



# Semi-Quantitative LC/ESI/MS Analysis Using Predictive Models of ESI Ionization Efficiencies

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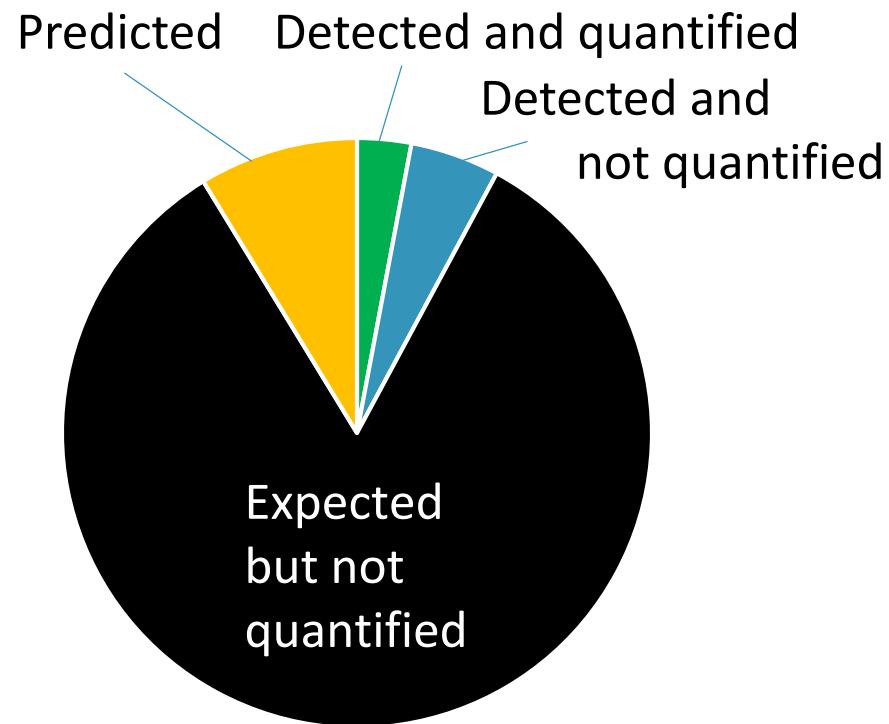
Janssen Pharmaceutica NV, Belgium



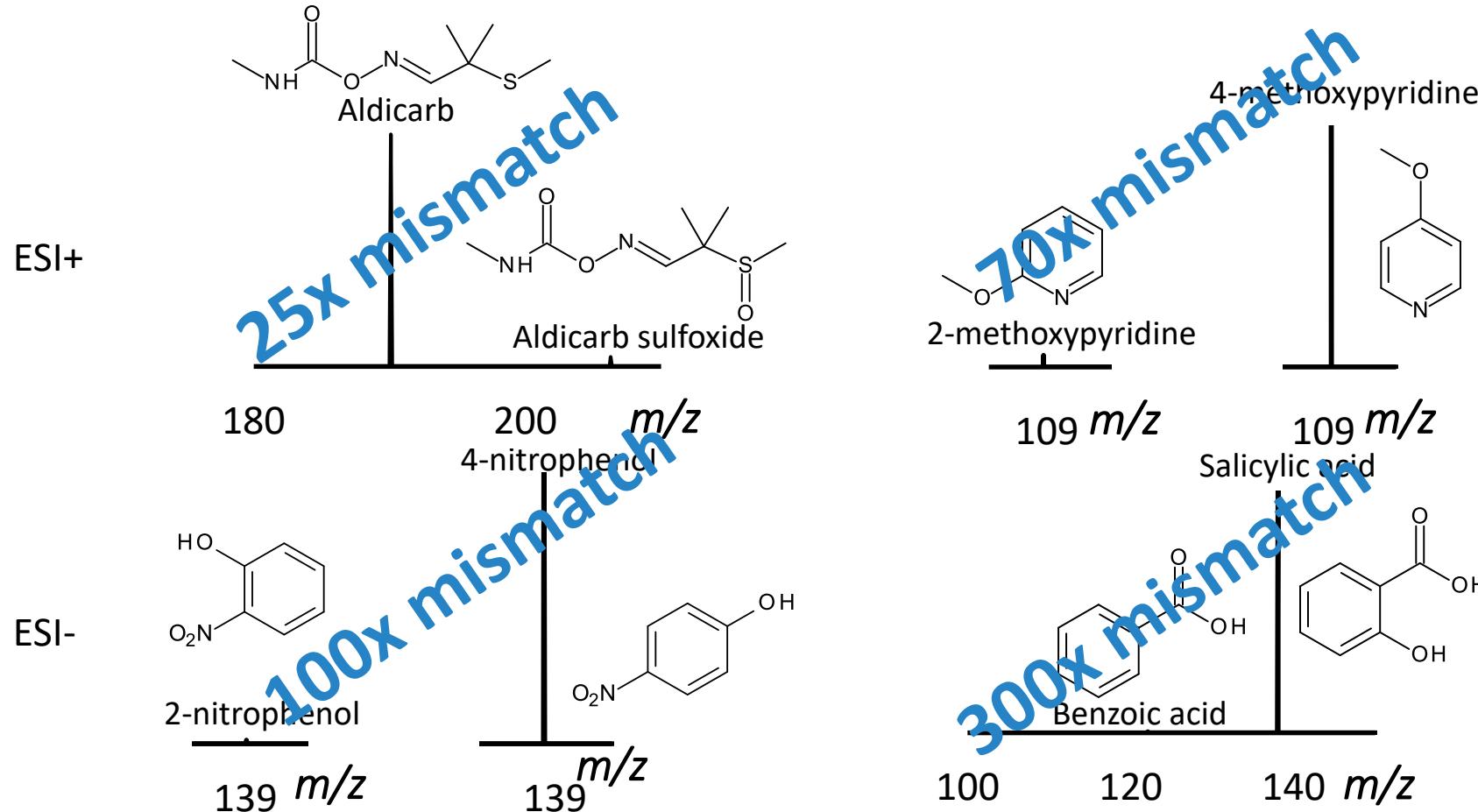
# Human Metabolome Database

- Quantified – 3% metabolome
- Identified – 8% metabolome

**How to obtain more  
(semi)quantitative  
information with  
existing information?**



# Standard substance free quantitation

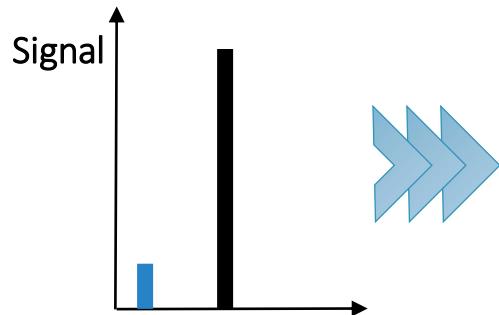
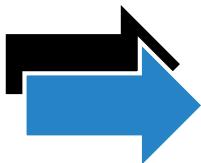


$$c_1 = c_2$$

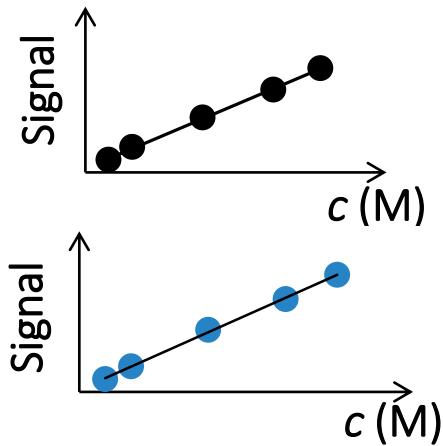
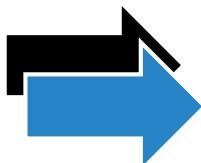
# Ionization efficiencies



$$C_1 = C_2$$

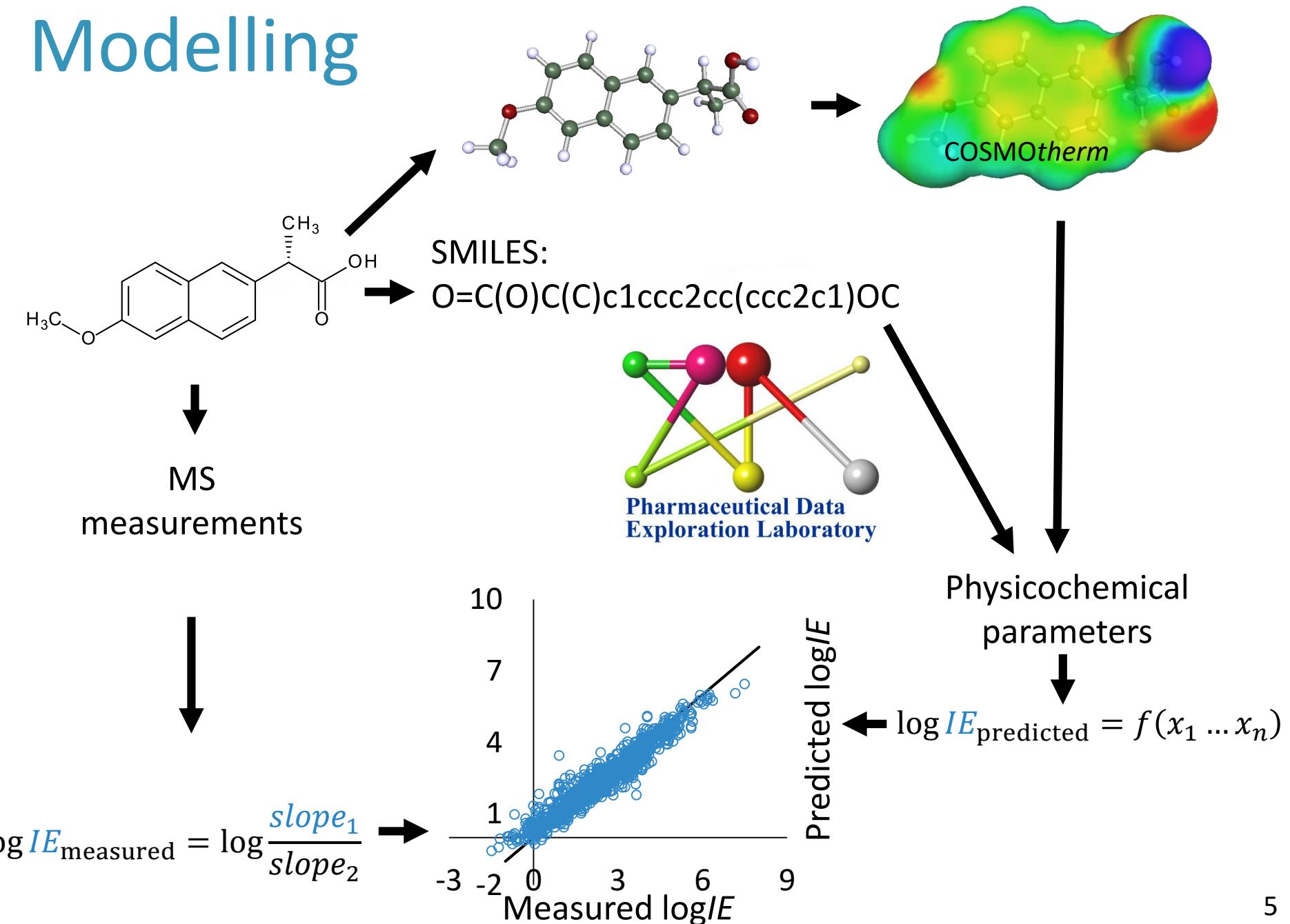


$$C_1 < C_2$$

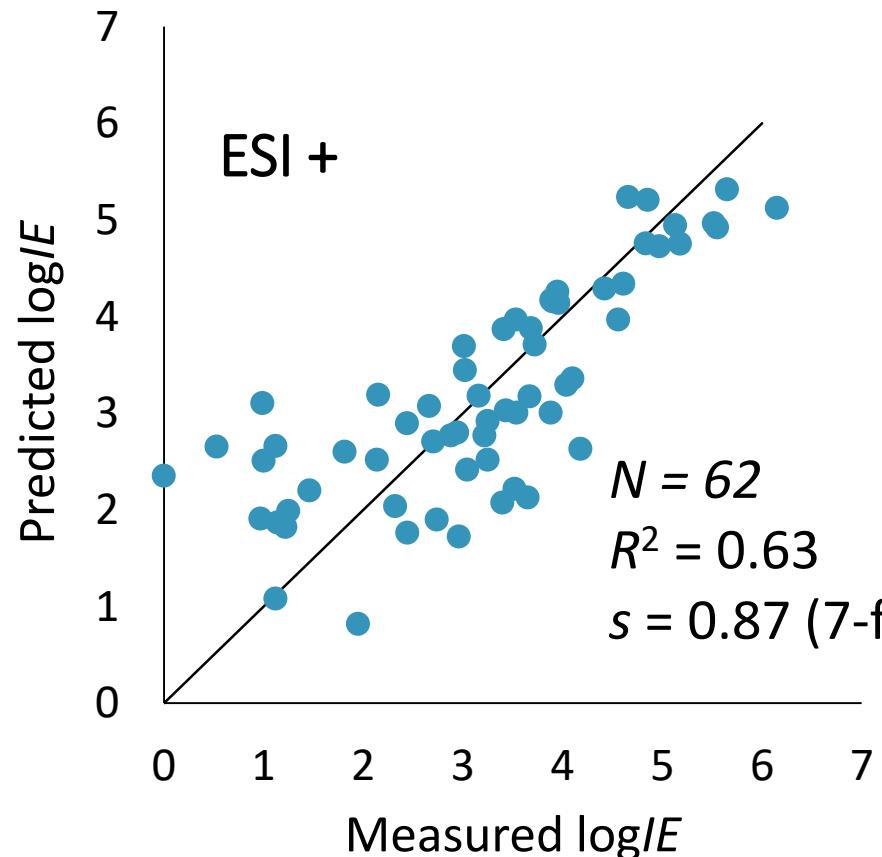


$$\log IE = \log \frac{slope_1}{slope_2}$$

# Modelling

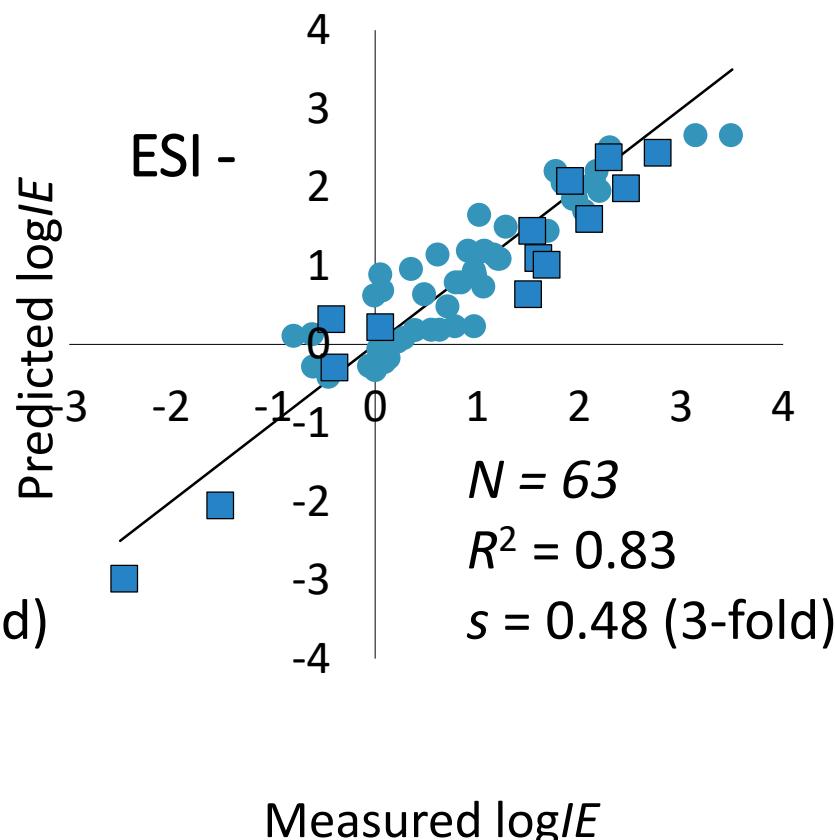


# First generation models



20/80 0.1% formic acid/acetonitrile

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



20/80 0.1% ammonia/acetonitrile

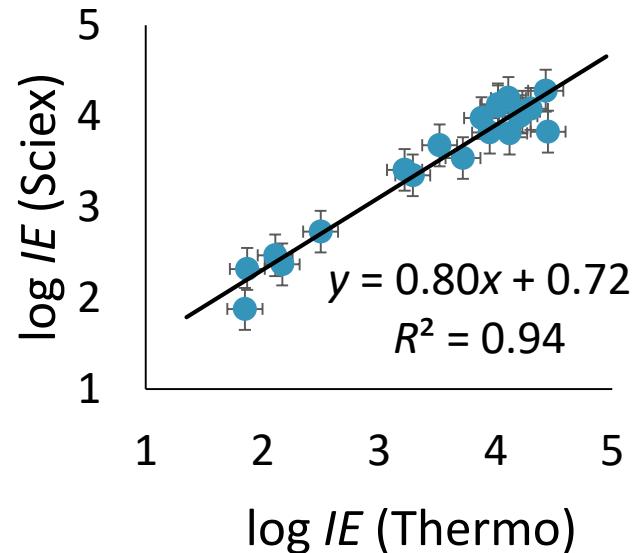
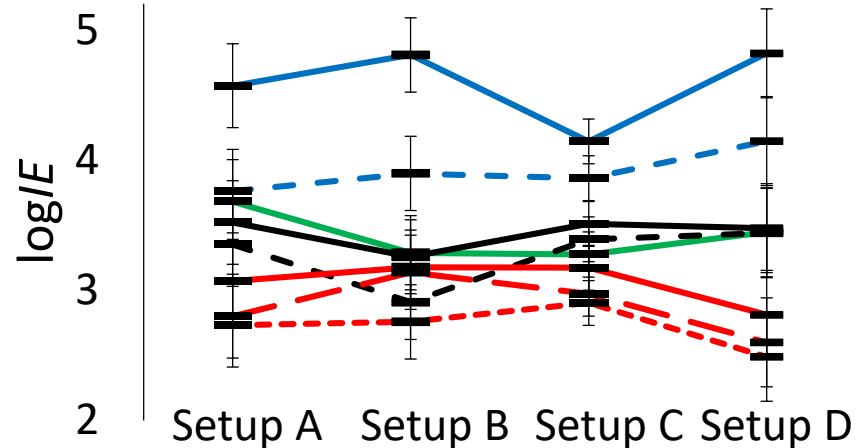
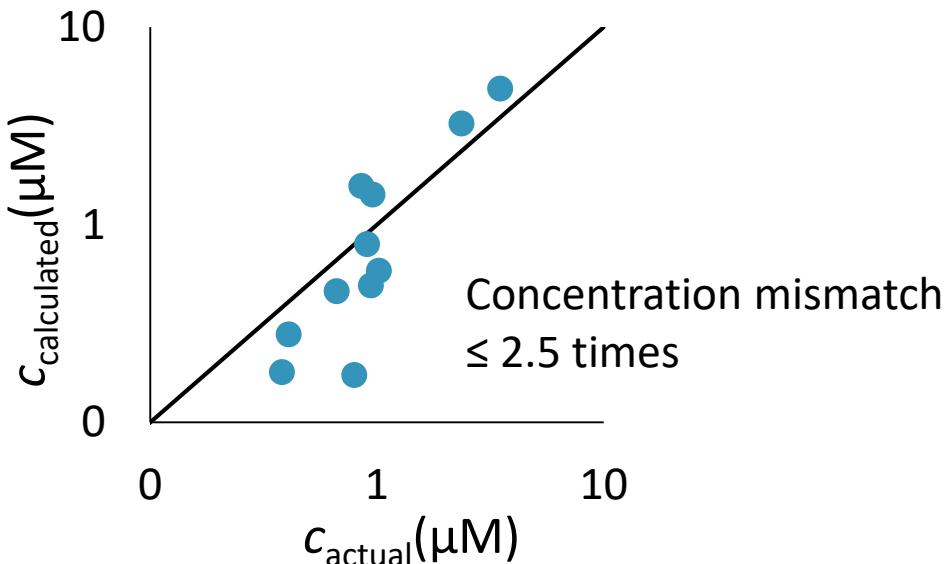
$$\log IE = 1.04 + 2.23 \cdot \alpha - 0.51 \cdot WAPS$$

# Transferability between instruments

11 setups

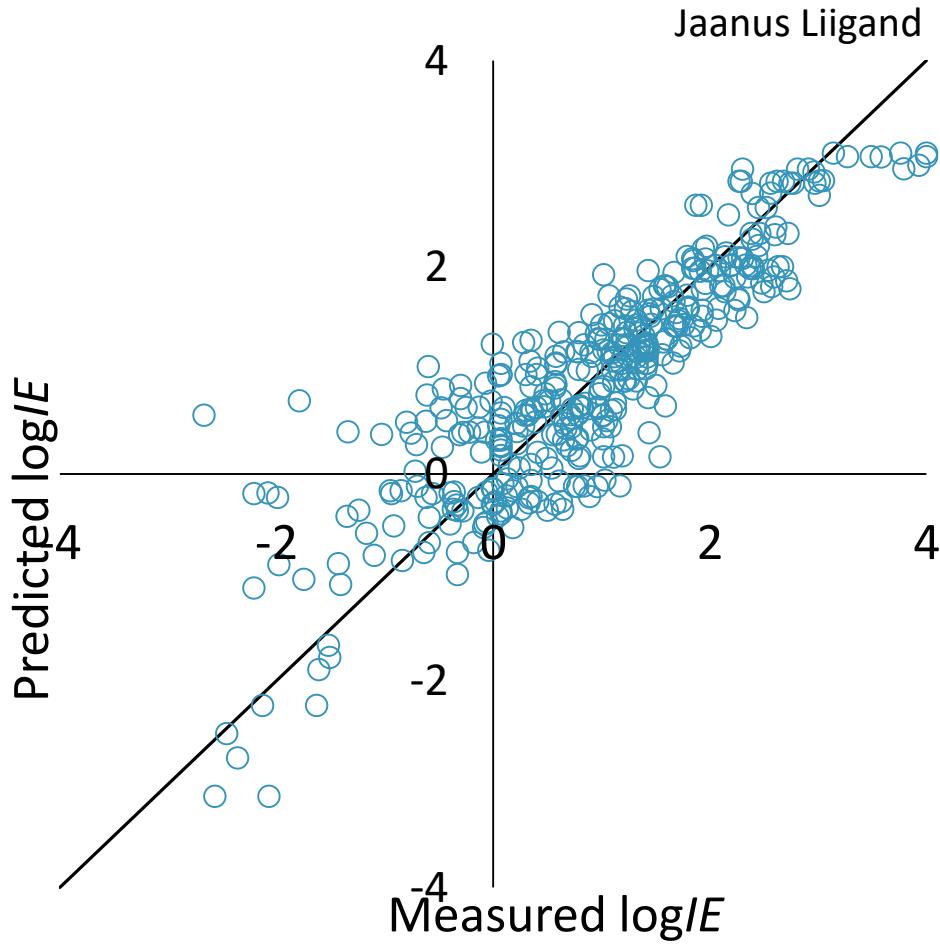
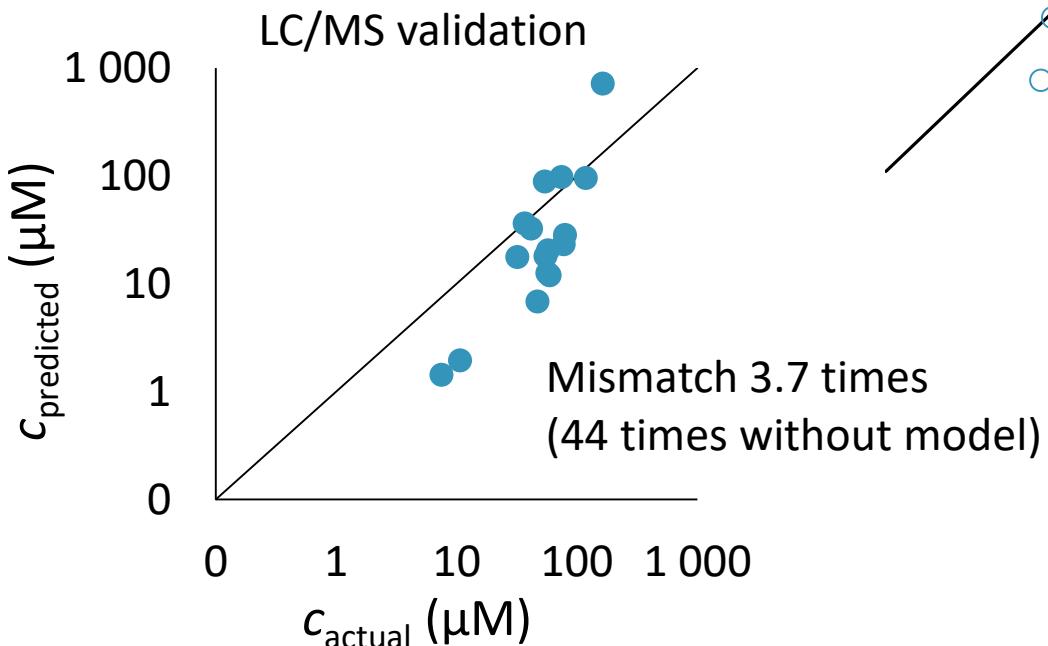


- Order of IEs independent of the instrument
  - $R^2 = 0.60 \dots 0.98$

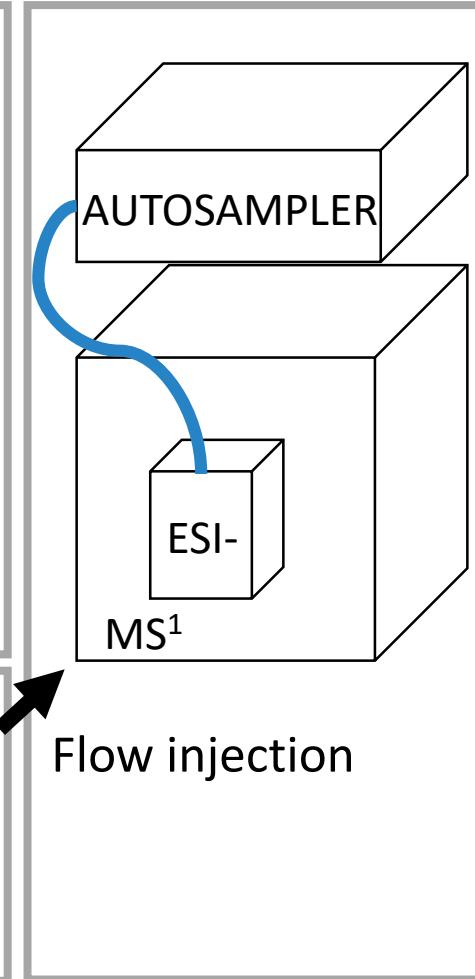
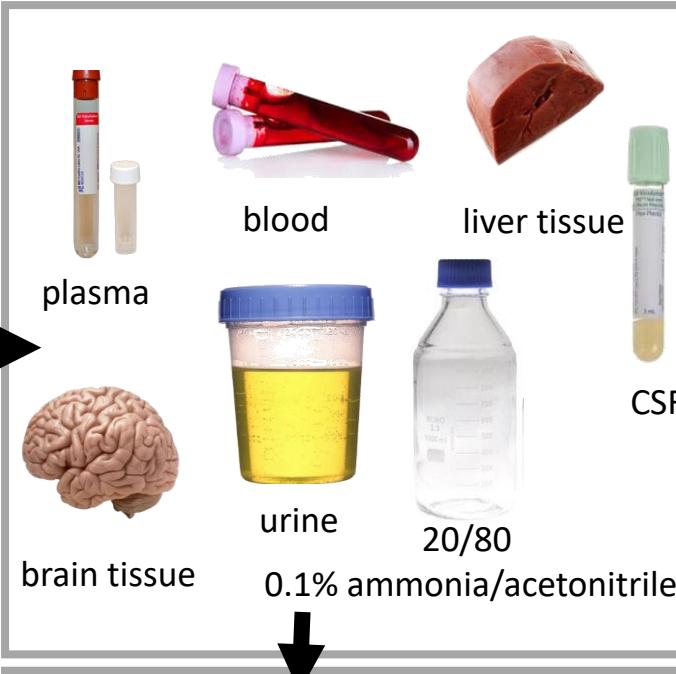
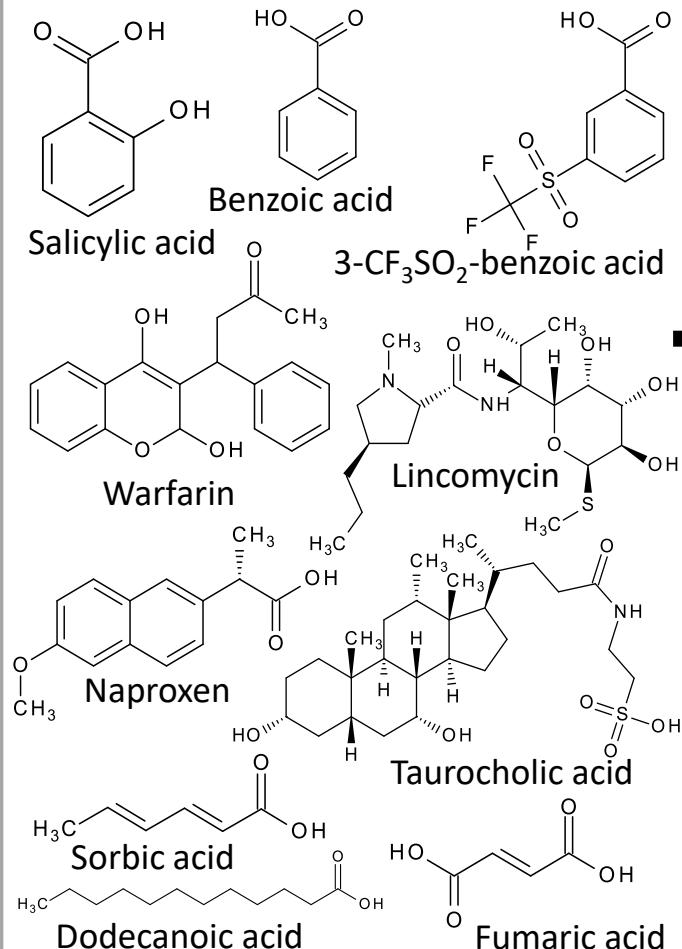


# Transferability between solvents

- ESI-
- 10 solvent compositions
- 62 compounds
- Flow injection (0.2 mL/min)



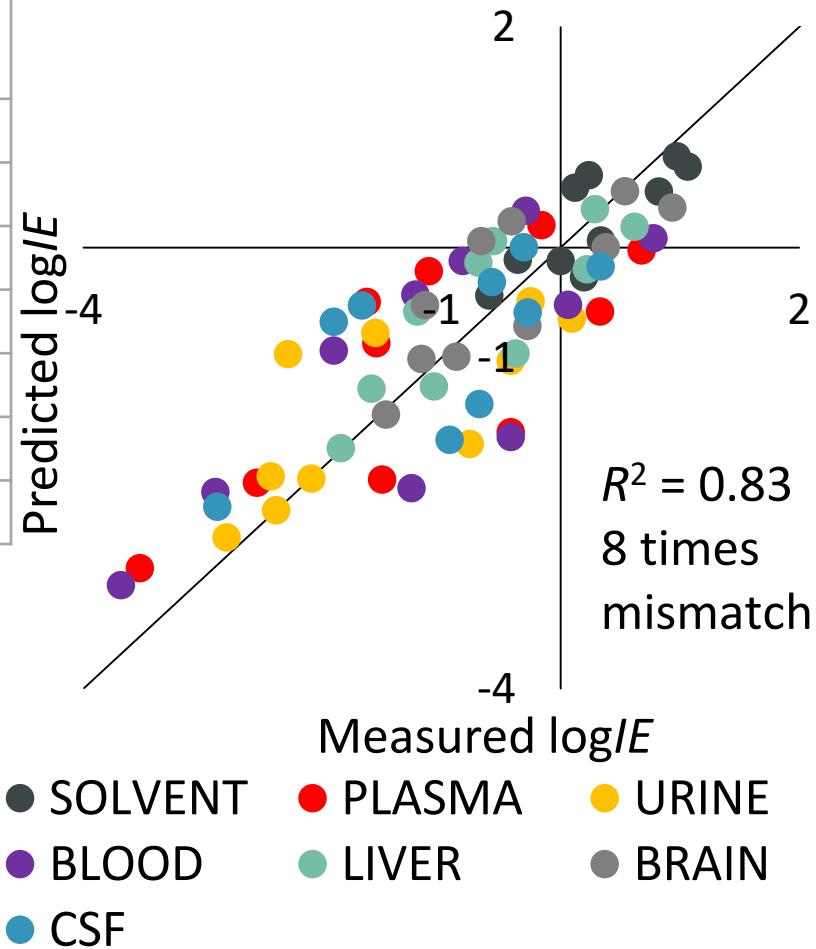
# Could this also work in biological matrices?



# Prediction of $\log IE$ in bio-matrices

$$\log IE = \text{coef}_{\text{WAPS}} \cdot \text{WAPS} + \text{coef}_\alpha \cdot \alpha + \text{intercept}$$

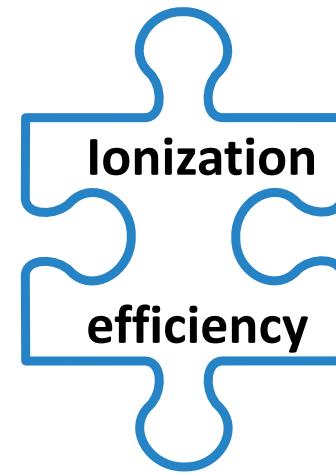
	$\text{coef}_{\text{WAPS}}$	$\text{coef}_\alpha$	$\text{intercept}$	$R^2$	mismatch
Solvent	-0.18	1.18		0.72	2
Urine	-0.31	2.04	-1.93	0.55	6
Plasma	-0.45	1.08		0.77	8
Blood	-0.50	1.29		0.78	8
Liver	-0.32	0.96		0.81	3
Brain	-0.30	1.08		0.73	3
CSF	-0.34	0.66		0.71	7



# Standard substance free quantitation

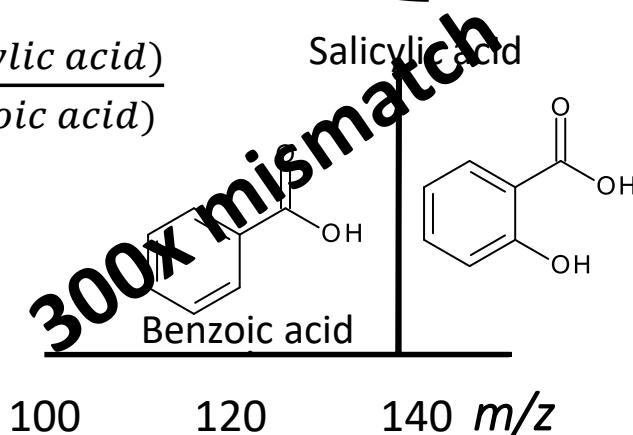
$$\log IE_{\text{blood}} = -0.50 \cdot WAPS + 1.29 \cdot \alpha$$

$\log IE(\text{benzoic acid}) = -2.22$     $\log IE(\text{salicylic acid}) = -1.72$   
 Signal (benzoic acid) =  $1 \cdot 10^2$    Signal (salicylic acid) =  $3 \cdot 10^4$



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

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



# Second generation prediction models - measurements

	ESI +	ESI -
Compounds	333	62
$\log P_{ow}$	-4.1 ... 7.7	-3.3 ... 6.3
$pK_a$	-15 ... 30	-4.5 ... 12
Eluents	21	10
Organic modifier	MeCN 0 – 100% MeOH 0 – 100%	MeCN 0 – 100%
Additive	HCOOH, TFA, NH <sub>3</sub> , formate buffer, acetate buffer, bicarbonate buffer, NH <sub>4</sub> F	HCOOH, NH <sub>3</sub> , acetate buffer, CH <sub>3</sub> NH <sub>2</sub>
pH	2.0 ... 10.7	2.7 ... 10.7

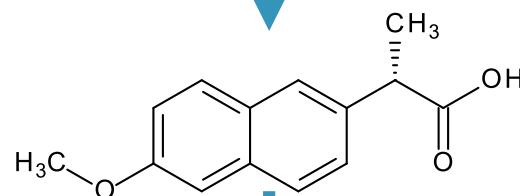
# Second generation prediction models - measurements

	ESI +	ESI -
Compounds	333	62
$\log P_{ow}$	-4.1 ... 7.7	-3 ... 6.3
$pK_a$	-15	-4.5 ... 12
Eluents	10	
Organic modifier	0% 100%	MeCN 0 – 100%
Additive	H <sub>3</sub> BO <sub>3</sub> , TFA, NH <sub>3</sub> , citrate buffer, acetate buffer, bicarbonate buffer, NH <sub>4</sub> F	HCOOH, NH <sub>3</sub> , acetate buffer, CH <sub>3</sub> NH <sub>2</sub>
pH	2.0 ... 10.7	2.7 ... 10.7

All together 2000 logE values!

# Second generation prediction models – model development

Identifier	Retention time	Area
naproxen	5.2	2345156



SMILES:  
O=C(O)C(C)c1ccc2cc(ccc2c1)OC



Eluent composition

Eluent descriptors:

- Viscosity
- polarity index
- surface tension
- pH

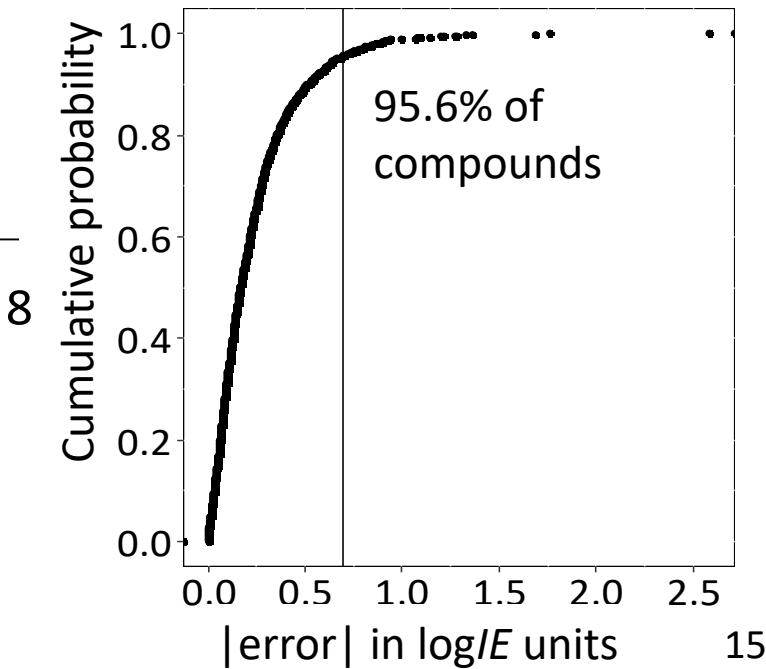
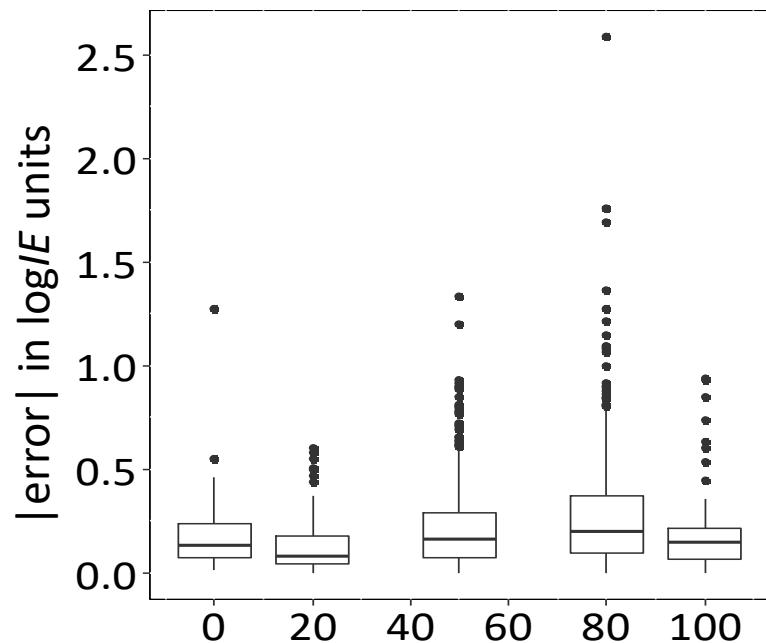
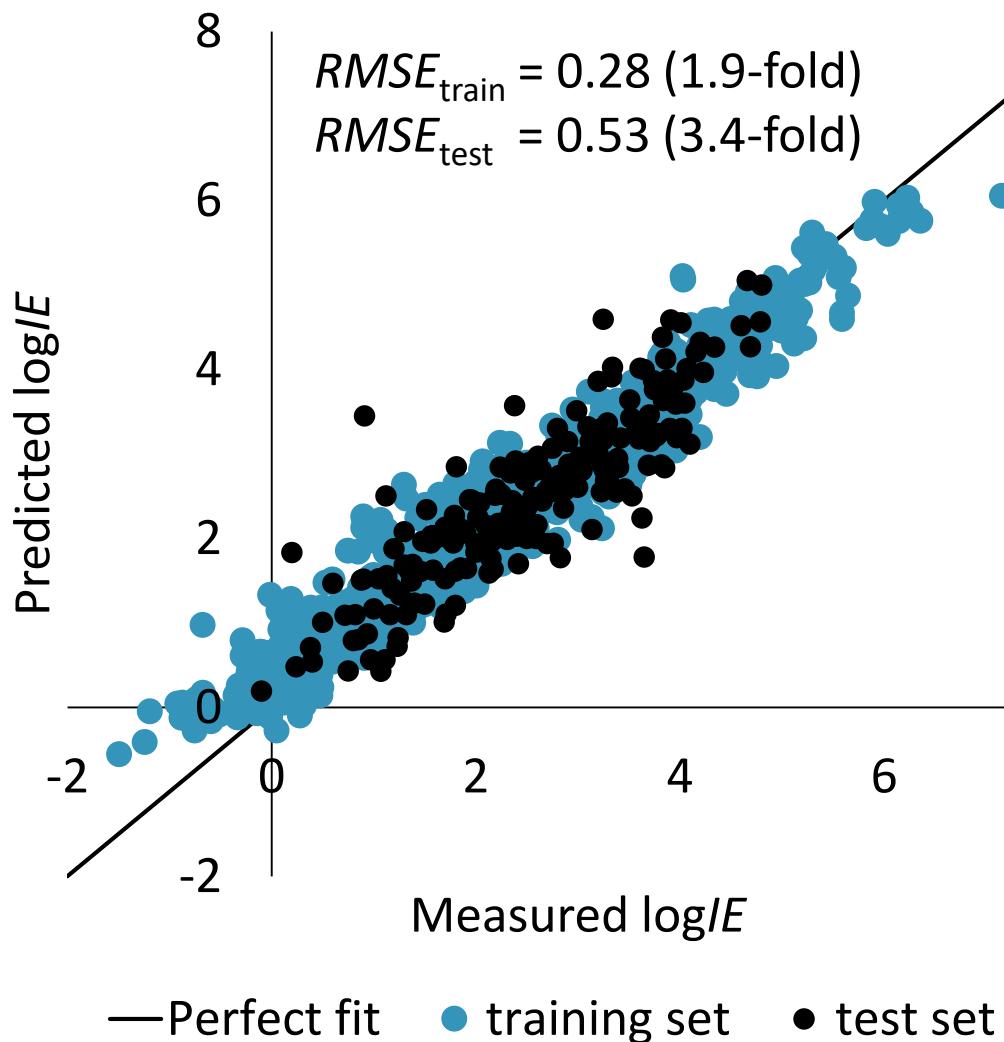
Compound descriptors

Concentration prediction

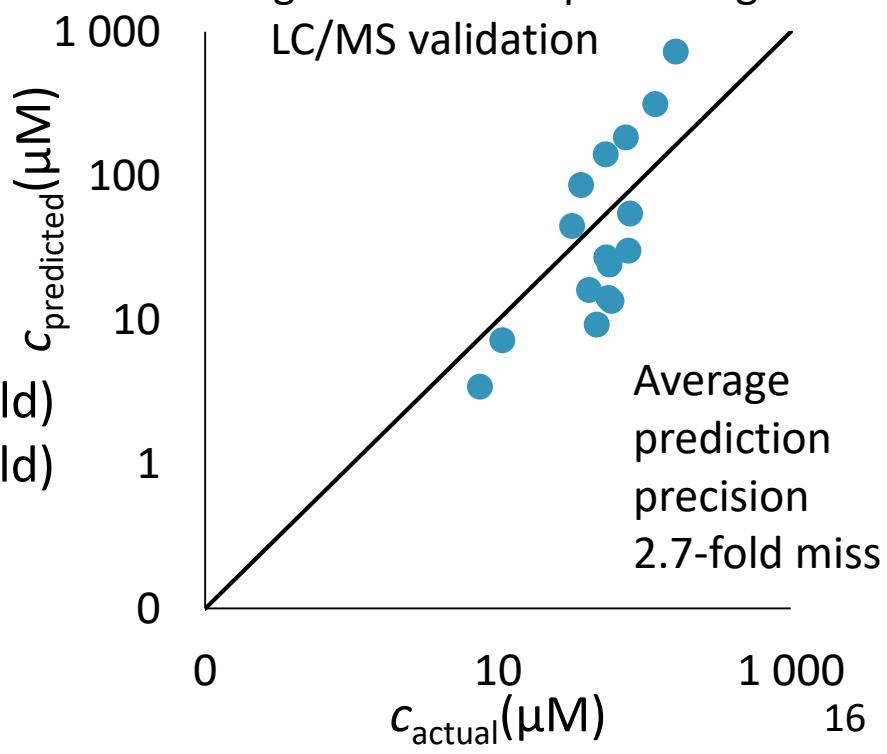
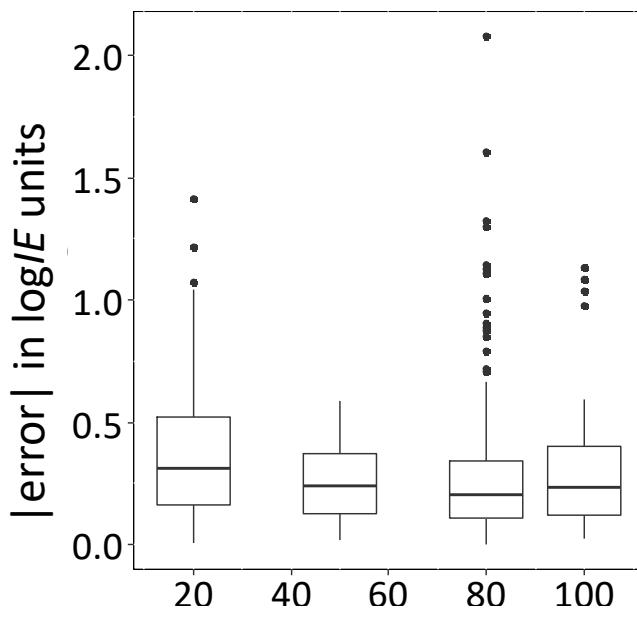
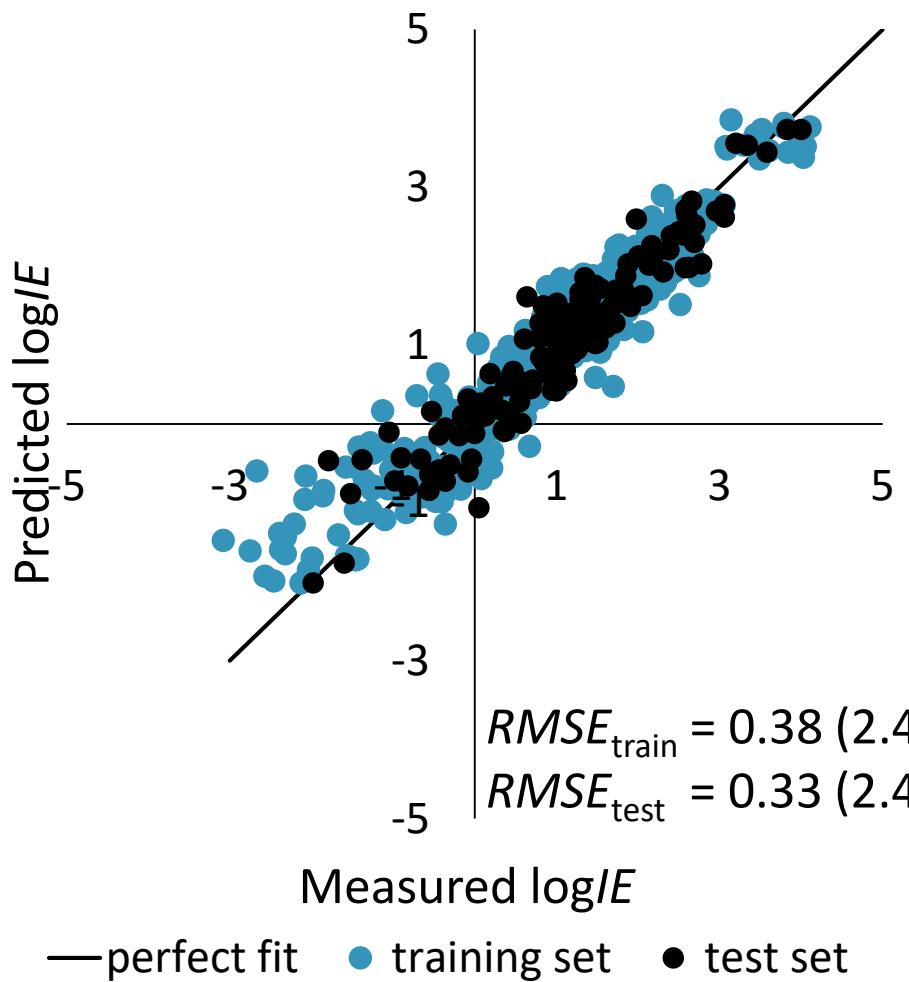
Prediction model



# ESI+ model



# ESI- model



# Achieved milestones

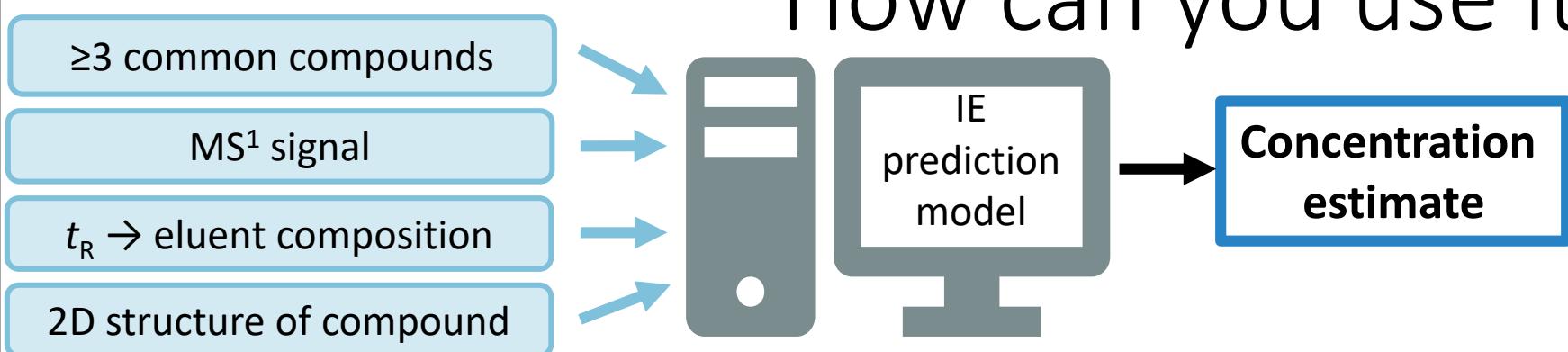
## Approach works!

- ESI+ and ESI-
- different setups
- different mobile phases
- biological matrices
- only 2D structures

# Outlook

- User-friendly software
  - automation
- LC, adducts
- More matrices
- **Self-improving**
  - community contributions

## How can you use it?





# Thank you!

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