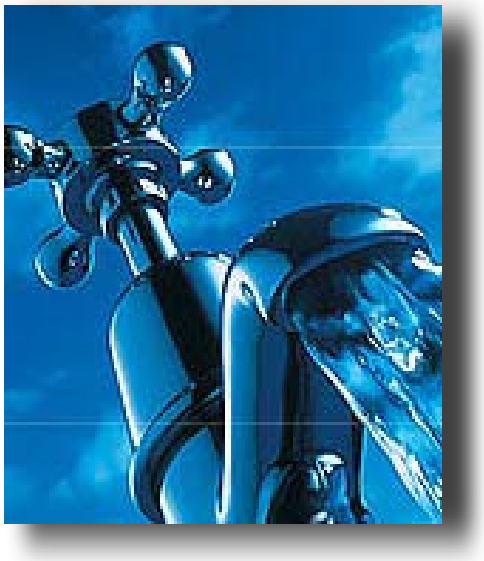
International Symposium for HPTLC
8. July 2011, Basel

Effect-directed analysis of landfill leachate using HPTLC/AMD with bioluminescence detection



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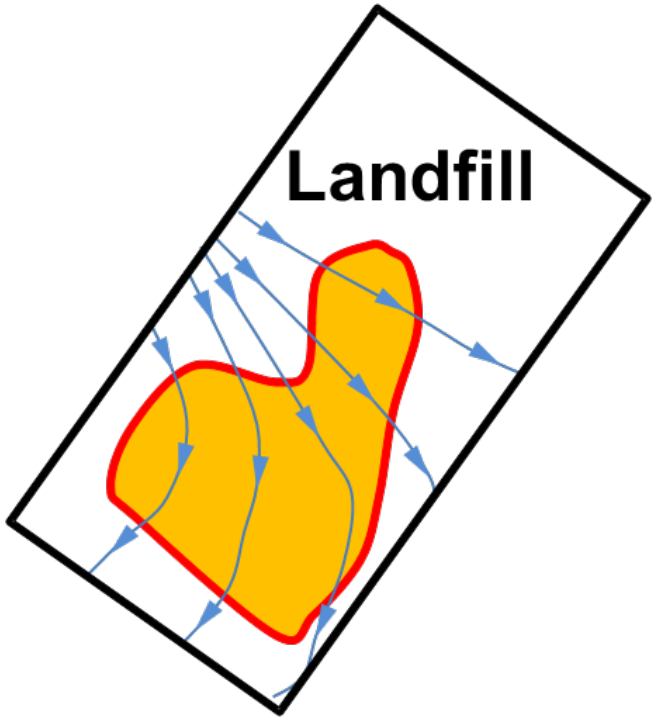
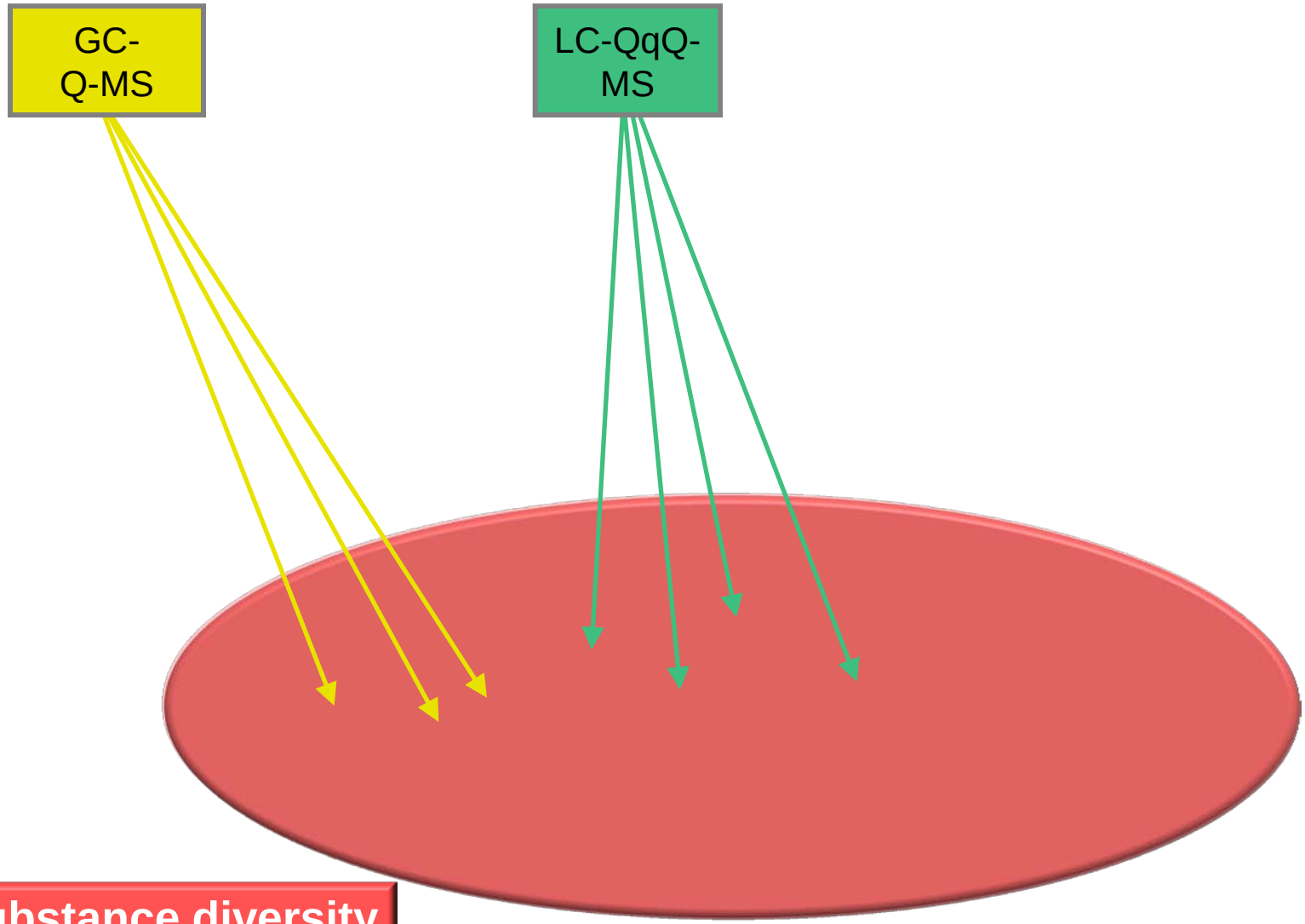
Exploration of contaminated sites



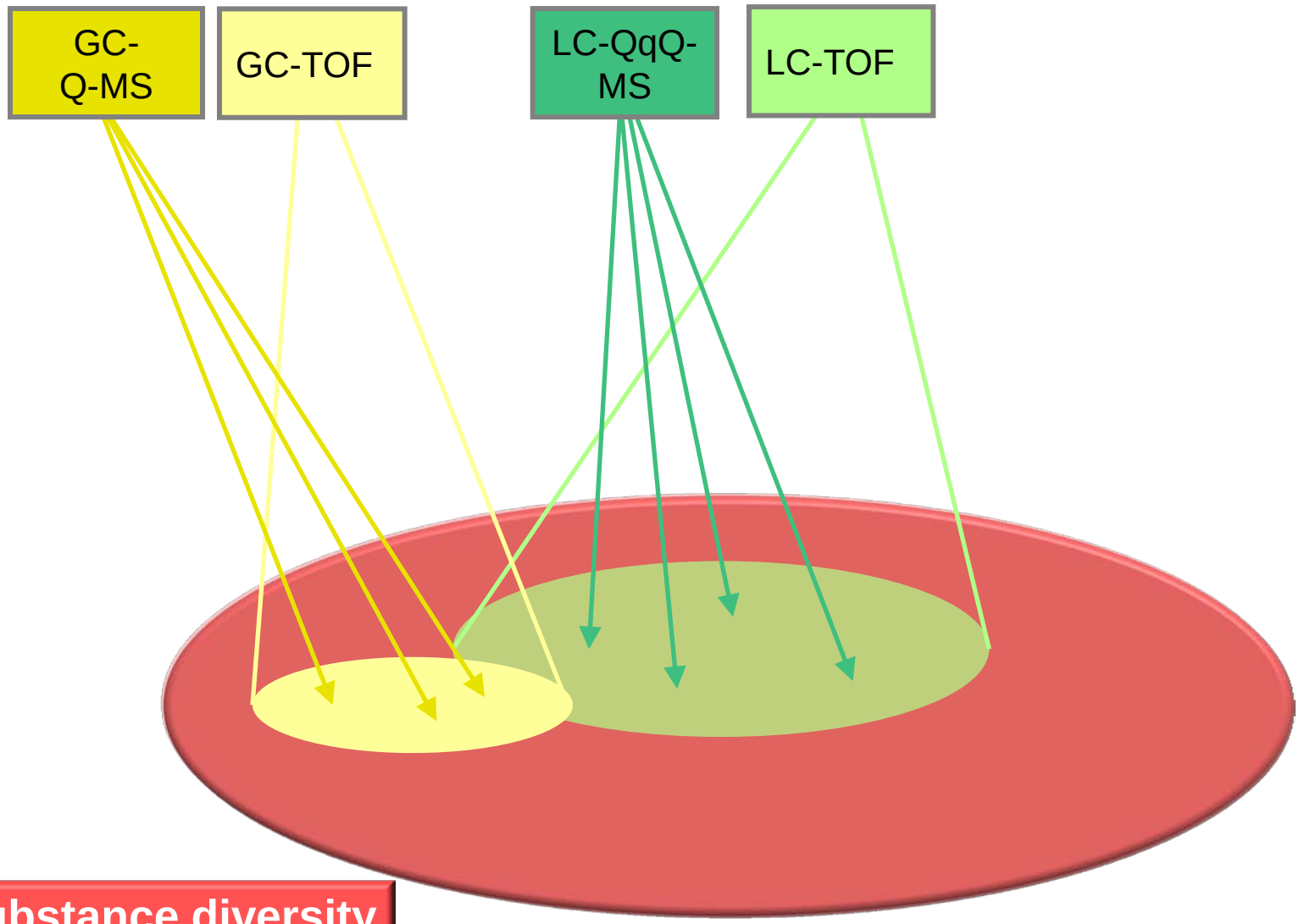
Definition of the problem

- How heavy polluted is this area?
- Which is the method of choice for evaluating the contamination?
- Which substances have a higher priority to be identified?

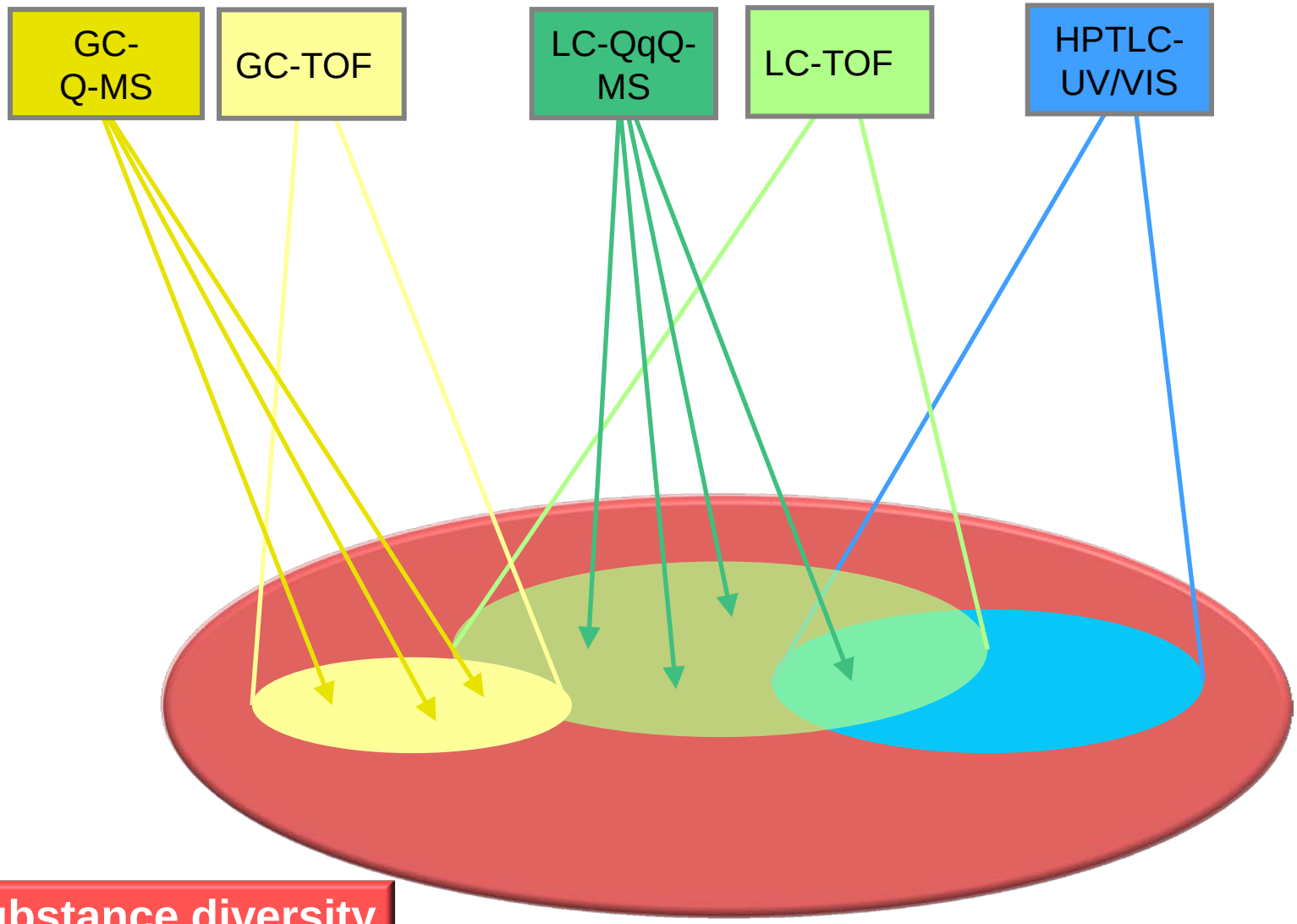
Strategy Target Analysis



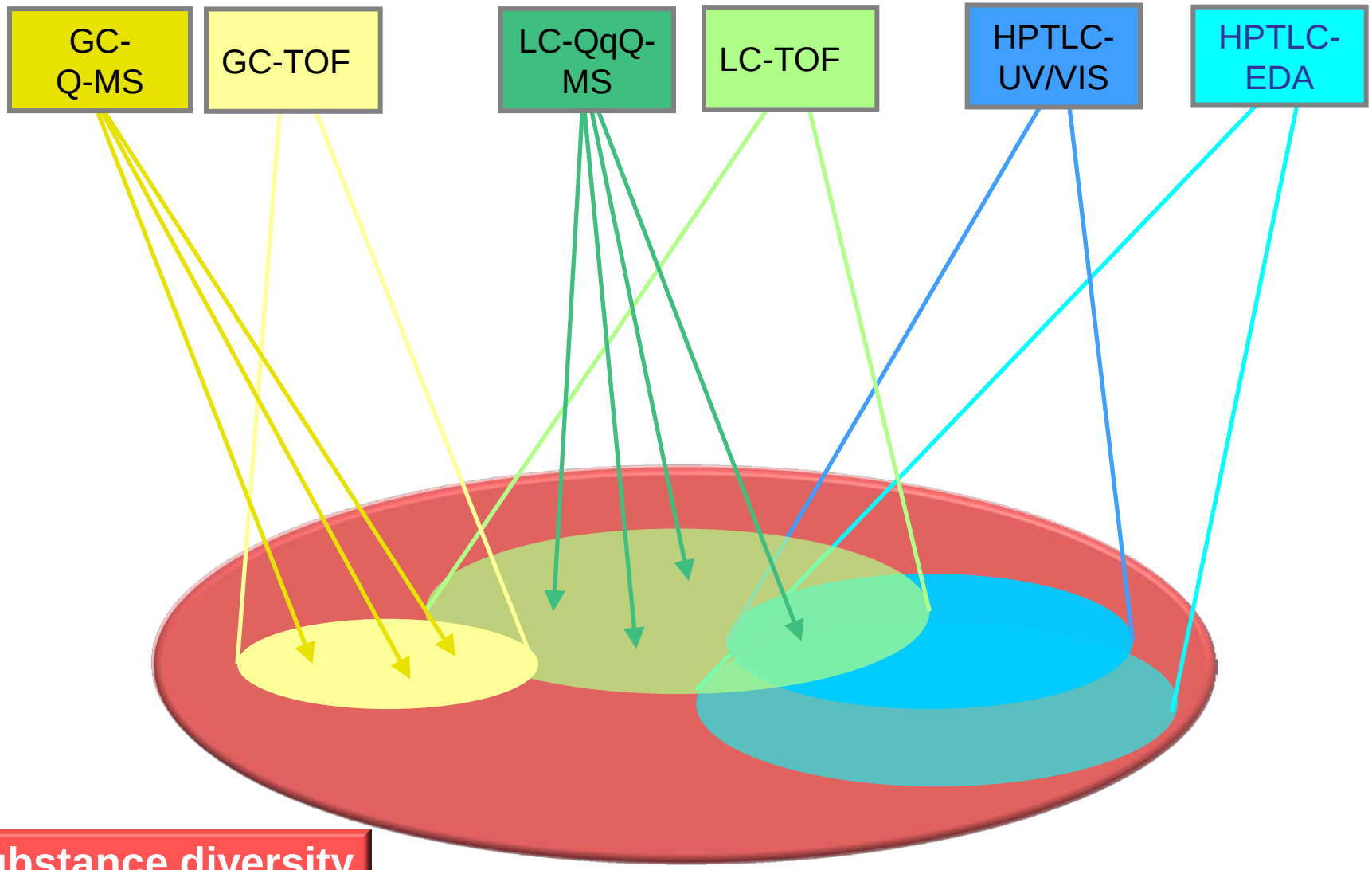
Strategy Non-Target-Screening



Strategy Non-Target-Screening



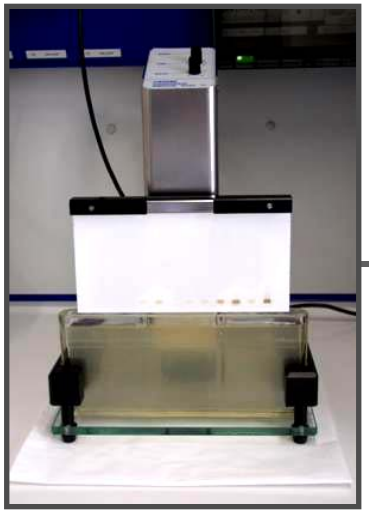
Strategy Non-Target-Screening with effect-directed analysis (EDA)



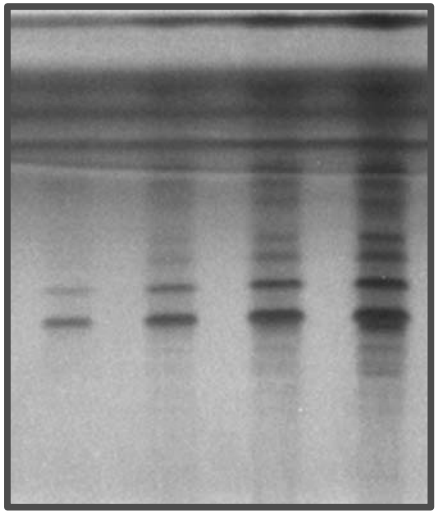
Schematic diagram of the HPTLC-AMD analysis with bioluminescence detection

Vibrio fischeri

Immersion

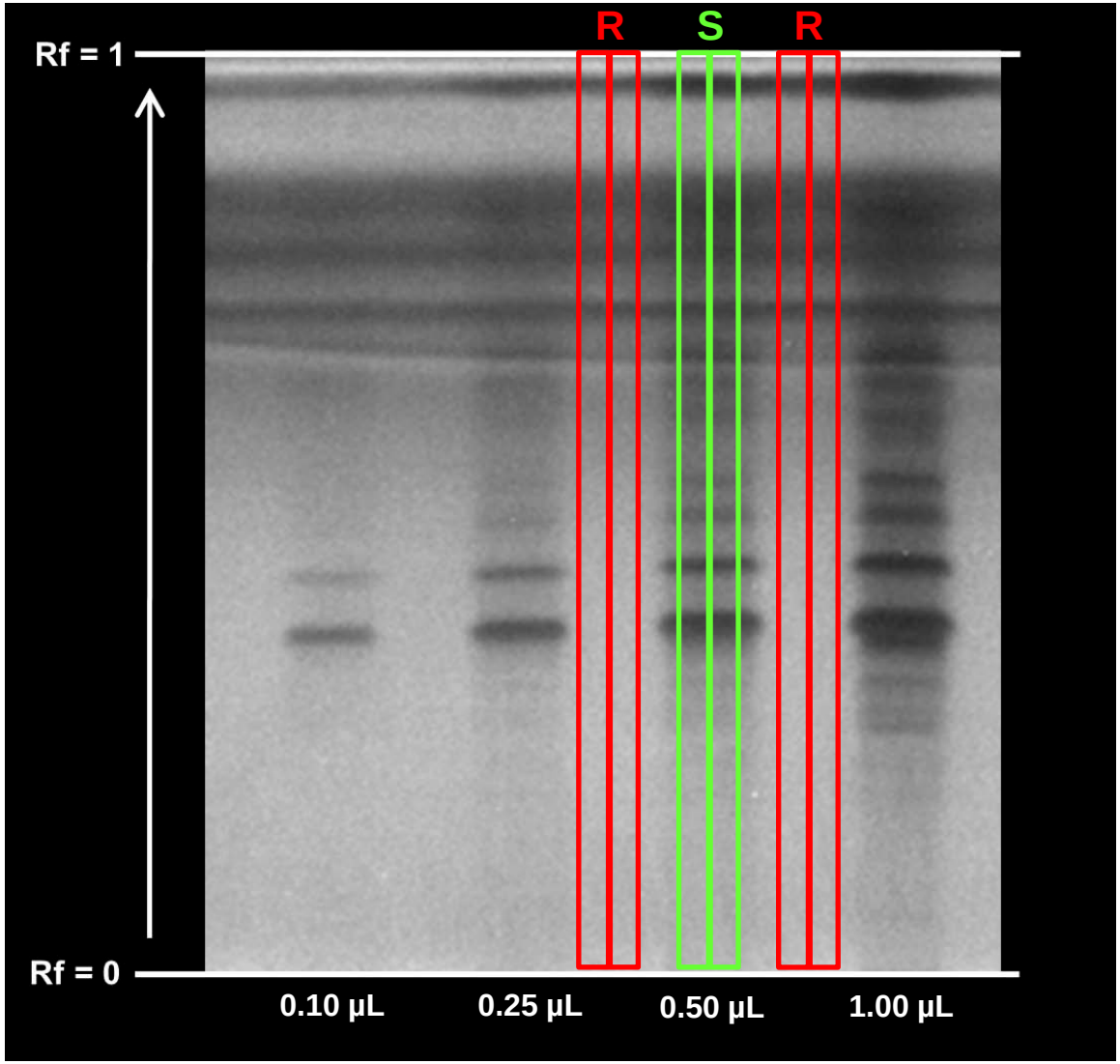


Detection



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.

Evaluation of the bioluminescence picture



Calculation of inhibition chromatogram

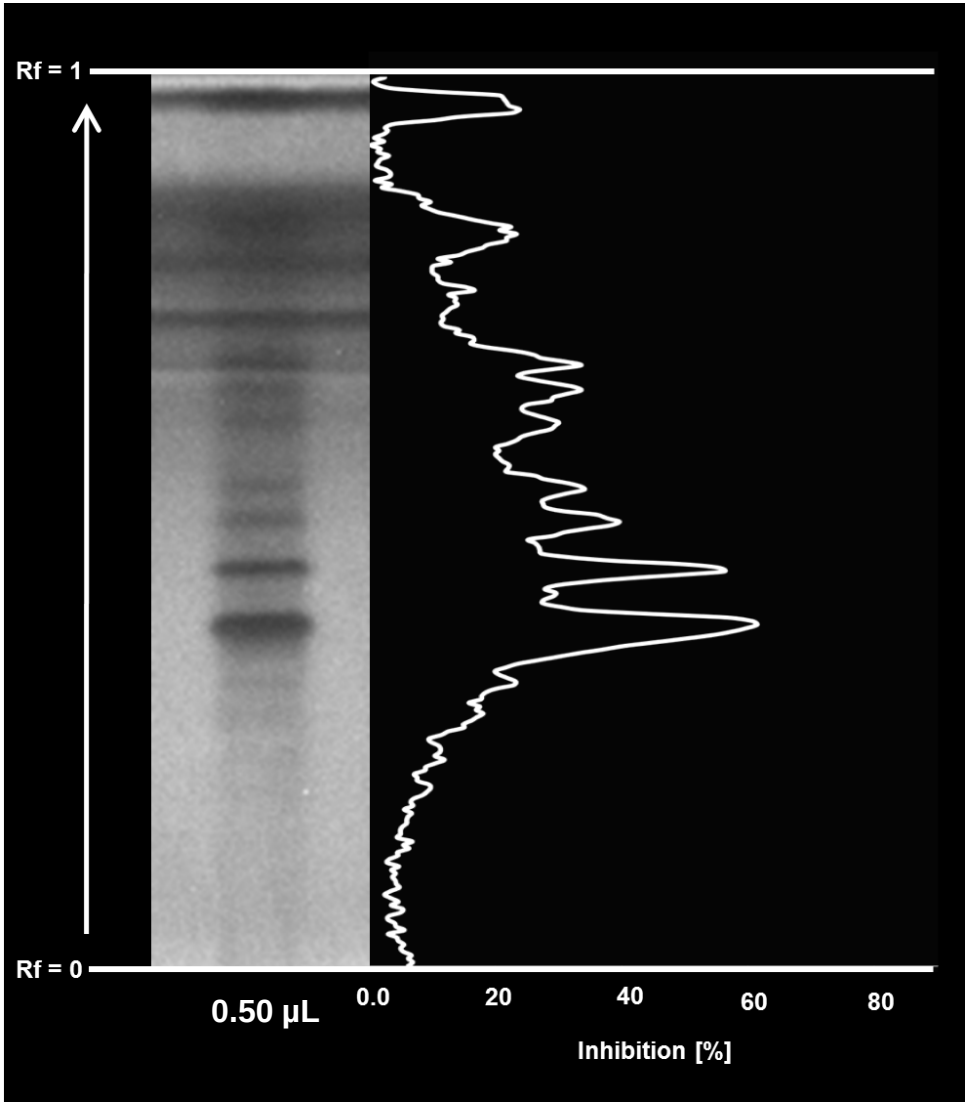
Pixel row	R left	Track	R right	Average R	Inhibition [%]
1	192,87	191,72	187,5	190,19	-0,81
2	193,1	191,97	188,35	190,73	-0,65
3	192,05	191,72	188,65	190,35	-0,72
4	195,05	192,47	190,75	192,90	0,22
5	196,15	192,69	192,1	194,13	0,74
6	197,8	189,25	193,7	195,75	3,32
7	197,05	184,14	193,65	195,35	5,74
8	198,2	177,42	193,7	195,95	9,46
9	199,4	171,47	195,35	197,38	13,12
10	199	168,53	195,95	197,48	14,66
11	199,25	167,58	196,65	197,95	15,34
12	200,25	166,64	197,6	198,93	16,23
13	201,75	168,14	199,35	200,55	16,16
⋮					⋮
233	159,35	153,31	154,55	156,95	2,32
234	153,15	144,92	146,45	149,80	3,26
235	144,05	137,28	139,55	141,80	3,19
236	137,75	132,97	134,9	136,33	2,46
237	136,45	133,89	133,35	134,90	0,75
238	139,35	137,83	136,95	138,15	0,23
239	146,4	146,19	146,1	146,25	0,04
240	154,75	154,36	155,8	155,28	0,59
241	162,5	162,92	163,8	163,15	0,14
242	169,95	170,97	171,55	170,75	-0,13

Grayscales

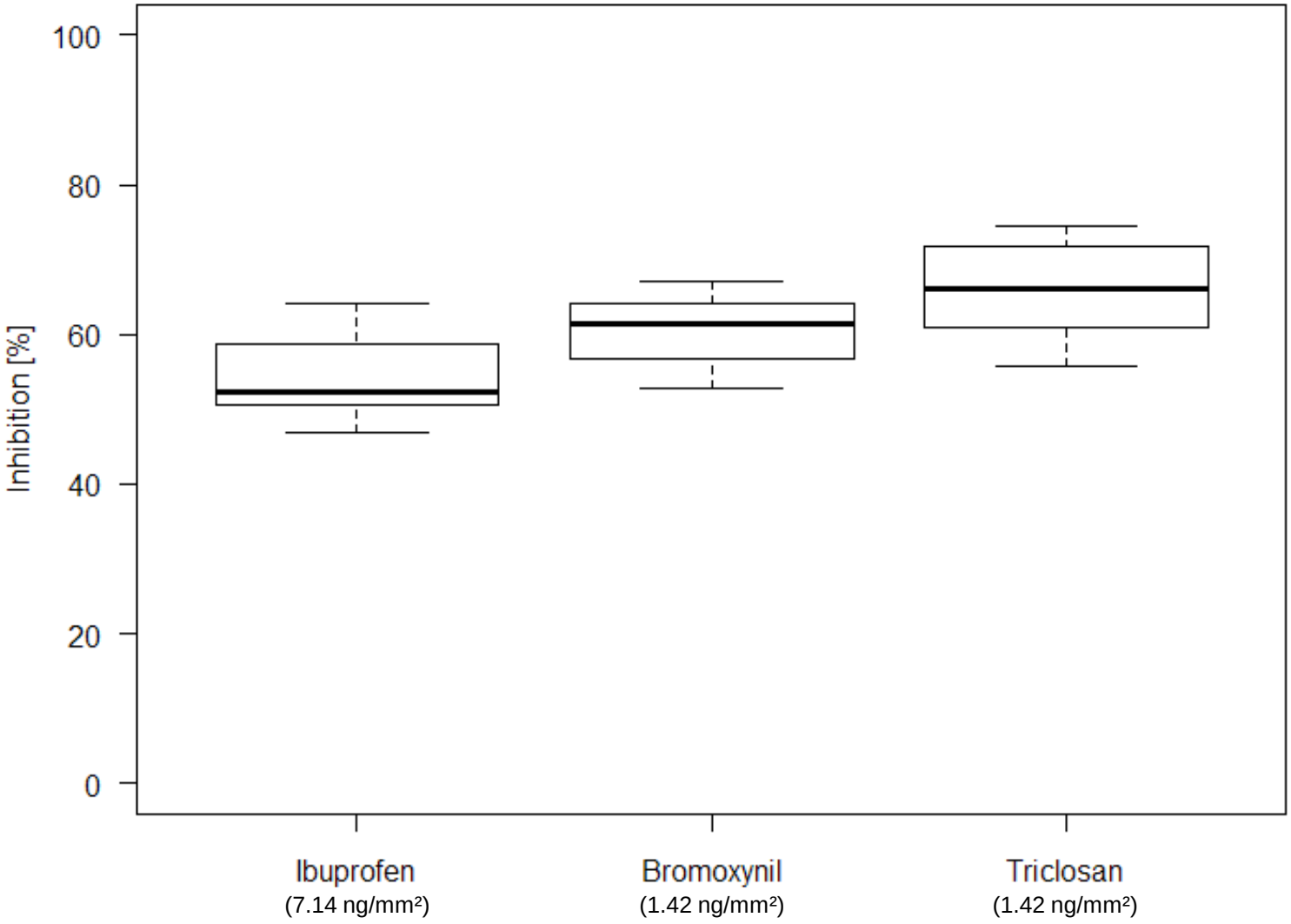
$$I^{S}_{n_k} = 1 - \frac{i^{S}_{n_k}}{i^{R}_{n_k}}$$

I = Inhibition
i = Light intensity
S = Sample
R = Reference
n = Pixel
k = row

Inhibition chromatogram



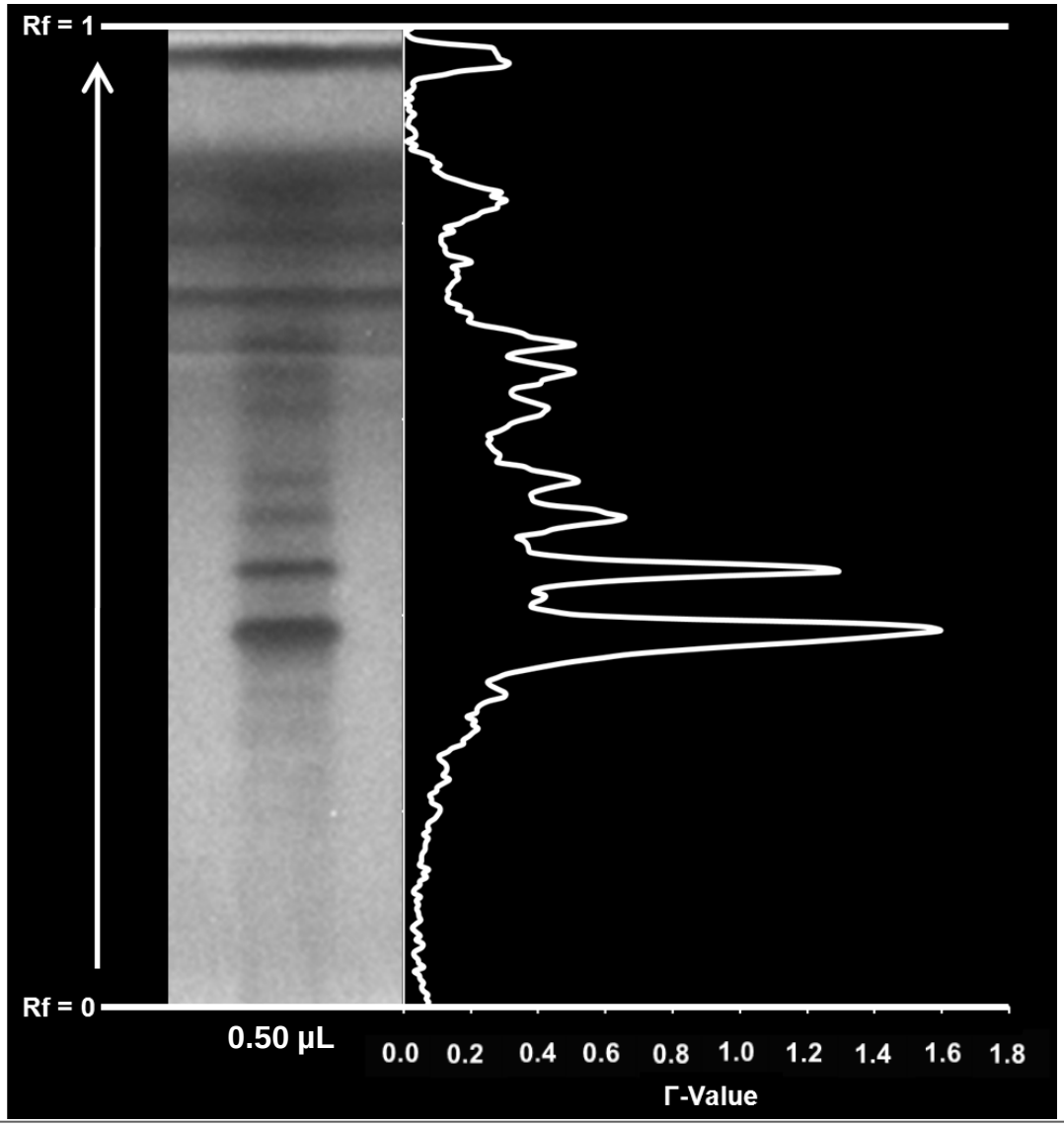
Boxplot for the inhibition of reference substances (N = 54 in a period of 2 months)



Gamma chromatogram

Gamma-Value:

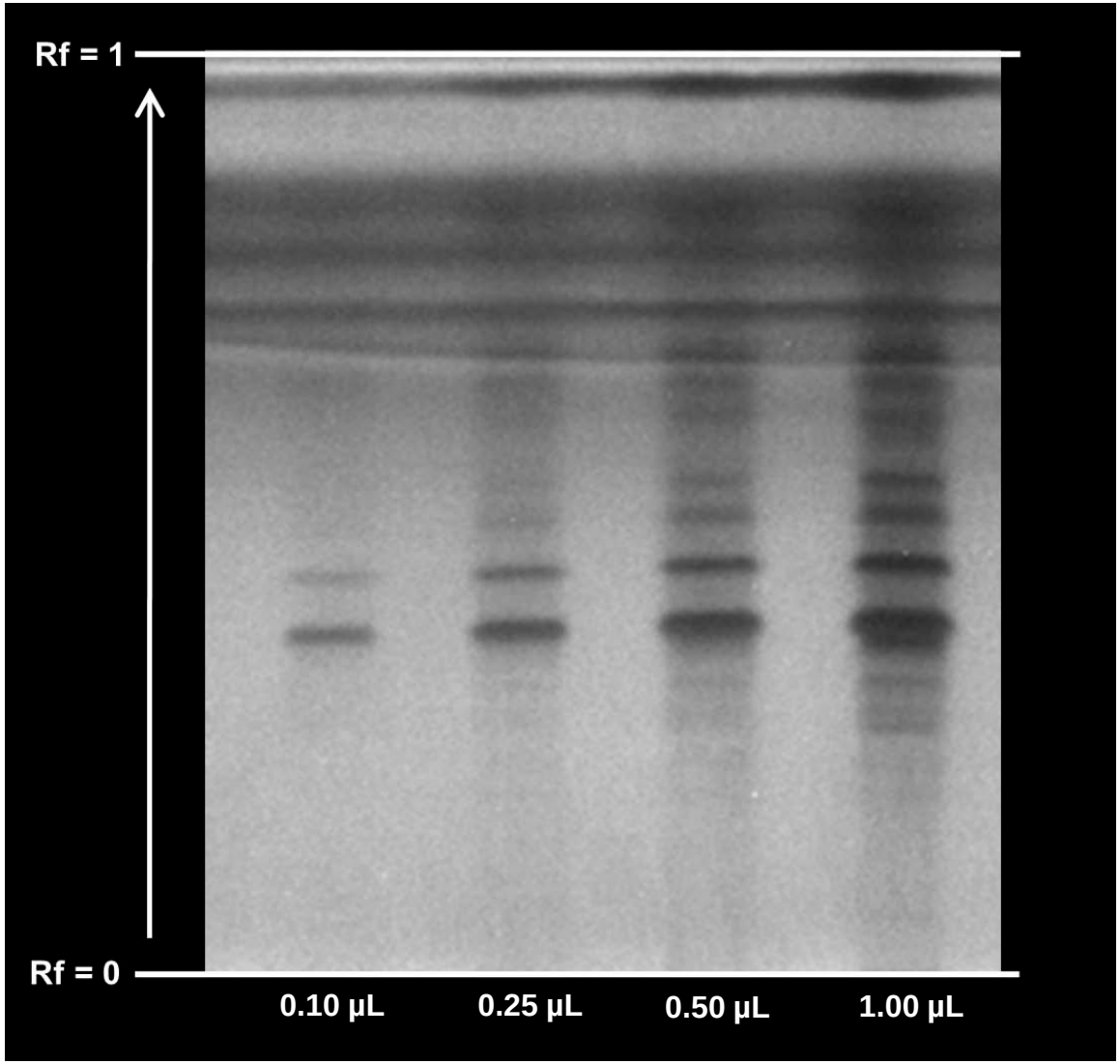
$$\Gamma = \frac{\text{Inhibition}\,[\%]}{100\,\% - \text{Inhibition}\,[\%]}$$



Schulz, W., Seitz, W., Weiss, S.C., Weber, W.H., Böhm, M., bioactivity in water analysis. JPC, 21 (6), 427-430

Flottmann, D. (2008): Use of *Vibrio fischeri* for screening for

Dose-effect relationship



Calculation of the iso-inhibition volume

Gamma value $\Gamma = \frac{I}{100 - I}$

Linearised dose-effect relationship $\lg \Gamma_{i,j} = a_{i,j} + b_{i,j} \cdot \lg V_{i,j}$

Effect concentration 50

$I = 50 \longrightarrow \Gamma = 1 \longrightarrow 0 = a_{i,j} + b_{i,j} \cdot \lg V_{i,j}(50)$

$\longrightarrow V_{i,j}(50) = 10^{-a_{i,j} / b_{i,j}}$ Iso-inhibition volume

$I_i \approx m_i = c_i \cdot V_i$ Inhibition is proportional to the applicated sample volume

$c_{i,1}(50) \sim \frac{1}{V_{i,1}(50)}$ Reciprocal iso-inhibition volume

I = *Inhibition (%)*

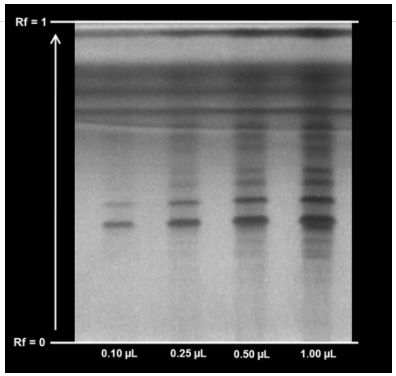
V = *Application volume (µl)*

$\Gamma_{i,j}$ = *Gamma value*

i = *Substance zone*

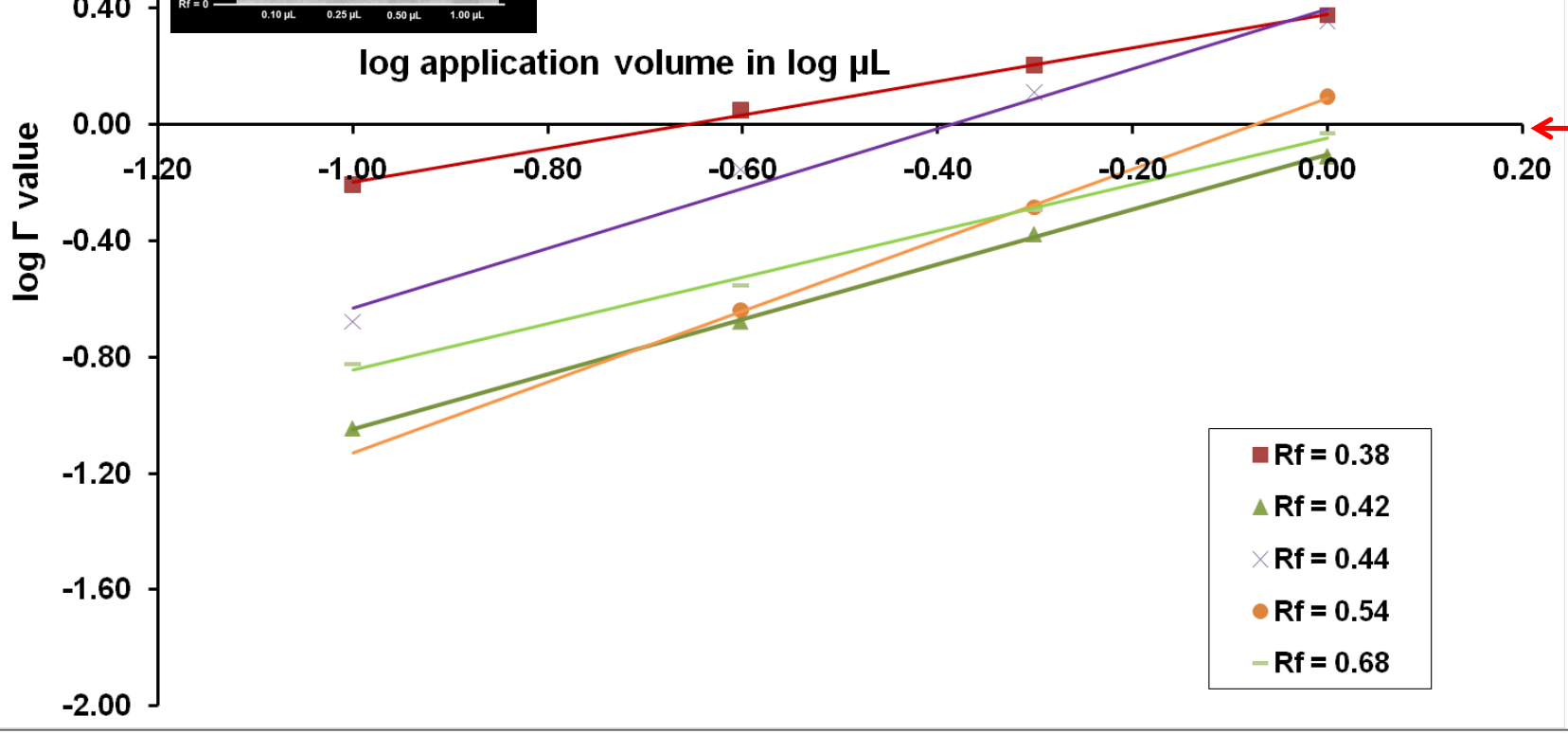
j = *Extract (sample)*

Linearised dose-effect relationship



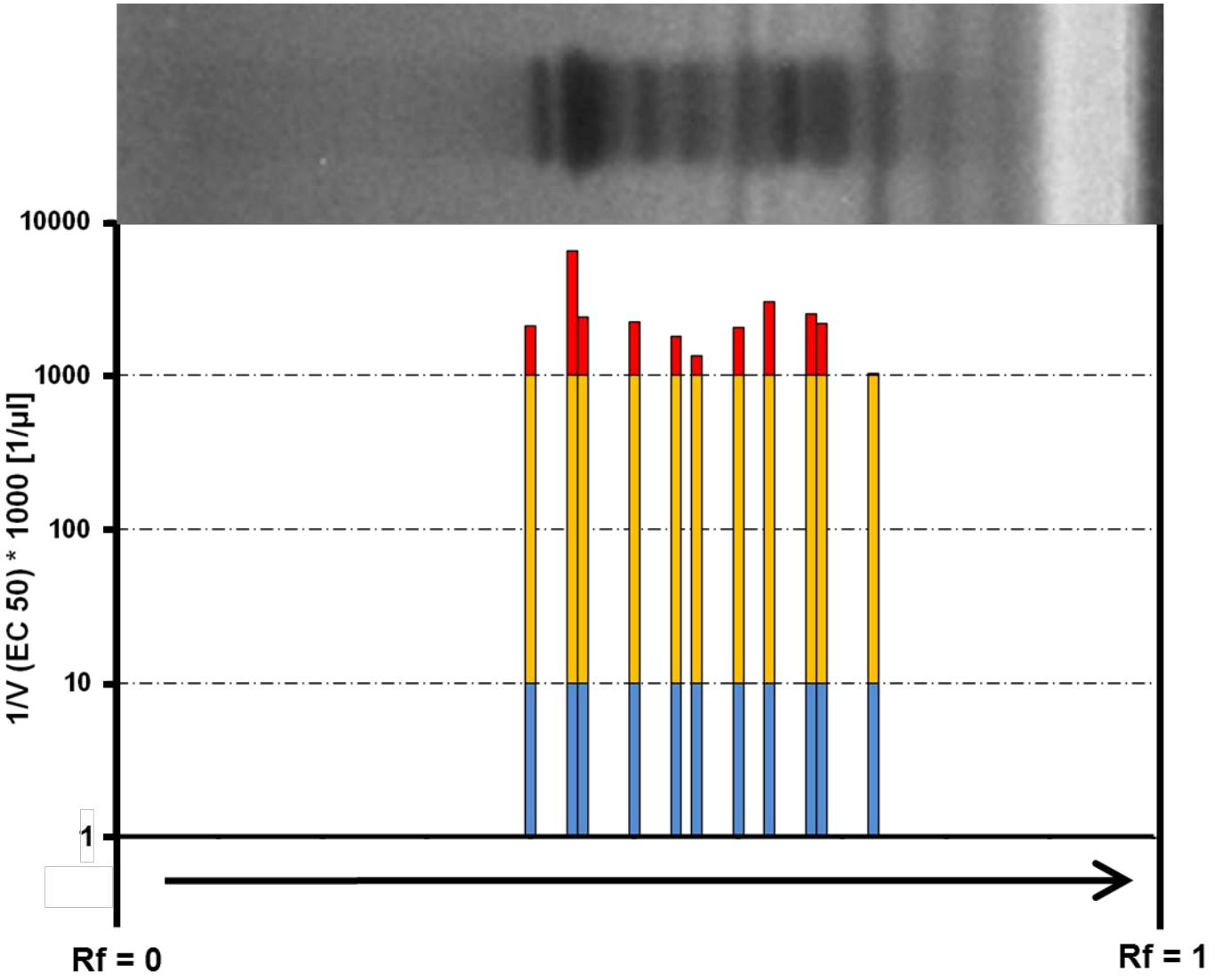
$$\Gamma = \frac{\text{Inhibition}\,[\%]}{100\,\% - \text{Inhibition}\,[\%]}$$

$$\lg \Gamma_{i,j} = a_{i,j} + b_{i,j} \cdot \lg V_{i,j}$$

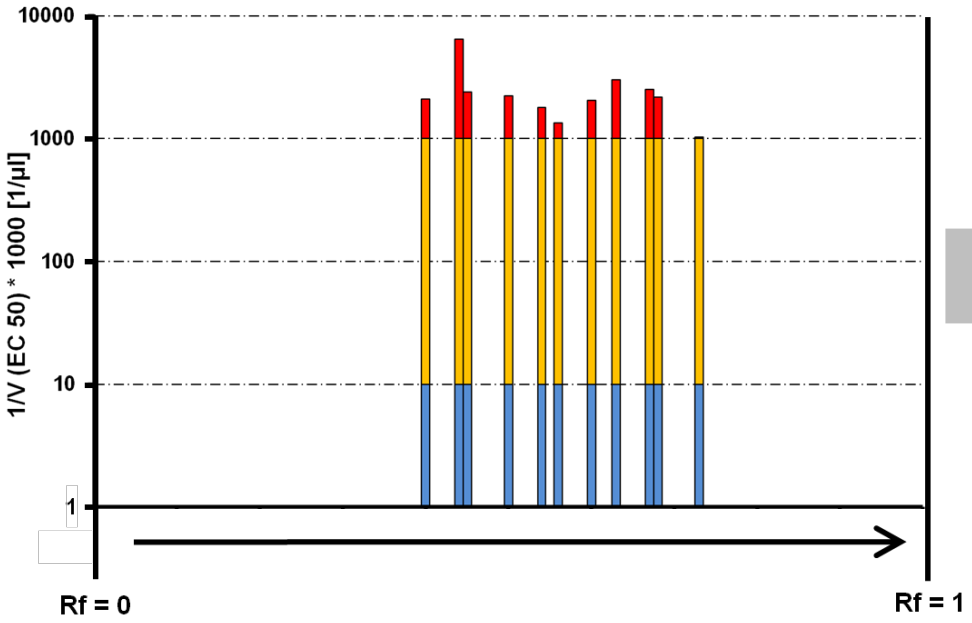


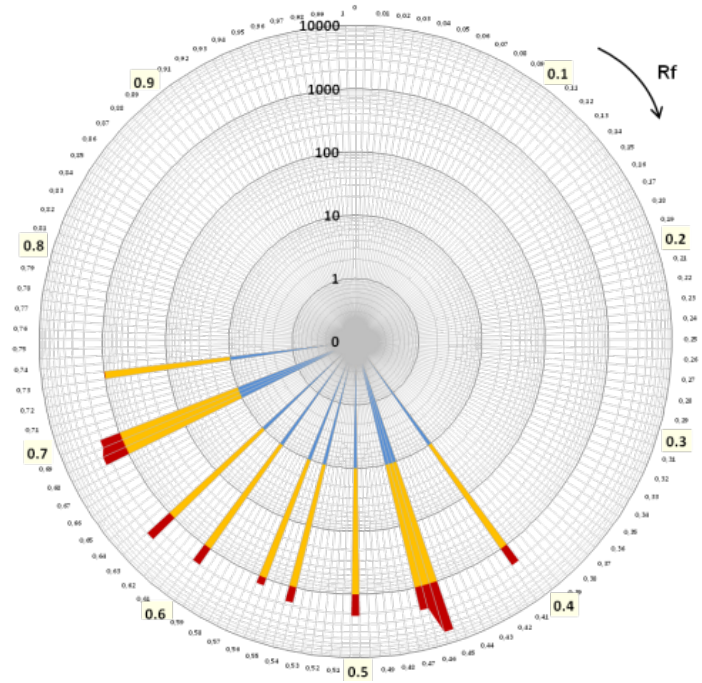
EC 50

Histogram of the reciprocal iso-inhibition volume (RIV)

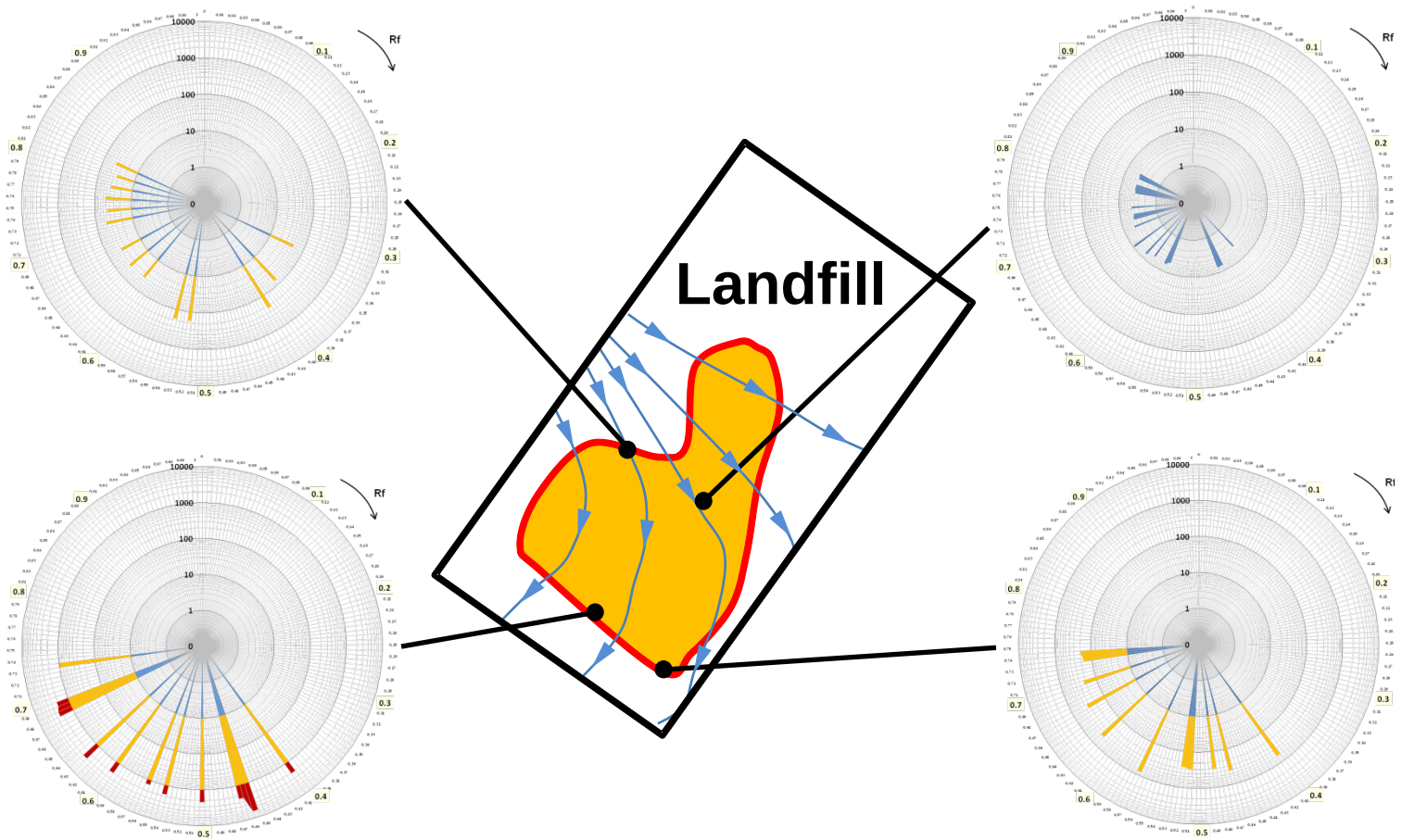


RIV Polar diagram



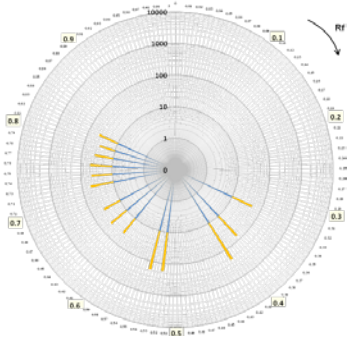


Comparison between the sampling points



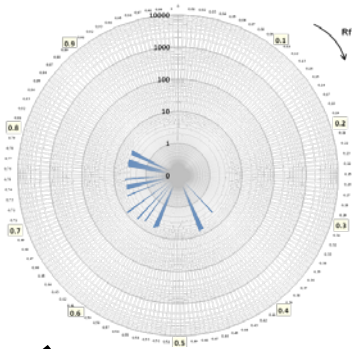
Goundwater flow

Comparison with other analysis data



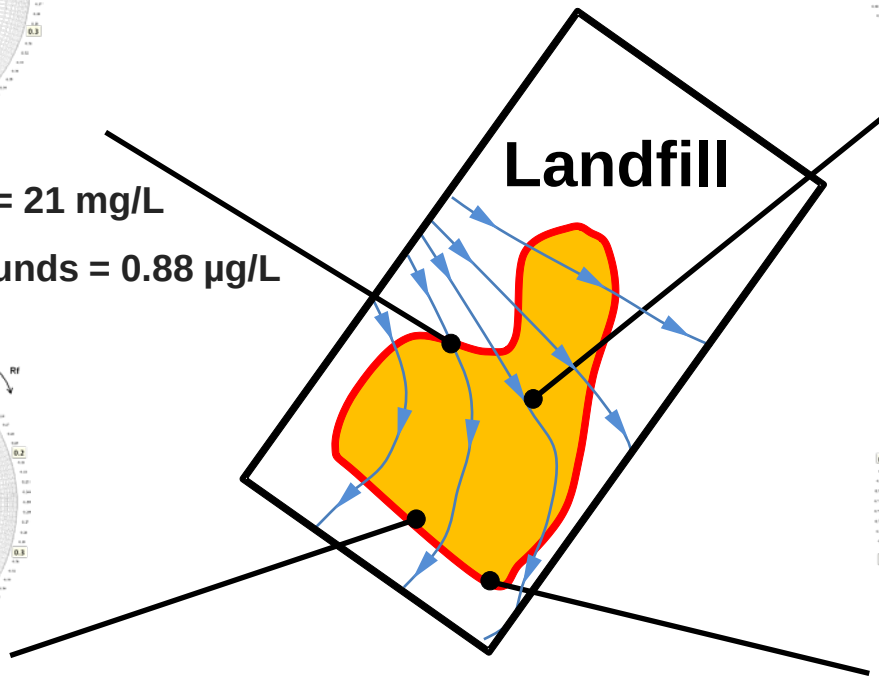
DOC = 21 mg/L

Σ Target compounds = 0.88 µg/L

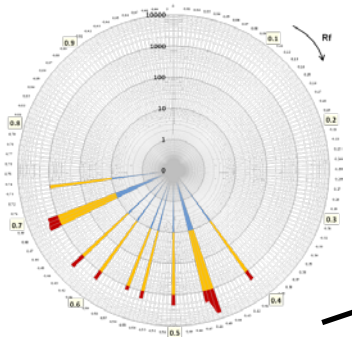


DOC = 2,3 mg/L

Σ Target compounds = 1,01 µg/L

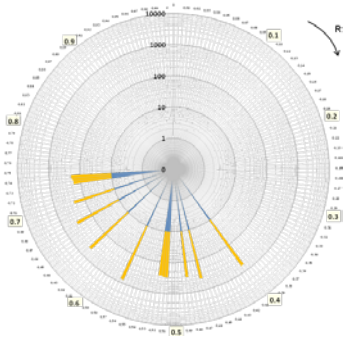


Goundwater flow



DOC = 290 mg/L

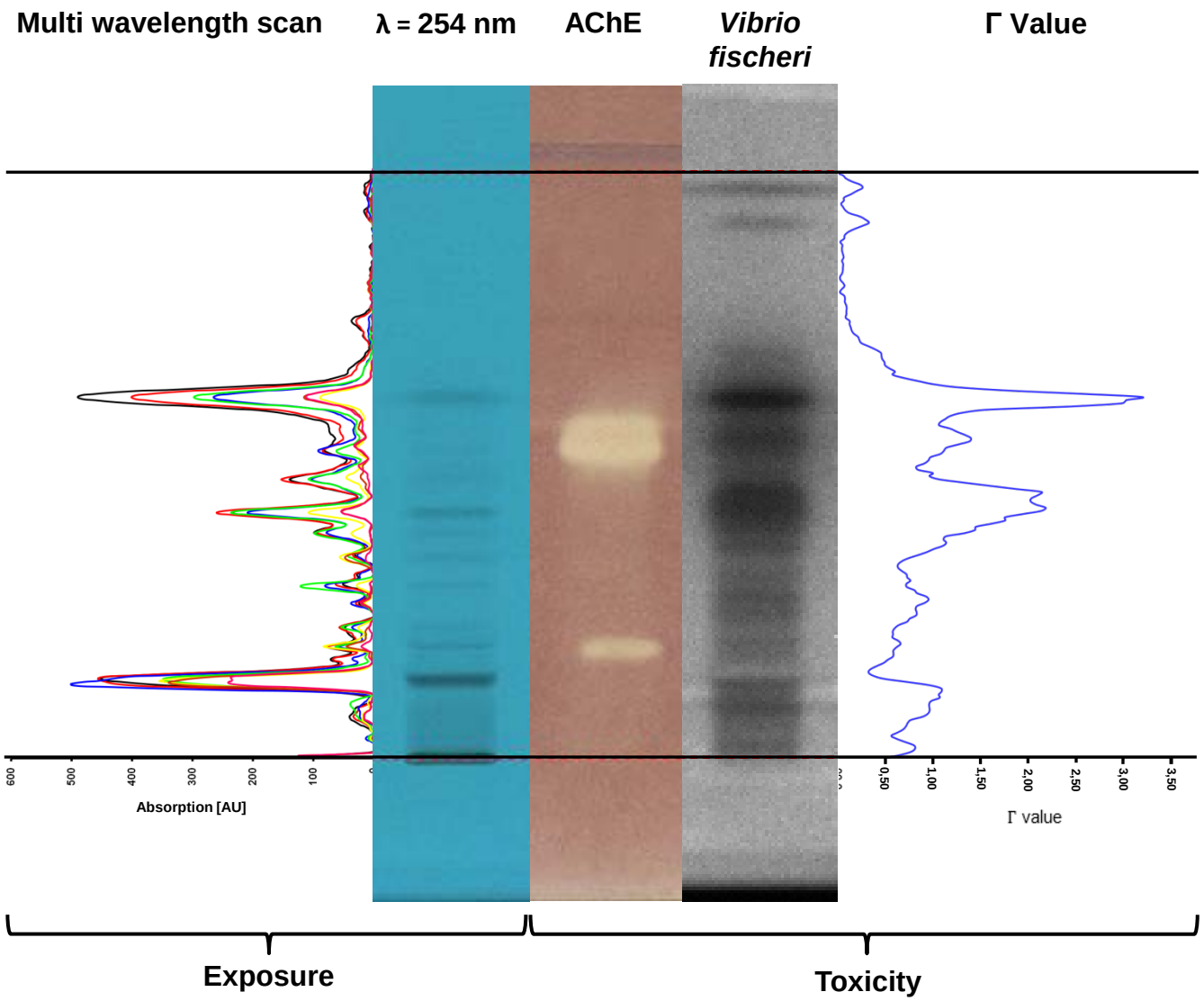
Σ Target compounds = 41,5 µg/L



DOC = 25 mg/L

Σ Target compounds = 4,11 µg/L

Comparison of the exposure with biological effects



Acknowledgments

Camag

Sarah Künzel
Nicole Jung

the member of staff at
the Laboratory for Operation Control and Research

Thank you very much for your attention!

Introduction of the reciprocal iso-inhibition volume (RIV)

$$m_{i,1}(50) = c_{i,1} \cdot V_{i,1}(50)$$
$$m_{i,2}(50) = c_{i,2} \cdot V_{i,2}(50)$$

$$m_{i,1}(50) = m_{i,2}(50)$$

The same mass for the same substance and the same inhibition

Comparison of substance i in two samples

$$c_{i,1} \cdot V_{i,1}(50) = c_{i,2} \cdot V_{i,2}(50)$$

c = *Concentration (ng/µl)*

V = *Application volume (µl)*

i = *Substance zone*

$$\frac{c_{i,1}}{c_{i,2}} = \frac{\frac{1}{V_{i,1}(50)}}{\frac{1}{V_{i,2}(50)}}$$

Reciprocal iso-inhibition volume