

Semiquantitative ESI/MS analysis of metabolites in biological matrices made feasible via prediction of ionization efficiencies

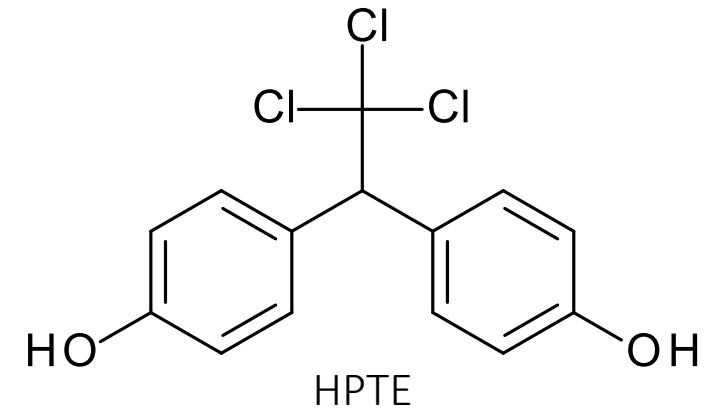
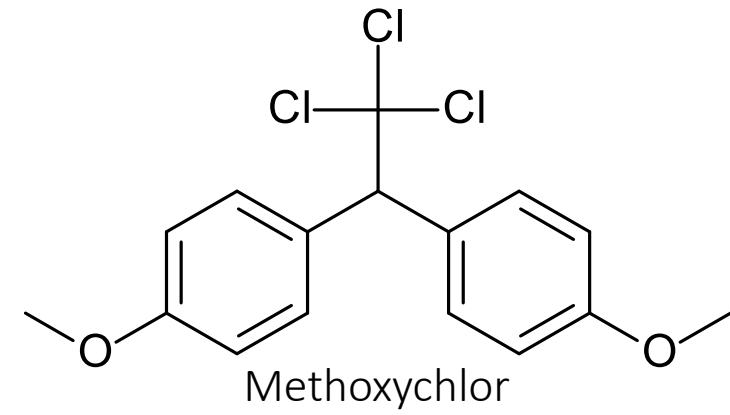
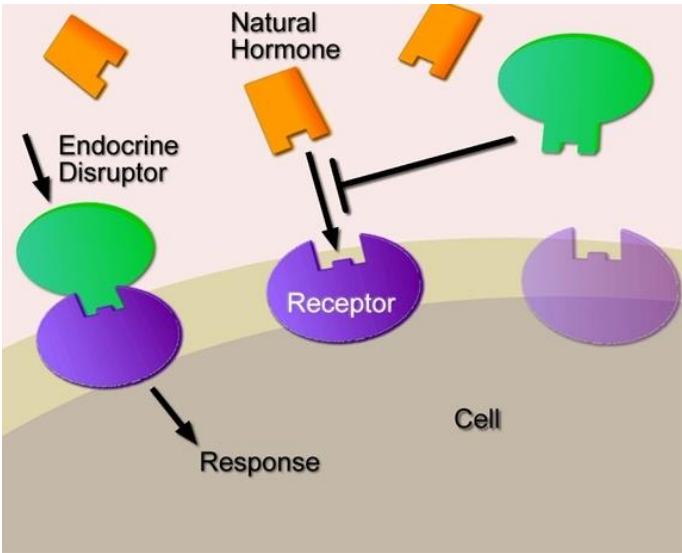
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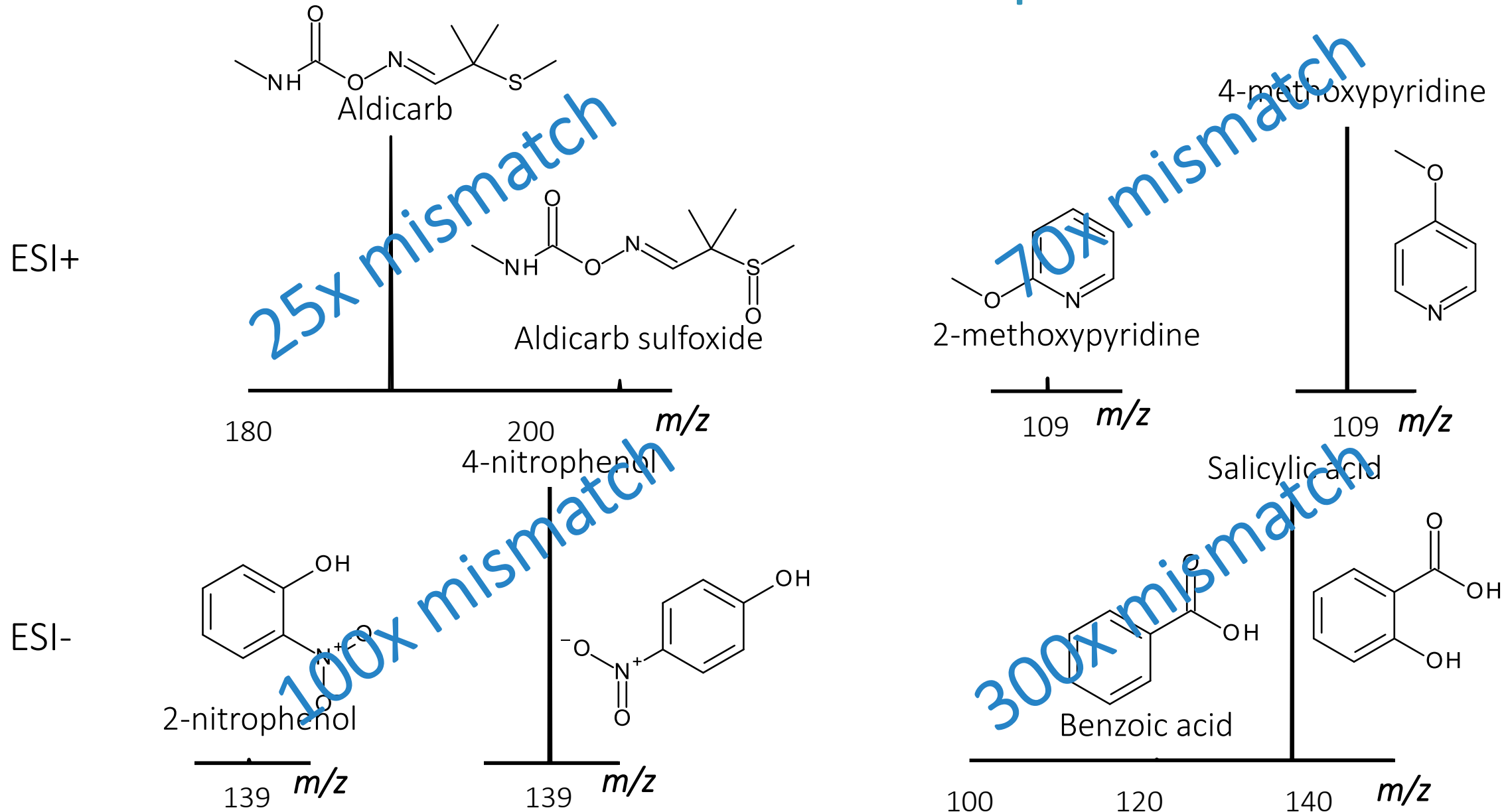




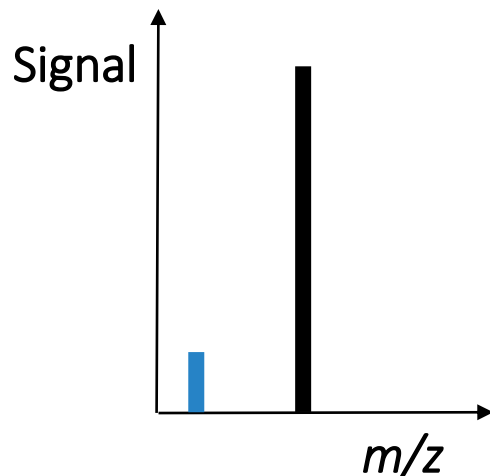
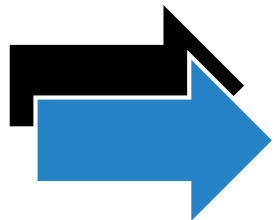
LC/MS

Stable isotope labelled internal standards

Standard substance free quantitation

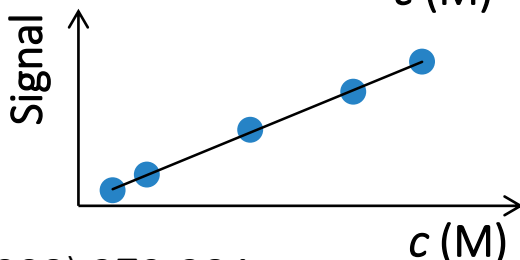
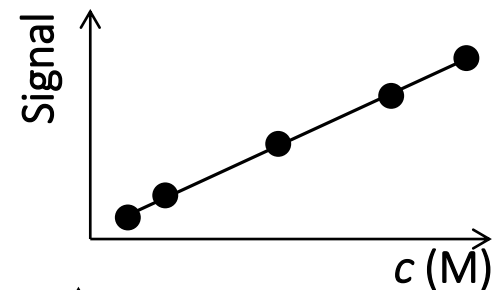
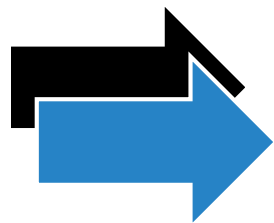


Ionization efficiencies



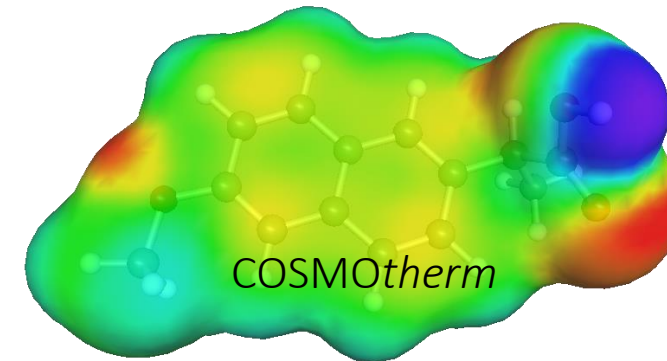
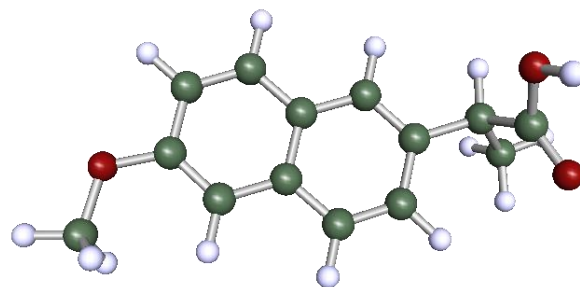
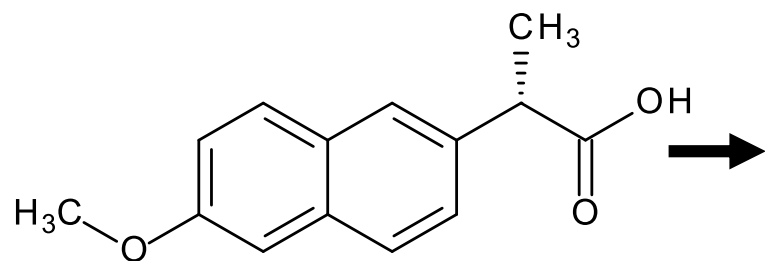
$$C_1 < C_2$$

$$\text{Signal}_1 < \text{Signal}_2$$



$$\log IE = \log \frac{\text{slope}_1}{\text{slope}_2}$$

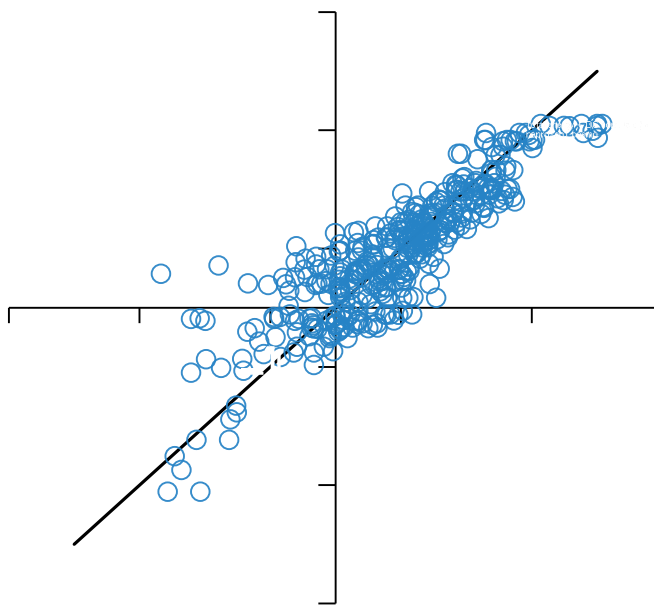
Modelling



↓
MS
measurements



$$\log IE_{\text{measured}} = \log \frac{\text{slope}_1}{\text{slope}_2}$$



Measured log IE



Predicted log IE

↓
Physicochemical
parameters

$$\log IE_{\text{predicted}} = f(x_1 \dots x_n)$$



ESI+

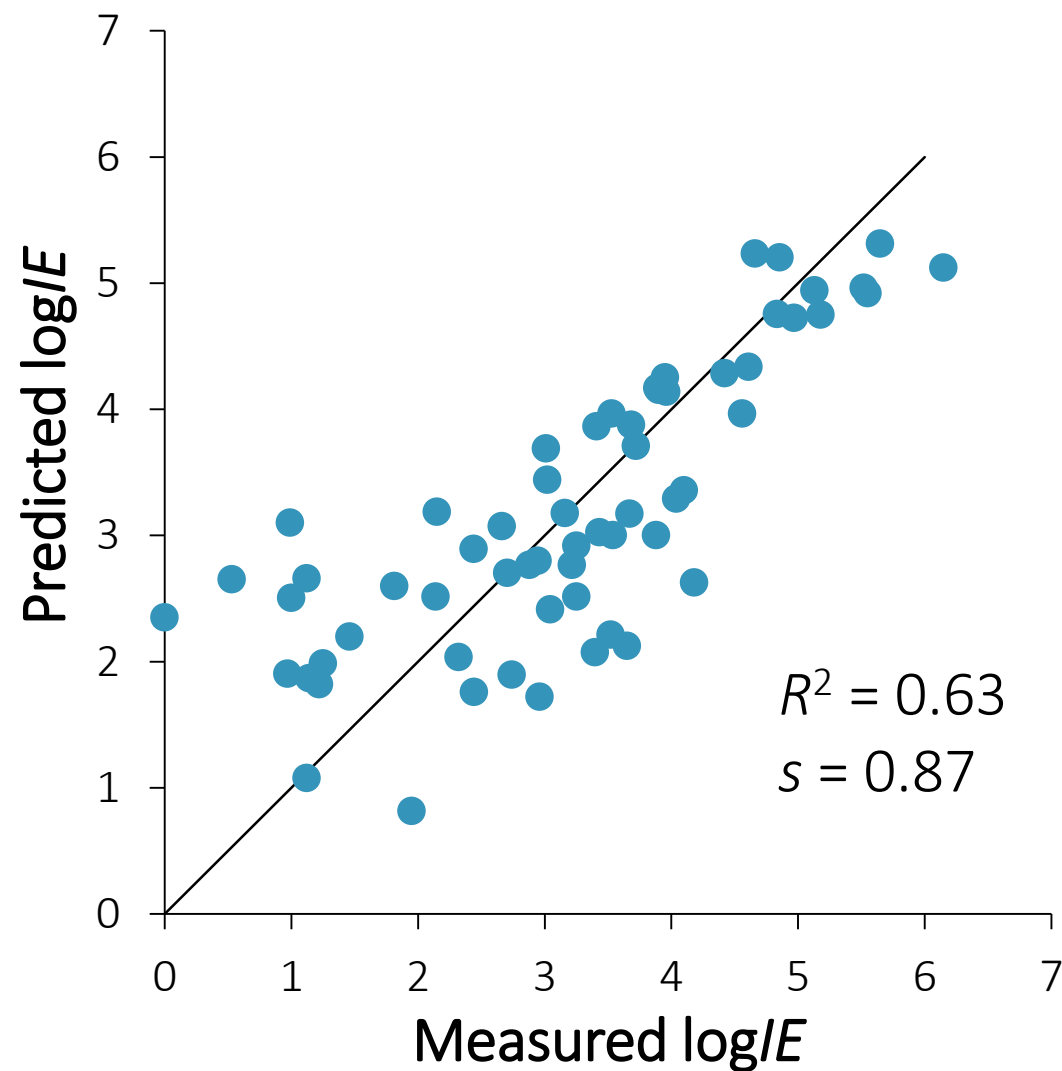
20/80 0.1% formic acid/acetonitrile

62 compounds:

$\log P = -22 \dots 6$; $pK_a(\text{aq}) = -15 \dots 30$

Mismatch 7 times

$$\log IE = 4.01 + 1.30 \cdot \alpha + 0.36 \cdot WANS$$



ESI-

20/80 0.1% ammonia/acetonitrile

$\log P = 1 \dots 18$; $pK_a(\text{aq}) = -5 \dots 12$

Model development

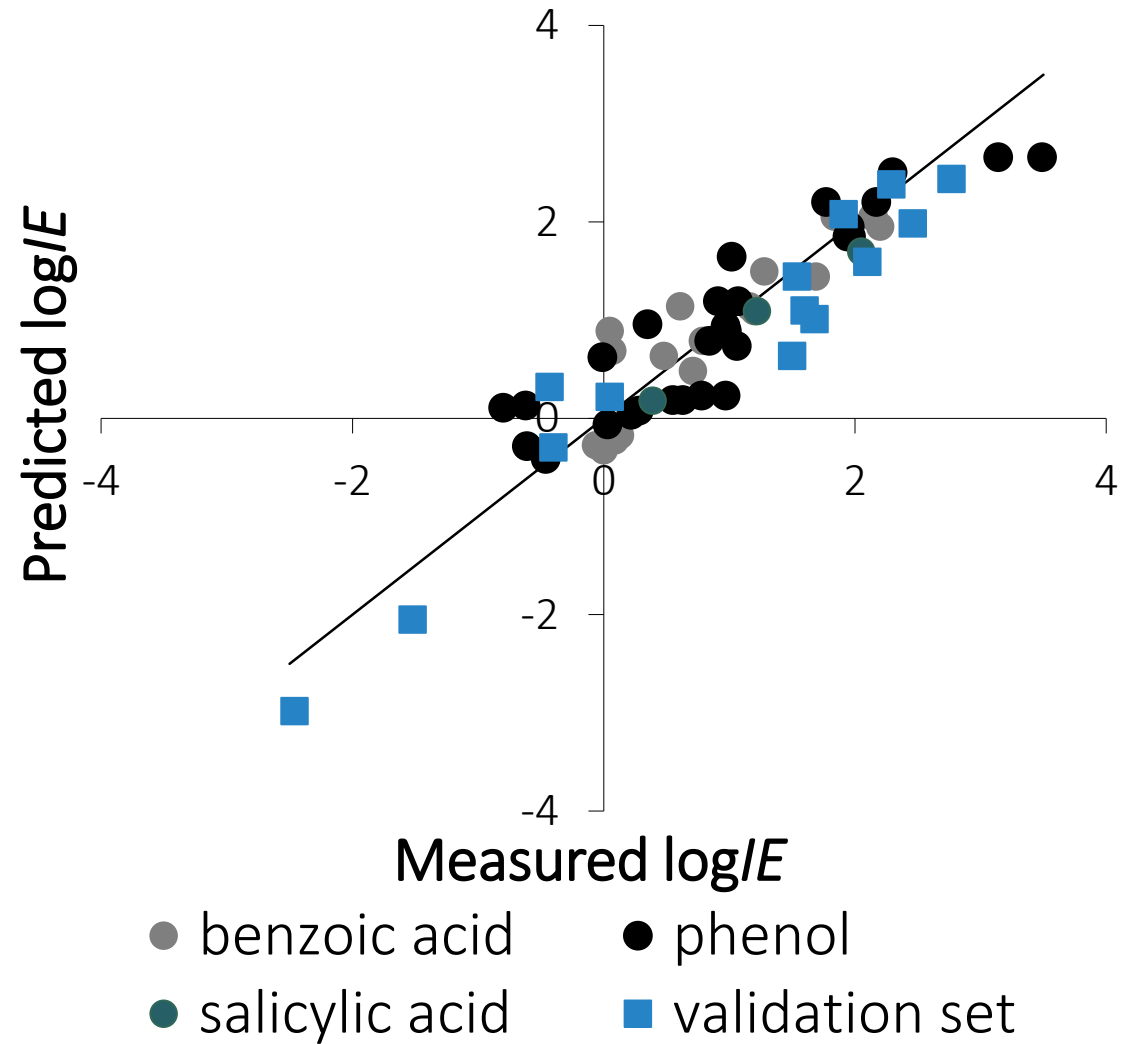
$R^2 = 0.83$

mismatch 2.5 times

Validation

mismatch 3 times

$$\log IE = 1.04 + 2.23 \cdot \alpha - 0.51 \cdot WAPS$$



Transferability between instruments

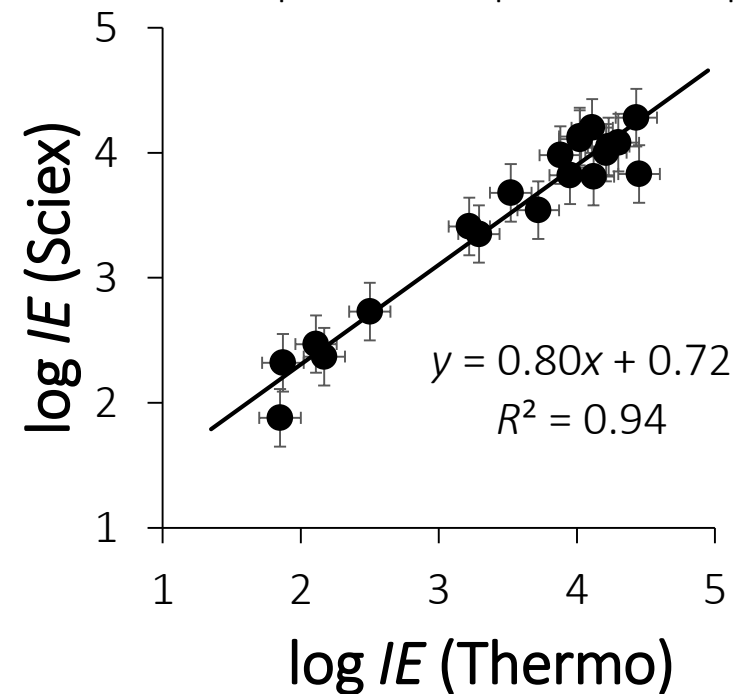
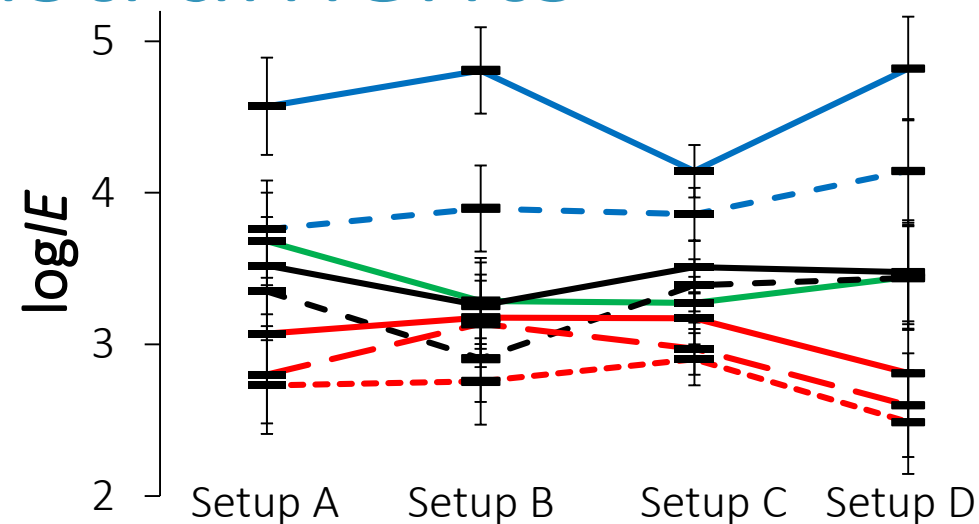
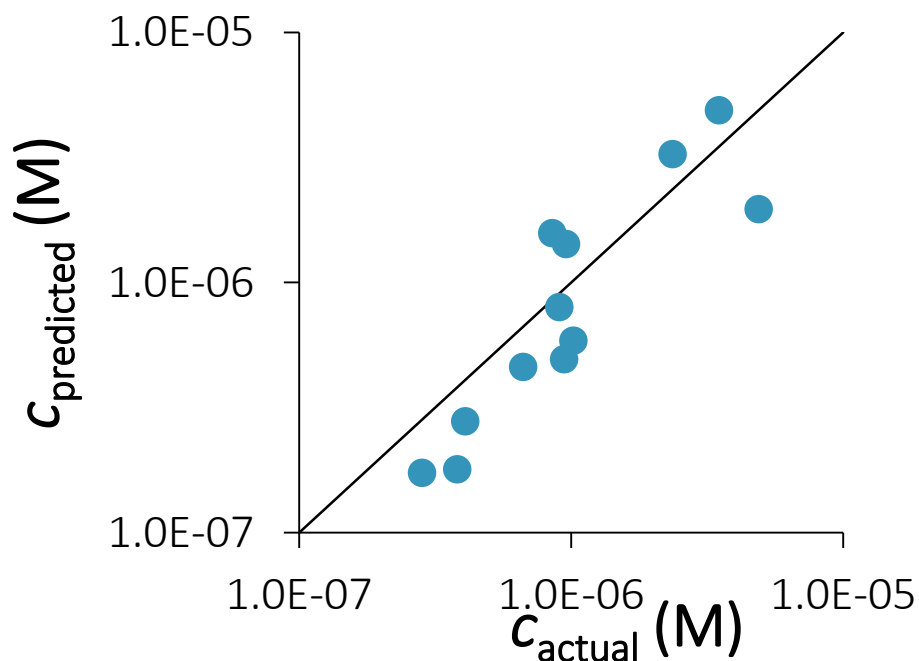
11 setups: 4 vendors, 3 ion sources, 5 mass-analyzers

Order of IEs independent of the instrument

$$R^2 = 0.60 \dots 0.98$$

Anchoring is recommended (calibration compounds)

Concentration mismatch lower than 2.5 times



Transferability between solvents

ESI-

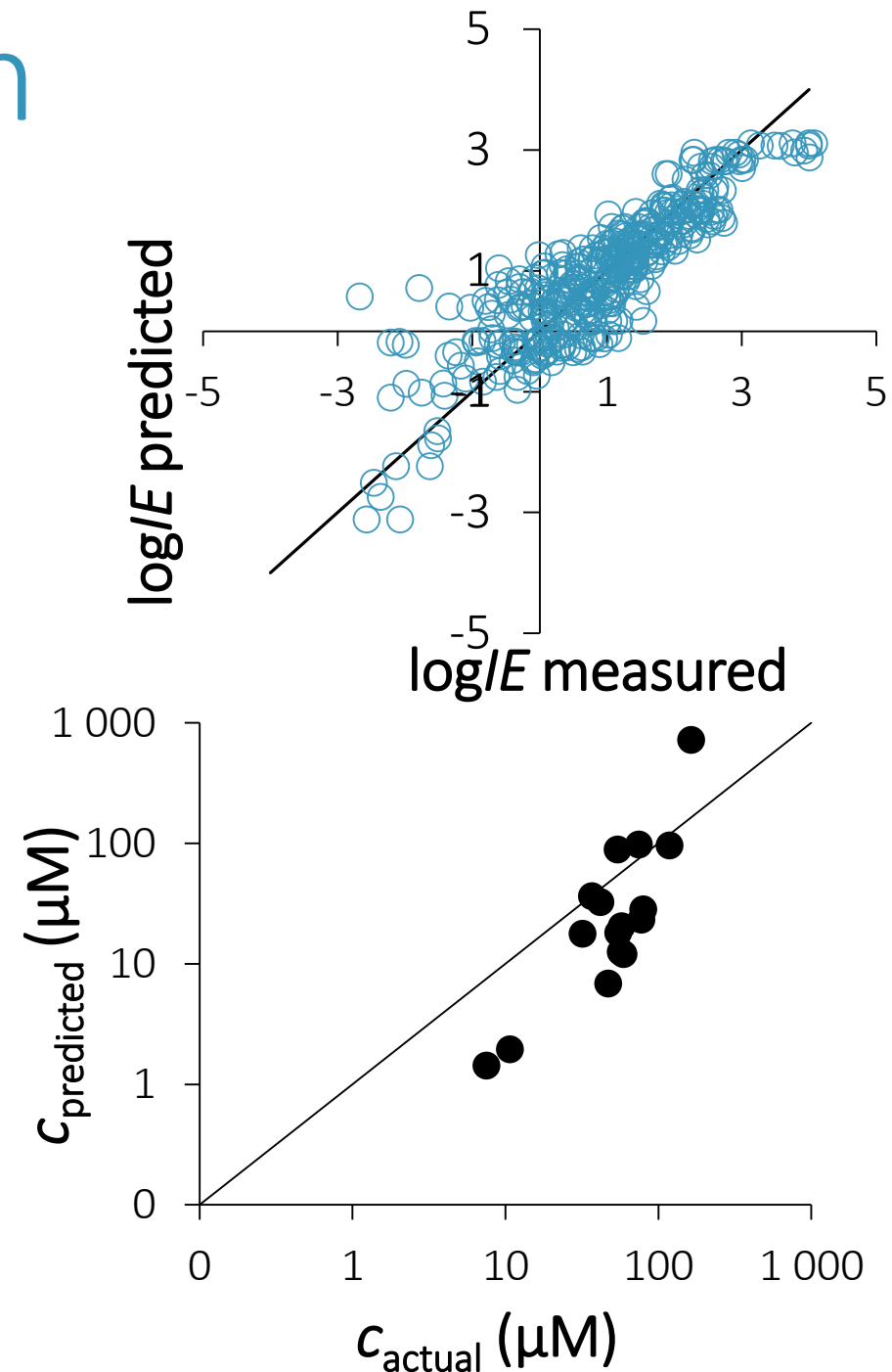
10 solvent compositions

62 compounds

Flow injection (0.2 mL/min)

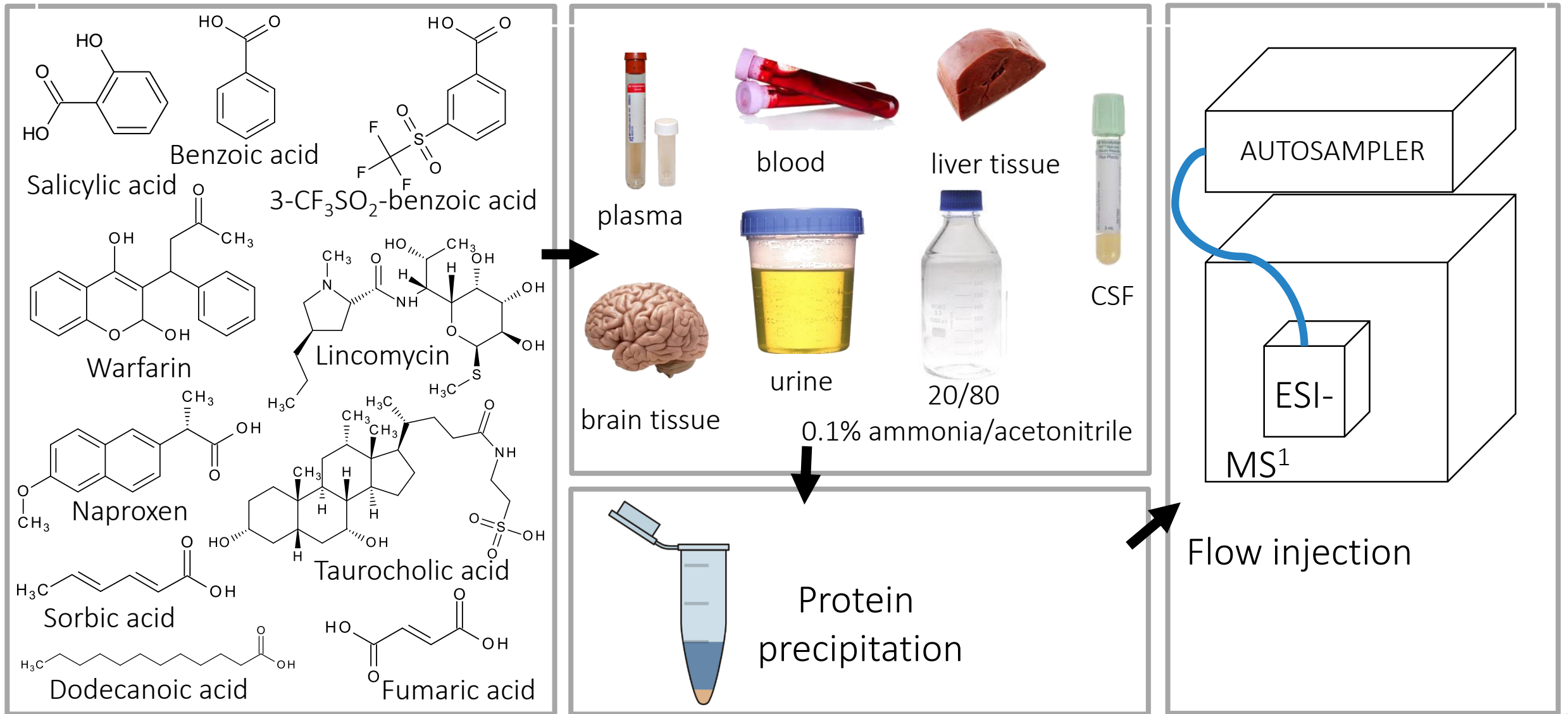
Validation with gradient LC/ESI/MS

Mismatch 3.7 times (44 times without model)

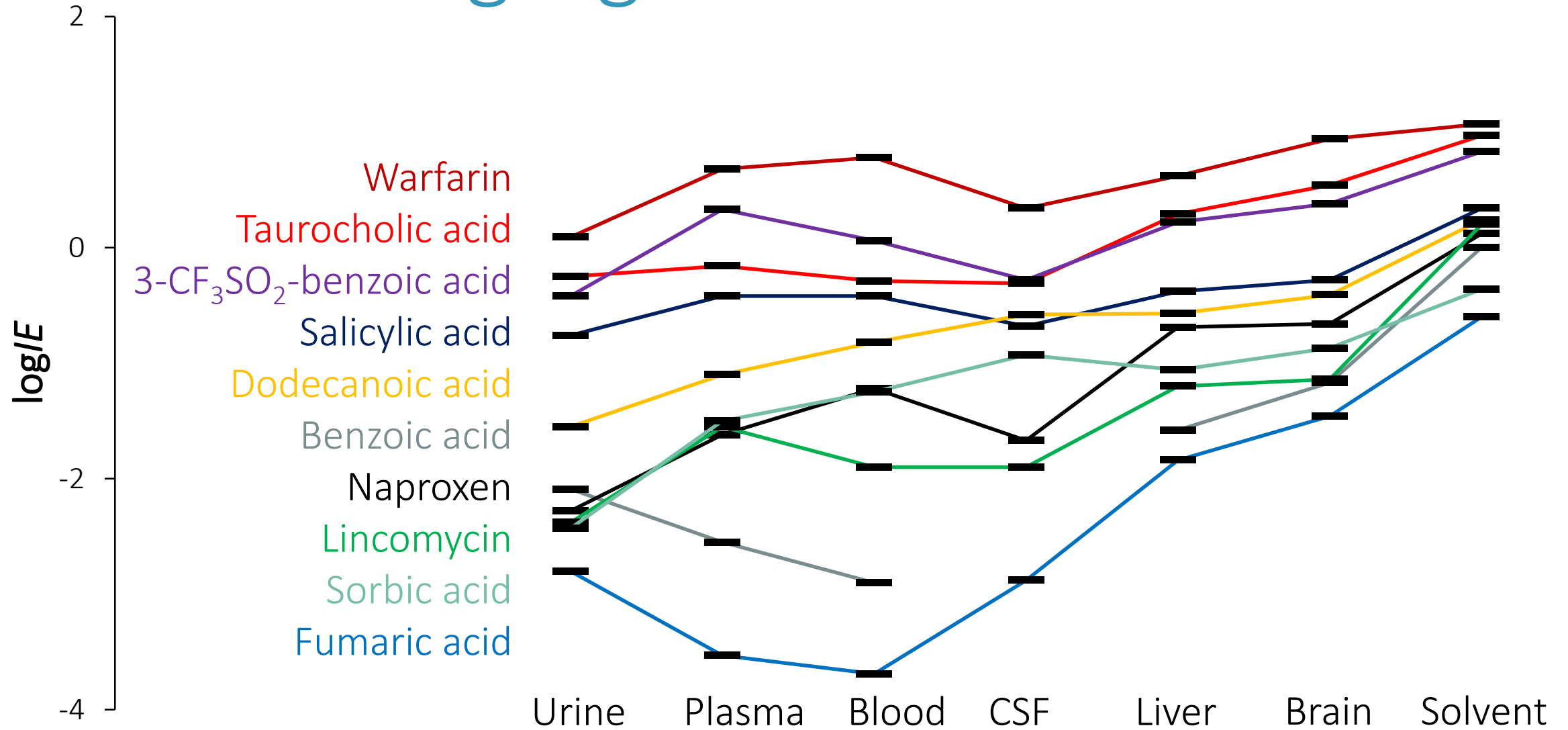


See poster WP 298 on Wednesday

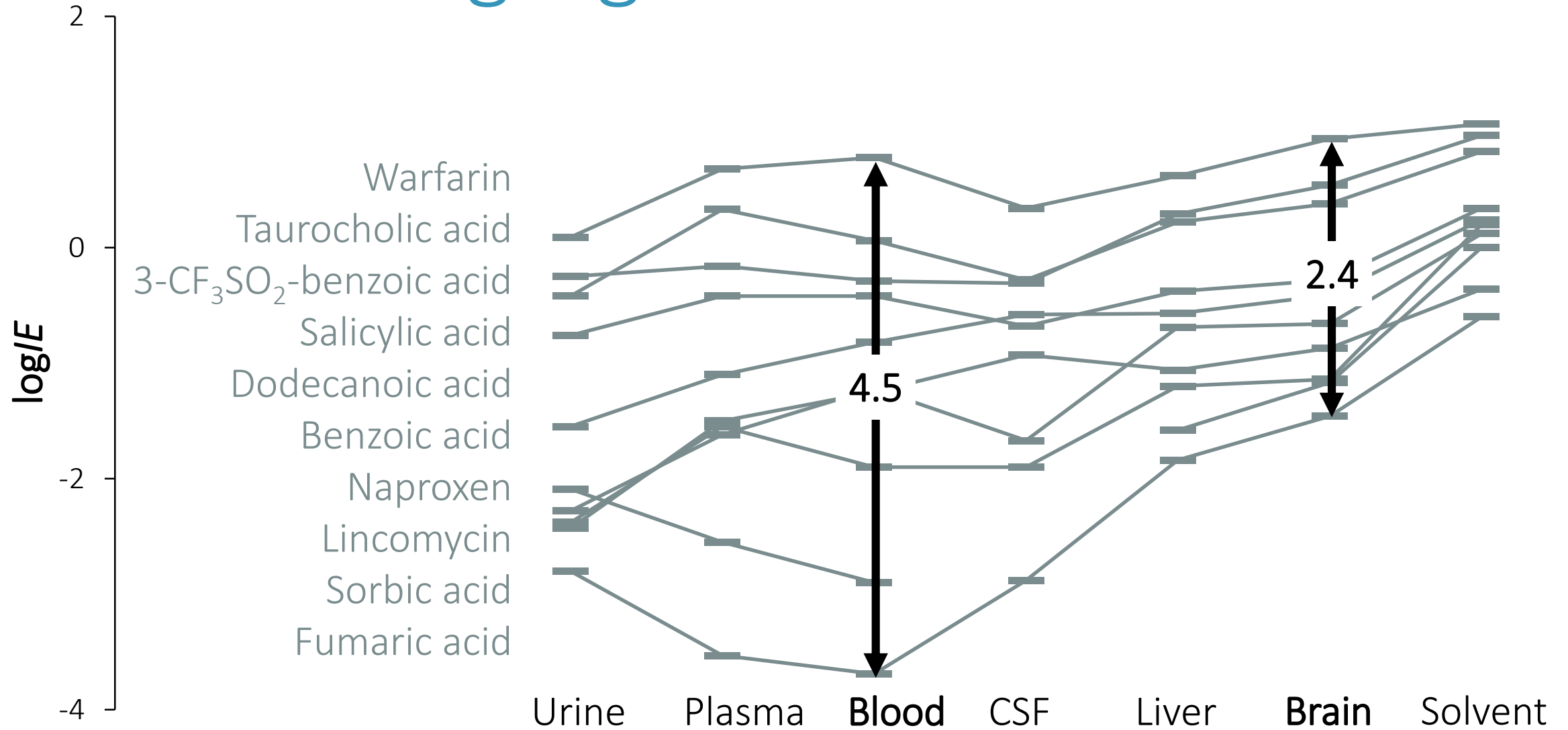
Could this also work in biological matrices?



I. Measuring \log/E in matrices



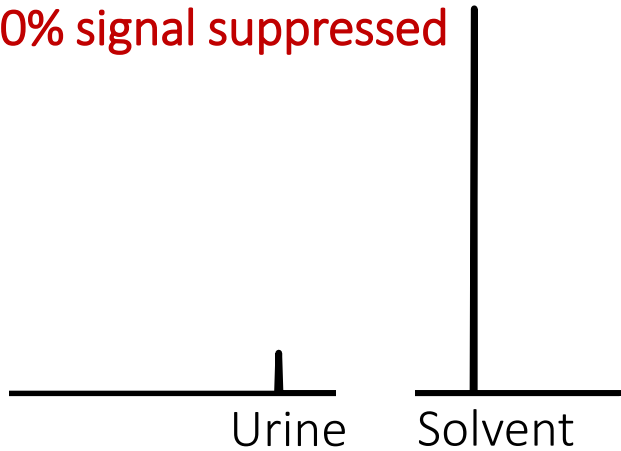
I. Measuring \log/E in matrices



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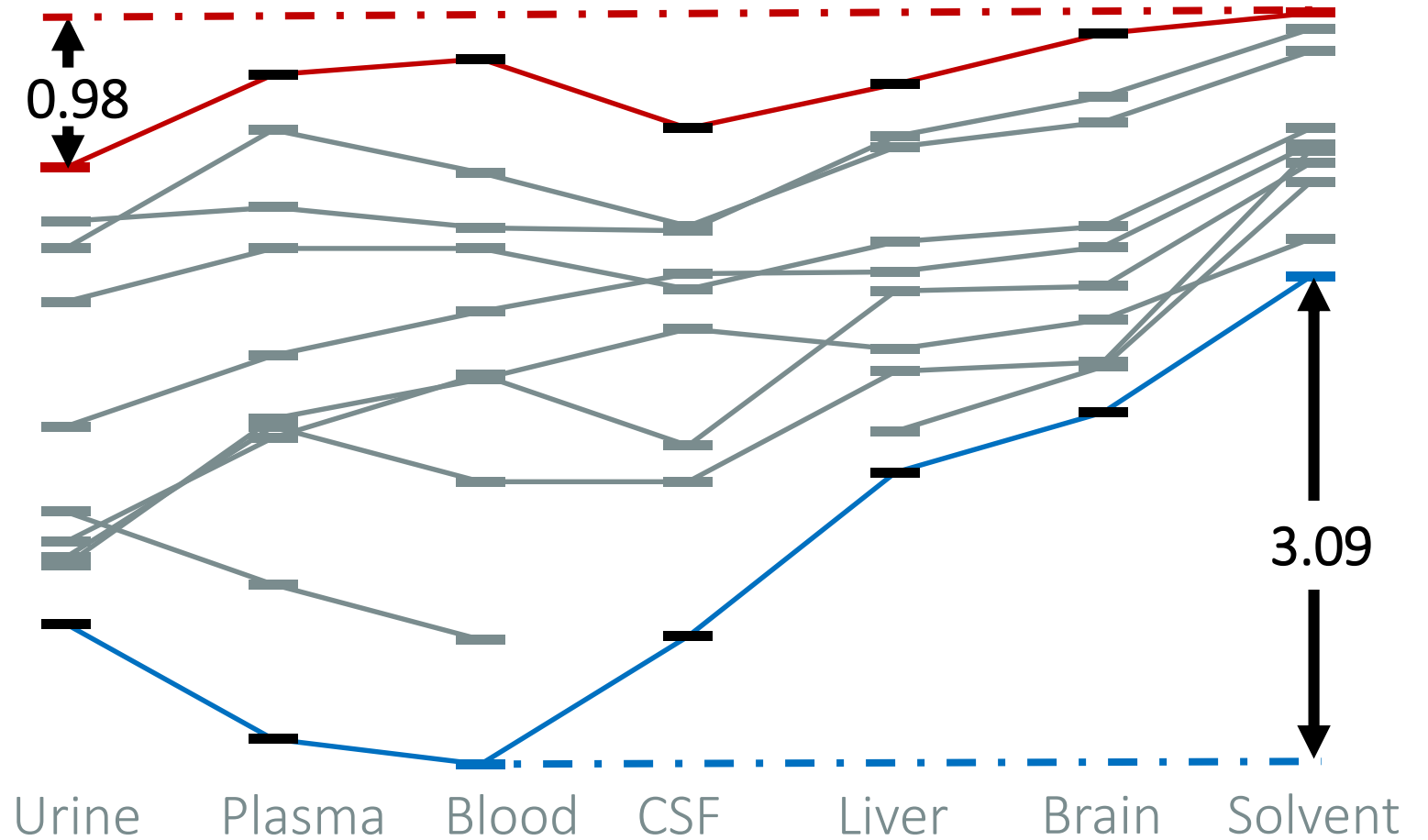
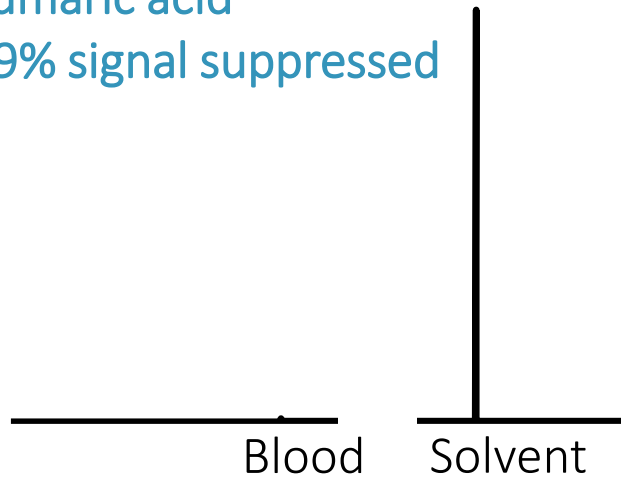
Warfarin

90% signal suppressed

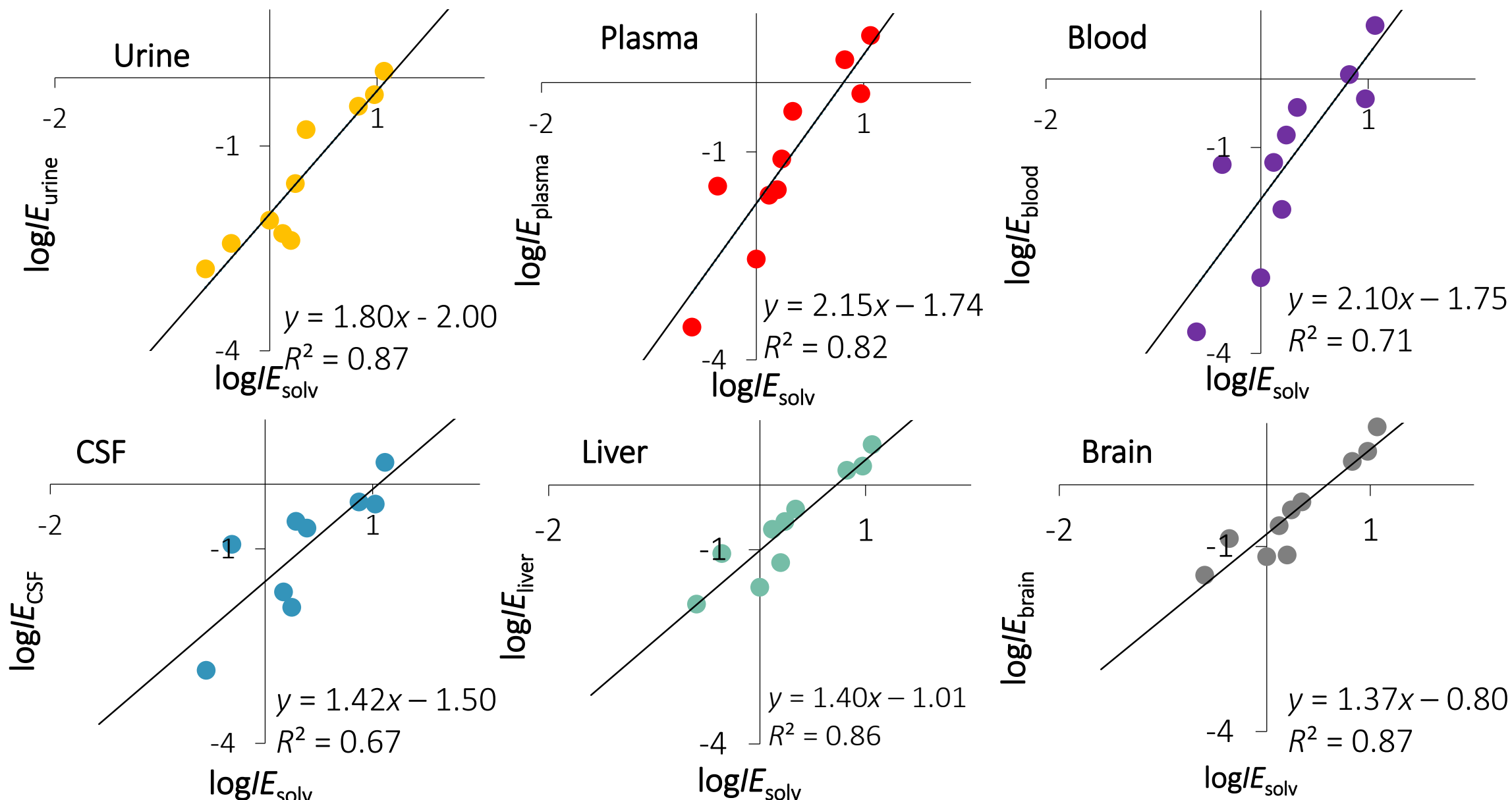


Fumaric acid

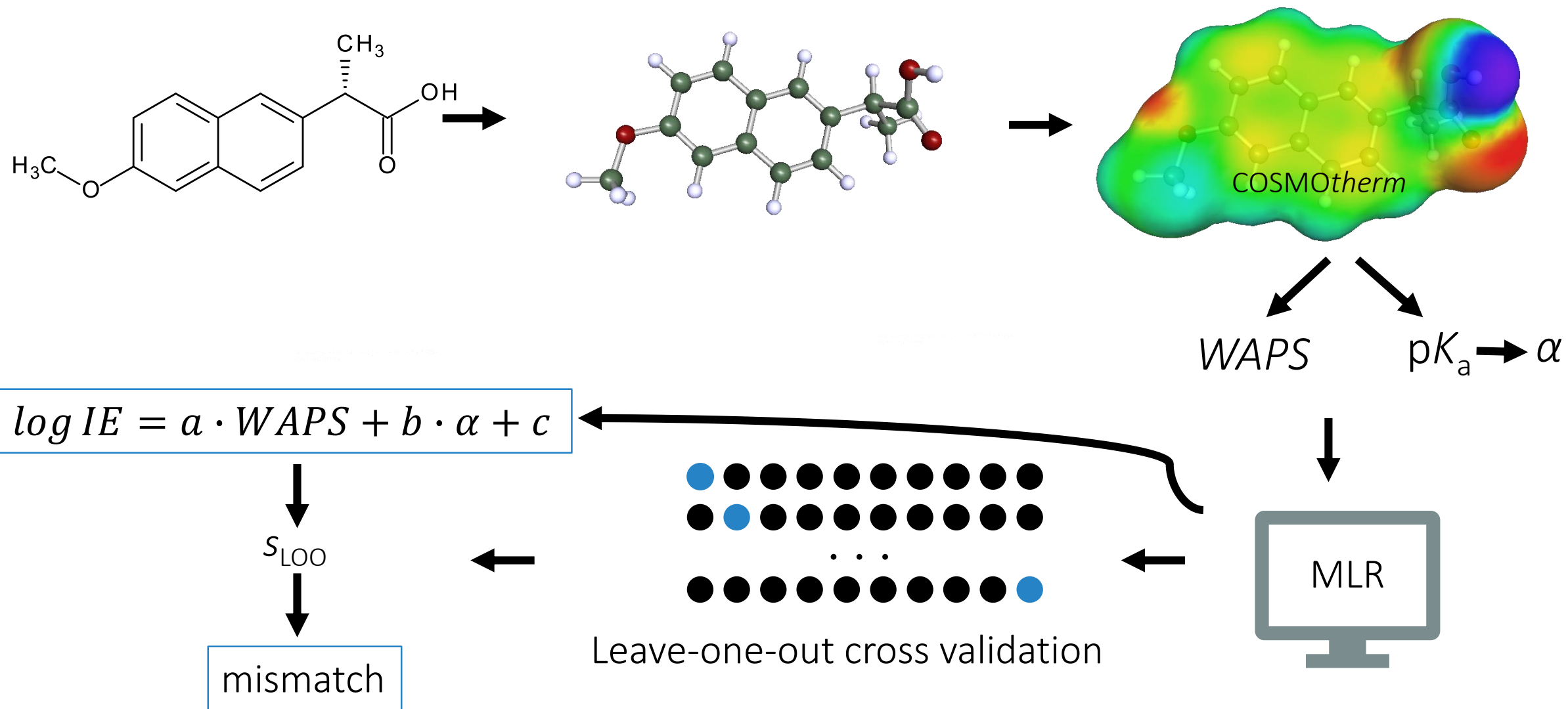
99% signal suppressed



II. Correlation with neat solvent



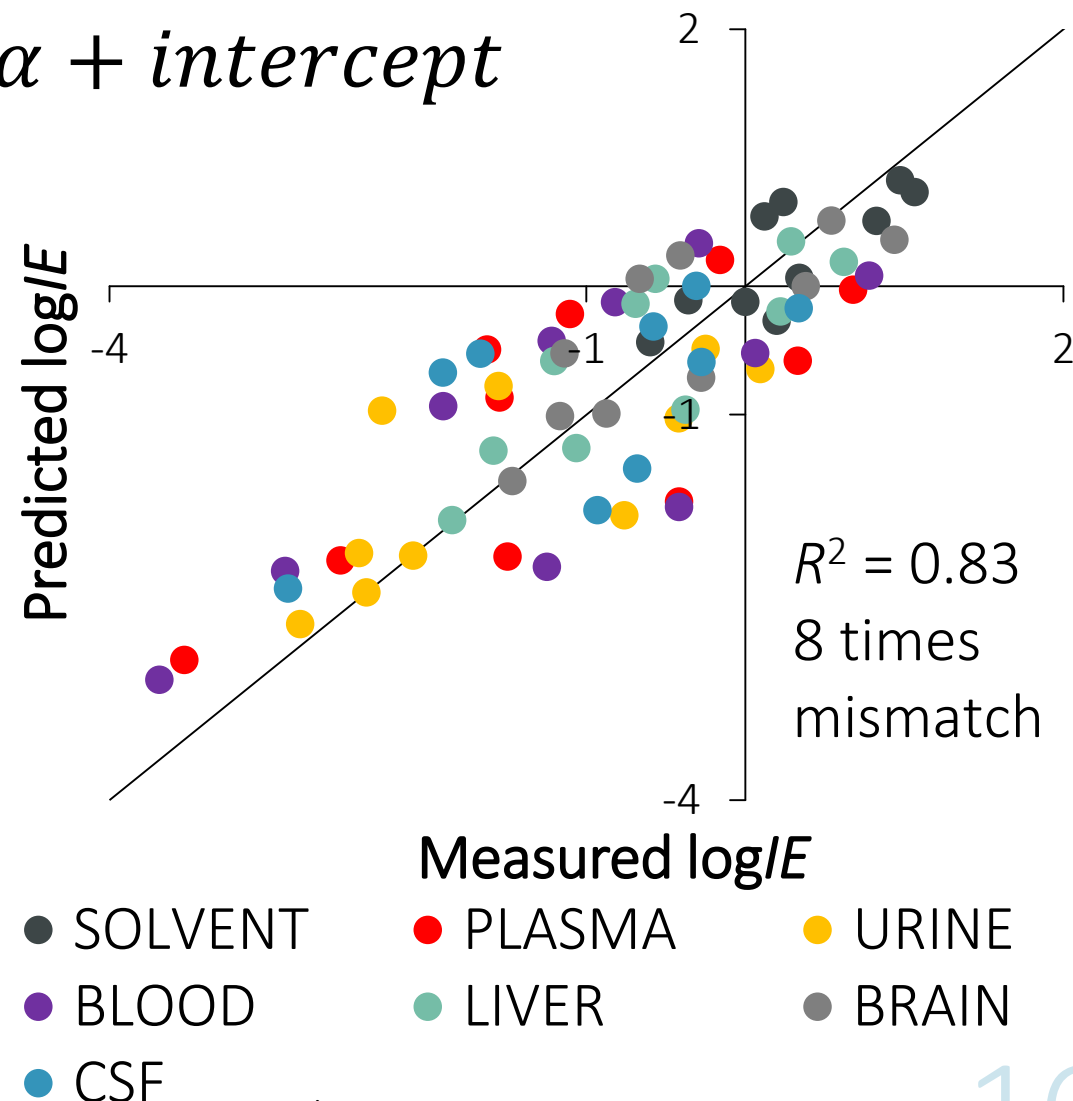
III. Model development



III. Model to predict log/E

$$\log IE = coef_{WAPS} \cdot WAPS + coef_{\alpha} \cdot \alpha + intercept$$

	$coef_{WAPS}$	$coef_{\alpha}$	$intercept$	R^2	mismatch
Solvent	-0.18	1.18		0.72	2
Urine	-0.31	2.04	-1.93	0.55	6
Plasma	-0.45	1.08		0.77	8
Blood	-0.50	1.29		0.78	8
Liver	-0.32	0.96		0.81	3
Brain	-0.30	1.08		0.73	3
CSF	-0.34	0.66		0.71	7



Standard substance free quantitation

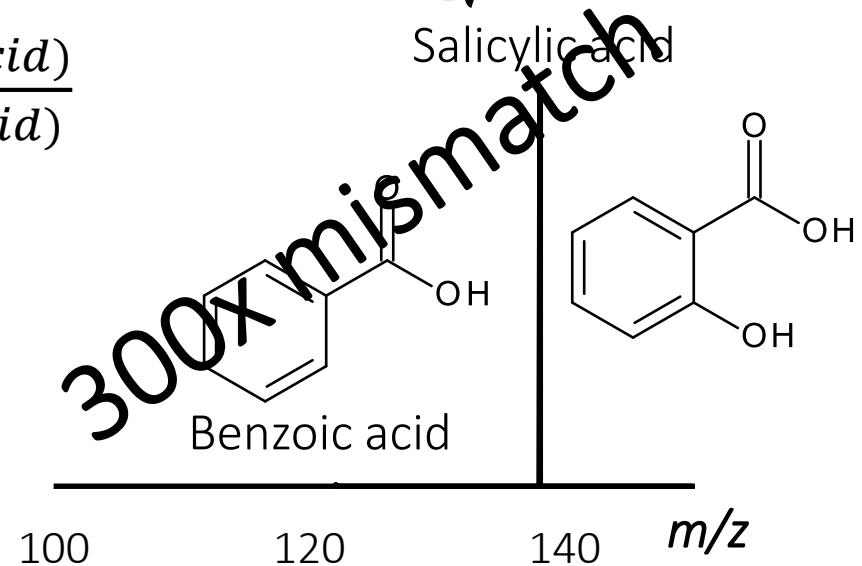
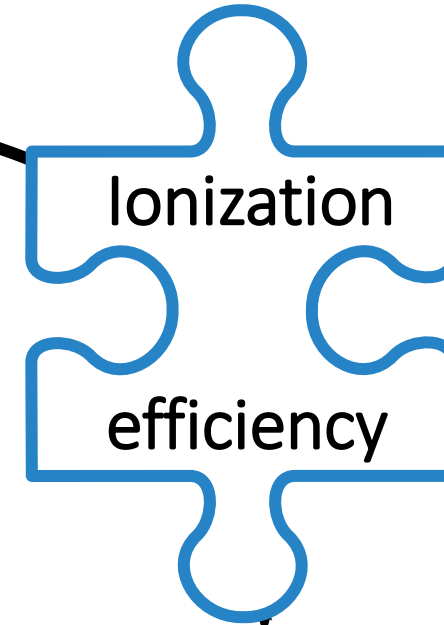
$$\log IE_{\text{blood}} = -0.50 \cdot WAPS + 1.29 \cdot \alpha$$

$$\log IE (\text{benzoic acid}) = -2.22$$
$$\text{Signal (benzoic acid)} = 1 \cdot 10^2$$

$$\log IE (\text{salicylic acid}) = -1.72$$
$$\text{Signal (salicylic acid)} = 3 \cdot 10^4$$

$$\frac{c(\text{salicylic acid})}{c(\text{benzoic acid})} = \frac{\text{signal (salicylic acid)}}{\text{signal (benzoic acid)}} \cdot \frac{IE(\text{salicylic acid})}{IE(\text{benzoic acid})}$$

$$\frac{c(\text{salicylic acid})}{c(\text{benzoic acid})} = \mathbf{8x \text{ mismatch}}$$



Proof-of-concept

Approach works!

ESI+ and ESI-,
different setups,
different mobile phases,
biological matrices

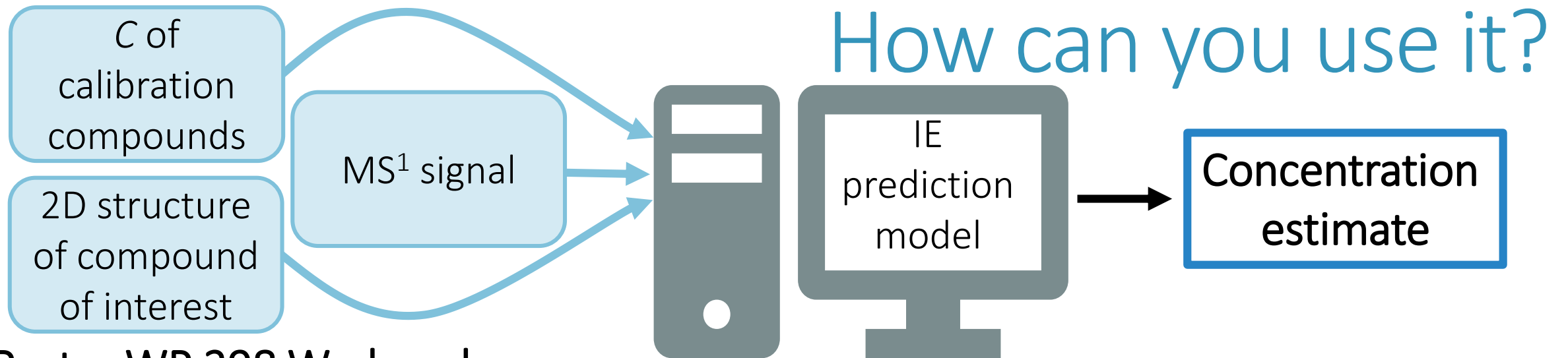
Technical aspects

Software (automation)

LC, adducts

More matrices (environmental,
food, botanics)

Self-improving



Poster WP 298 Wednesday

Conclusion

Predicting ESI ionization efficiencies

Different ESI modes

Different mobile phase compositions

Transferable between different instruments

Biological matrices

Semiquantitative analysis

prediction mismatch ≤ 8 times





Poster WP 298 on Wednesday

Thank you!

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