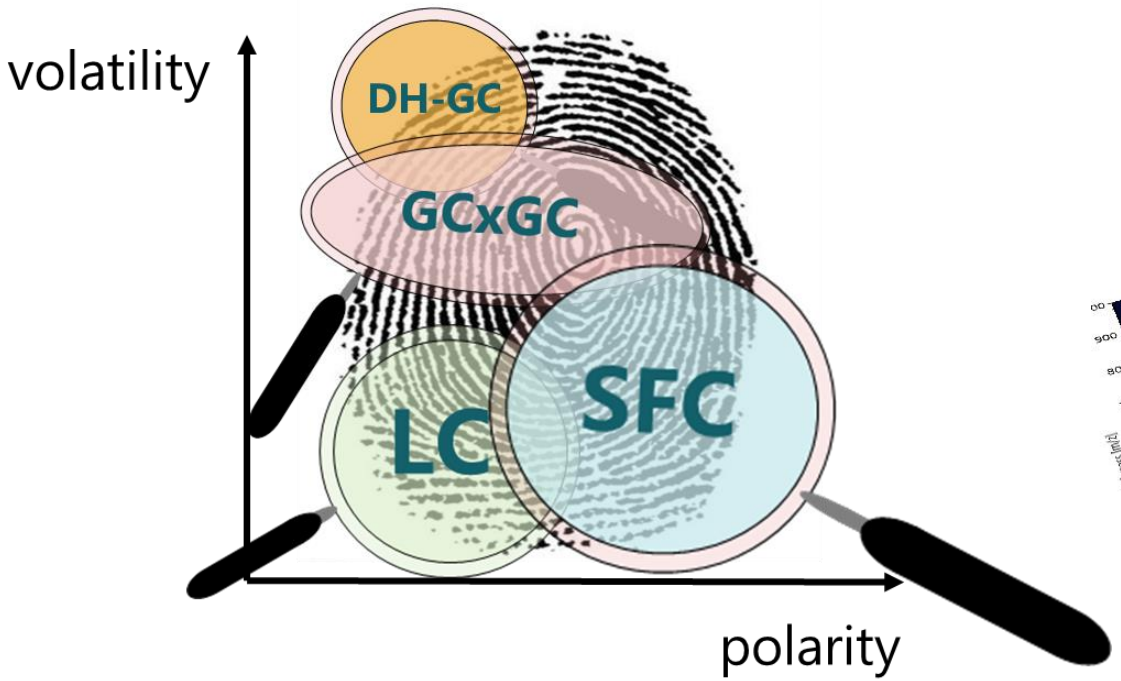
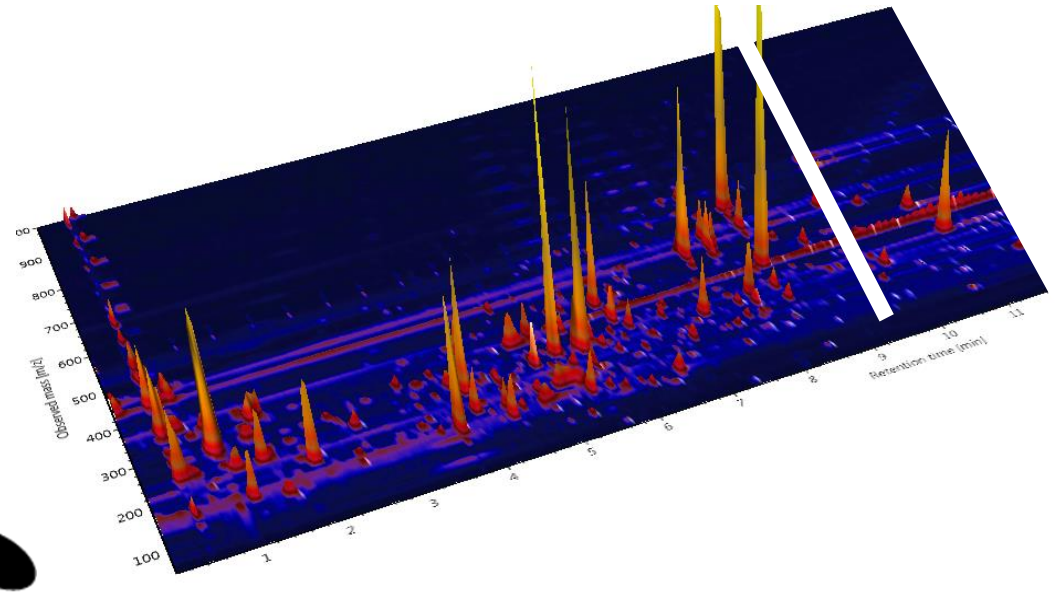
UNIVERSITY OF COPENHAGEN

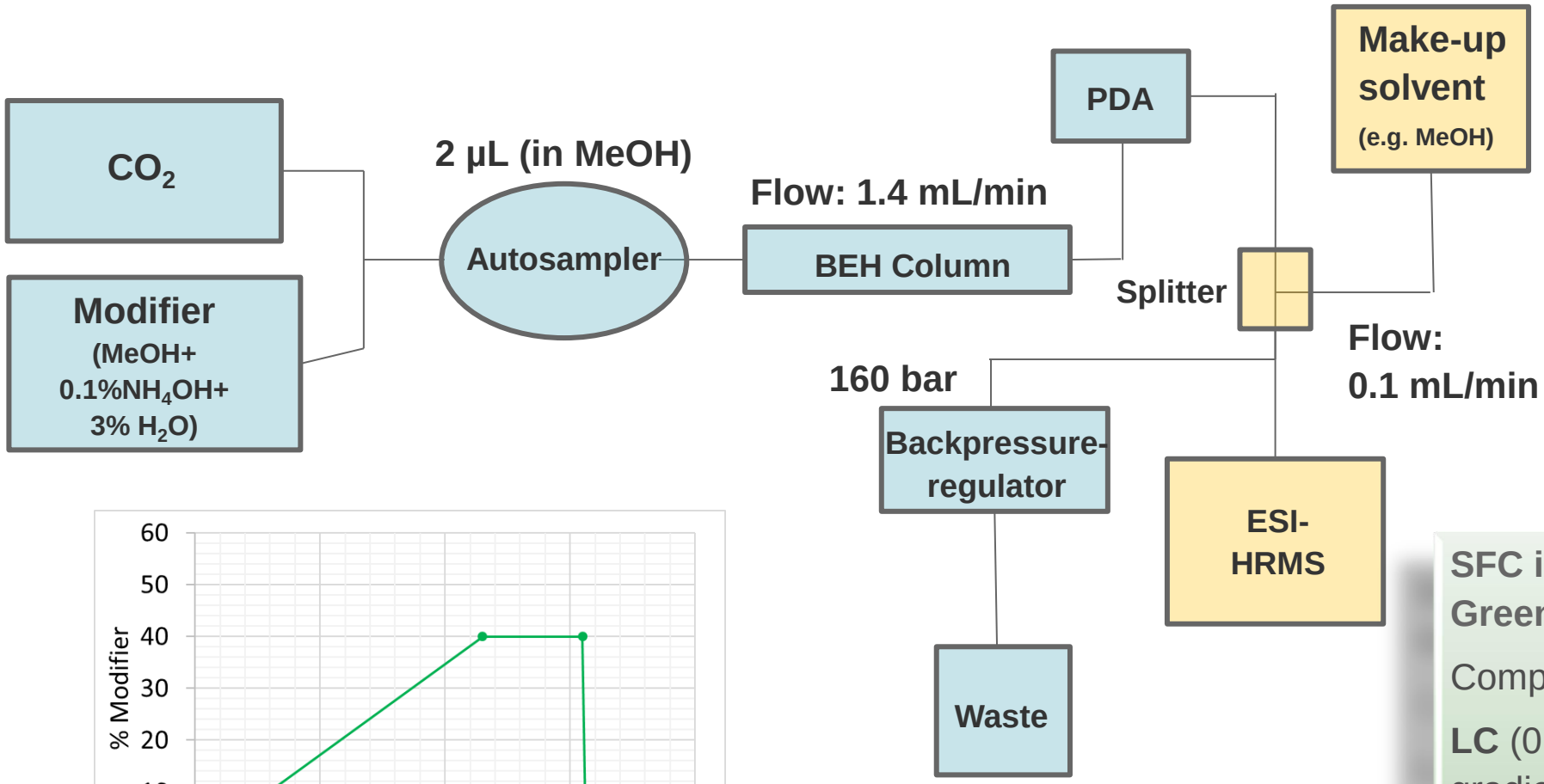
SFC: Expanding Polarity in Environmental Non-Target Screening





Selina Tisler, University of Copenhagen

Set-up of the SFC for the analysis of polar contaminants



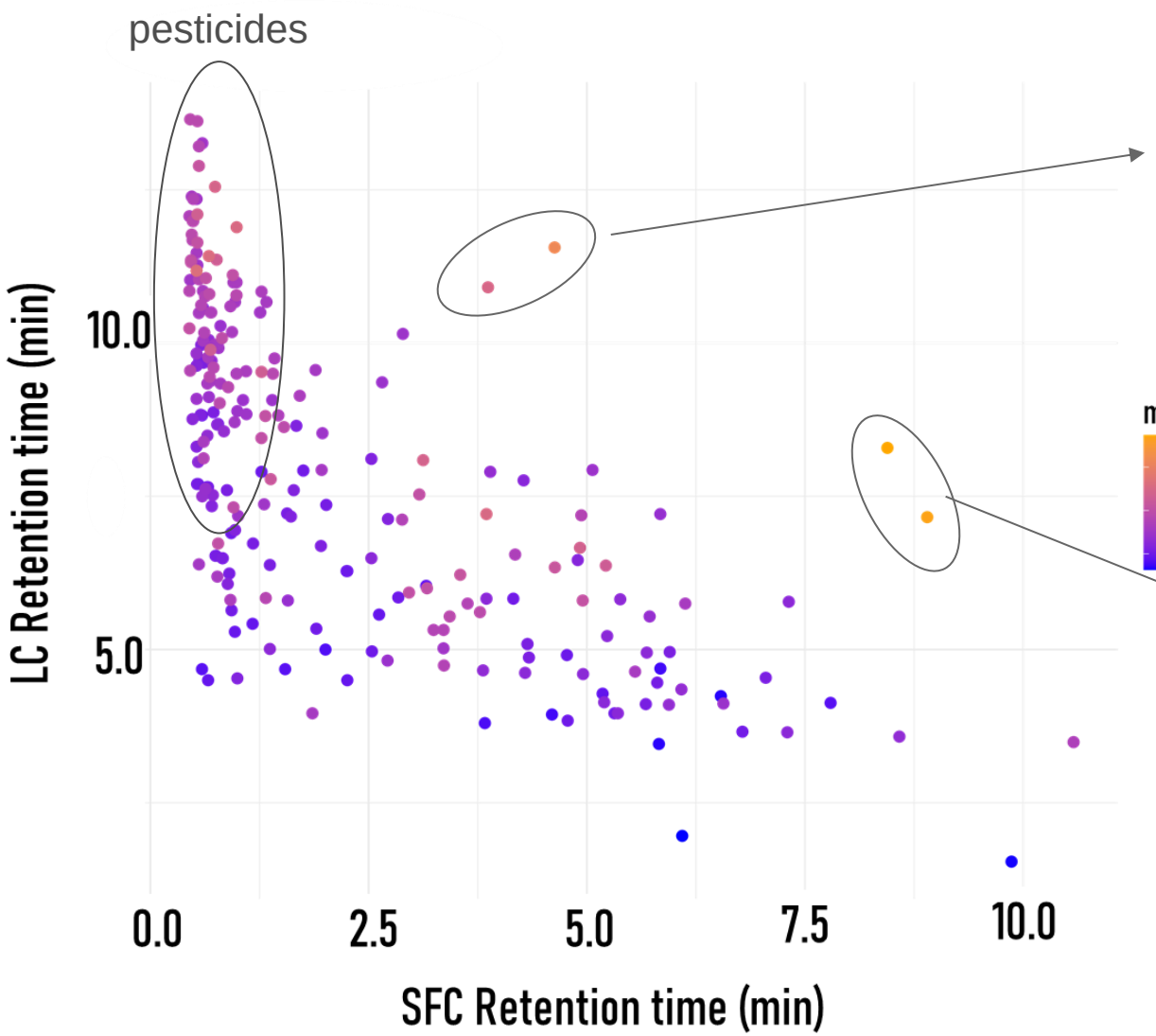
SFC in Environmental Analysis-Green Technology?

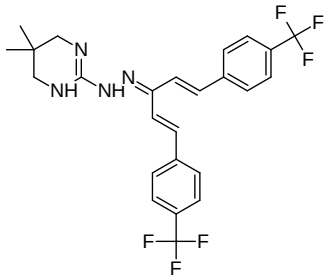
Comparison:

LC (0.3 ml/min, 24 min run-time with gradient → **3.6 mL ACN**

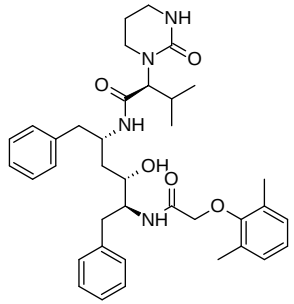
SFC (1.4 ml/min, plus make-up solvent 0.1 ml/min) → **7.2 mL MeOH**

LC/SFC Retention Time comparison for 218 Standards

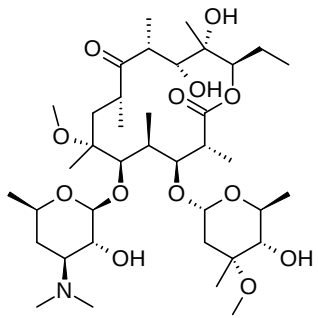




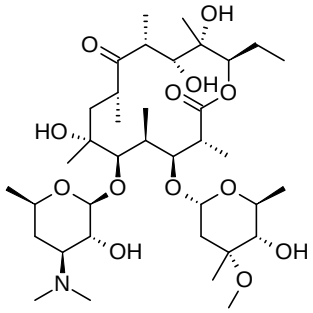
Hydramethylnon



Lopinavir



Clarithromycin



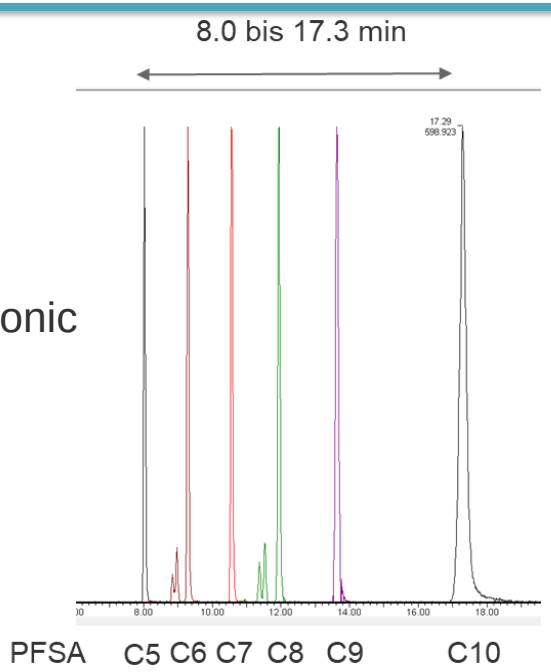
Erythromycin

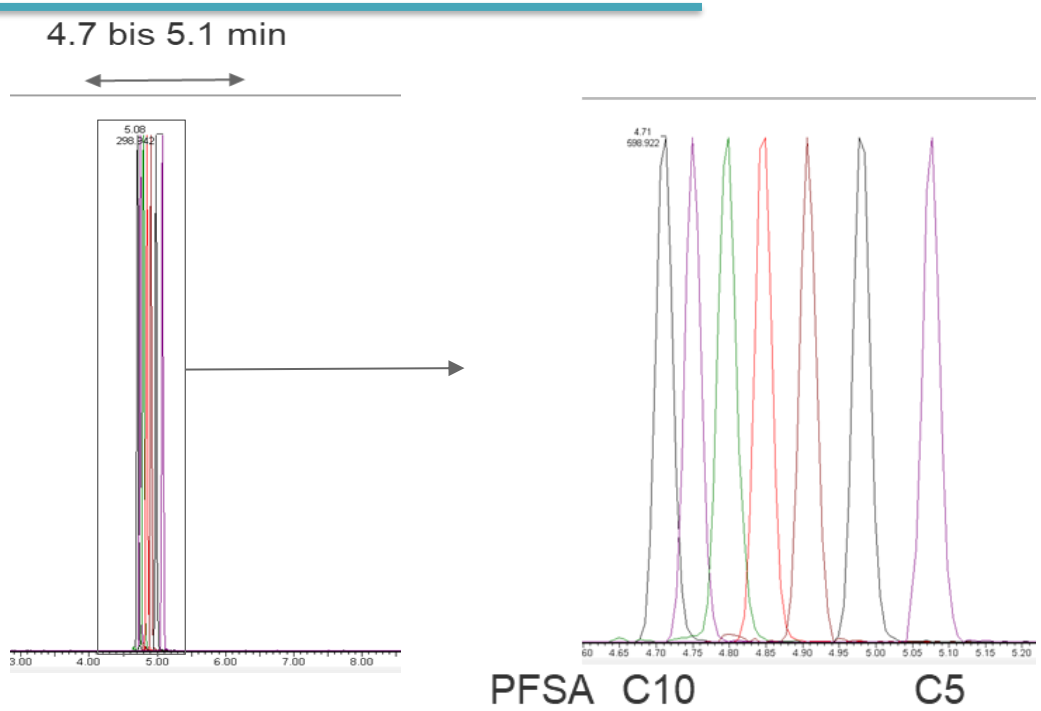
Polarity trend for SFC as well-

However not the only driving factor

Example PFAS for LC/SFC Retention Time comparison

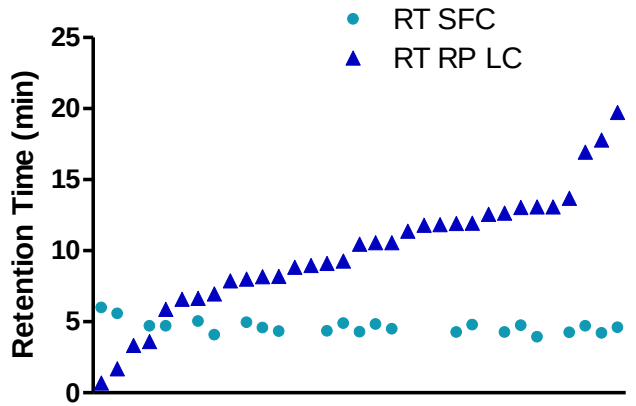
Perfluorosulfonic acids





Comparison of 33 PFAS

(chain length C1 to C13)



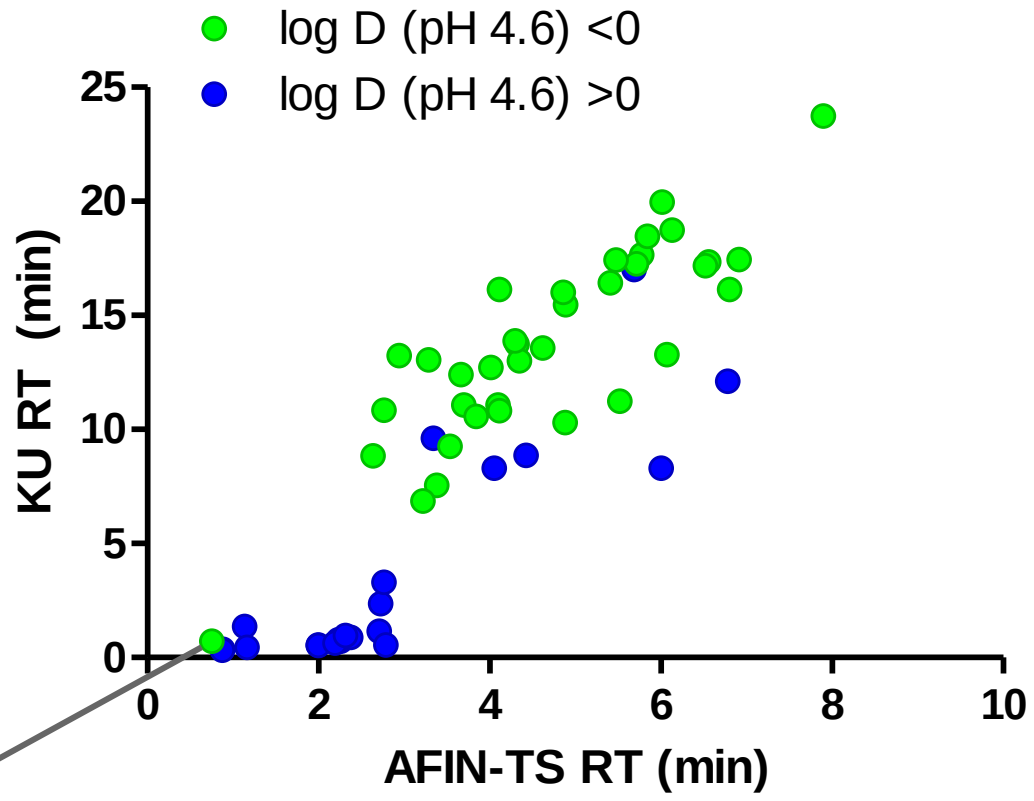
Retention time is highly influenced by functional group and less by chain length

Retention Time comparison across Laboratories

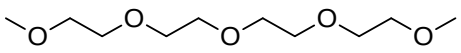
KU (Waters)

Säule: BEH (Silika)

Gradient: 10 min von 5 % zu 40 % MeOH+ 3% H2O+ 0.1 % NH_4OH



Highly polar compounds show similar retention time trends across two methods using different columns.



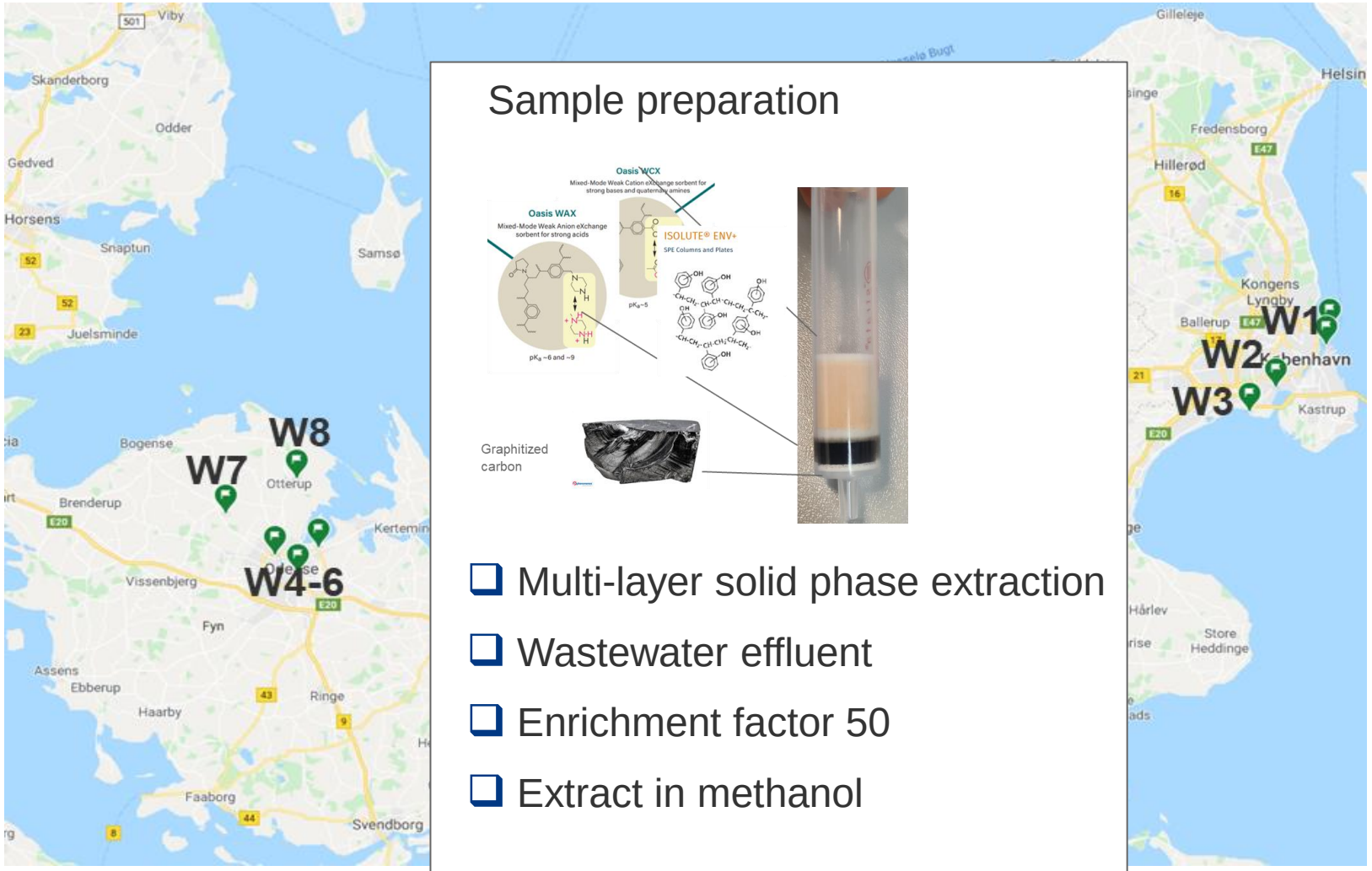
Tetraethylene glycol dimethylether

AFIN-TS (Agilent)

Säule: HILIC Zwitterionic modification (Ammonium – sulfonic acid)

Gradient: 6 min von 2 % zu 60 % MeOH+ 20 mM NH_4Ac

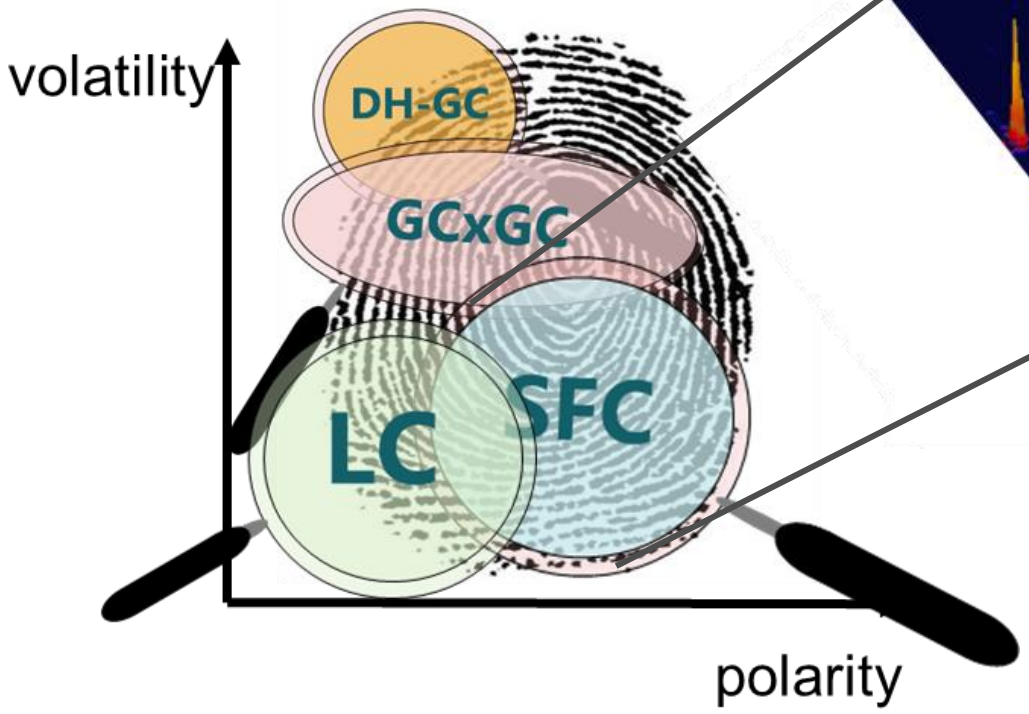
Case study: SFC for the analysis of wastewater effluent via Non-target Screening

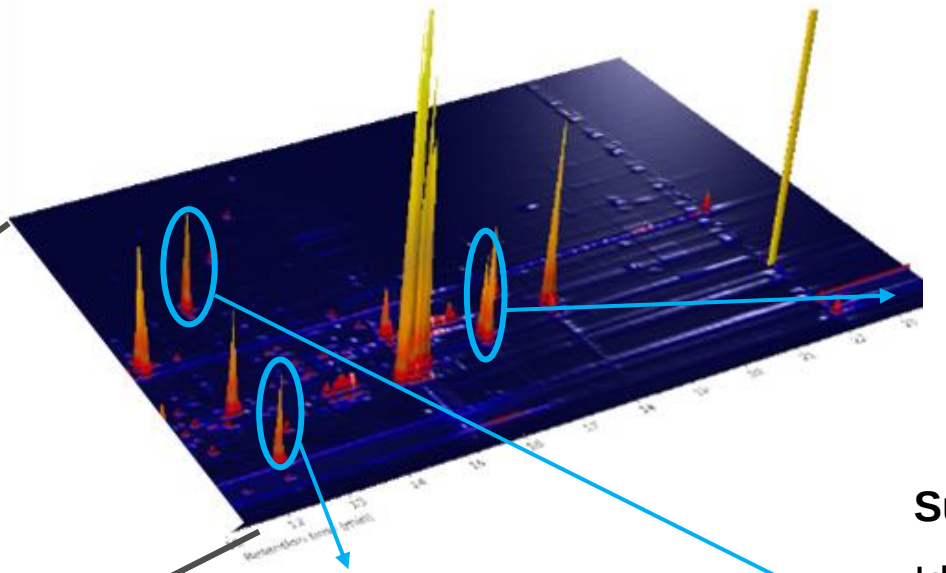


Sample preparation

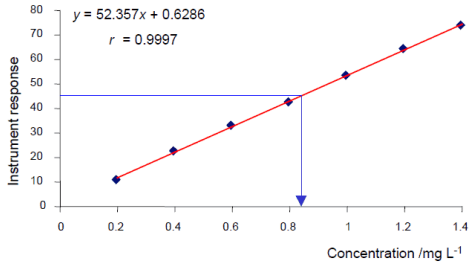
- Multi-layer solid phase extraction
- Wastewater effluent
- Enrichment factor 50
- Extract in methanol

Case study: SFC for the analysis of wastewater effluent via Non-target Screening



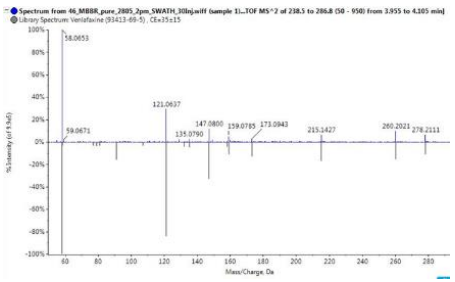


Target screening: quantification of known



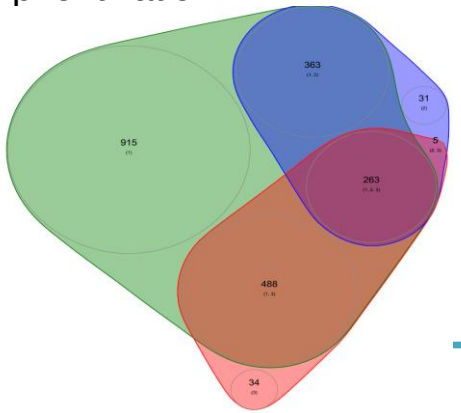
Suspect screening:

Identify compounds by comparison with databases

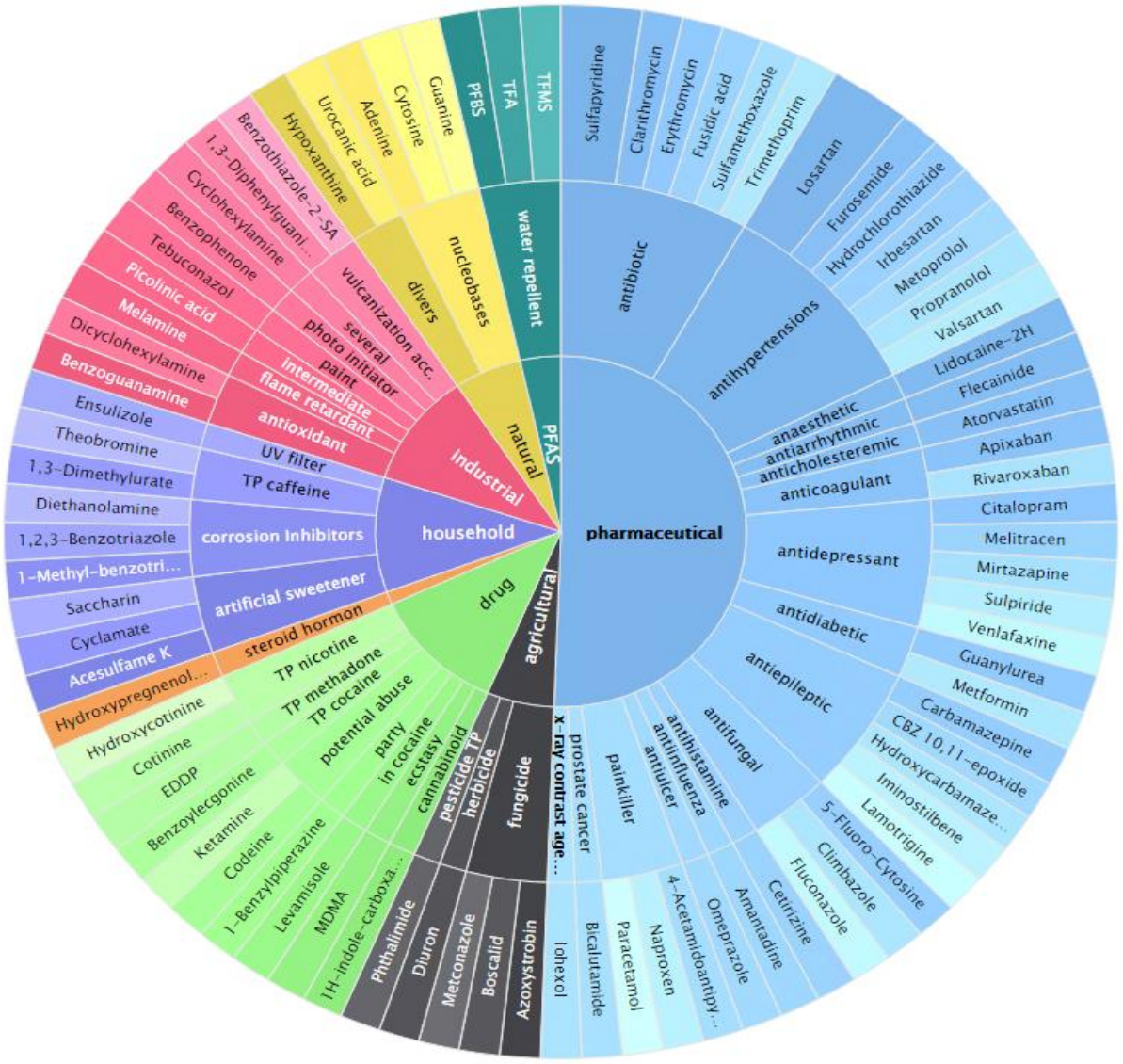


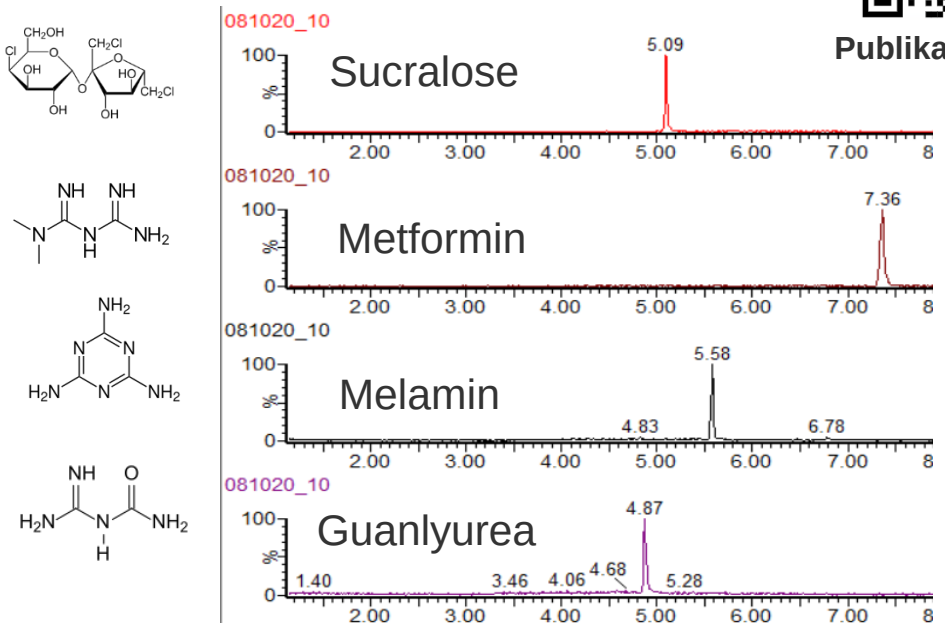
Non-target screening:

Identifying unknowns by prioritization



Suspect screening with SFC





Publikation in ES&T

- 85 compounds identified in wastewater effluent - log D (pH=7) range from -5.6 (Metformin) to 4.93 (Boscalid)
- 30 compounds not identified with C18 LC
- Compound classes similar to LC- but less pesticides and more drugs of abuse

Comparison SFC and LC (RP) HRMS Analysis of Wastewater

SFC

- BEH column
- Eluent **A**: CO_2;
- Eluent **B**: MeOH+additives
- 2 µL injection
- Make-up: 0.1 ml/min MeOH

LC

- BEH C18 column
- Eluent **A**: H2O+0.1% FA;
- Eluent **B**: ACN+0.1% FA
- 2 µL injection

Electrospray ionization with quadrupole time of flight mass spectrometer (**ESI-QTOF**)

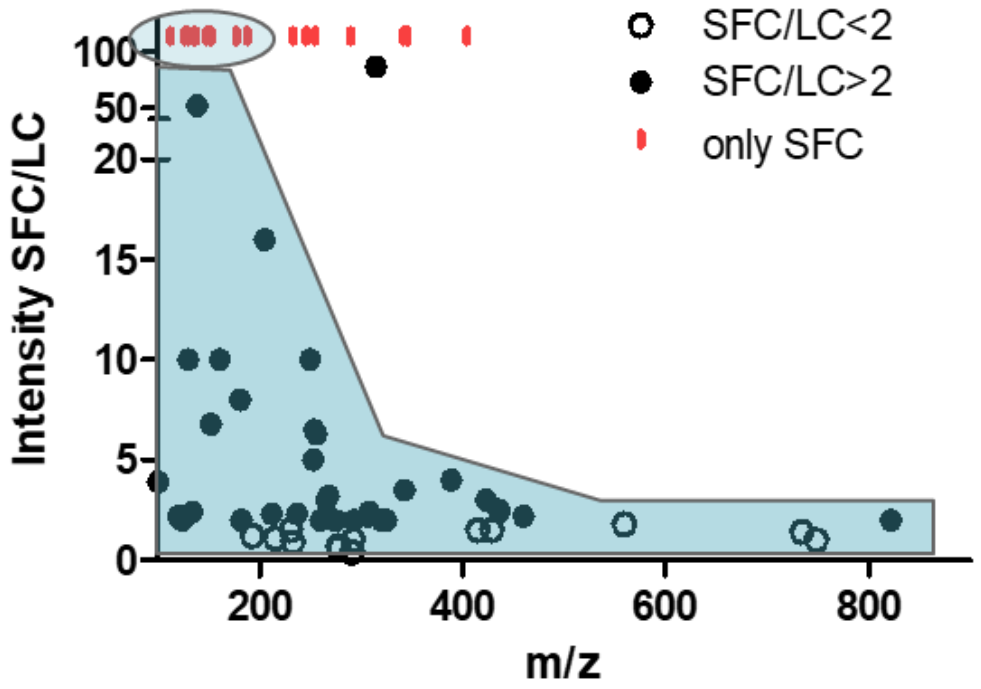
Mass range m/z 50-1000 with “standard” HRMS parameters

(system calibration parameters)

Comparison: ionization efficiency

Comparison of suspect screening compounds

- 48 compounds detected with LC and SFC
- 44 showed higher peak intensity with SFC (38 substances > Faktor 2)
- 22 further compounds detected with only SFC

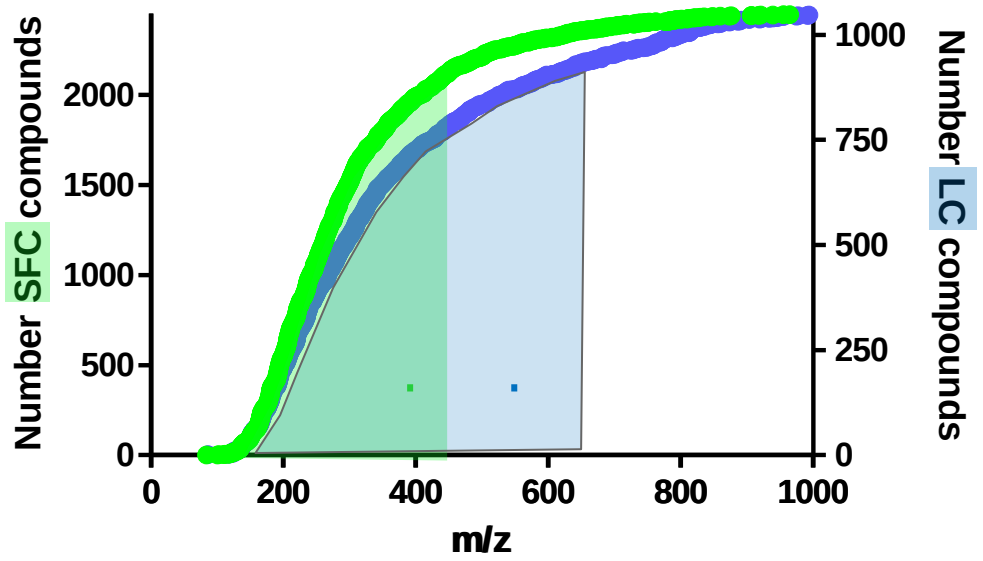


Compounds m/z> 300 showed the highest increase in response factor with SFC

Non-Target Screening: Comparison of numbers of detected compounds

Data for pooled wastewater effluent samples

→ filtered: triplicate (3 out of 3) and 50x higher as blank



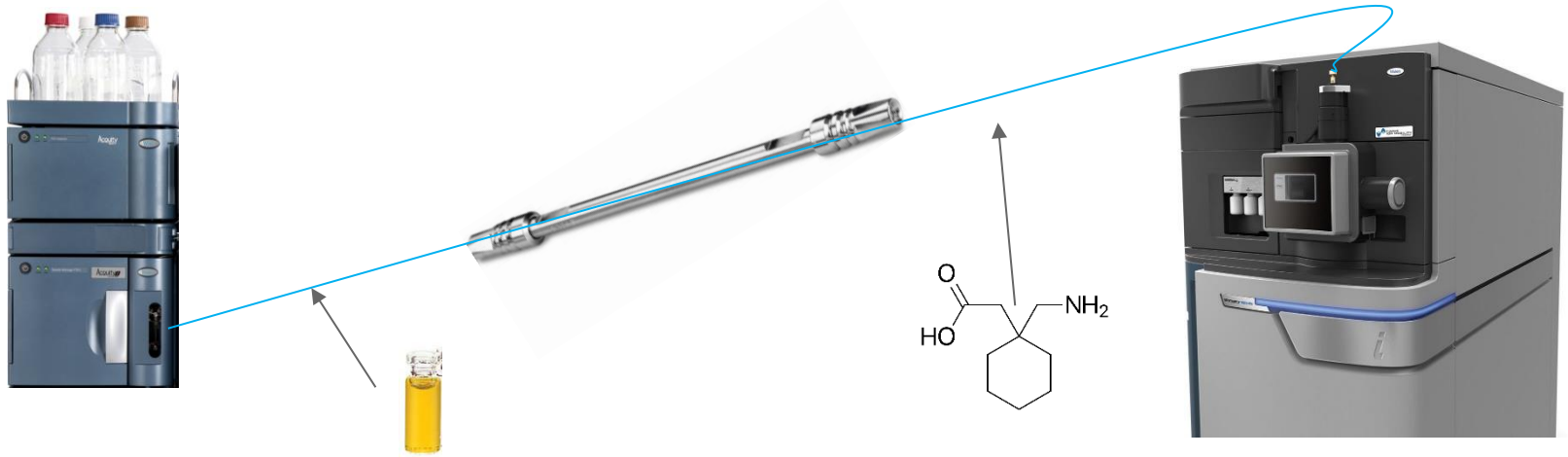
- 2300 compounds detected with SFC and 1100 compounds detected with LC
- 90 % of the compounds < m/z 483 for SFC and <m/z 655 for LC

Double as many compounds detected with LC than SFC

Smaller molecules detected with SFC

Matrix Effect evaluation by “post-column infusion”

Publikation in Analytical Chemistry

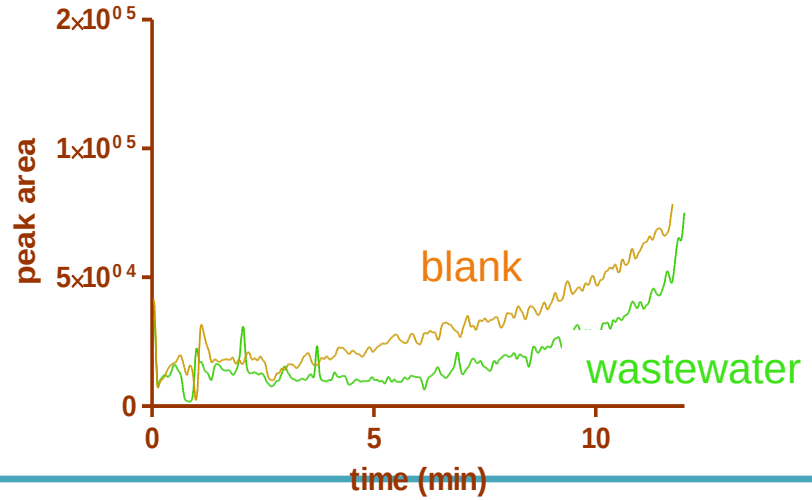


Pre-column injection:

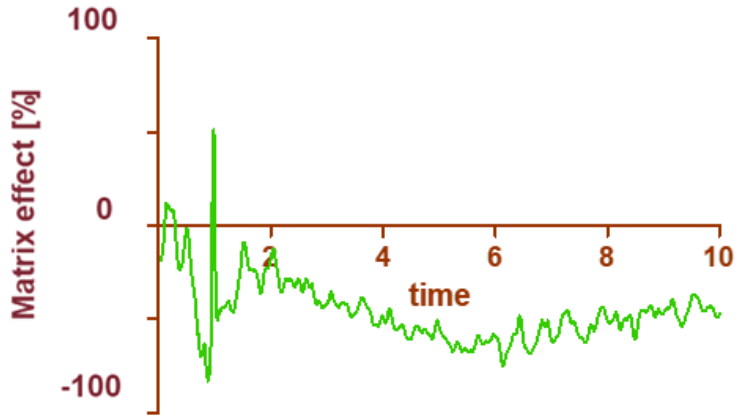
Sample (wastewater)

Post-column infusion (flow):

Specific compounds



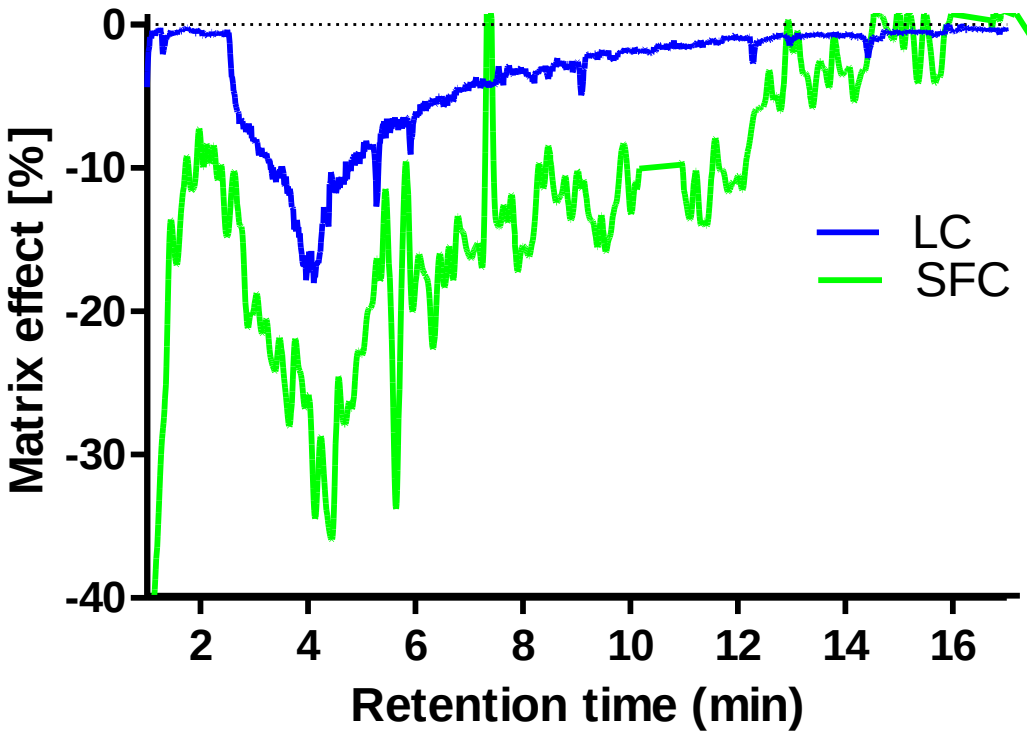
$$Matrix\ effect = \left(\frac{EIC^{j}_{matrix}}{EIC^{j}_{blank}}\right)$$



Tisler et al. (2021), Analytical Chemistry, 10.1021/acs.analchem.1c00357

Comparison matrix effect LC/SFC

Pooled wastewater effluent sample (enrichment factor 50)

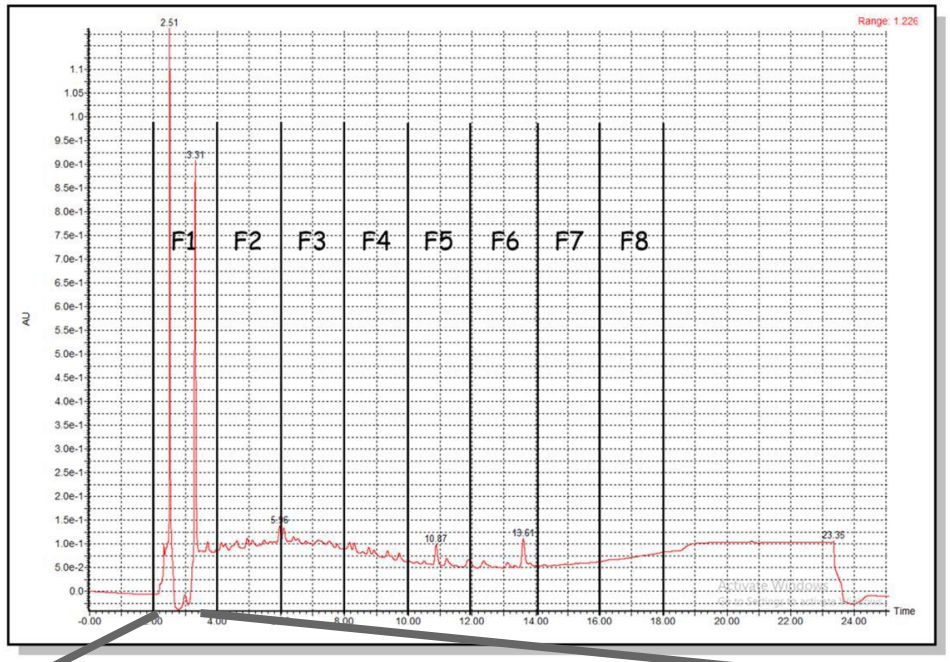


- LC matrix effect normally < -10%
- SFC matrix effect between -10 und -37 %

Better ionization→ Detection of more compounds→ higher matrix effect

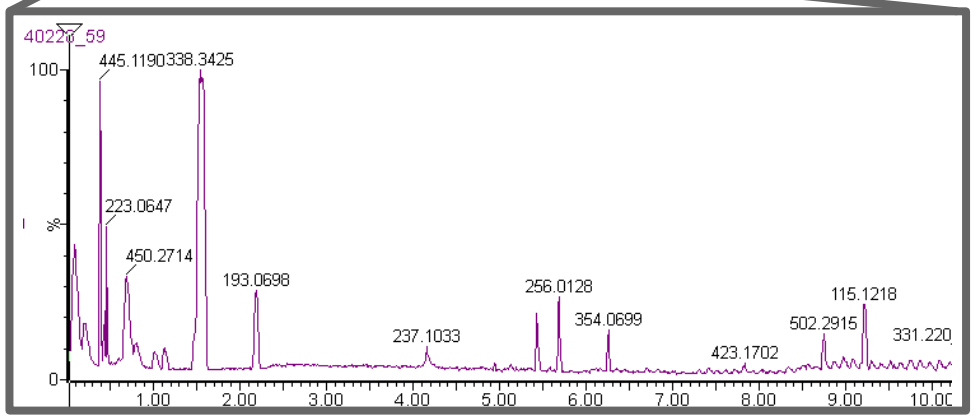
More effort to produce reliable data with SFC

Combination of RP-LC und SFC for wastewater screening



RP-LC

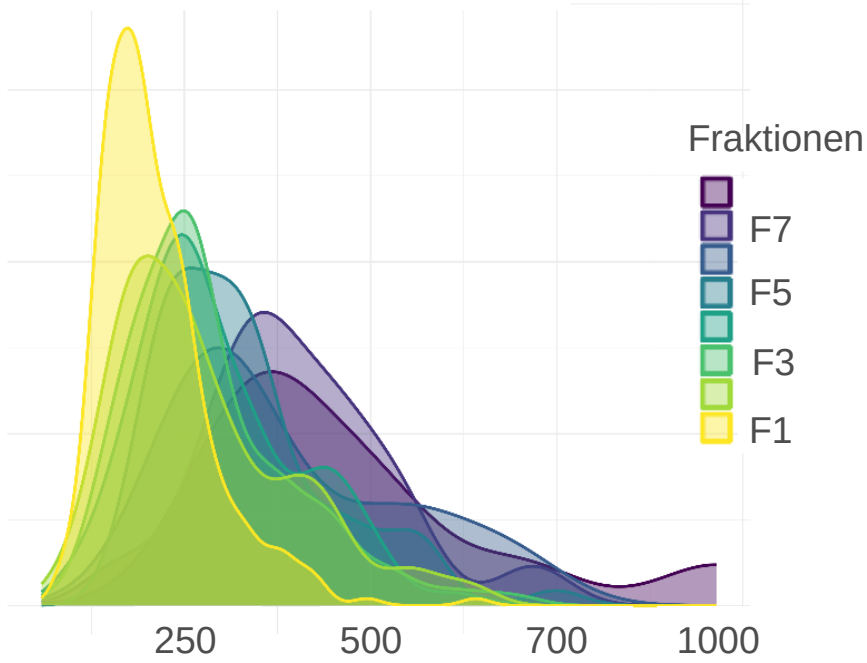
Fractionation of wastewater effluent



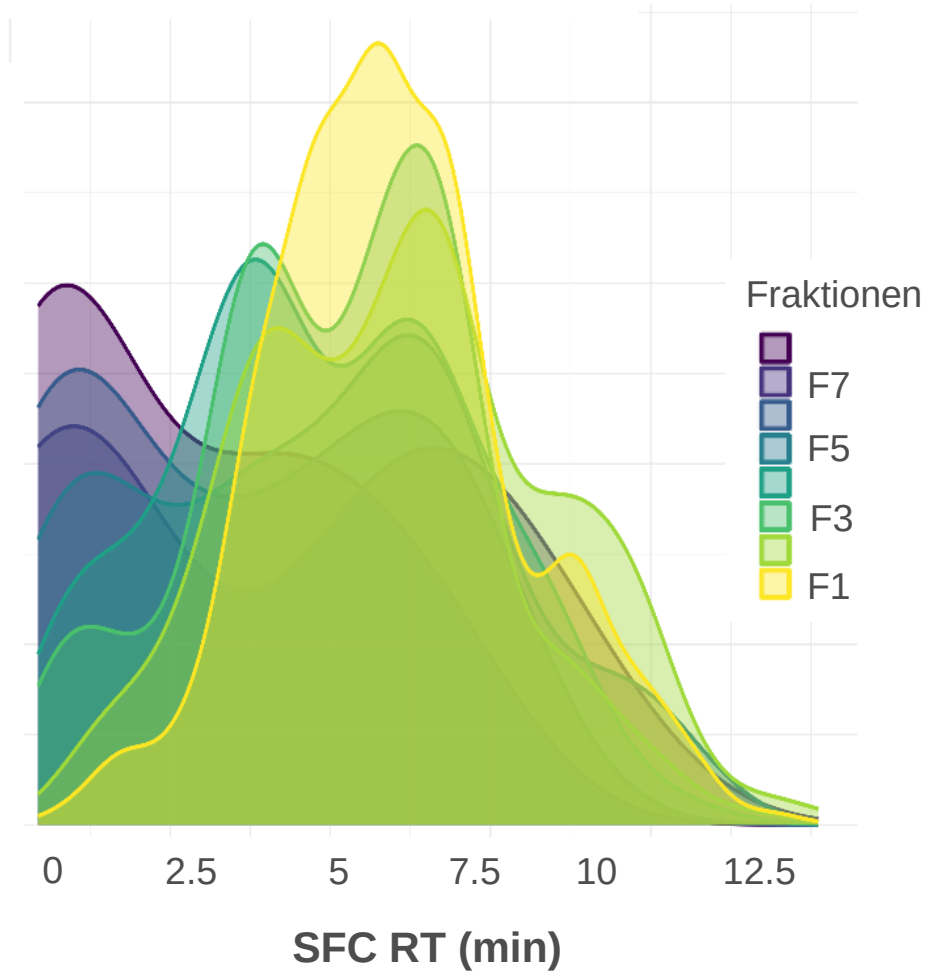
SFC

Analysis of fractions

m/z distribution of the compounds in each fraction



SFC Retention time distribution for LC fractions



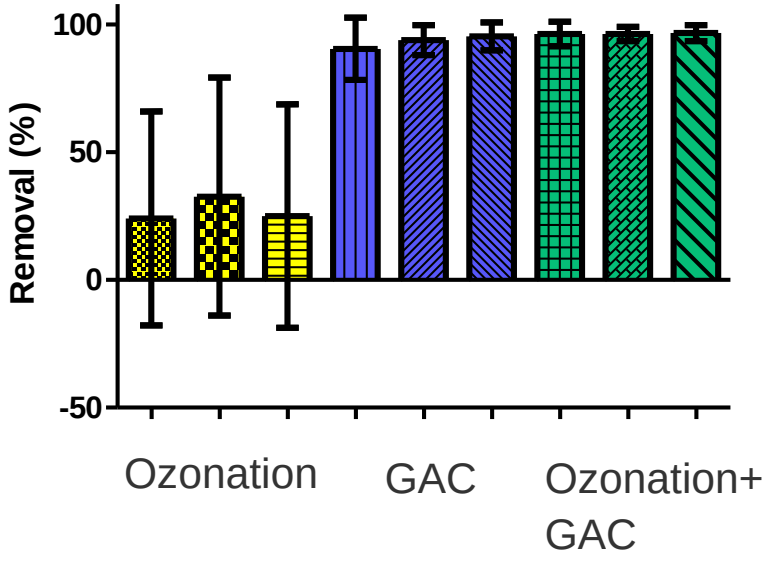
Number of detected compounds in each fraction

- 250 F1 → injection peak with LC
- 300 F2
- 200 F3
- 110 F4
- 57 F5
- <50 F6 bis F8

- Most polar compounds of the wastewater effluents are eluting between 3 and 10 min (SFC)
- Enables 'targeted non-target screening' of highly polar substances that are neglected in RP LC

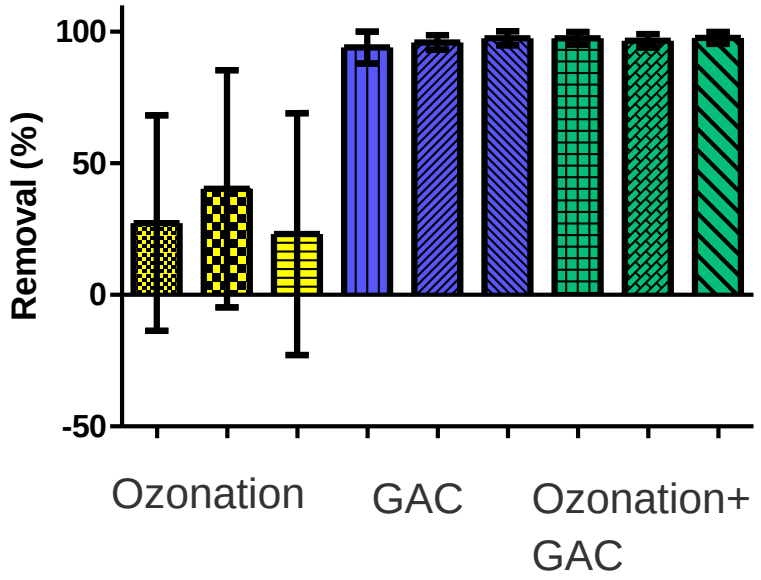
Lab-experiment: removal of contaminants in wastewater effluent

All compounds detected with SFC



- 490 compounds detected
- >90 % removed by GAC

Polar compounds (RP LC F1) detected with SFC

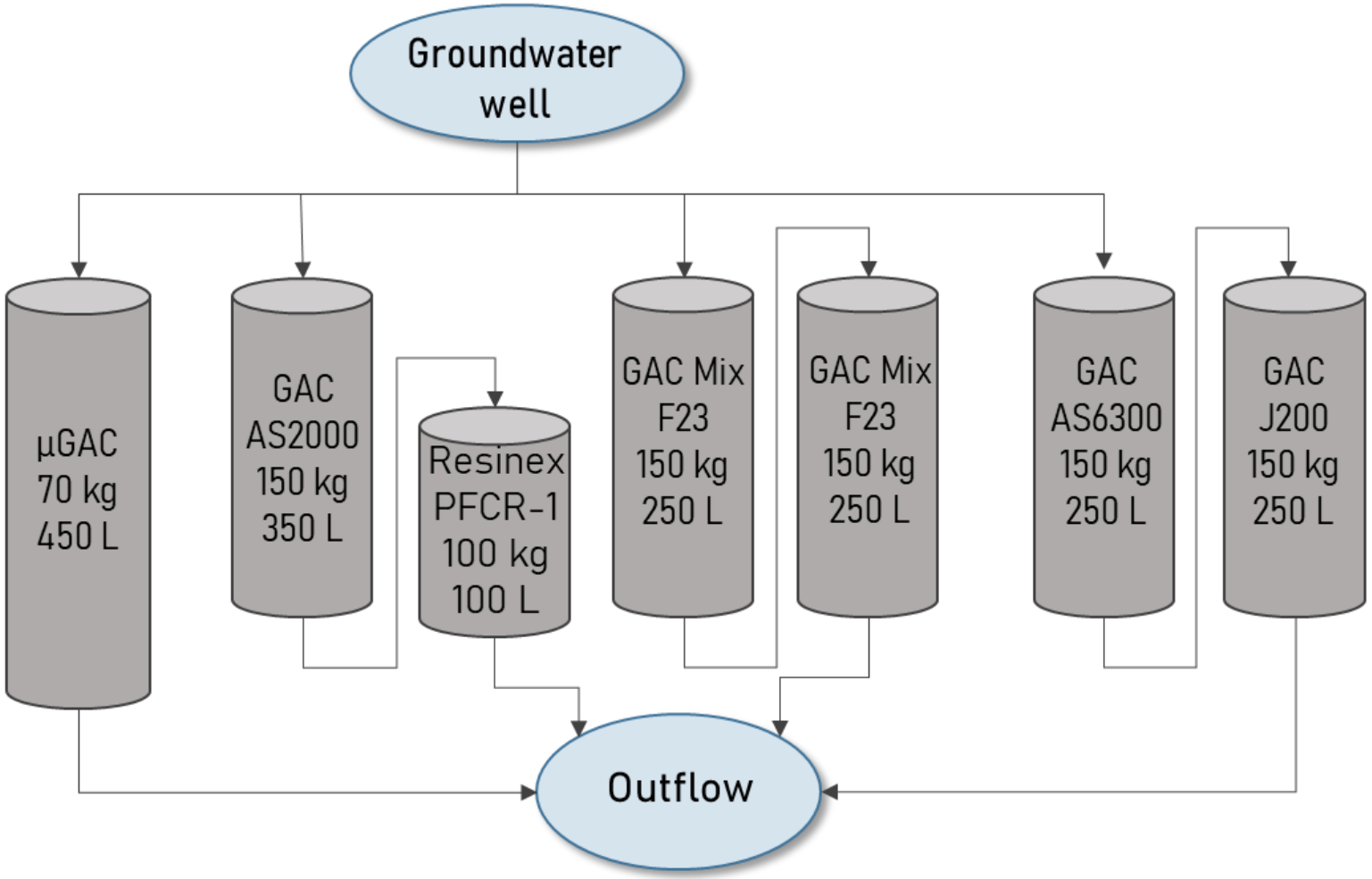


- 48 polar compounds were identified
- Average removal similar to wide polarity range

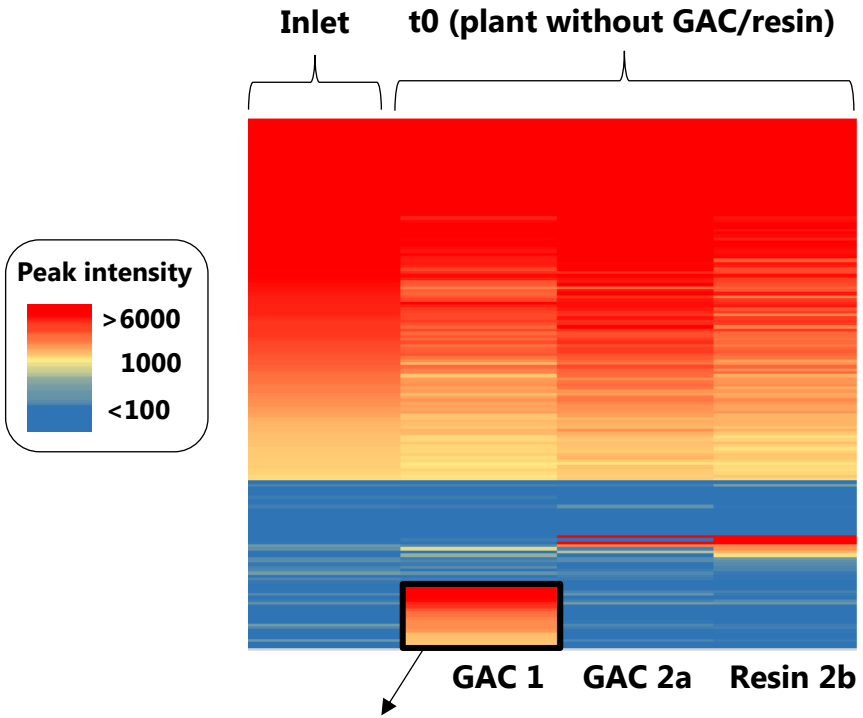
Case study: Non-target Screening Groundwater

Pilot Scale Treatment

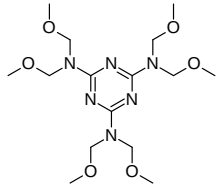
Granular Activated Carbon (GAC) and Anionic Exchange Resin



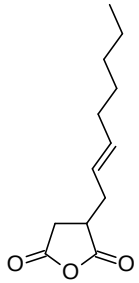
Treatment evaluation of GAC and anionic exchange resin for groundwater with SFC



features migrating from container

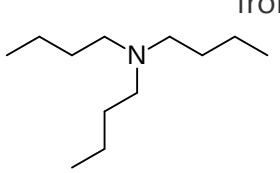


hexa(methoxymethyl)melamine

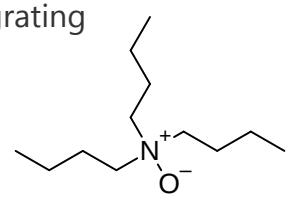


octenylsuccinic anhydride

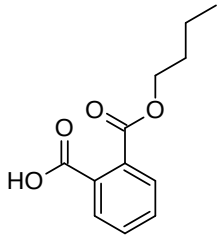
features migrating from resin



tributylamine derivates



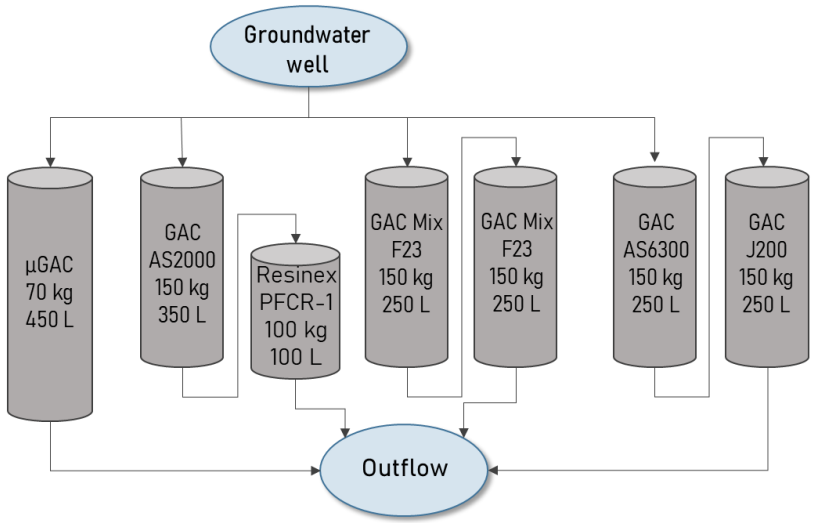
N-oxide tributylamine



phthalates

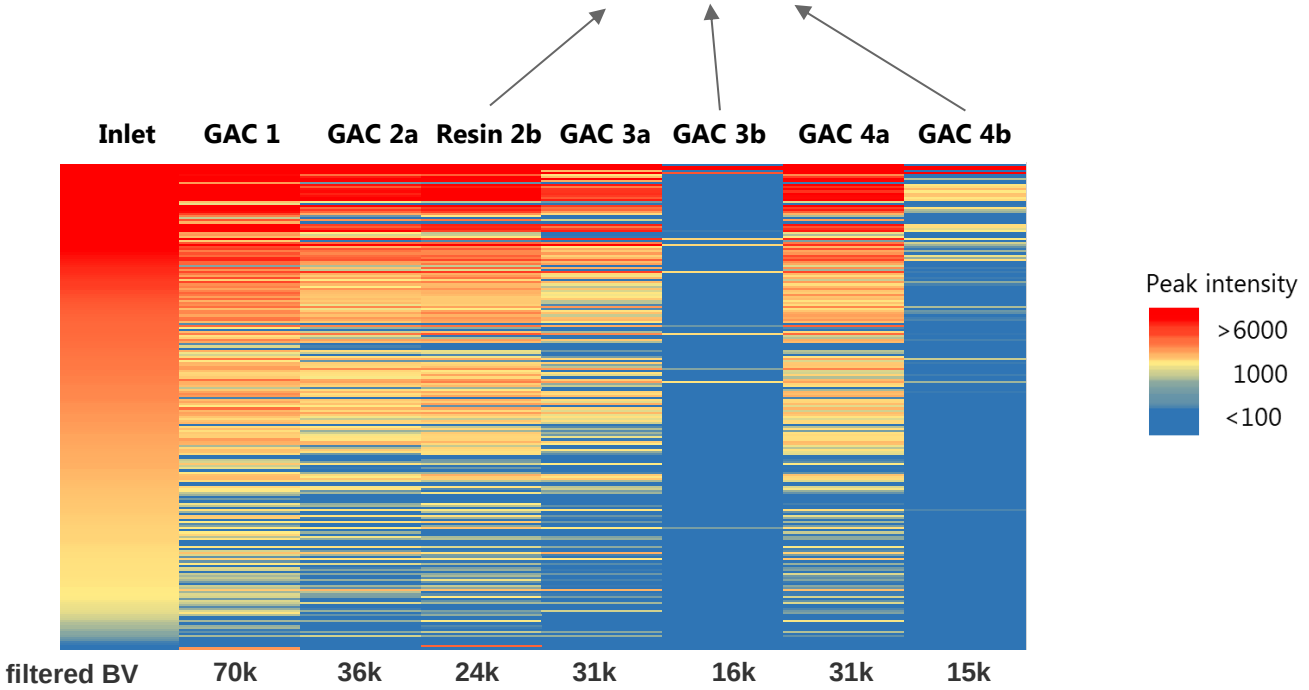
Treatment evaluation of GAC and anionic exchange resin for groundwater

7 month and >10,000 m^3 later



Equally efficient in removing PFAS

(removal >95 % for PFAS >C5)



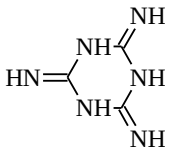
Even though GAC and Resin seem equally good in removing PFAS, differences in overall cleaning efficiency are observed

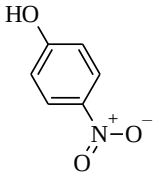
Suspect Screening in Groundwater

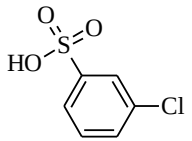
SFC

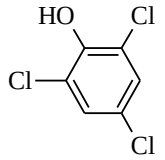
SFC/LC

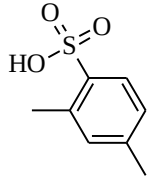
LC

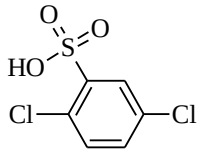

Melamine

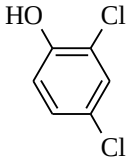

Nitrophenol


4-Chlorobenzene-sulfonic acid

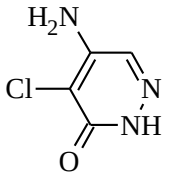

2,4,6-Trichlorophenol

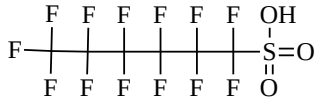

Dimethylbenzene-sulfonic acid


2,5-Dichlorobenzene-sulfonic acid


2,4-Dichlorophenol


Pyrimidinol


Chloridazon-desphenyl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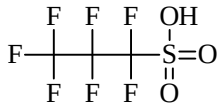
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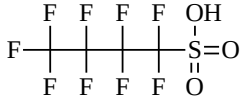
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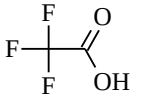
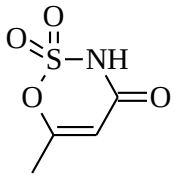
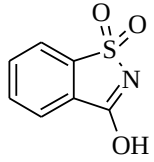
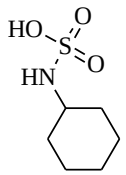
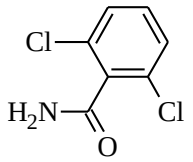
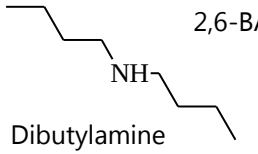

Trifluoroacetic acid

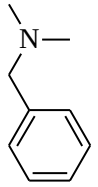

Acesulfame

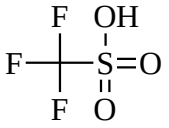

Saccharin

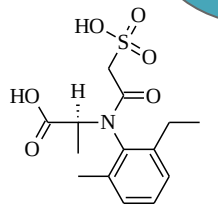

Cyclamate

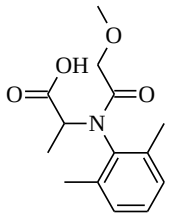

2,6-BAM

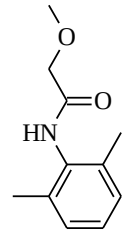

Dibutylamine

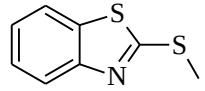

Benzyldimethylamine

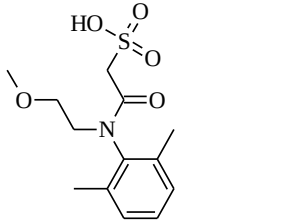

Trifluoromethanesulfonic acid

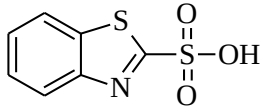

Metolachlor NOA 413173

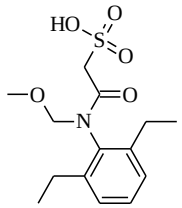

Metalaxyl acid


TP from Metalaxyl


2-(Methylthio)-benzothiazole


Dimethachlor ESA


Benzothiazole-2-sulfonate


Alachlor ESA

Tisler et al. (2022), Environmental Pollution, 10.1016/j.envpol.2022.119758

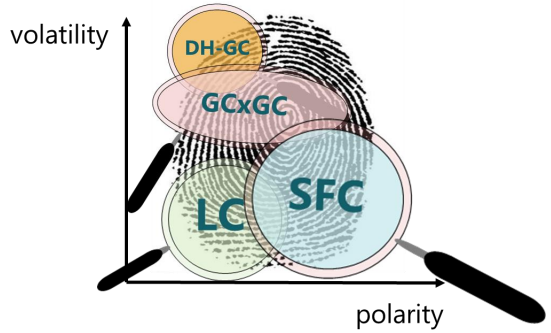
SFC is effective for water analysis over a broad polarity range

Challenges SFC in comparison to LC

- ❑ Higher matrix effect
- ❑ Retention time prediction for increasing confident needs improvement
- ❑ Direct injection of water limited

Advantages of SFC in comparison to LC

- ❑ More compounds can be screened
- ❑ Higher intensity → better identification of precursor and fragments
- ❑ Broader polarity spectra can be screened, especially very polar compounds



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