EURACHEM 2019 TARTU

QUANTITATIVE NON-TARGETED ANALYSIS

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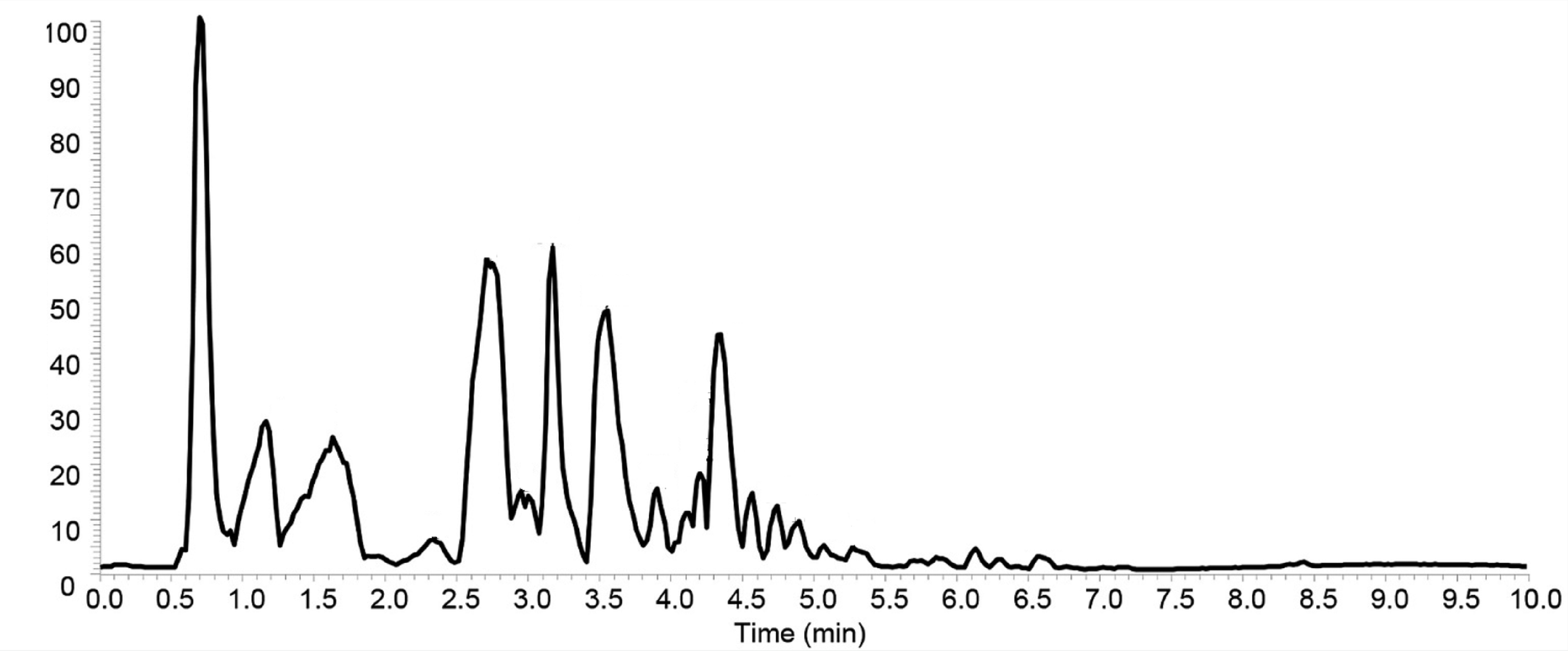
Green tea is one of the most commonly consumed beverages worldwide and is claimed to possess numerous health-protective qualities.

Metabolomic study of green tea

LC/HRMS

aims to correlate changes in the chemical profile of a sample with a corresponding shift in macroscopic phenotype



Non-targeted analysis

LC/ESI/HRMS

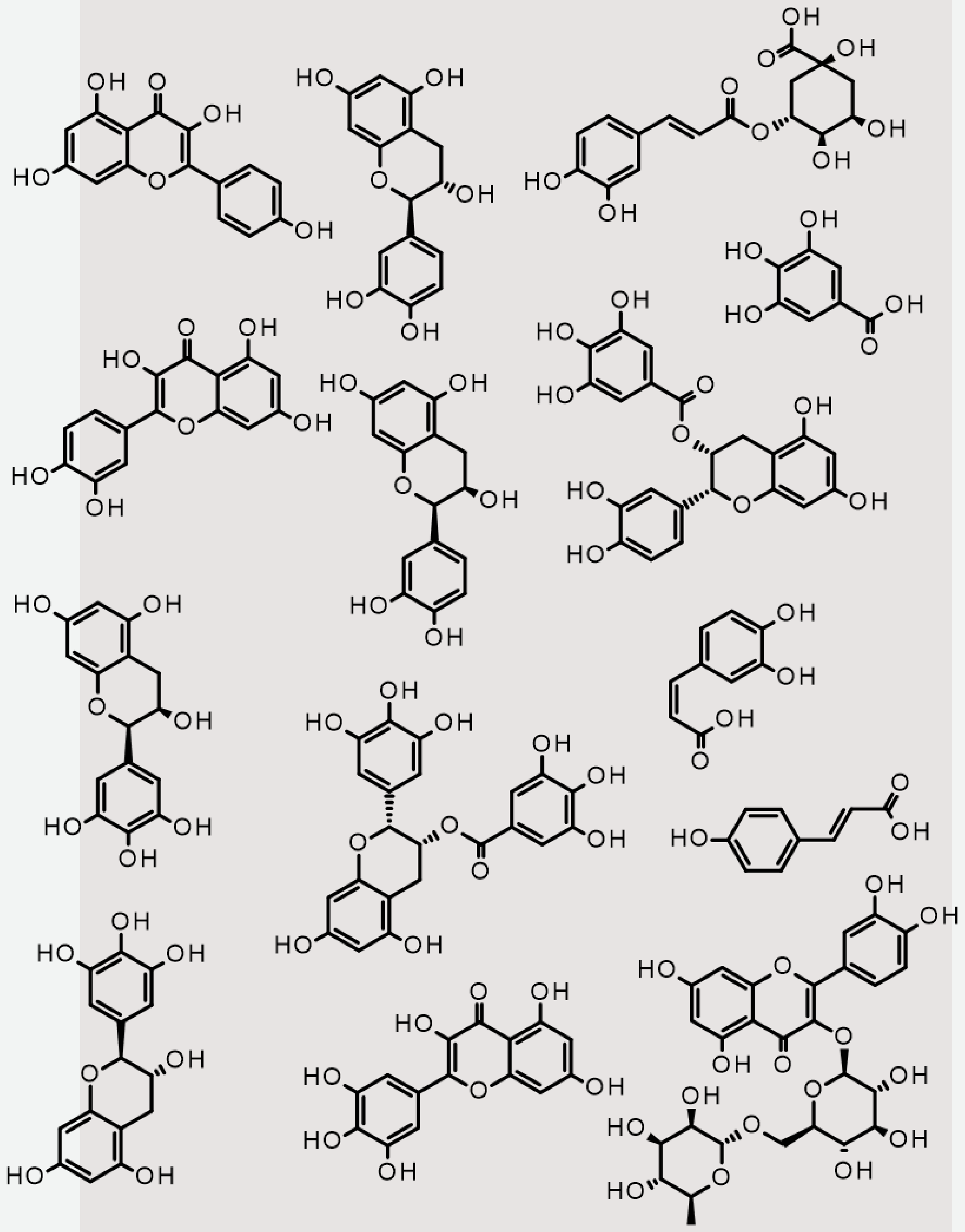
2270 marker ions
38 tea samples
19 identified compounds

QUALITATIVE

stop after identification

QUANTITATIVE

purchase or synthesize the standard substances



Quantification

Preliminary assignment

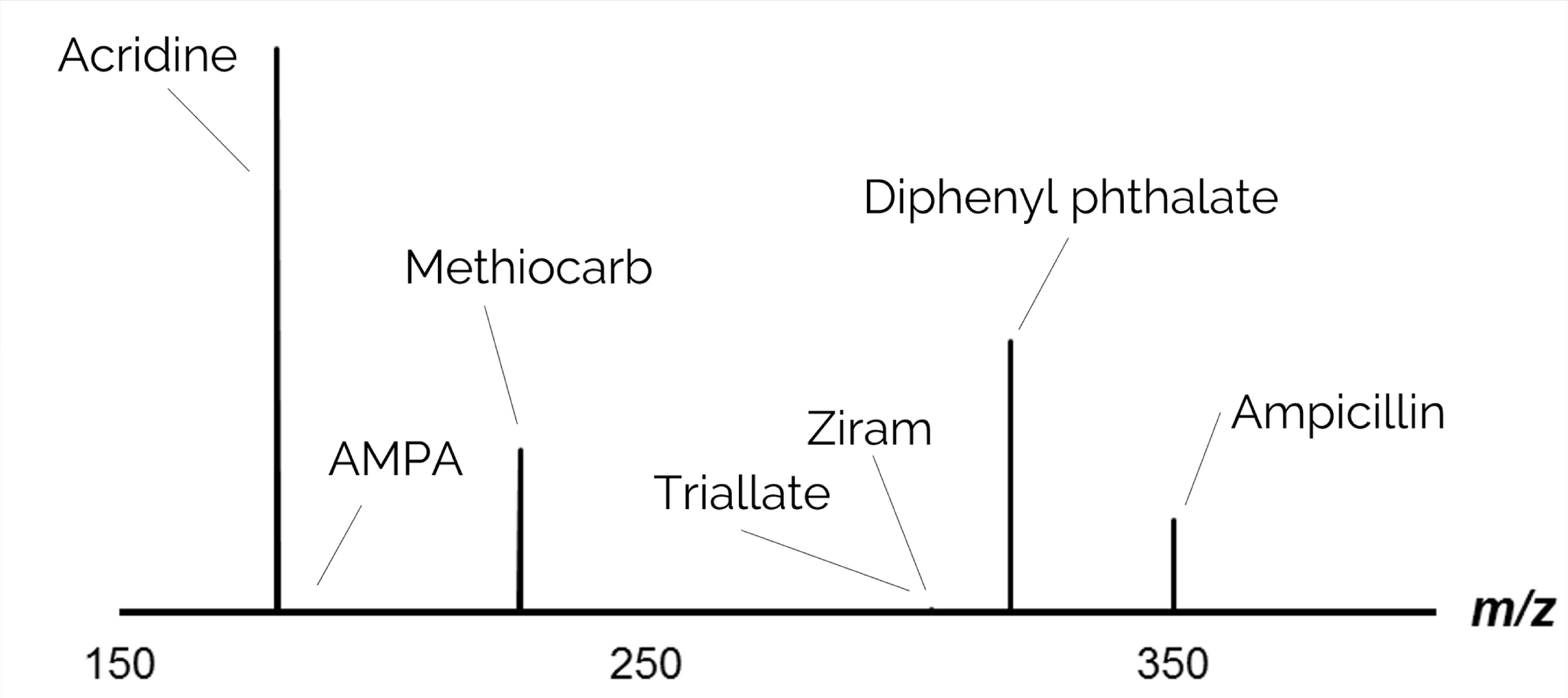
with non-targeted LC/HRMS

Standard substances

were available for 14 compounds

Quantification possible

with calibration graph method



Mixture of seven compounds at equal concentrations.

Climbing the way to quantitative non-targeted analysis

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Ionization efficiency

for ESI source

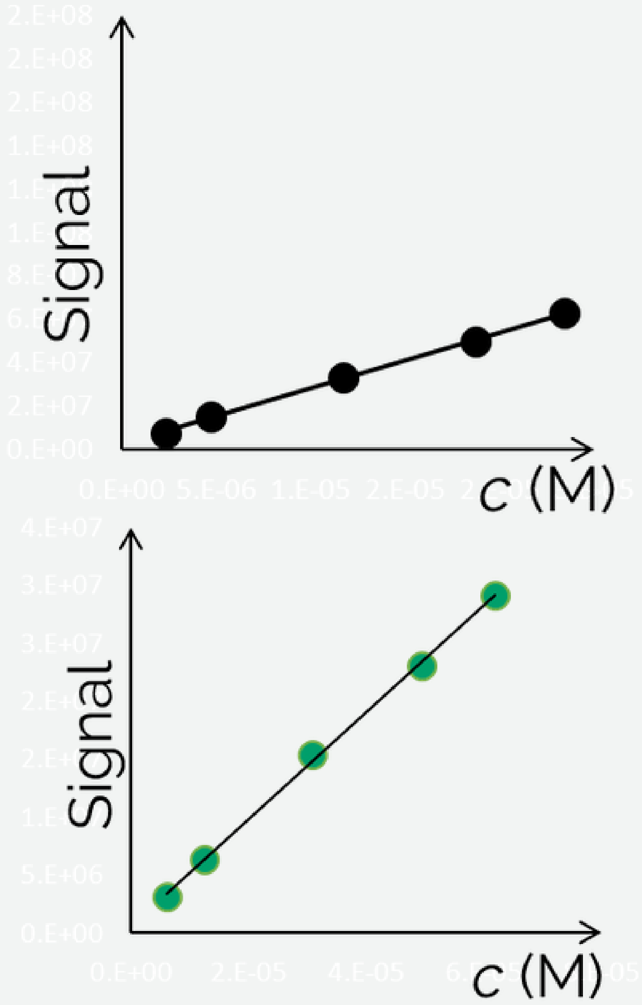
Ionization efficiency

RELATIVE MEASUREMENTS

Flow injections

Calibration graph

Calculation

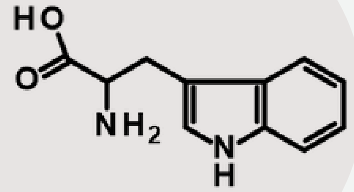


$$\log \frac{slope_1}{slope_2} \rightarrow \log IE$$

Kruve et al. DOI: 10.1021/ac404066v

Ionization efficiency

IS AFFECTED BY



Compound structure

hydrophobicity, basicity/acidity

Eluent

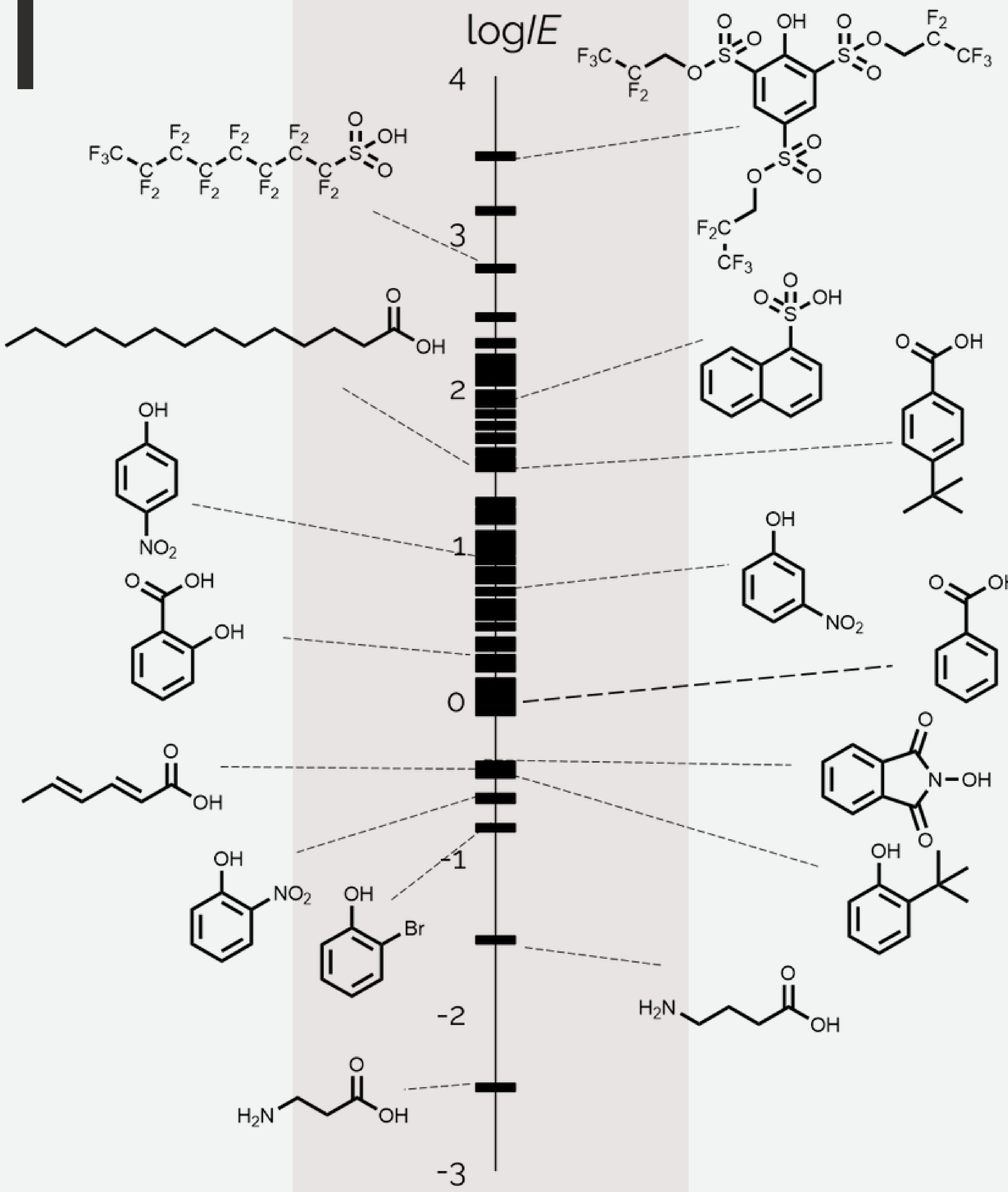
organic modifier %, pH, additive type

Instrument

source design, mass analyzer

Matrix

ionization suppression/enhancement



Kruve et al. DOI: 10.1021/ac404066v

Structure

Hydrophobicity

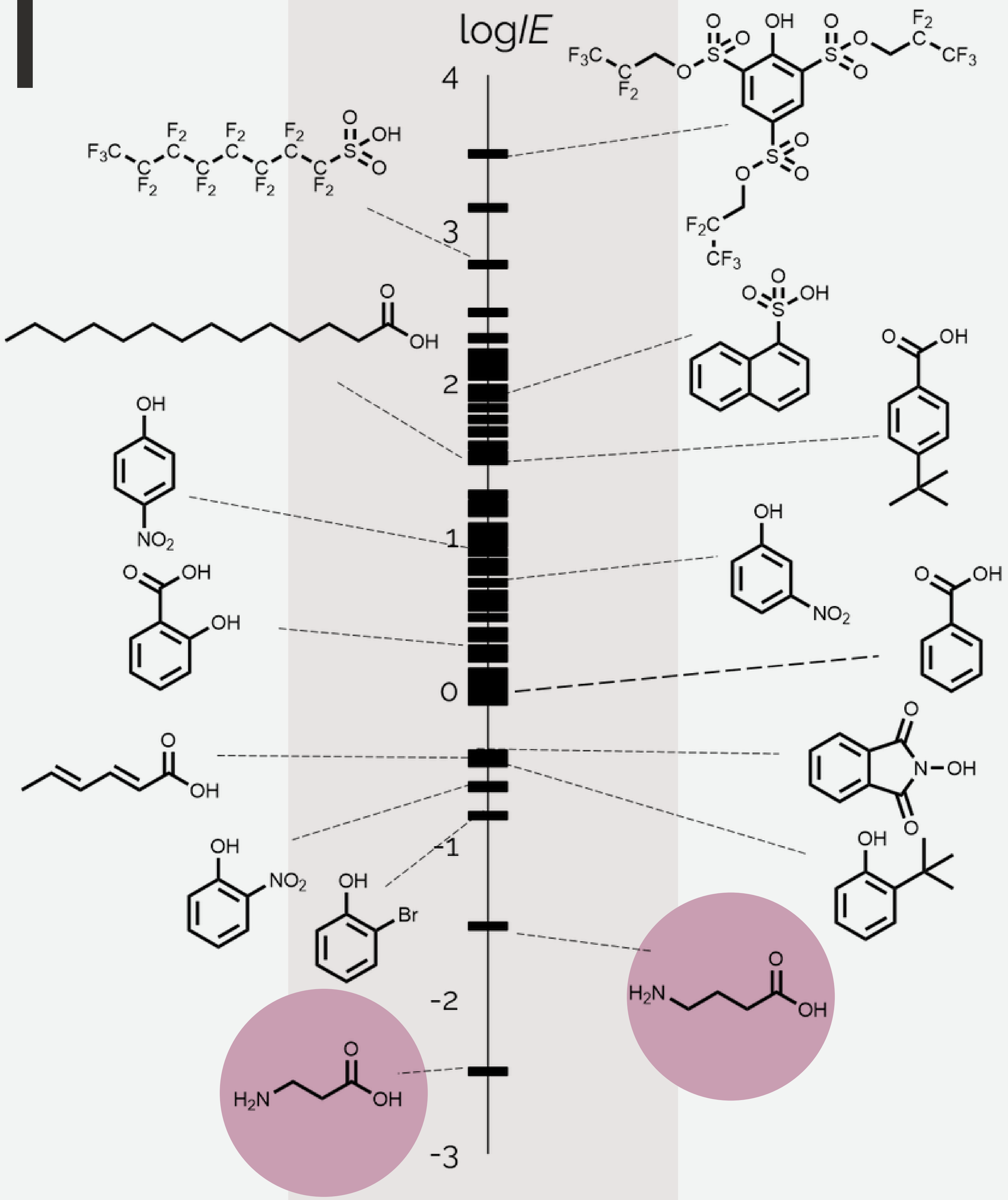
affinity for the droplets surface

Acidity/Basicity

protonation/deprotonation in the solution

Interplay between properties

no single ESI mechanism



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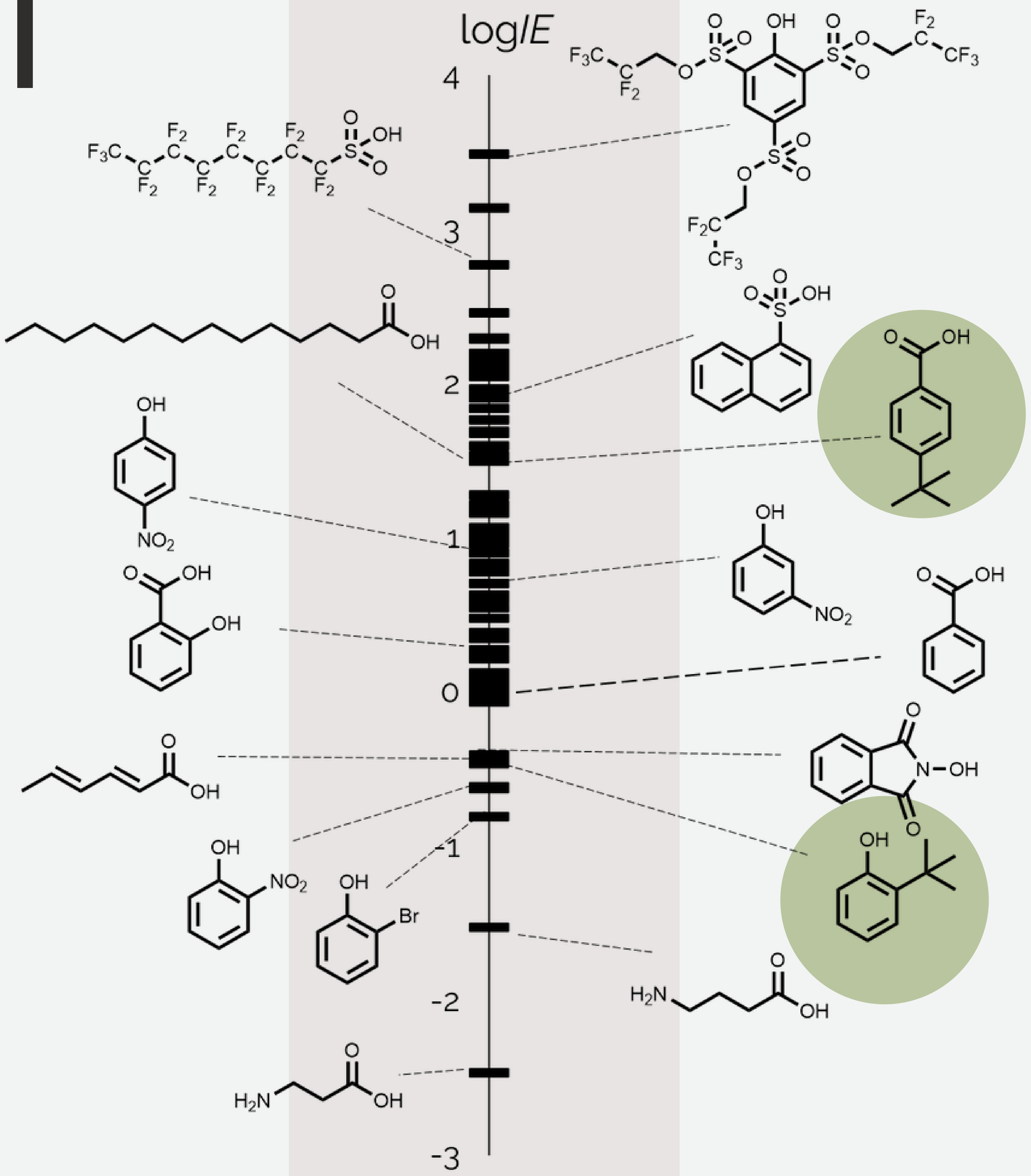

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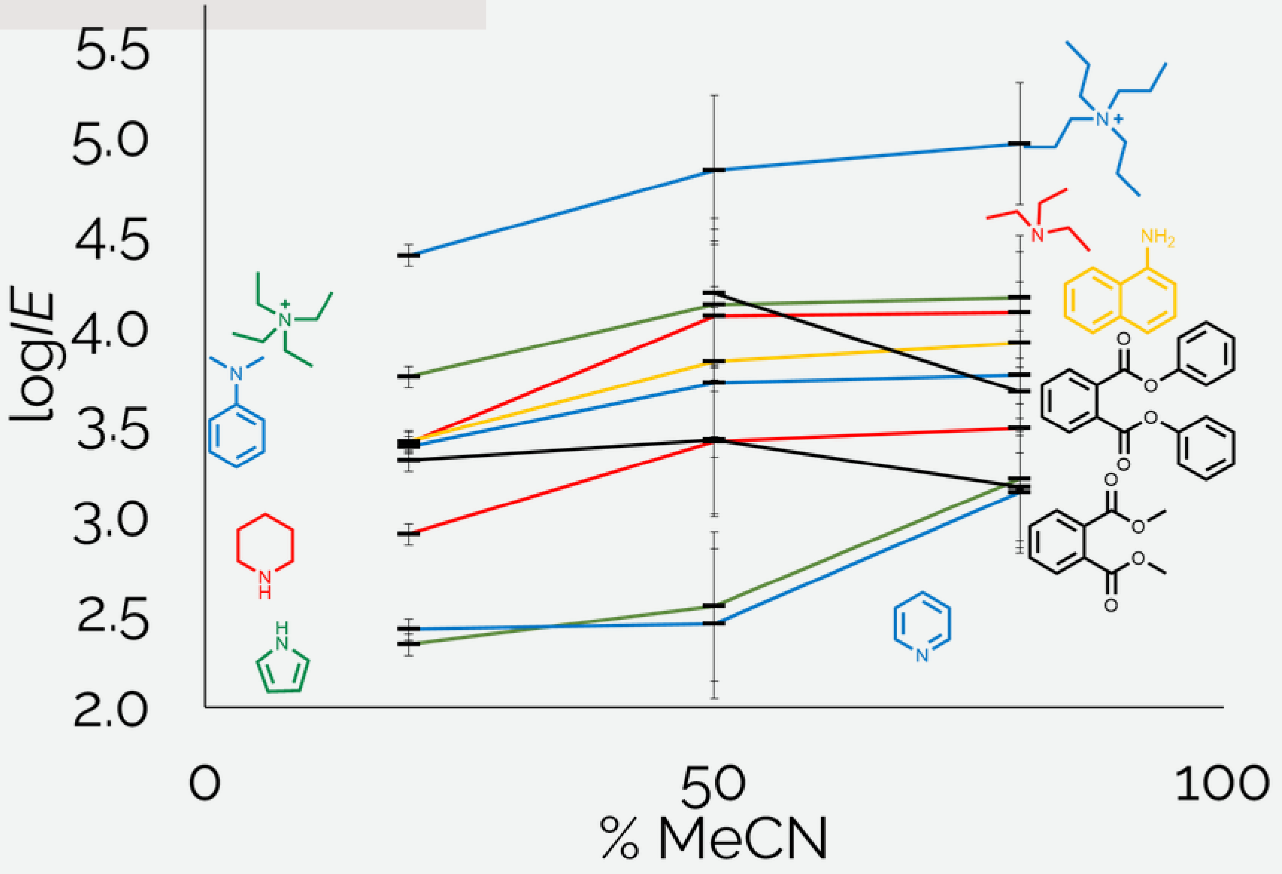
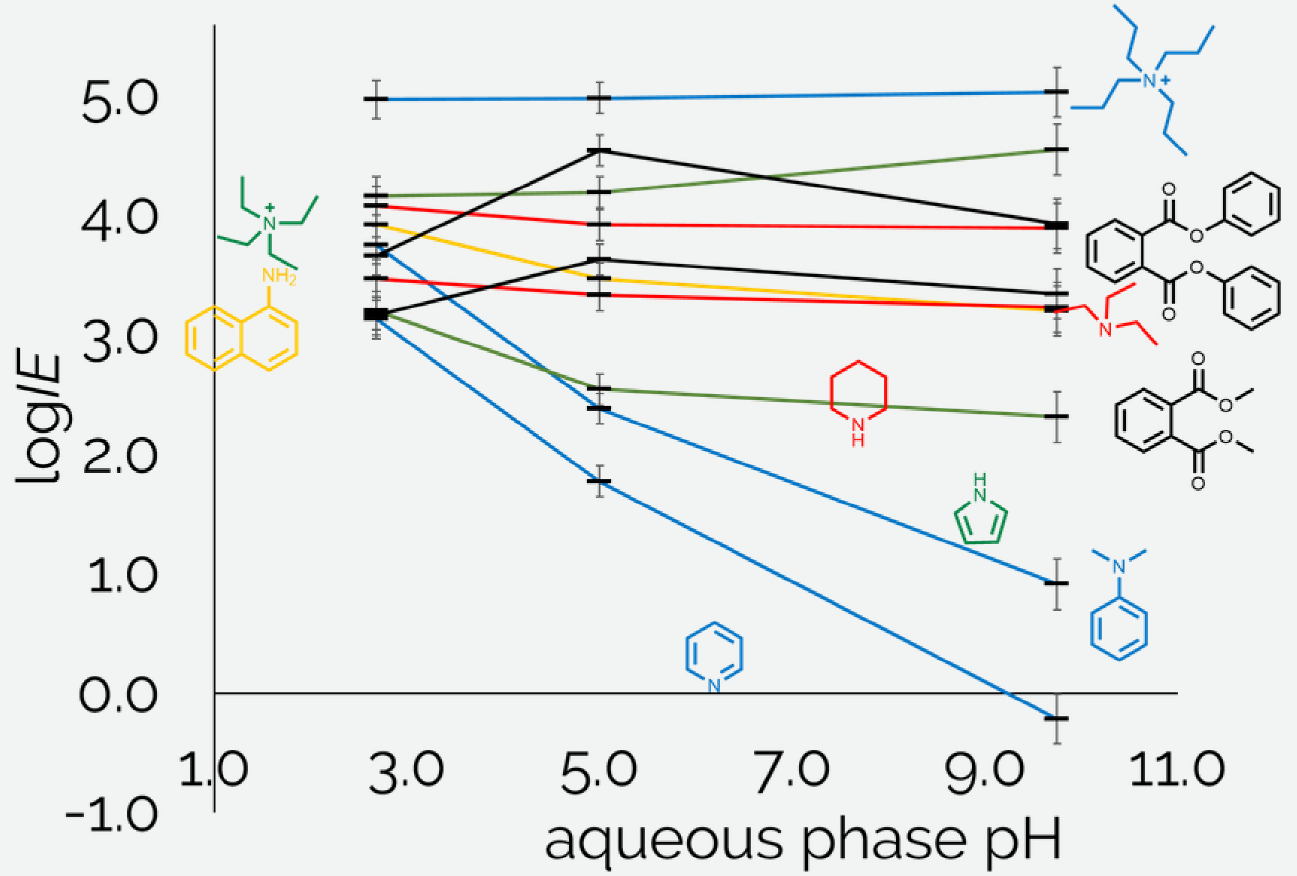
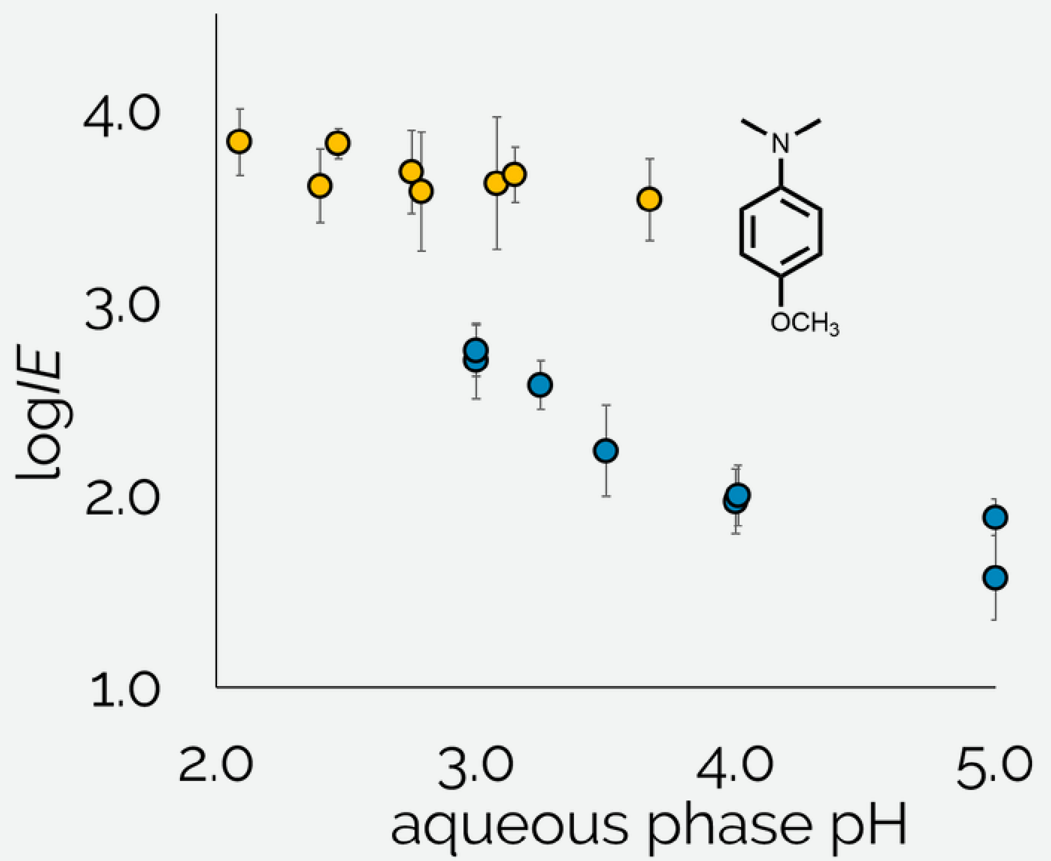






Organic modifier

higher organic modifier content increases ionization efficiency by >10x

pH

change in ionization efficiency >1000x

Additive type

same pH, different buffer: 20 to 50x different ionization efficiencies

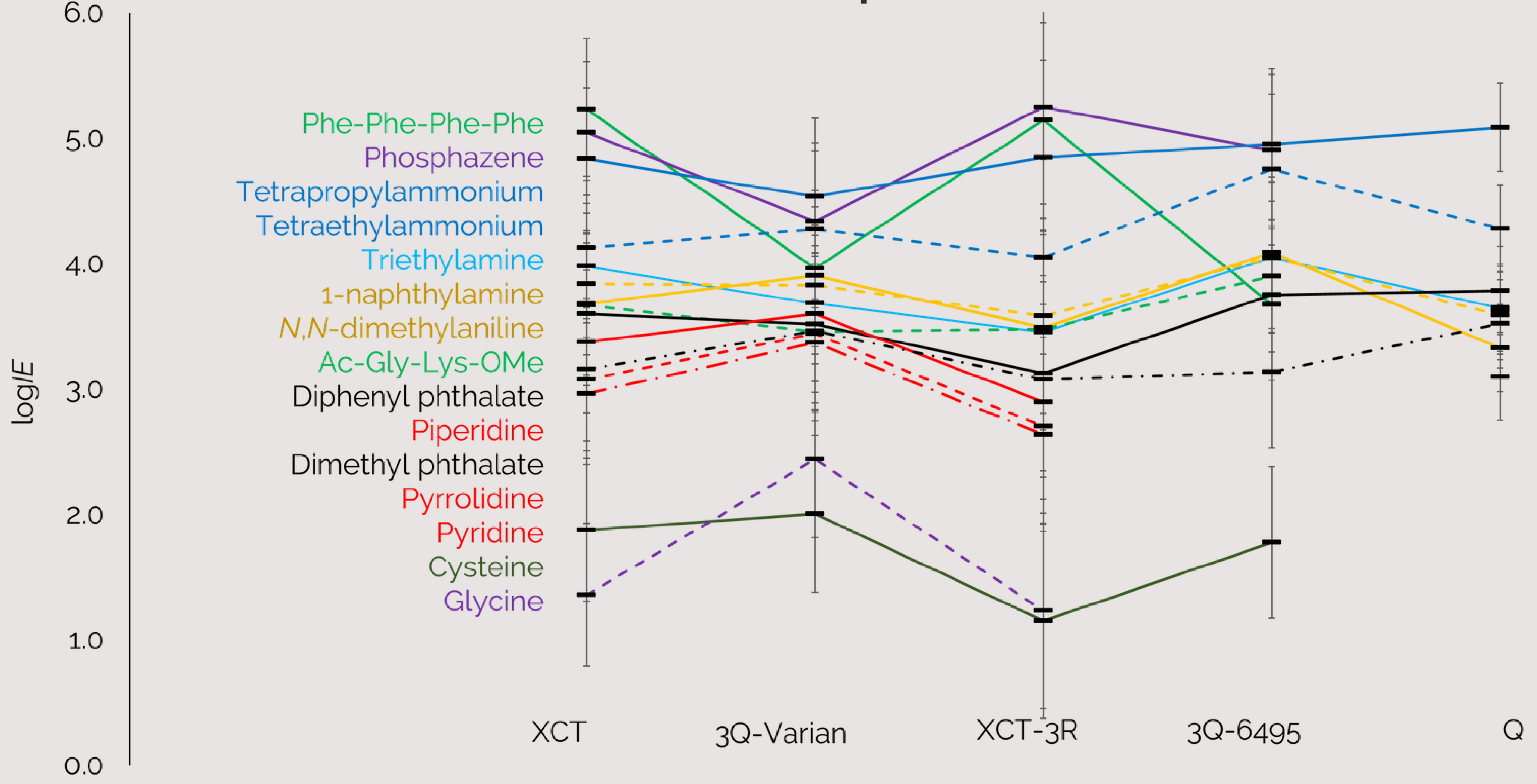
Liigand et al. DOI: 10.1007/s13361-014-0969-x
Liignad et al. DOI: 10.1007/s13361-016-1563-1
Ojakivi et al. DOI: 10.1002/slct.201702269.

DIFFERENT INSTRUMENTS

7 different instruments
5 vendors
4 countries

IONIZATION EFFICIENCY

order of compounds is similar
good correlation

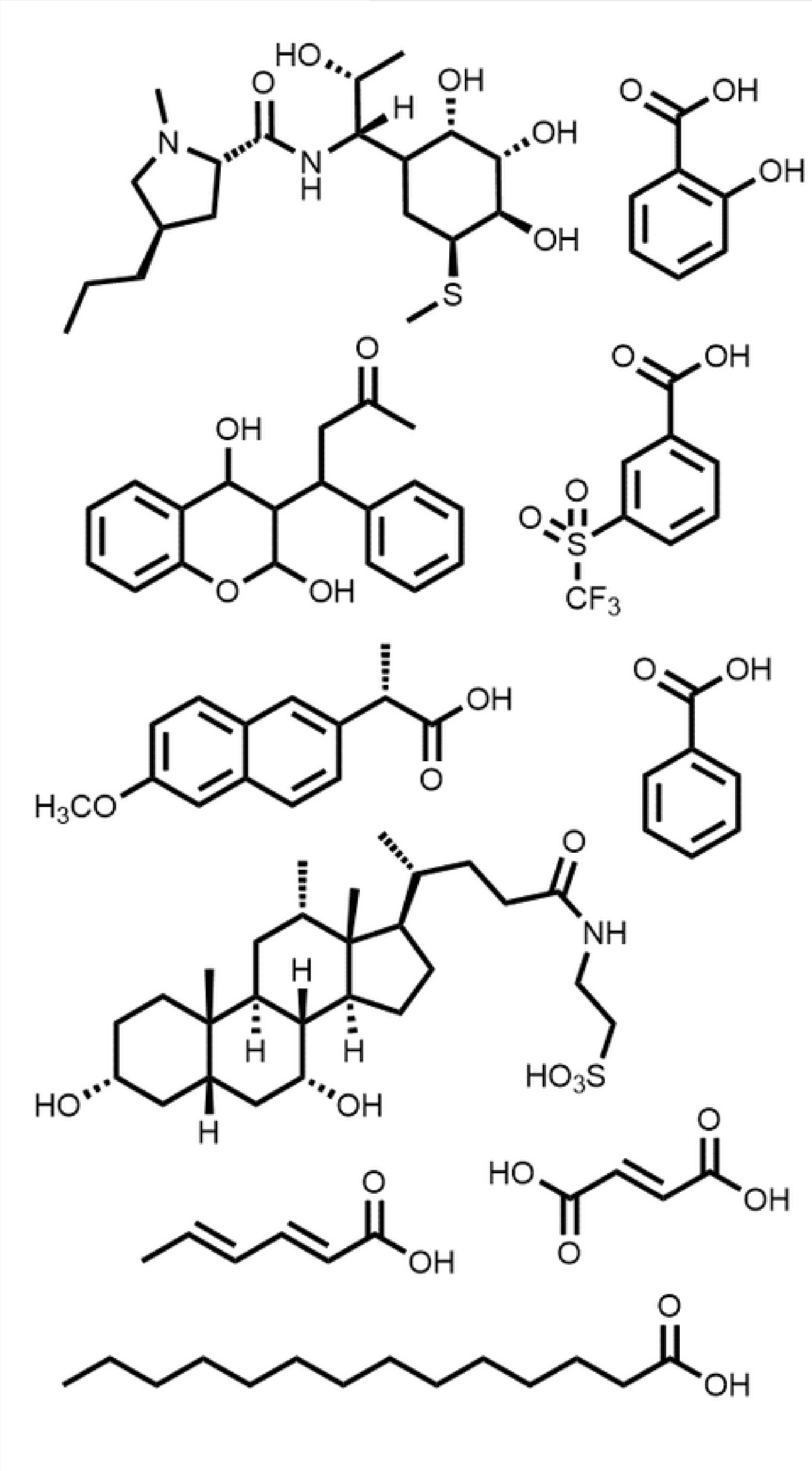


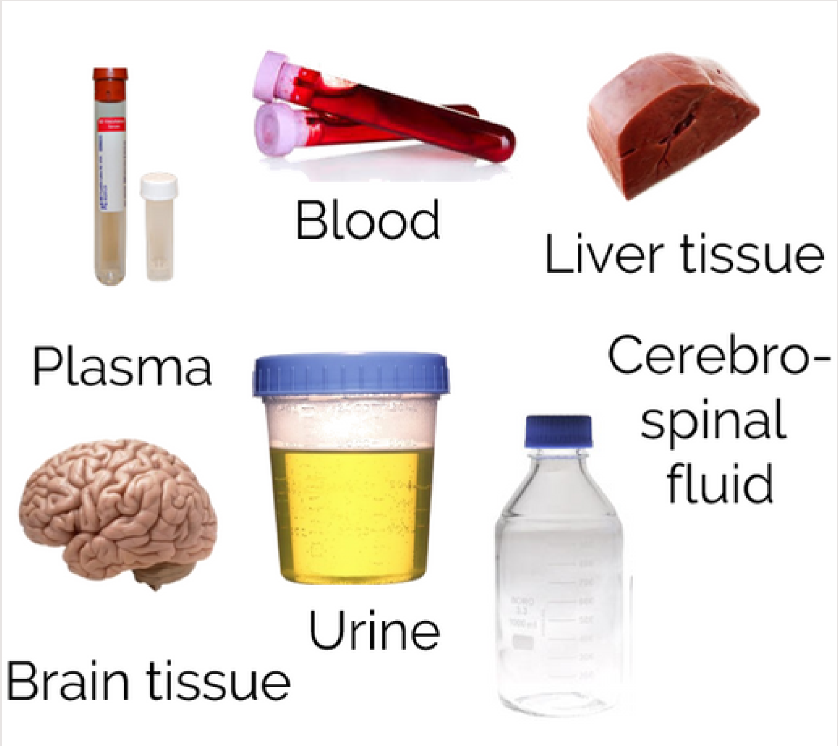
Liigand et al. DOI: 10.1007/s13361-015-1219-6

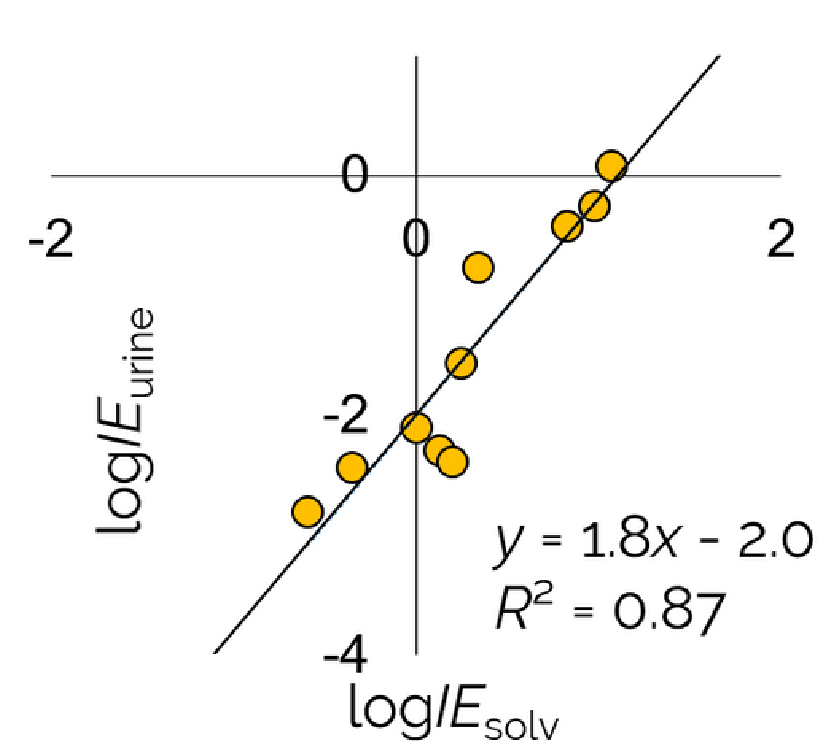
Matrix effect in ESI

BIOLOGICAL SAMPLES

order of compounds is the same
good correlation







Liignad et al. DOI: 10.1016/j.aca.2018.05.072

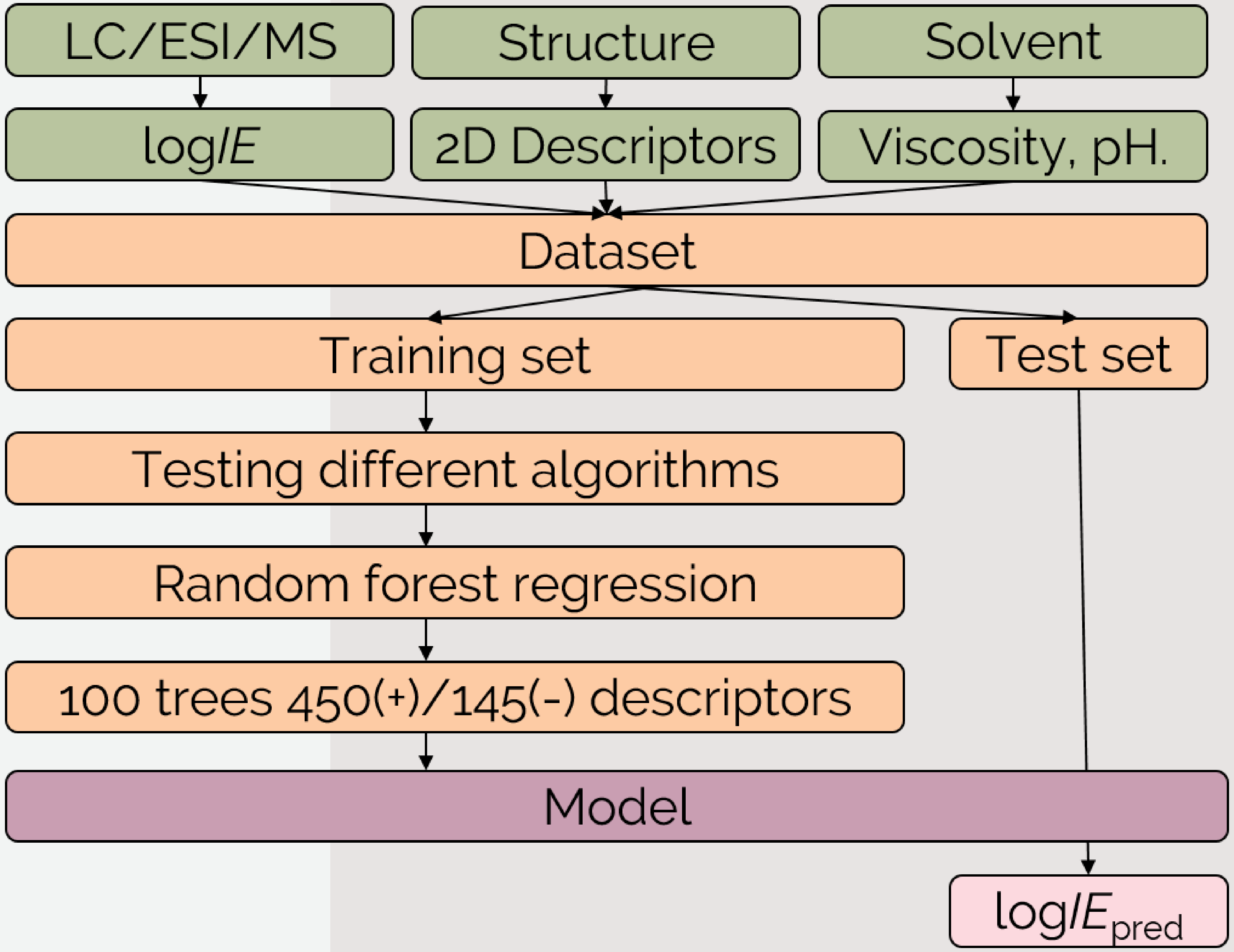
Modelling

bringing previous knowledge together

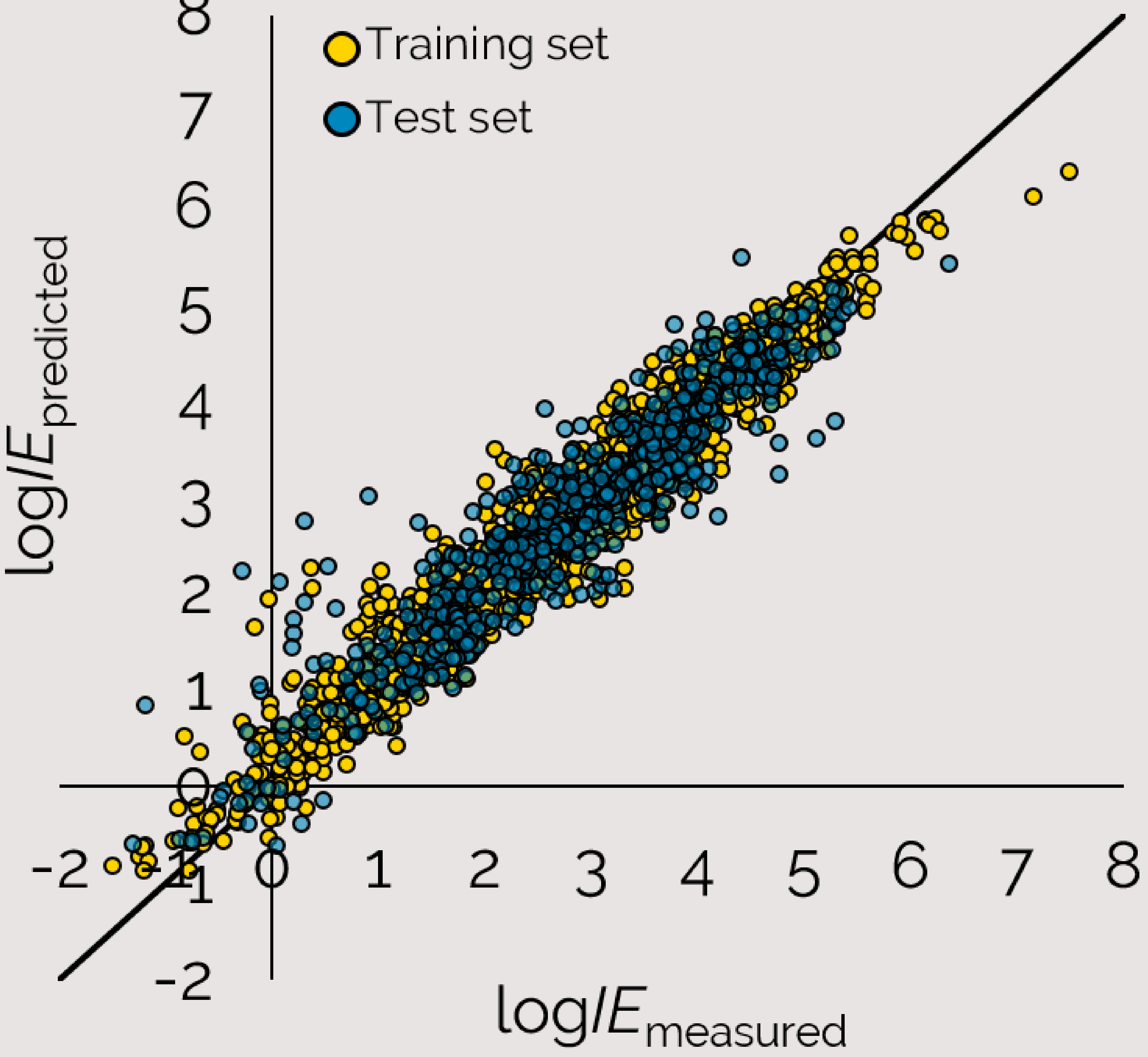
Modelling of ionization efficiency

TRAINING & VALIDATION

different machine learning approaches tested



Liignad et al. submitted for publication



Liignad et al. submitted for publication

ESI+

3139 data points
353 small molecules

106 eluent compositions
MeCN/MeOH 0 – 100%
pH = 2.0 – 10.7

Average prediction error
Training set 1.9x
Test set 3.0x

ESI-

1286 data points

101 small molecules

33 eluent compositions

MeCN/MeOH 0 – 100%

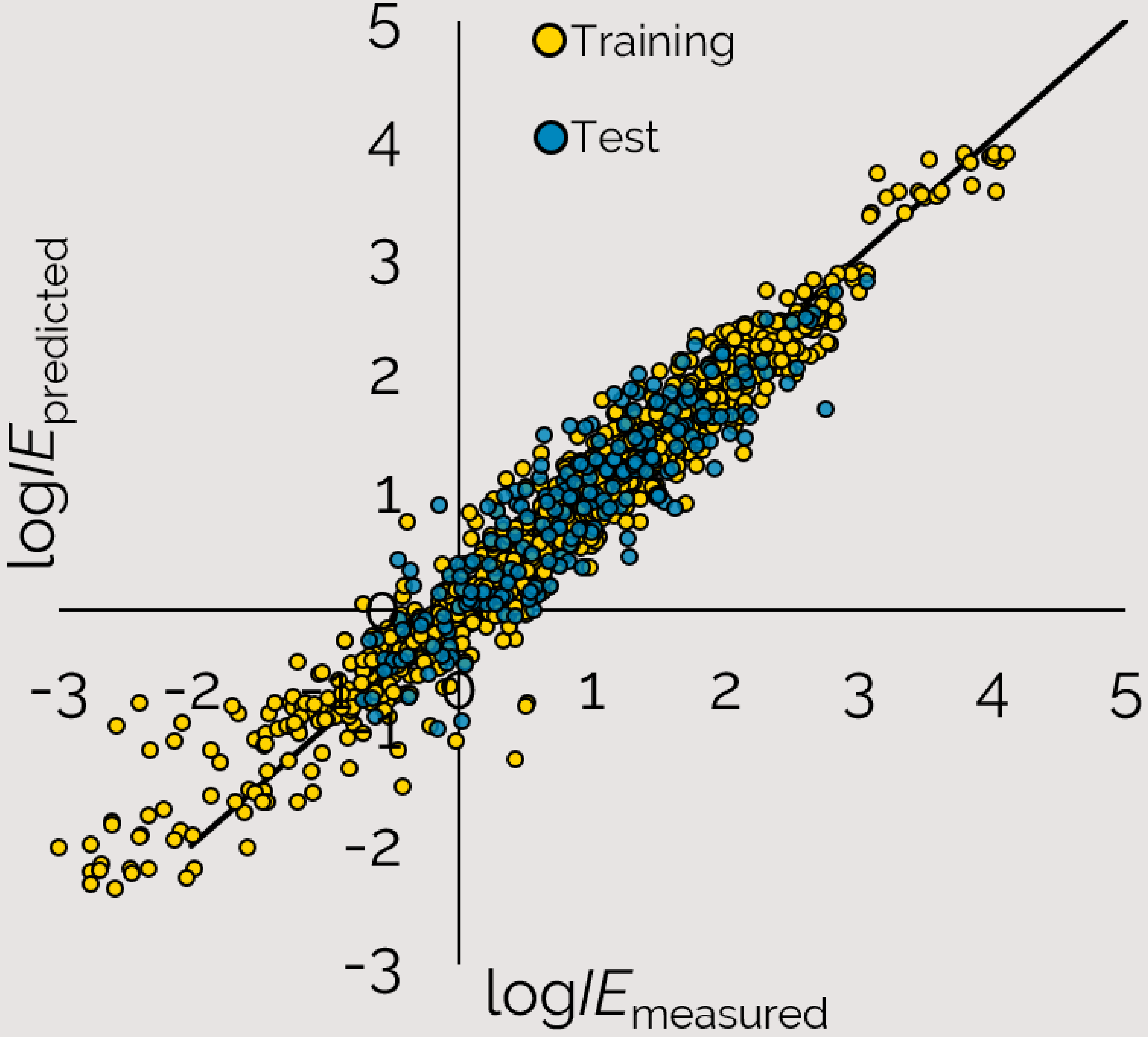
pH = 2.0 – 10.7

Average prediction error

Training set 2.0x

Test set 2.3x

Liignad et al. submitted for publication



Concentrations

application for green tea

How to use it for quantification?

Step 1

predict ionization efficiencies

Step 2

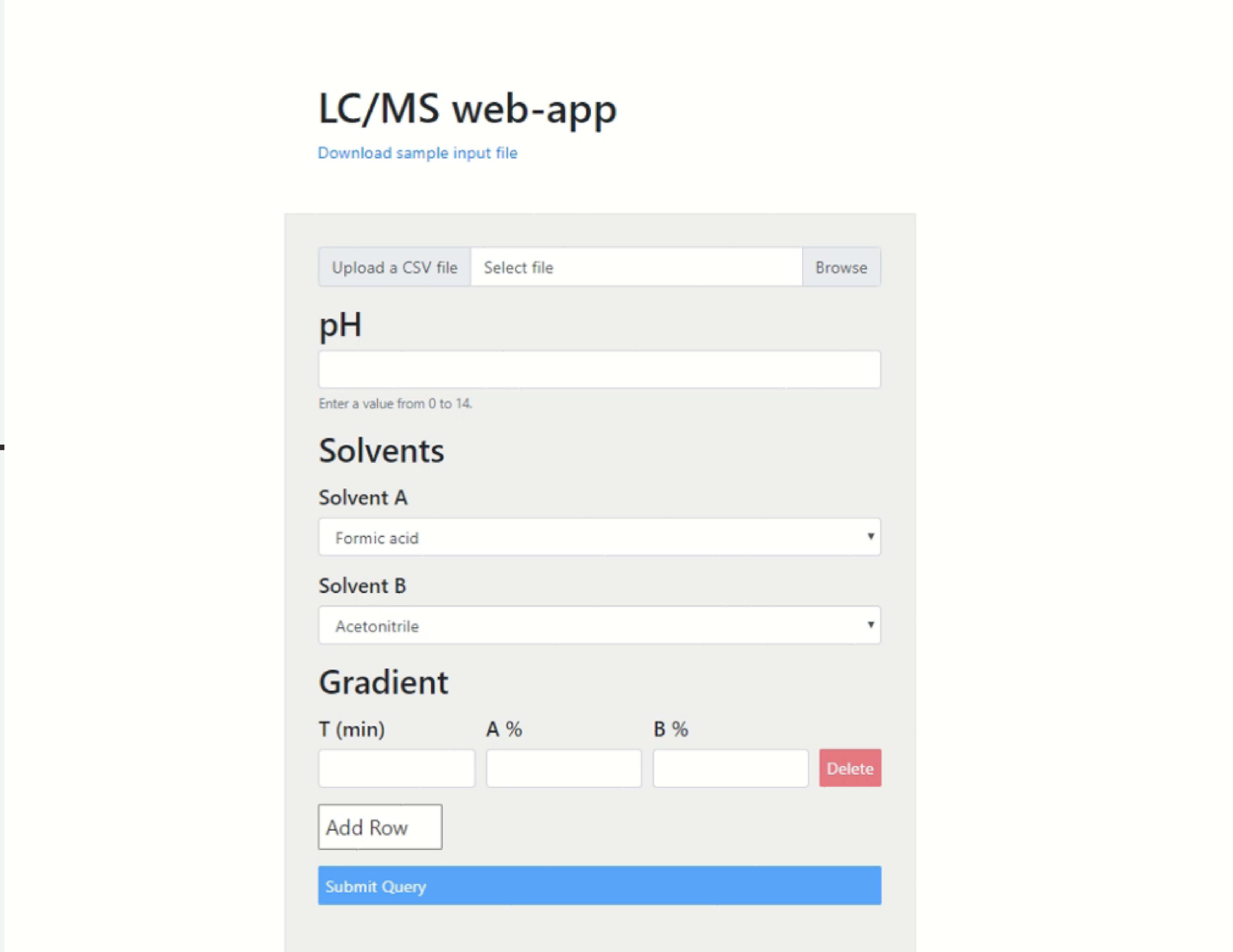
transfer to specific setup

Step 3

calculate

How does it work in practice?

(1) SMILES for identified compounds
(2) 5-6 compounds with known concentration
(3) gradient parameters
READY...SET...GO



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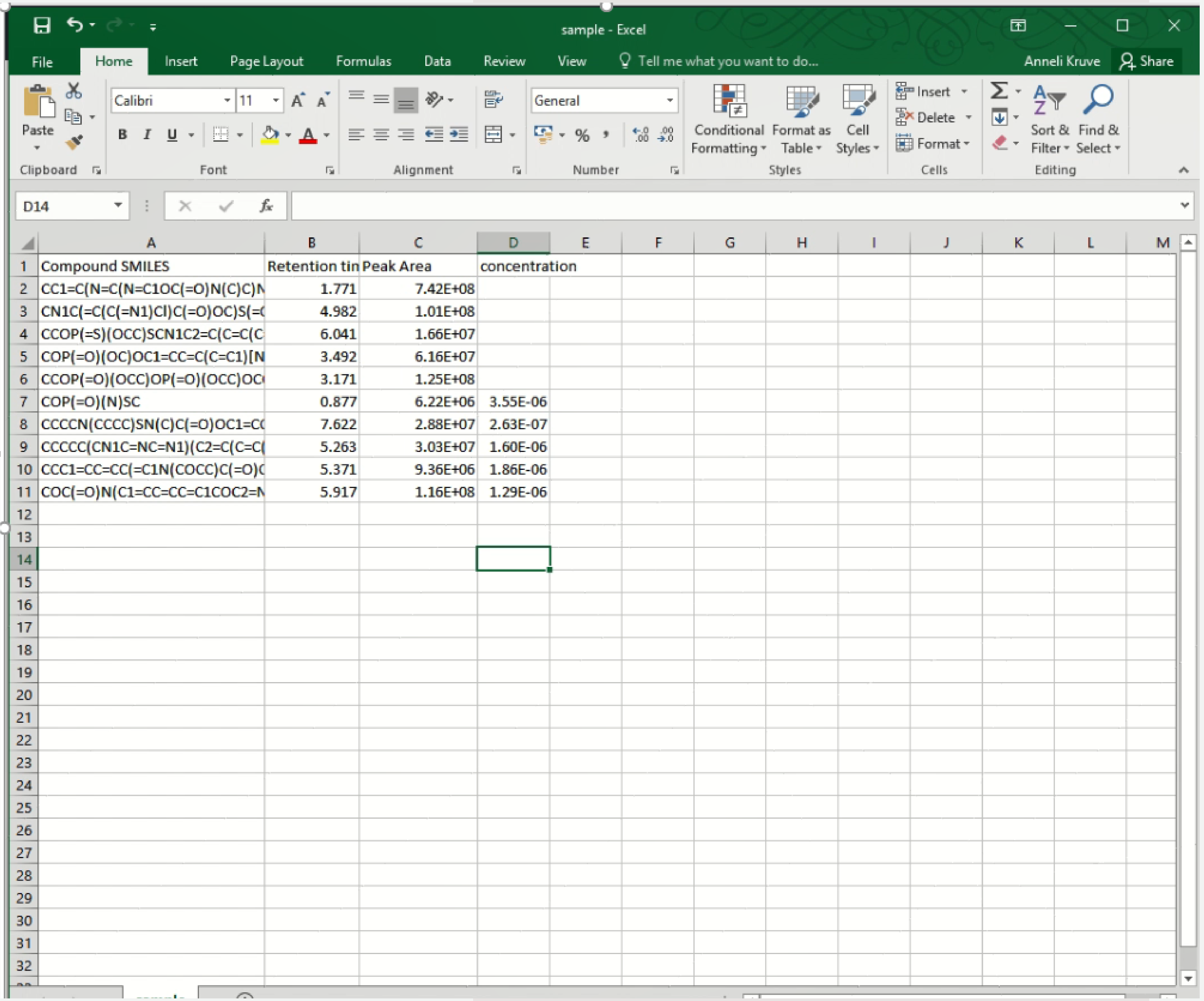

	A	B	C	D
1	Compound SMILES	Retention tin	Peak Area	concentration
2	CC1=C(N=C(N=C1OC(=O)N(C)C)N	1.771	7.42E+08	
3	CN1C(=C(C(=N1)Cl)C(=O)OC)S(=(	4.982	1.01E+08	
4	CCOP(=S)(OCC)SCN1C2=C(C=C(C	6.041	1.66E+07	
5	COP(=O)(OC)OC1=CC=C(C=C1)[N	3.492	6.16E+07	
6	CCOP(=O)(OCC)OP(=O)(OCC)OC	3.171	1.25E+08	
7	COP(=O)(N)SC	0.877	6.22E+06	3.55E-06
8	CCCCN(CCCC)SN(C)C(=O)OC1=C(	7.622	2.88E+07	2.63E-07
9	CCCCC(CN1C=NC=N1)(C2=C(C=C(	5.263	3.03E+07	1.60E-06
10	CCC1=CC=CC(=C1N(COCC)C(=O)C	5.371	9.36E+06	1.86E-06
11	COC(=O)N(C1=CC=CC=C1COC2=N	5.917	1.16E+08	1.29E-06

(1) SMILES for identified compounds

(2) 5-6 compounds with known concentration

(3) gradient parameters

READY...SET...GO

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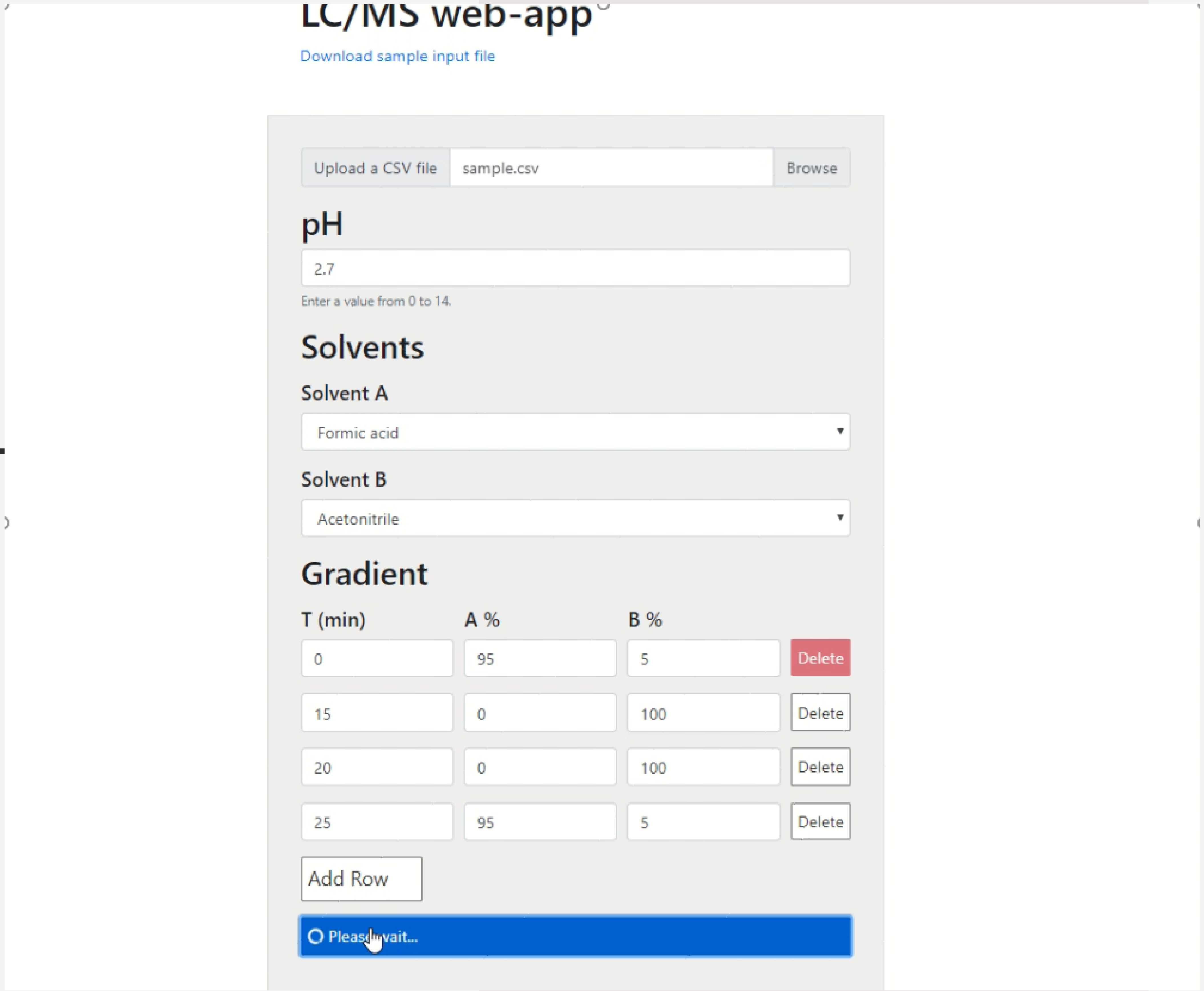

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(1) SMILES for identified compounds
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READY...SET...GO

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Results

Download results

Substance	Concentration
CC1=C(N=C(N=C1OC(=O)N(C)C)NC)C	4.39e-05
CN1C(=C(C(=N1)Cl)C(=O)OC)S(=O)(=O)NC(=O)NC2=NC(=CC(=N2)OC)OC	1.83e-05
CCOP(=S)(OCC)SCN1C2=C(C=C(C=C2)Cl)OC1=O	2.64e-06
COP(=O)(OC)OC1=CC=C(C=C1)[N+](=O)[O-]	2.36e-05
CCOP(=O)(OCC)OP(=O)(OCC)OCC	1.7e-05
COP(=O)(N)SC	3.55e-06
CCCCN(CCCC)SN(C)C(=O)OC1=CC=CC2=C1OC(C2)(C)C	2.63e-07
CCCCC(CN1C=NC=N1)(C2=C(C=C(C=C2)Cl)Cl)O	1.6e-06
CCC1=CC=CC(=C1N(COCC)C(=O)CCl)C	1.86e-06
COC(=O)N(C1=CC=CC=C1COC2=NN(C=C2)C3=CC=C(C=C3)Cl)OC	1.29e-06

Prediction for green tea

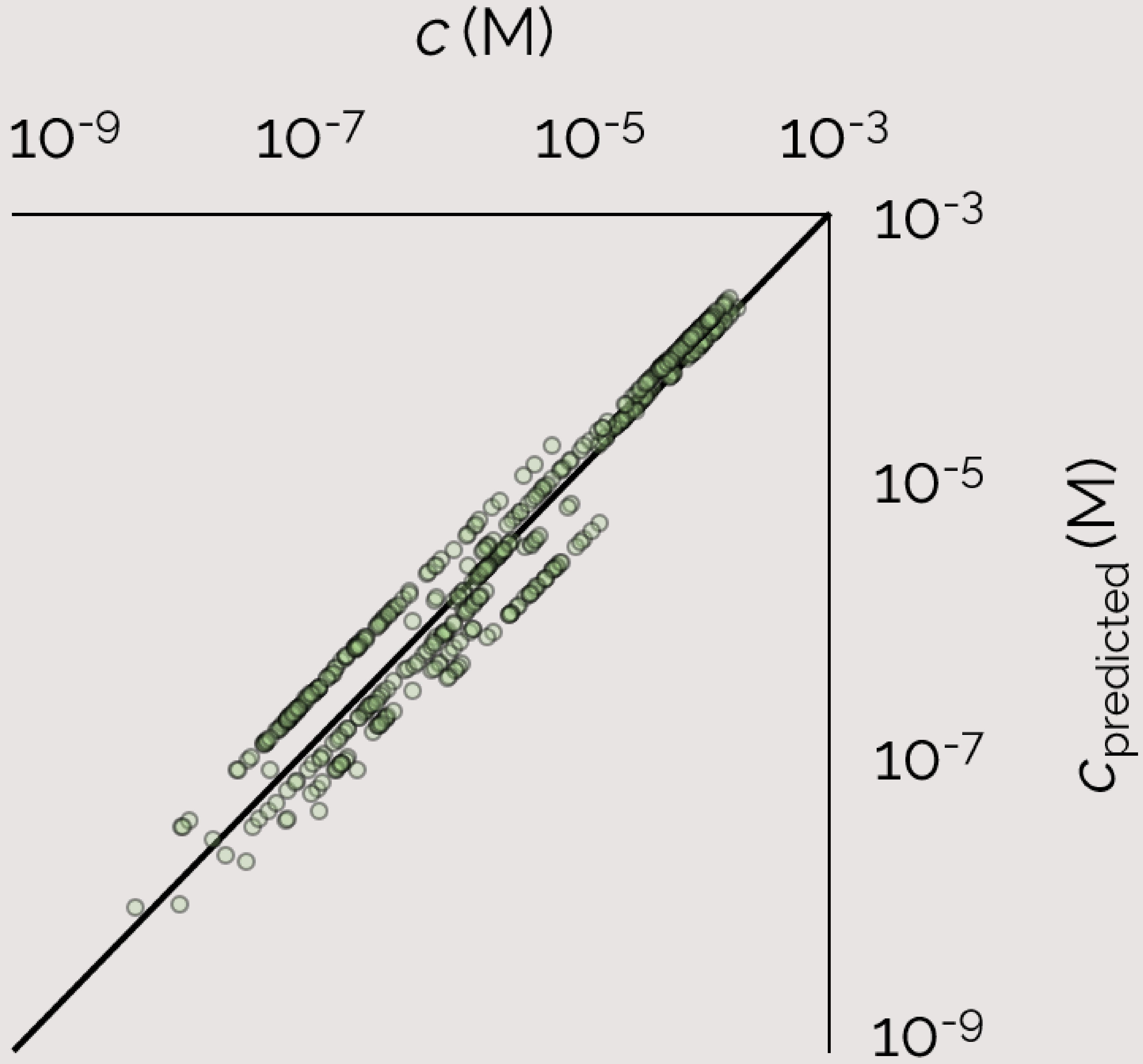
Measurements

UNCG (USA) in 2017

Calculations

UT (Estonia) in late 2018

Liignad et al. submitted for publication

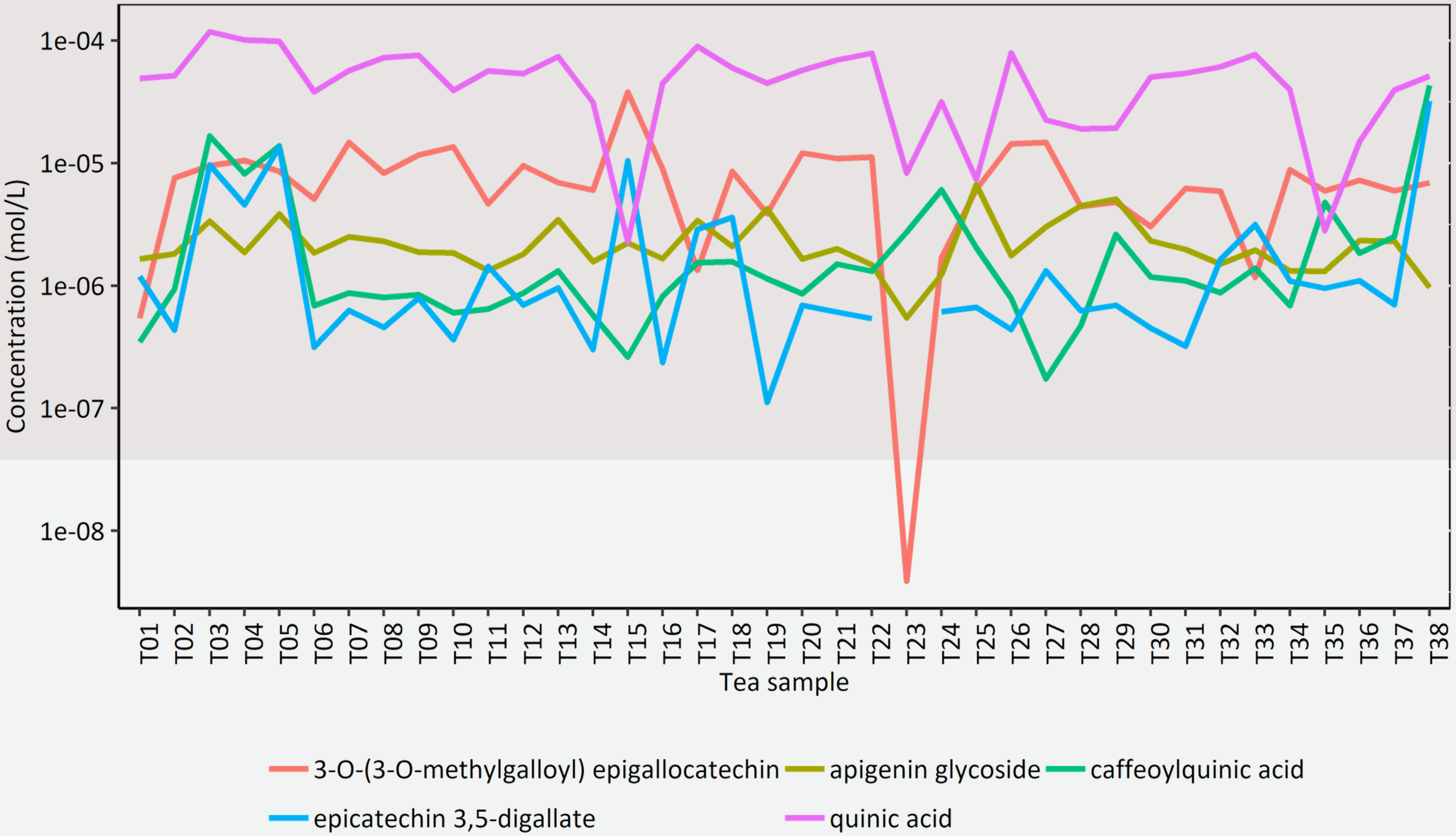


The average prediction error was

1.7X

predicted concentration of quercetin 0.8 µmol/L the confidence interval 0.5 to 1.4 µmol/L

Can the predicted concentrations reveal anything about different tea samples?



Indeed, we can identify the non-green tea samples!

Pesticides in cereals

More than 1000 pesticides and mycotoxins registered in EU.

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Cereal samples

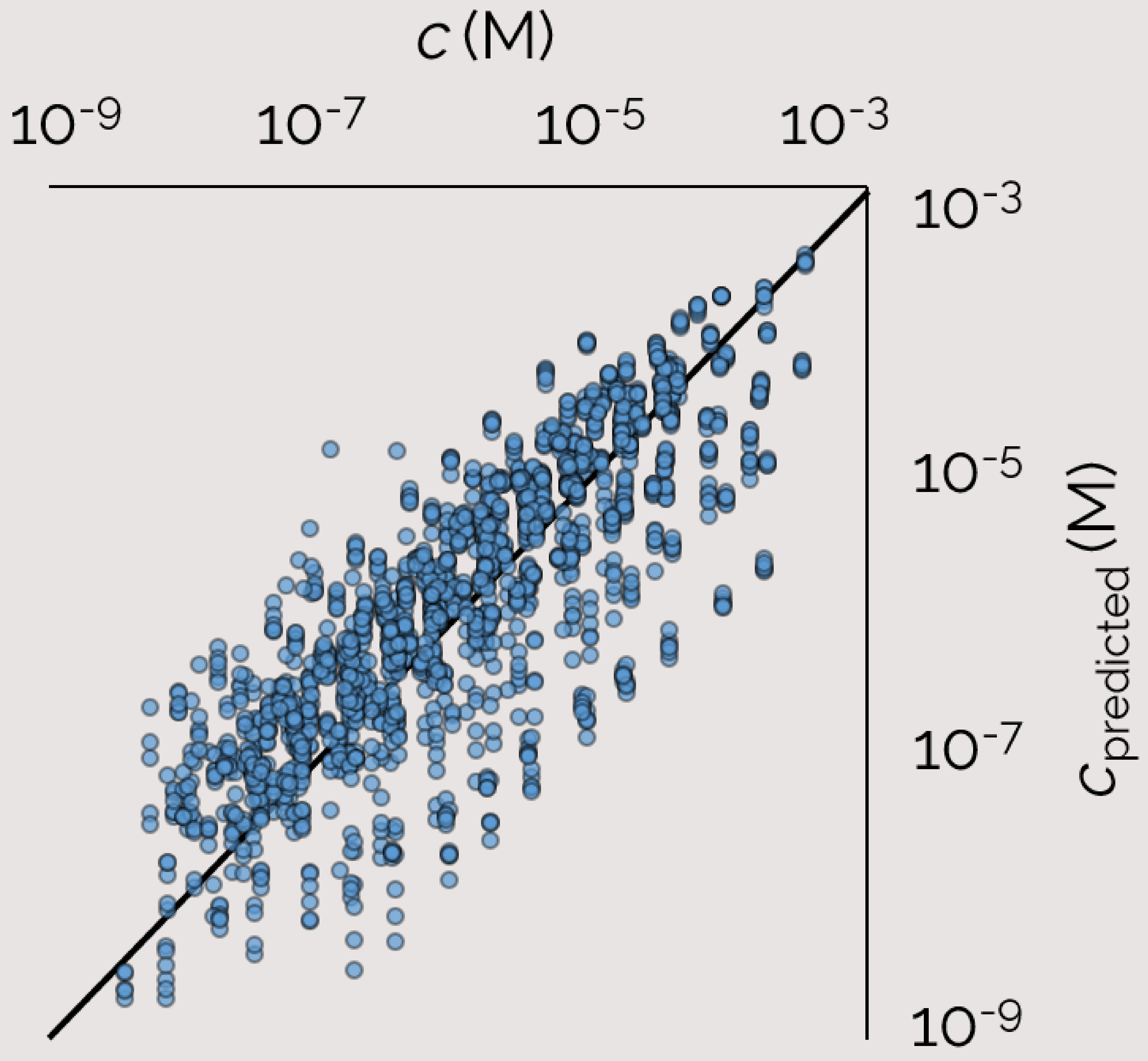
PESTICIDES AND MYCOTOXINS

35 compounds & 6 transformation compounds
3.6 nM to 0.35 mM
oat, barley, rye, wheat, rice, maize

Cereal samples

PESTICIDES AND MYCOTOXINS

2233 data points
average prediction error 5x.



Wang et al. submitted for publication

7 out of 10

prediction error <5x.

9 out of 10

prediction error <10x.

What can we see now?

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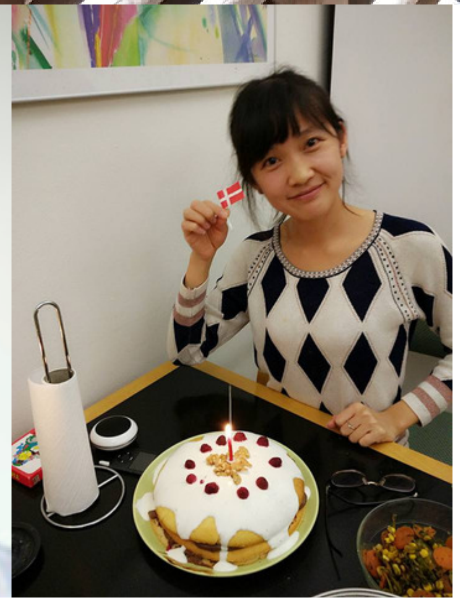
What does the future hold for non-targeted analysis?

- Quantitative results
- Directly comparable data from different labs
- Retrospective analysis









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