Ascona, 29. May - 3. June, 2016

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Non-target screening using HPLC-HRMS in combination with Effect-Directed Analysis to prioritize contaminants in the aquatic environment

- Non-target real world applications -

Wolfgang Schulz, Stefan C. Weiss, Lena Stütz, Tobias Bader, Thomas Lucke and Rudi Winzenbacher

Zweckverband Landeswasserversorgung
Laboratory for Operation Control and Research

Langenau, Germany

outline

- **Non-Target screening in drinking water analysis**
- **Introduction to HPTLC-EDA***
- **Reciprocal iso-inhibition volume (RIV)**
- **Linkage of HPTLC-EDA with HPLC-MS**
- **Conclusion**

*HPTLC: High Perfomance Thin Layer Chromatography

EDA: Effect-directed analysis

River water abstracted from the Danube

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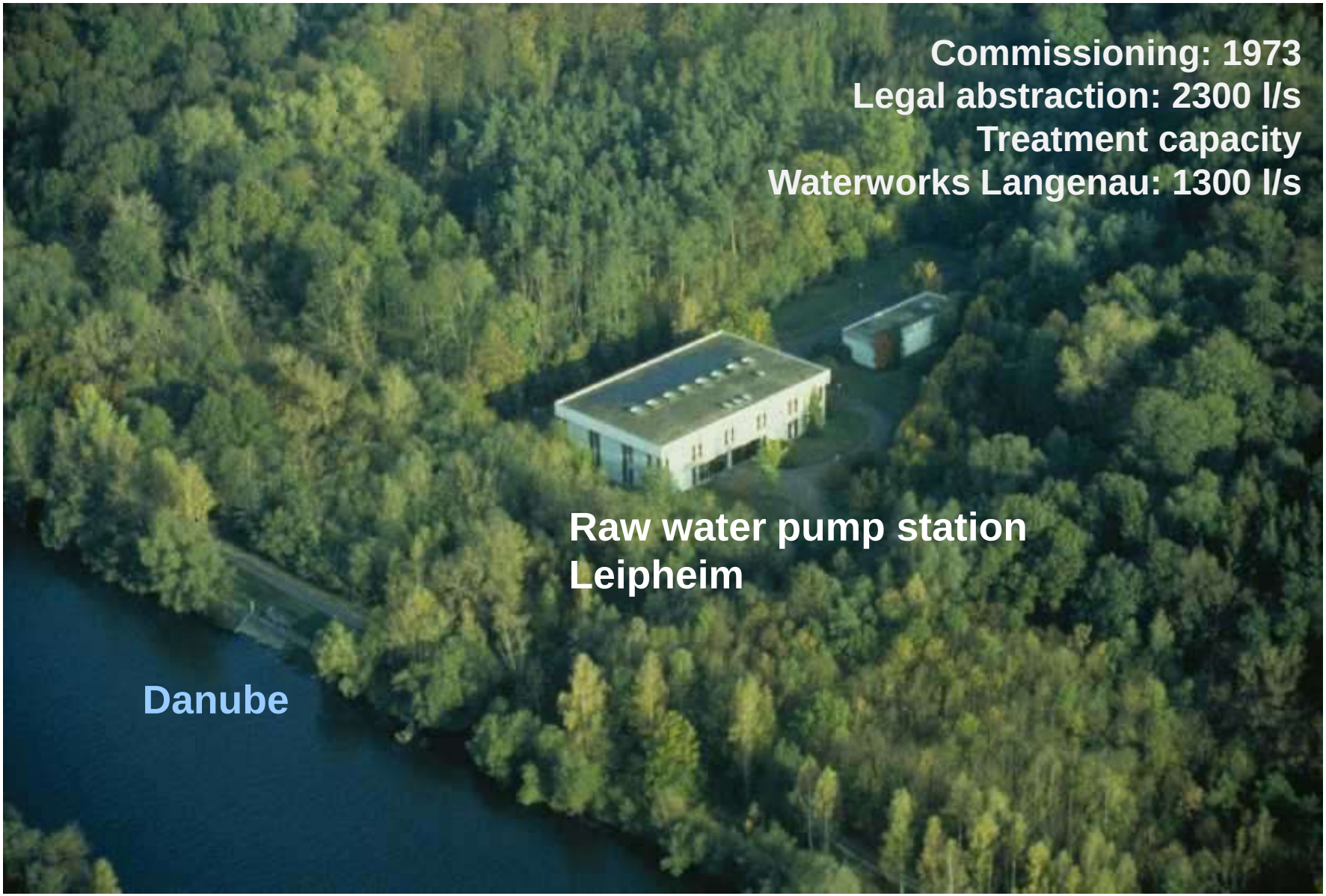

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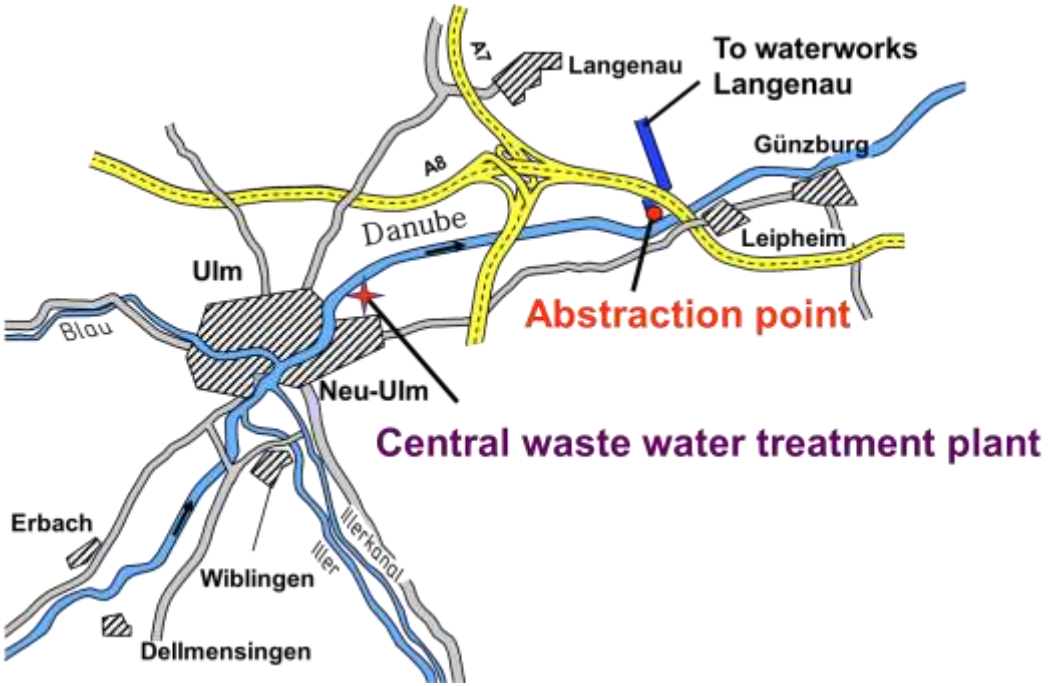
surface water
river danube

treatment (process)

drinking water



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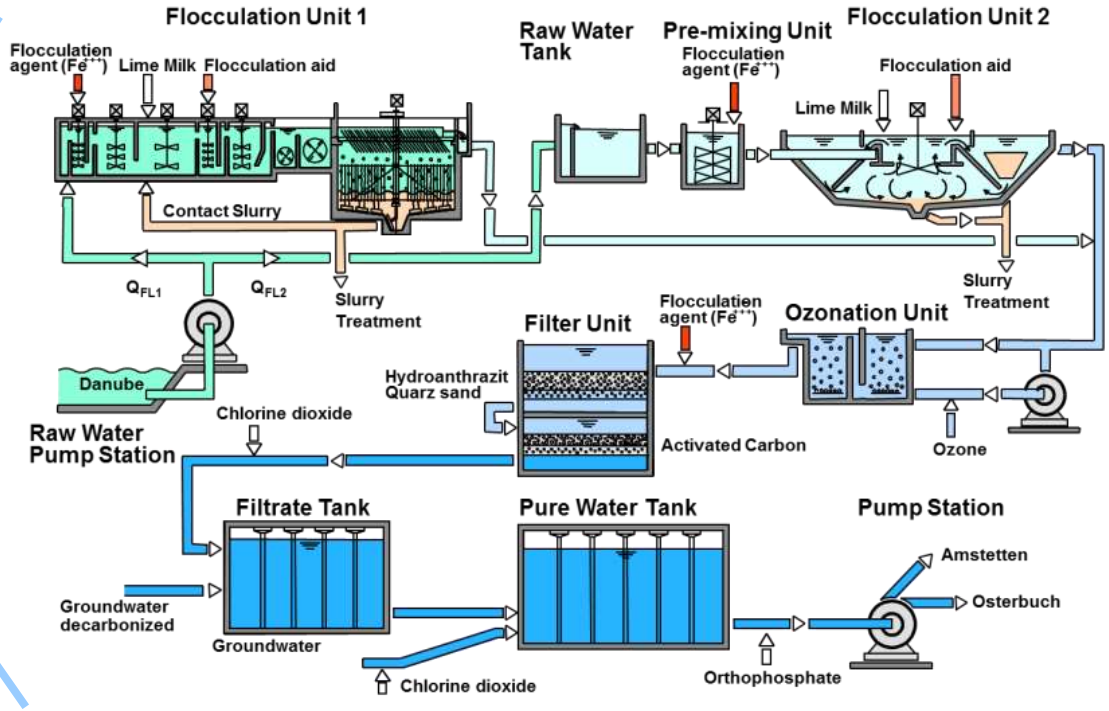
surface water river danube

treatment (process)

drinking water



Treatment of Danube water at Langenau waterworks

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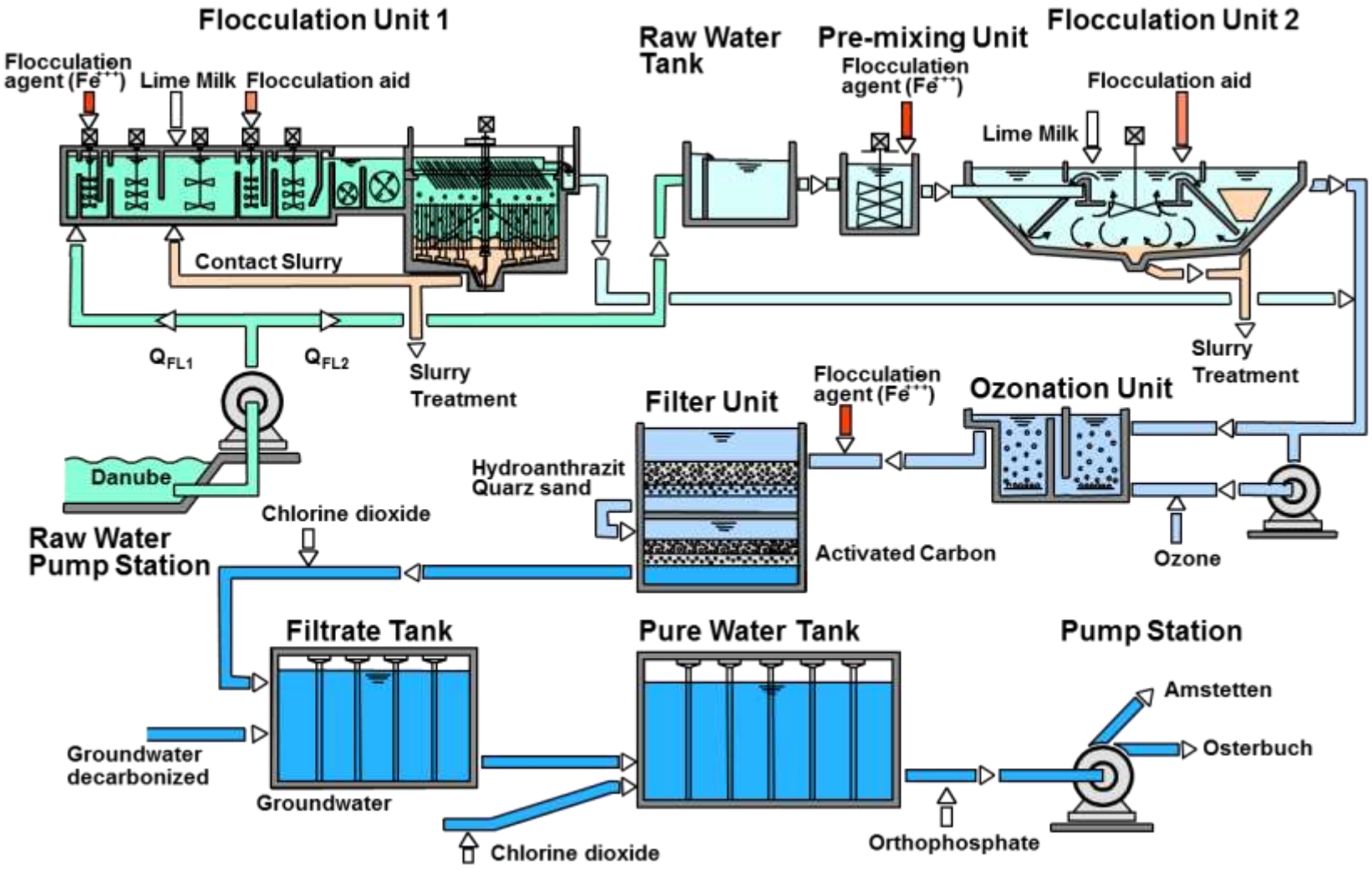

TIC of HPLC-HRMS Chromatogram river Danube

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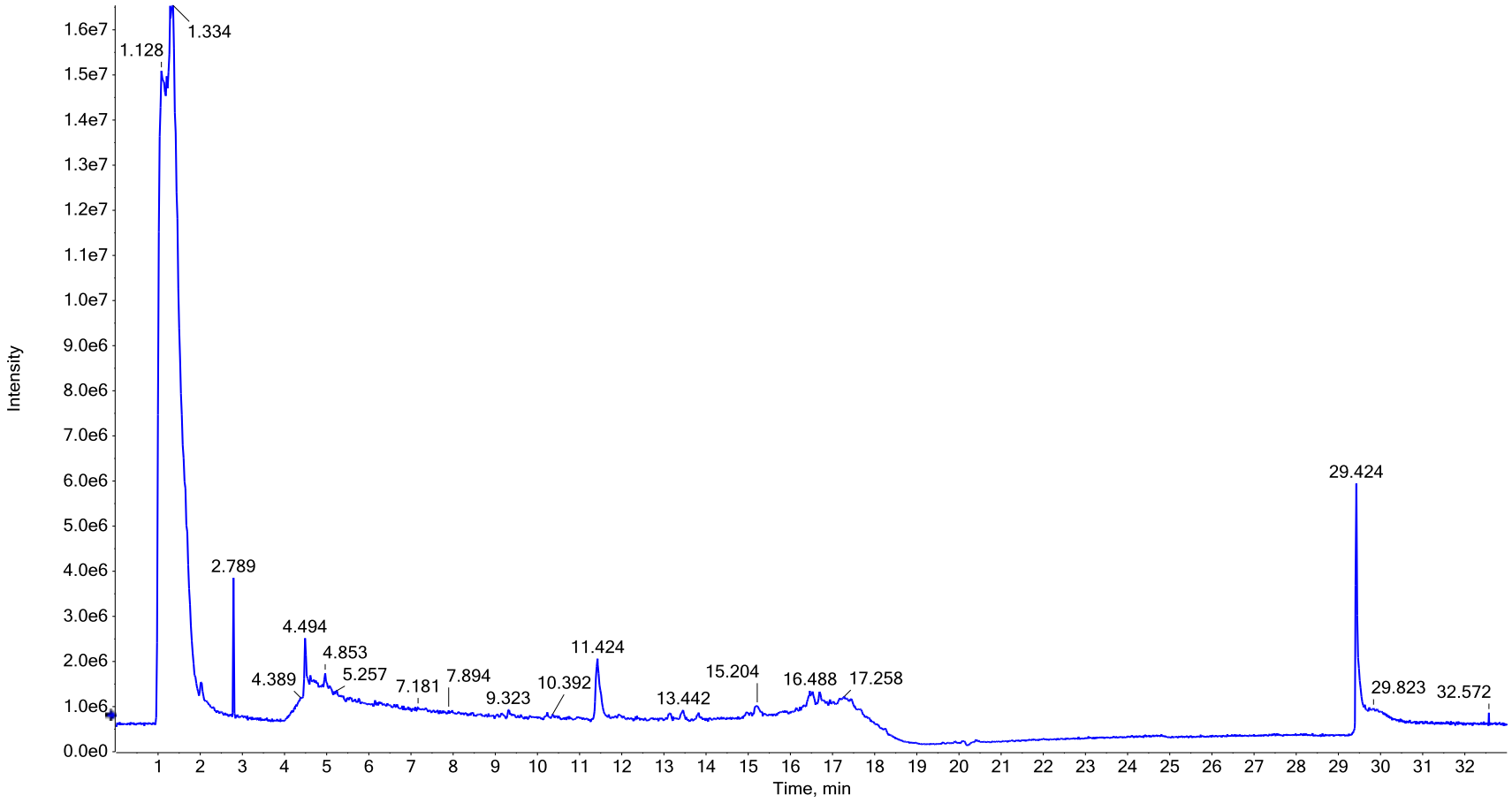

Non-Target-Screening Workflow

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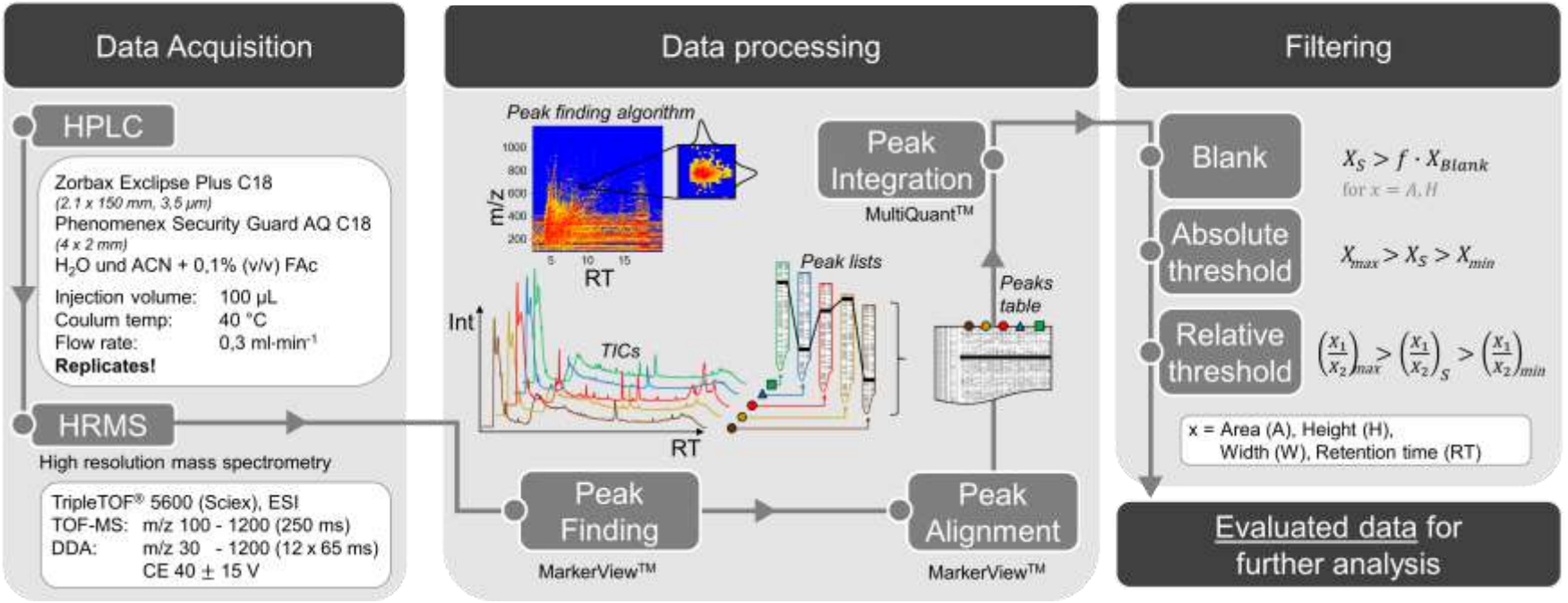

Talk Tobias Bader
Tuesday

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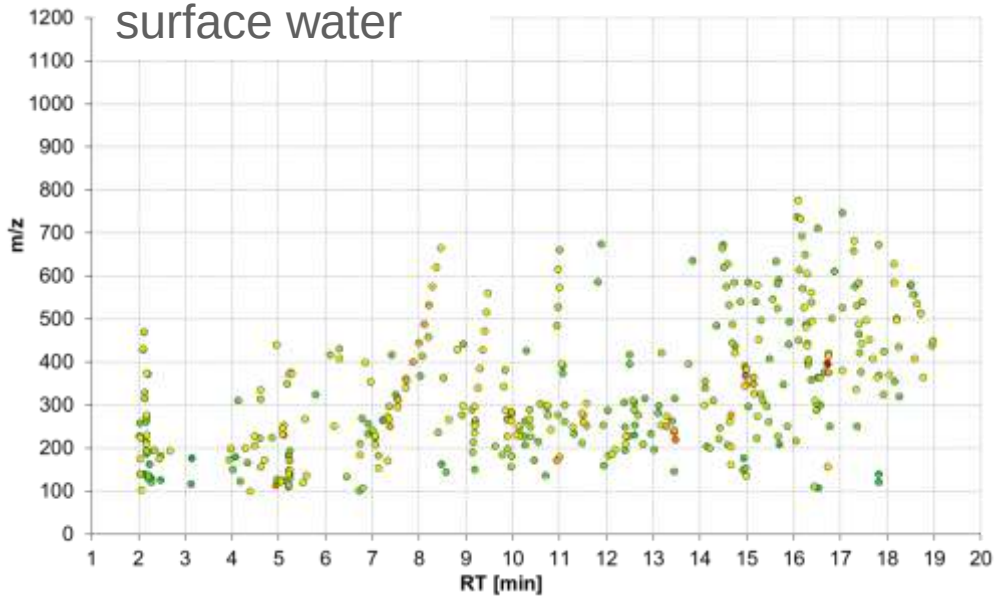
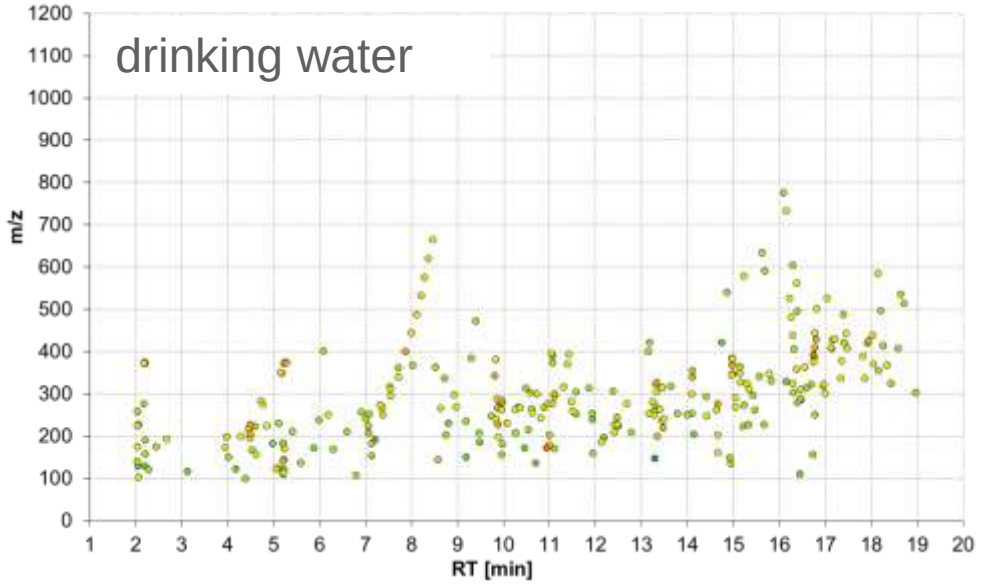
surface water
river danube

treatment
(process)

drinking water





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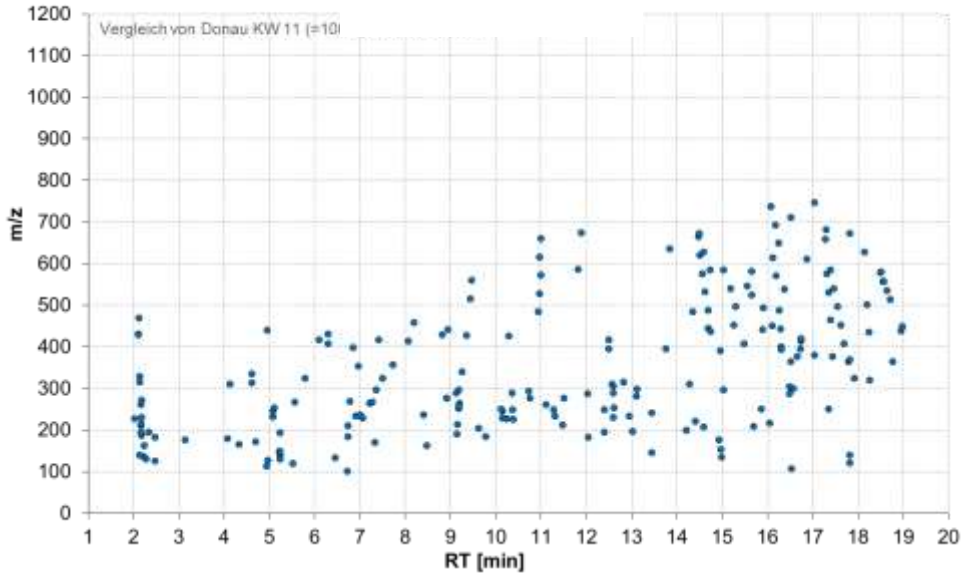

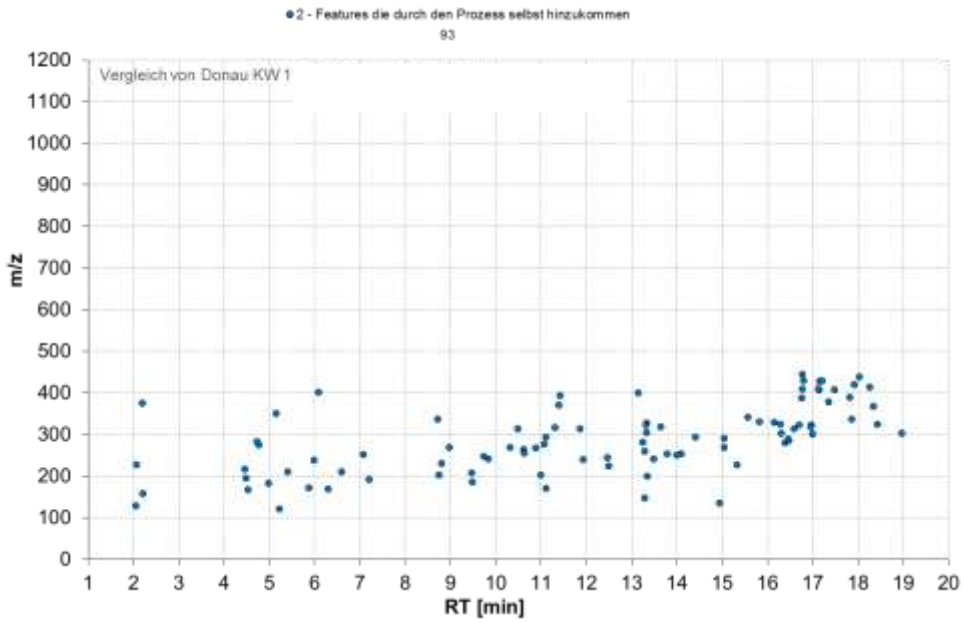

Total number **Inlet**
399 81.1%

Removed by process
211 42.9%

Common compounds
188 38.2%

93 18.9%
Formed by process

281 57.1%
Total number **Outlet**

Strategy for Prioritization

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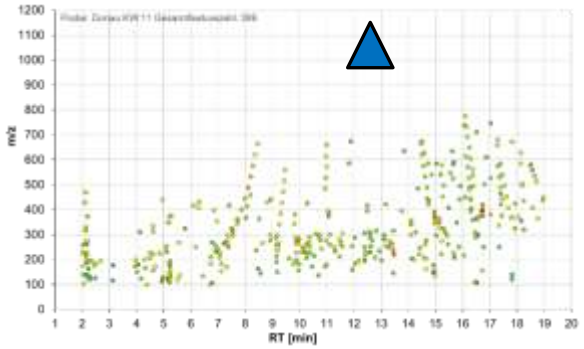
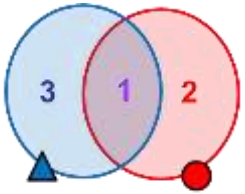
water sample input → process (waterworks) → water sample output

LC-HRMS

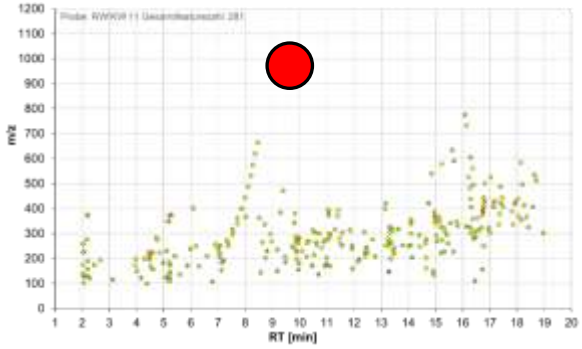
Non-Target Screening



Prioritization



Venn diagram



Identification

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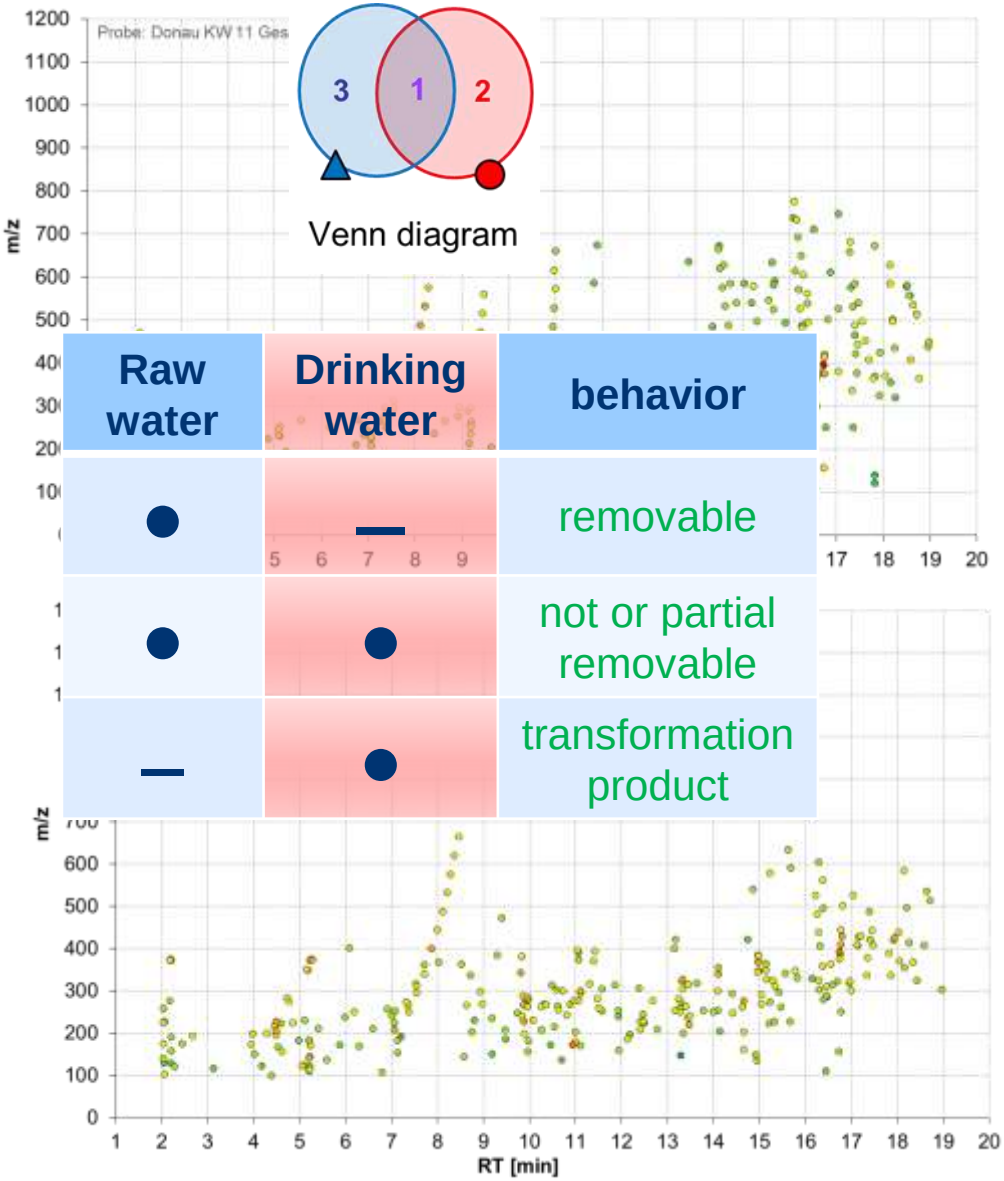
Landeswasserversorgung

surface water
river danube

treatment
(process)

drinking water



Raw water	Drinking water	behavior
●	—	removable
●	●	not or partial removable
—	●	transformation product

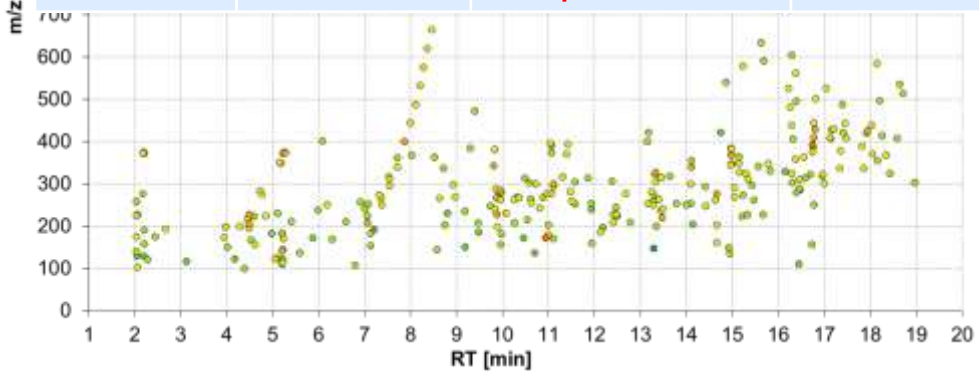
Zweckverband
Landeswasserversorgung

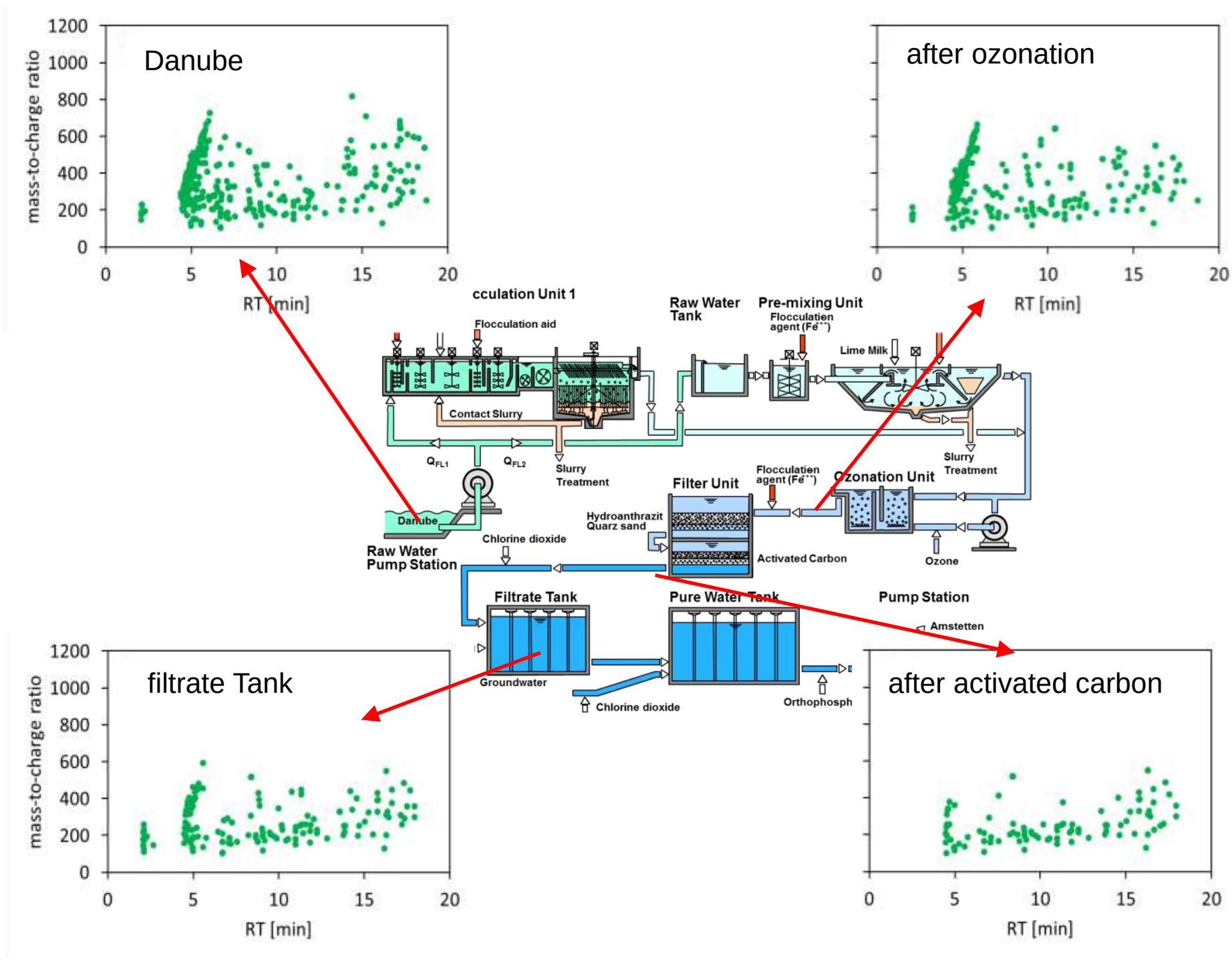
surface water
river danube

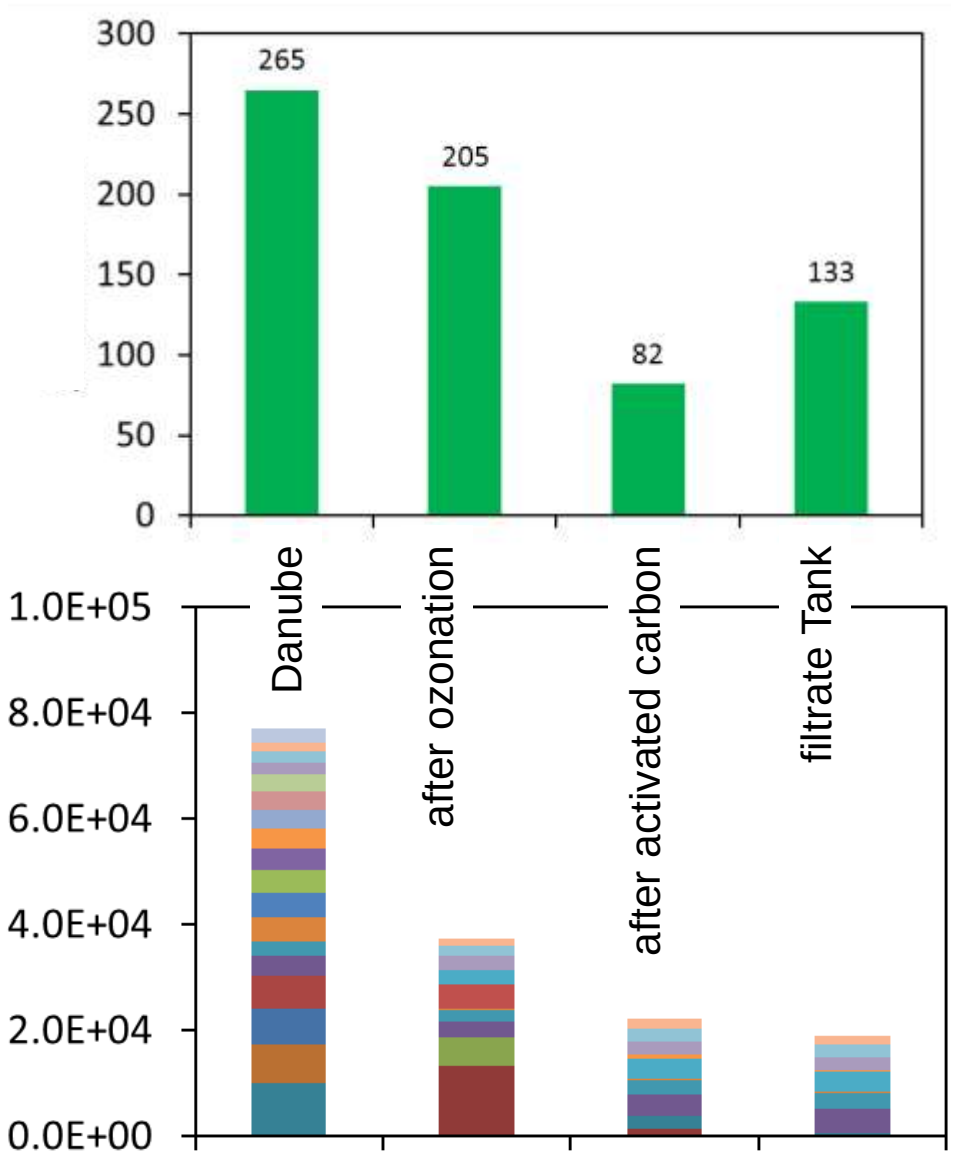
treatment (process)

drinking water

Raw water	Drinking water	behavior	Prioritization
●	—	removable	2
●	●	not or partial removable	1
—	●	transformation product	1




Danube
after ozonation
filtrate Tank
after activated carbon
mass-to-charge ratio
RT [min]
cculation Unit 1
Flocculation aid
Contact Slurry
Slurry Treatment
Raw Water Tank
Pre-mixing Unit
Flocculation agent (Fe++)
Lime Milk
Filter Unit
Hydroanthrazit
Quarz sand
Activated Carbon
Ozonation Unit
Ozone
Chlorine dioxide
Raw Water Pump Station
Filtrate Tank
Pure Water Tank
Pump Station
Amstetten
Groundwater
Orthophosph



Number of Features

Intensity of the
TOP 25 features
in the Danube

overview of sampling points

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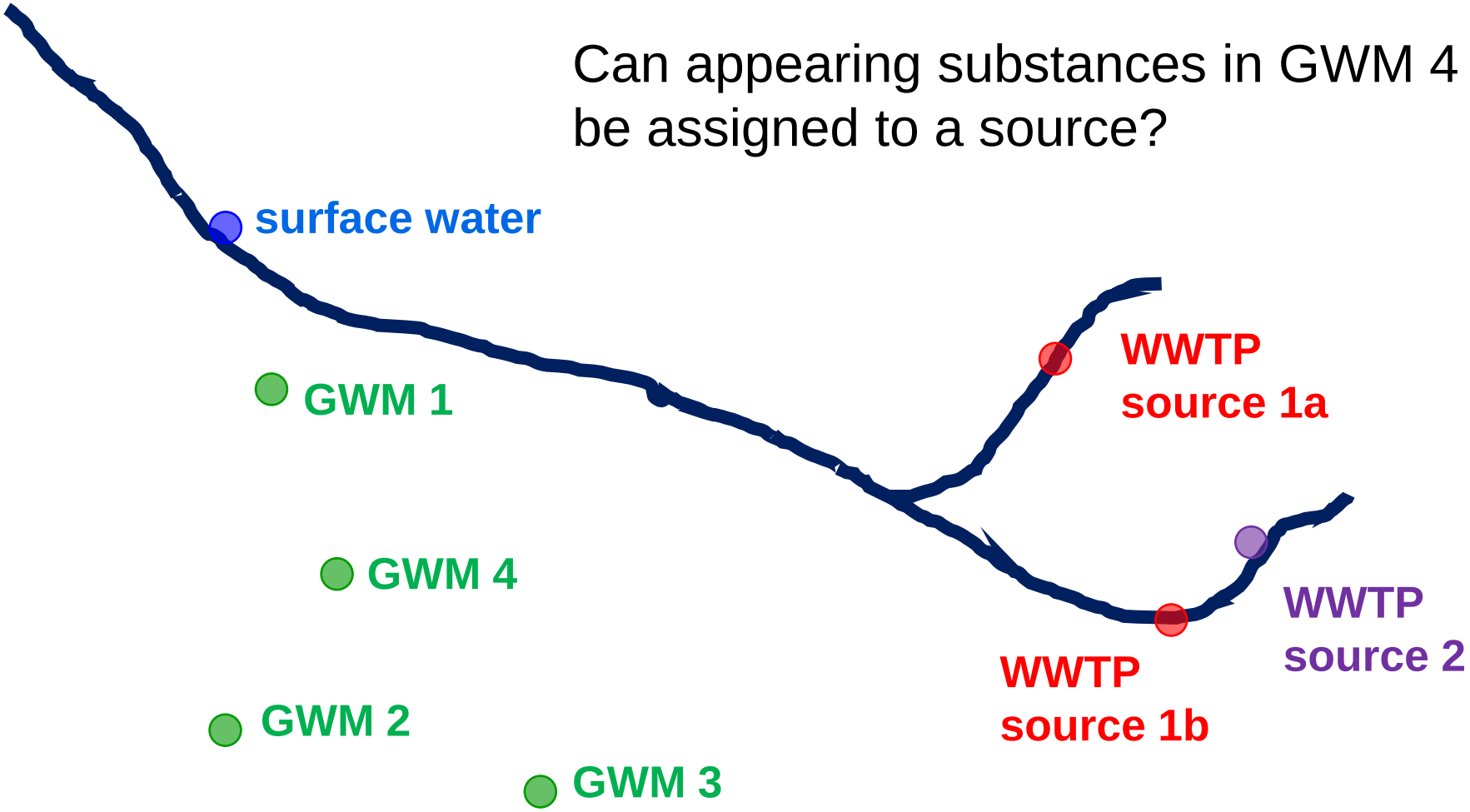
Can appearing substances in GWM 4 be assigned to a source?



GWM: groundwater monitoring wells
WWTP: waste water treatment plant

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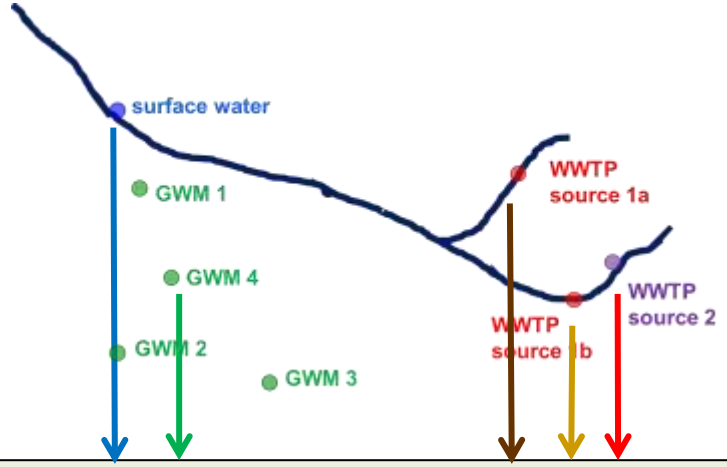
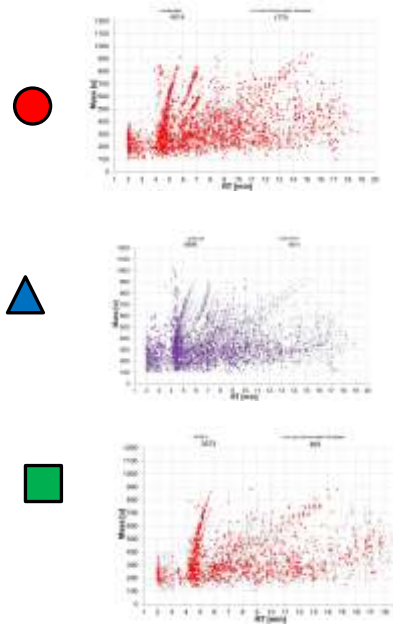
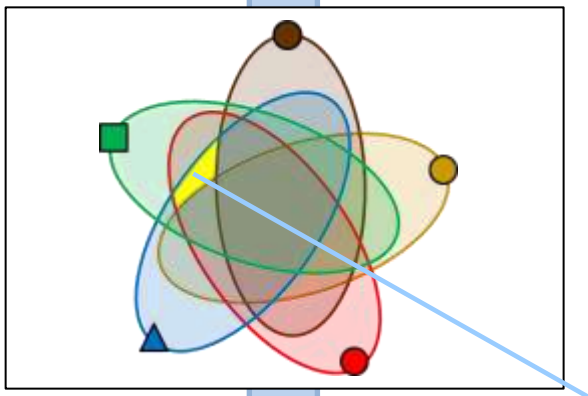


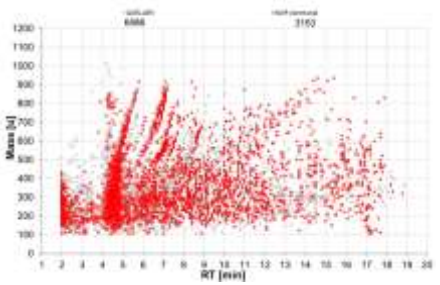
process (infiltration into groundwater)

LC-HRMS

Non-Target Screening







$(\bullet \cup \bullet)$

$(\bullet \cap \blacktriangle \cap \blacksquare) \setminus (\bullet \cup \bullet)$

Set theory

Identification

Distinction of sources
specific features for the sources

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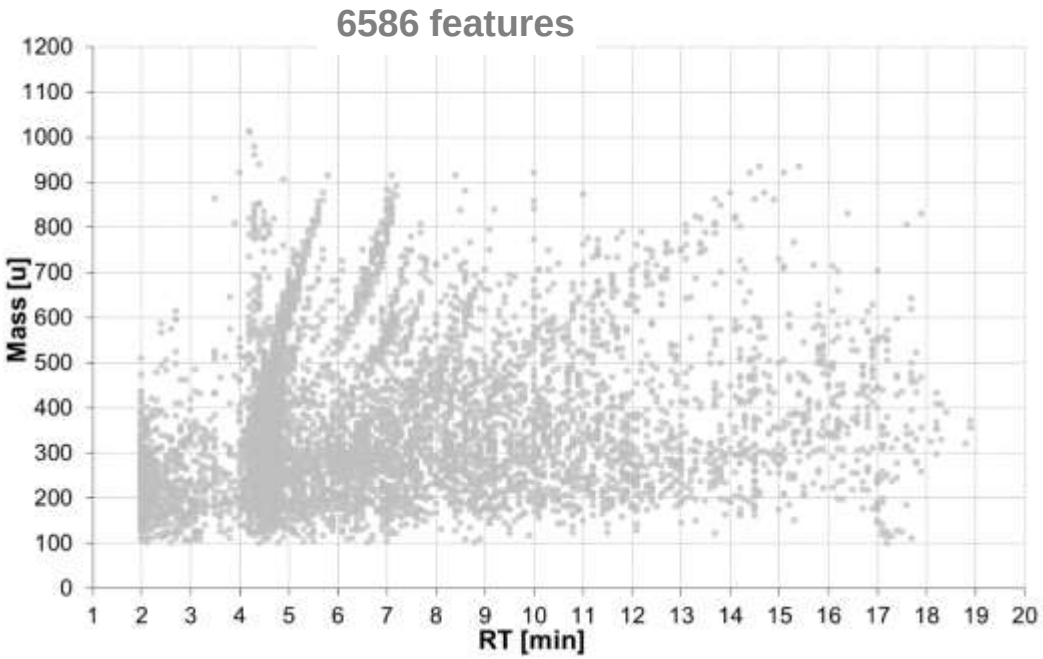

source 1a

source 1b

source 2

Mass-RT scatterplot of all features in the sources 1a, 1b, 2

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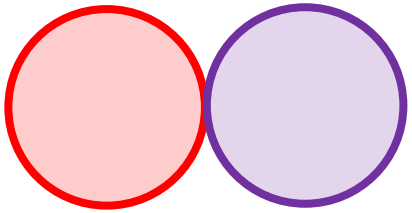
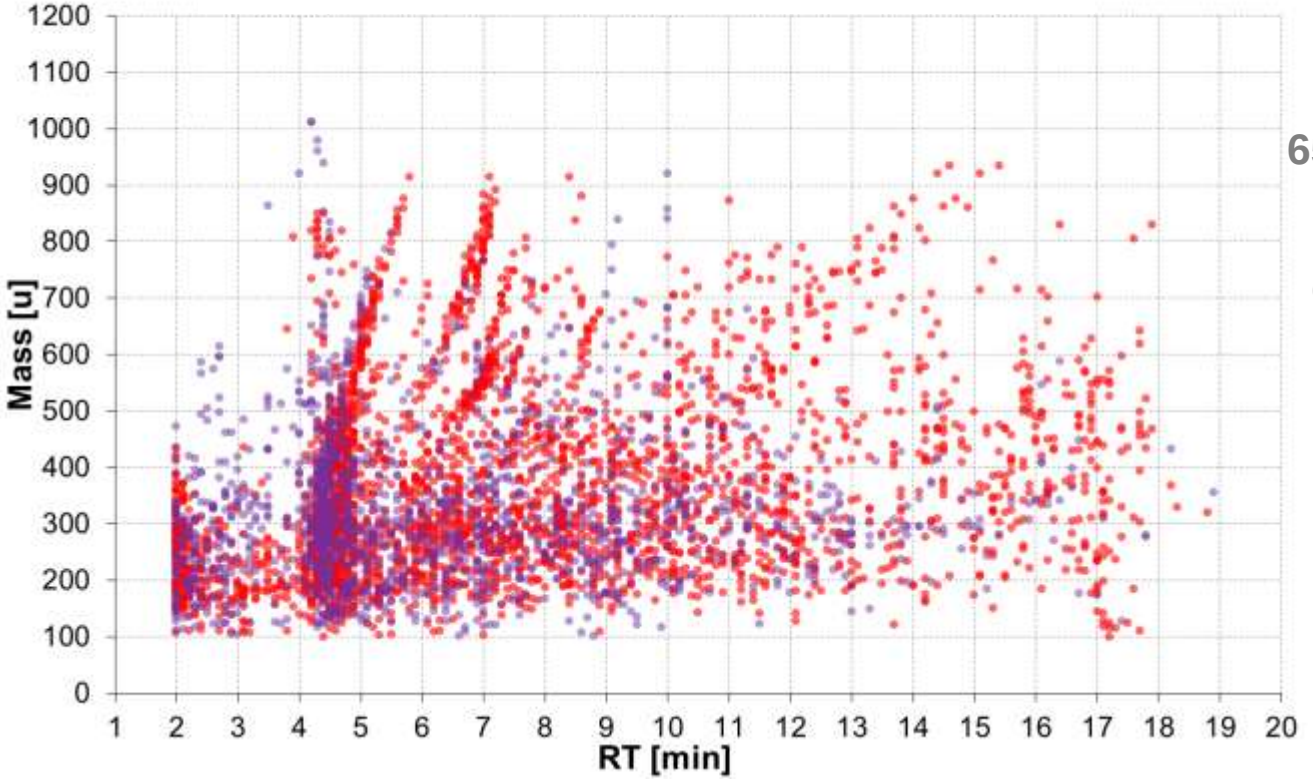
Source 1a/1b



Source 2

no interception

6586 total **3153 source 1a/1b** **1611 source 2**



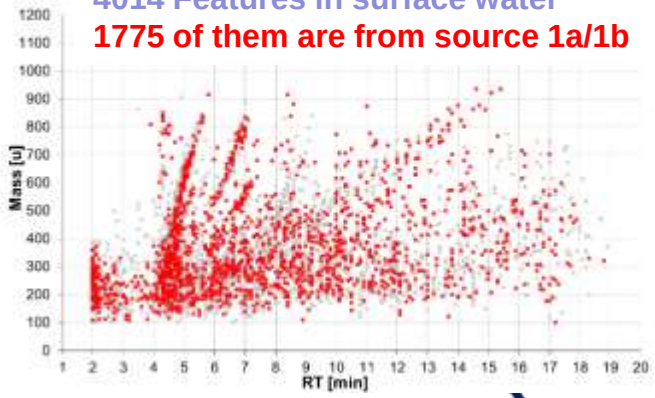
6586 - 3153 - 1611 = 1822

1822
common features in all sources

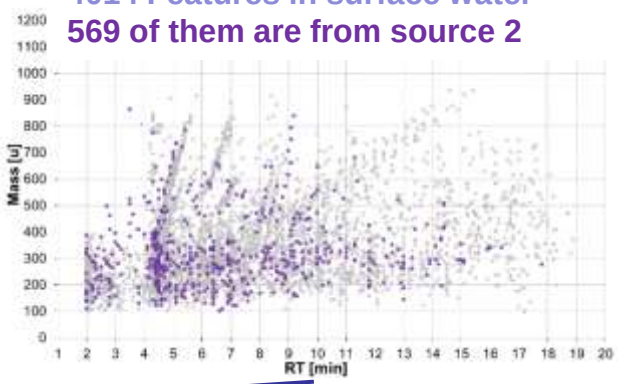
only from source 1a and 1b

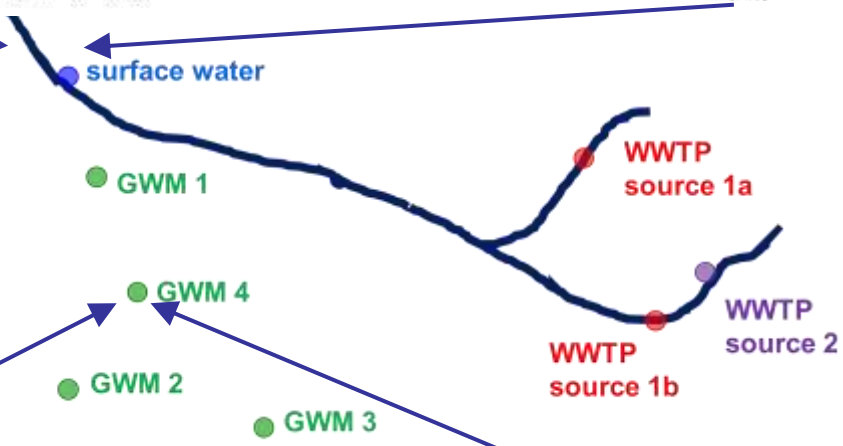
only from source 2

4014 Features in surface water
1775 of them are from source 1a/1b



4014 Features in surface water
569 of them are from source 2

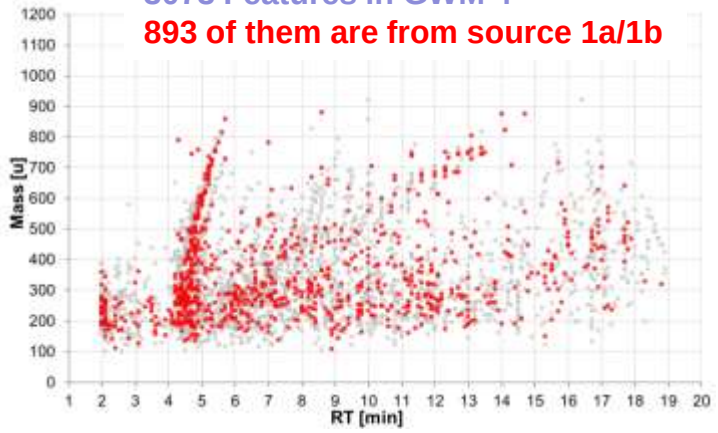




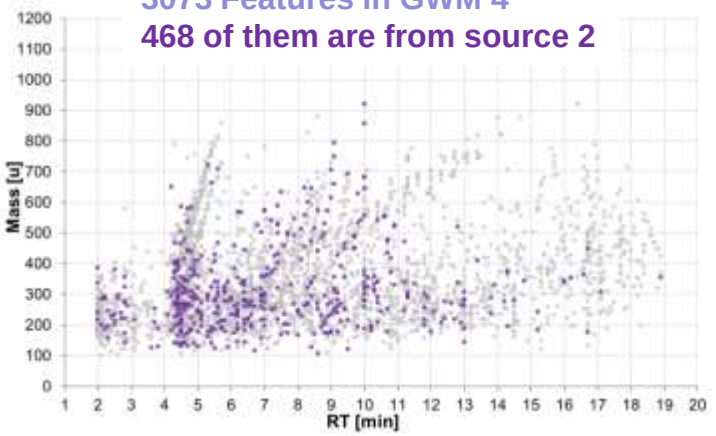
Source 1a/1b

Source 2

3073 Features in GWM 4
893 of them are from source 1a/1b



3073 Features in GWM 4
468 of them are from source 2



Identification of some Features from source 2 present in GWM 4

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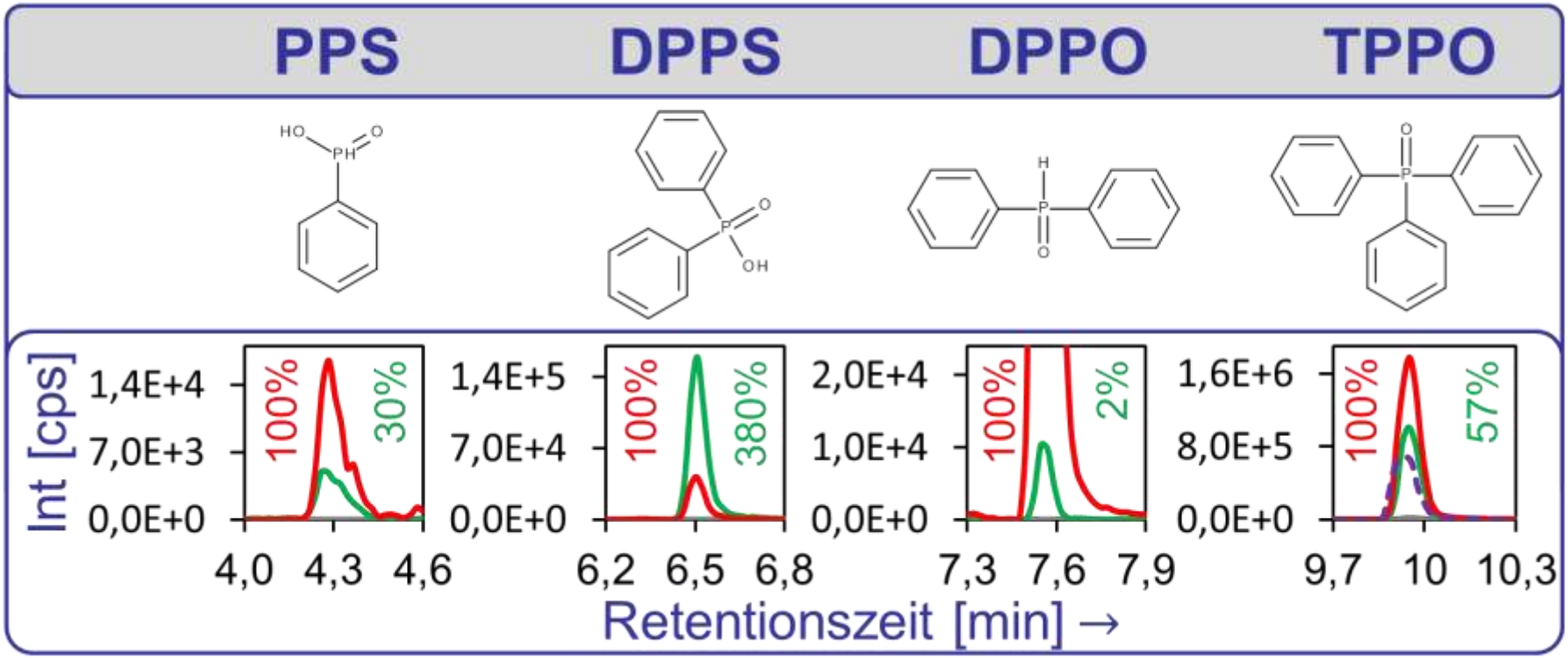


Source 2 GWM 4 Blank standard: 5 µg·L^{-1}

TPPO Triphenylphosphineoxid

Talk: Uwe Kunkel, Friday
Quarternary triphenylphoshonium compounds

Non-Target Screening

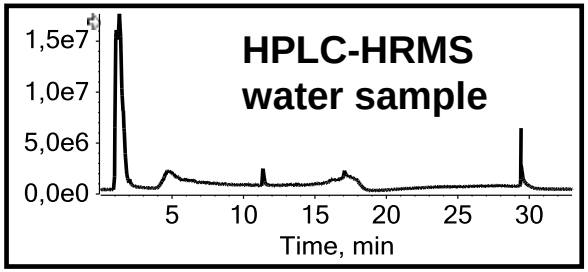
Search for

$$max(Var_i) \gg Q_{0.8}(Var_i)$$

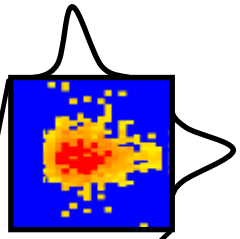
m/z @ RT

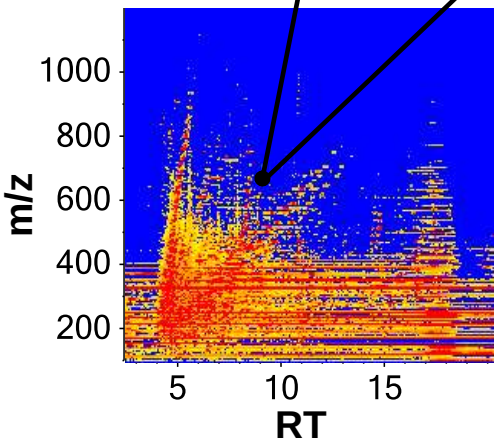
$$\begin{pmatrix} A_{1,1} & \cdots & A_{1,k} \\ \vdots & \ddots & \vdots \\ A_{n,1} & \cdots & A_{n,k} \end{pmatrix} \; Var_i$$

week

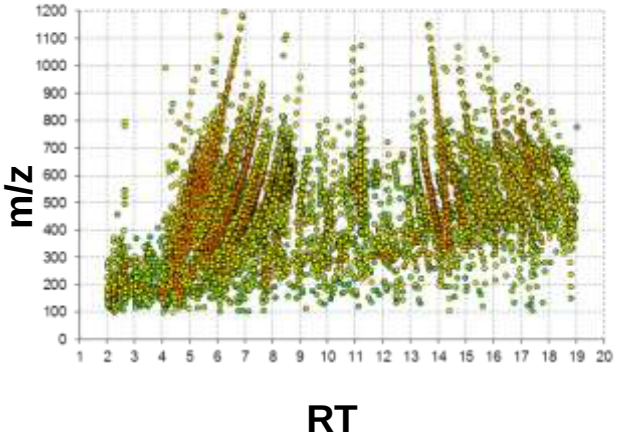


Peak finding algorithm





Feature Extraction



Non-Target Screening

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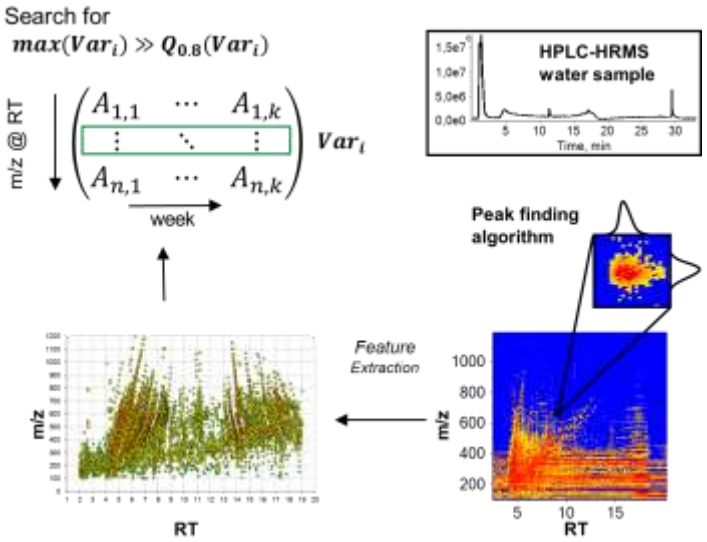
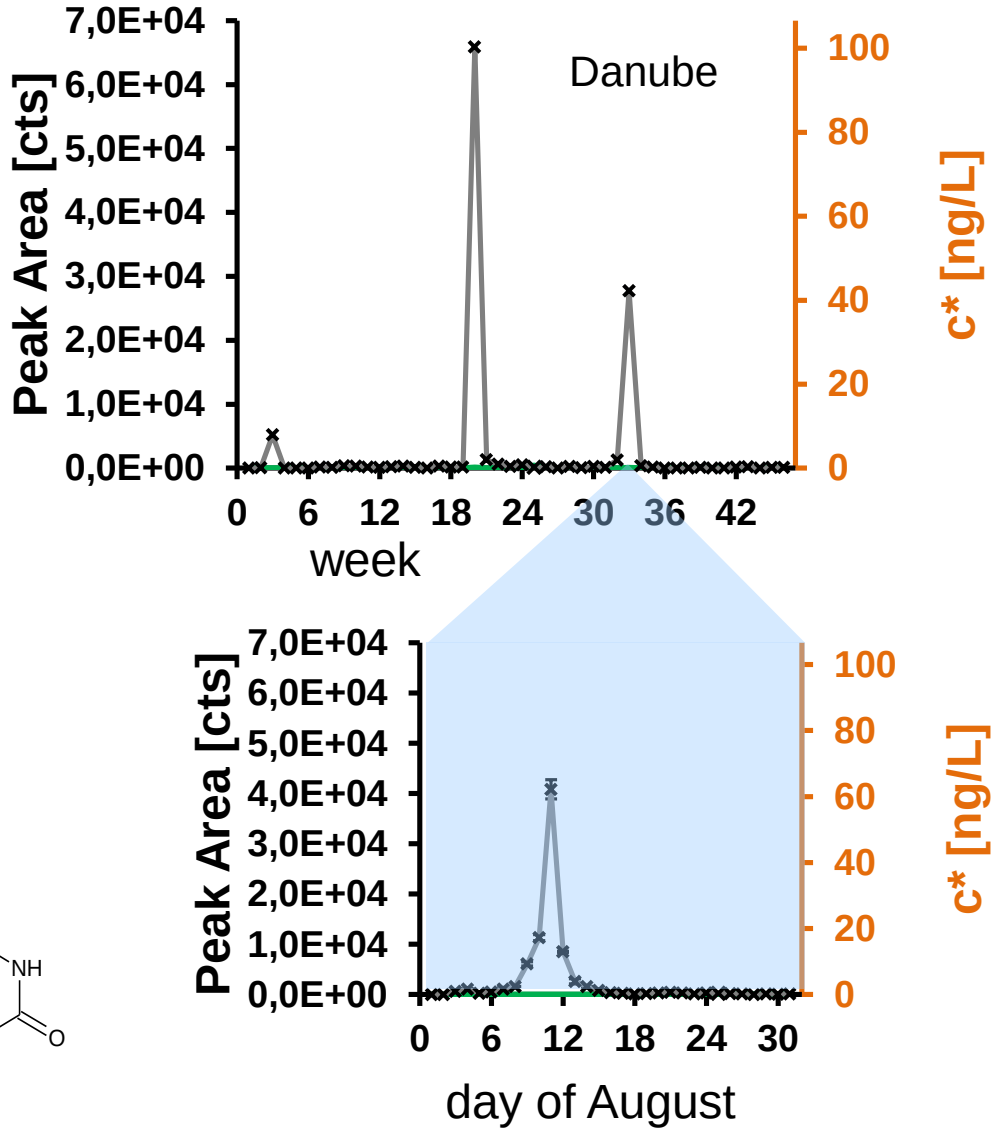
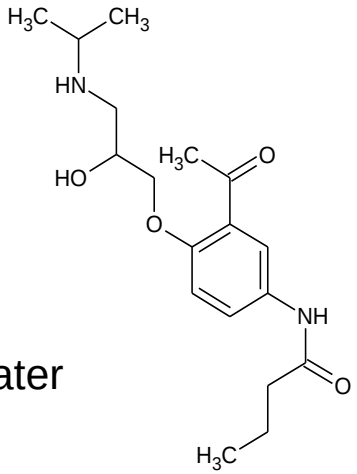




Acebutolol

337.212@5.4min



Not found in drinking water

PCA-DA

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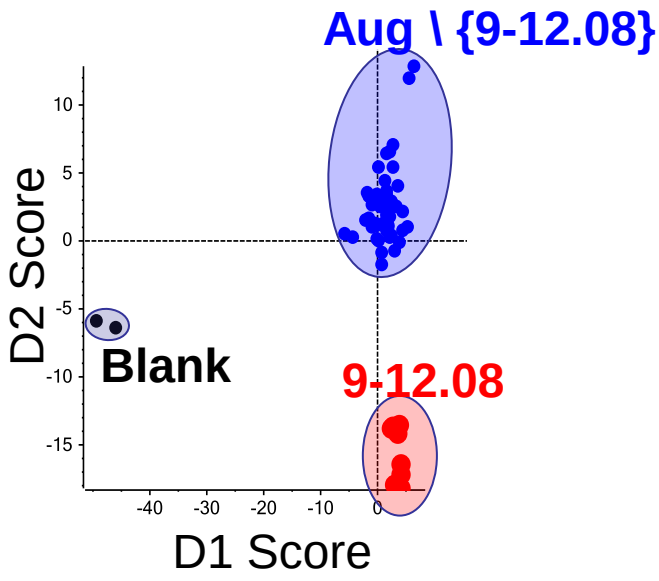
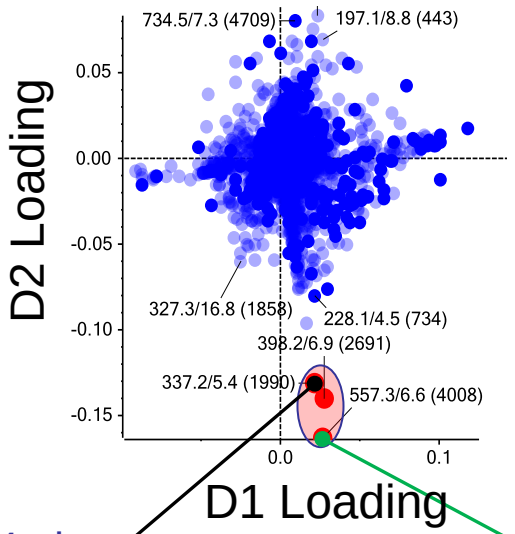
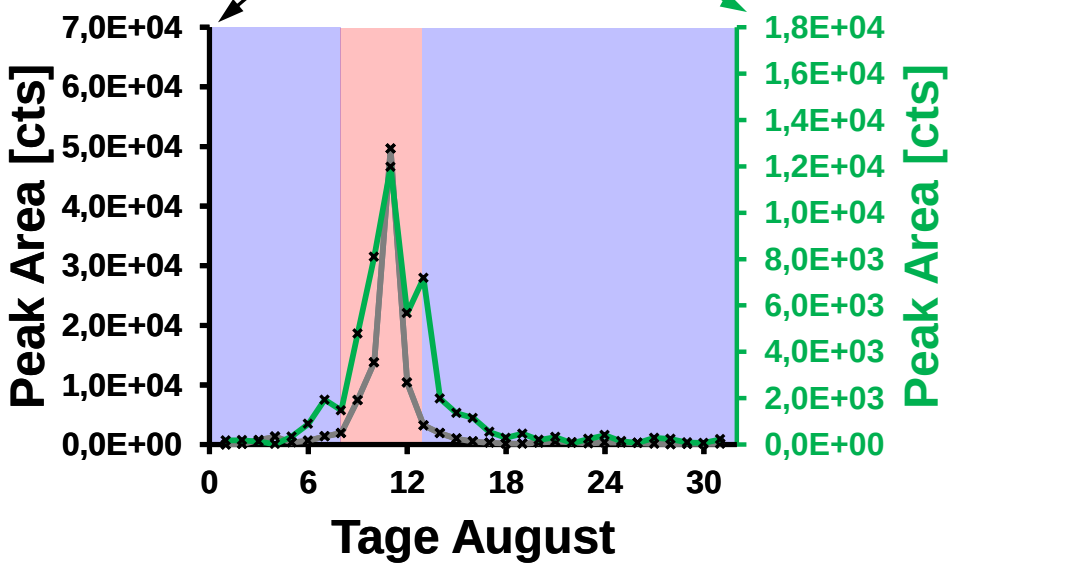




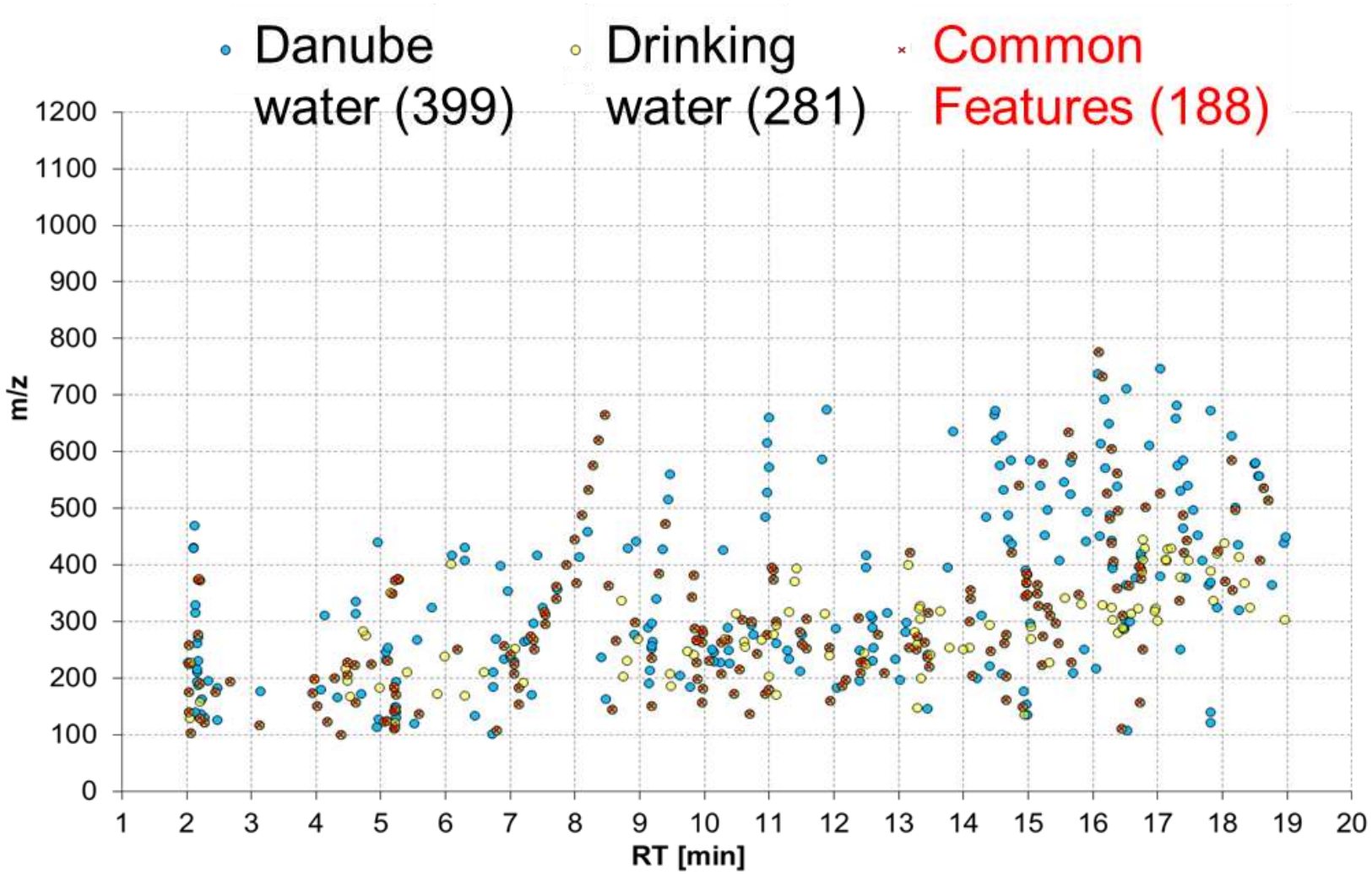
?

337.212@5.4min

557.255@6.6min



Principal component analysis and discriminant analysis (PCA–DA)


Danube water (399)
Drinking water (281)
Common Features (188)
m/z
RT [min]
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

Thin-layer chromatography (TLC)

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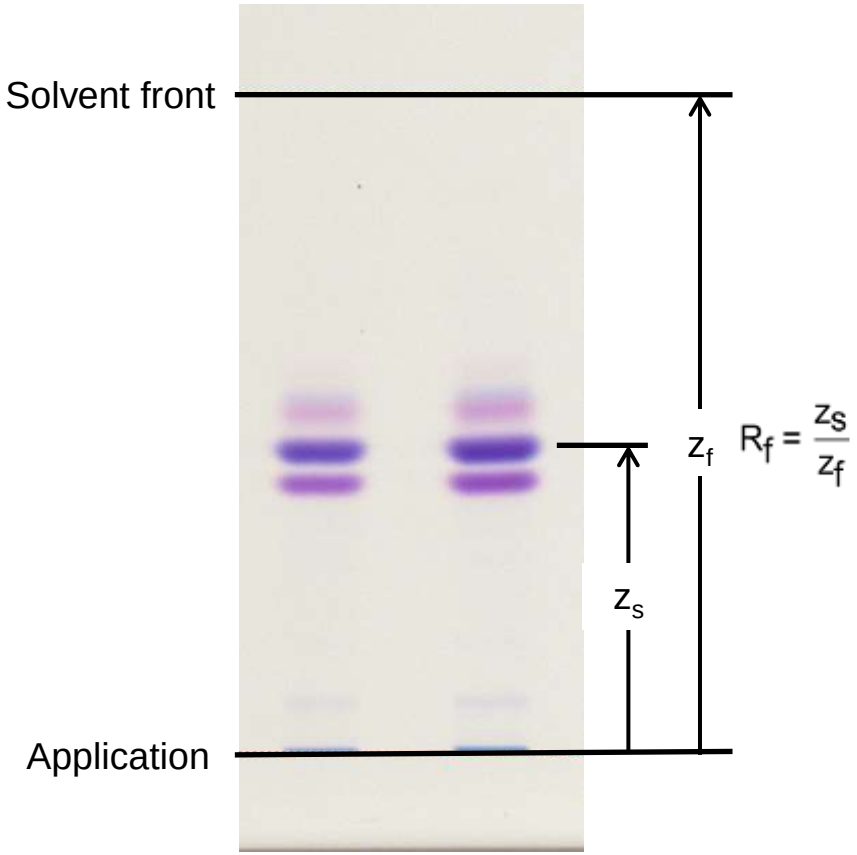

Advantages:

- Substances which remain on the application zone were gathered
- Solvents have no influence on the biological tests
- Large choice of solvents

Disadvantages:

- Lower separation efficiency as e.g. HPLC
- Not fully automated
- Limited separation distance

Overview of effect-directed analysis (EDA) with thin-layer chromatography (TLC)

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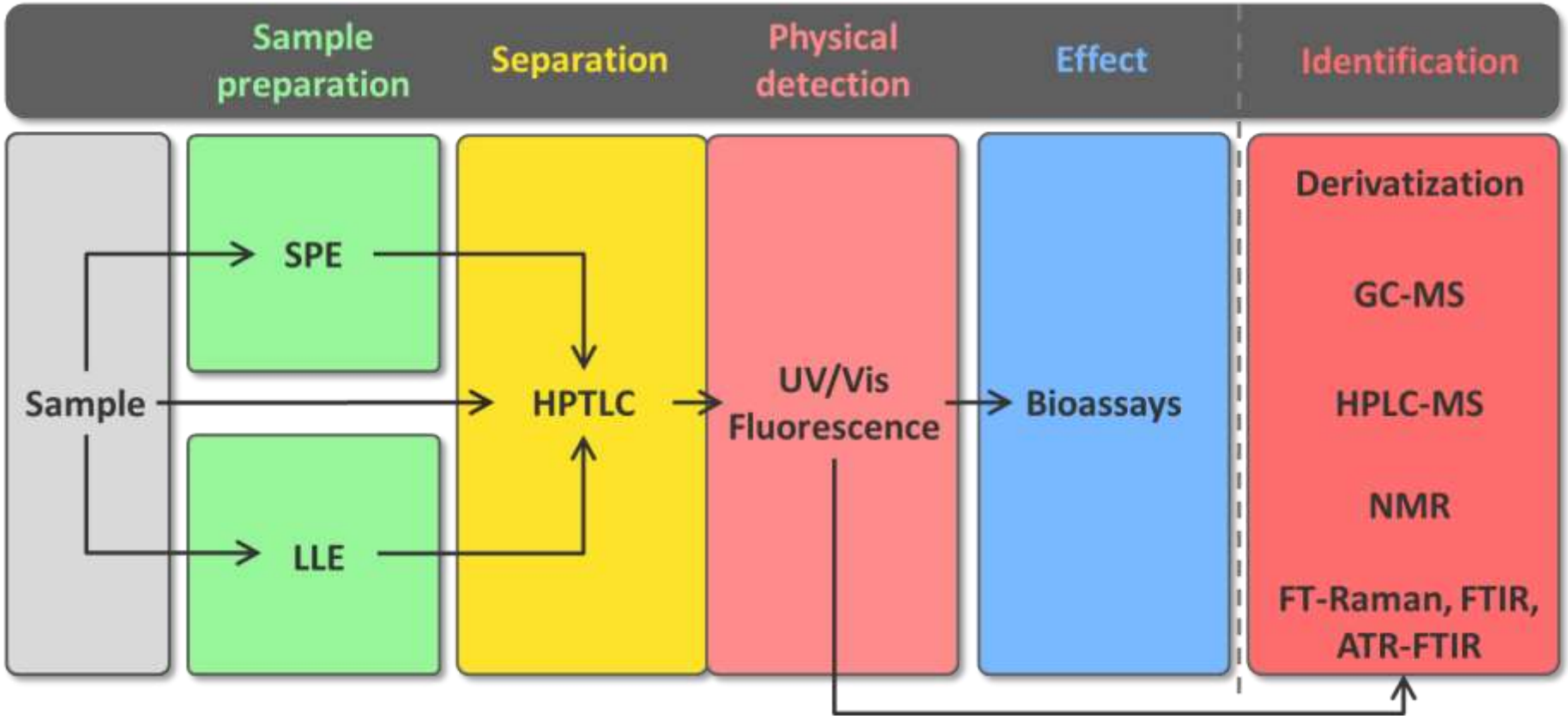

Procedure of EDA/HPTLC-analysis with *Aliivibrio fischeri* inhibition assay

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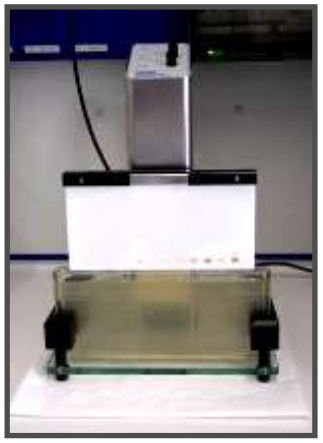
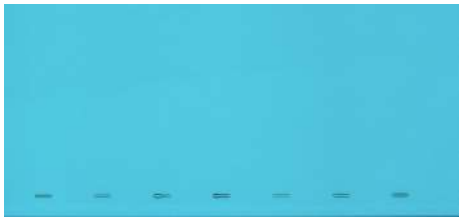
application

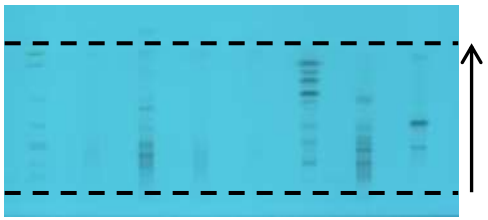
separation

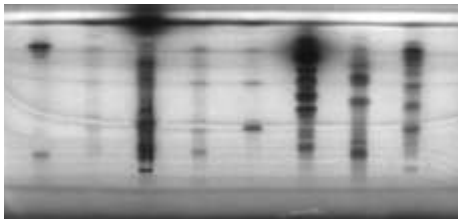
immersion



detection

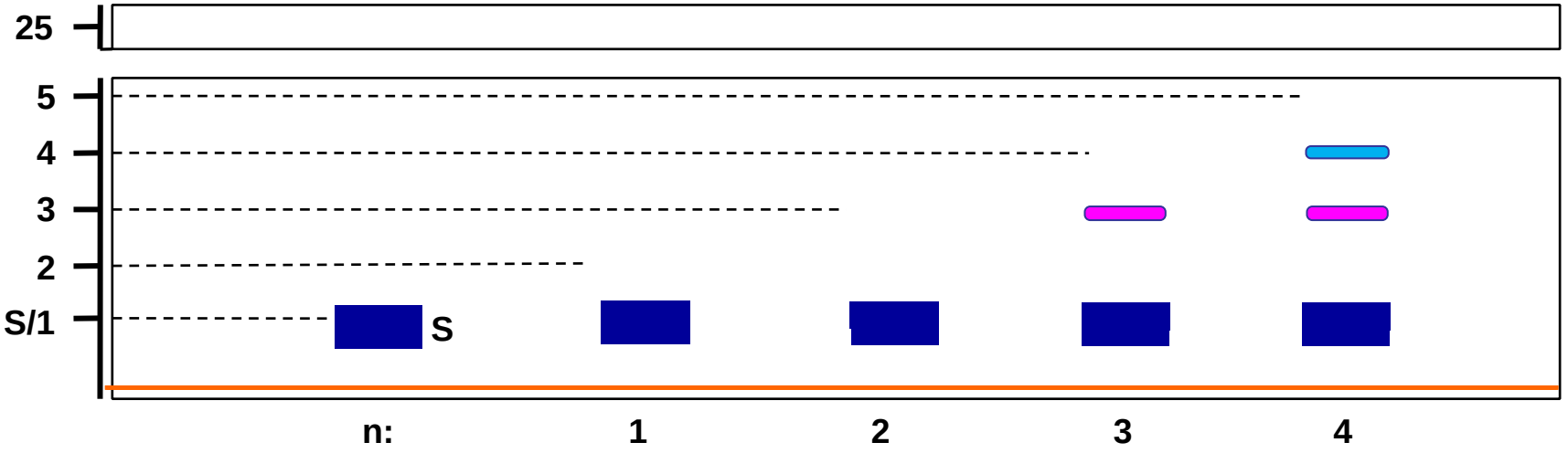


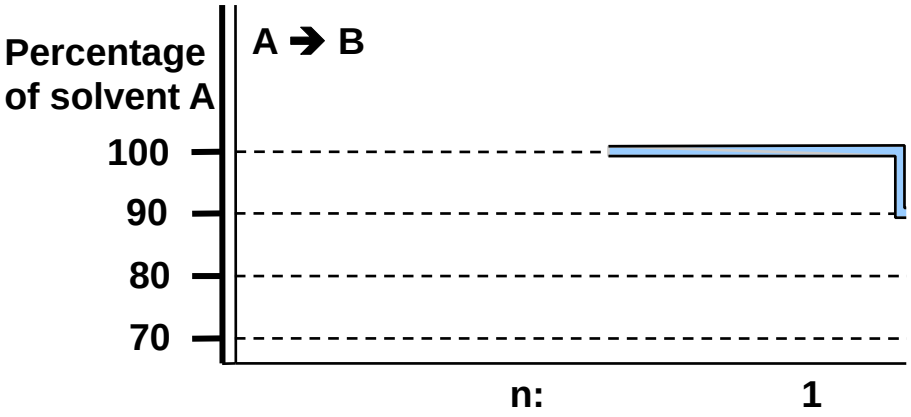




Procedure of HPTLC-analysis with automated multiple development (AMD)

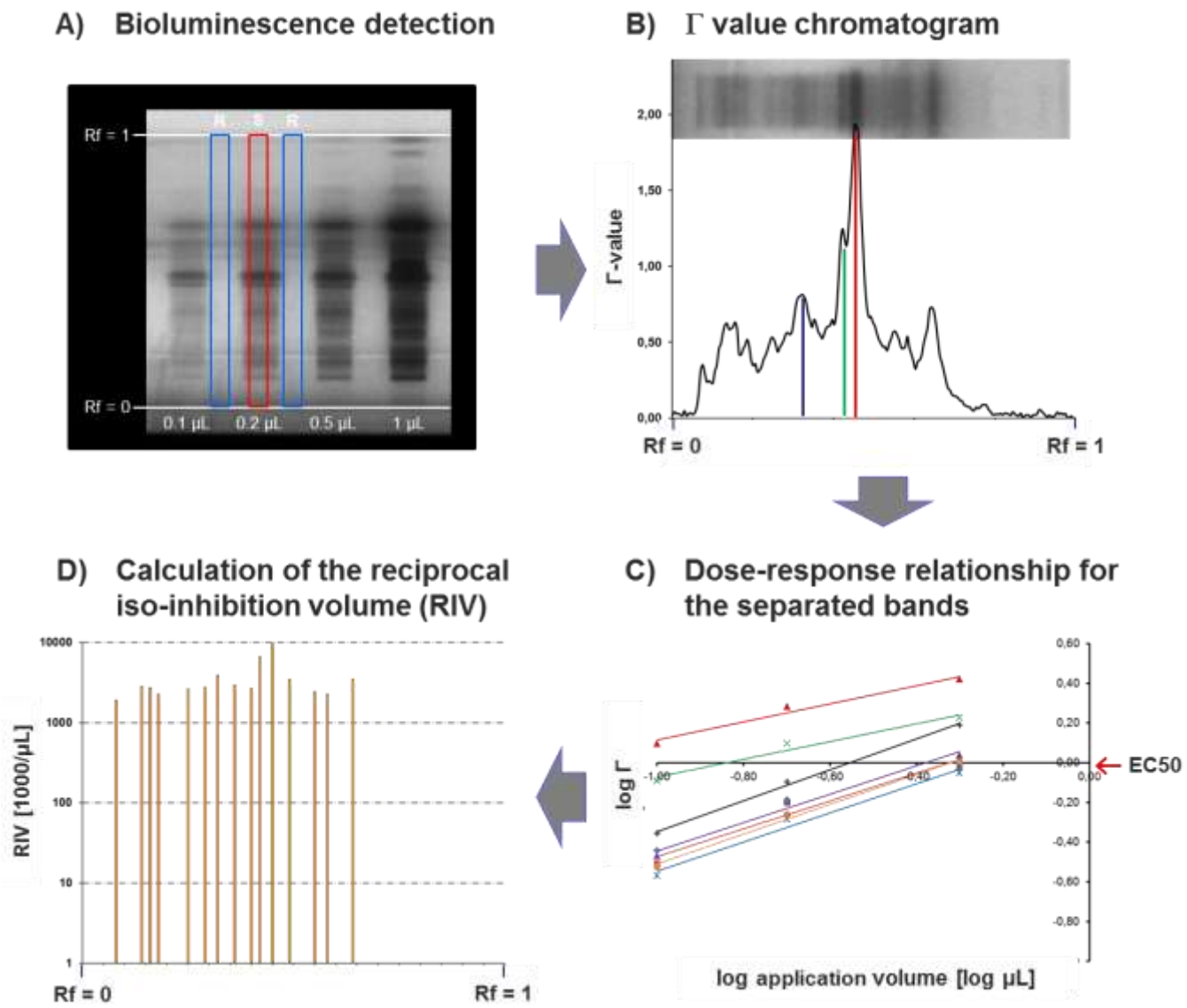
Zweckverband
Landeswasserversorgung





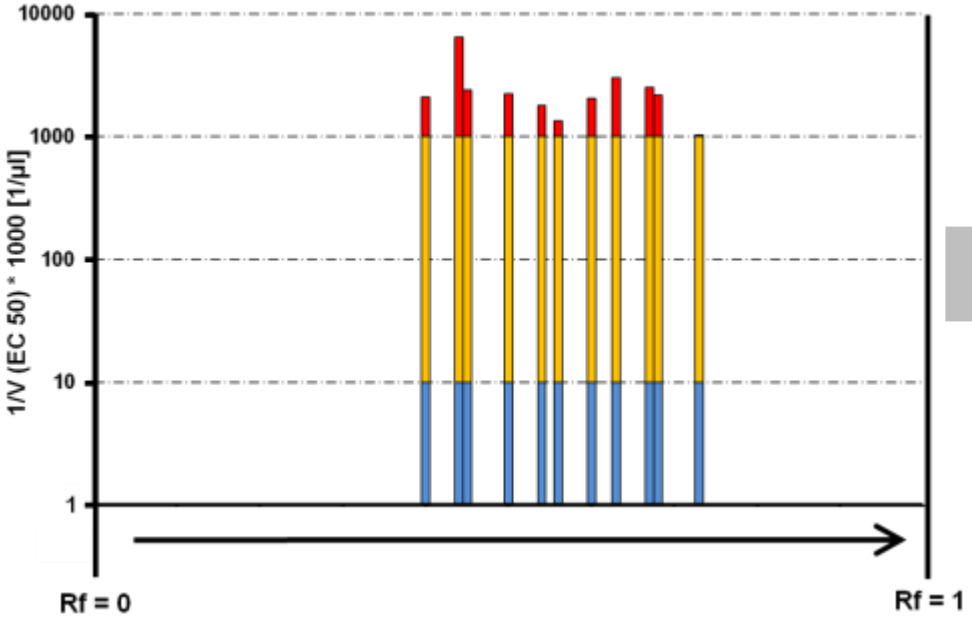
Reciprocal iso-inhibition volume (RIV)

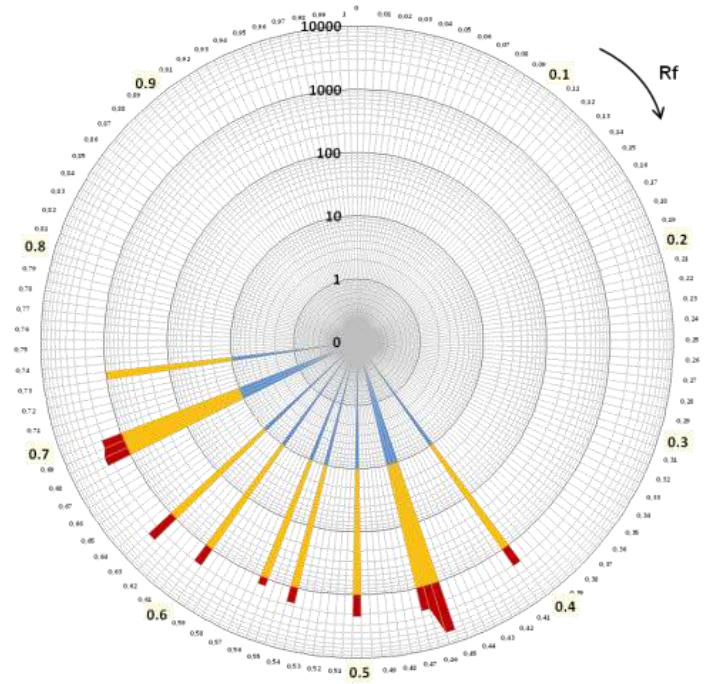
Zweckverband
Landeswasserversorgung



Reciprocal iso-inhibition volume (RIV)
Polar diagram

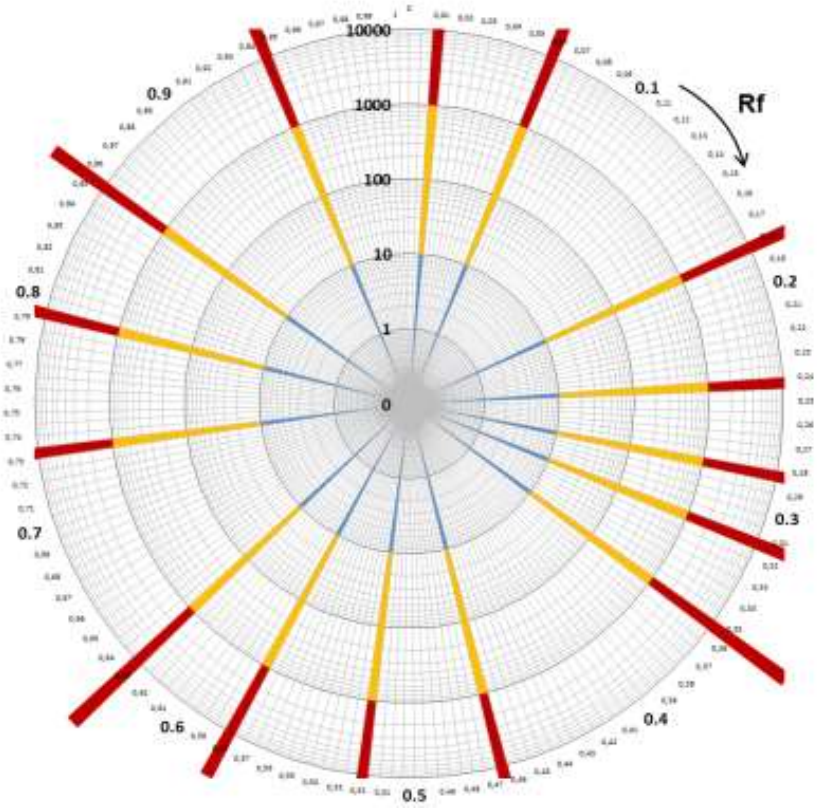
Zweckverband
Landeswasserversorgung

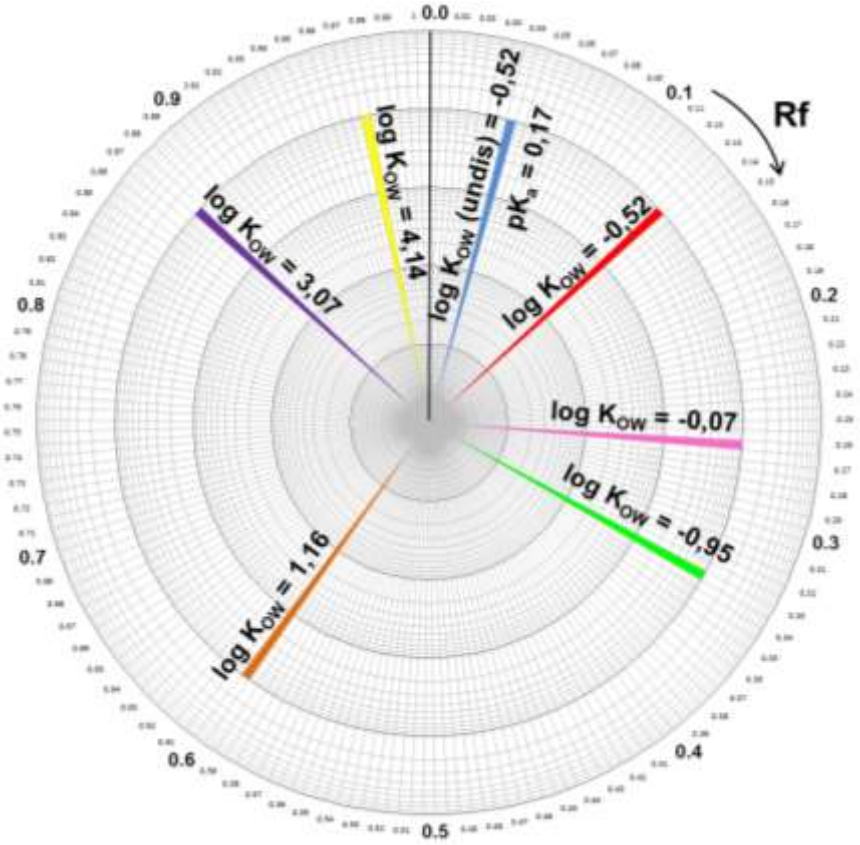




Estimation of polarity

Zweckverband
Landeswasserversorgung

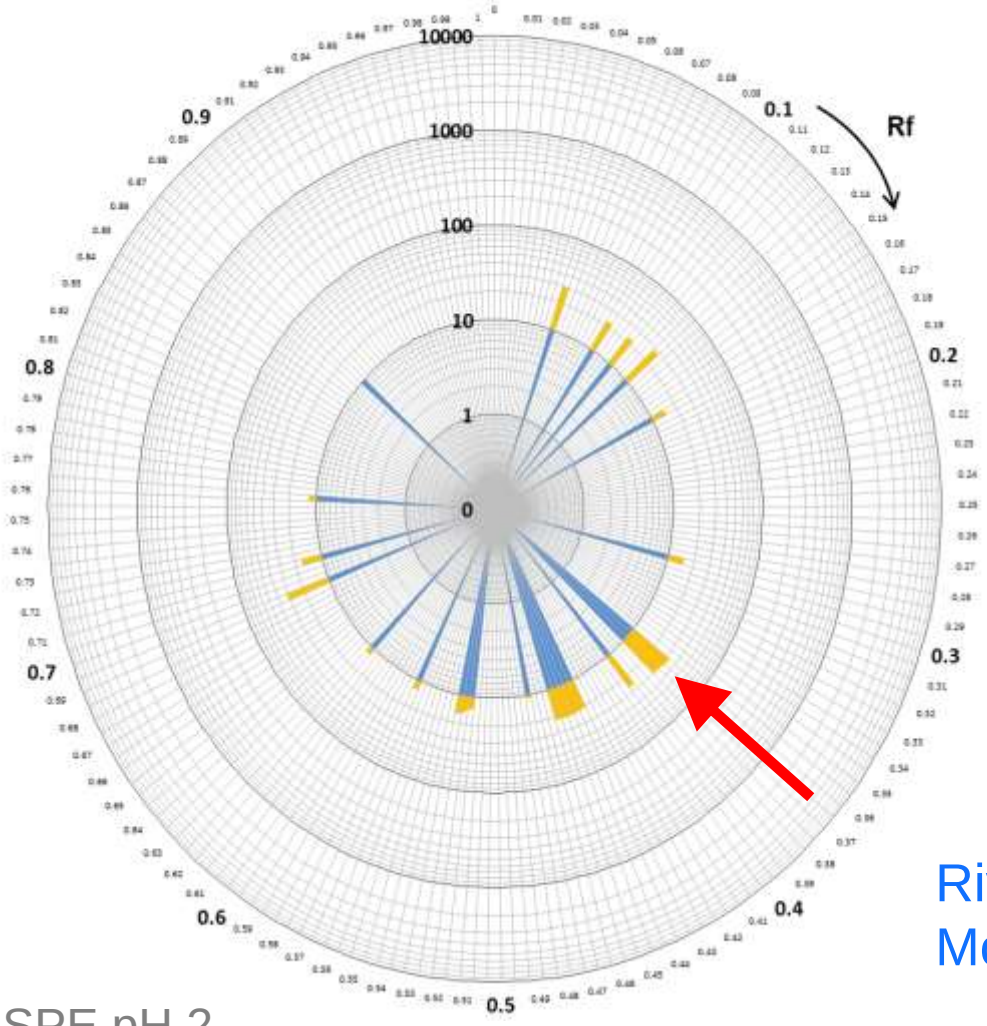




- 1 - Naphthalene sulfonic acid
- Theobromine
- Caffeine
- Thiourea
- Acetanilide
- Benzanilide
- Methyl yellow

EDA/HPTLC-analysis with Aliivibrio fischeri inhibition assay

Zweckverband Landeswasserversorgung

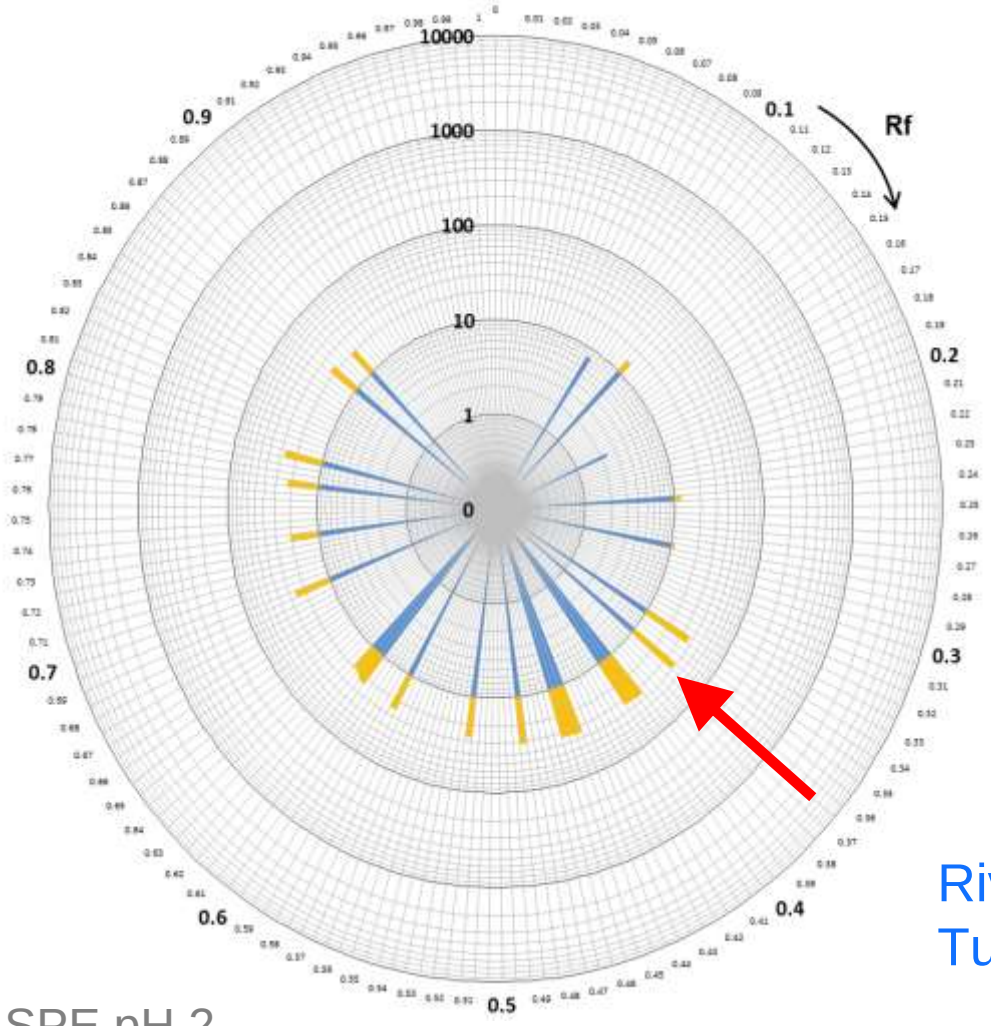


River Danube
Monday, March 7th, 2016

SPE pH 2

EDA/HPTLC-analysis with Aliivibrio fischeri inhibition assay

Zweckverband
Landeswasserversorgung

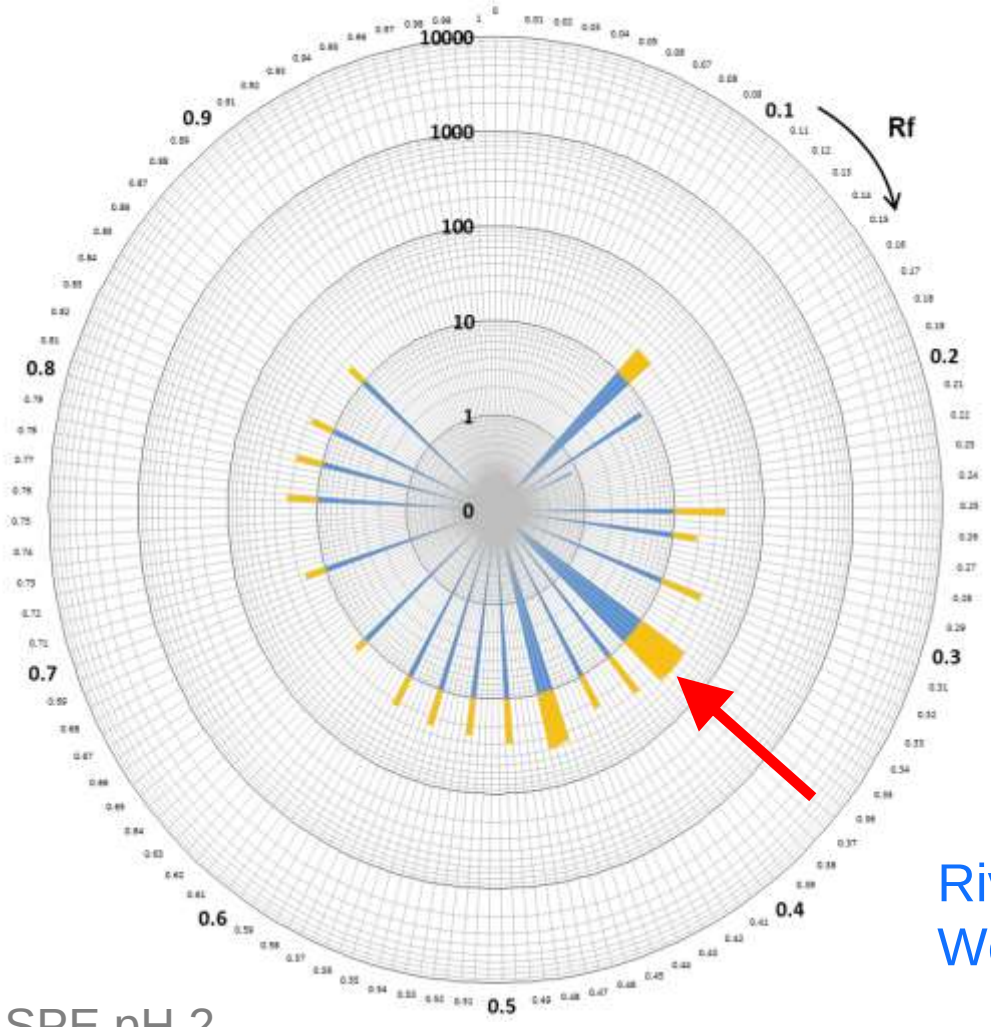


River Danube
Tuesday, March 8th, 2016

SPE pH 2

EDA/HPTLC-analysis with Aliivibrio fischeri inhibition assay

Zweckverband
Landeswasserversorgung

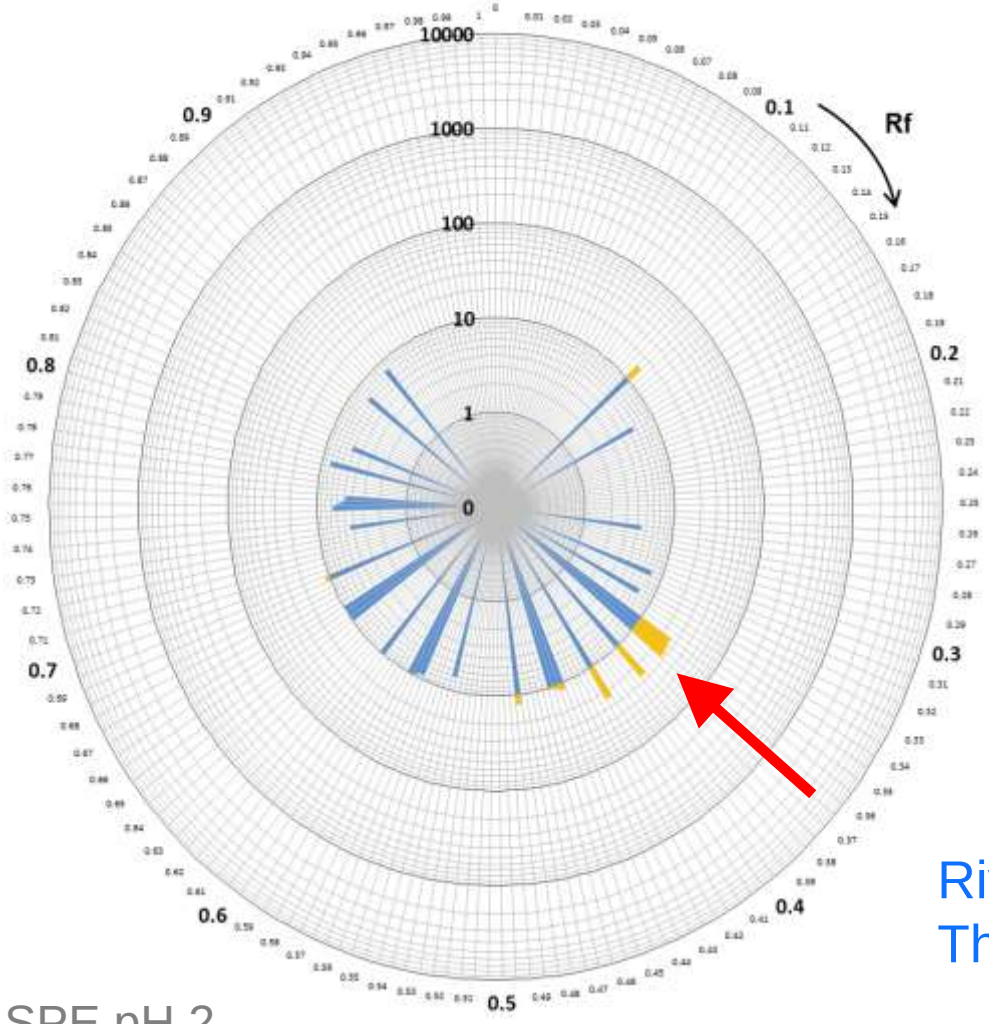


River Danube
Wednesday, March 9th, 2016

SPE pH 2

EDA/HPTLC-analysis with Aliivibrio fischeri inhibition assay

Zweckverband Landeswasserversorgung

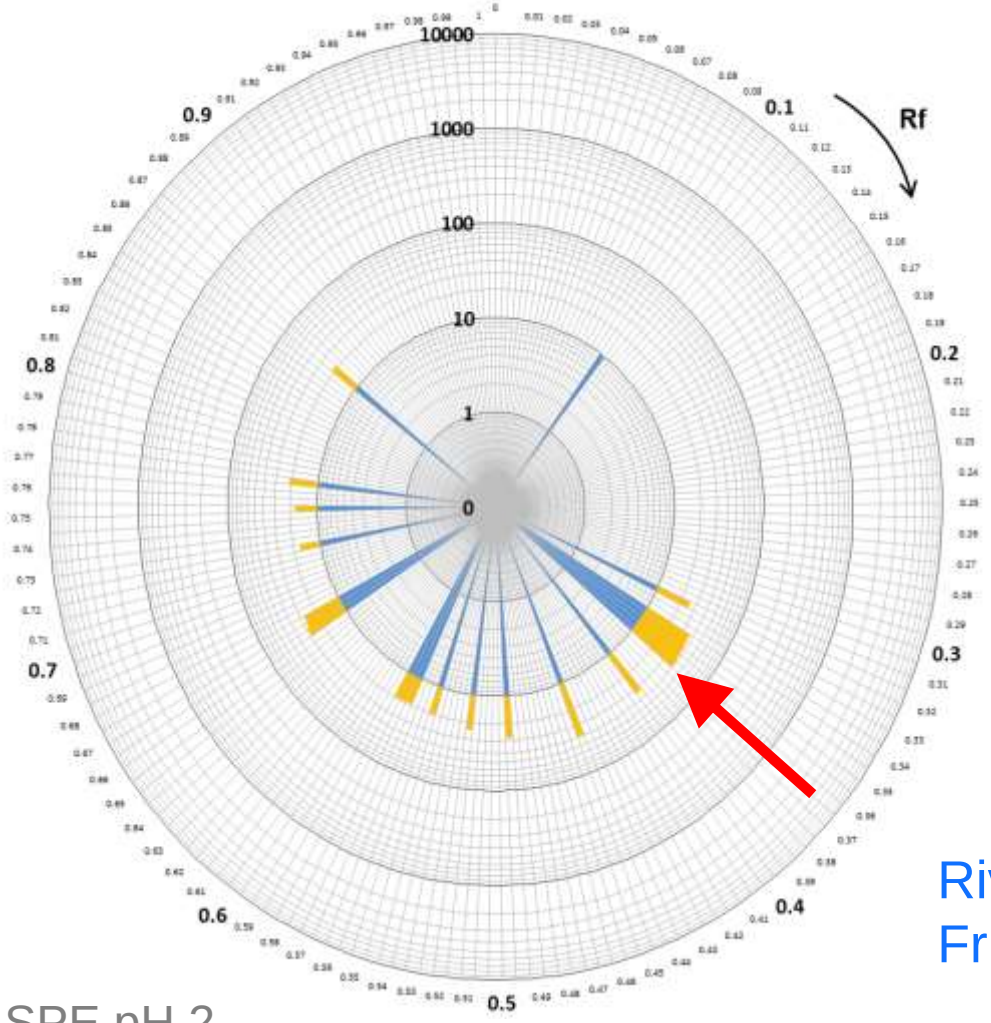


River Danube
Thursday, March 10th, 2016

SPE pH 2

EDA/HPTLC-analysis with Aliivibrio fischeri inhibition assay

Zweckverband
Landeswasserversorgung



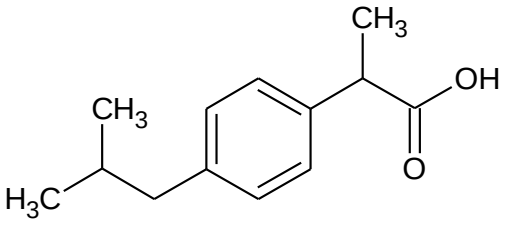
River Danube
Friday, March 11th, 2016

SPE pH 2

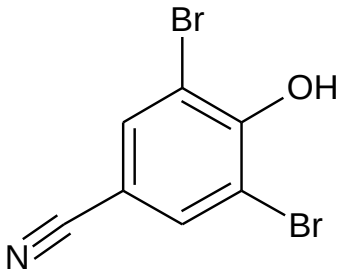
Repeatability of *Aliivibrio fischeri* inhibition (N = 80)

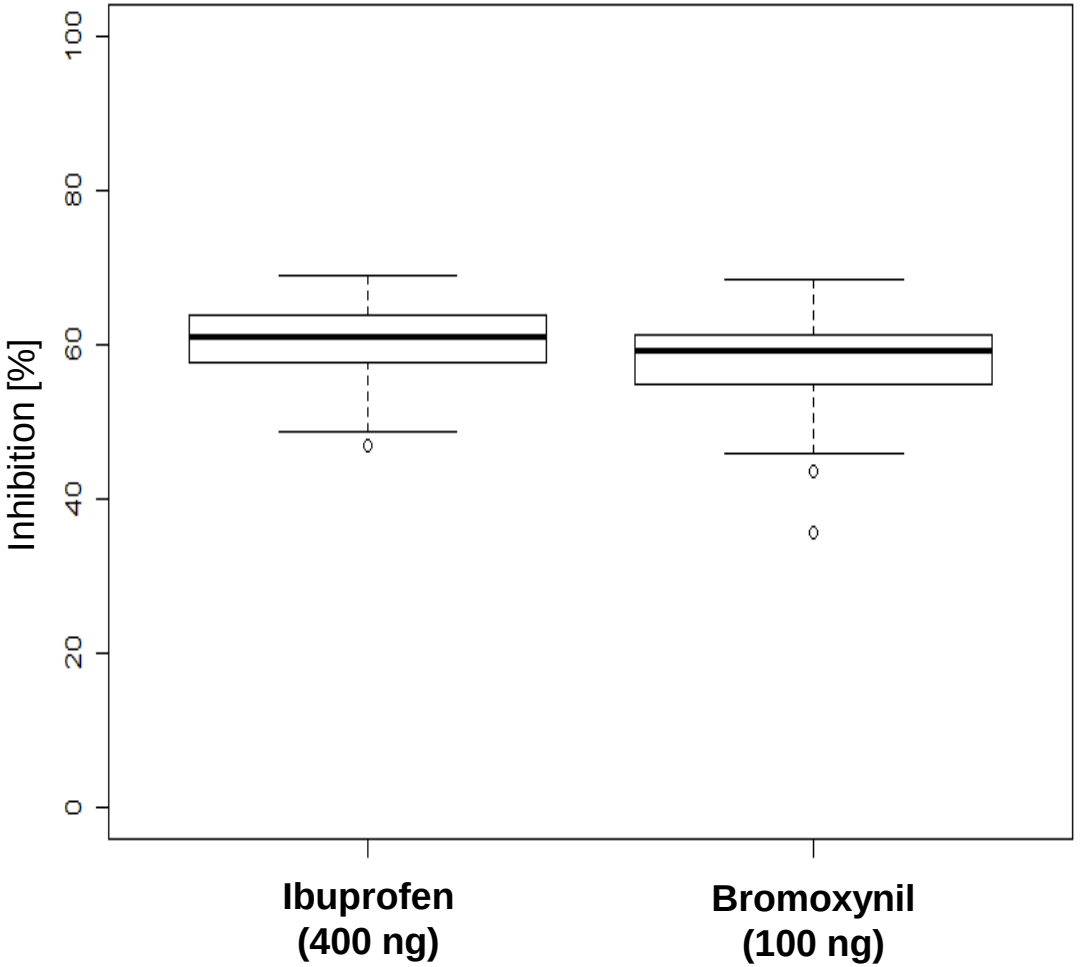
Zweckverband
Landeswasserversorgung

Ibuprofen



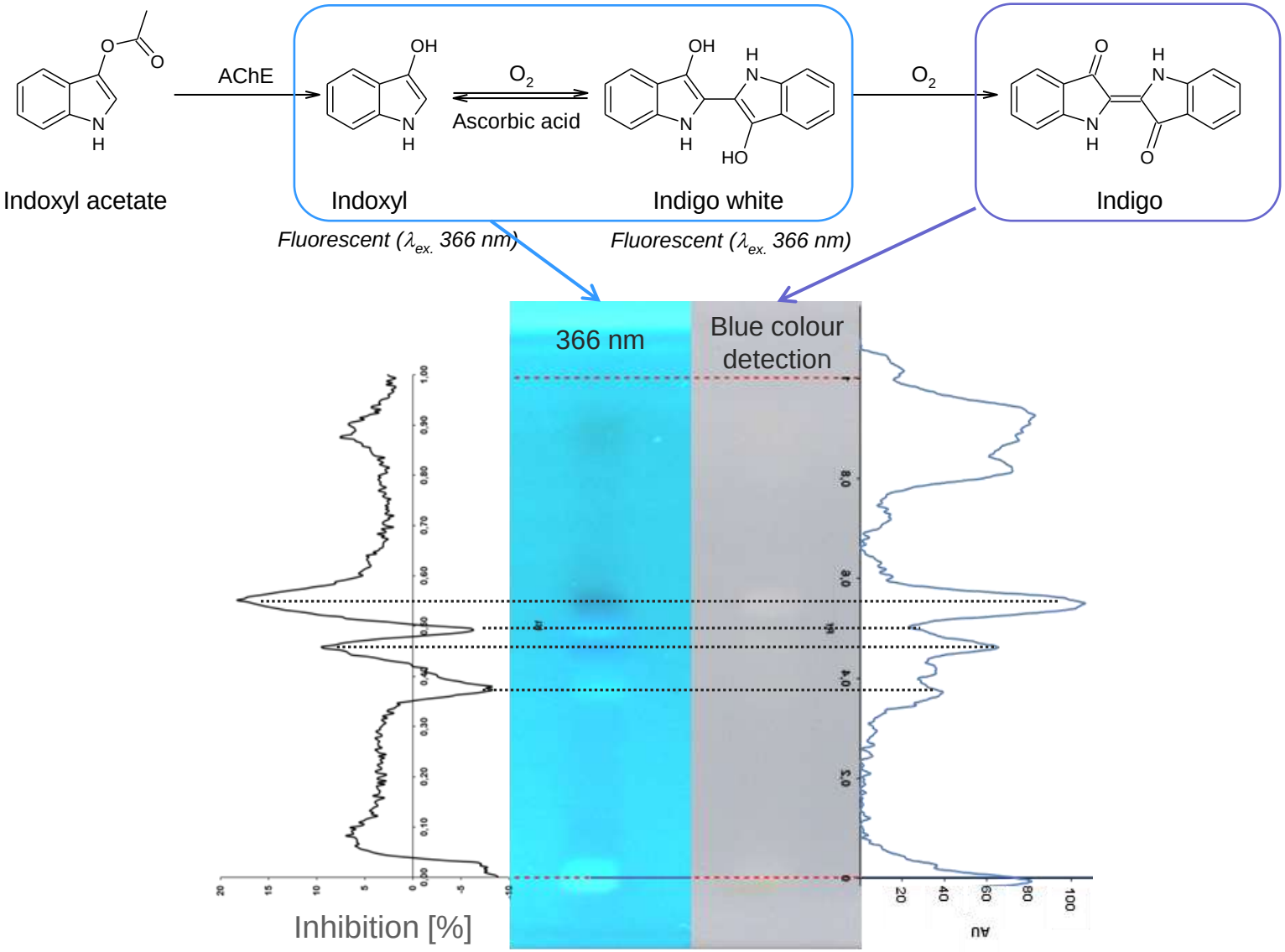
Bromoxynil





Detection of the inhibition of AChE

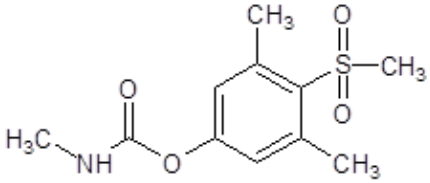
Zweckverband
Landeswasserversorgung



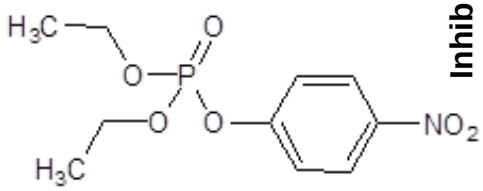
Repeatability of Acetylcholinesterase inhibition (N = 40)

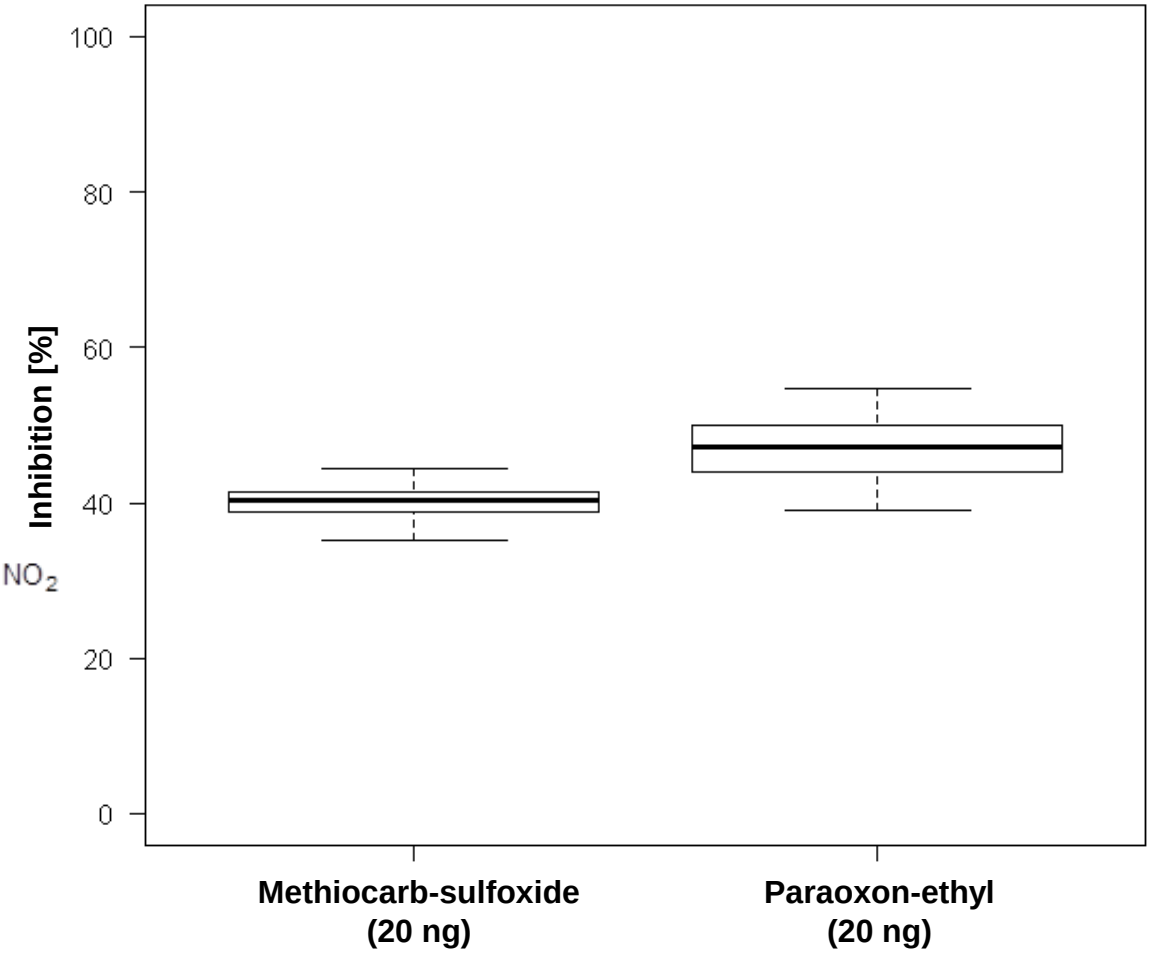
Zweckverband Landeswasserversorgung

Methiocarb-sulfoxide



Paraoxon-ethyl



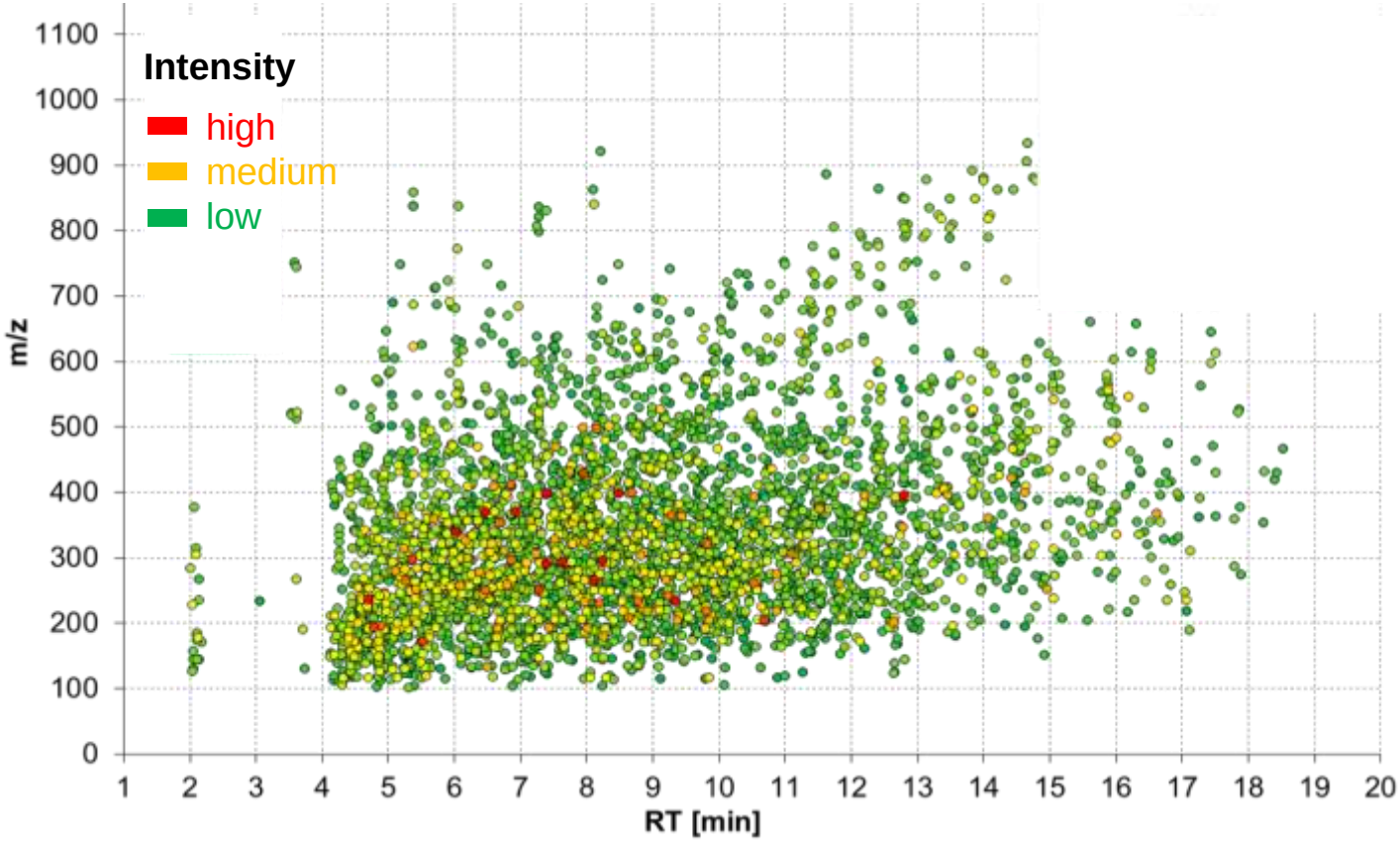


Effluent of the industrial sewage treatment plant

Zweckverband Landeswasserversorgung

Luminescence bacteria

AChE



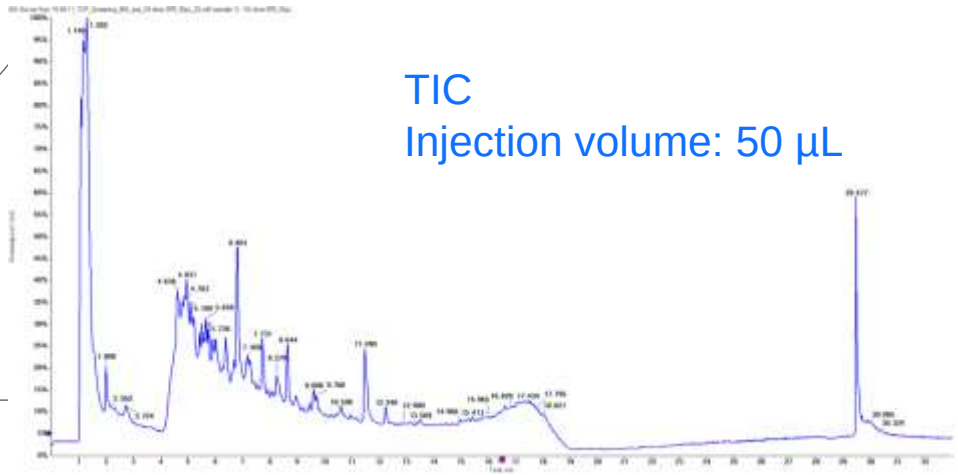
Treatment of wastewater from a contaminated site

Zweckverband
Landeswasserversorgung

Contaminated site

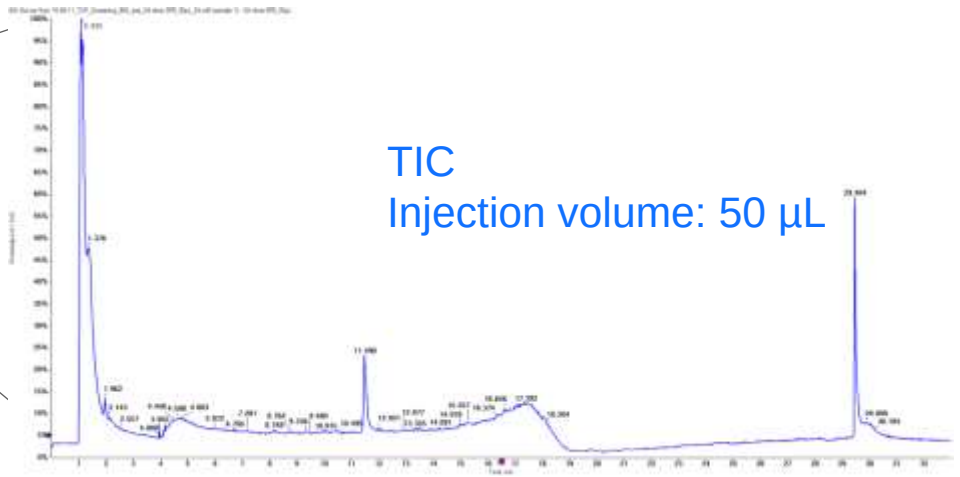
Treatment step

original sample before treatment



TIC
Injection volume: 50 µL

original sample after treatment



TIC
Injection volume: 50 µL

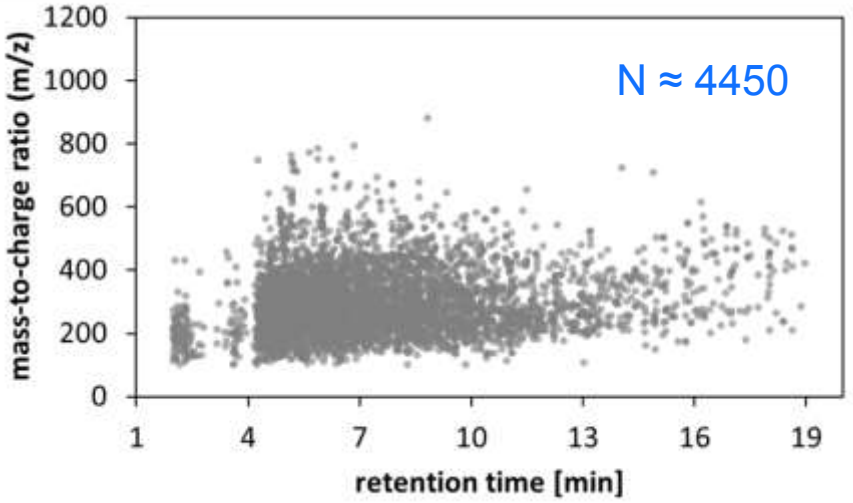
Treatment of wastewater from a contaminated site

Zweckverband Landeswasserversorgung

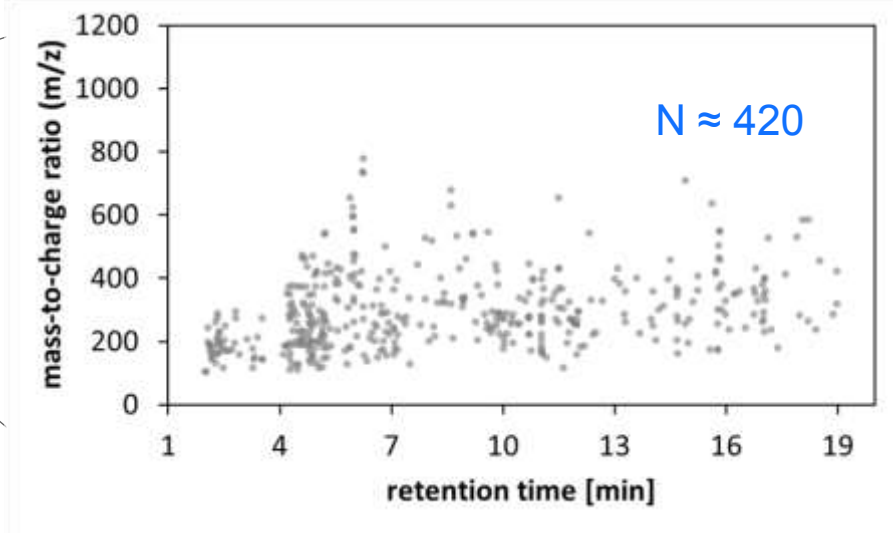
Contaminated site

Treatment step

Mass-RT scatterplot original sample before treatment

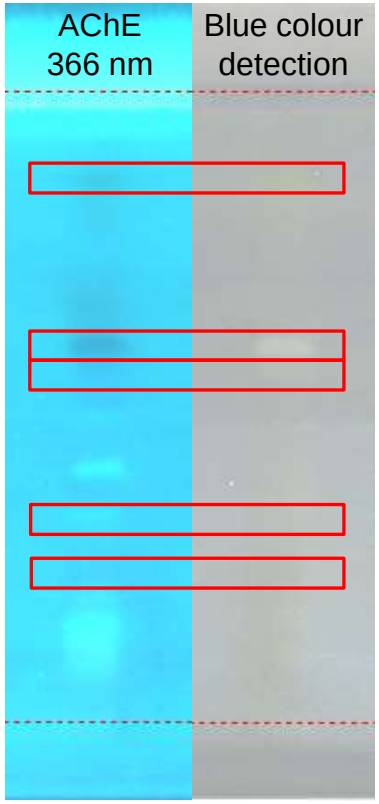


Mass-RT scatterplot original sample after treatment



Extraction for HPLC-HRMS

Zweckverband
Landeswasserversorgung



Effect-directed analysis

TLC-MS Interface

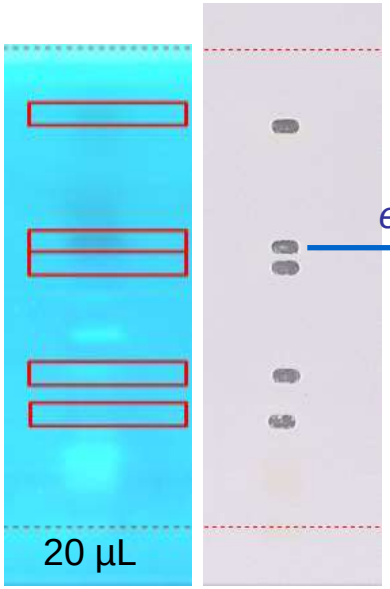
HPLC-HRMS

same chromatography
for water sample
and TLC extract

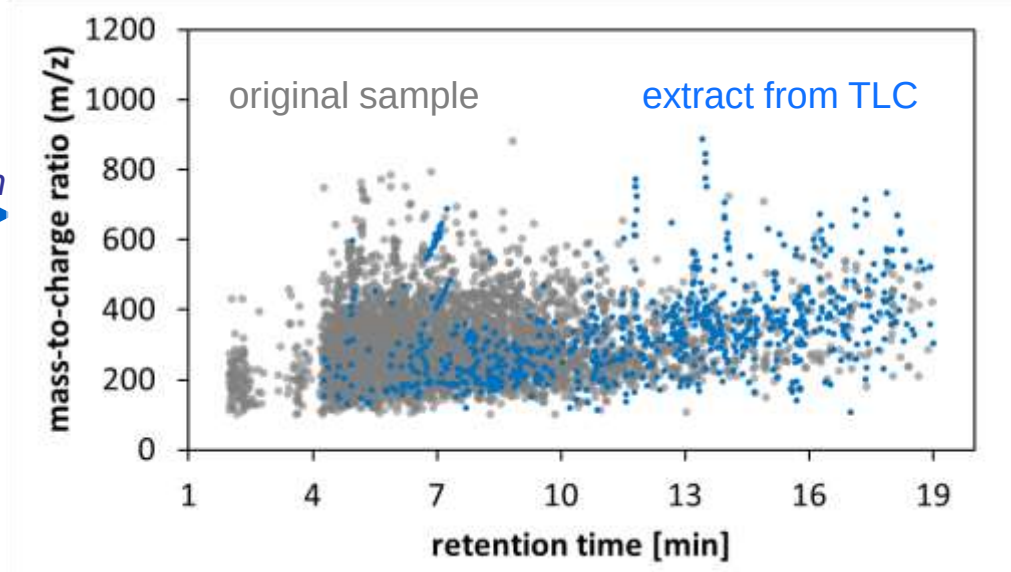
Extraction for HPLC-HRMS

Zweckverband
Landeswasserversorgung

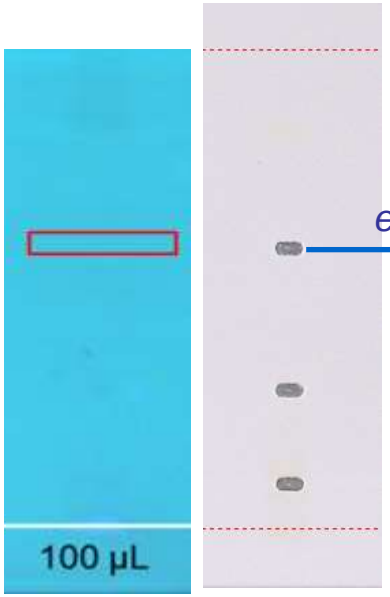
before treatment



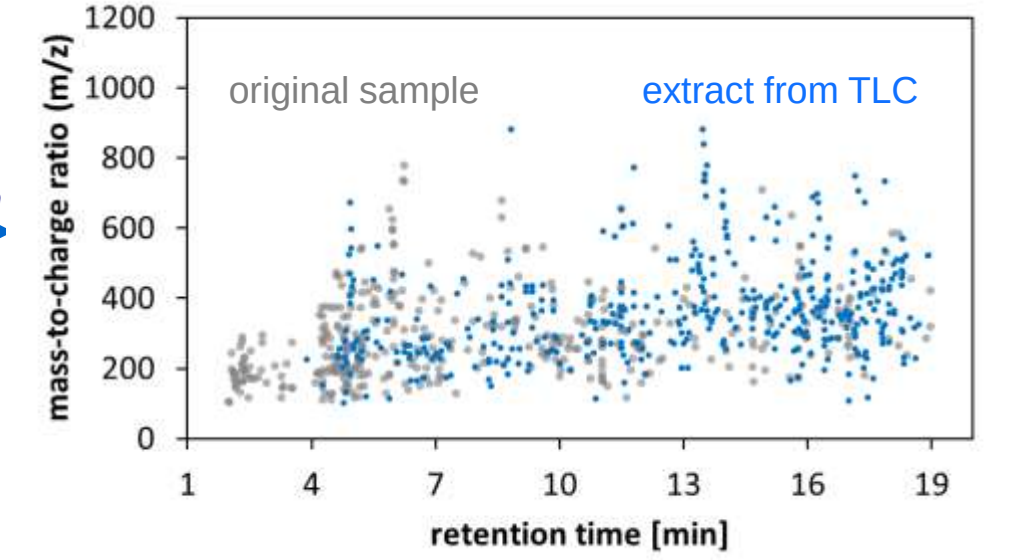
extraction



after treatment

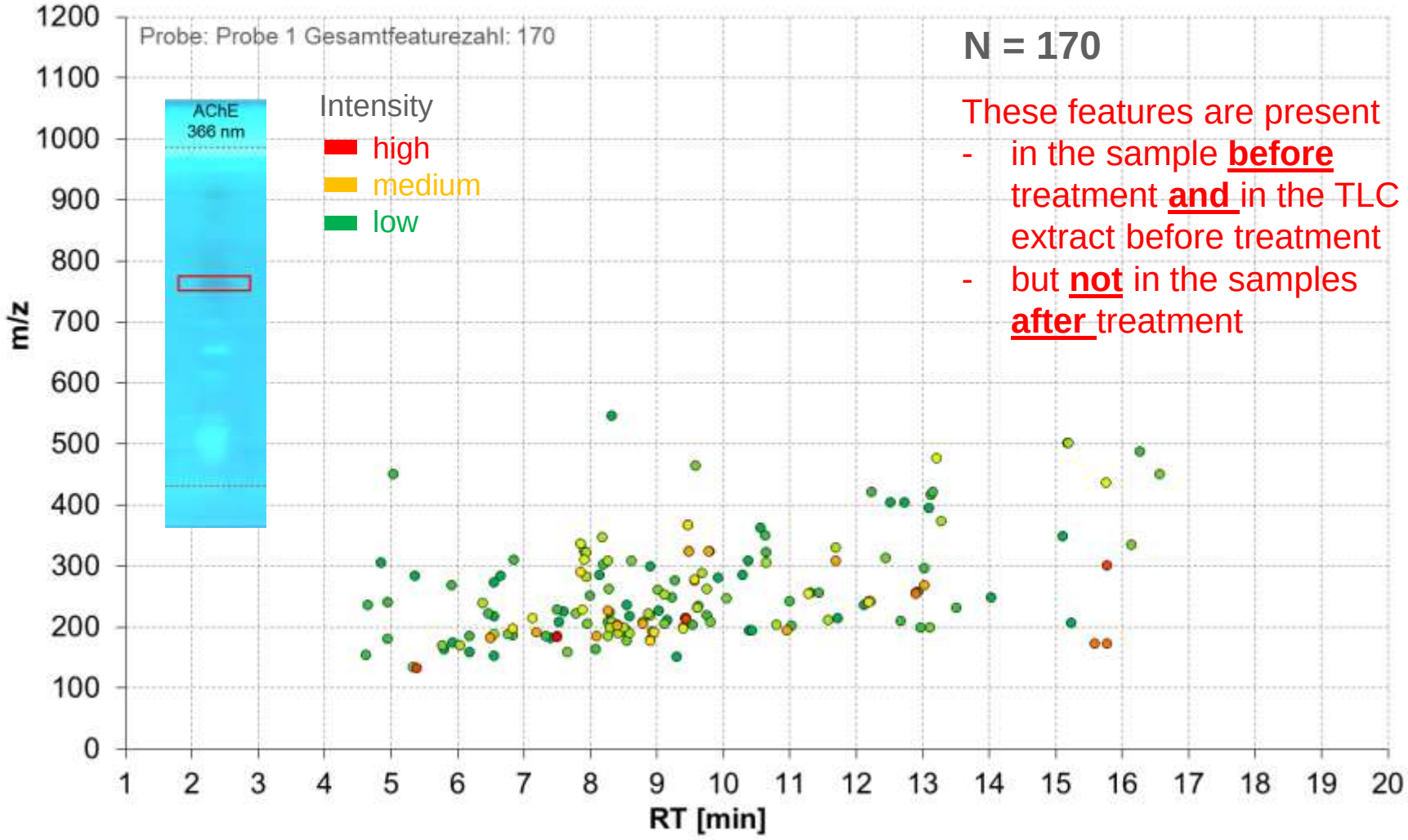


extraction



RT-m/z scatterplot of AChE inhibition zone

Zweckverband
Landeswasserversorgung



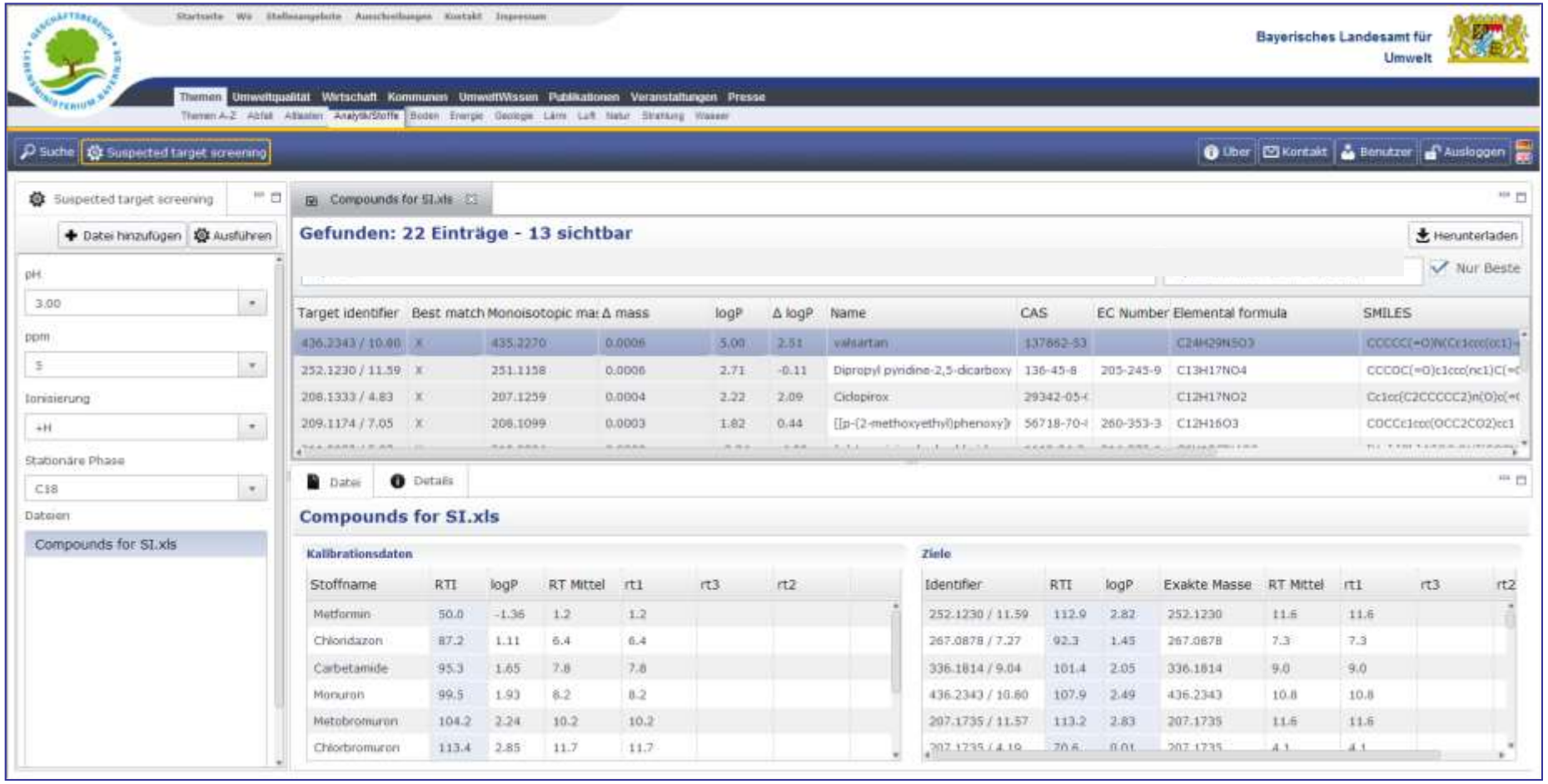
N = 170

These features are present

- in the sample **before** treatment **and** in the TLC extract before treatment
- but **not** in the samples **after** treatment

Stoff-Ident Database Search

Zweckverband
Landeswasserversorgung

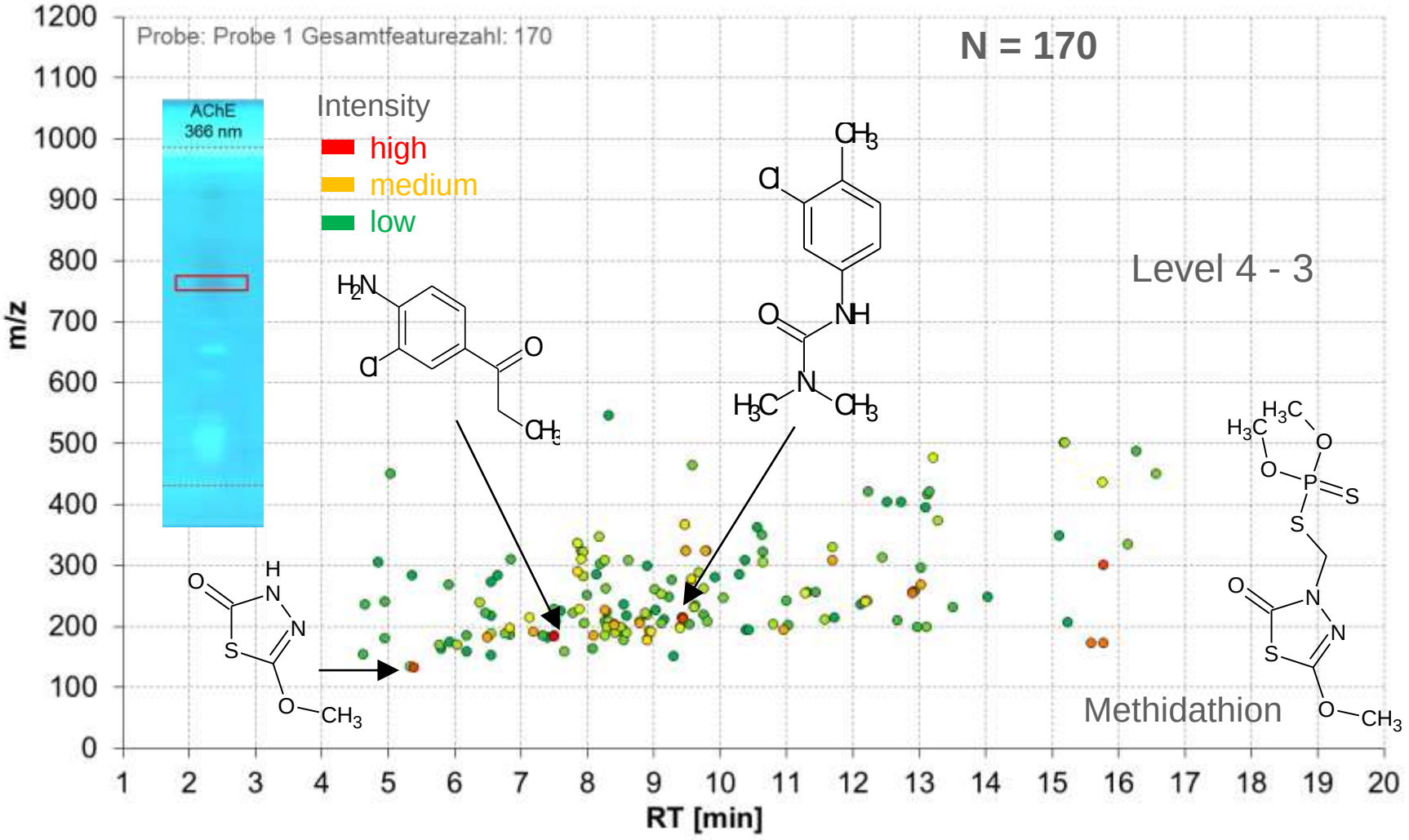


RISK IDENT Identifizieren | Bewerten Handeln | Kommunizieren

gefördert vom: Bundesministerium für Bildung und Forschung

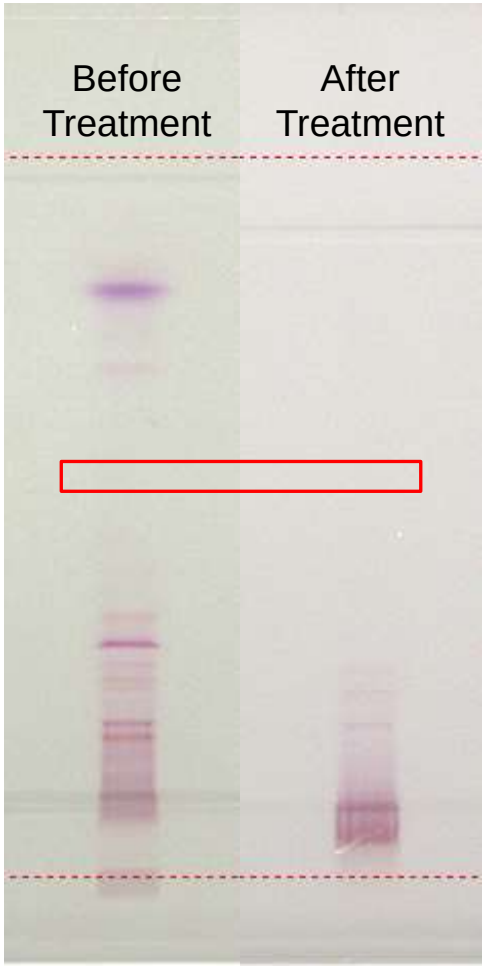
RT-m/z scatterplot of AChE inhibition zone

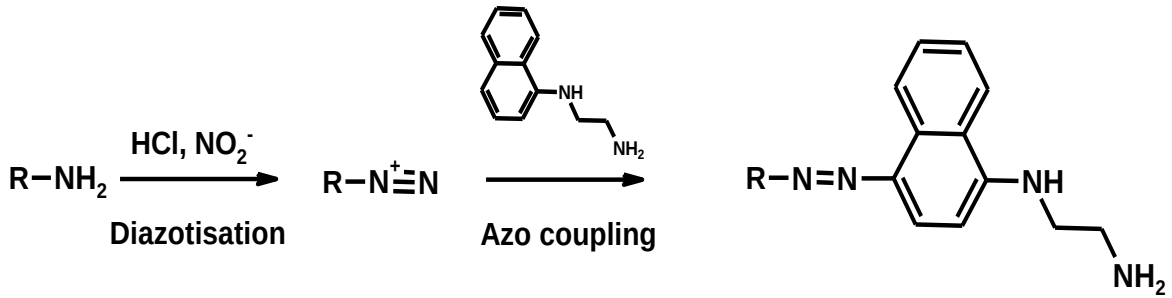
Zweckverband
Landeswasserversorgung

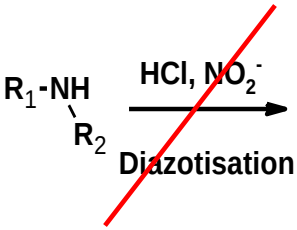


Detection of primary amino groups with Bratton-Marshall reagent

Zweckverband Landeswasserversorgung

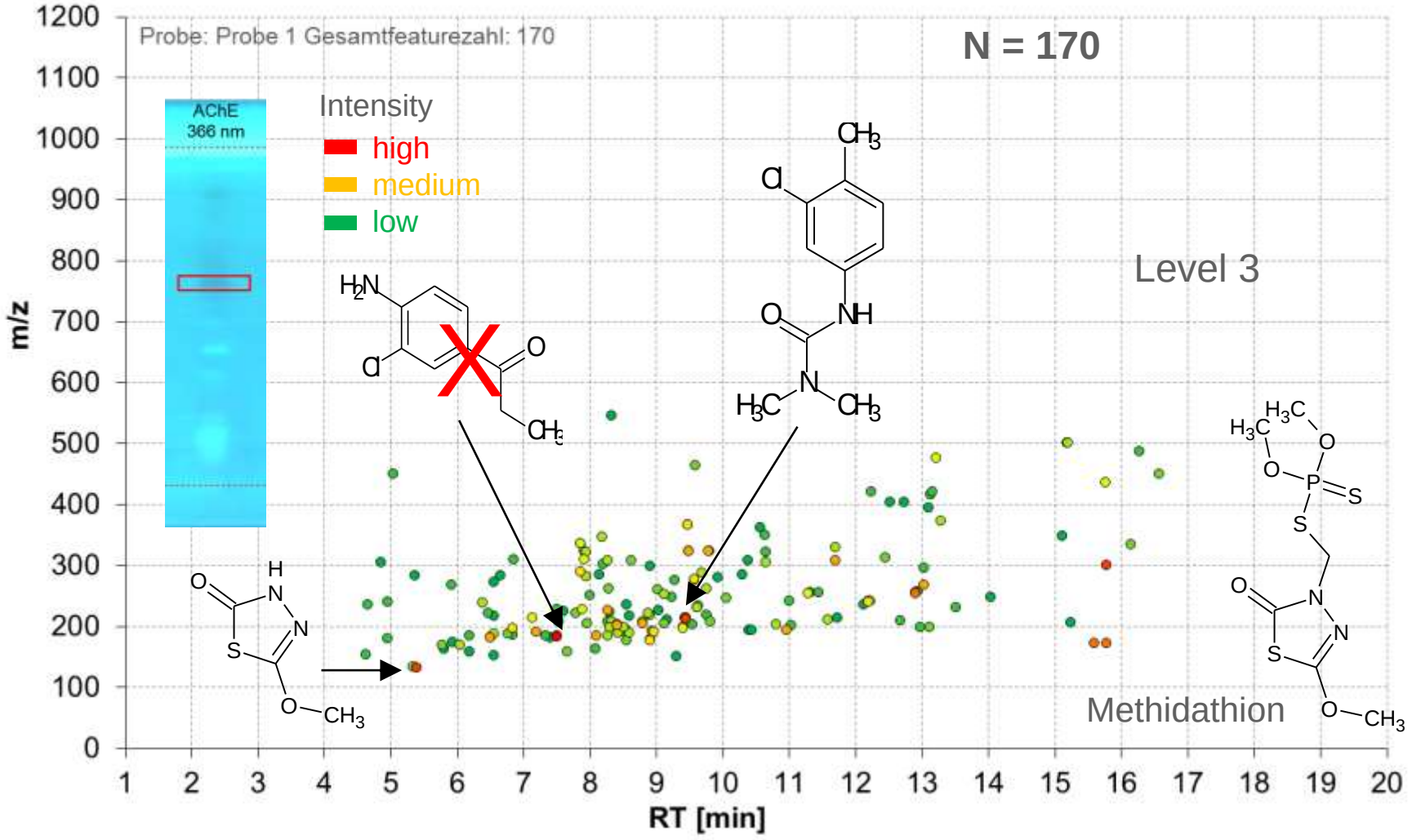






RT-m/z scatterplot of AChE inhibition zone

Zweckverband
Landeswasserversorgung



Conclusions

- Application of set theory with the Mass-RT scatterplot is a tool for prioritization of features (i.e. Process description, identifying relevant sources of contamination)
- EDA in combination with TLC is a second dimension to distinguish samples
- EDA with TLC and different endpoints is a powerful tool for Prioritization in non-target analysis
- Non-target analysis in combination with EDA makes the gap between detection and assessment smaller

Zweckverband
Landeswasserversorgung

Thank you
for your attention!