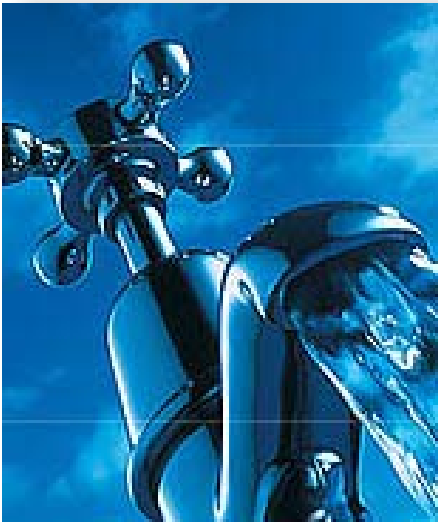


Club de CCM - 15<sup>ème</sup> année  
6 Juin 2013

# HPTLC/AMD-EDA - HPLC-qTOF/MS in water and environment analysis



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# Content

- Introduction
- Identification of pesticides in ground water
- Ozonation reaction products
- Effect-Directed Analysis with *Vibrio fischeri*
- Ground water contamination from synthetic turf
- Conclusion

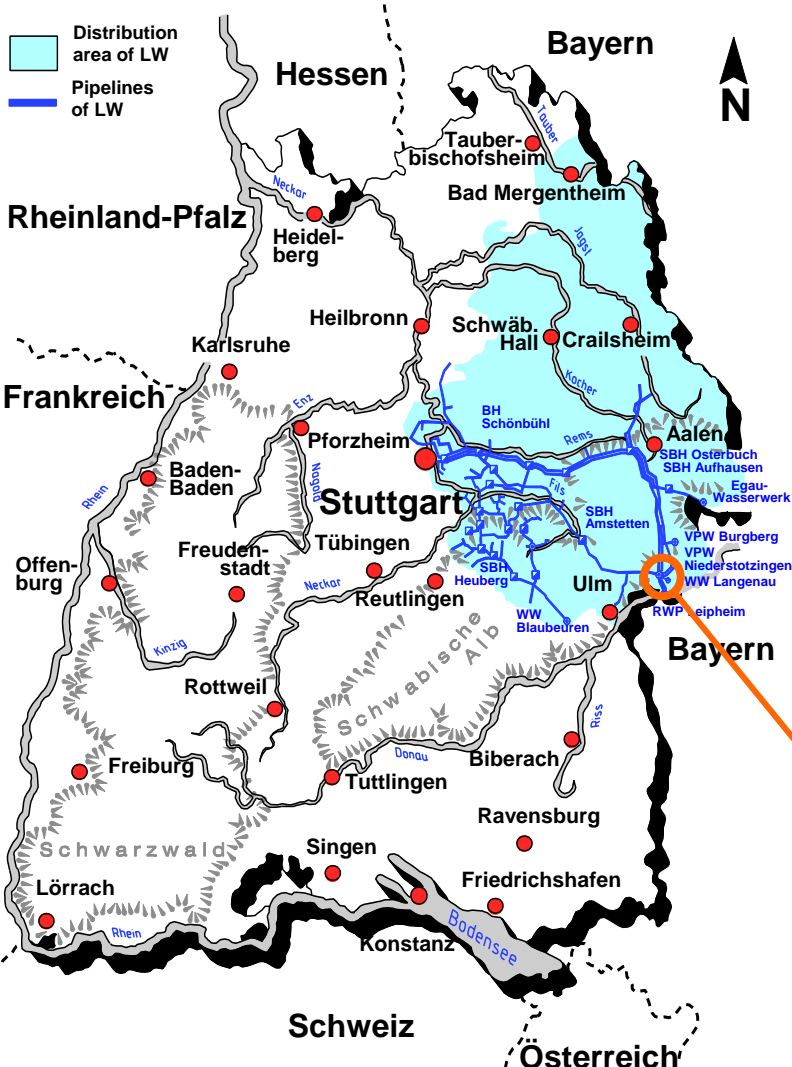
# Content

- **Introduction**
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- Conclusion

# Landeswasserversorgung is situated in South Germany



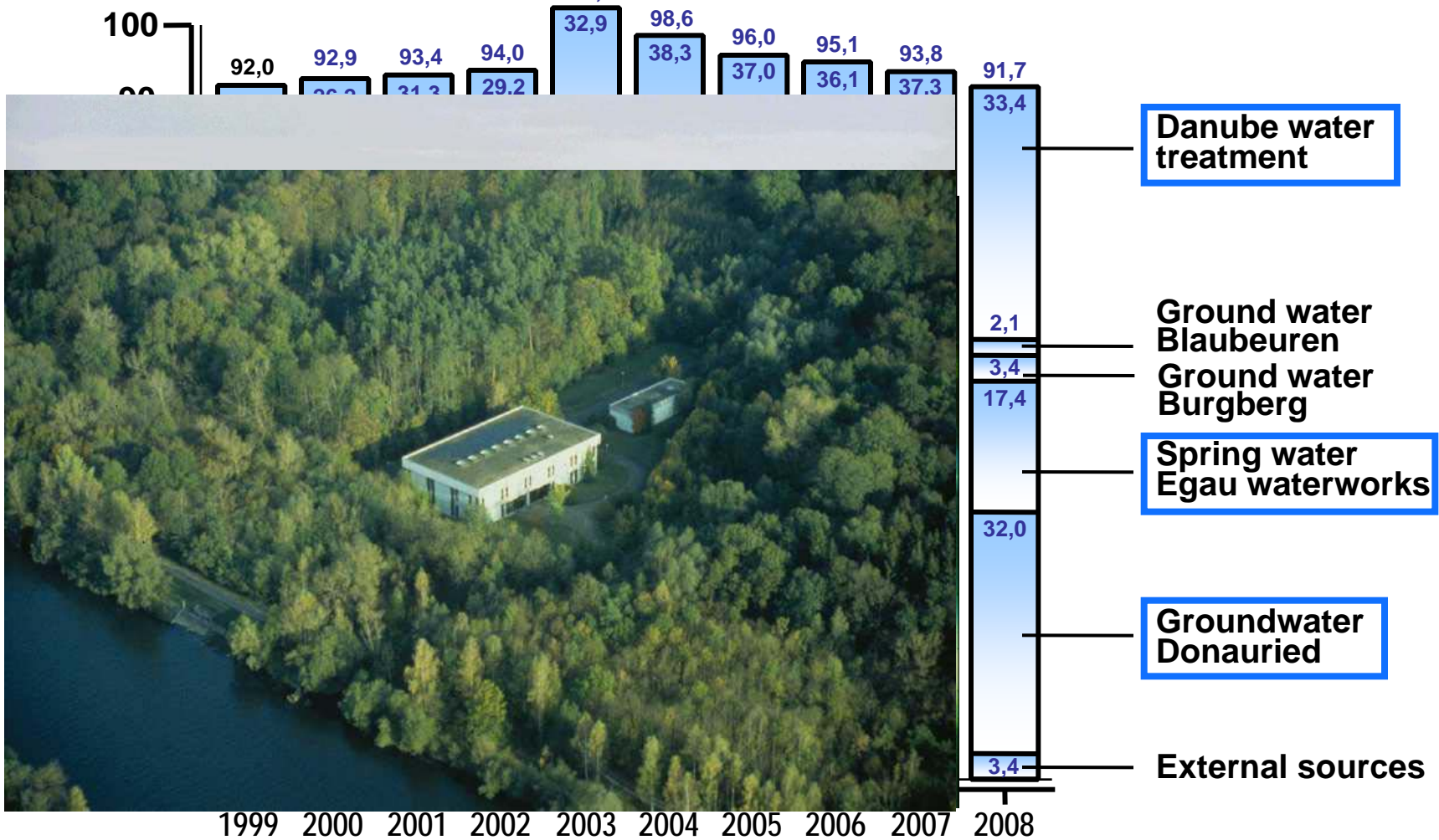
# Distribution area of LW in South West Germany



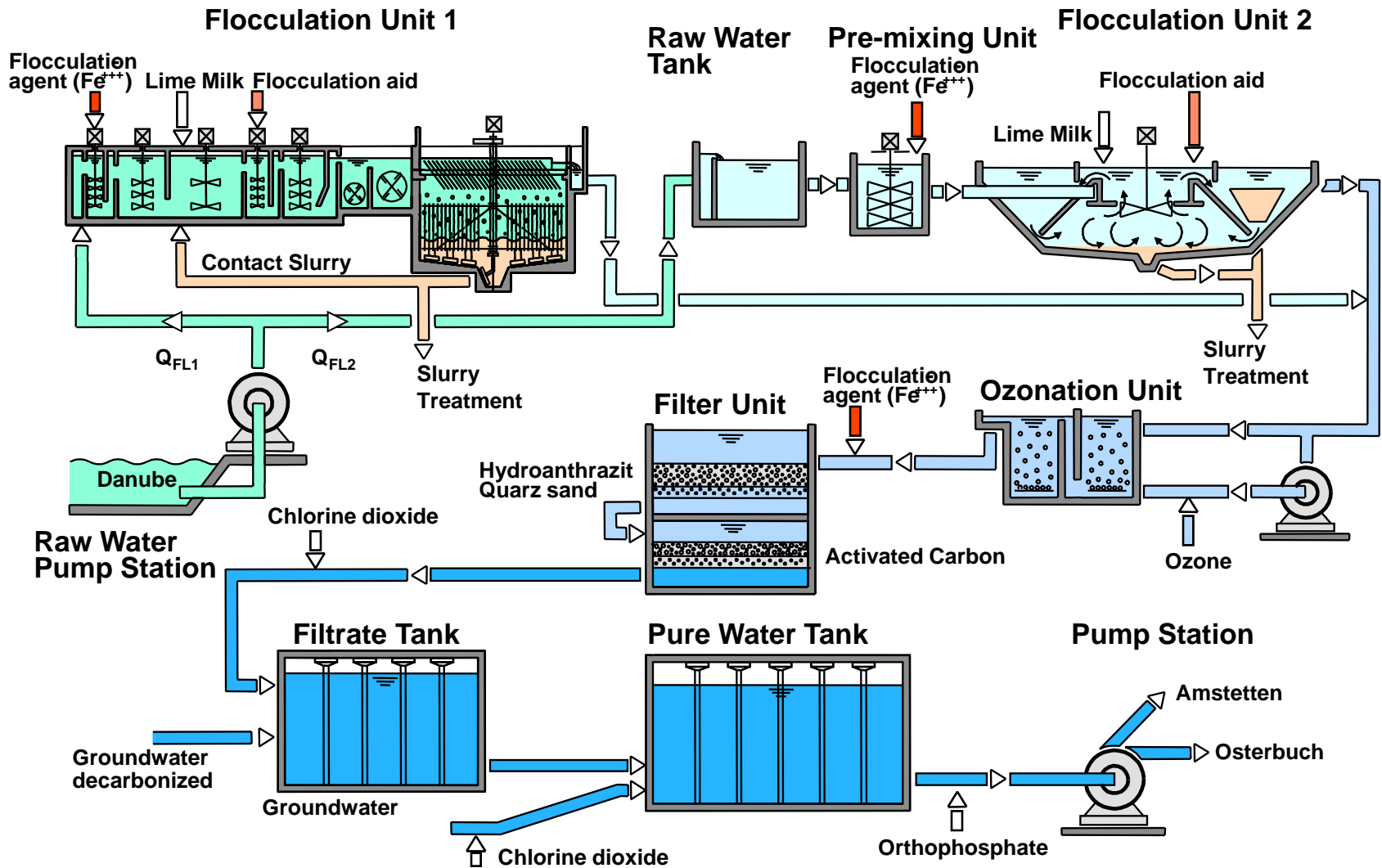
- Distribution area in South West Germany
  - 3 Million customers within distribution area
  - Long-distance water fraction approx. 50%
- Langenau Waterworks**

# Water treatment from different resources

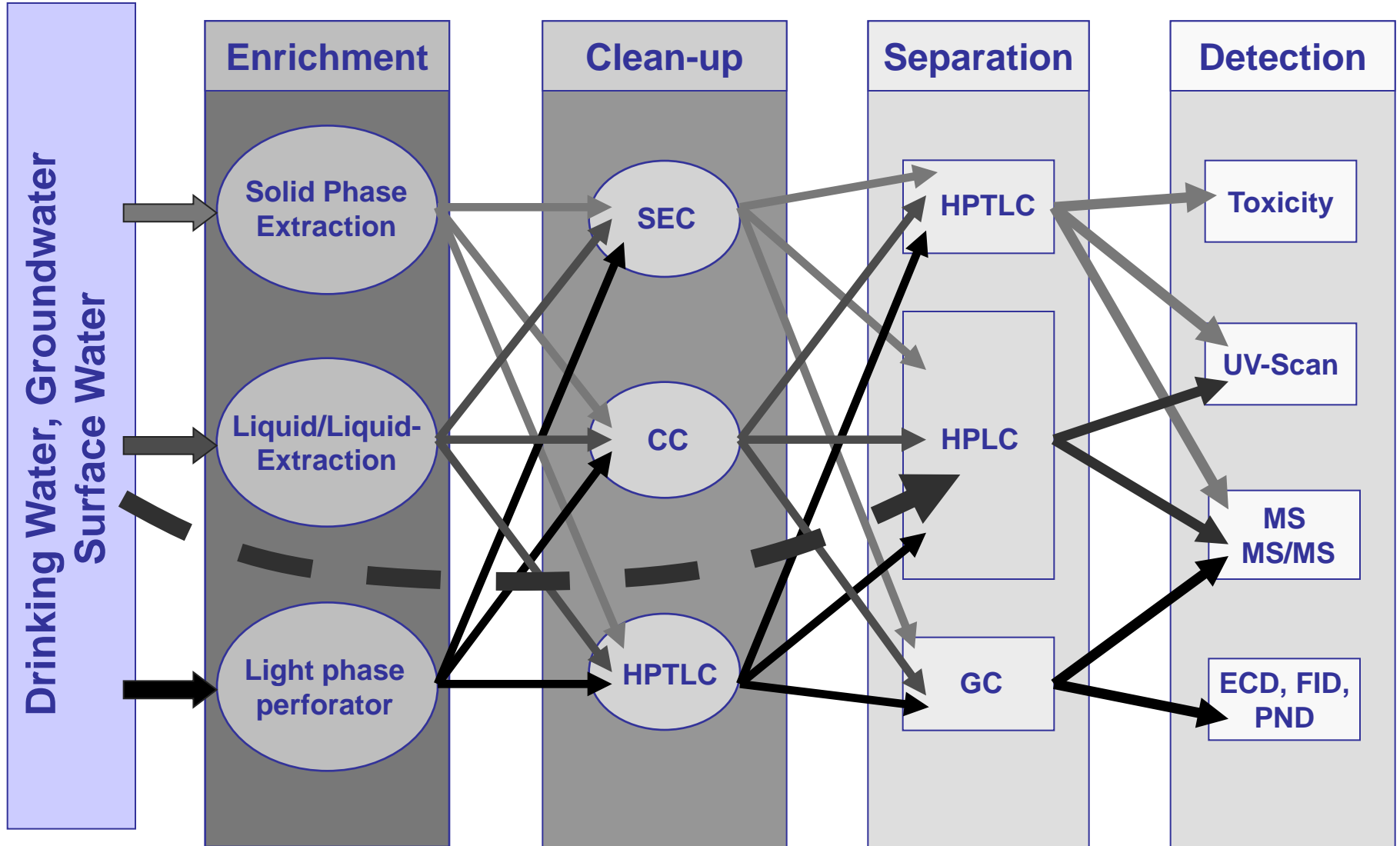
Million m<sup>3</sup> / Year



# Treatment of Danube water at Langenau waterworks

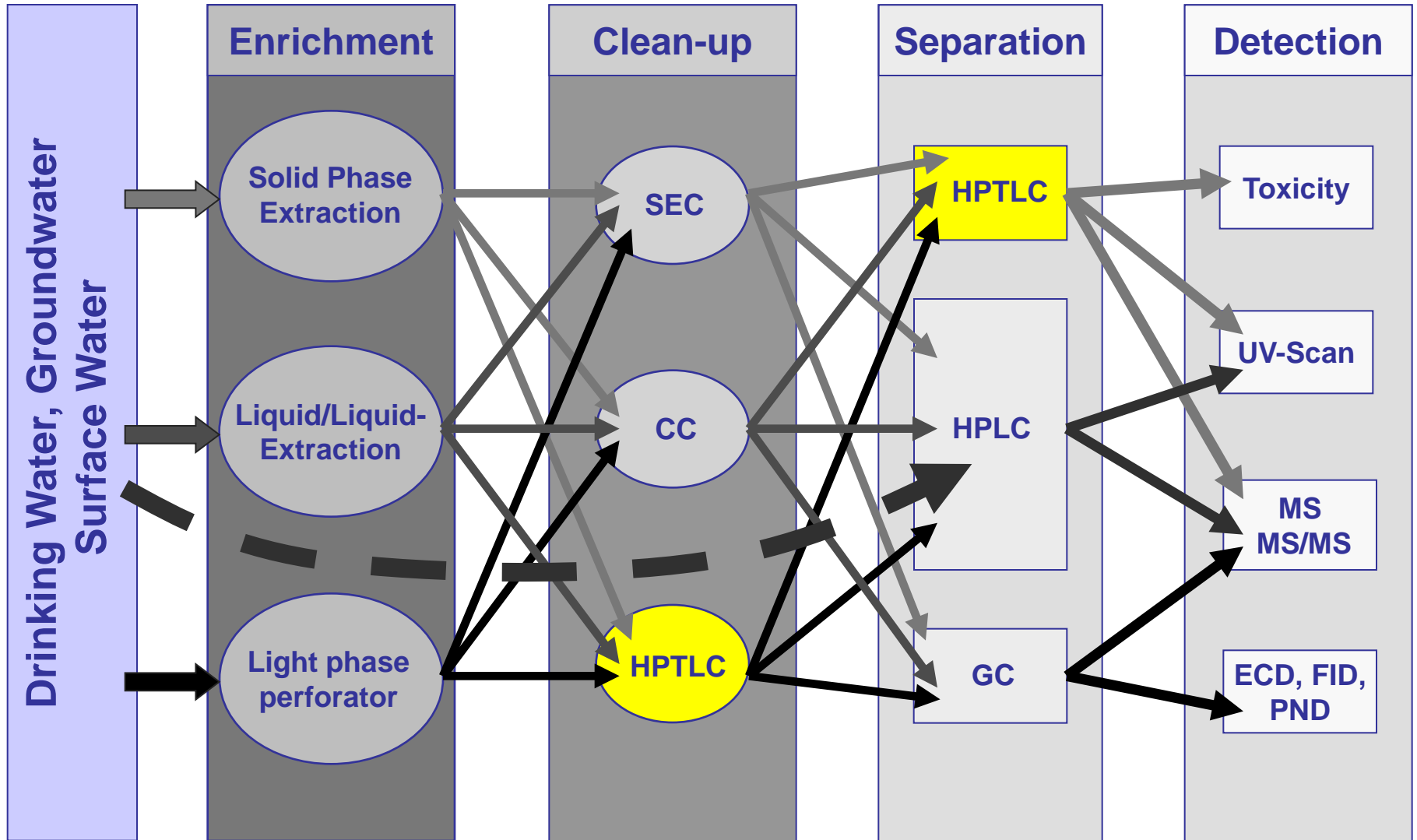


# „Multidimensional Screening“





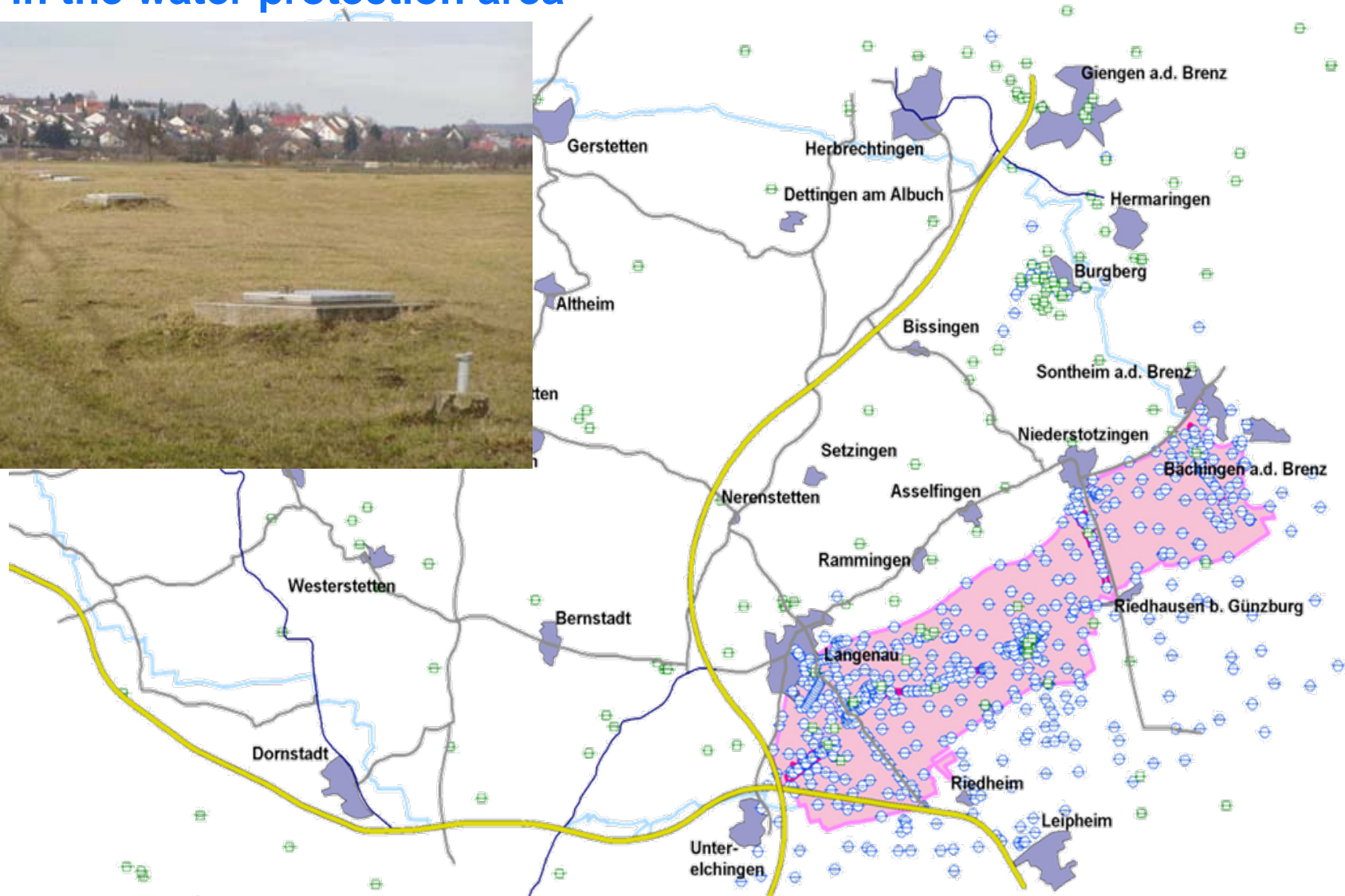
# „Multidimensional Screening“



# Content

- Introduction
- **Identification of pesticides in ground water**
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# Map of ground water monitoring wells in the water protection area



# Schematic diagram of the HPTLC/AMD analysis

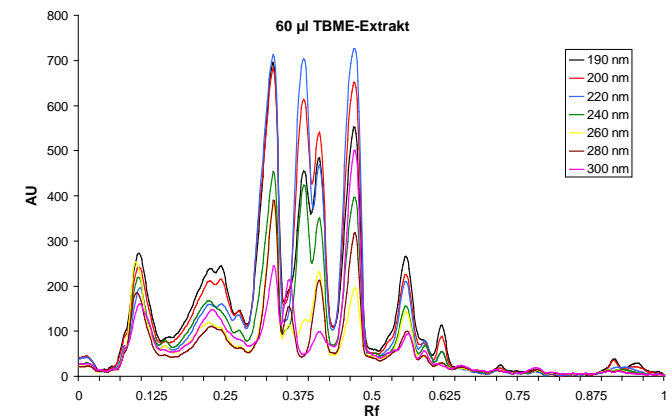
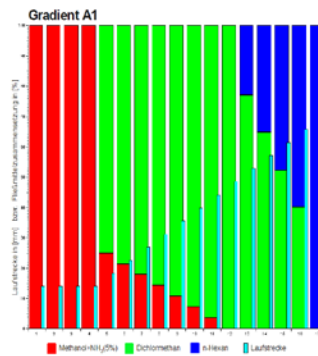
**Application**  
TLC Sampler 4



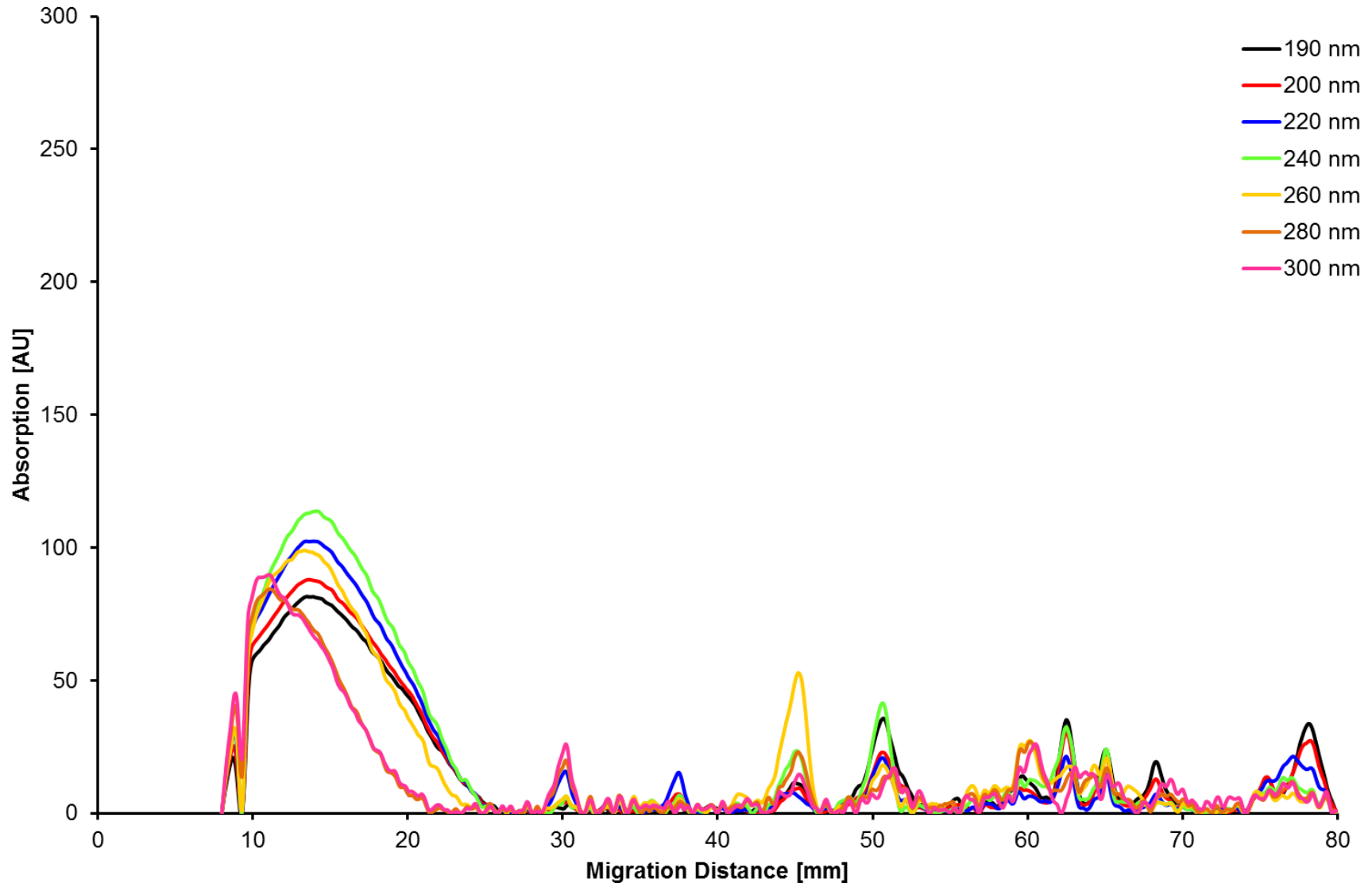
**Development**  
AMD 2



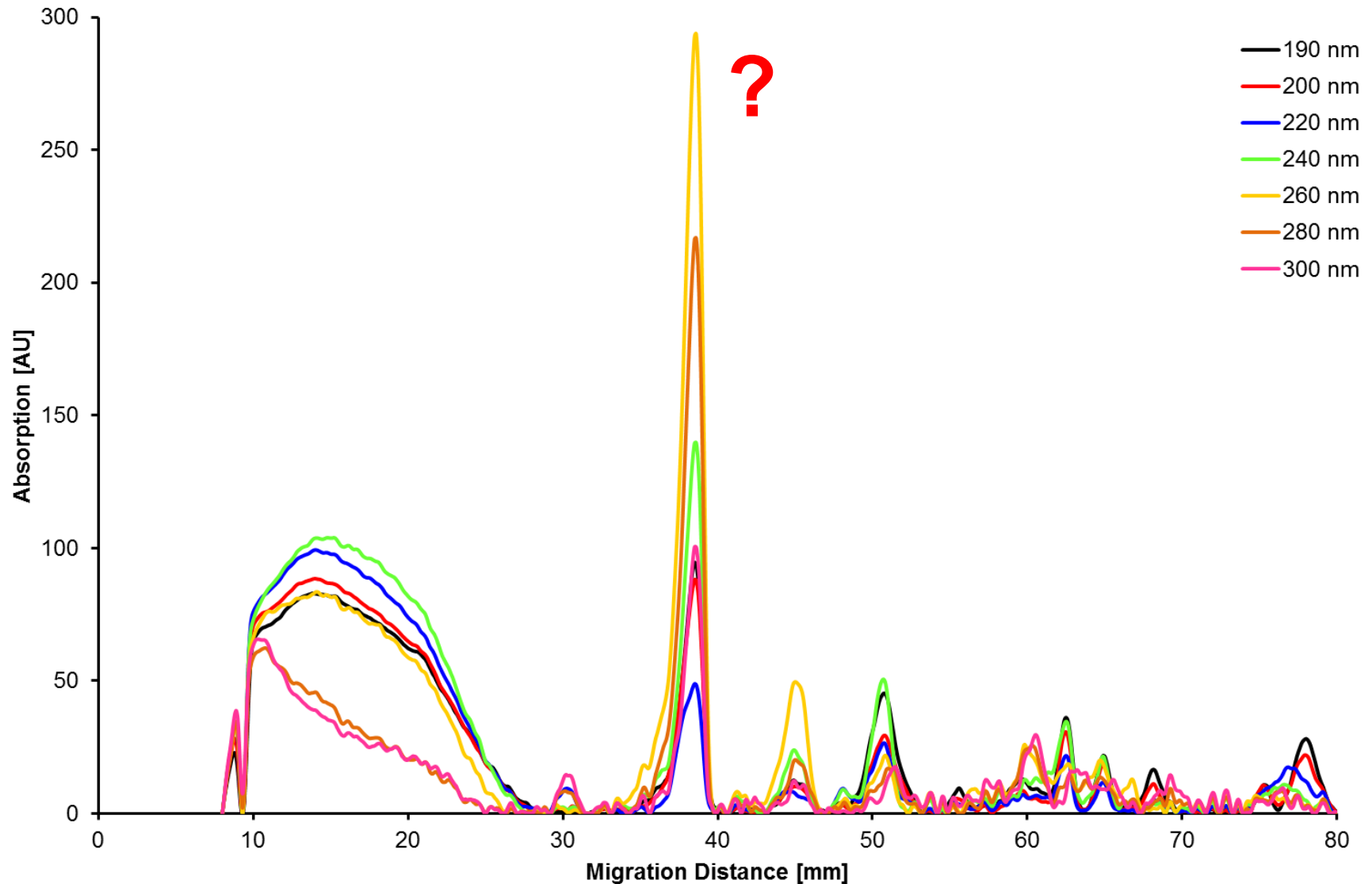
**Detection**  
Scanner 3



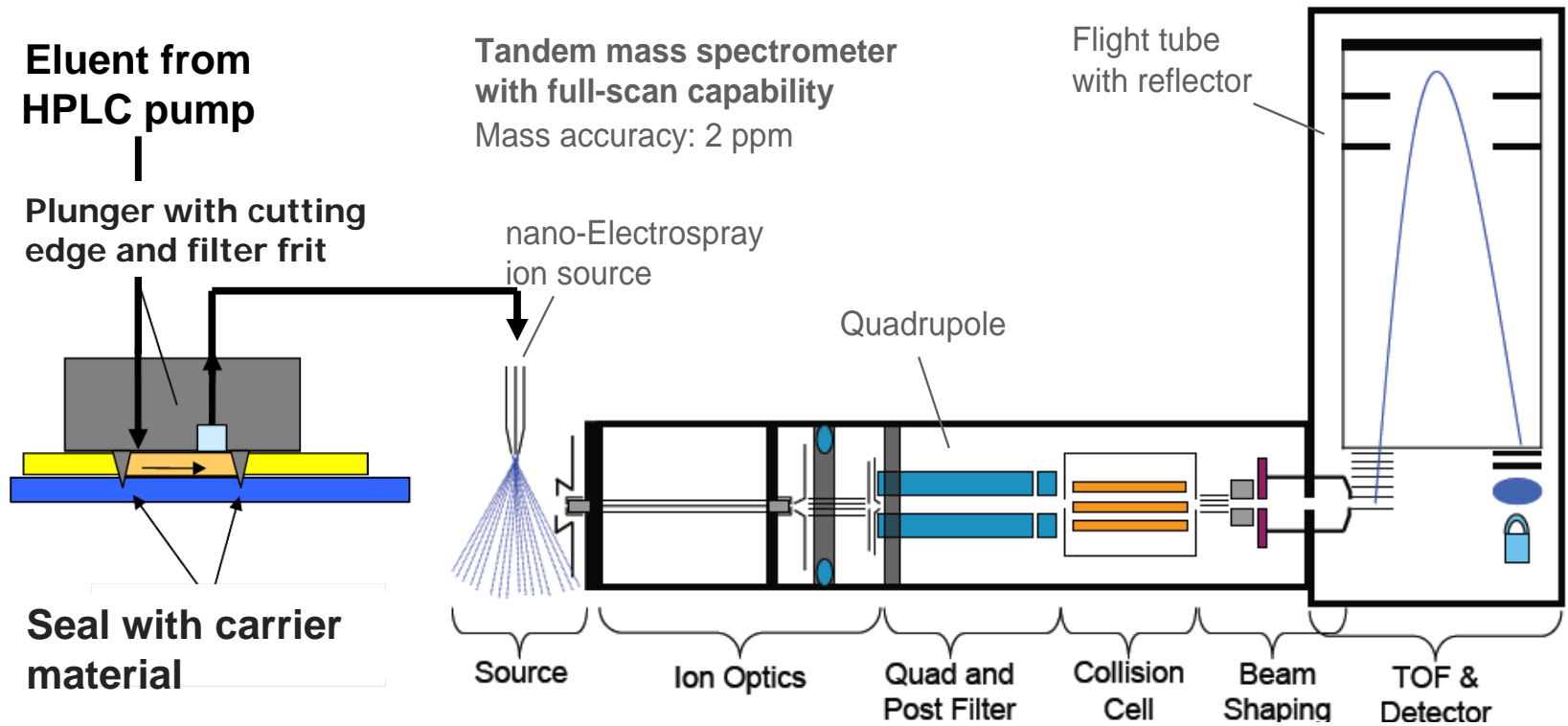
# HPTLC/AMD fingerprint of a ground water sample



# HPTLC/AMD fingerprint of a ground water sample

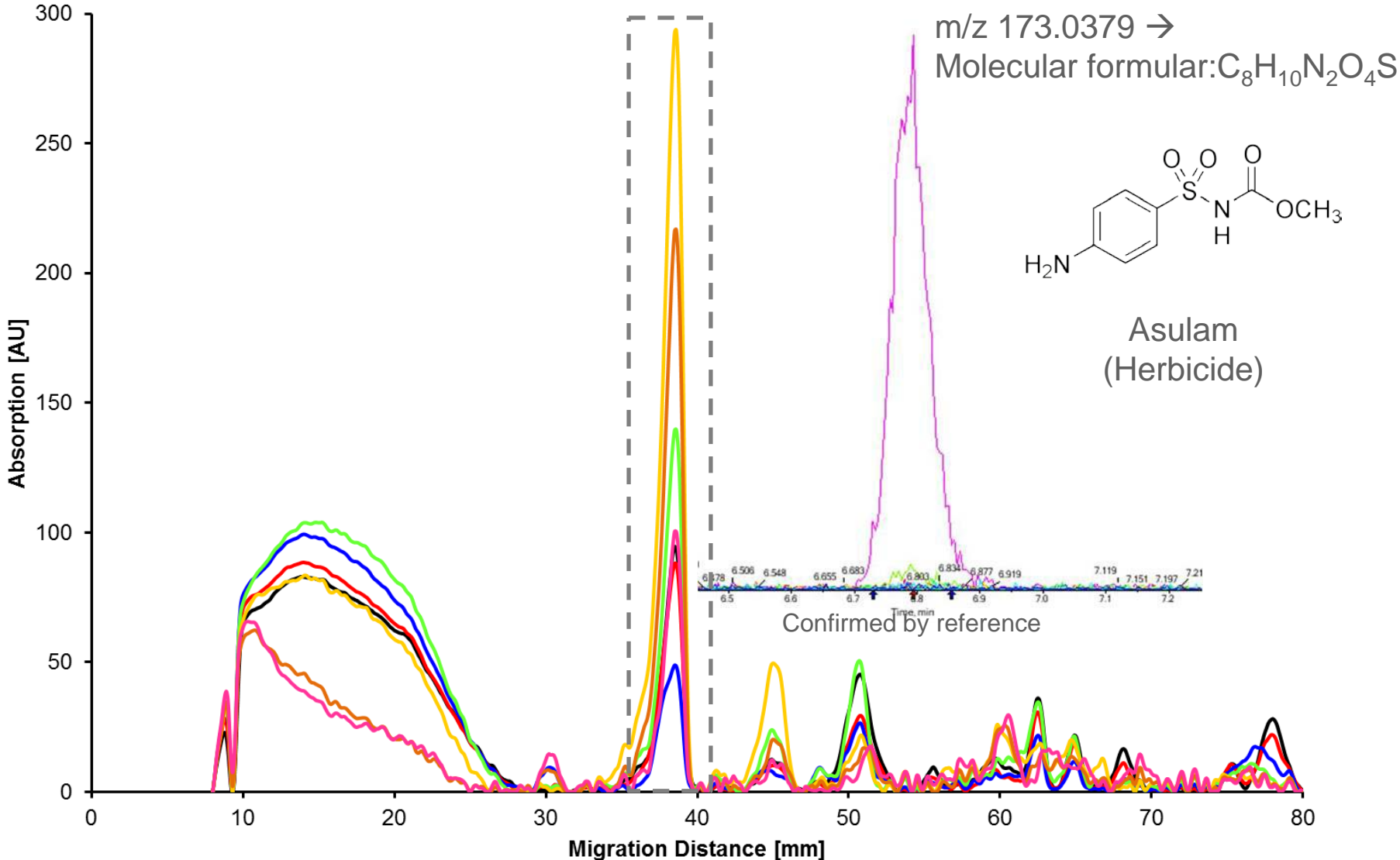


# Transfer of substances via HPTLC-MS interface and MS analysis



H. Luftmann (2004) Anal. Bioanal Chem., 378, 964

# Identification via HPLC-qTOF/MS

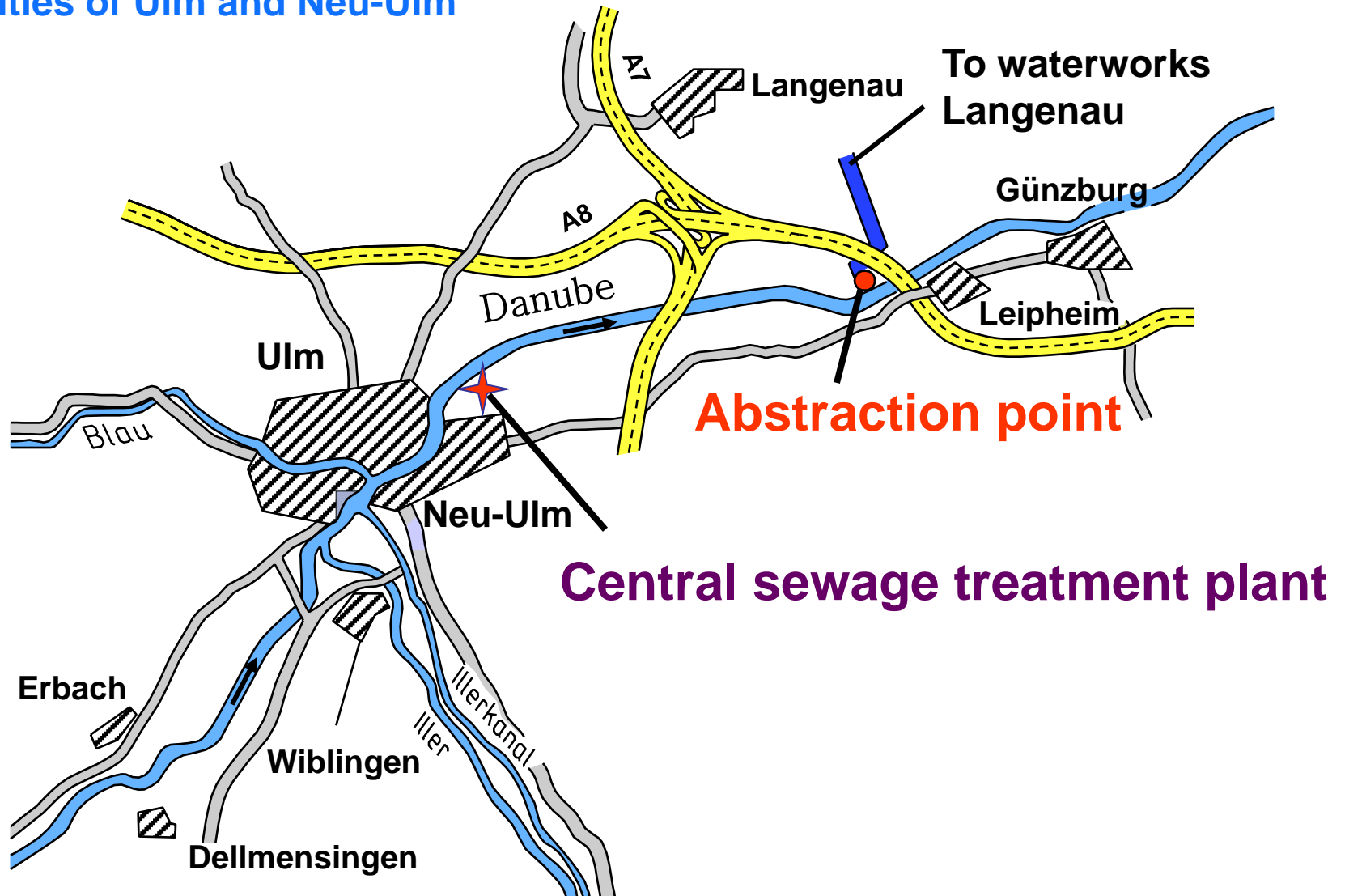




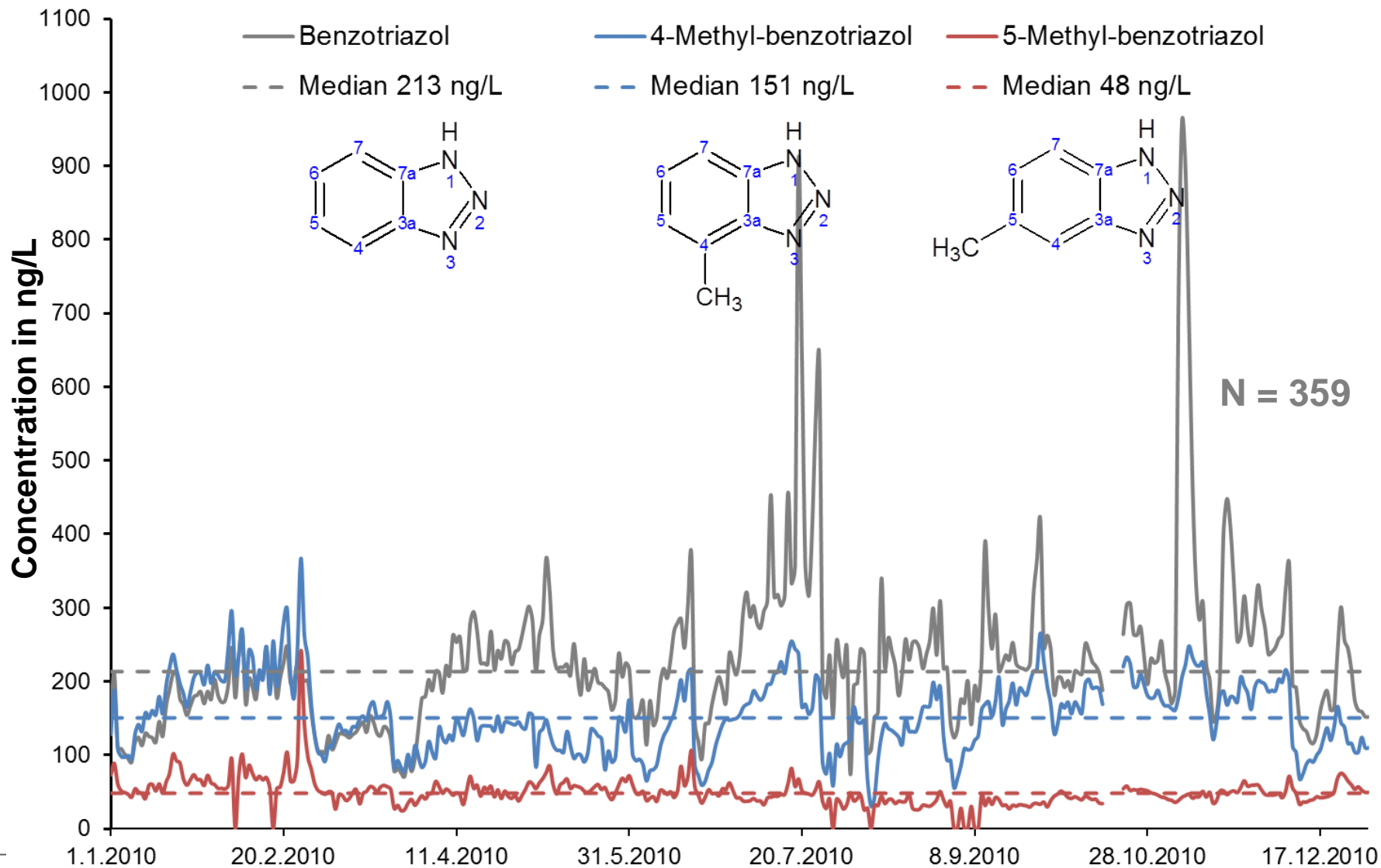
# Content

- Introduction
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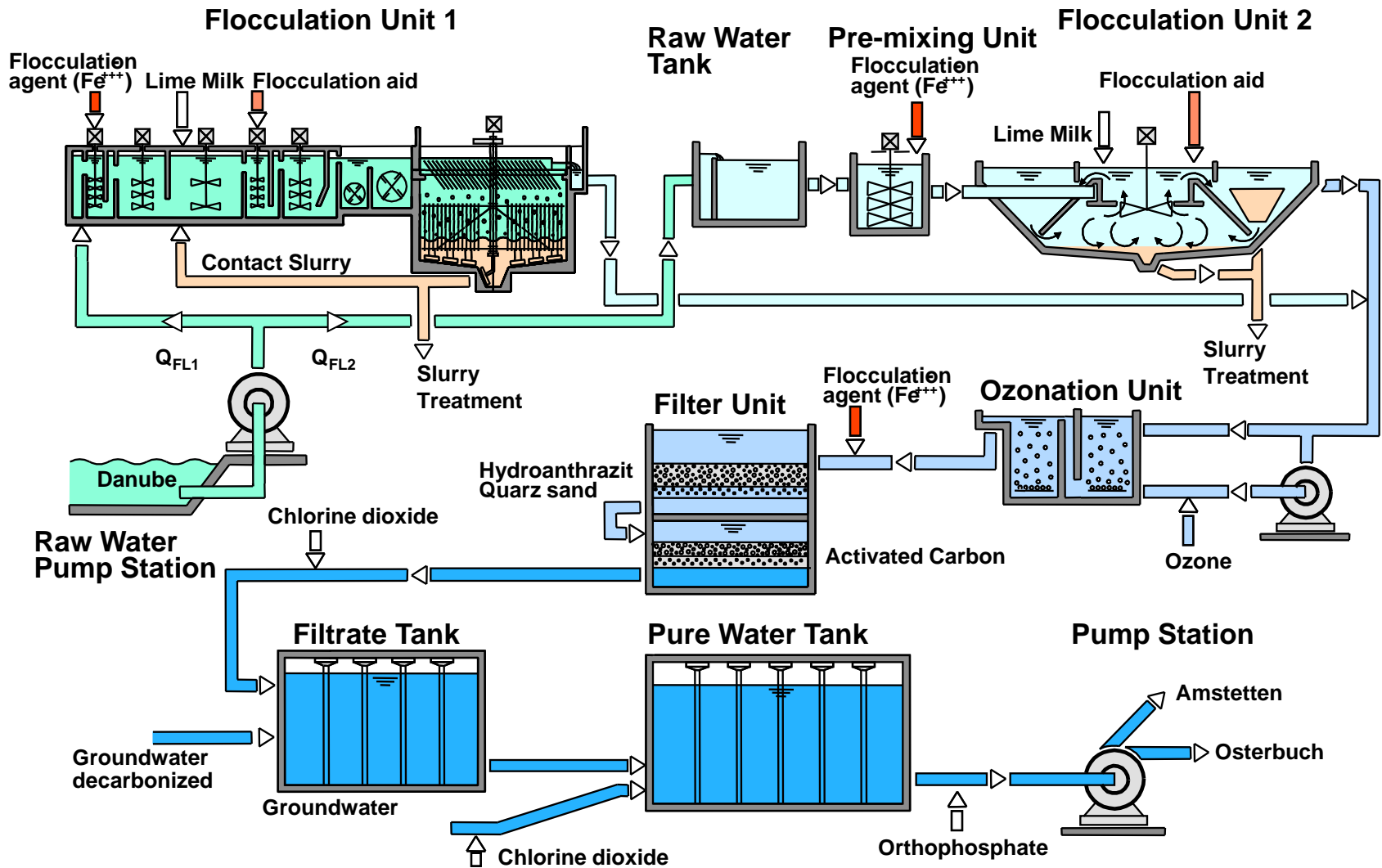
## Direct abstraction of Danube water in the downstream of the Cities of Ulm and Neu-Ulm



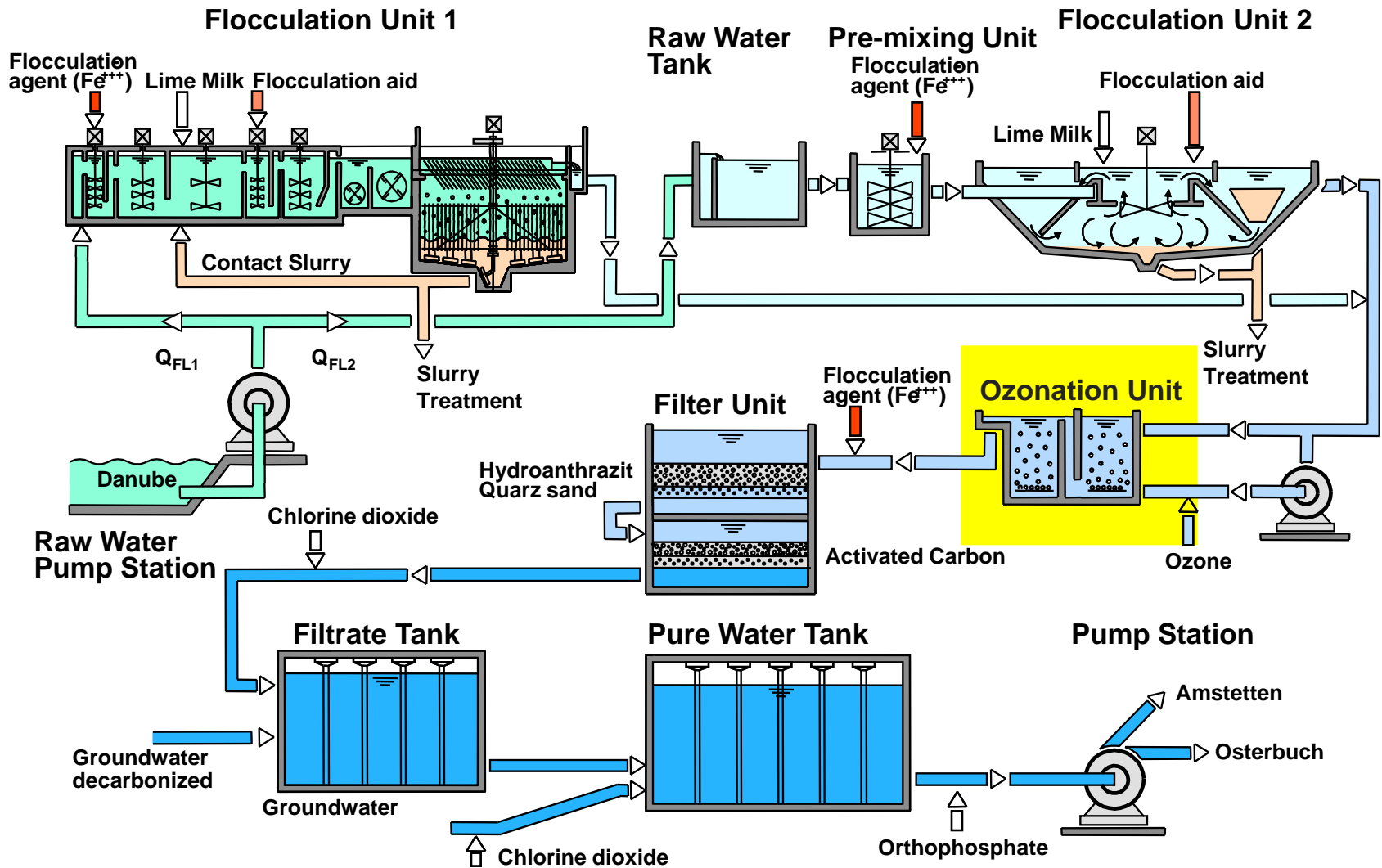
# Time variation curve of Benzotriazoles in the Danube (Leipheim, 2010)



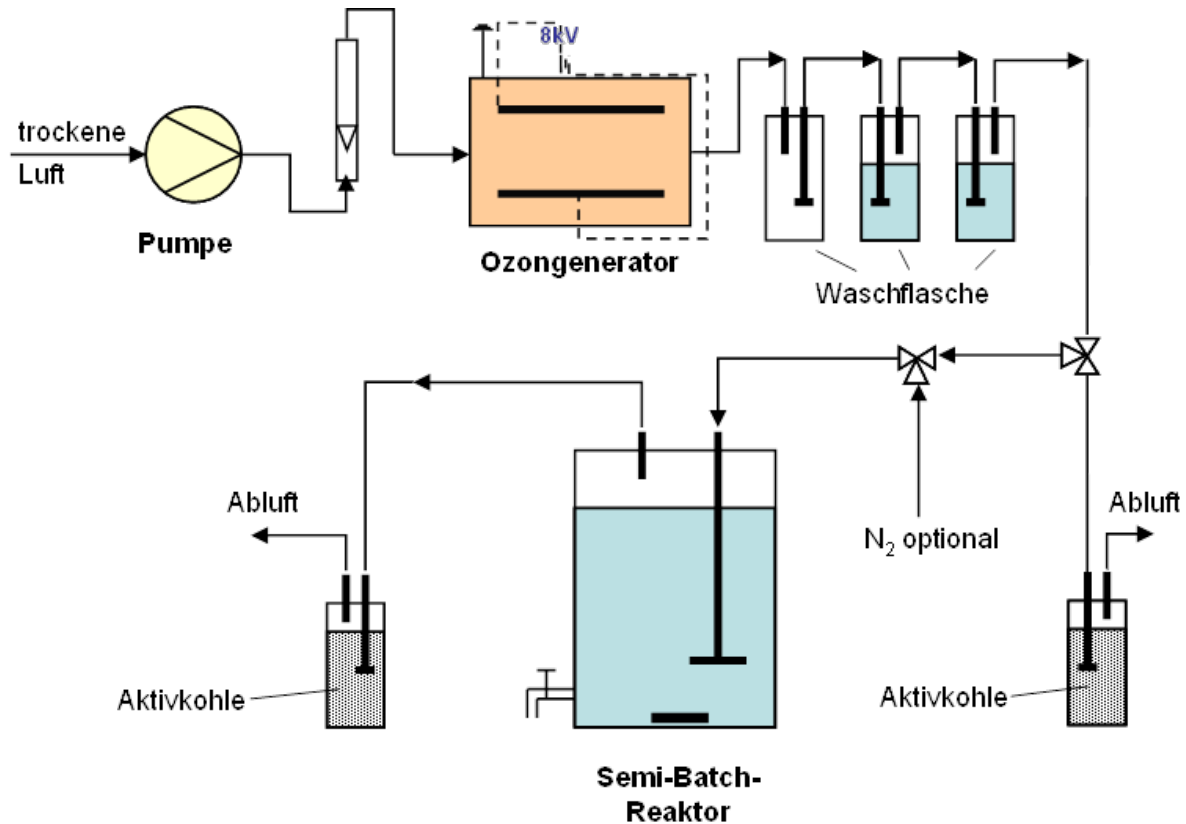
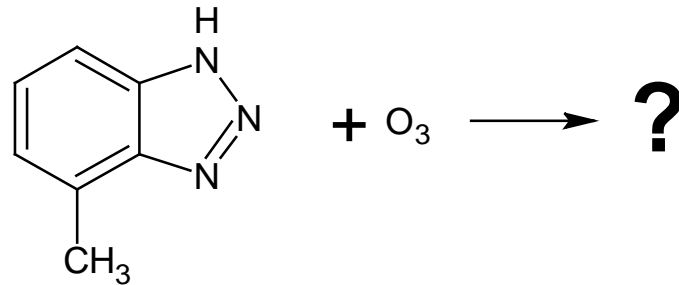
# Treatment of Danube water at Langenau waterworks



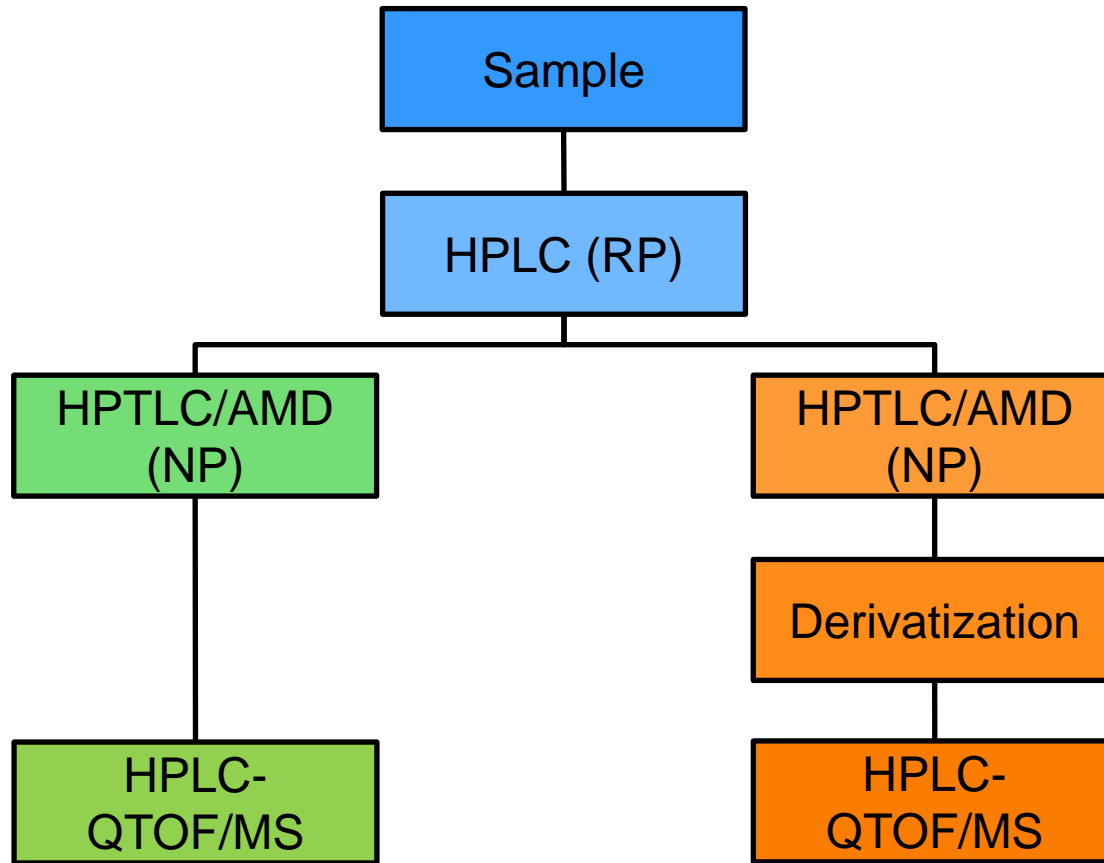
# Treatment of Danube water at Langenau waterworks



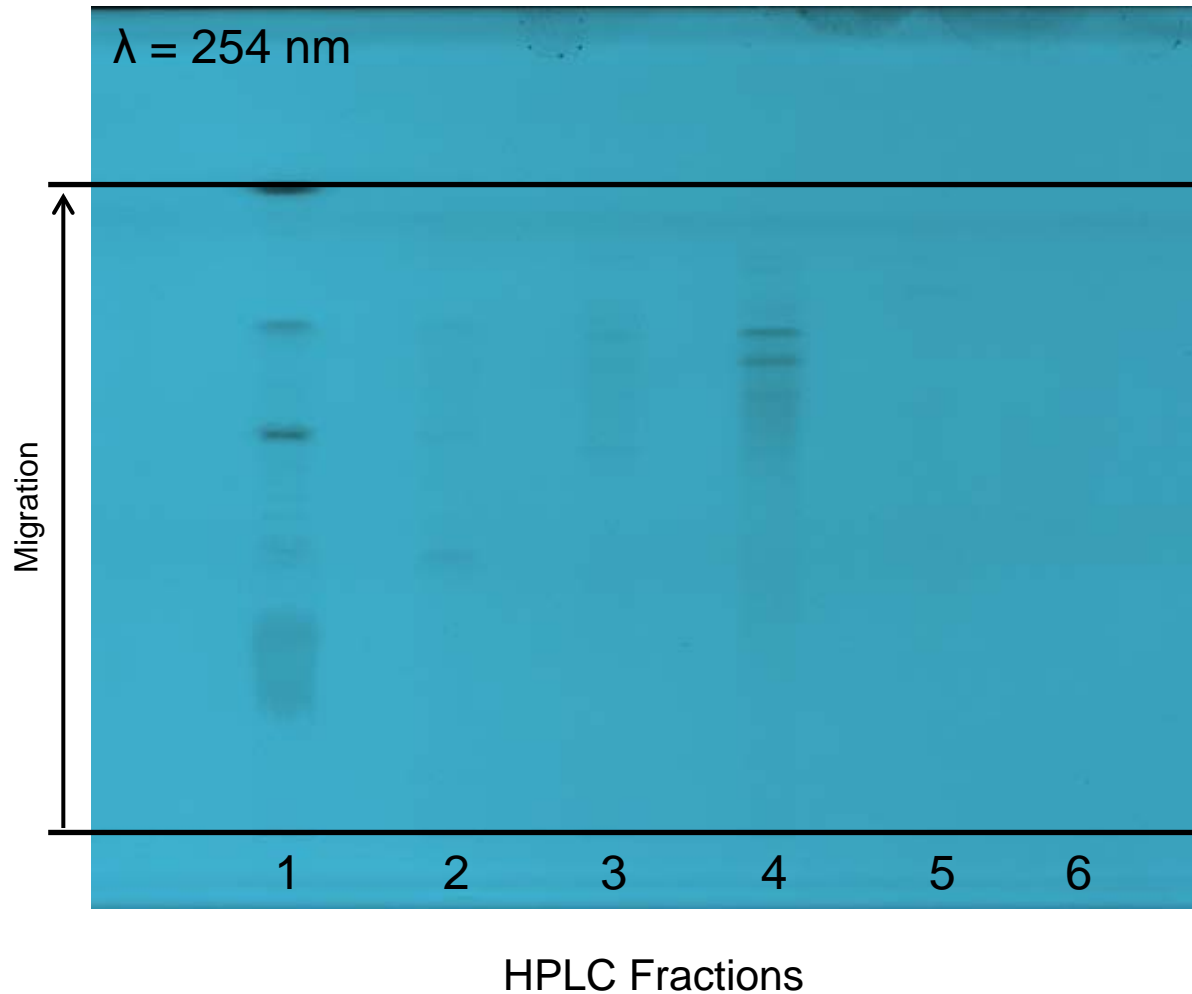
# Schematic illustration of the experimental ozonation reactor



# Separation of the main ozonation products via „2D-chromatography“



## Separation of HPLC-fractions via HPTLC/AMD ( $\lambda = 254 \text{ nm}$ )





# Schematic diagram of the HPTLC-MS coupling

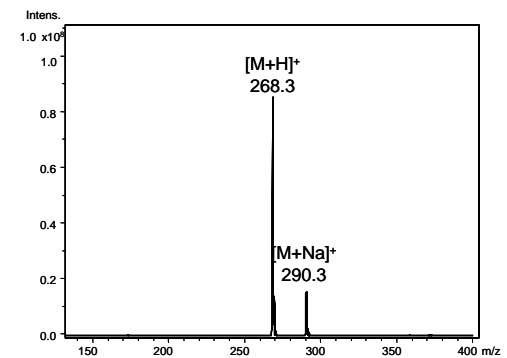
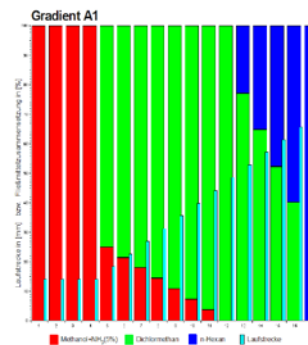
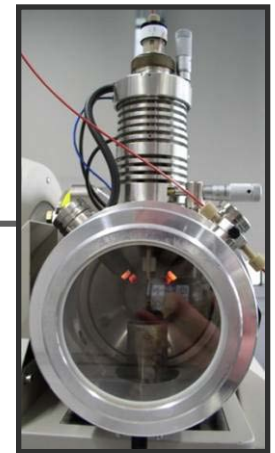
**Application**  
TLC Sampler 4



**Development**  
AMD 2

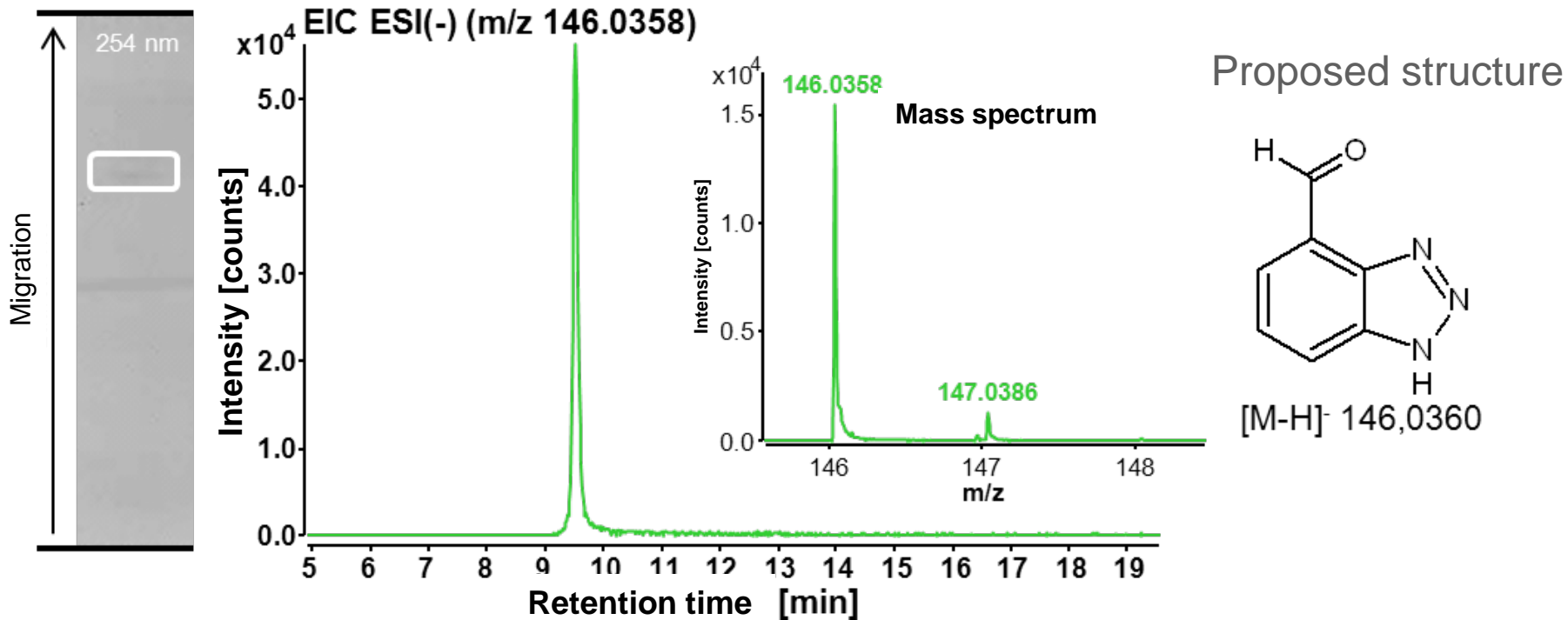


**Transfer and Detection**  
TLC-MS interface and ESI-MS

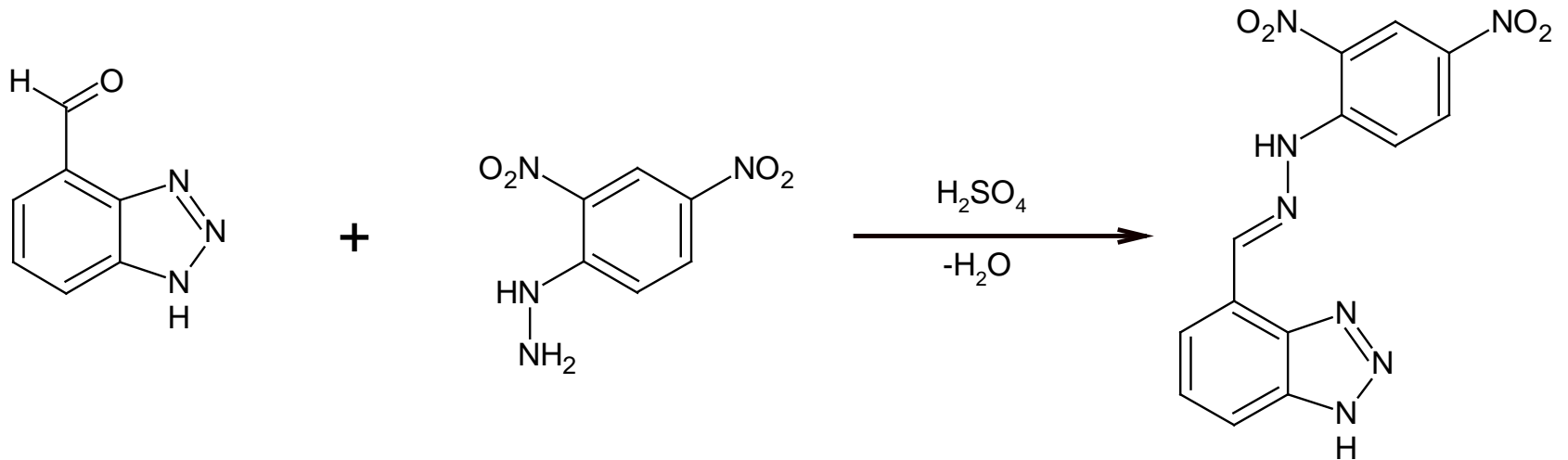


H. Luftmann (2004), Anal. Bioanal Chem., 378, 964

# Localisation of the postulated oxidation product M147 on the developed HPTLC plate



# Confirmation of the carbonyl group with 2,4-DNPH\*



M = 147,0433 Da  
[M-H]<sup>-</sup> = 146,0360 Da

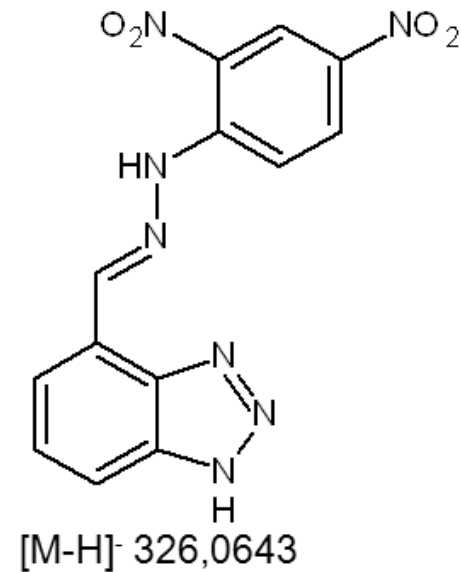
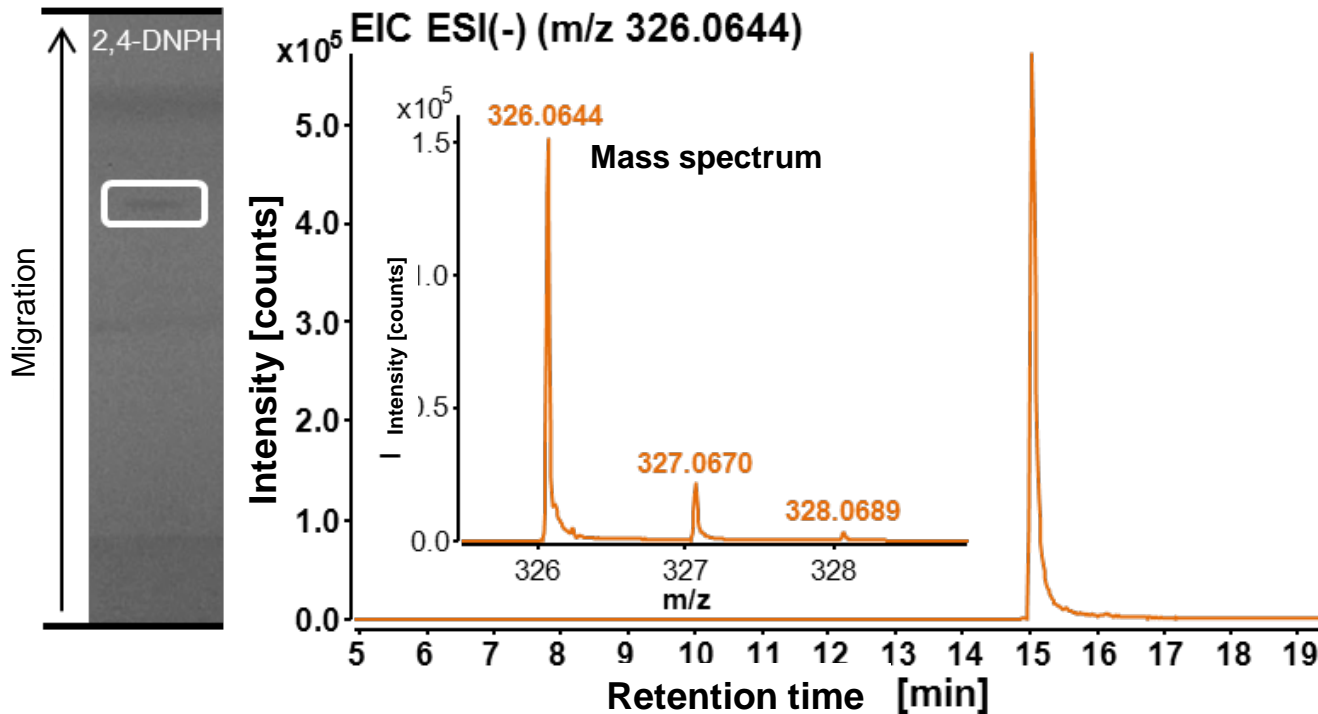
M = 198,0389 Da  
[M-H]<sup>-</sup> = 197,0316 Da

M = 327,0716 Da  
[M-H]<sup>-</sup> = 326,0643 Da

\* 2,4-Dinitrophenylhydrazin

# Confirmation of the carbonyl group with 2,4-DNPH

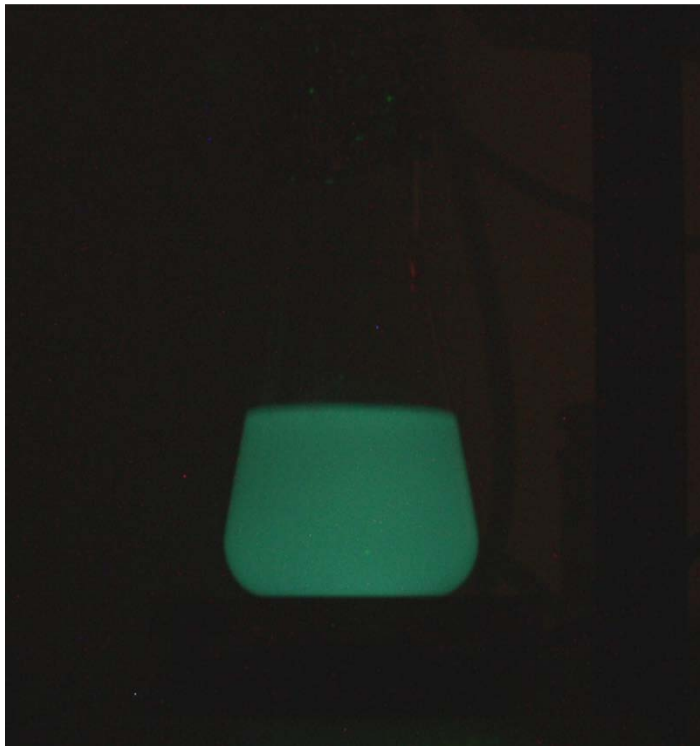
Extraction of the derivatization product (m/z 326) for the oxidation product M147



# Content

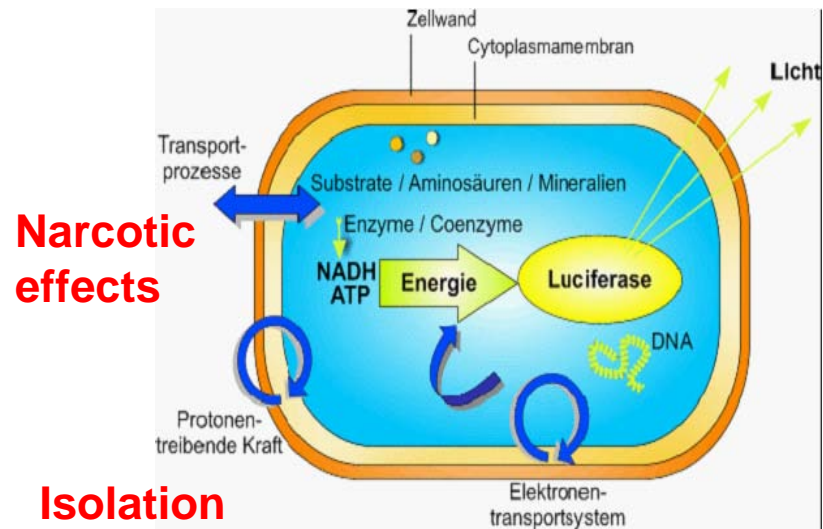
- Introduction
- Identification of pesticides in ground water
- Ozonation reaction products
- **Effect-Directed Analysis with *Vibrio fischeri***
- Ground water contamination from synthetic turf
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# Luminescence bacteria *Vibrio Fischeri*

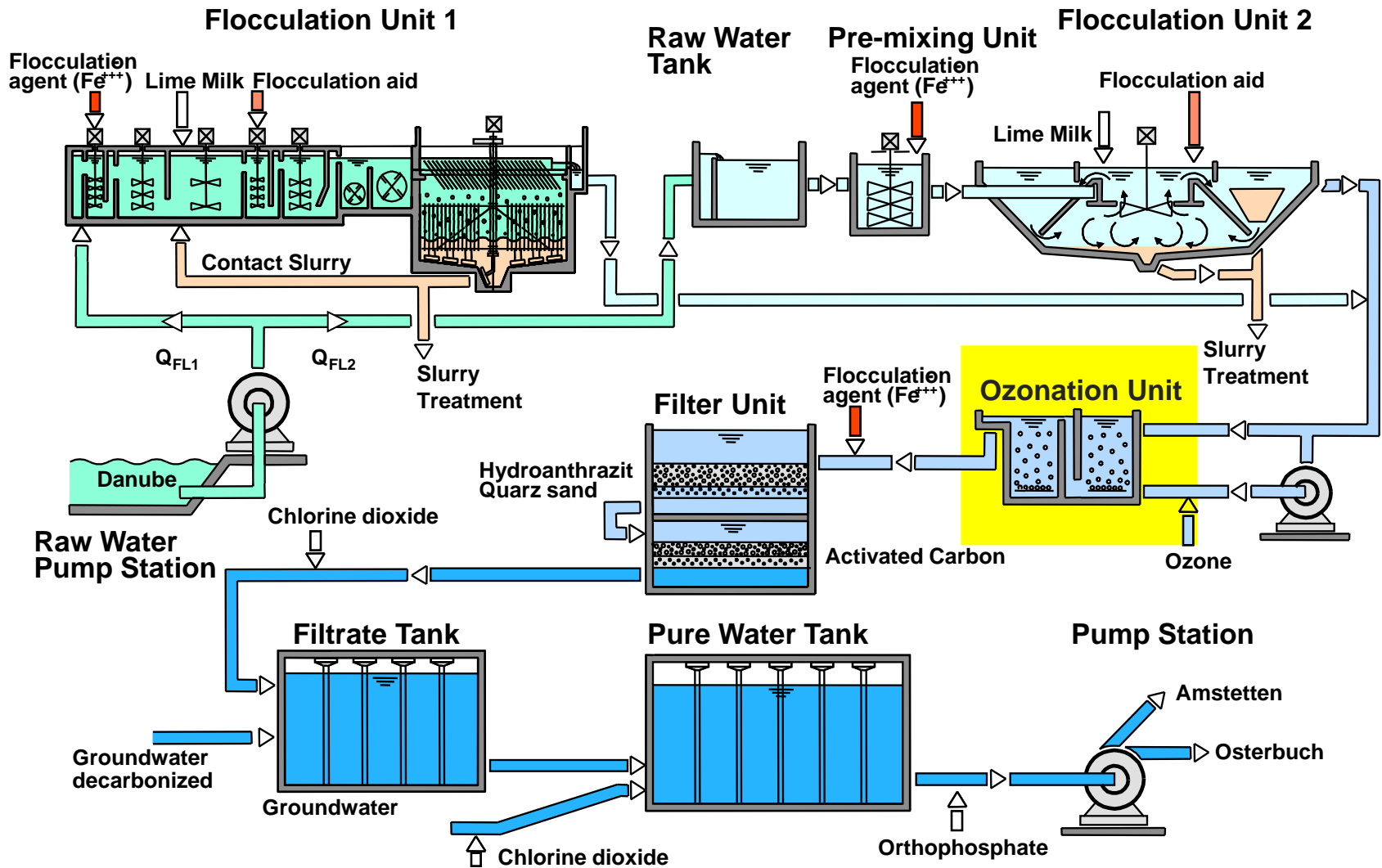


Suspension of *Vibrio fischeri*  
in water

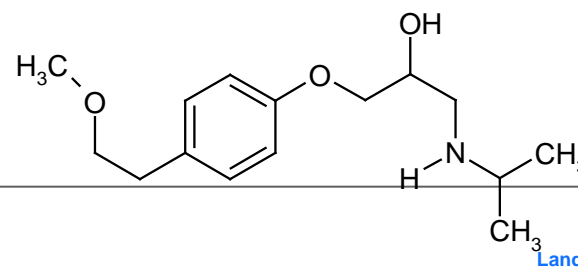
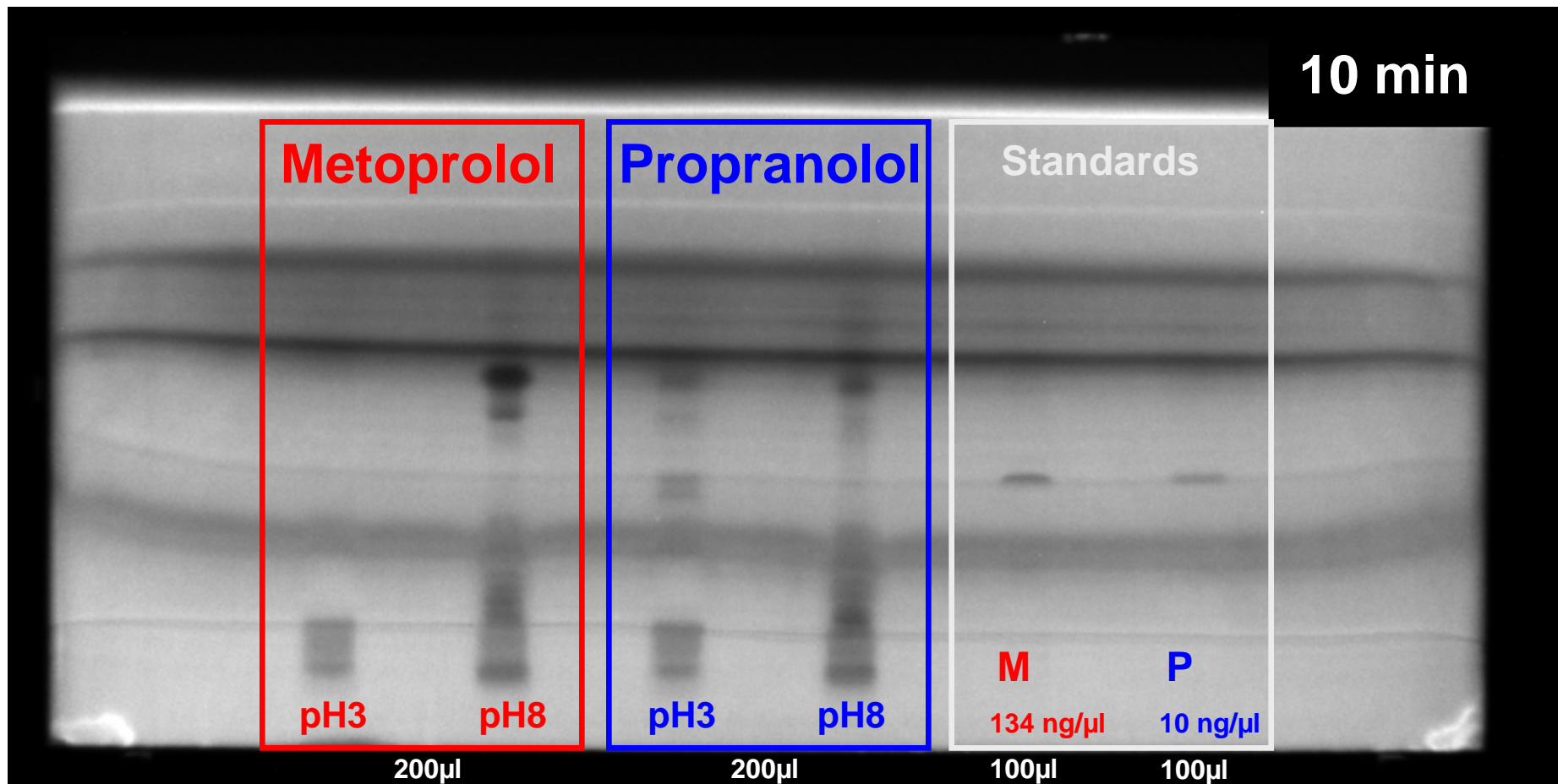
- Marine bacterium
- Lives in symbiosis with marine life forms
- Continuous bioluminescence
- Bioluminescence is coupled to energy metabolism



# Treatment of Danube water at Langenau waterworks



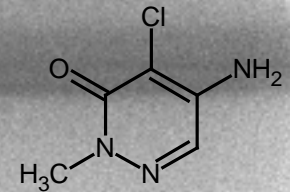
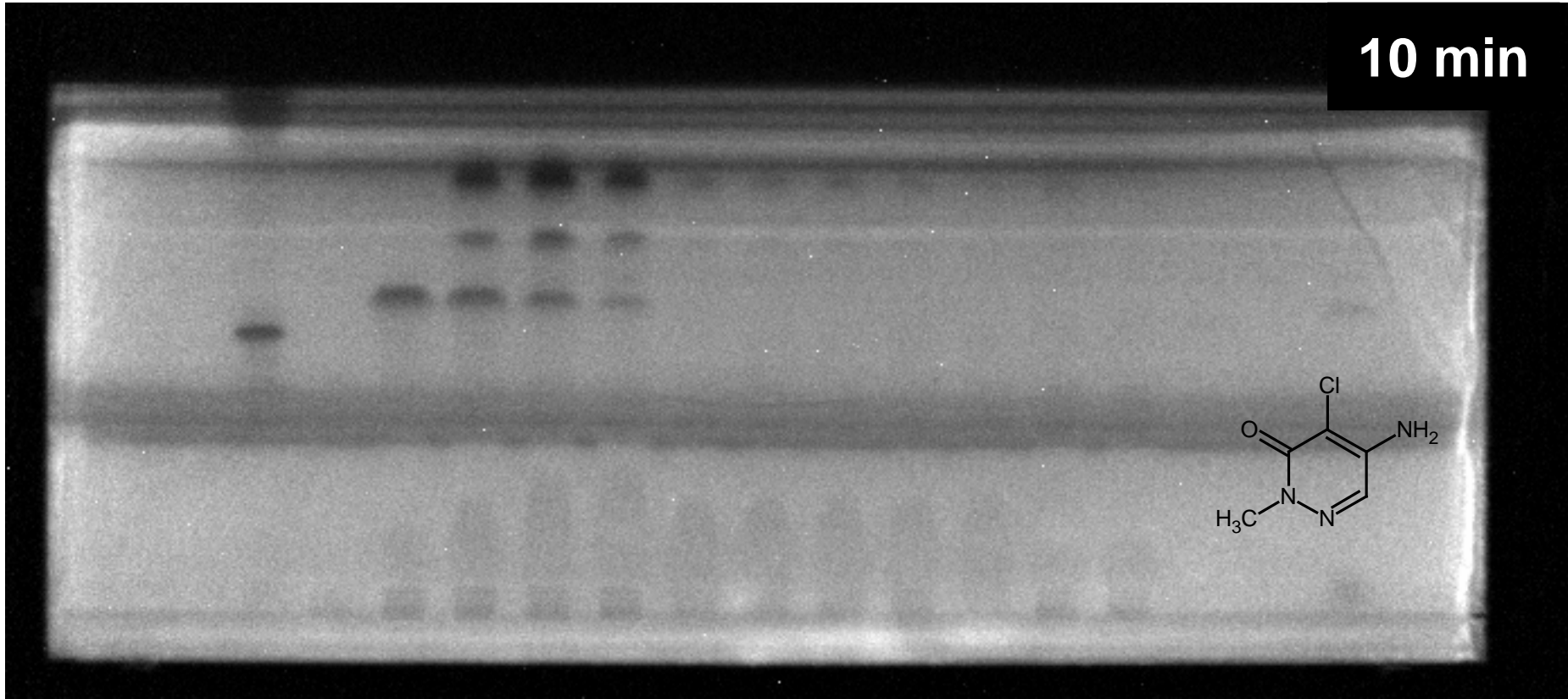
# Ozonation by-products of $\beta$ -blockers





# Ozonation by-products of the main metabolite of the pesticide Chloridazon

10 min



Controll Mix  
Blank

t = 0.5 min

t = 5 min

t = 10 min

t = 15 min

t = 20 min

t = 25 min

t = 30 min

t = 40 min

t = 50 min

t = 60 min

t = 85 min

Methyl-desphenyl-  
chloridazon

# Potential ground water contamination by synthetic turfs

## Leaching test:

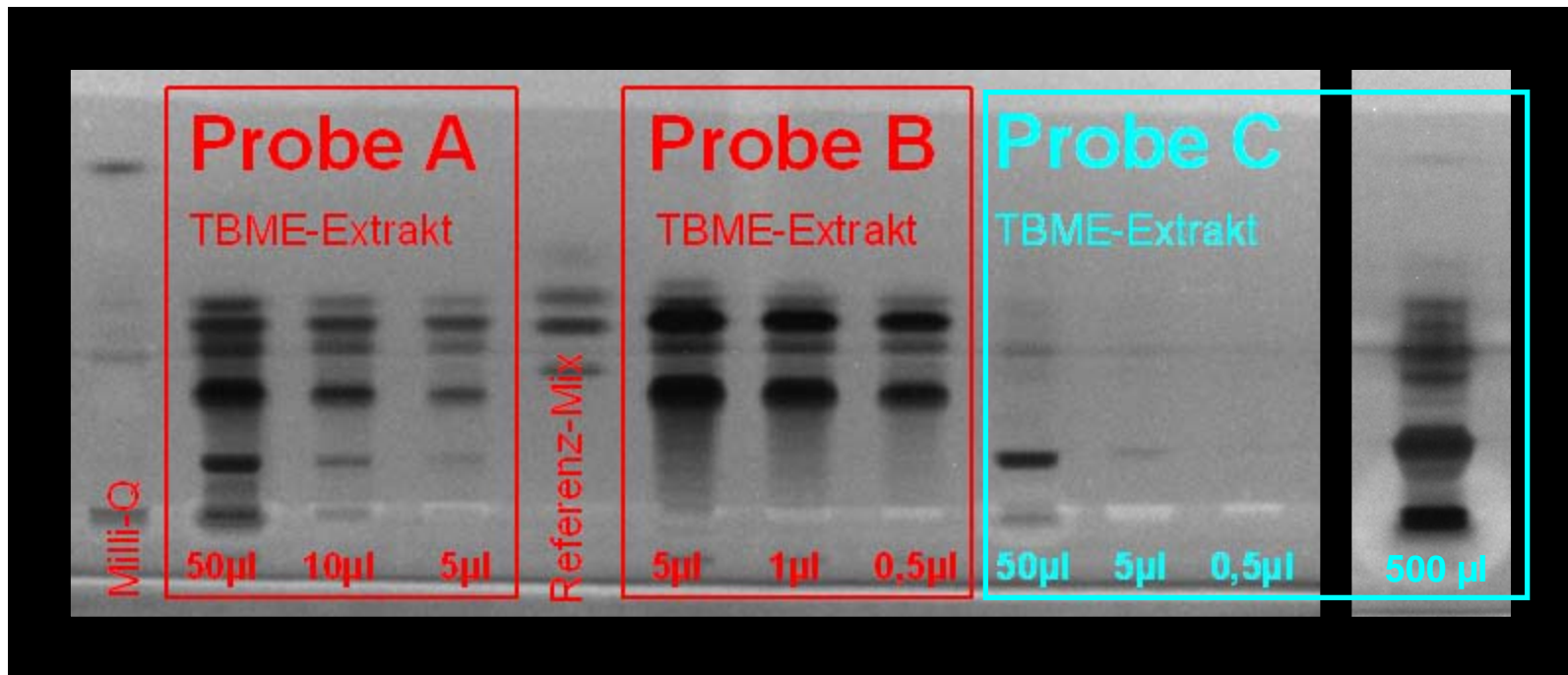
(analog DIN 38414-S4)

Elution of 40 g sample with 80 g water

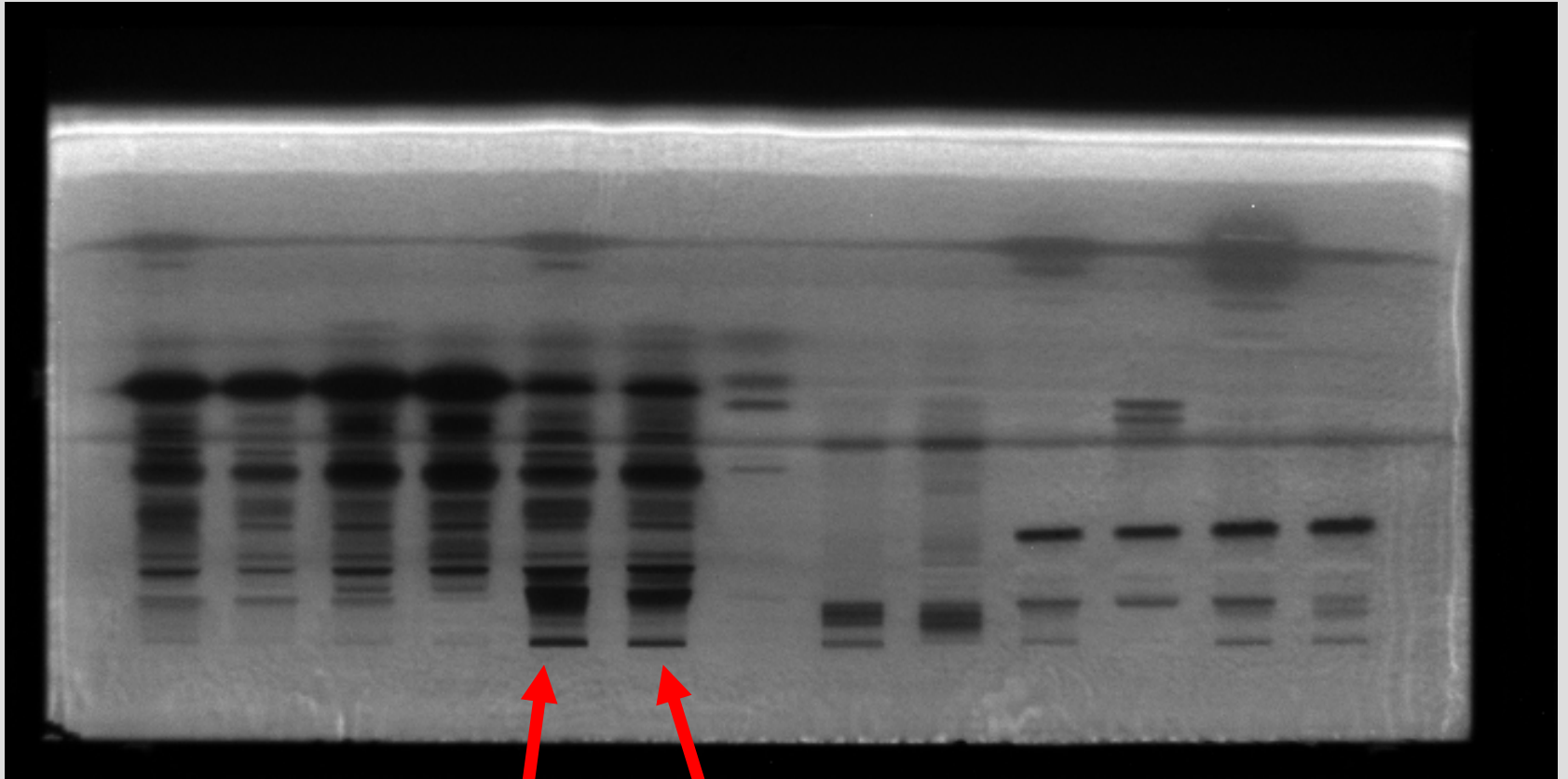
Duration: 24 h

Liquid/liquid extraction of the eluate

Extraction solvent: MTBE Blank: Ultra pure water



## Fingerprint of waste water samples ( $\Delta t = 6$ month)



**1. Sample series**

**2. Sample series**

# Content

- Introduction
- Identification of pesticides in ground water
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## Aerial photo of the region of interest



Ref.: Google Maps

# Simulation of leaching synthetic turf with water

## Synthetic turf:

PUR rubber granulate (elastic layer)



## Leaching test:

(analog DIN 38414-S4)

Elution of 40 g sample with 80 g water

Duration: 24 h

Liquid/liquid extraction of the eluate

Extraction solvent: Tert-butyl-methyl-ether

Blank: Ultra pure water



# Schematic diagram for the HPTLC-AMD analysis with bioluminescence detection

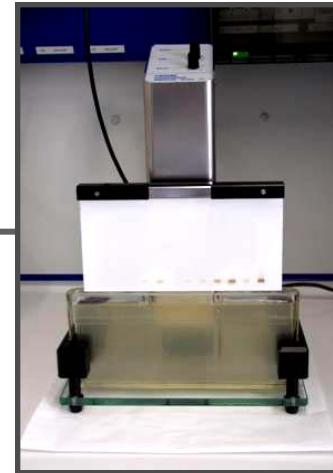
**Application**  
TLC Sampler 4



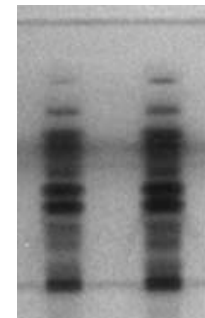
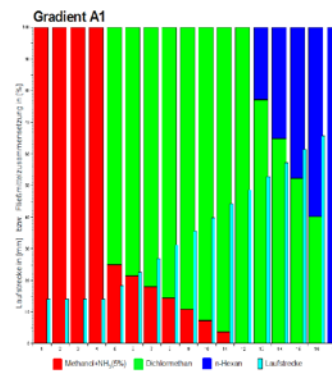
**Development**  
AMD 2



**Immersion**  
*Vibrio fischeri*



**Detection**  
Bioluminizer

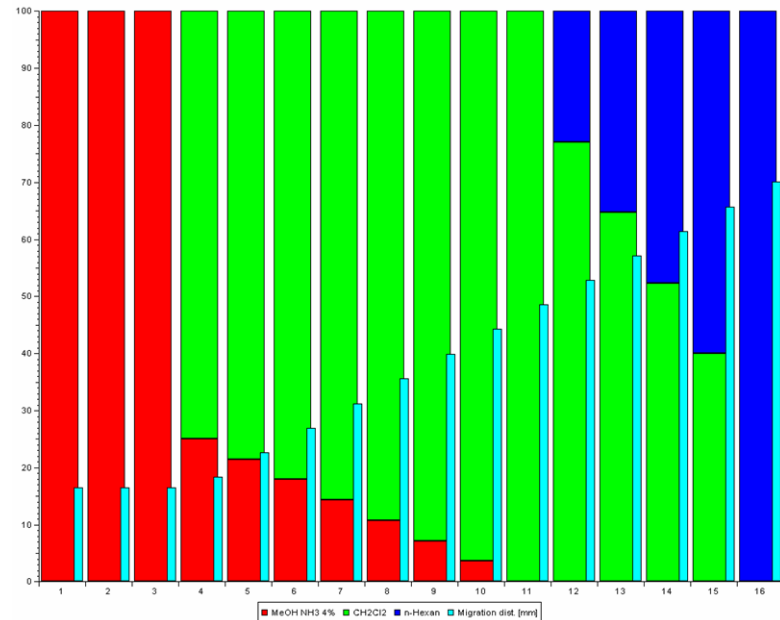


Weisemann, C., Kreiss, W., Rast, H-G., Eberz, G.; "Analytical Method for Investigating Mixtures for Toxic Components."  
European Patent No: EP 0 588 139 B1.

# HPTLC/AMD analysis

## Chromatography:

Eluents: MeOH:NH<sub>3</sub> (4%)  
Dichloromethane  
n-Hexane

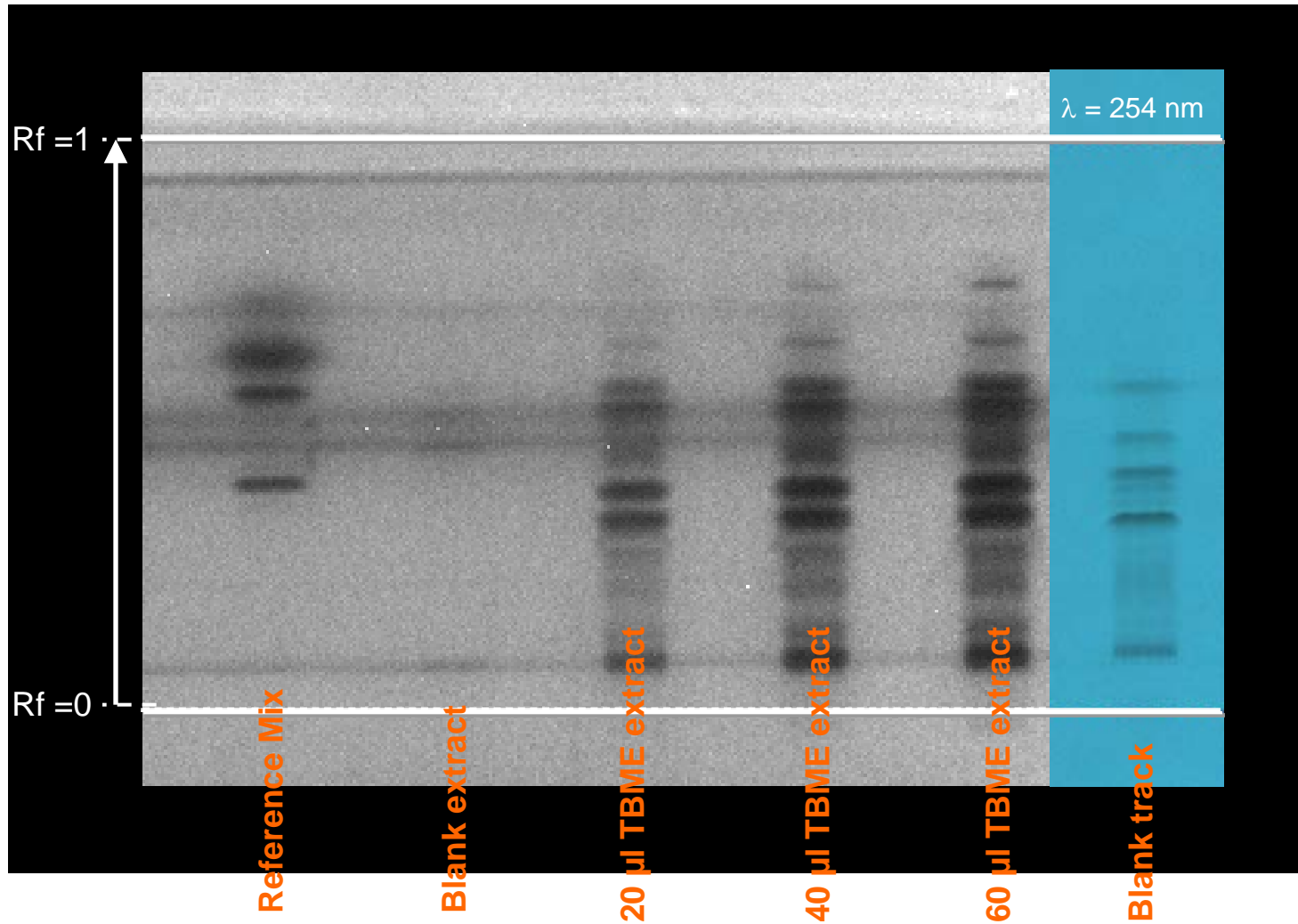


## Detection:

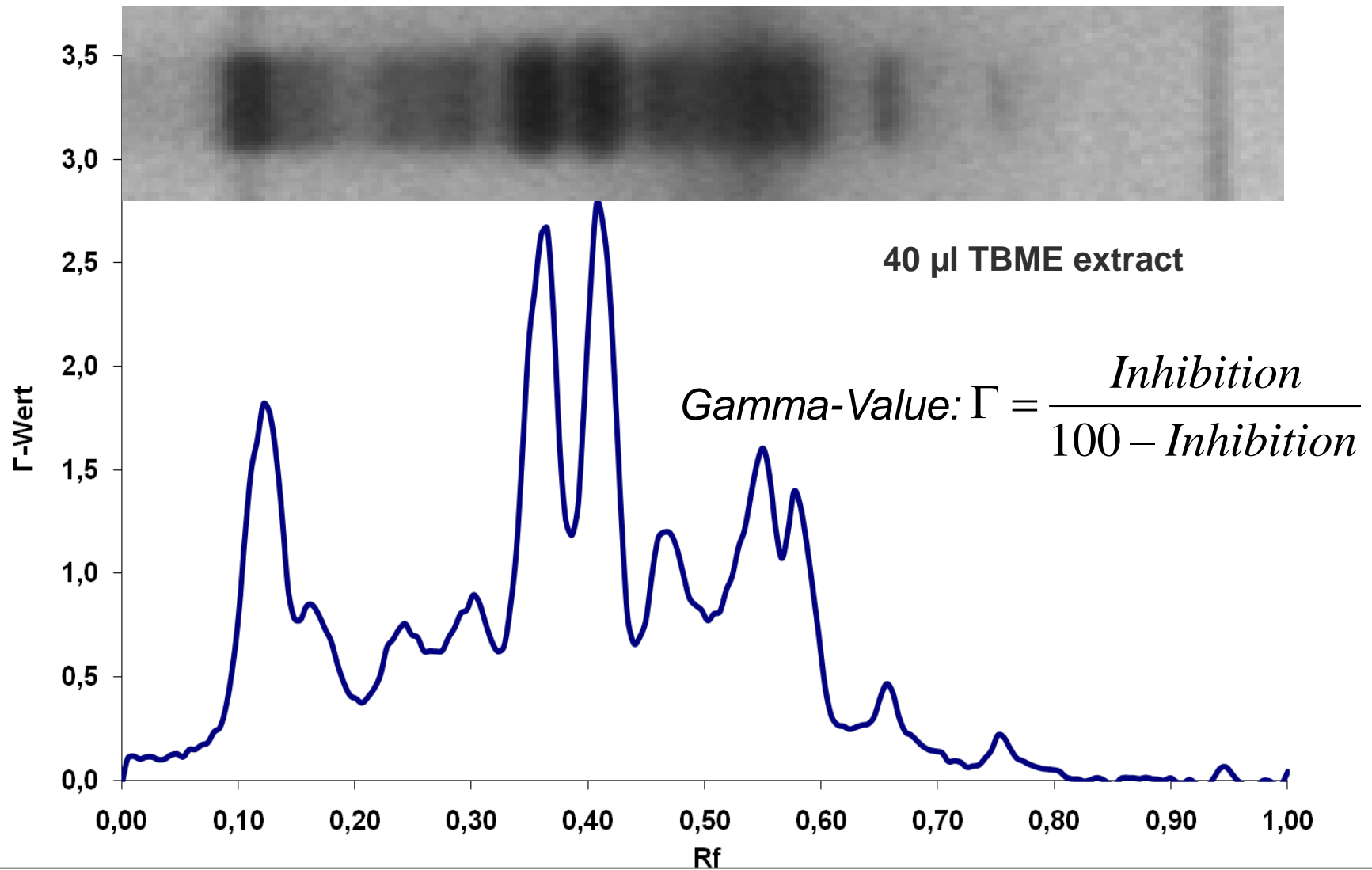
- UV absorption (multiple wavelength scan)
- Fluorescence quenching at 254 nm
- Fluorescence, excitation at 366 nm
- Luminescence inhibition test



# Detection of luminescence inhibition (*Vibrio fischeri*)

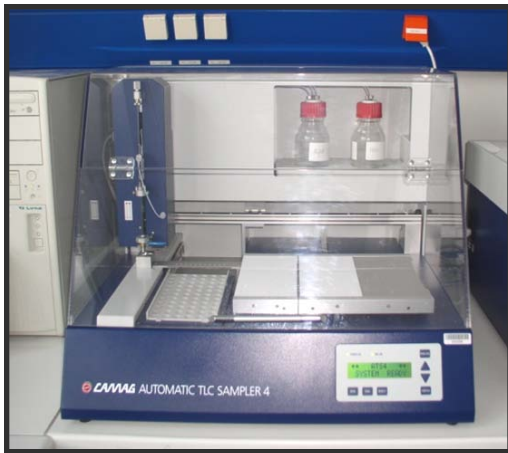


## Gamma value chromatogram (proportional to luminescence inhibition)



# Schematic diagram of the HPTLC-MS coupling

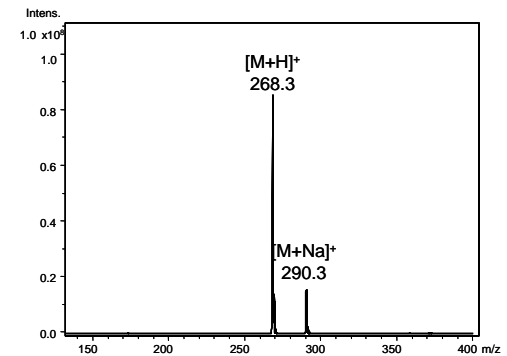
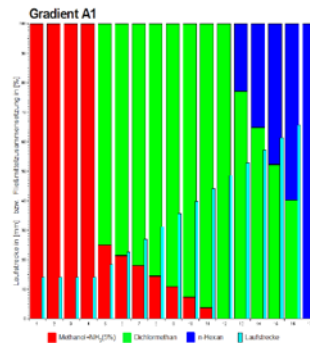
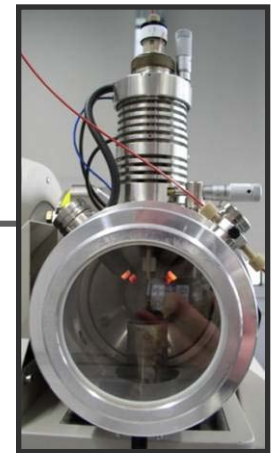
**Application**  
TLC Sampler 4



**Development**  
AMD 2



**Transfer and Detection**  
TLC-MS interface and ESI-MS

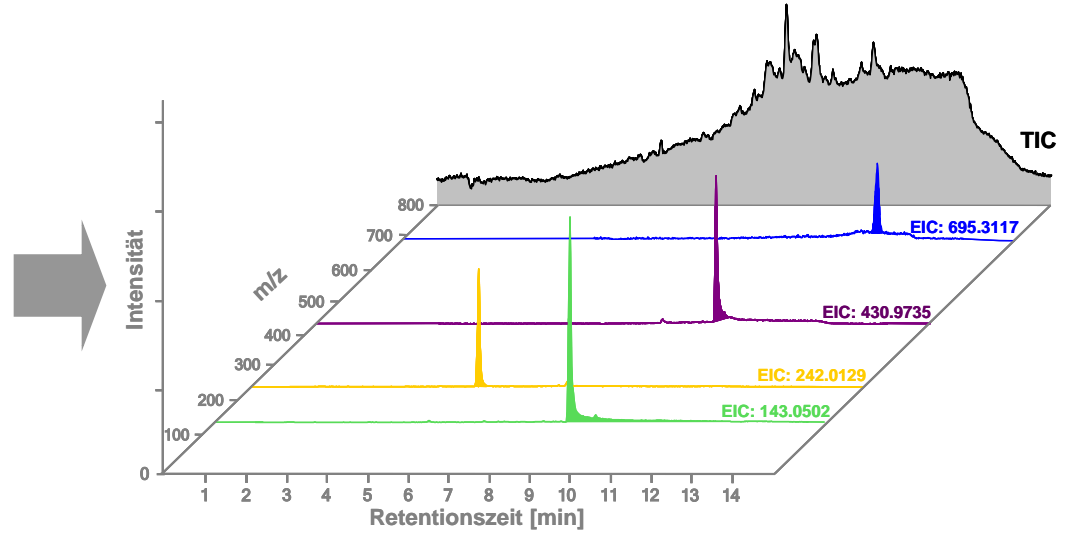


H. Luftmann (2004), Anal. Bioanal Chem., 378, 964

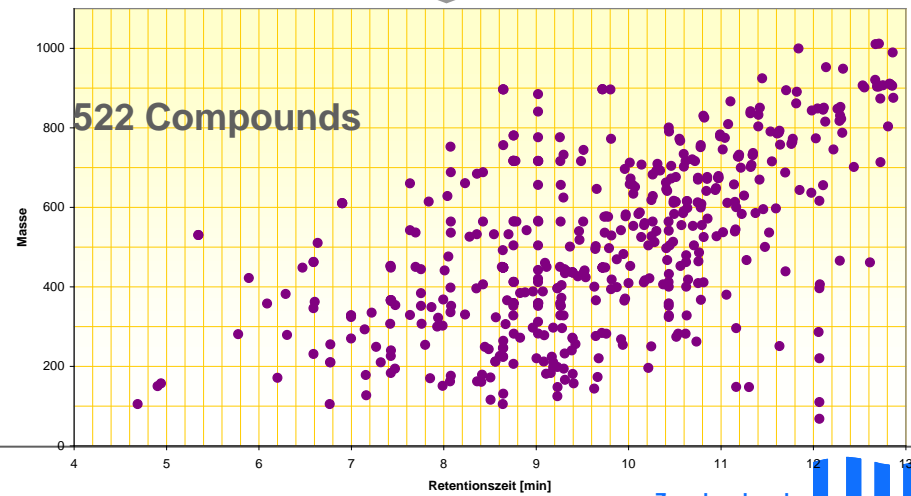
# Non-Target-Screening via LC-QTOF



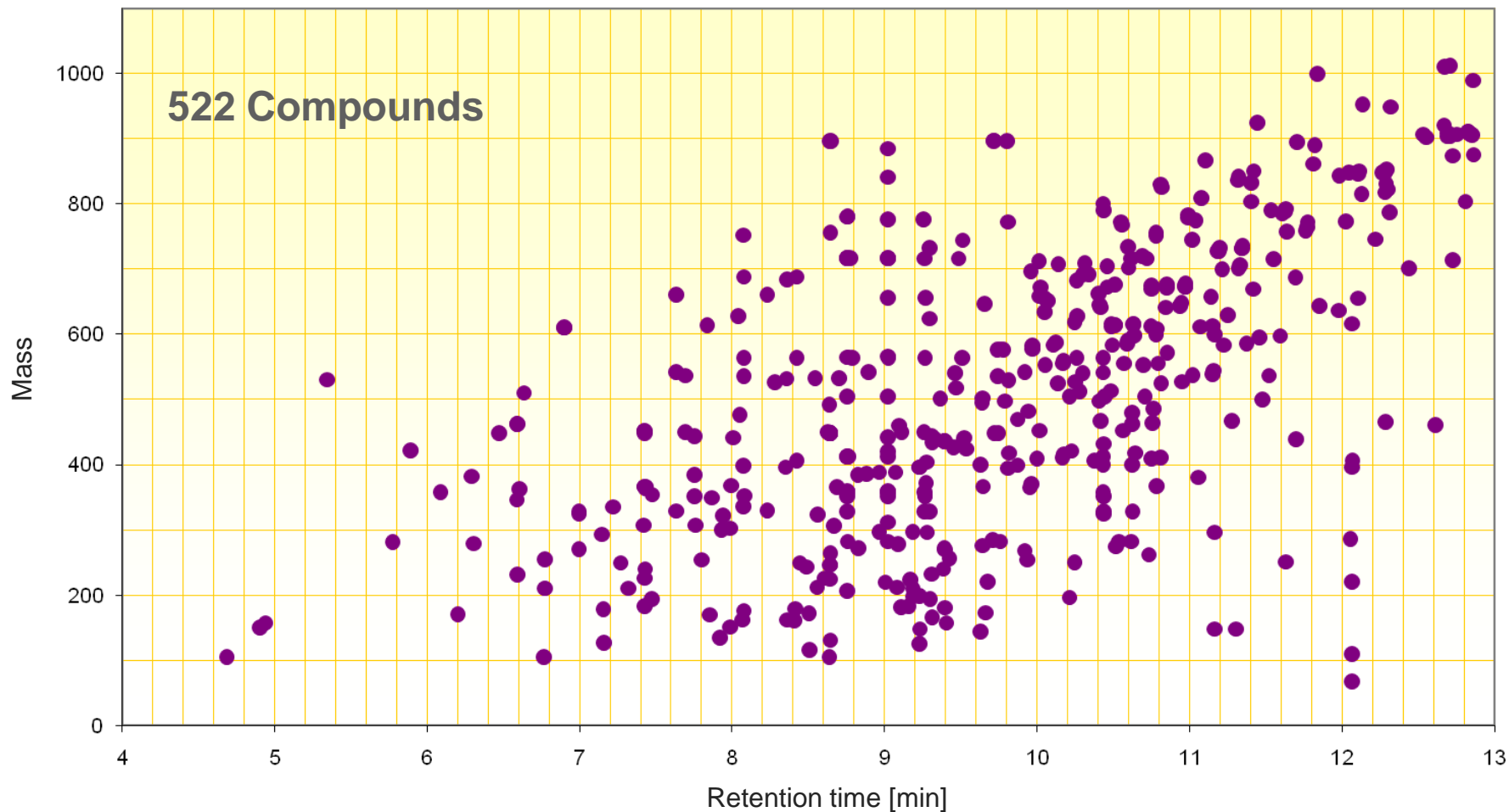
Nano-LC



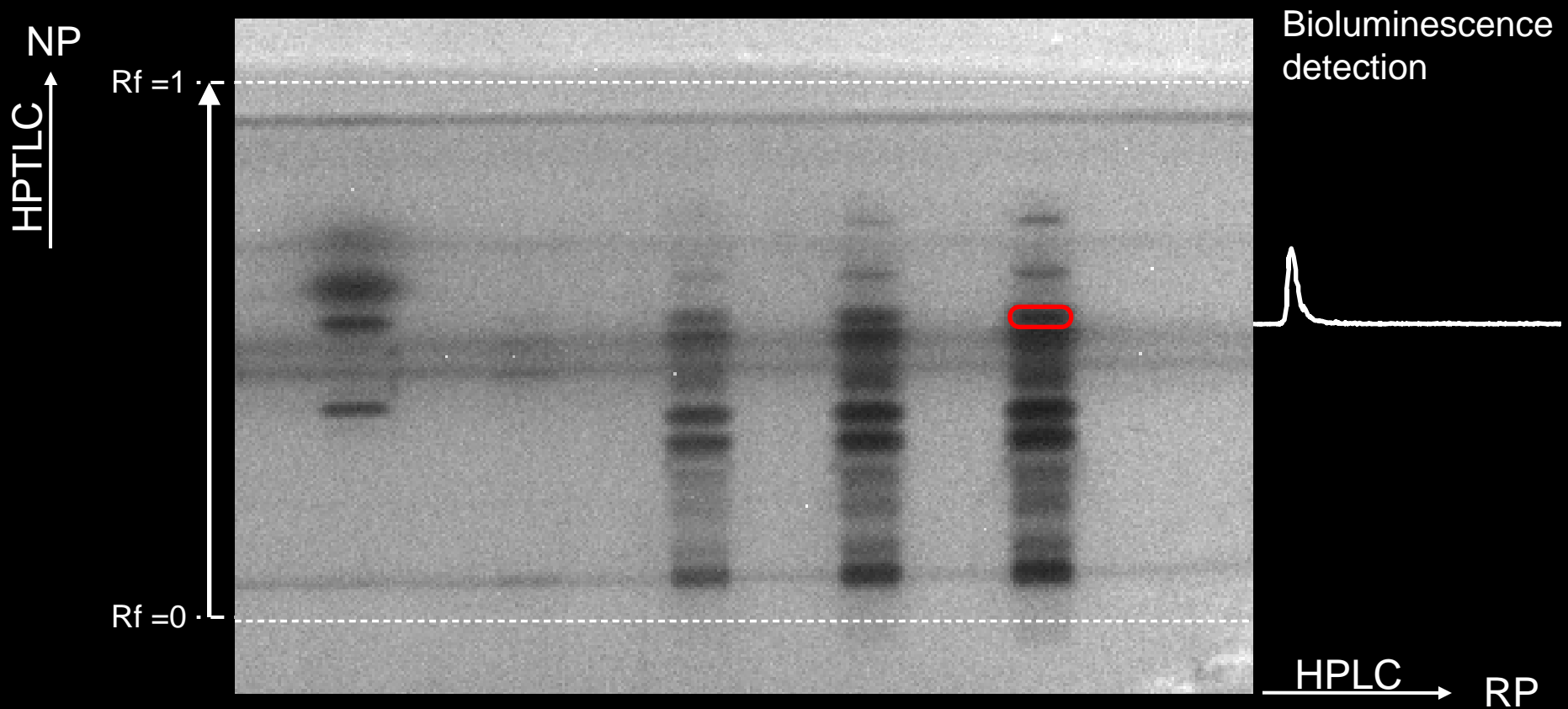
Top view



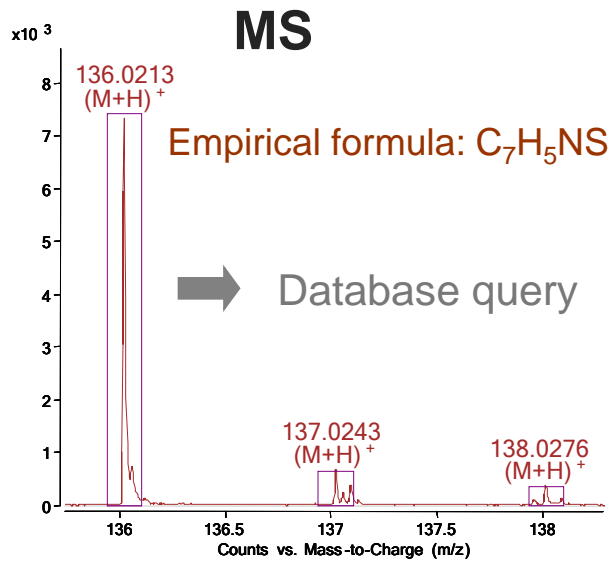
# Mass/Retention time diagram of all compounds in the eluate



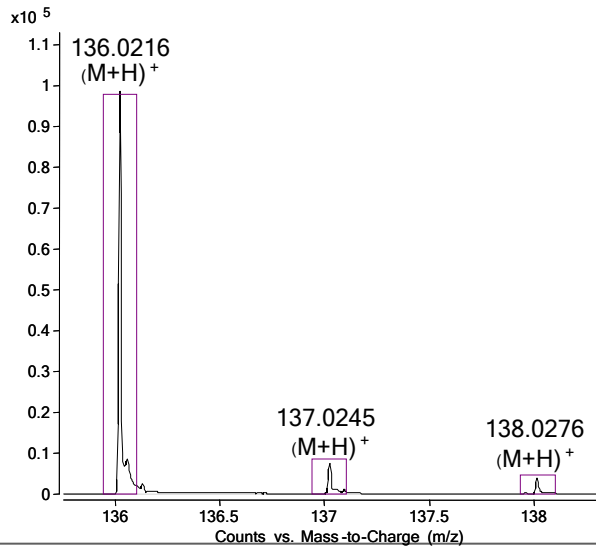
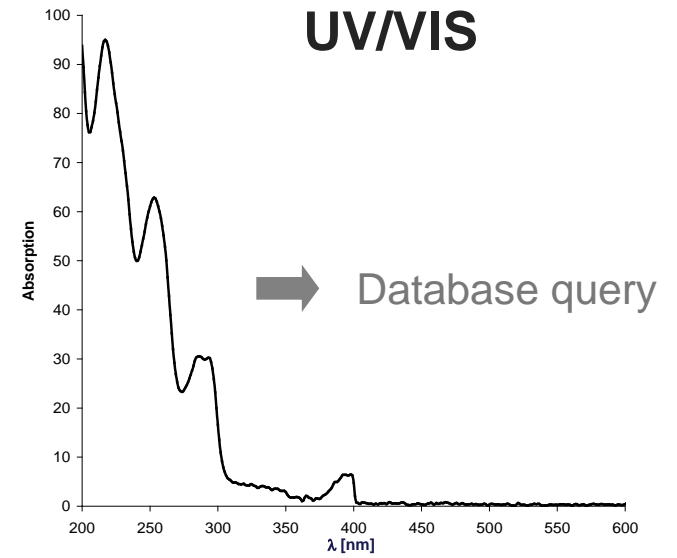
# Detection of luminescence inhibition (*Vibrio fischeri*) and transfer of the substances via HPTLC-MS interface to MS



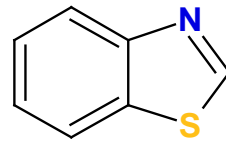
# Identification of the unknown compound



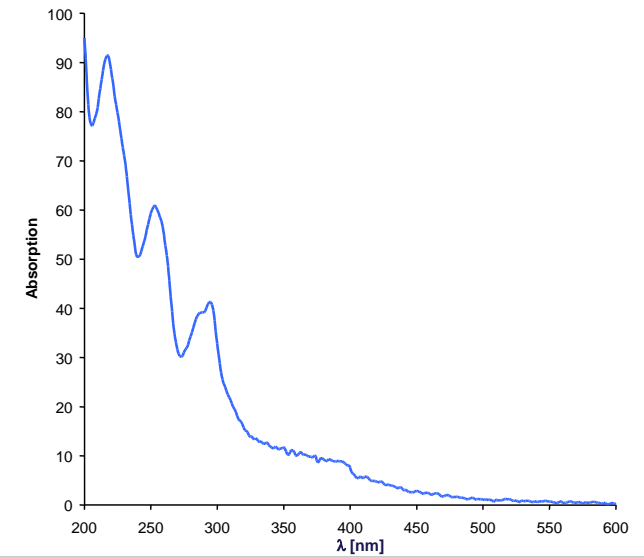
Sample



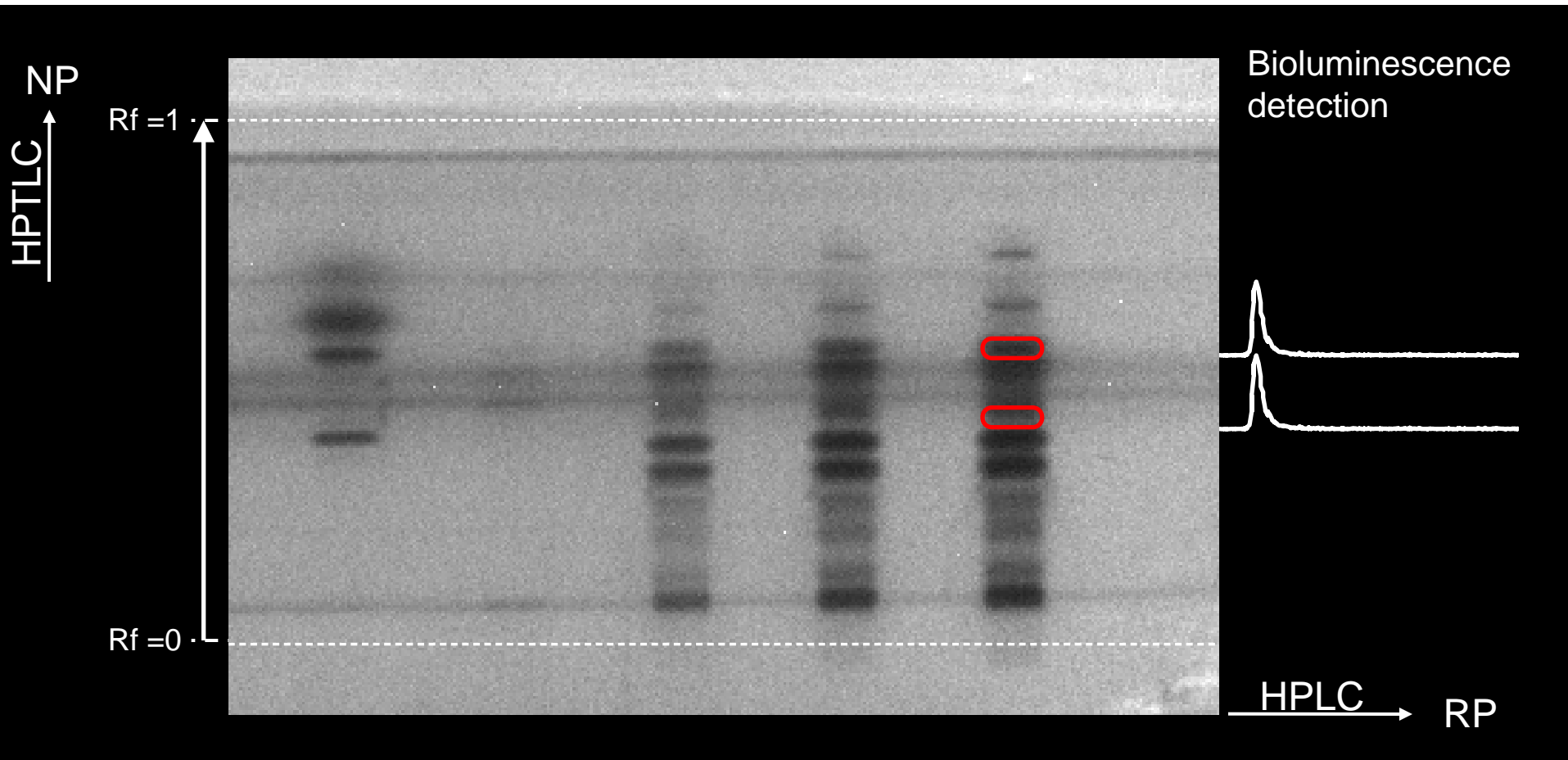
Reference



Benzothiazole

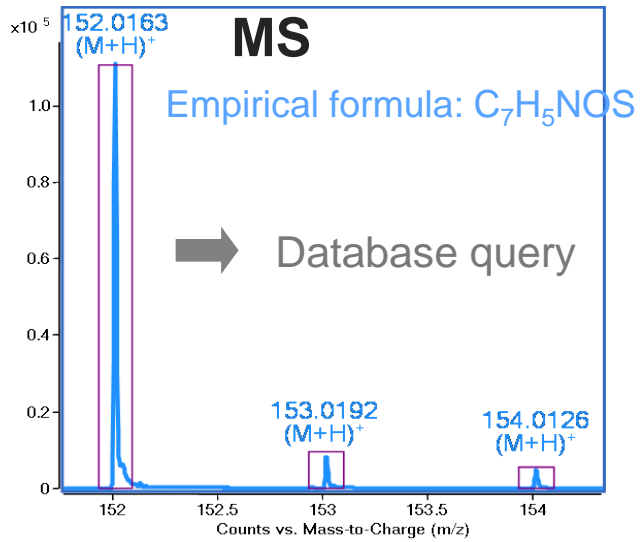


# Detection of luminescence inhibition (*Vibrio fischeri*) and transfer of the substances via HPTLC-MS interface to MS

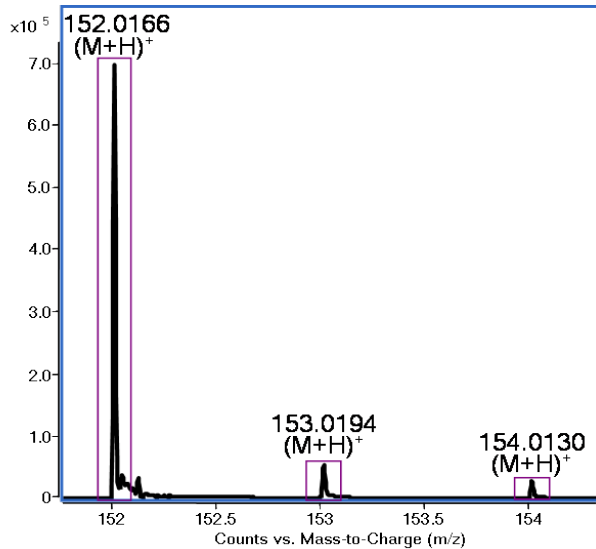
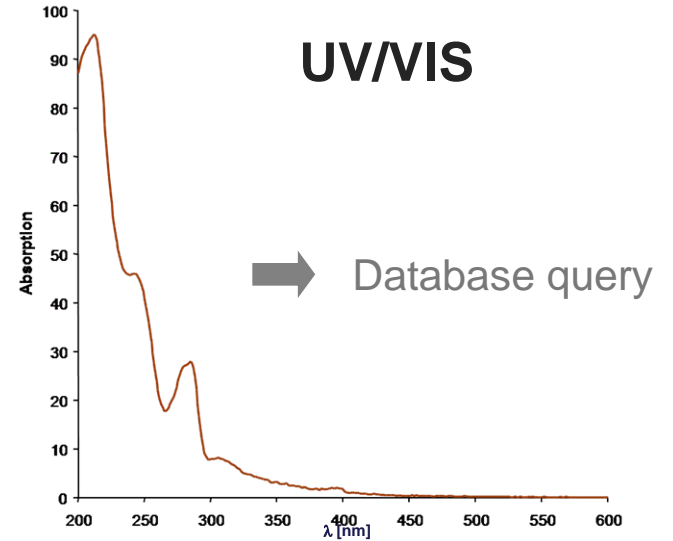




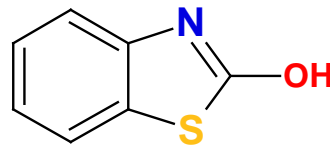
# Identification of the unknown compound (II)



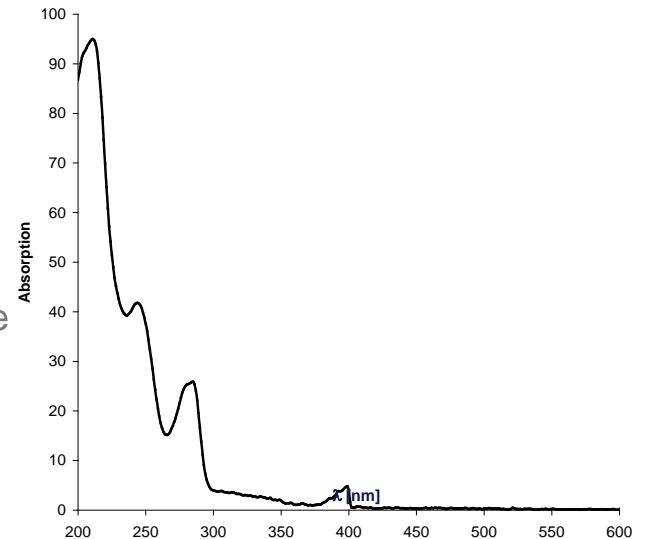
Sample



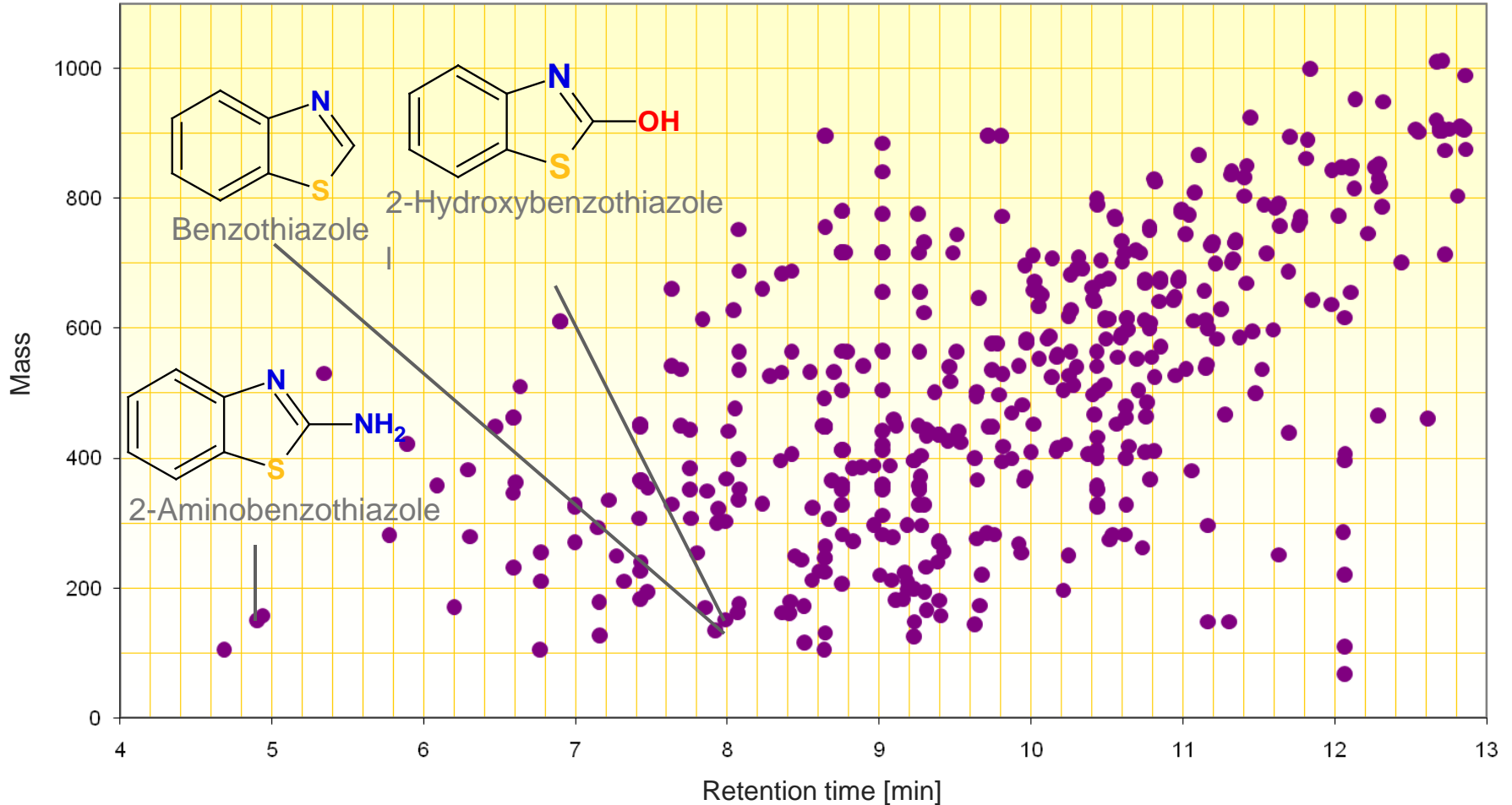
Reference



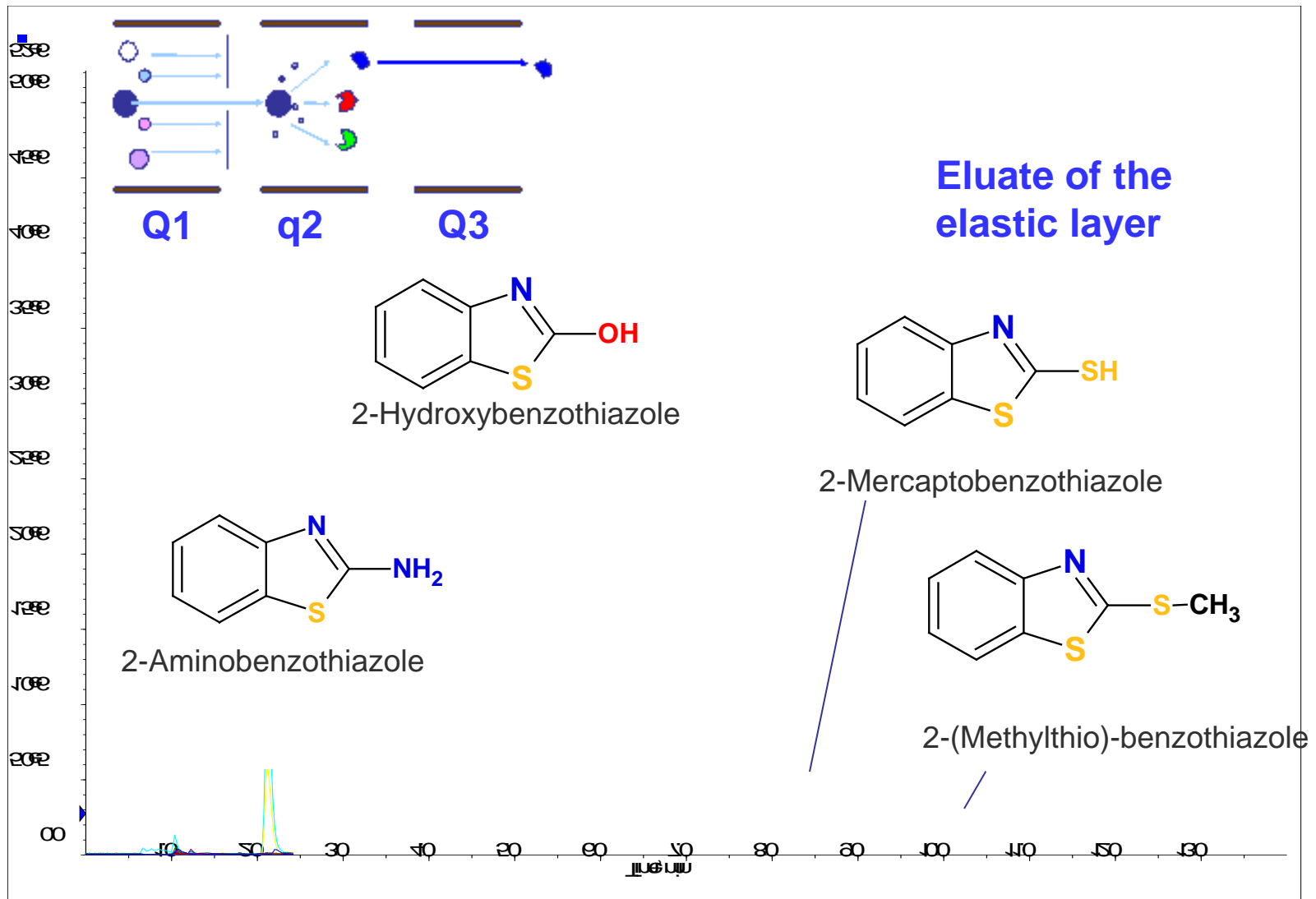
2-Hydroxybenzothiazole



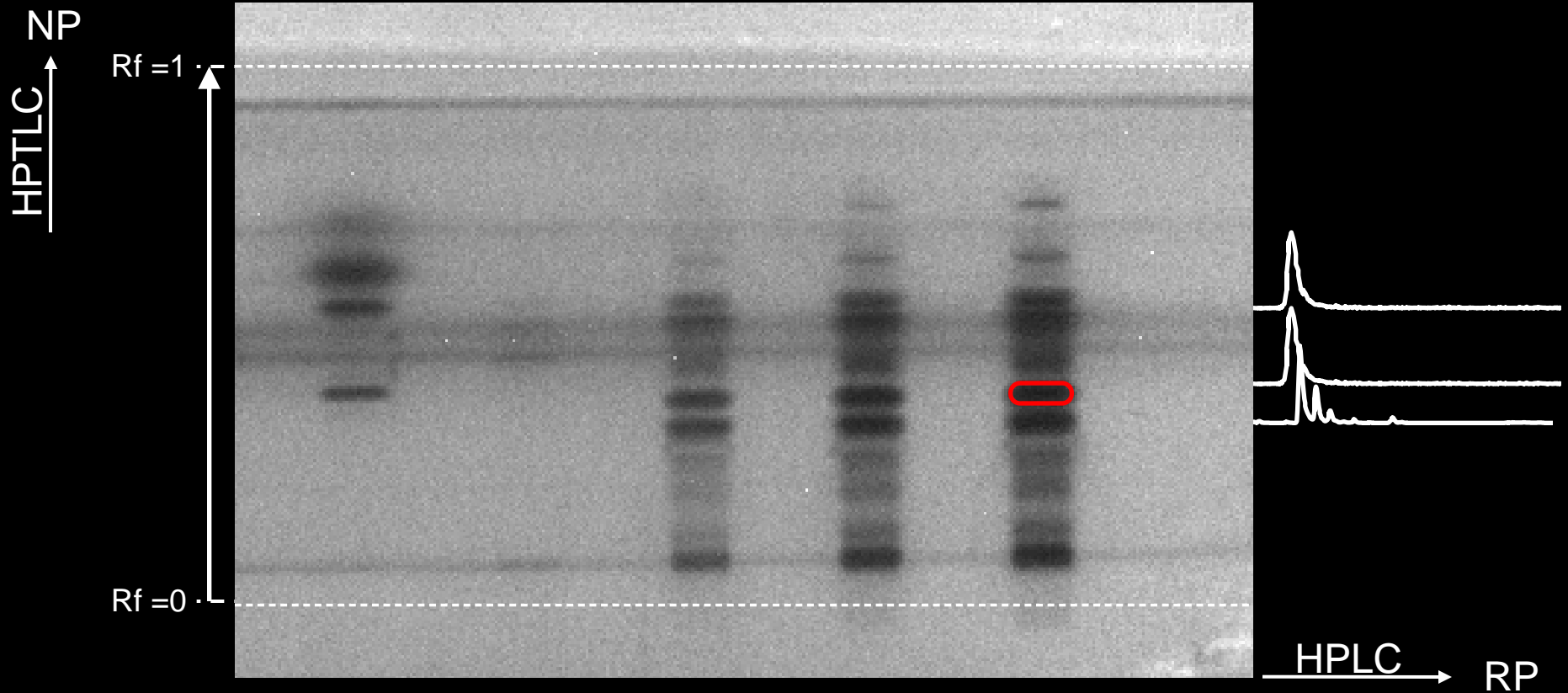
# Mass/Retention time diagram of all compounds in the eluate



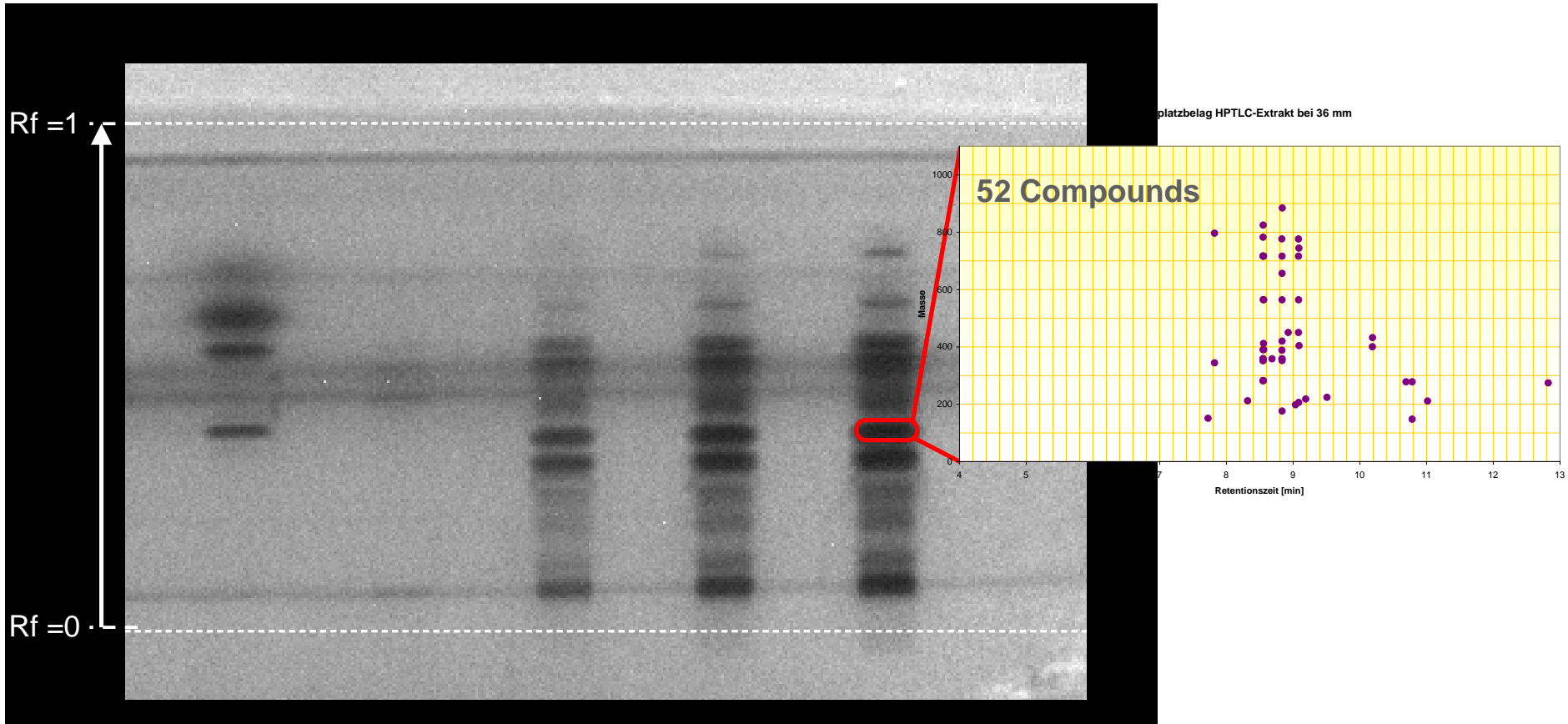
# Target Analysis for Benzothiazole via Triple Quadrupol MS



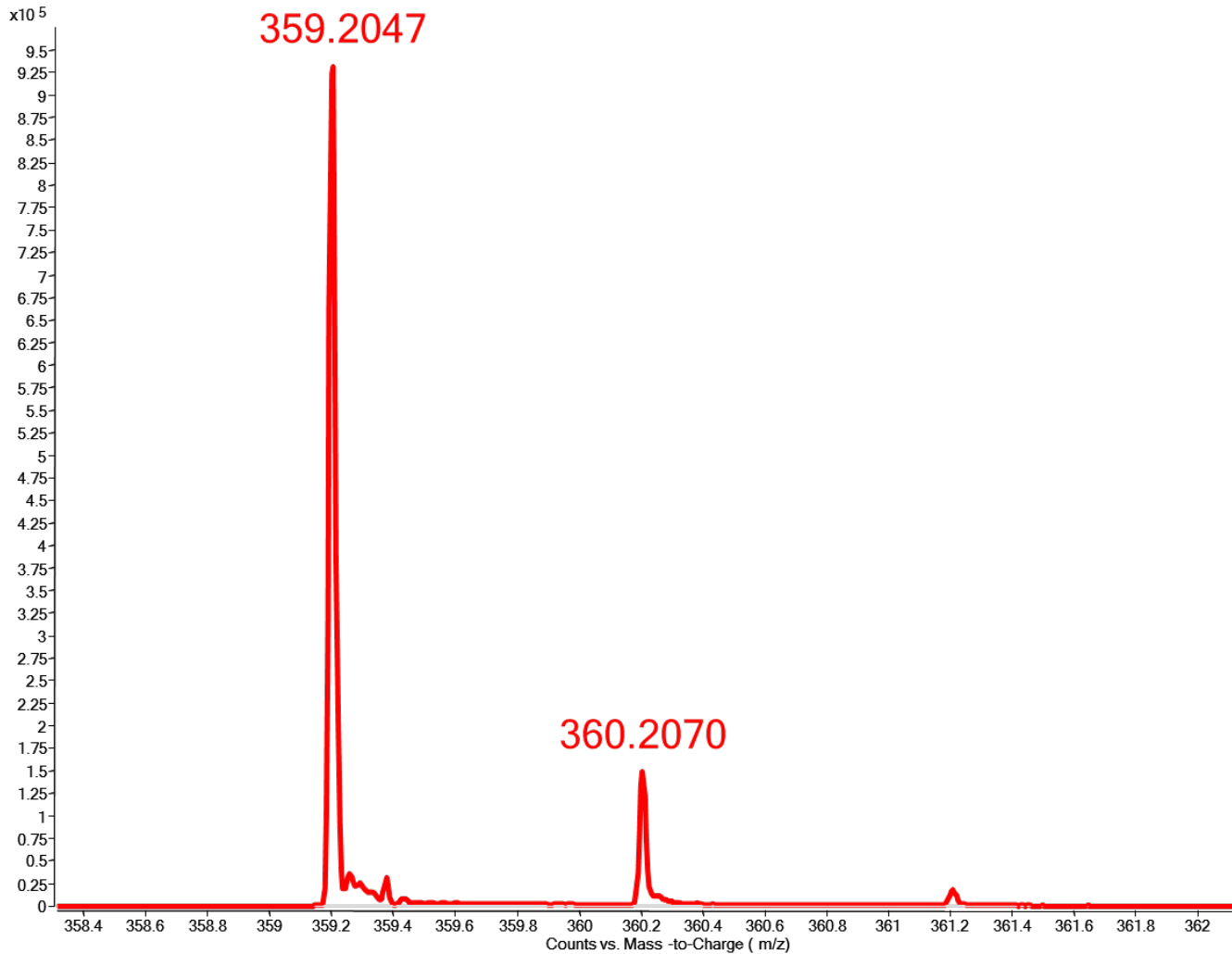
# Detection of luminescence inhibition (*Vibrio fischeri*) and transfer of the substances via HPTLC-MS interface to MS



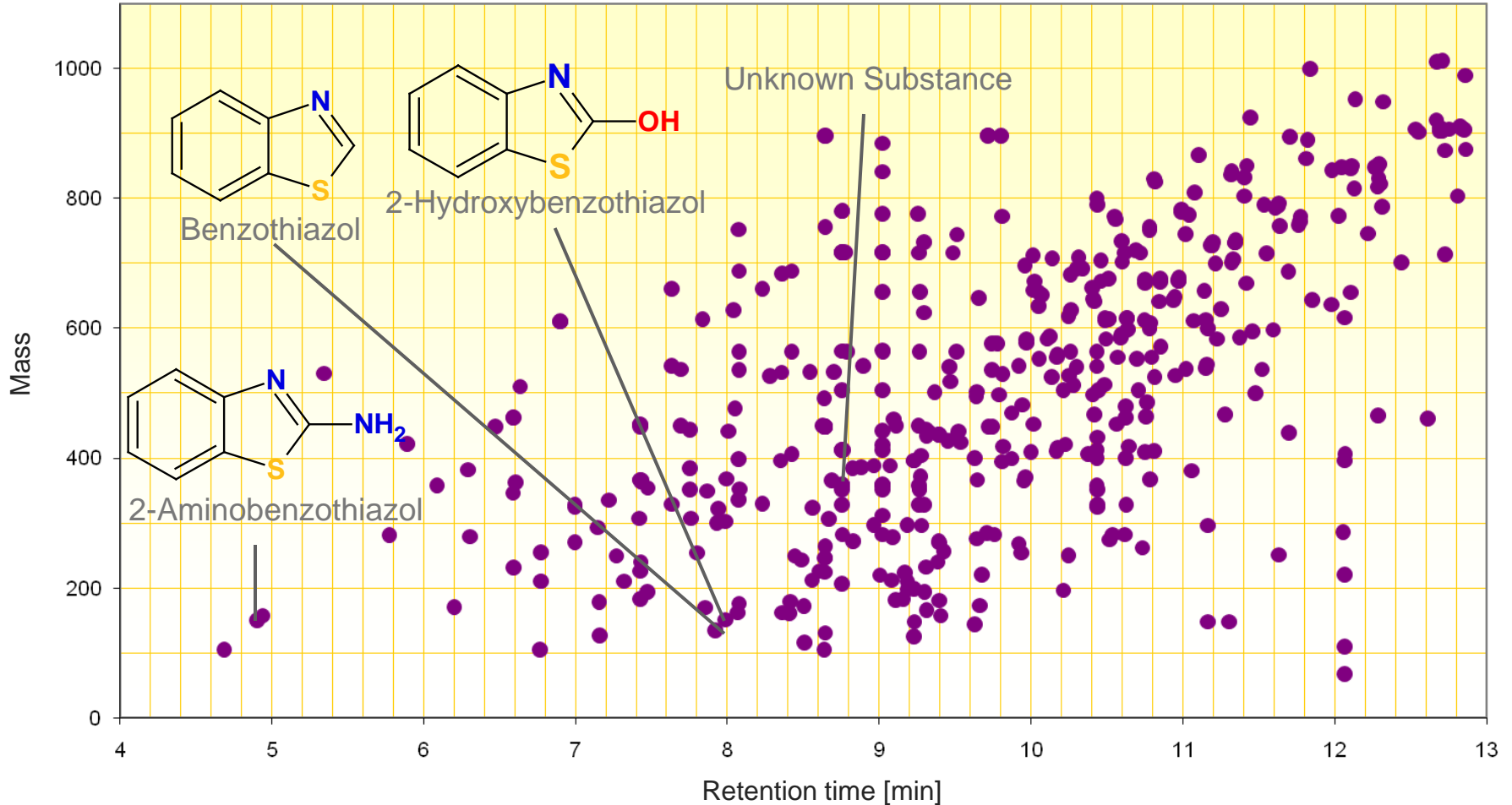
# Combination of the Non-Target-Screening Strategies



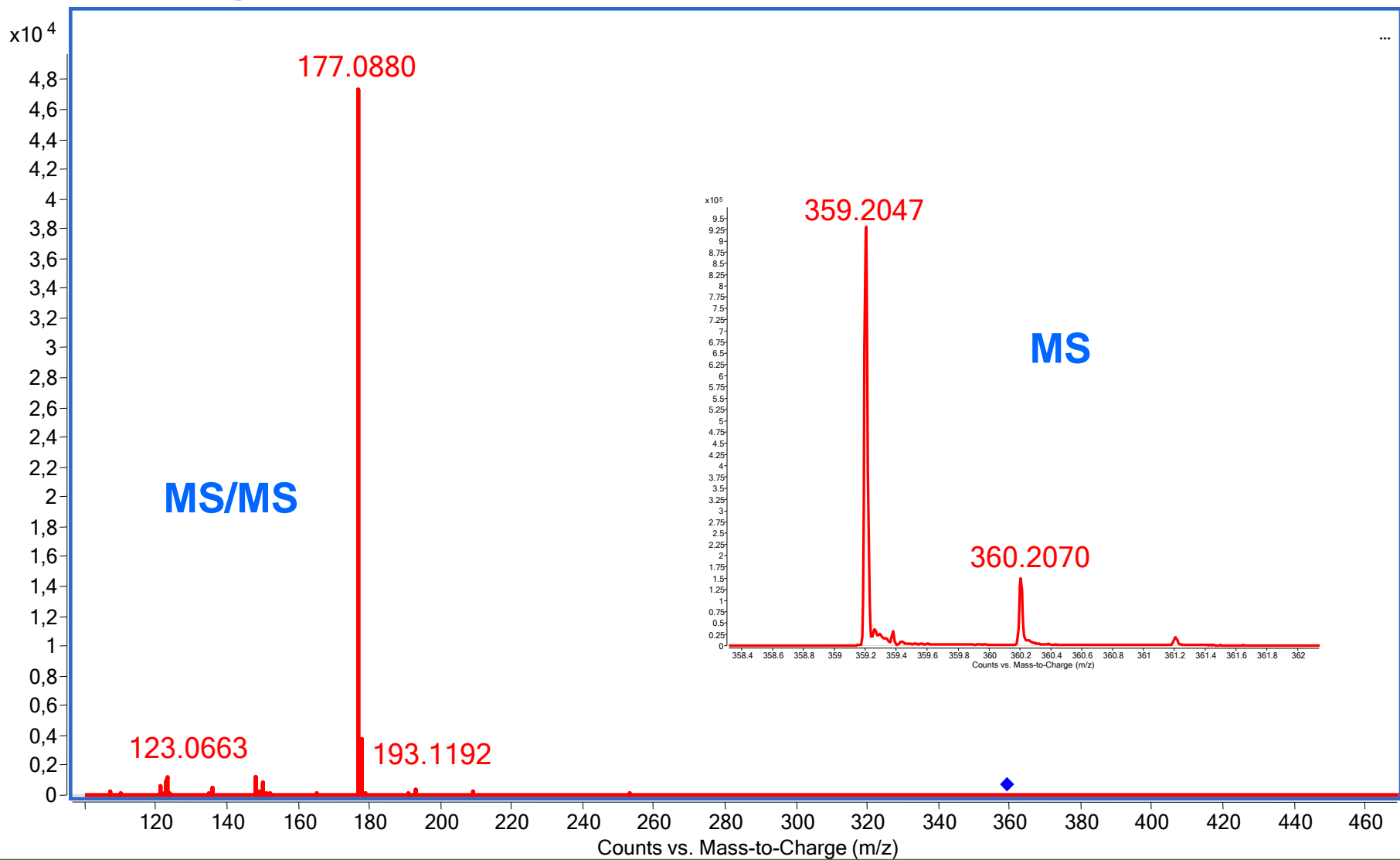
# Mass spectra from the HPTLC zone with a migration distance of 36 mm



# Mass/Retention time diagram of all compounds in the eluate

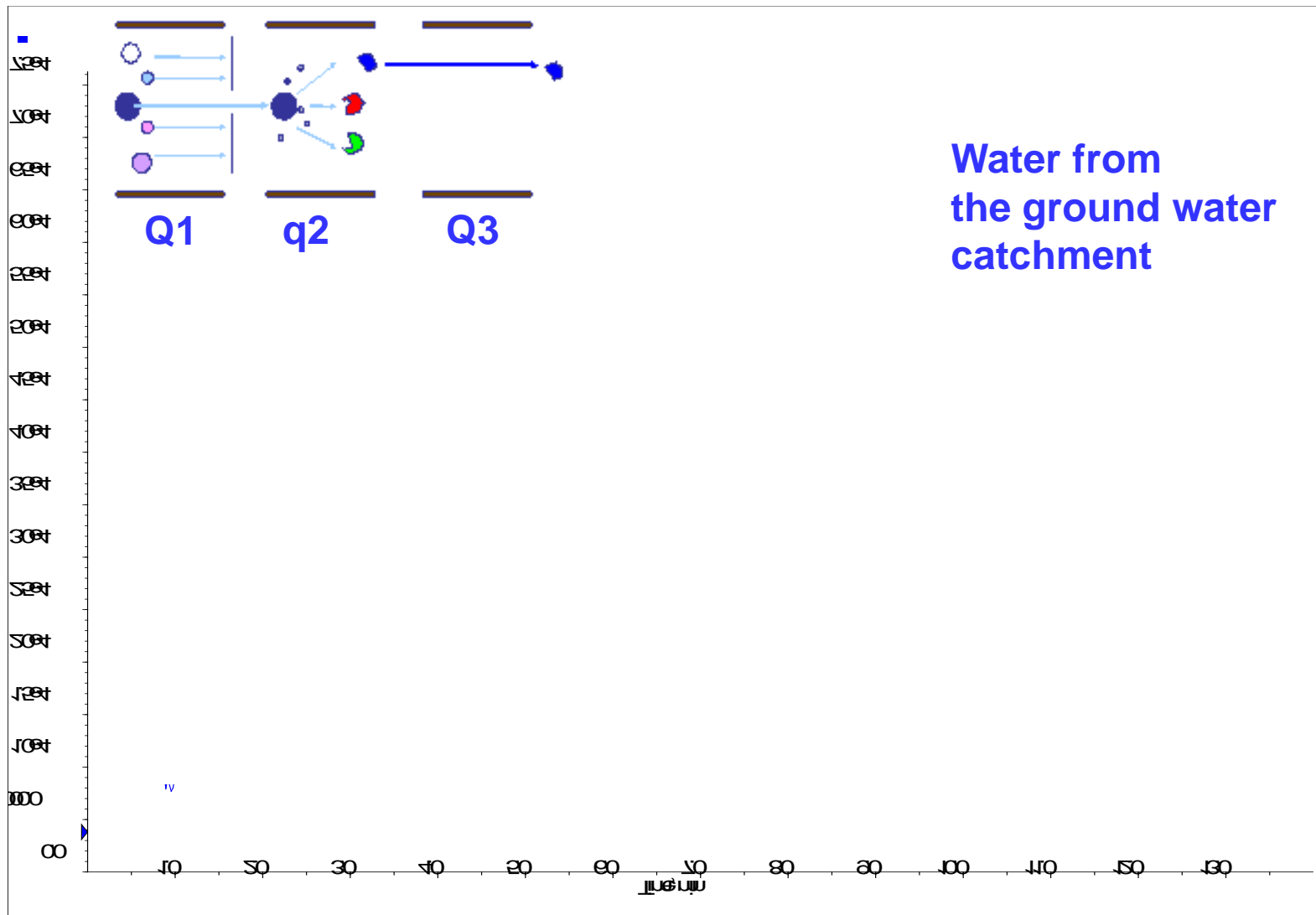


# MS/MS from the HPTLC zone with a migration distance of 36 mm

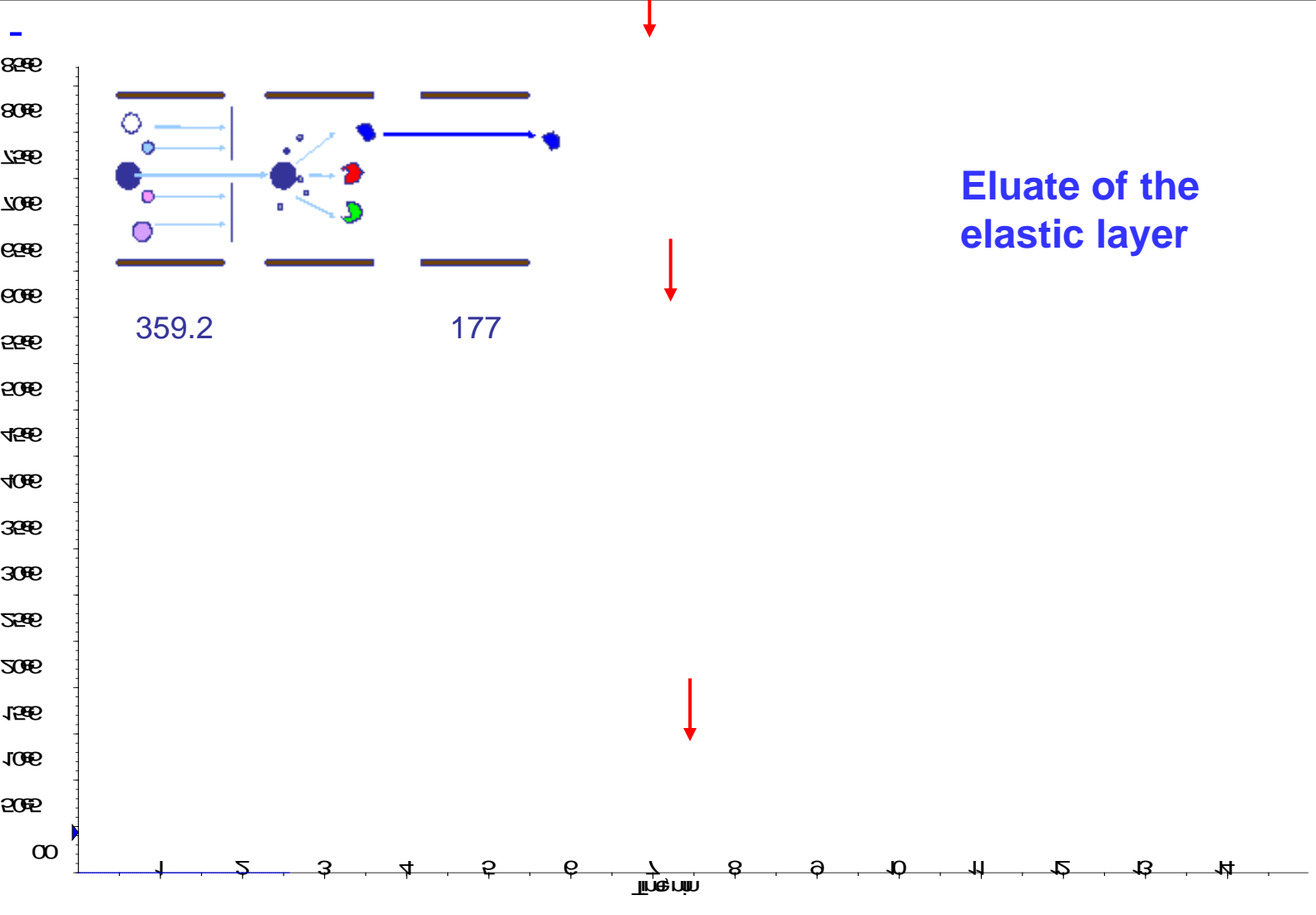




# Target Analysis for Benzothiazole via Triple Quadrupol MS

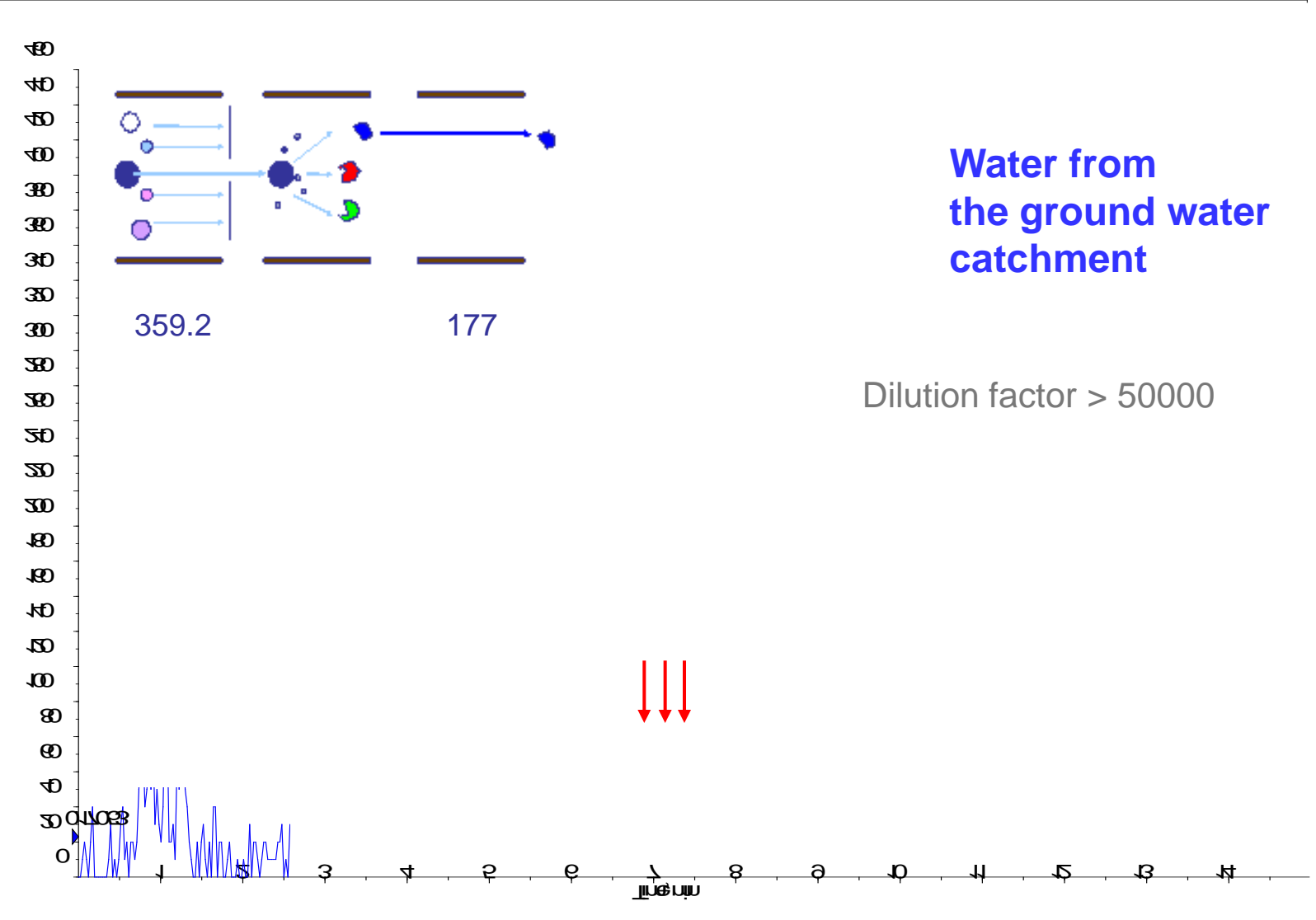


# Mass transition 359.2 / 177



Eluate of the elastic layer

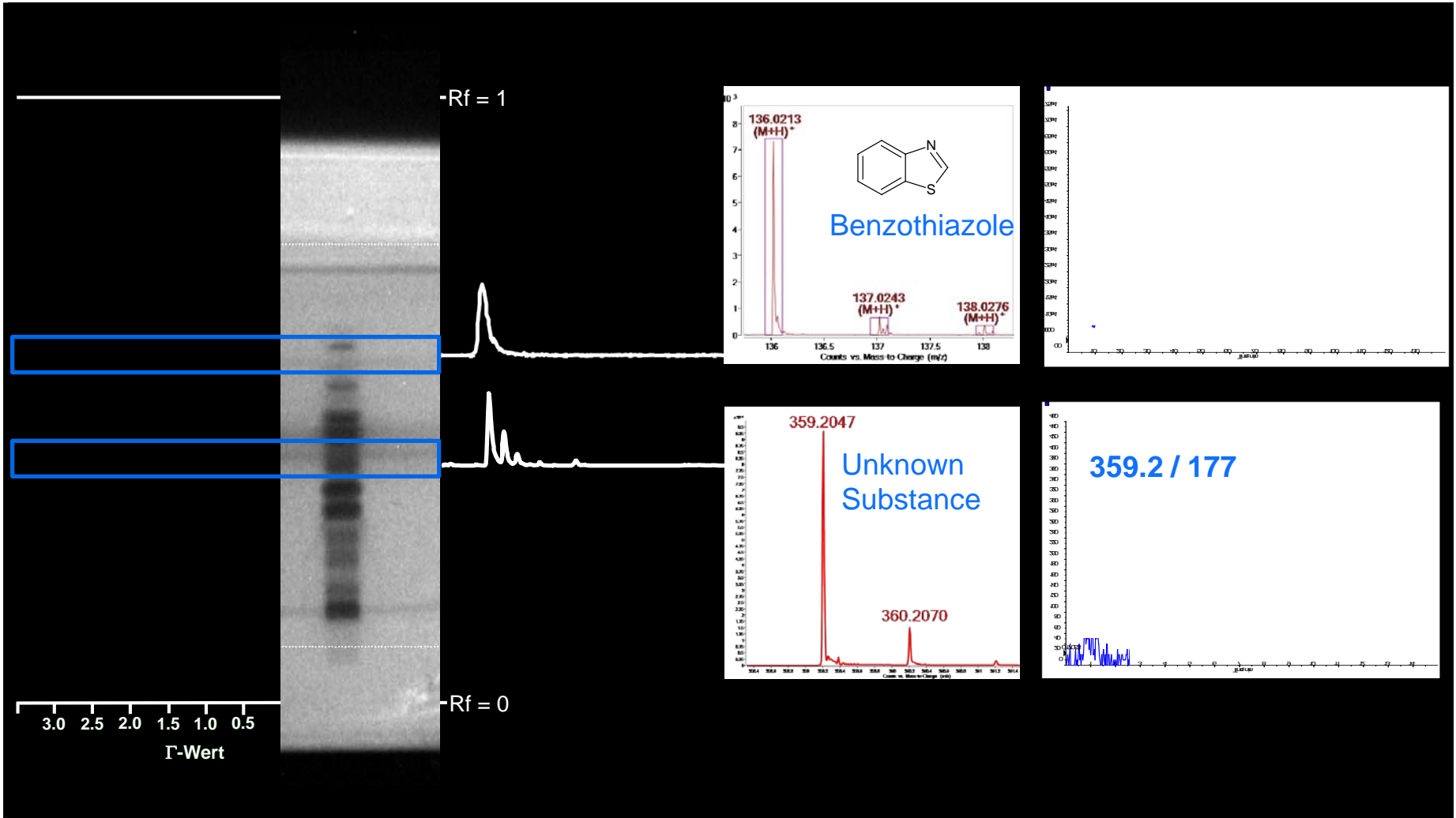
# Mass transition 359.2 / 177



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- Introduction
- Identification of pesticides in ground water
- Ozonation reaction products
- Effect-Directed Analysis with *Vibrio fischeri*
- Ground water contamination from synthetic turf
- Conclusion

# Conclusion





# Control and Research



**Thank you for your attention!**

