Characterization of Effluent Organic Matter (EfOM) Using Size Exclusion Chromatography (SEC) Using Quadrupole Time-of-Flight Mass Spectrometry (Q-TOF-MS) and Diode Array Detector (DAD)-Fluorescence Detector (FLD)

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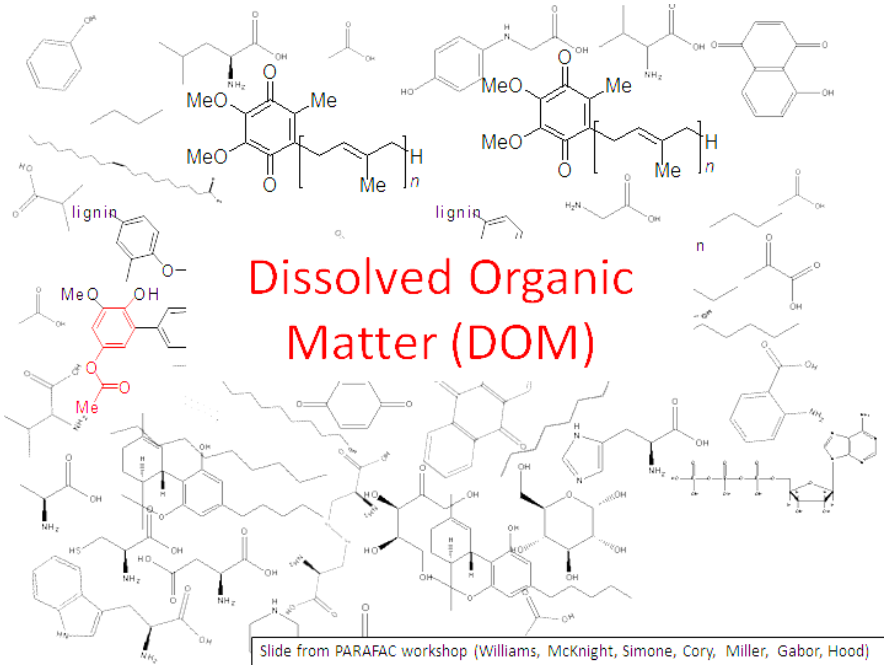
Natural organic matter (NOM)

- Refractory organic matter in soils, sediments, and natural water.
- NOM is a complex mixture of aromatic and aliphatic hydrocarbon structures that have attached functional groups.



Slide from PARAFAC workshop (Williams, McKnight, Simone, Cory, Miller, Gabor, Hood)

Effluent organic matter (EfOM)

EfOM

: Organic matter originated from WWTP effluents

NOM

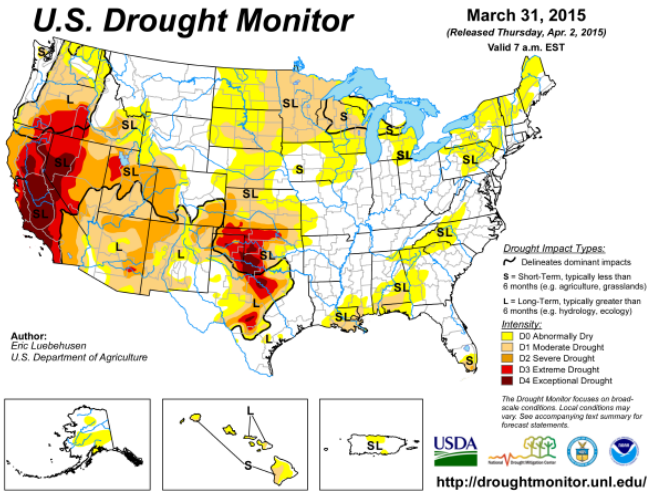
Transformation products by biological treatments

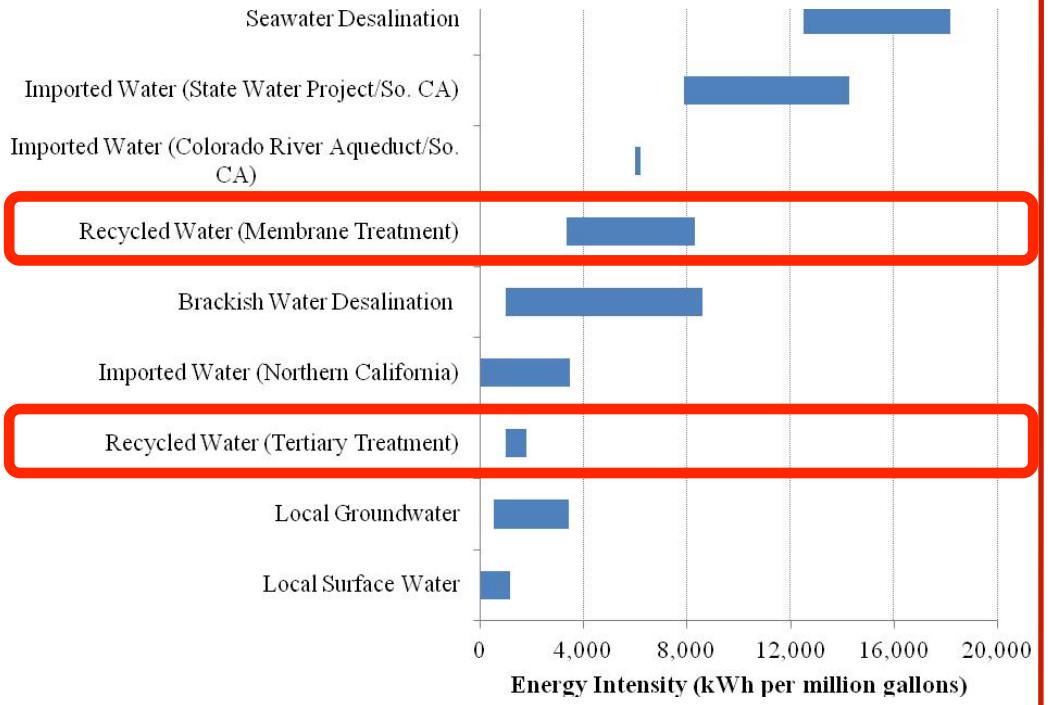
Anthropogenically driven OM

Micropollutant

Water Reuse

- Moving forward to potable water reuse





http://pacinst.org/desal-and-energy-use-should-we-pass-the-salt/

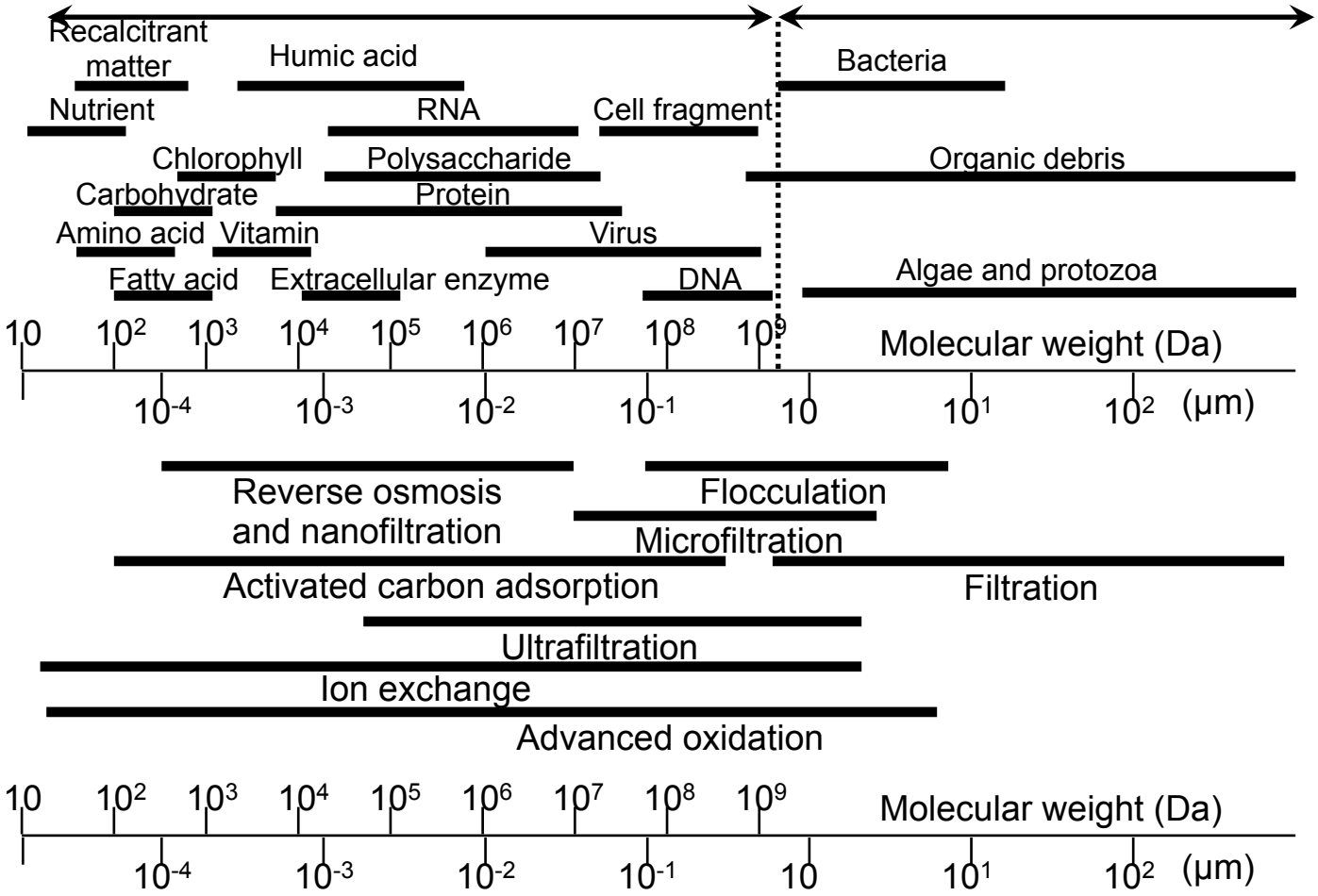
Water Reuse

- Severe climatological droughts around the US increases the demands on reliable and dependable water resources.

Adverse effects of EfOM on water treatment processes

- Precursor for disinfection by-product formation.
- Exerts higher coagulant and oxidant demands.
- Fouls adsorbents and membranes.
- Causes corrosion problems.
- Supplies substrate for biomass growth in water distribution networks

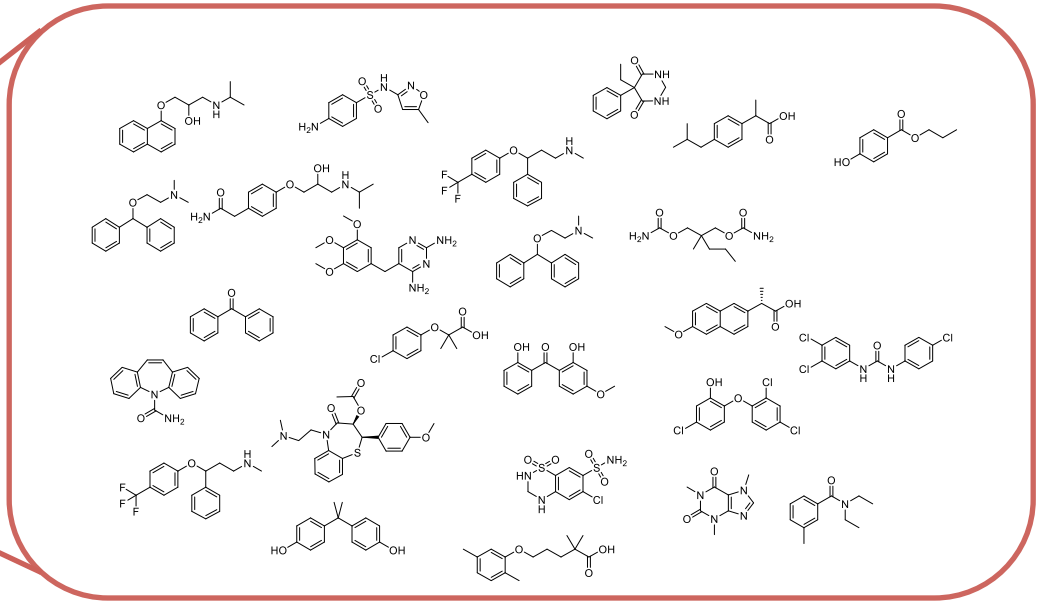
OM size and treatment processes



Shon, H.K., Vigneswaran, S. and Snyder, S.A. (2006) Effluent Organic Matter (EfOM) in Wastewater: Constituents, Effects, and Treatment. Critical Reviews in Environmental Science and Technology 36(4), 327-374.

Trace organic compounds (TOrCs)

- EfOM contains anthropogenically driven compounds such as pharmaceutical and personal care products (PPCPs) and endocrine disrupting compounds (EDCs)



Objectives

Size separation

SEC

Accurate mass

QTOF-MS

Non-selective detectors (bulk properties)

DAD/FLD

- To investigate **elemental compositions** of EfOM
- To **hyphenate bulk properties** (i.e., UV abs and FL) with **elemental compositions** of EfOM.
- To provide **non-target screening of TOrCs**
- To provide **fingerprinting** of EfOM

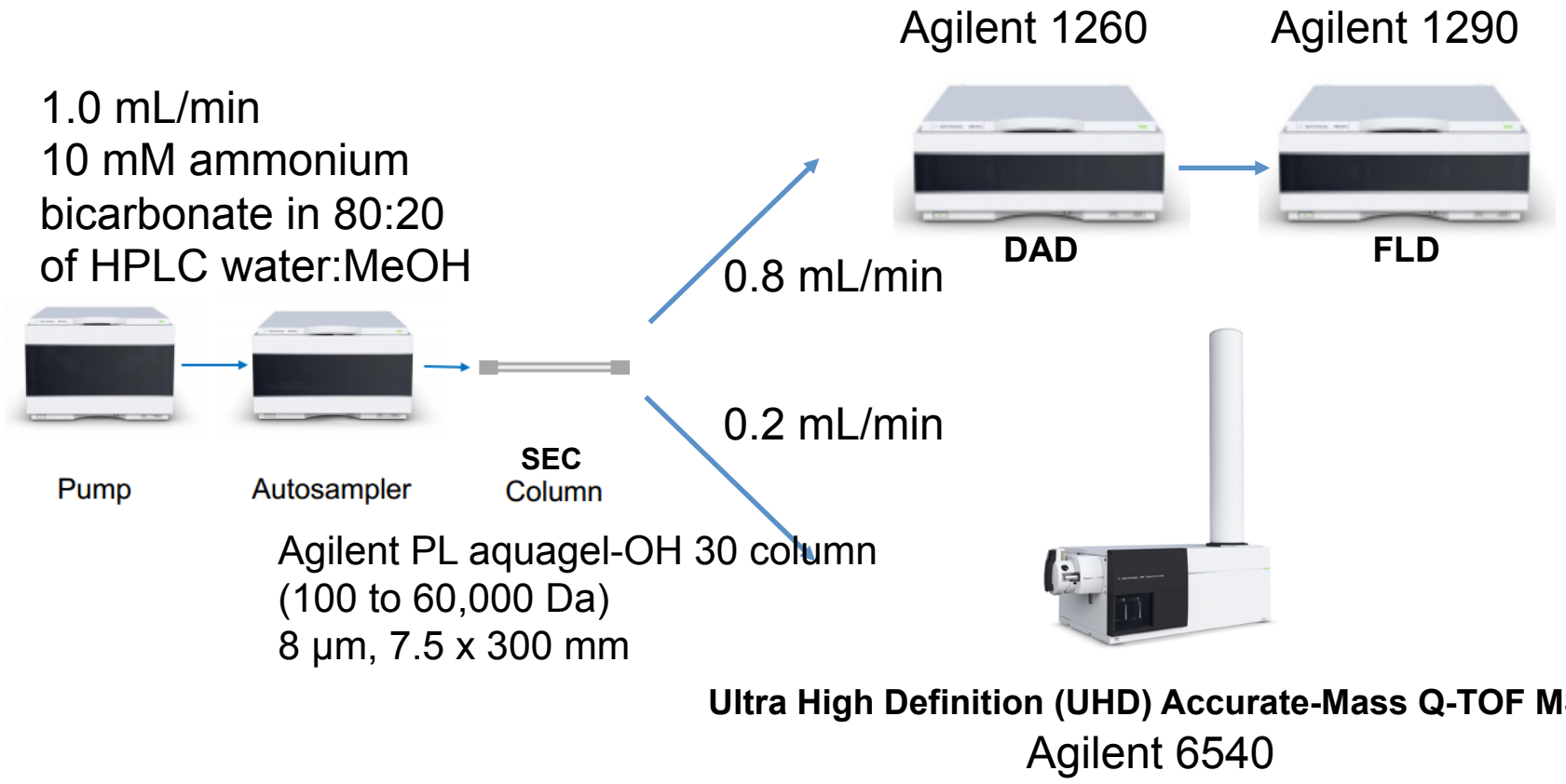
EXPERIMENTAL

Sample collection & Preparation

- Water samples were extracted using Dionex Autotrace 280 Soild-phase extraction instrument was employed for the extraction.
- Agilent Bond Elut PPL cartridge (500 mg bed mass) was used.

	Solvent	Flow	Volume/Time
Condition	MeOH	5 mL/min	5 mL
	DI water with pH2	5 mL/min	10 mL
Load	Sample	10 mL/min	200 mL
Rinse	DI water with pH2	10 mL/min	10 mL
Dry	N_2 gas		60 min
Elute	MeOH	5 mL/min	12 mL

SEC-LC-DAD-FLD/ESI-QTOF-MS



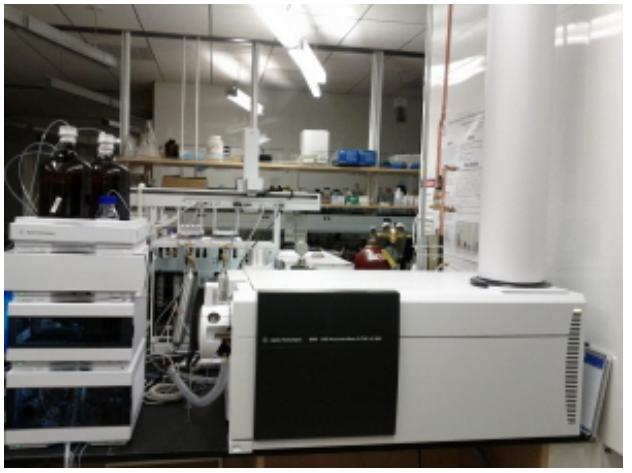
Acquisition Methods

QTOF

Ion mode	ESI negative
Scan range	50-3000 (m/z)
MS/MS scan range	50-3000 (m/z)
Scan rate	1.2 spectra/second
Gas temp	200 °C
SheathGas temp	350 °C
Nebulizer	35 psi
Fragmentor	120V

SEC

Column	PL Aquagel-OH 30 (7.5*300mm, 8µm)
Flow	1 mL/min (split: 1:4)
Col Temp.	40 °C
Injection	100 uL
Mobile Phase	10mM ammonia bicarbonate (20% Methanol)



DAD

Wavelength	254 nm

FLD

Wavelength	Ex/Em= 220nm/295nm
	Ex/Em= 220nm/340nm
	Ex/Em= 220nm/440nm
	Ex/Em= 280nm/340nm
	Ex/Em= 335nm/440nm

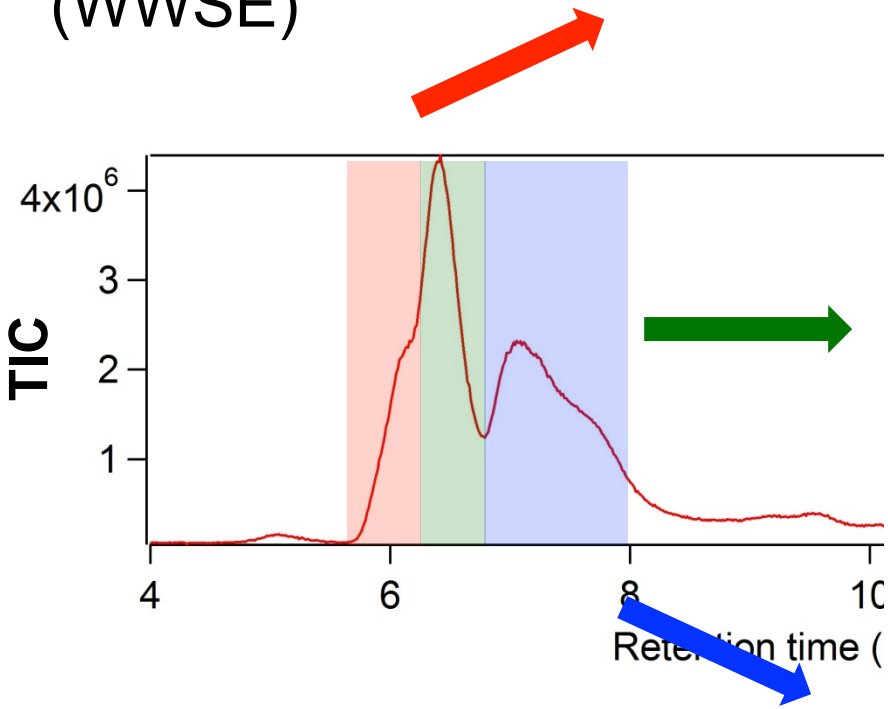
Data process

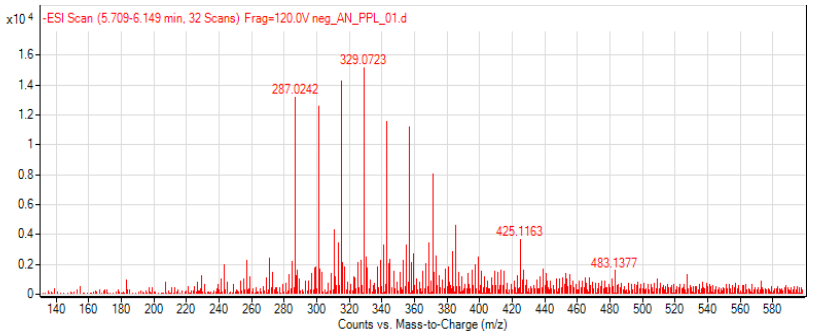
- Data mining and statistical analysis
  - Agilent Mass Profiler Professional (MPP, Ver. 12.1)
- Analysis of chromatogram and mass spectra
  - Agilent Mass Hunter Qualitative Analysis (Ver. B.07.00)
- Database search
  - Agilent Mass Hunter Qualitative Analysis
  - Databases
    - Forensic/Toxicology (ForTox) accurate mass DB
    - METLIN metabolite accurate mass DB

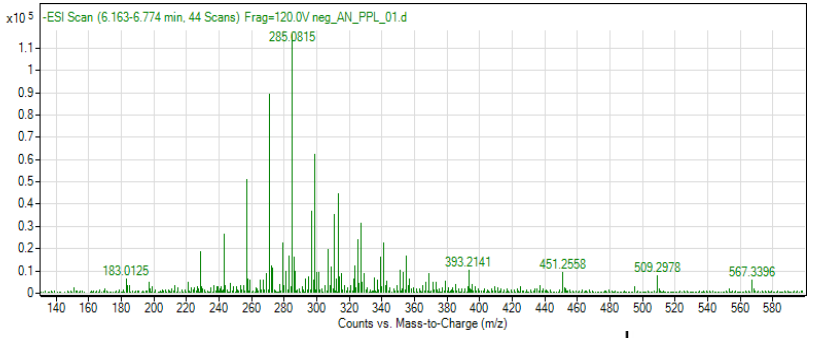
RESULTS AND DISCUSSION

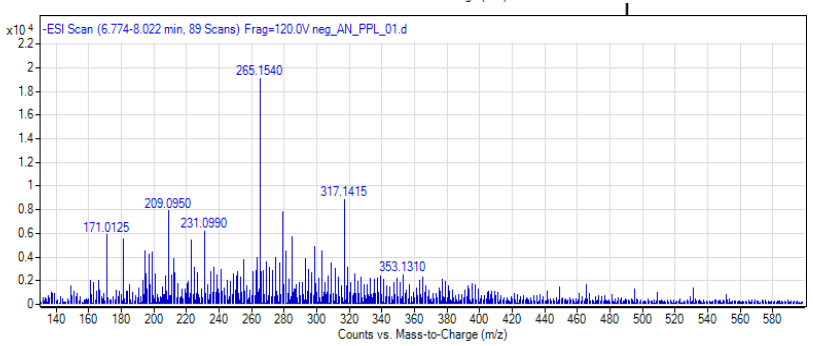
Size exclusion chromatography

- Wastewater secondary effluent (WWSE)



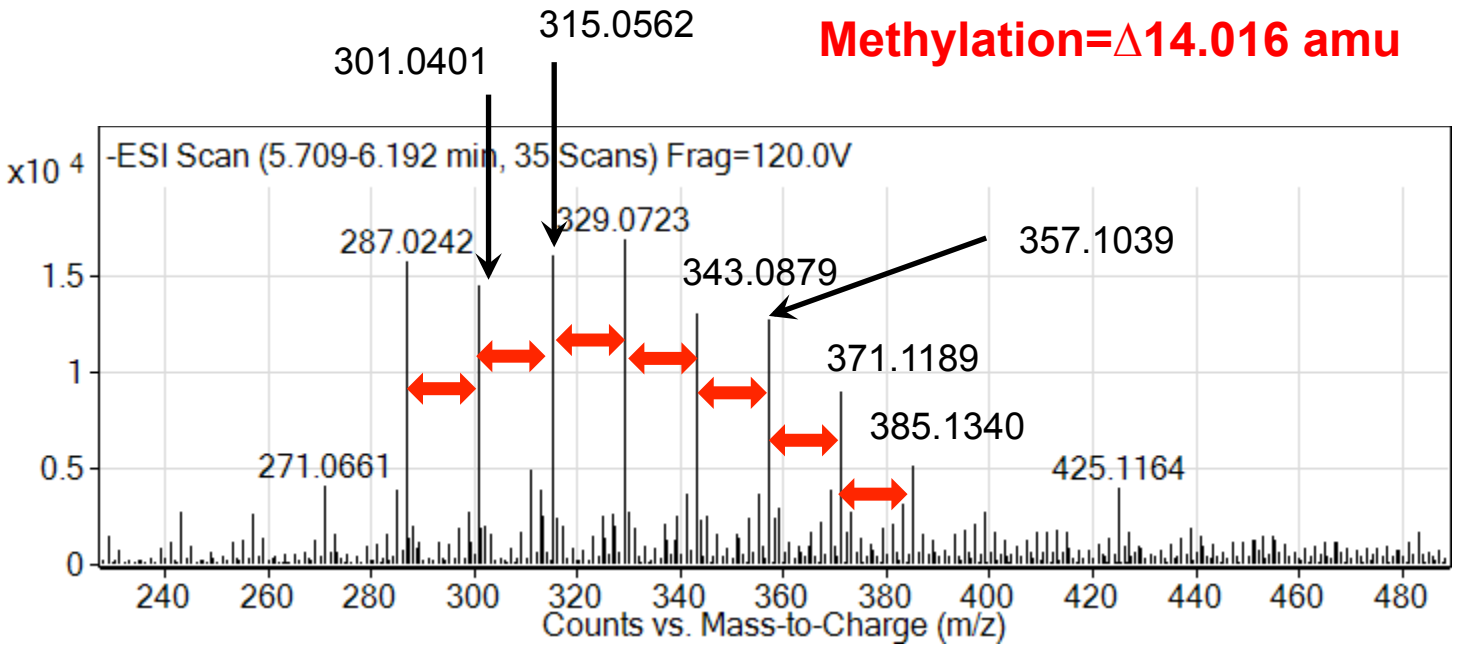






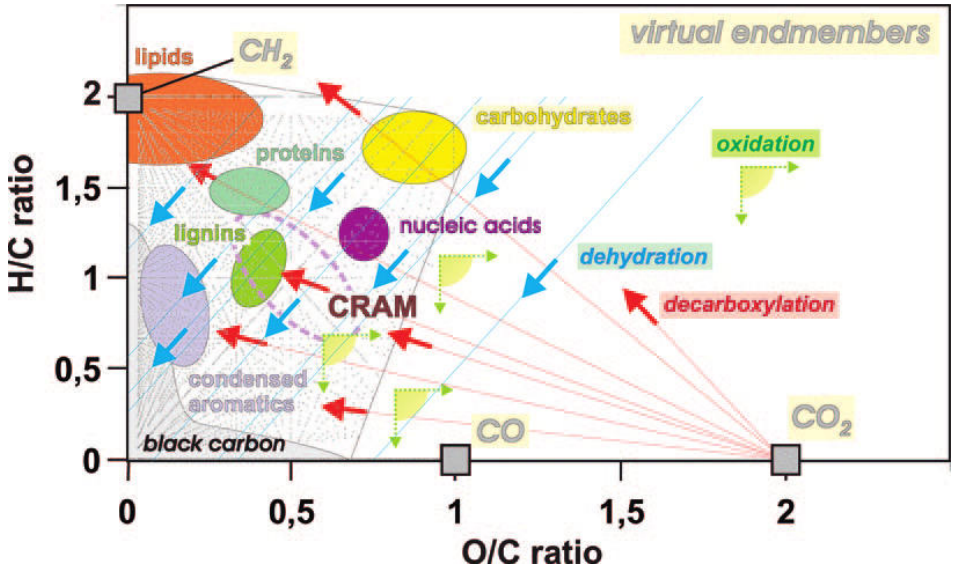
Elemental composition of EfOM

- Homologues series of organic matter was found in WWSE1.



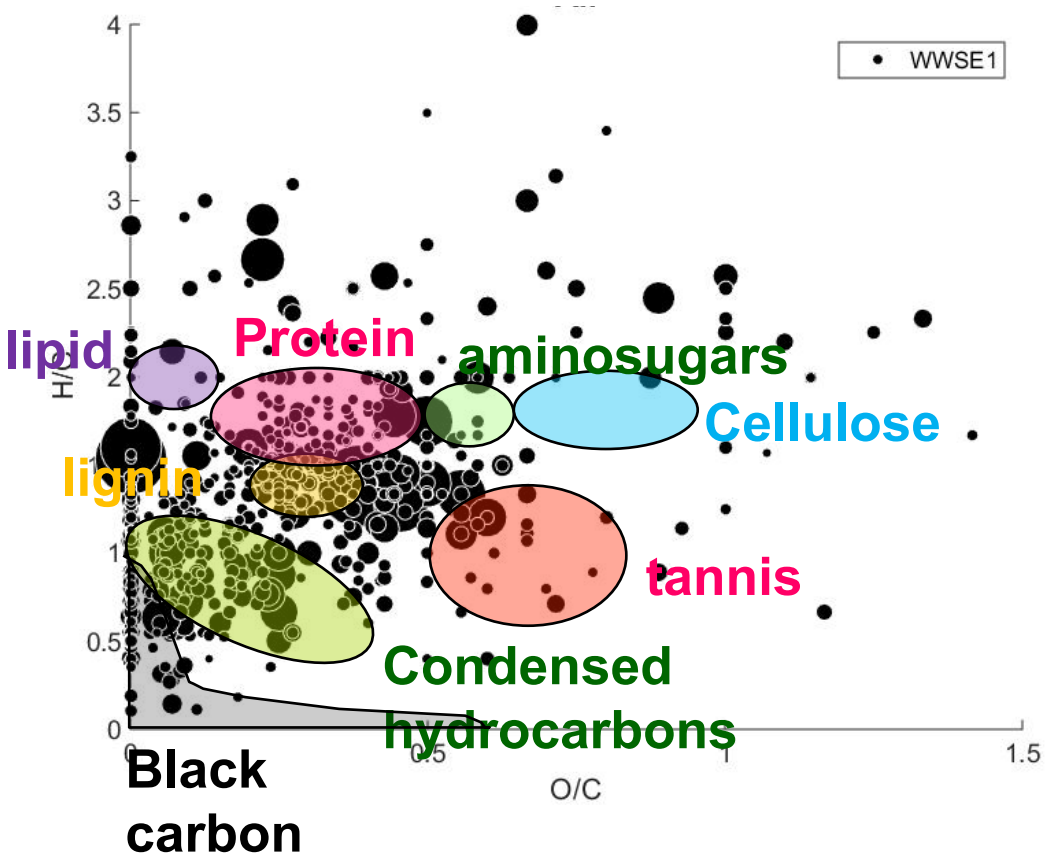
Van Krevelen diagram

- Van Krevelen diagram is a plot of the molar ratio of hydrogen to carbon (H/C ratio) and the molar ratio of oxygen to carbon (O/C).



Hertkorn, N., Frommberger, M., Witt, M., Koch, B.P., Schmitt-Kopplin, P. and Perdue, E.M. (2008) Natural Organic Matter and the Event Horizon of Mass Spectrometry. Analytical chemistry 80(23), 8908-8919.

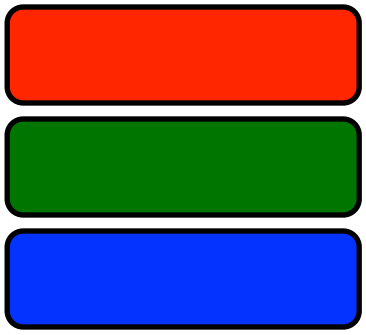
Van Krevelen diagram



Aromaticity index (AI)

- Aromaticity index (AI)
  - a measure for C–C double-bond density and considers the contribution of p-bonds by heteroatoms

$$\mathrm{AI} = \frac{1 + C - O - S - 0.5H}{C - O - S - N - P}$$



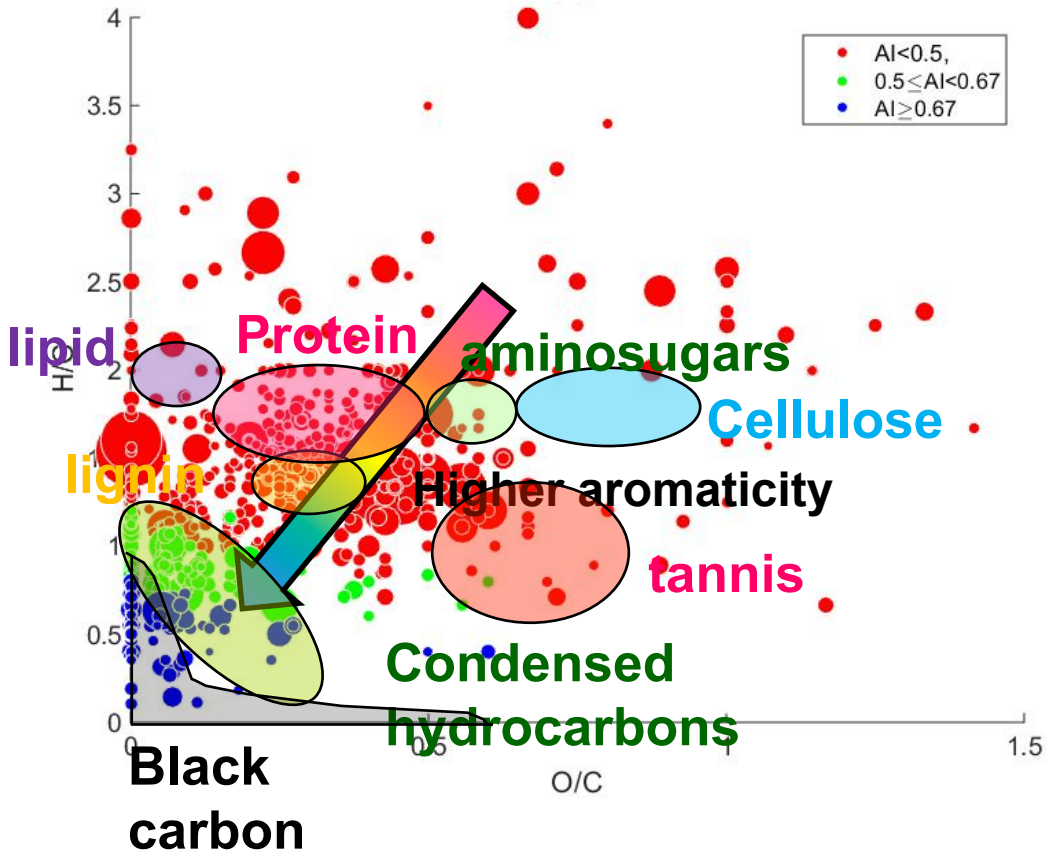
AI≤0.5

0.5<AI<0.67 (Aromatic structure)

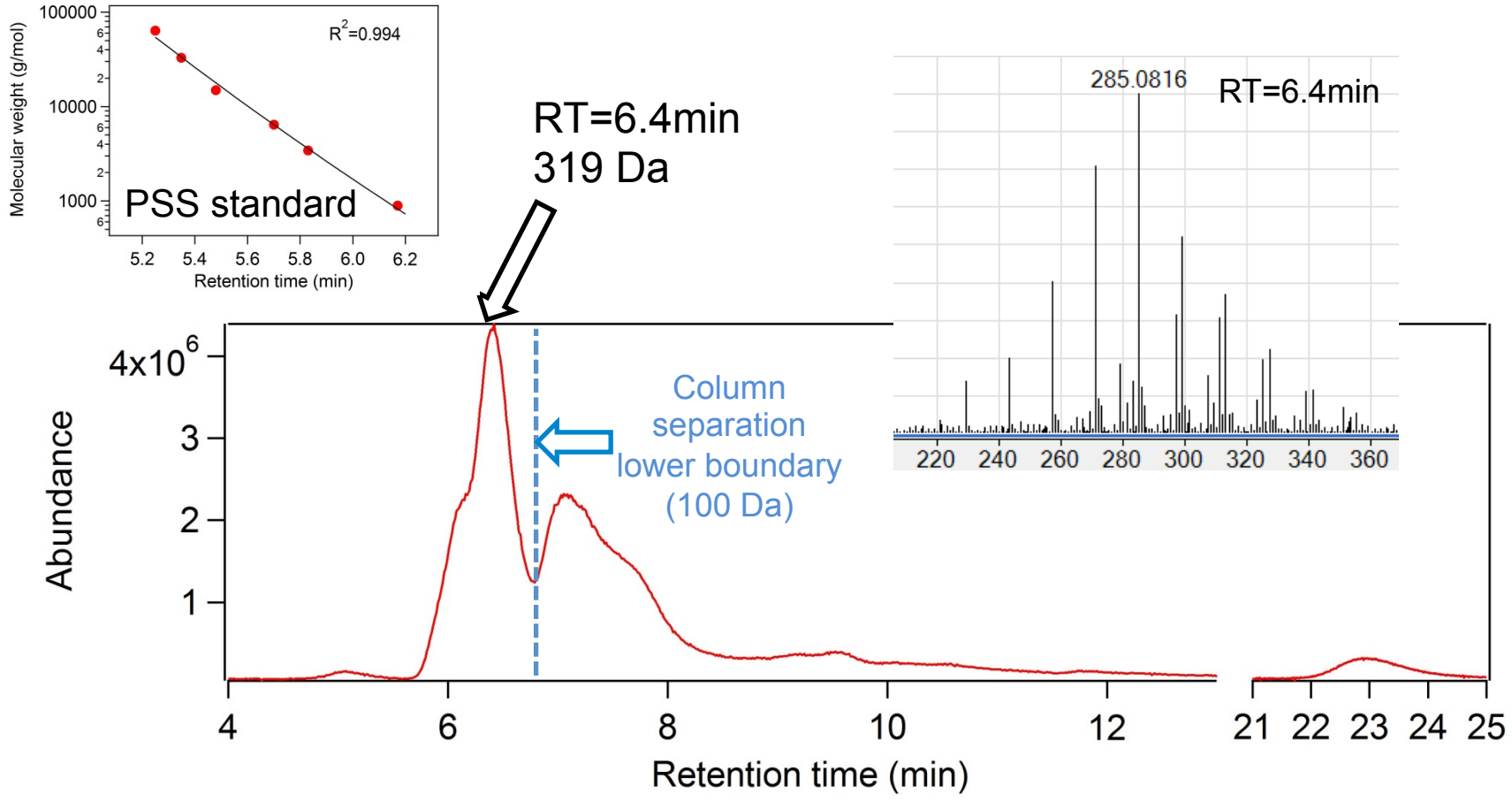
AI≥0.67 (Condensed aromatic structure)

AI with van Krevelen diagram

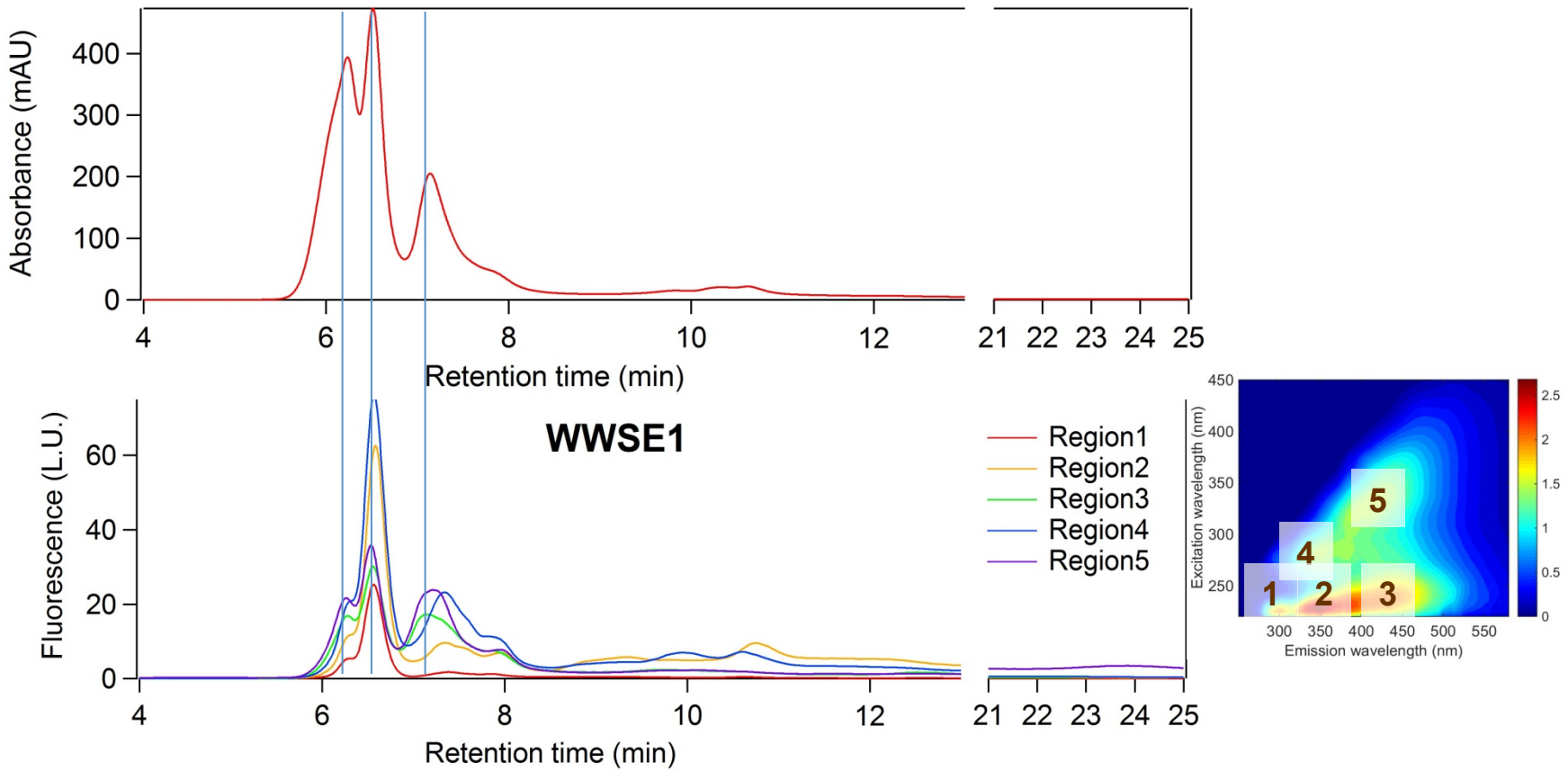
- EfOM contains aromatic compounds (AI>0.5)



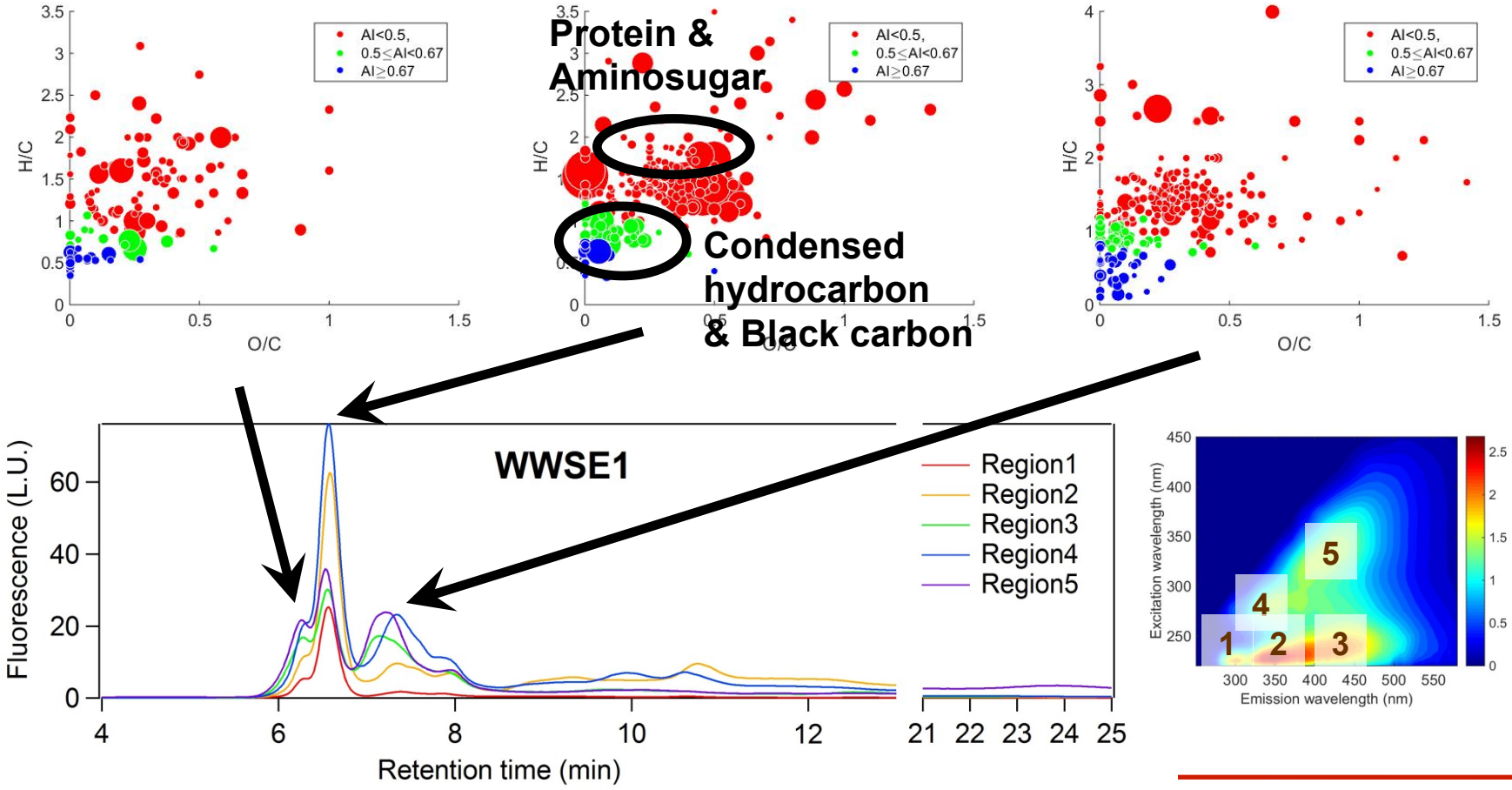
Molecular weight standards



UV254 and FL with SEC

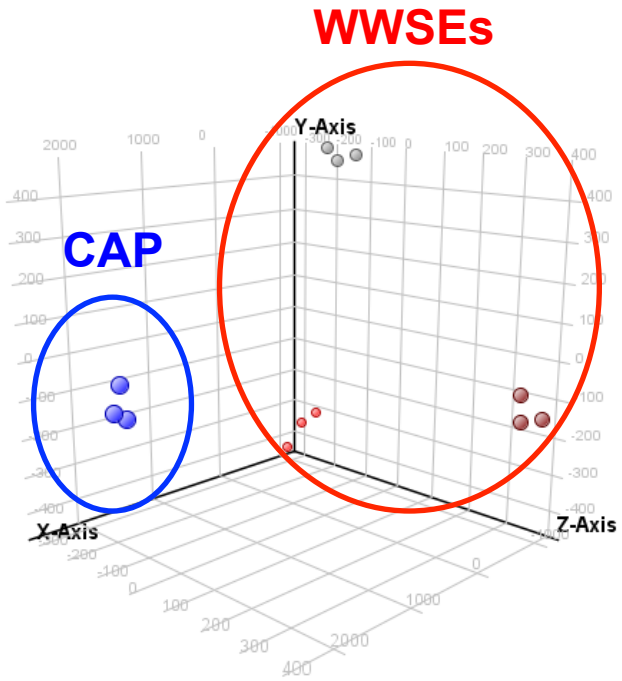


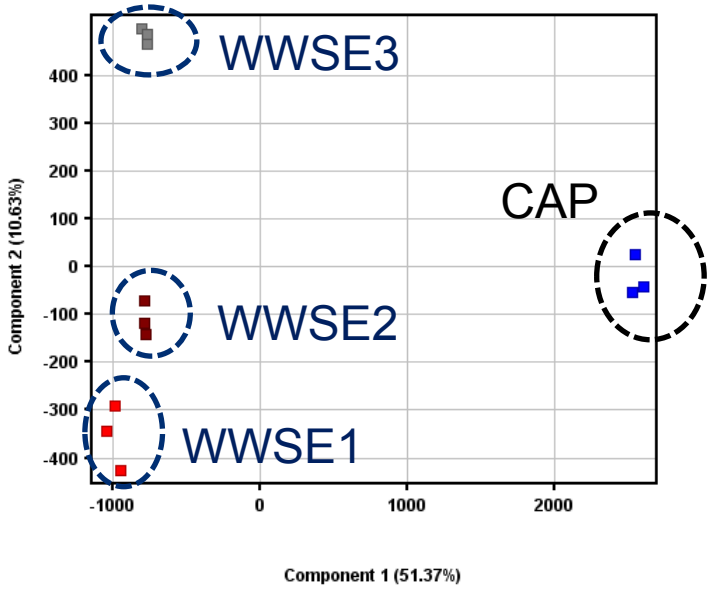
van Krevelen diagrams at different RTs



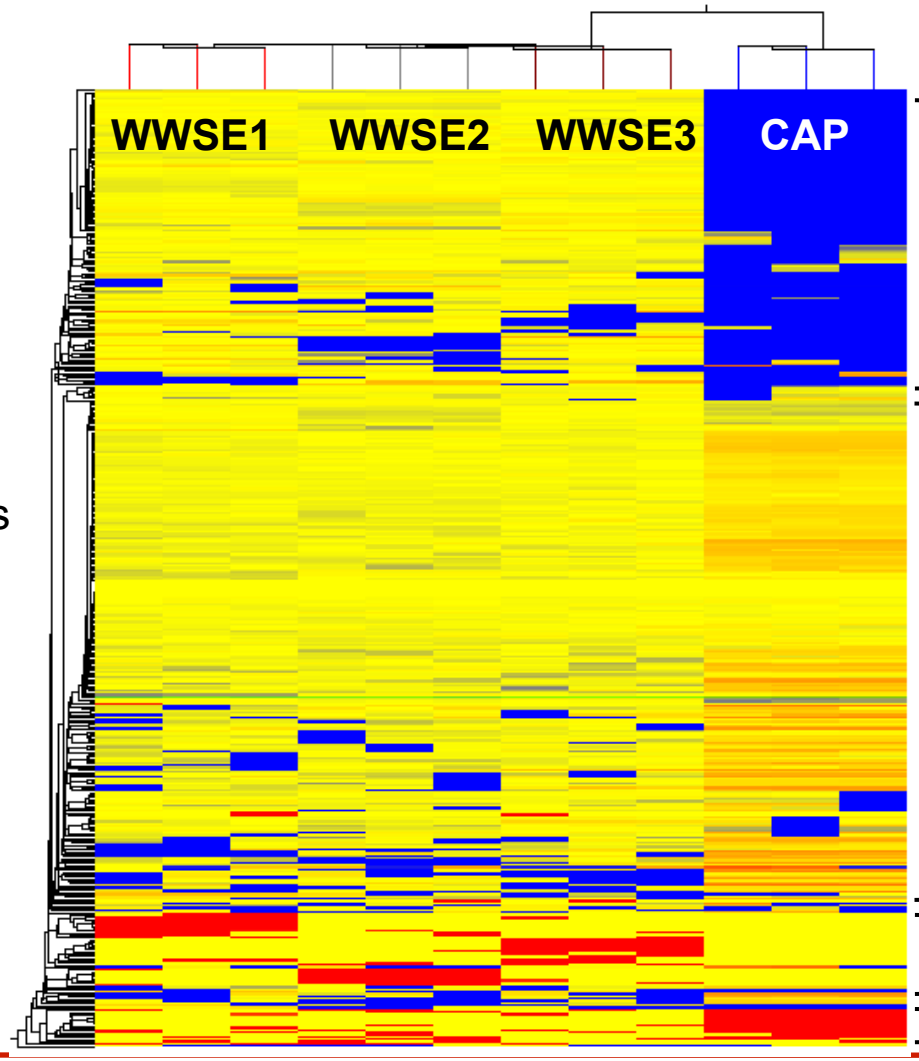
Principle component analysis

- Statistical analyses including PCA and clustering were conducted using Agilent Mass Profiler Professional (MPP) software.
- WWSE1 and WWSE2 are statistically analogous.
- CAP water has different variance from the three WW effluents.





Clustering & Heatmap



Color by normalized abundance

-11.1 -0 11.1

Ions

Triplicates

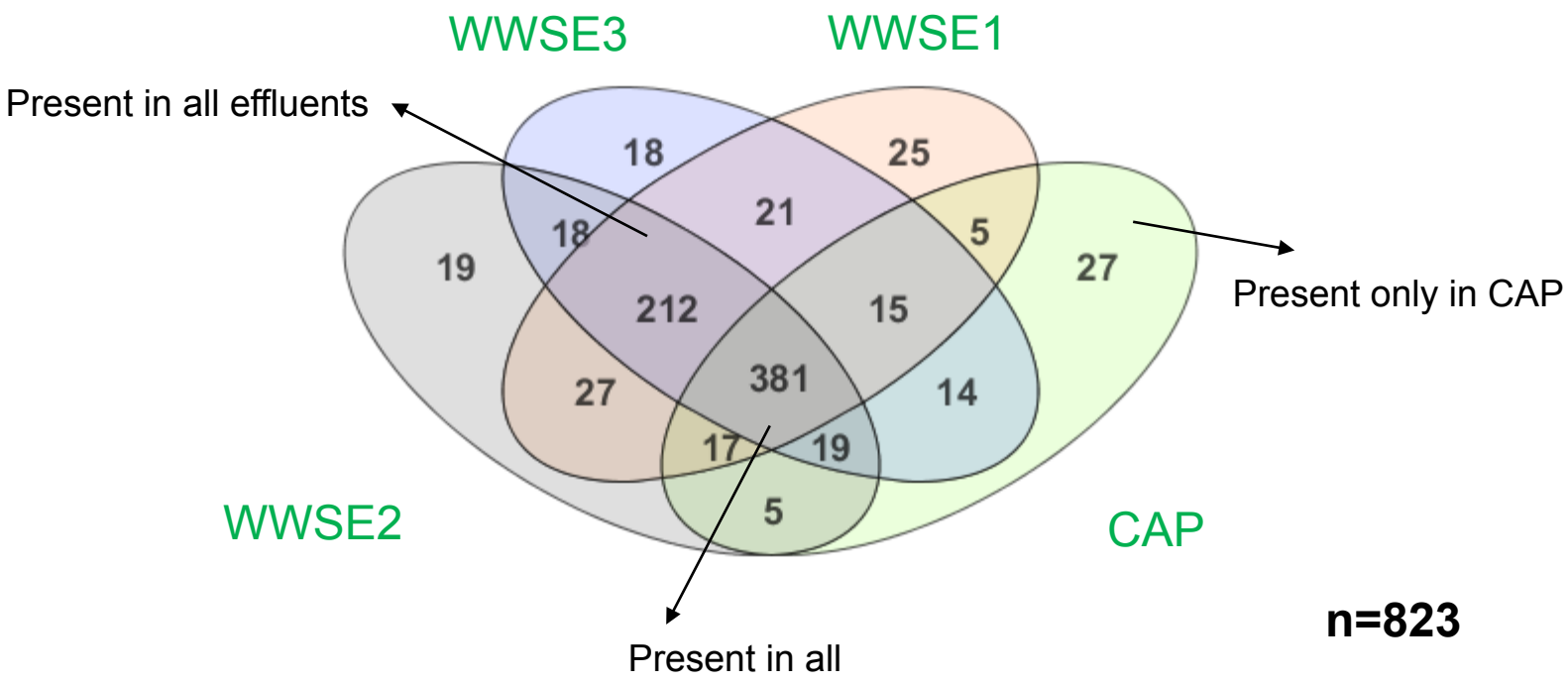
Commonly present in effluents, but not in CAP water

Commonly present in all waters with similar abundance

Highest abundance in the effluents

Highest abundance in CAP water

Venn Diagram



n=823

Generate Formula & Database Search

Formula generation

Elements and limits

Element	Minimum	Maximum
C	3	60
H	0	120
O	0	30
N	0	30
S	0	3
P	0	3
Cl	0	3
Br	0	3
I	0	3

Contribution to overall score

Mass score	100.00
Isotope abundance score	60.00
Isotope spacing score	50.00
Retention time score	100.00

Expected data variation

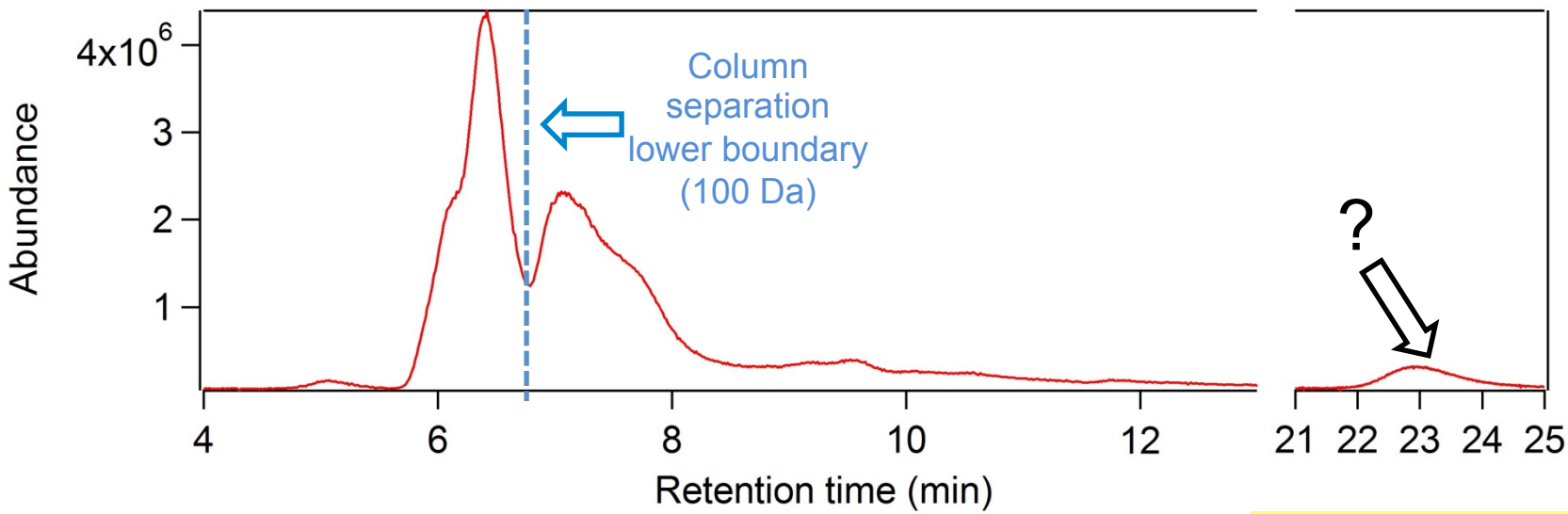
MS mass:	2.0	mDa	+	5.6	ppm
MS isotope abundance:				7.5	%
MS/MS mass:	5.0	mDa	+	7.5	ppm
Retention time:				0.115	min

Overall score cutoff: 50

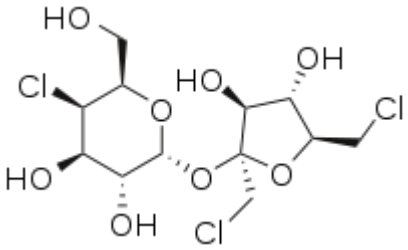
Database Search:

- Forensic/Toxicology (ForTox) accurate mass database
- METLIN metabolite accurate mass database

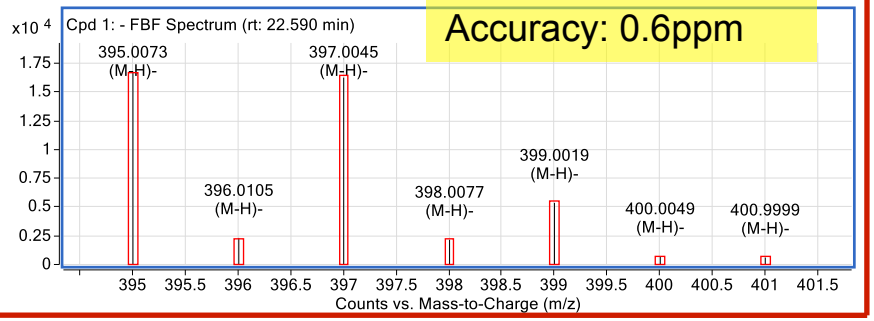
Compound identification using database search



Sucralose

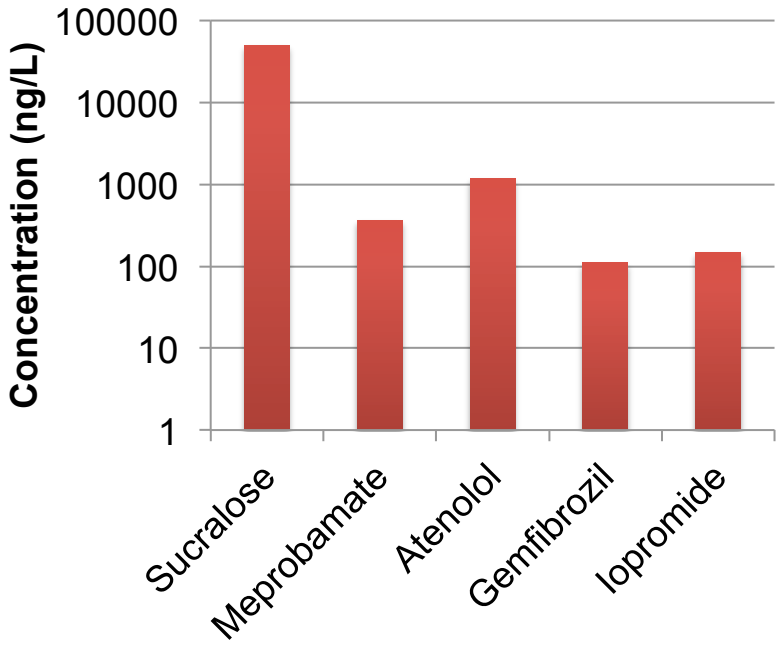


Match Score: 99.8
Accuracy: 0.6ppm



Compound identification using database search

Target analysis

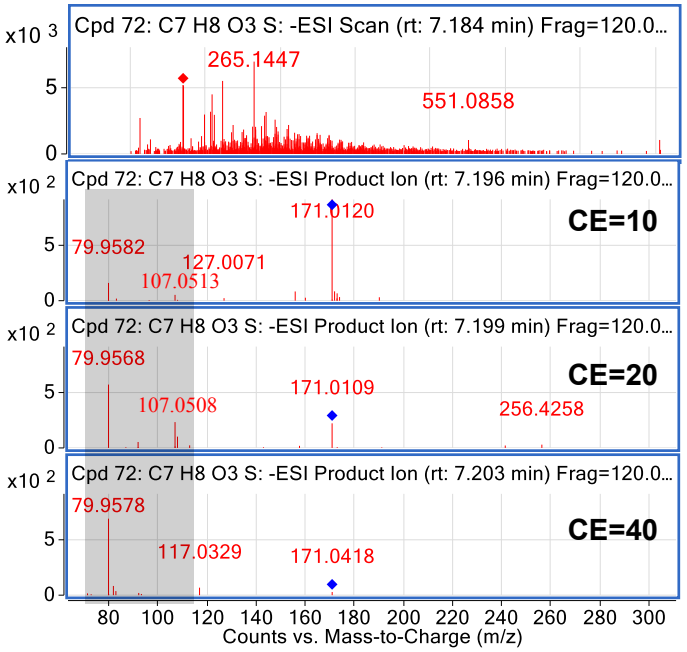


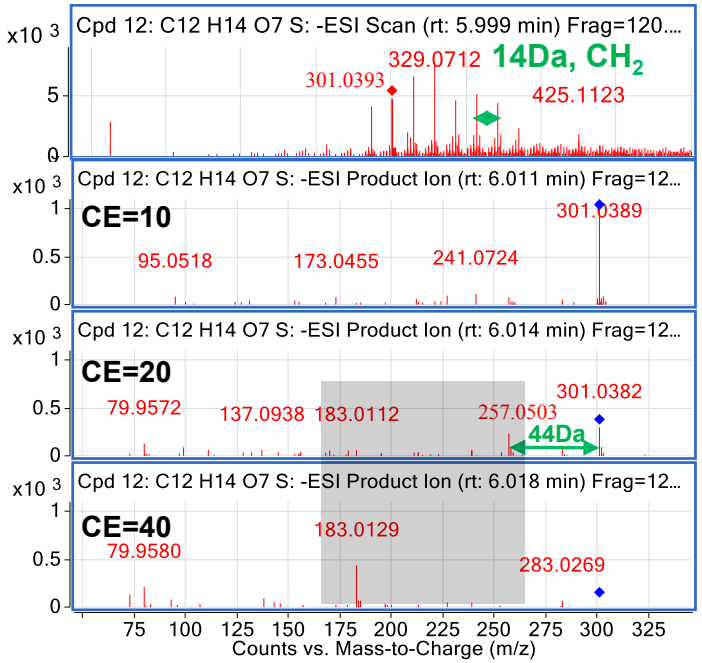
Database Search (non-target screening)

Name	Formula	Mass	Score
Amifloxacin	C16 H19 F N4 O3	334.144	99.91
Sucralose	C12 H19 Cl3 O8	396.0145	99.80
Meprobamate	C9 H18 N2 O4	218.1265	99.75
Iomeprol	C17 H22 I3 N3 O8	776.8537	98.71
Difenamizole	C20 H22 N4 O	334.1788	97.92
Atractyligenin	C19 H28 O4	320.1982	97.85
Hydrocortisone aceponate	C26 H36 O7	460.247	97.72
Atenolol	C14 H22 N2 O3	266.1623	95.60
Protiofate	C12 H16 O6 S	288.068	88.87
Nitrophenol, 4-	C6 H5 N O3	139.027	87.71
Gabapentin	C9 H17 N O2	171.1261	86.79
TBPA / Tributylphosphate	C12 H27 O4 P	266.1646	86.41
Gemfibrozil	C15 H22 O3	250.1571	85.93
Cymoxanil (Curzate)	C7 H10 N4 O3	198.0755	85.77
Embelin	C17 H26 O4	294.1836	85.28
Hydroxystilbamidine	C16 H16 N4 O	280.1319	84.56
Hydroxystilbamidine	C16 H16 N4 O	280.132	84.43
Meralluride- degradation product	C9 H16 N2 O5	232.1061	84.34
Renanolone	C20 H30 O4	334.2146	83.81
Nitracrine	C18 H20 N4 O2	324.1589	83.77
Hexyl hydroxybenzoate	C13 H18 O3	222.1256	83.62
Zearalenone	C18 H22 O5	318.1475	83.09
Iopromide	C18 H24 I3 N3 O8	790.8692	82.72

Find by Auto MS/MS

WWSE1 effluent: 147 ions were generated with MS/MS spectrum

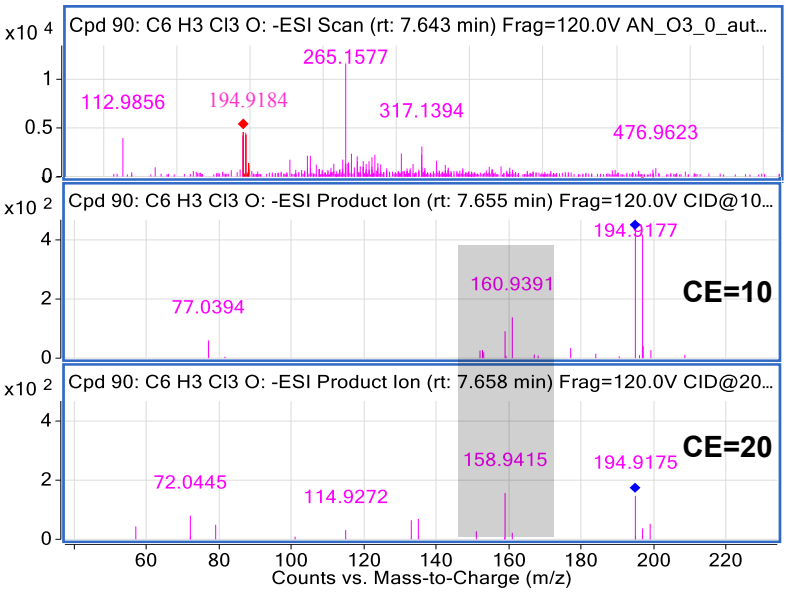


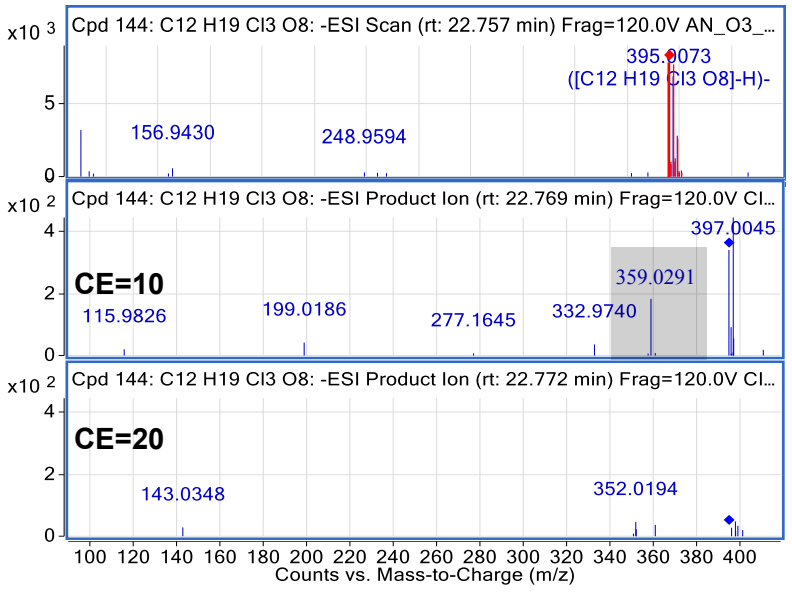


- Two product ions: 79.9582, 107.0513
- MFG: $C_7H_8O_3S$, match score: 97.1
- *p*-Toluenesulfonic acid?

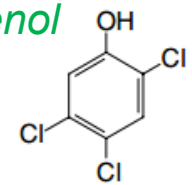
- Two product ions: 257.0503, 183.0129
- Δ=43.99 Da, neutral loss, CO_2

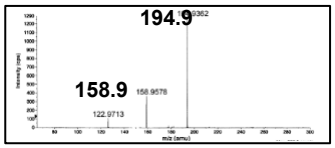
Find by Auto MS/MS





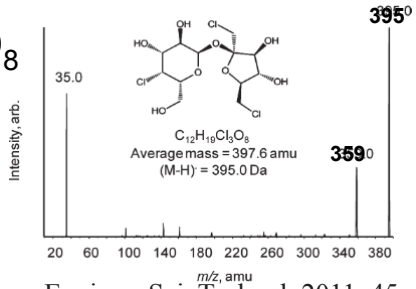
- Product ion: 158.9415
- MFG: $C_6H_3Cl_3O$, match score: 91
- *Triclophenol*





Chromatographia 2004, 59

- Sucralose, $C_{12}H_{19}Cl_3O_8$
- Product ion: 359.0291



Environ. Sci. Technol. 2011, 45

Summary

Size separation

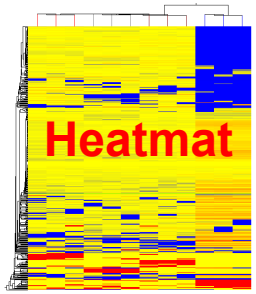
SEC

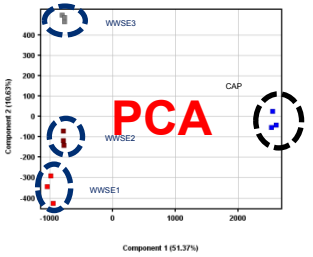
Accurate mass

QTOF-MS

Non-selective detectors (bulk properties)

DAD/FLD





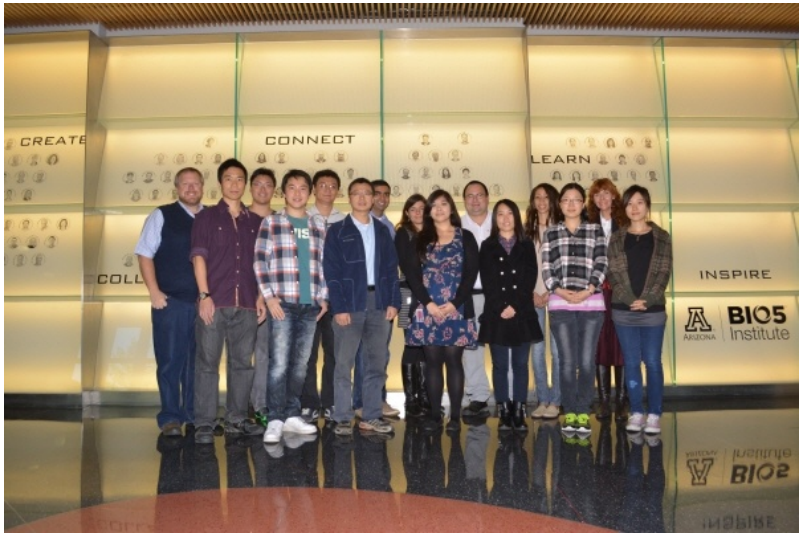
CONCLUSIONS

Conclusions

- QTOF-MS allows identification of elemental composition of EfOM.
- Hyphenation of SEC with DAD/FLD with QTOF-MS
- Accurate mass obtained by QTOF-MS enables non-target screening of TOrCs based on database search.
- Statistical analyses including PCA and clustering are able to provide fingerprints of different origin of organic matter (i.e., NOM and EfOM)

Acknowledgement

- Snyder Research group (University of Arizona)
  - Dr. Shane A. Snyder
  - Dr. Ai Jia



Agilent Technologies

NEMC

Q&A

Questions???