bfg Bundesanstalt für Gewässerkunde

Characterization of surface and wastewater samples using the planar Yeast Estrogen Screen (pYES)

Two sides of the coin

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Bundesministerium für Umwelt, Naturschutz, Bau und Reaktorsicherheit

Two sides of the coin

Unknown compounds with same MOA ?

Chemistry

Compound (mixtures)

Biology

(Sum-) effect

Which compounds contribute to the observed Effect ?

Two sides of the coin

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Which compounds contribute to the observed Effect ?

- Possibilities to implement effect based tools in WFD
- WFD-watch list candidates (E2, EE2, E1)

Two sides of the coin

Chen **ogy**

Comp m-)

(mixt ect

- Possibilities to implement effect based tools in WFD
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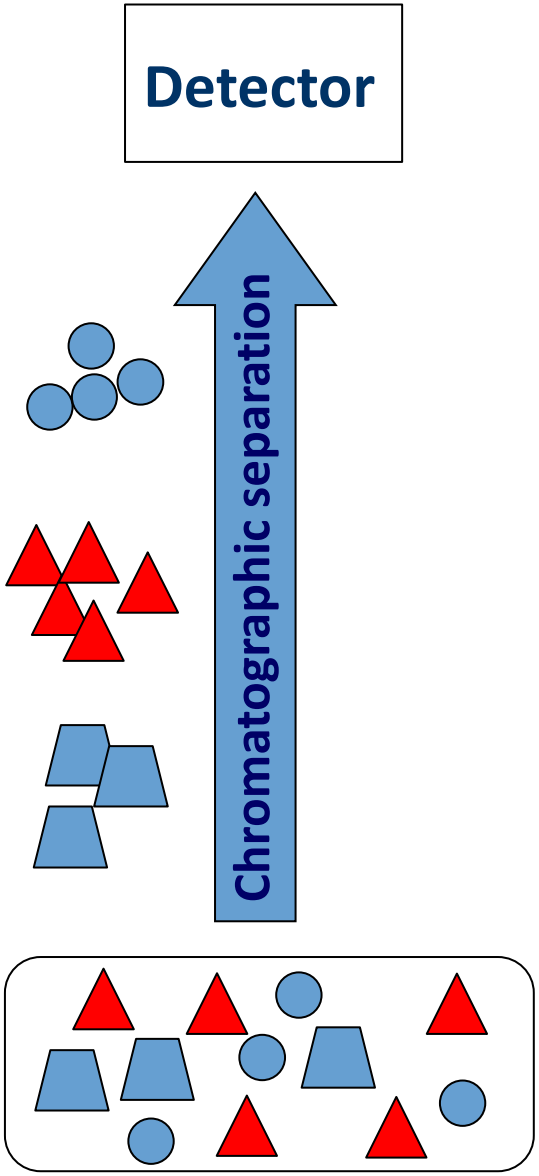
Two sides of the coin

Chemistry

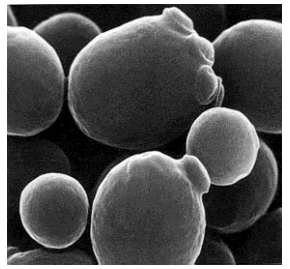
Mass-spectrometry

↓

Molecular weight



Biology



Whole cell Biosensor (YES)

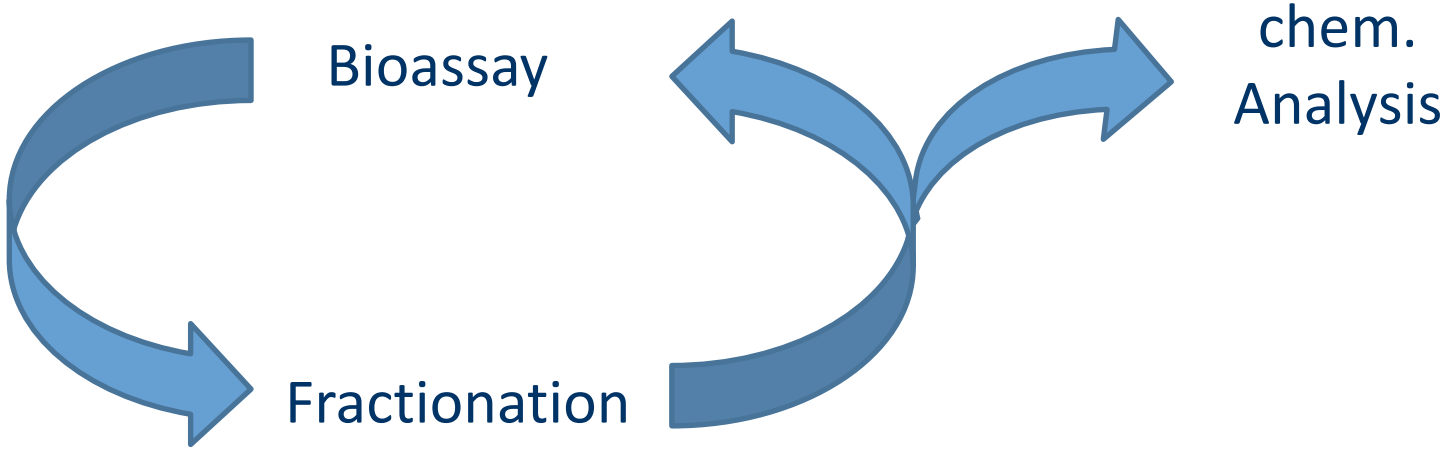
↓

Effect (e.g. estrogenicity)

planar **Y**east **E**strogen **S**creen (pYES)

Motivation

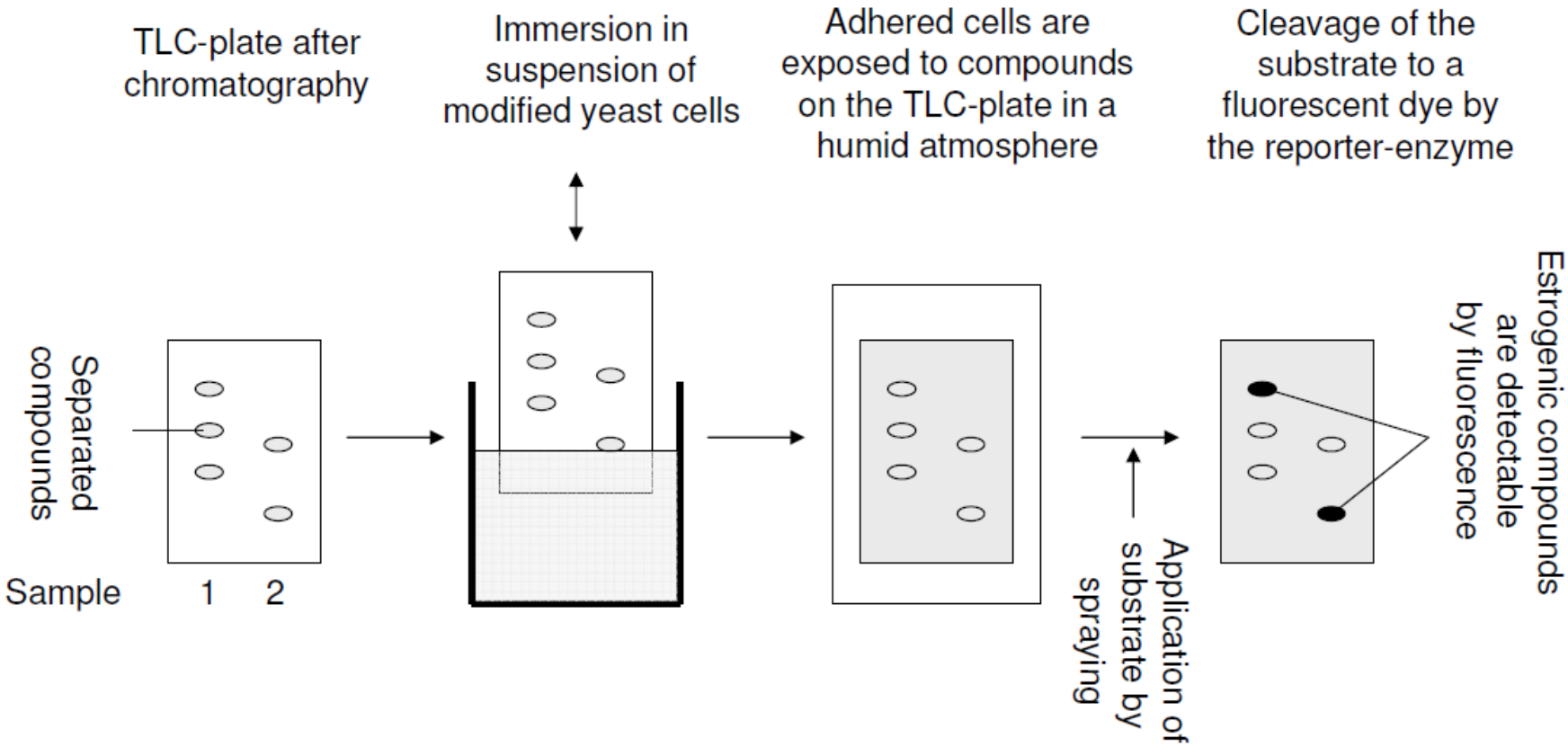
EDA-Approach



	HPLC	HPTLC
Separation	+	-
Number of Tests	-	+
Biocompatibility	-	+

Can we use a direct combination of TLC with a specific bioassays?

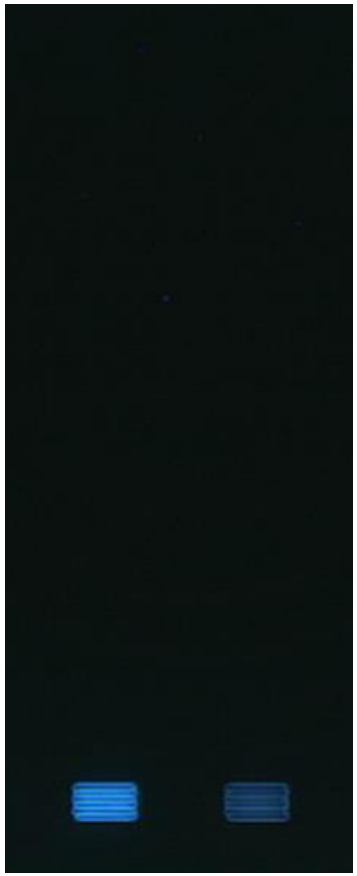
pYES
Workflow



Spira D., Reifferscheid G., Buchinger S. (2013)
Journal of planar chromatography, 26(5), p 395
Buchinger S., Spira D., Bröder K., Schlüsener M., Ternes T.
Reifferscheid G. (2013) Anal. Chem., 85, p 7248

Planar Yeast Estrogen Screen (pYES)

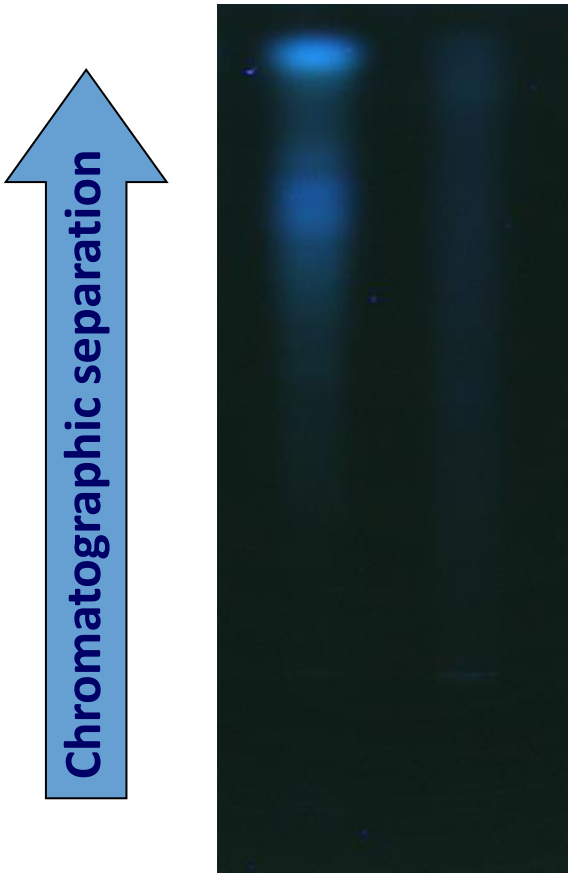
TLC-plate

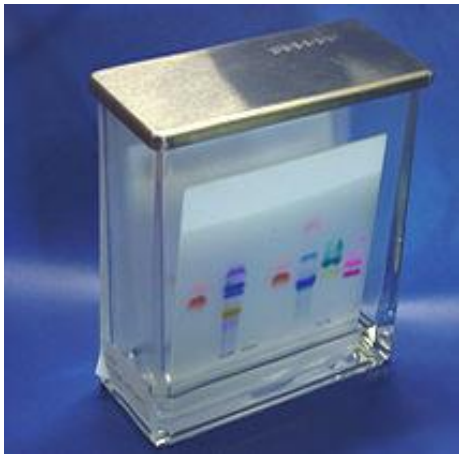


Sample
(Mixture)

Planar Yeast Estrogen Screen (pYES)

TLC-plate





„single“ compounds

Planar Yeast Estrogen Screen (pYES)

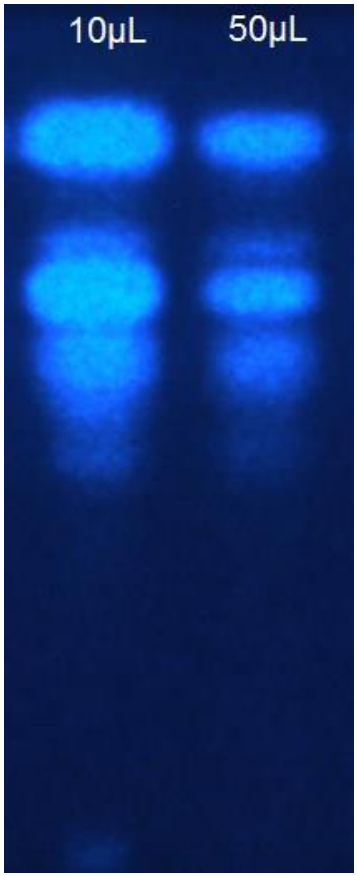
Use of

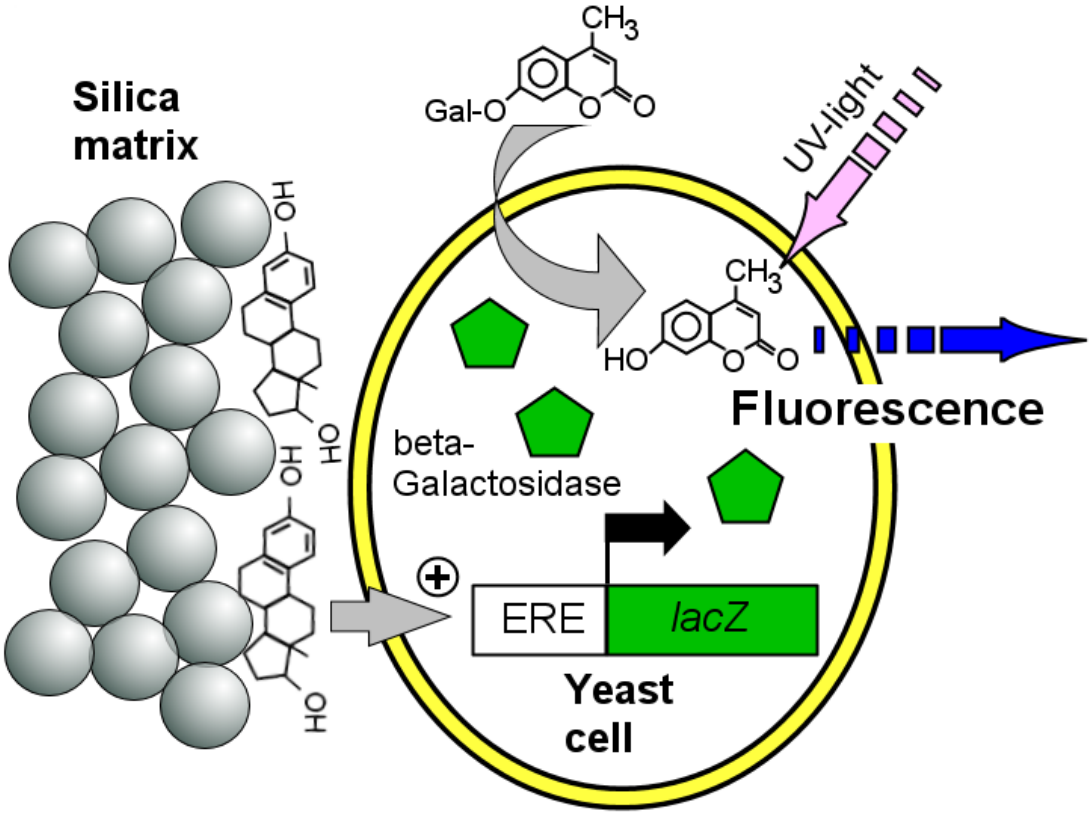
In-vitro Bioassay

directly coupled to

planar chromatography

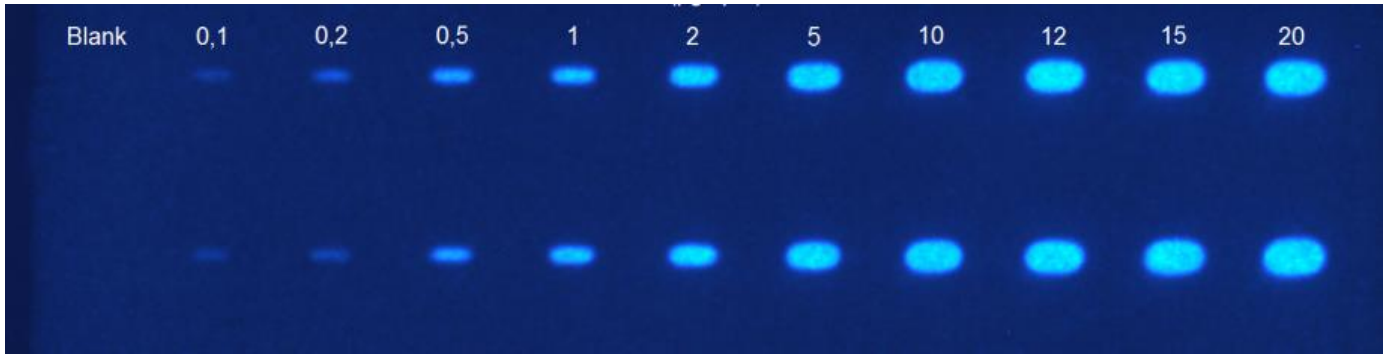
TLC-plate



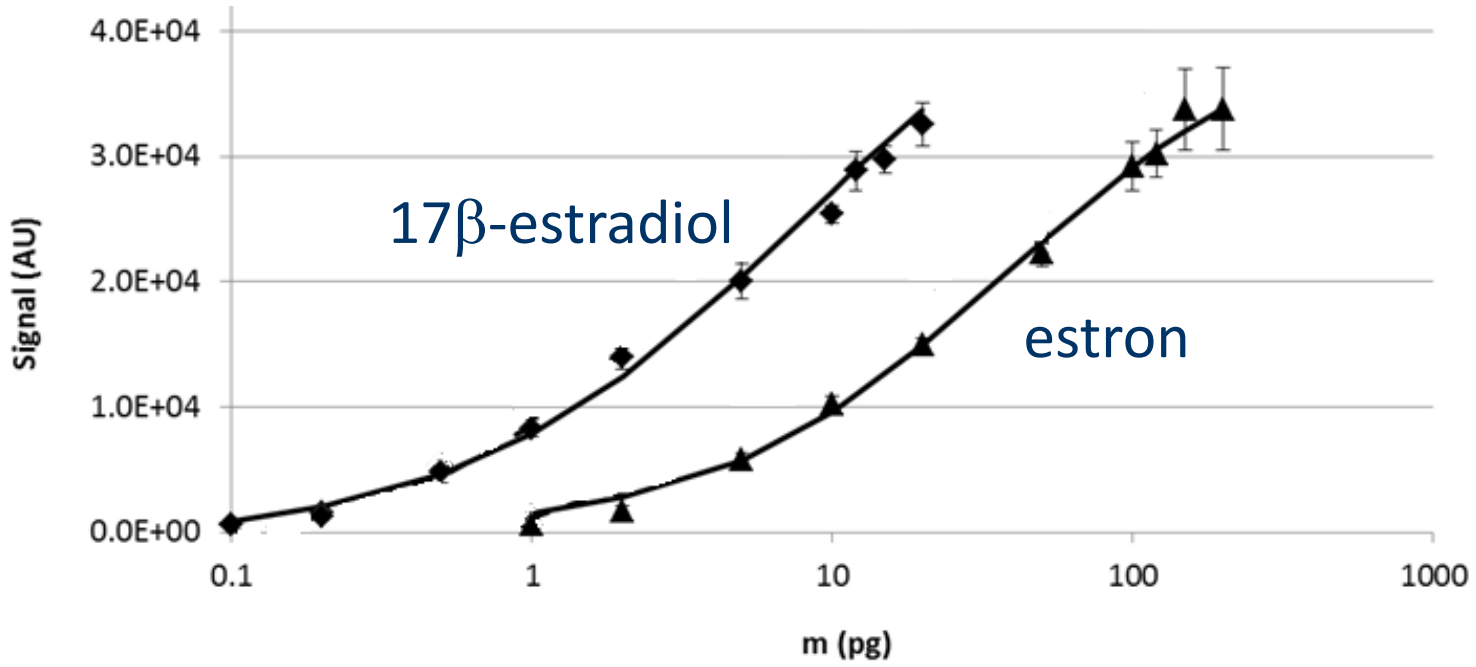


„single“ compounds with estrogenic potential

Planar Yeast Estrogen Screen (pYES) - sensitivity

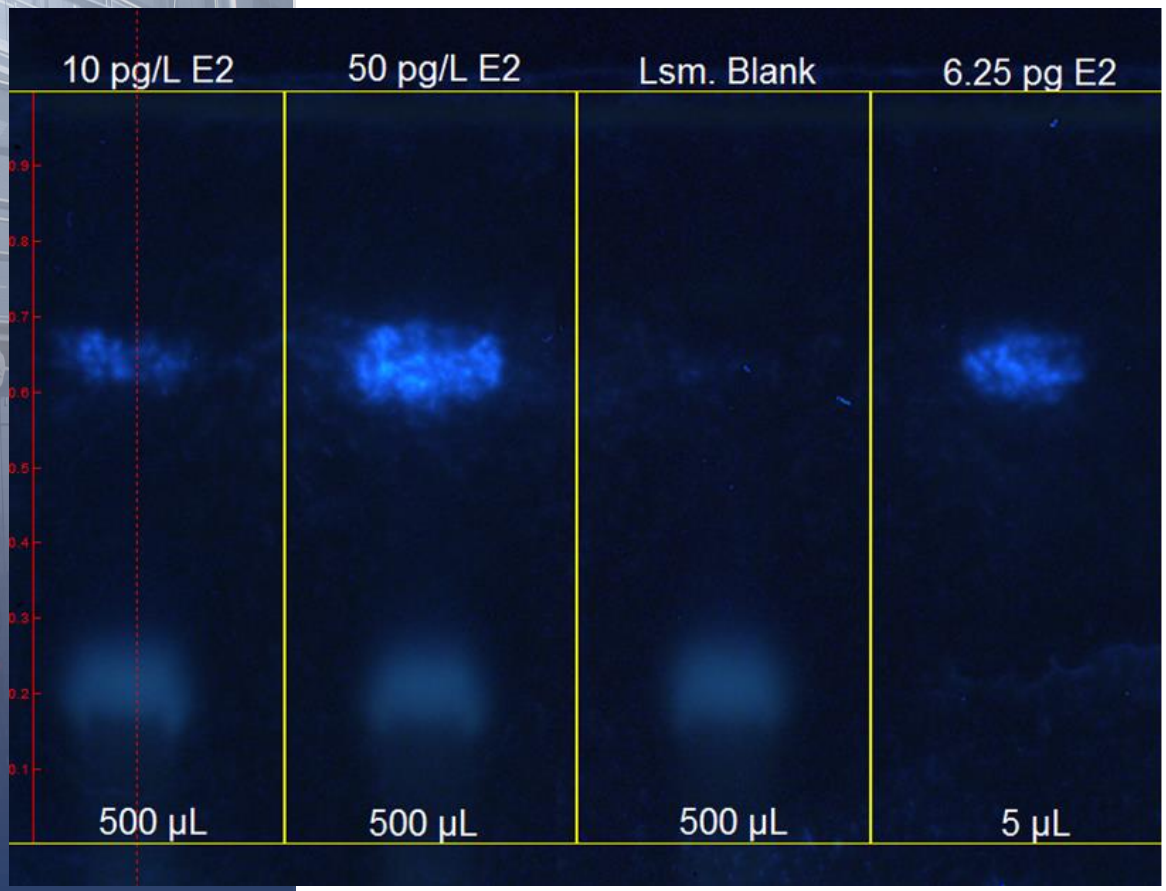


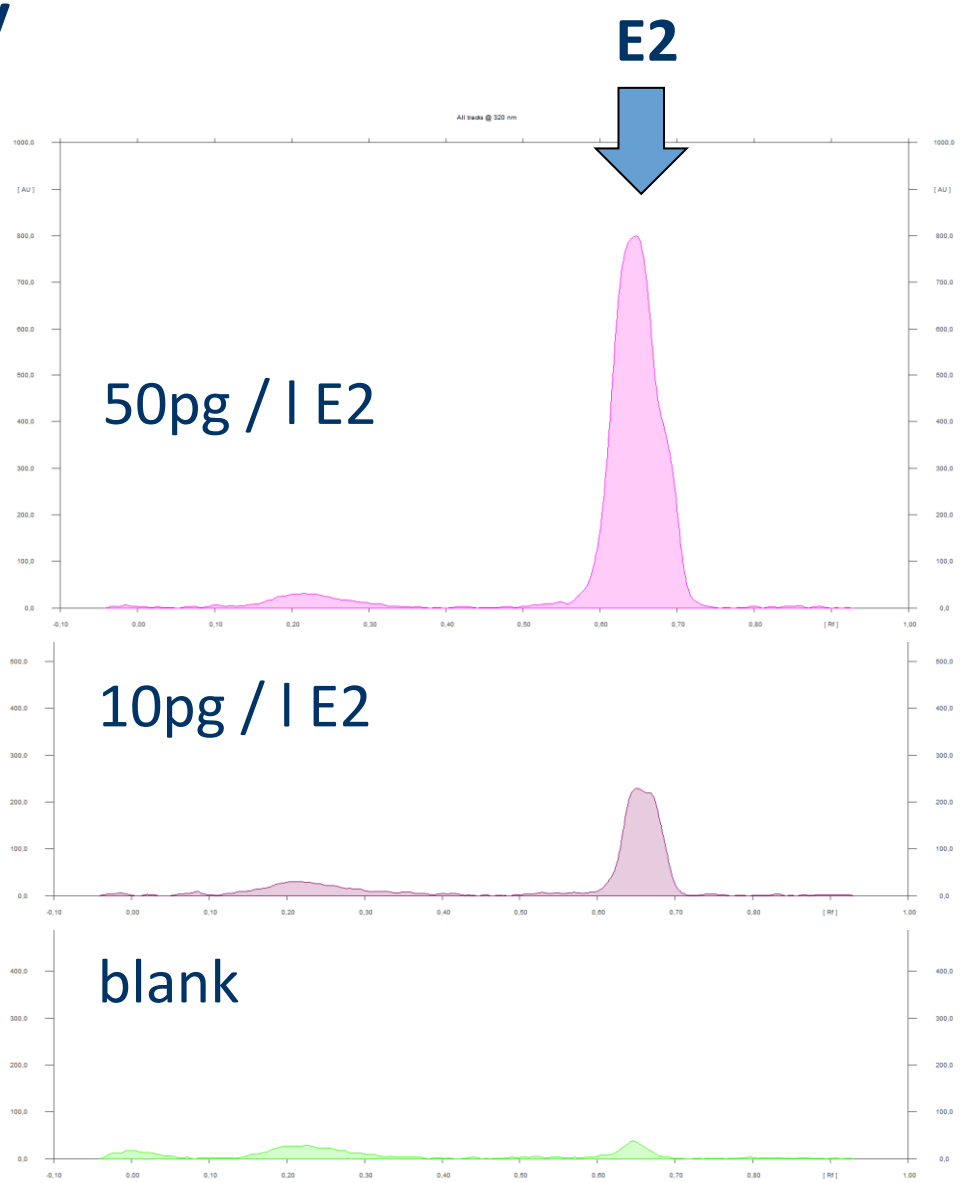
17β-estradiol
LOQ ~ 0.1 pg



Planar Yeast Estrogen Screen (pYES) - sensitivity

500 µl SPE-extrac: 1000-fold concentrated

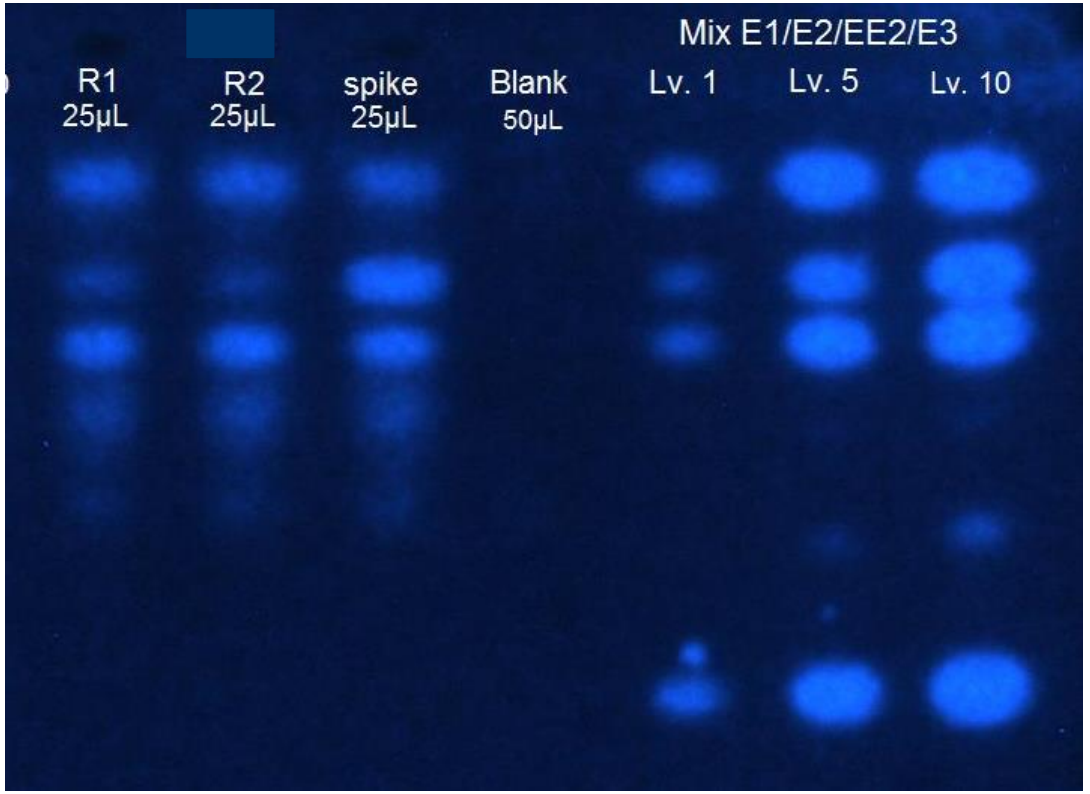




Compound contribution ?

Samples References

Chromatographic separation



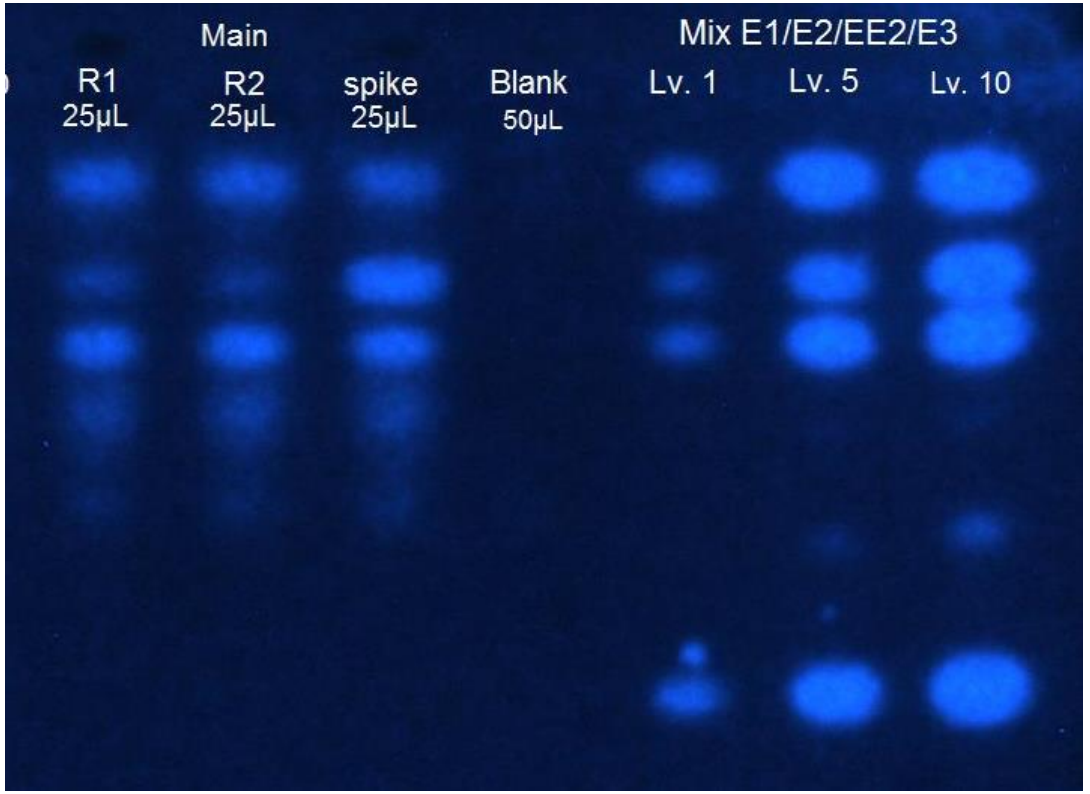
	c [pg/l]		
E1	350	400	260
EE2	< 20	< 20	98
E2	45	52	39
X	Na	Na	Na

m (pg)			
10	50	100	E1
1	5	10	EE2
1	5	10	E2
100	500	1000	E3

Unknowns with same MoA ?

Samples References

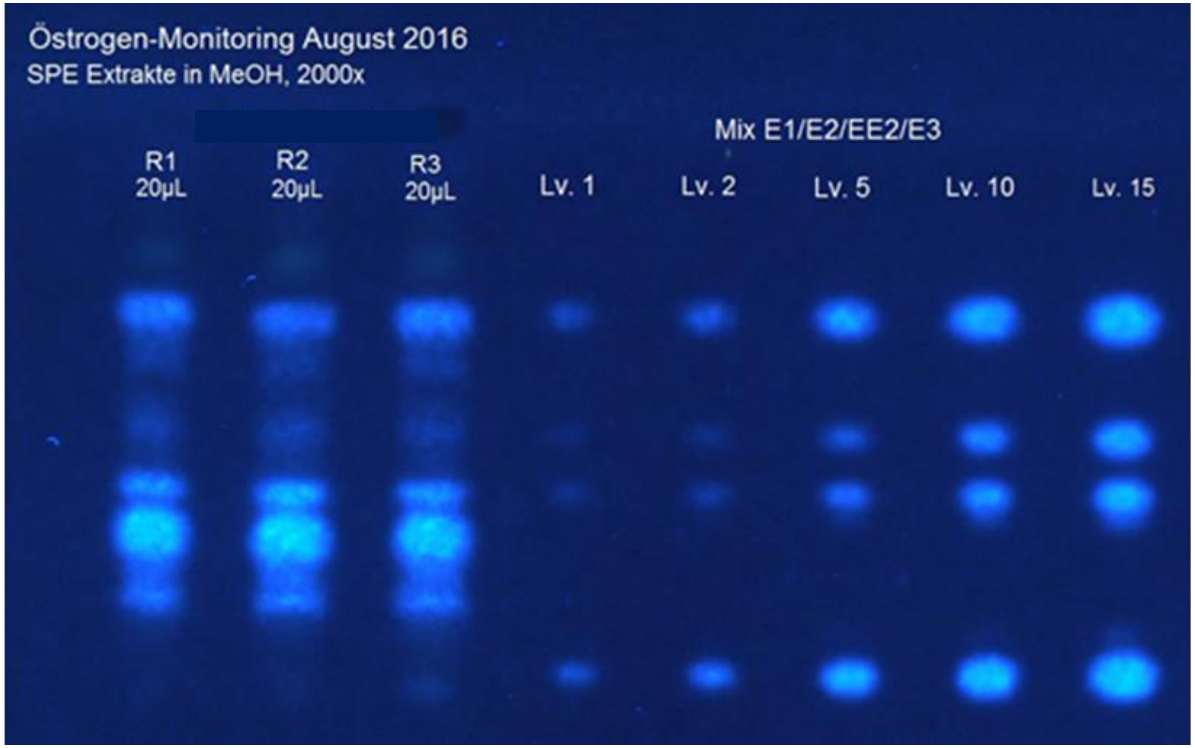
Chromatographic separation



	EEQ [pg/l]		
E1	56	60	46
EE2	10	6	70
E2	45	52	39
X	16	18	10
Σ	127	136	165

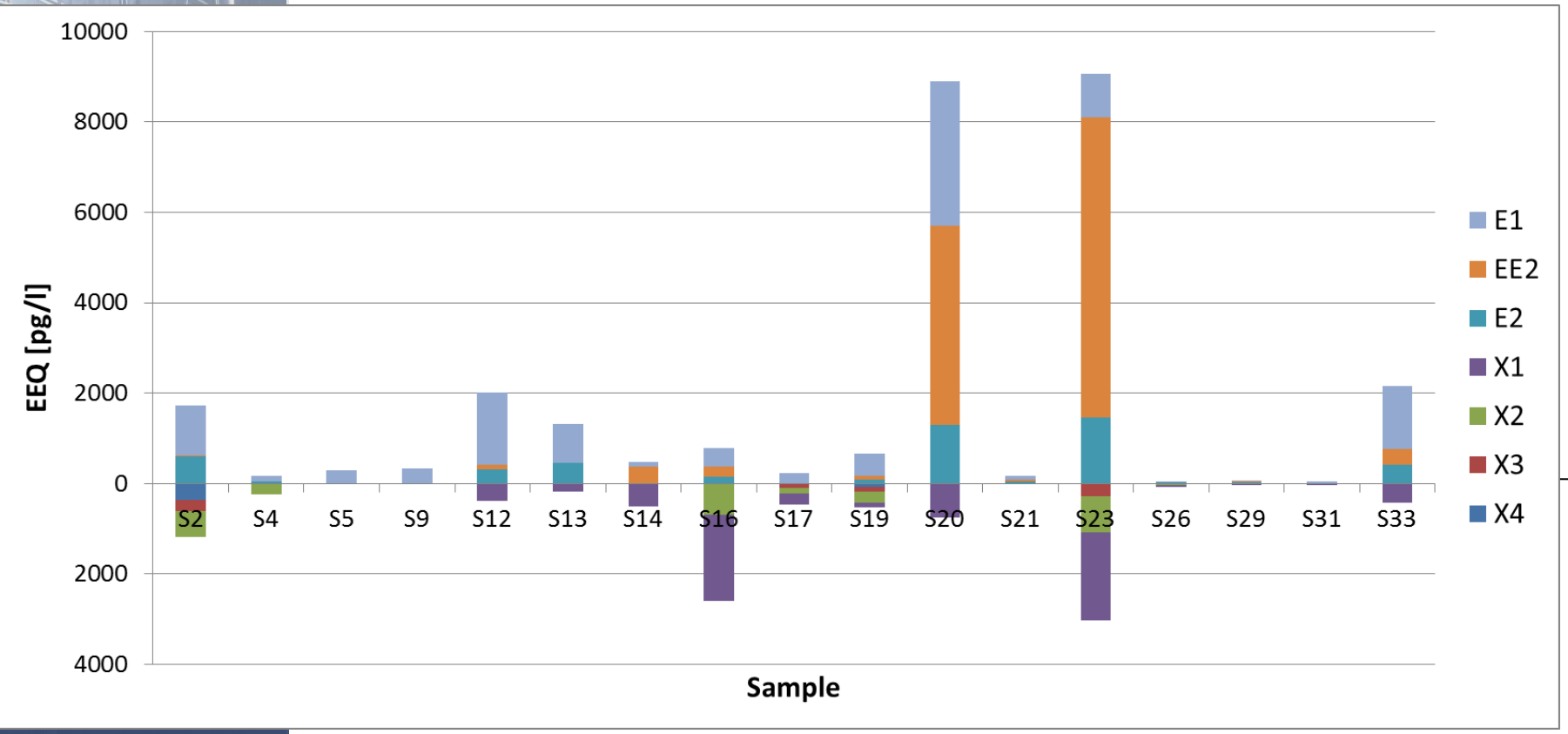
m (pg)			
10	50	100	E1
1	5	10	EE2
1	**5**	**10**	**E2**
100	500	1000	E3

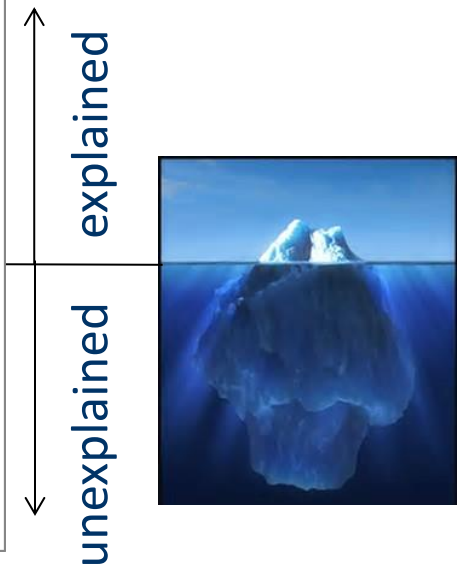
Unknowns with same MoA ?



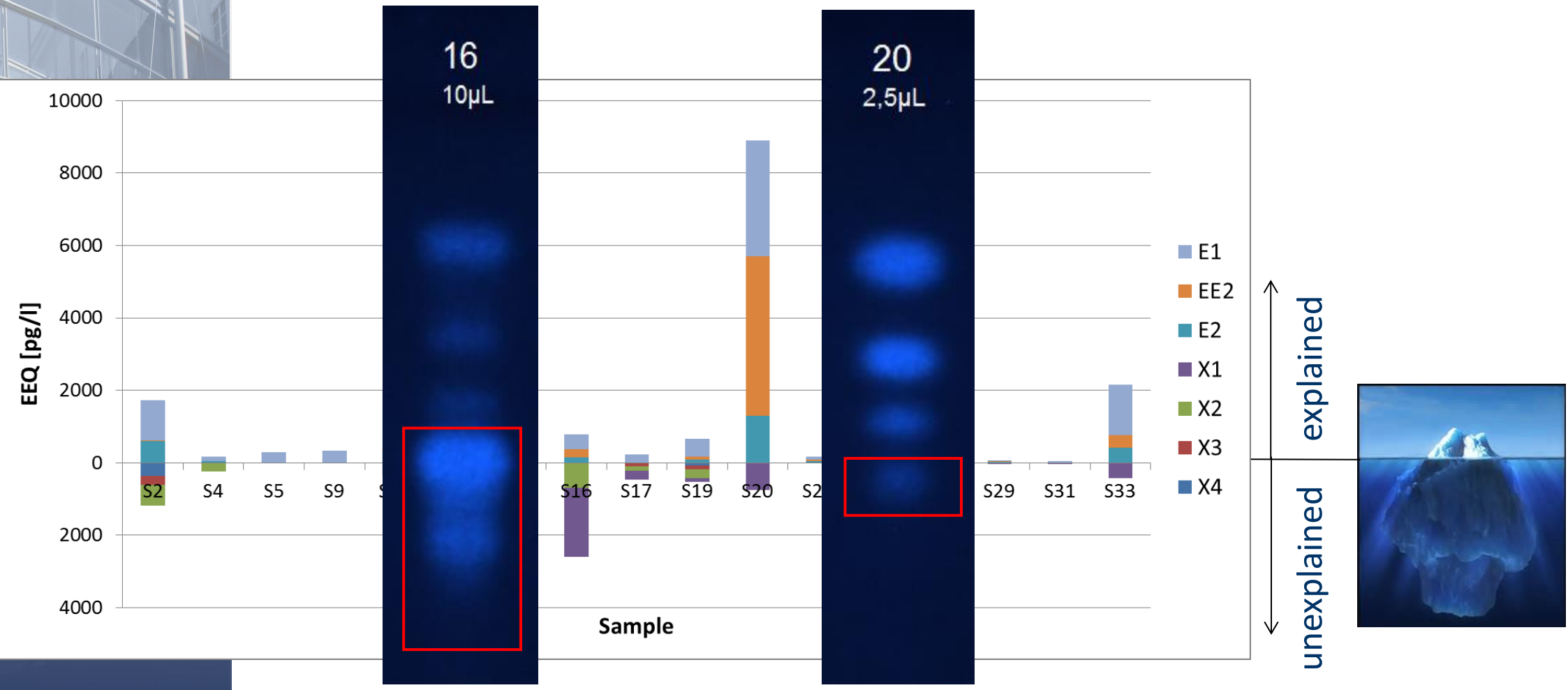
Subst.	EEQ pg/l	%
E1	490	20
x1	70	3
EE2	50	2
E2	310	12
x2	**1300**	**52**
x3	280	11
Σ	2500	

Unknowns with same MoA ? Wastewater

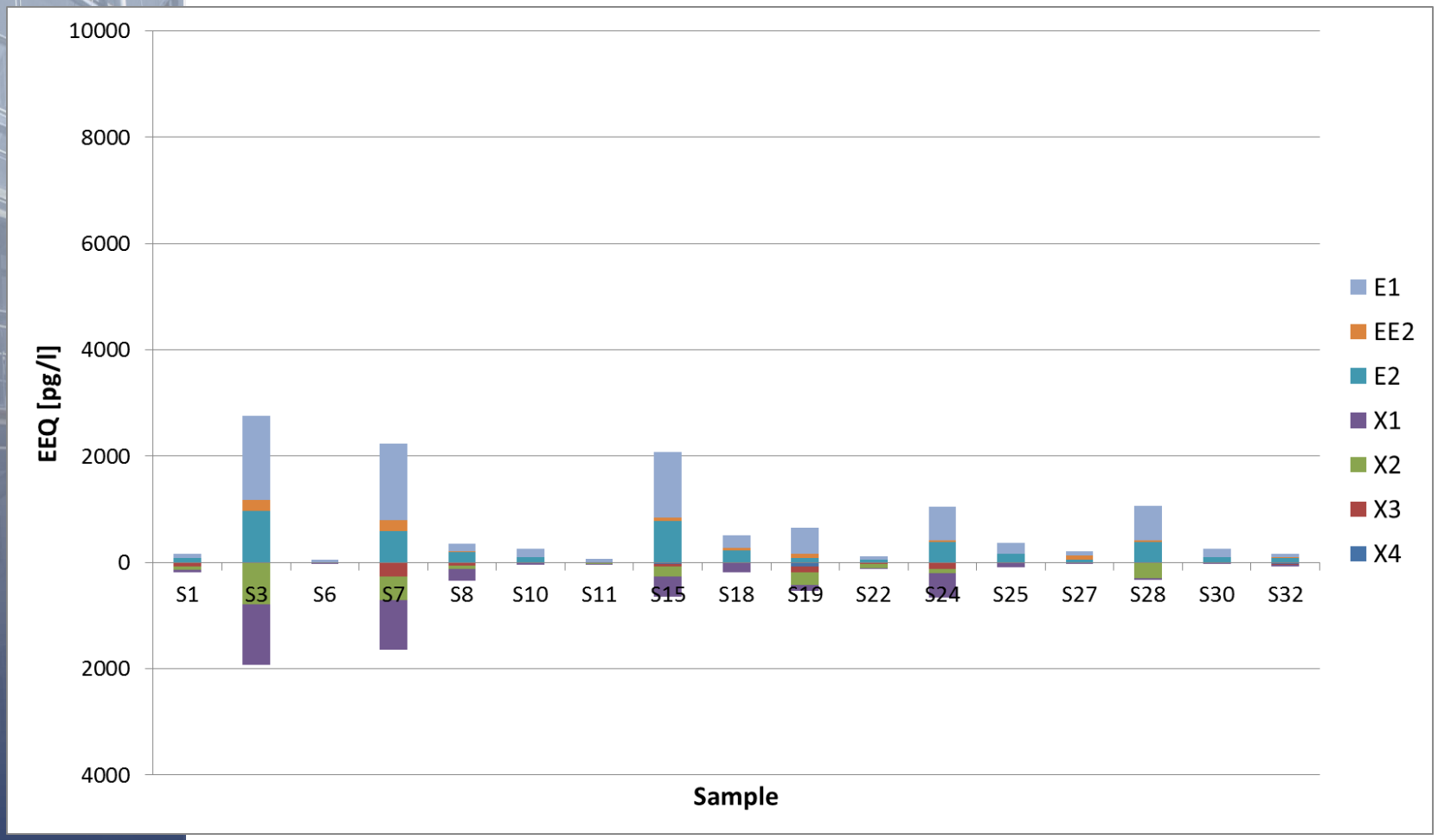


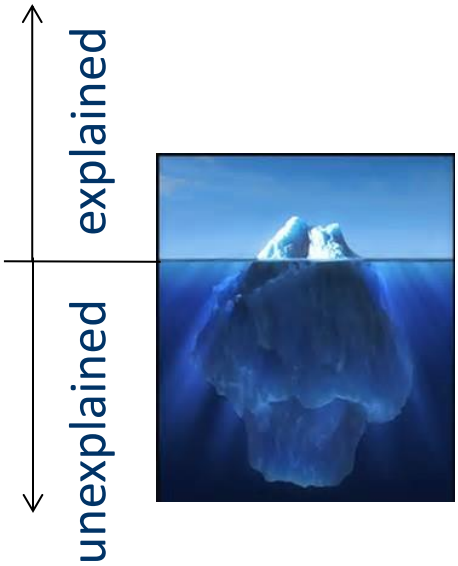


Unknowns with same MoA ? Wastewater

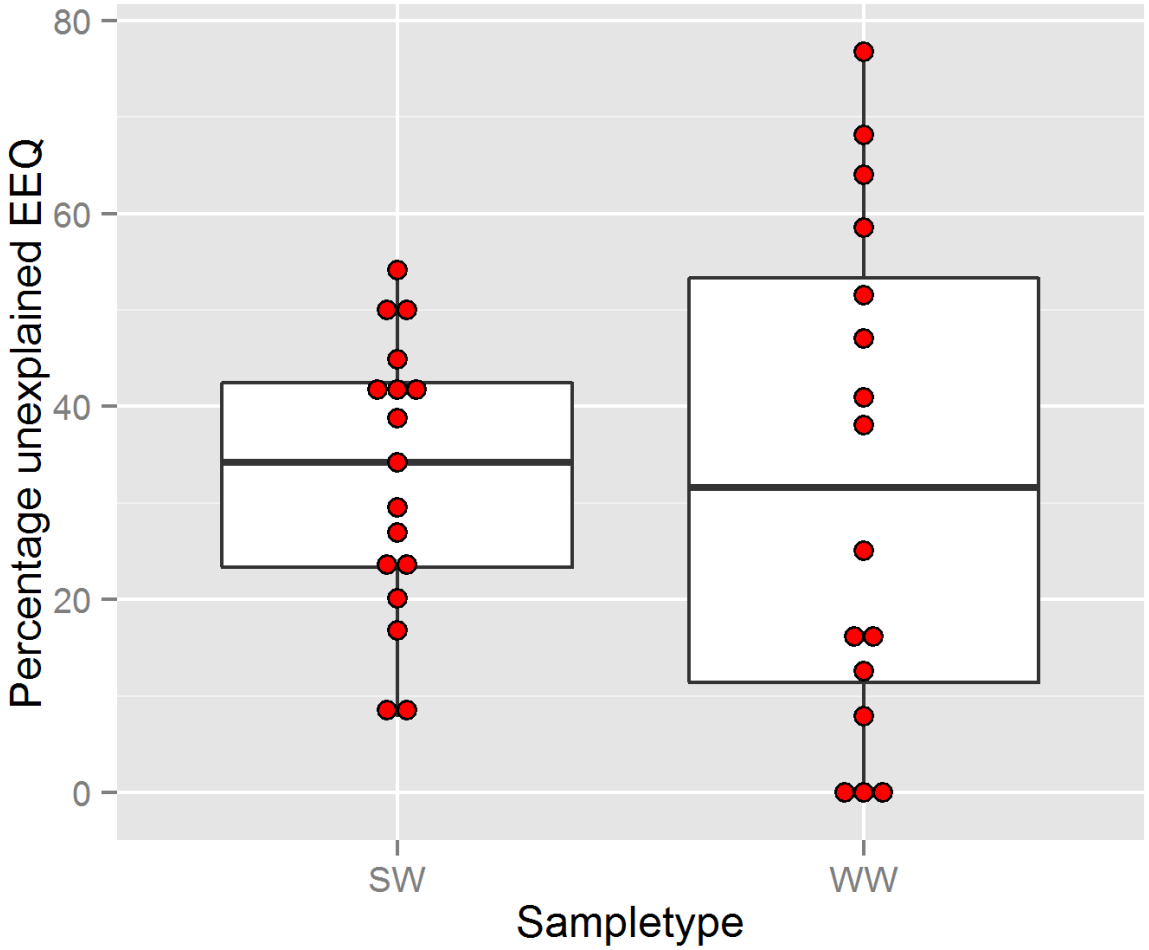


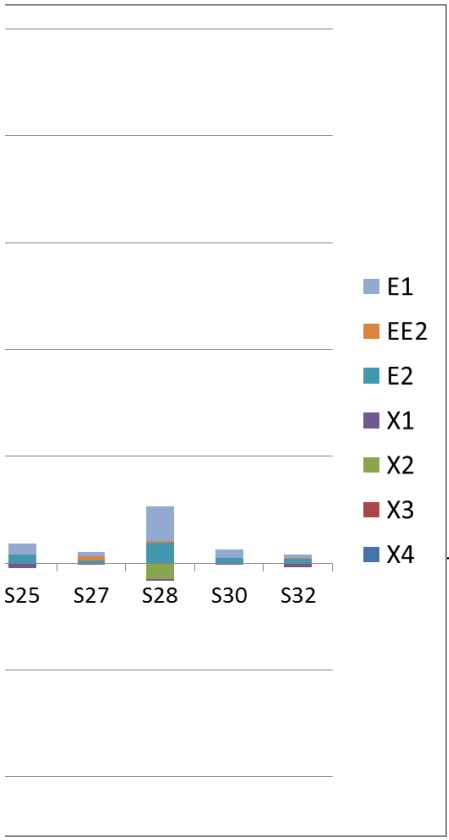
Unknowns with same MoA ?
Surfacewater





Unknowns with same MoA ?

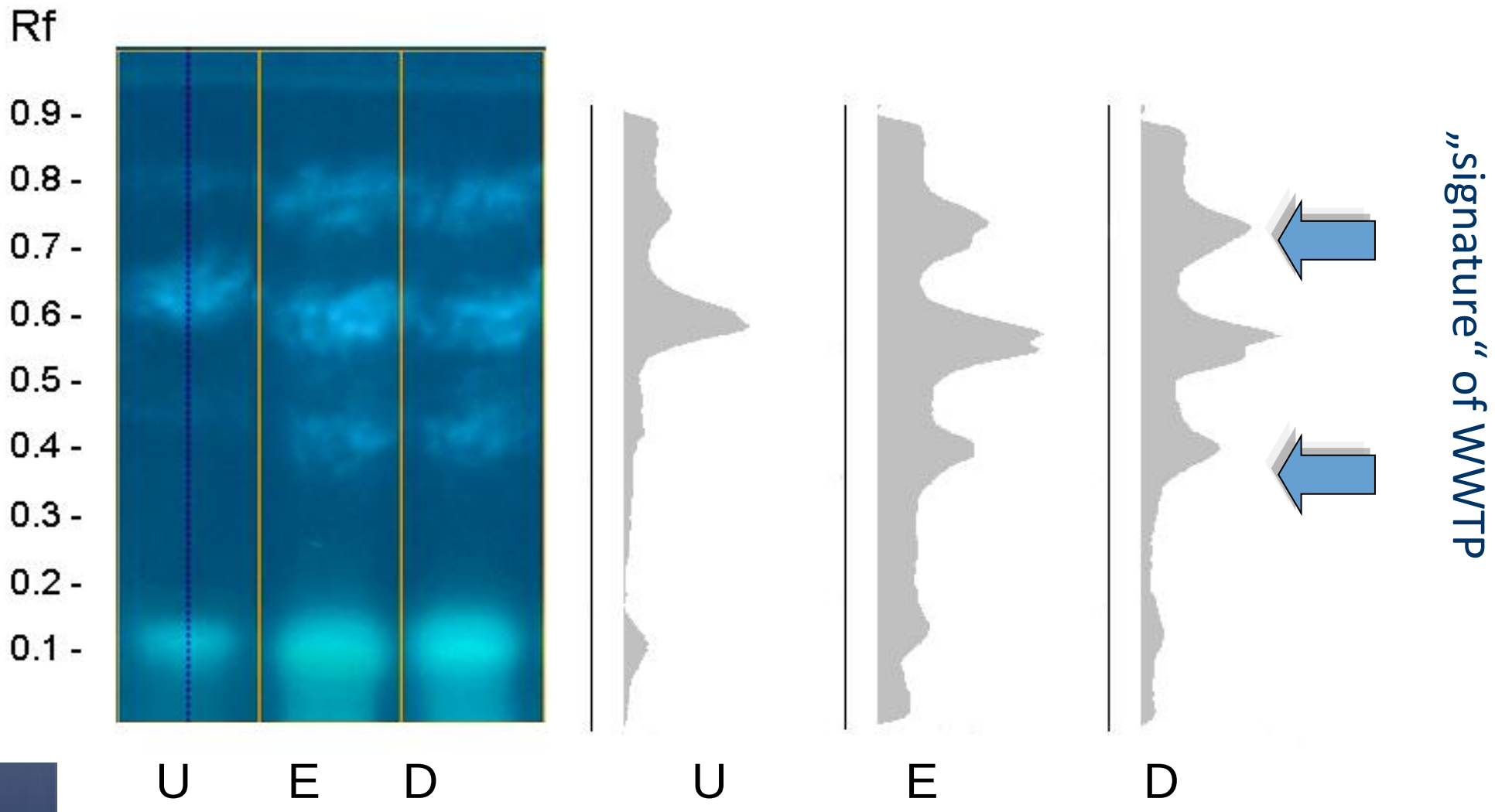




explained

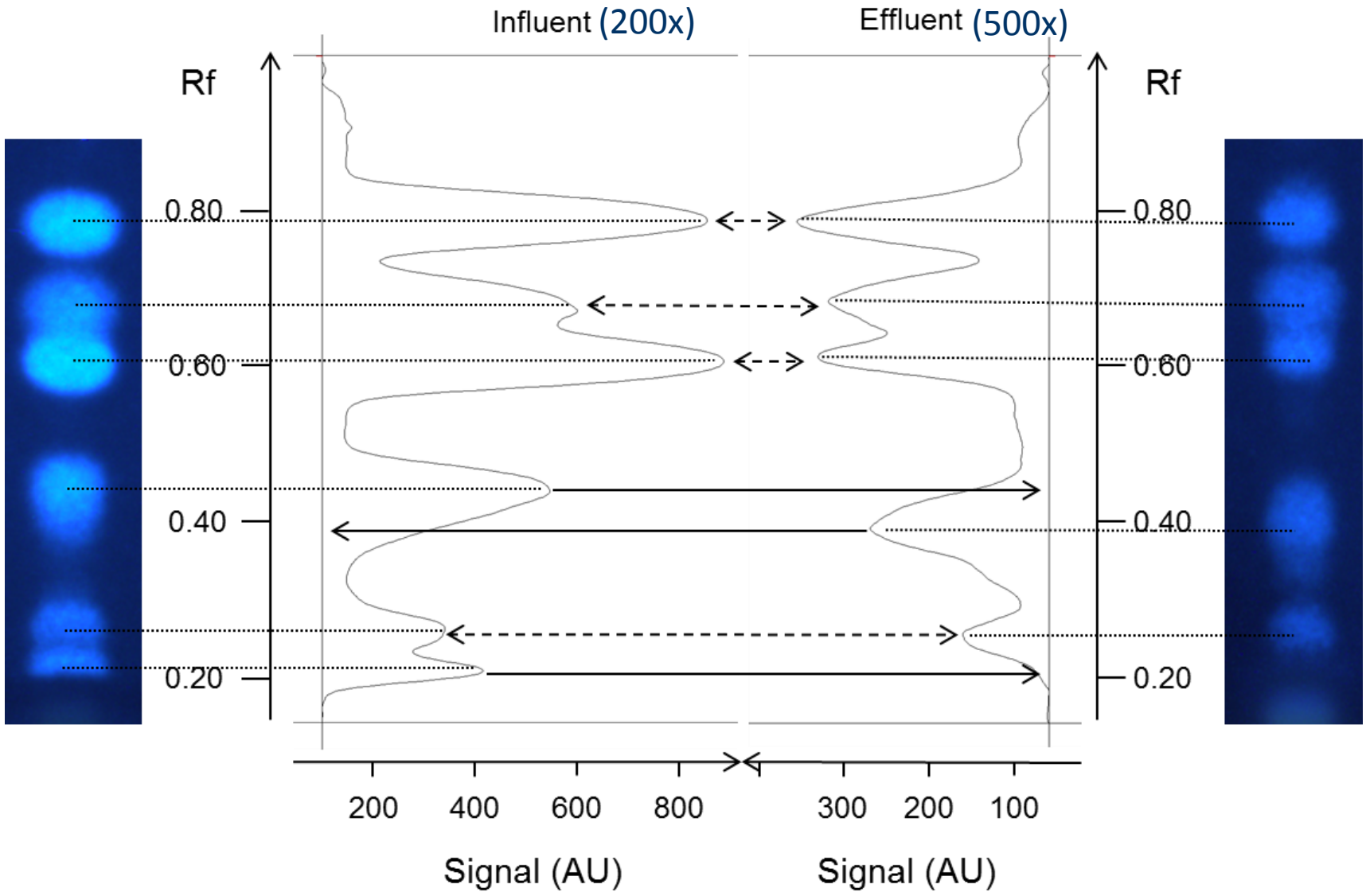
unexplained

Unknowns with same MoA ?
- Effect profiles



10 µl SPE-extract: 1000-fold concentrated
(U: upstream, E: effluent, D: downstream)

Unknowns with same MoA ?
- Effect profiles

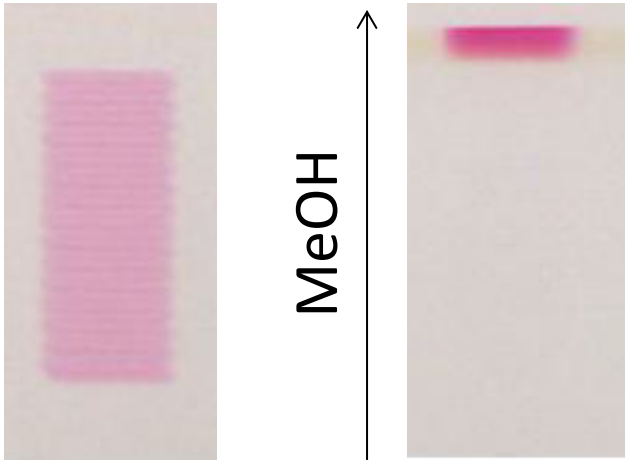


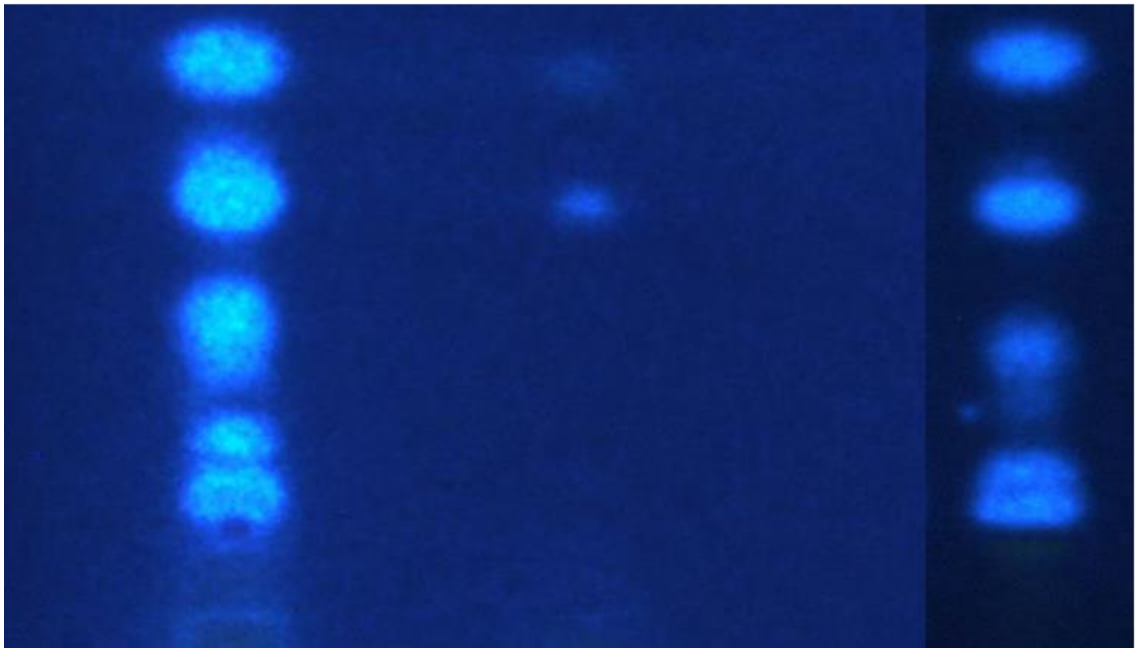
Direct testing of water samples

Sample → C18 SPE

- STP influent 1000 µl
- SPE flow through 1000 µl
- 200 fold SPE extract 5 µl

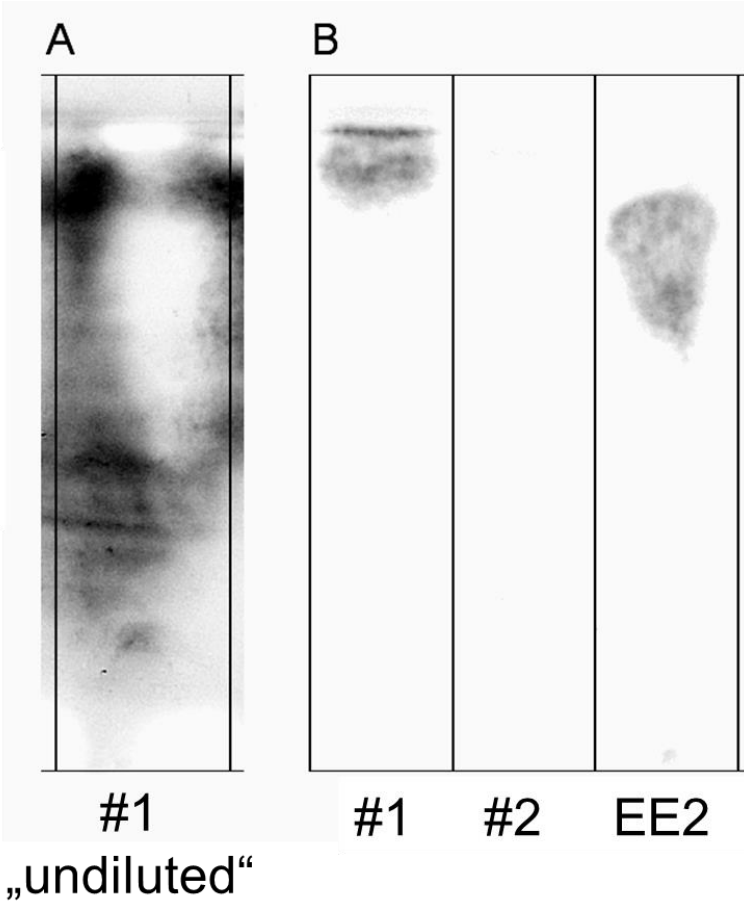
Focussing after large volume application

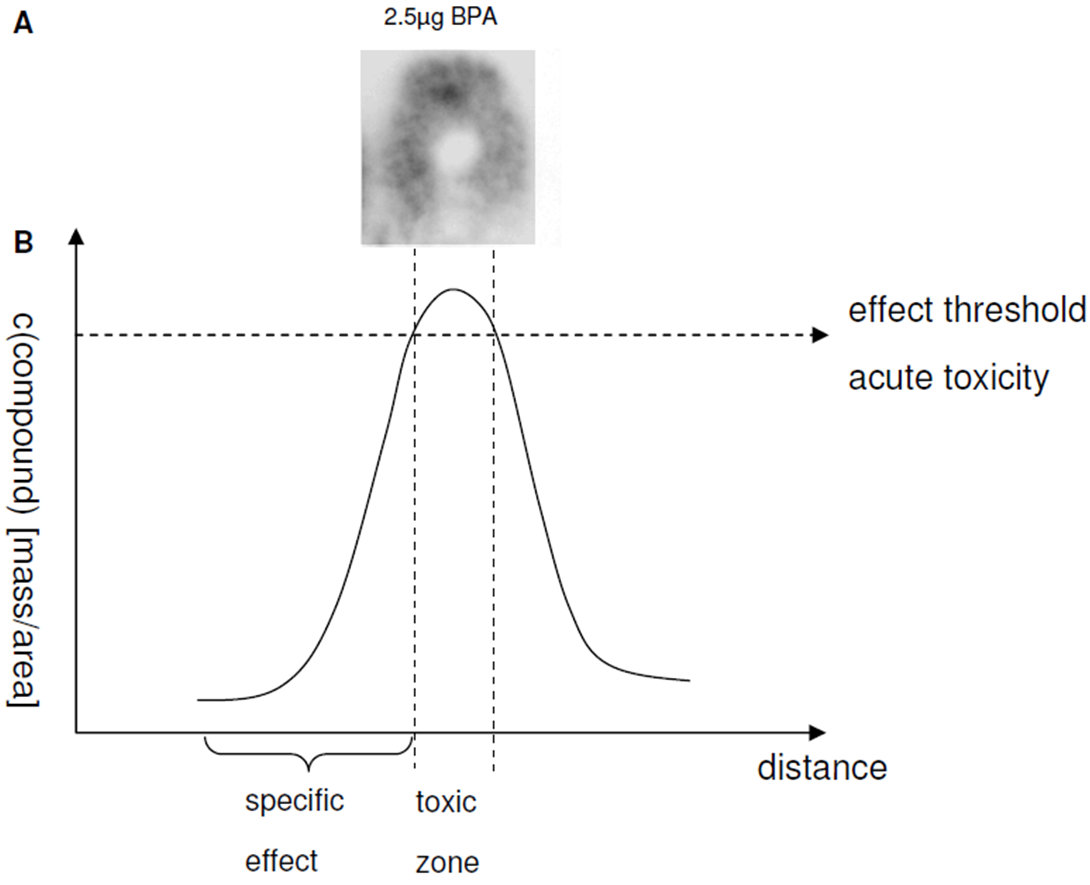




Challenging (toxic) samples

Sunscreens





Summary and Outlook

In depth EDA

- Samples with high percentage of unexplained activity

chem. Analysis

- On plate
- Off plate

- estrogenic effects „on plate“
- Quantification limit for e.g. EE2 ~ 0.1 pg
- high correlation with hr LC/MS
- Detection of compounds with same MoA
  → Generation of effect profiles allows
  - source tracking
  - process characterization
- Comparably fast (~ 20 sample / day)

further ‚endpoints‘

- AR
- AhR
- TR
- RAR / RXR
- …

Comparative in vivo-exposures

- Samples with different activity profiles

Acknowledgement :

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