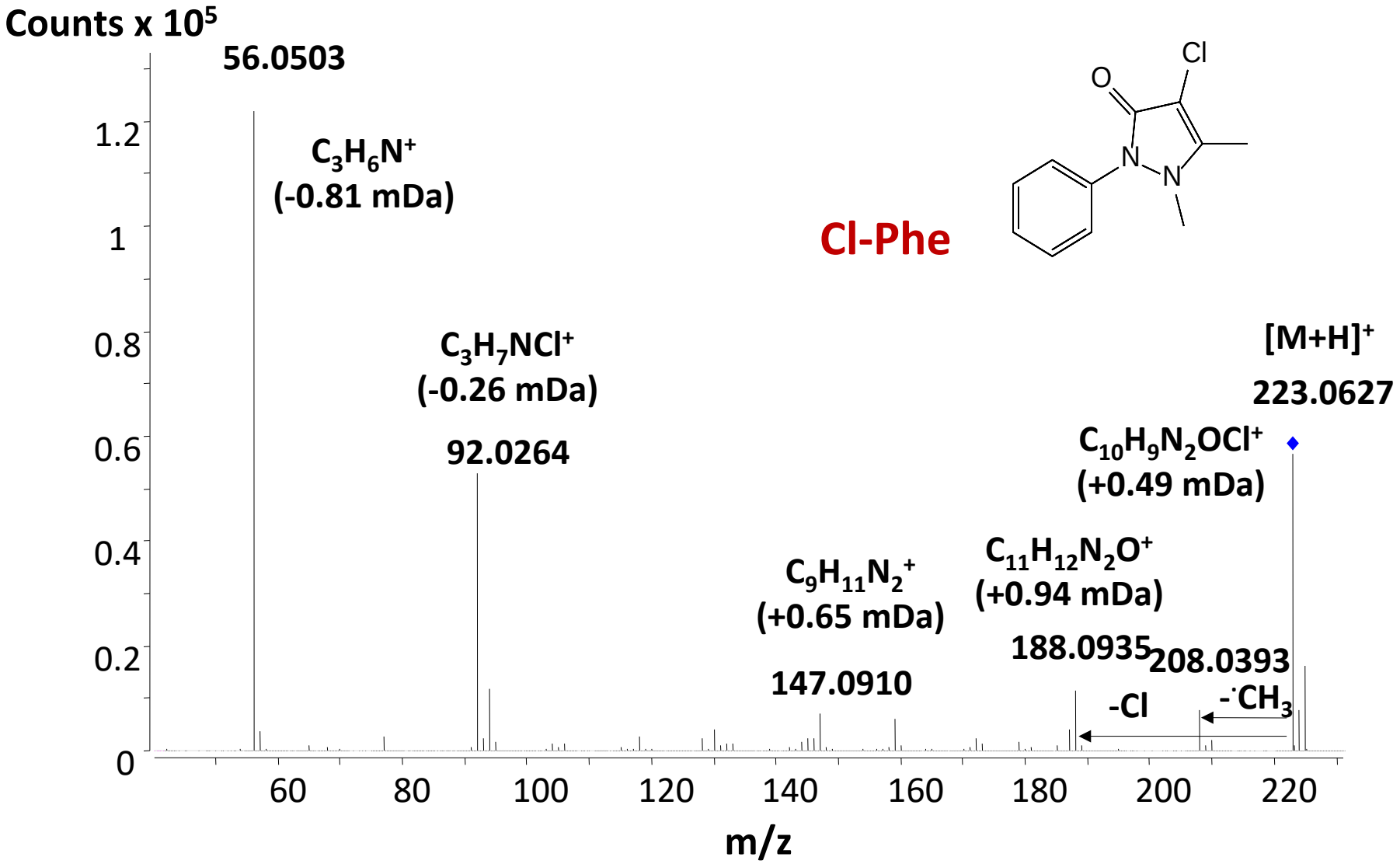
Mass accuracy

isotopic distribution

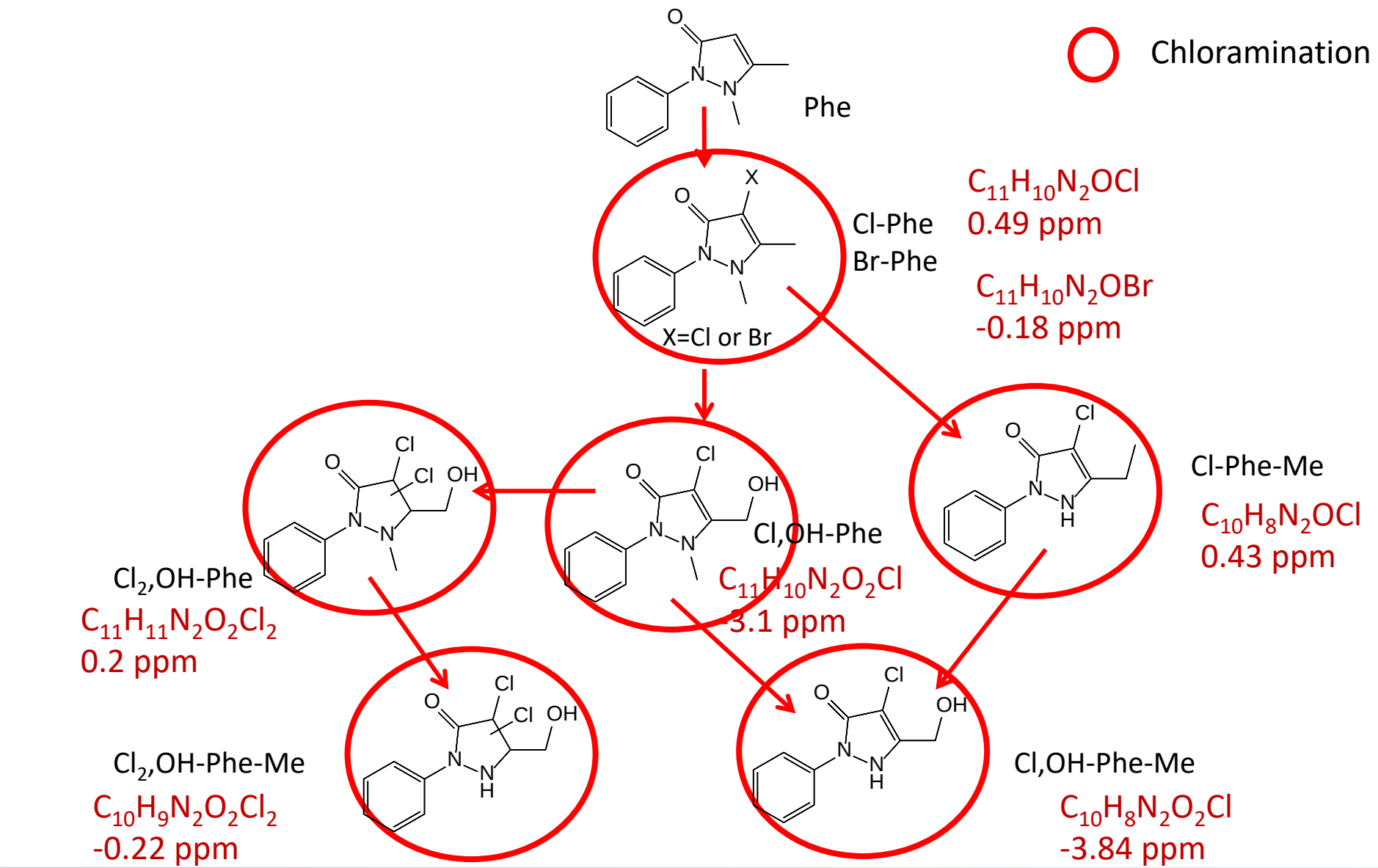
spacing between isotopes



Chasing the TPs



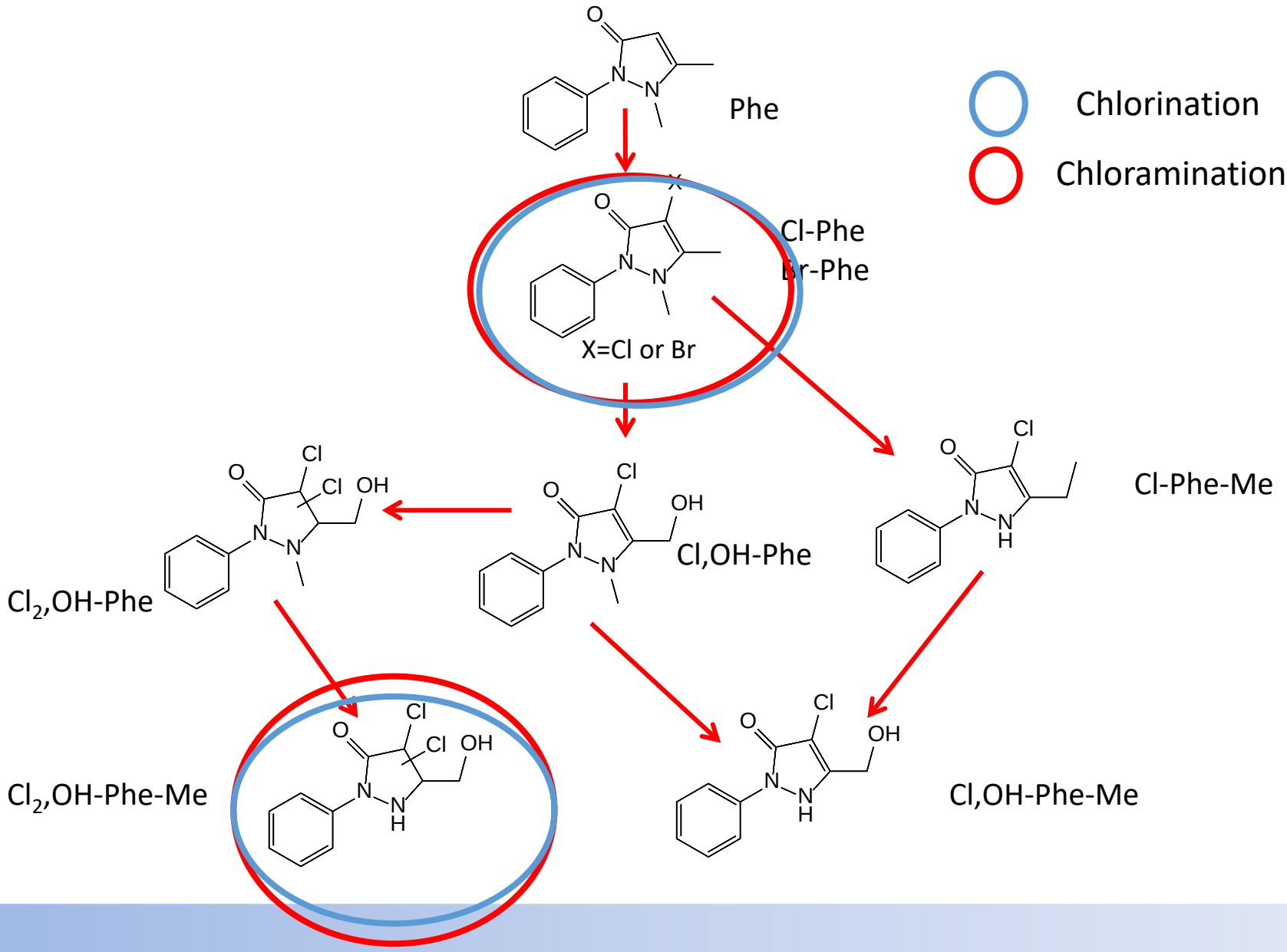
Phenazone TPs



QSAR Toxicity – phenazone TPs

Compound	Ecotoxicological data predicted by US EPA TEST	
	Fathead Minnow (96h LC_{50} mg/L)	Daphnia magna (48h LC_{50} mg/L)
Phe	36.82	41.36
Cl-Phe	10.67	24.45
Br-Phe	9.64	17.33
Cl, OH-Phe	18.34	50.83
Cl2,OH-Phe	N/A	N/A
Cl2,OH-Phe-Me	N/A	N/A
Cl-Phe-Me	34.61	24.95
Cl,OH-Phe-Me	53.71	131.74

Reaction in real river water



PROMOTE Workshop

www.promote-water.eu

Workshop

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Linking science, technology and regulation to protect drinking water quality

23 - 24 November 2017, Leipzig, Germany

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THANK YOU!