Laboratory of Analytical Chemistry,
Department of Chemistry
National and Kapodistrian
University of Athens

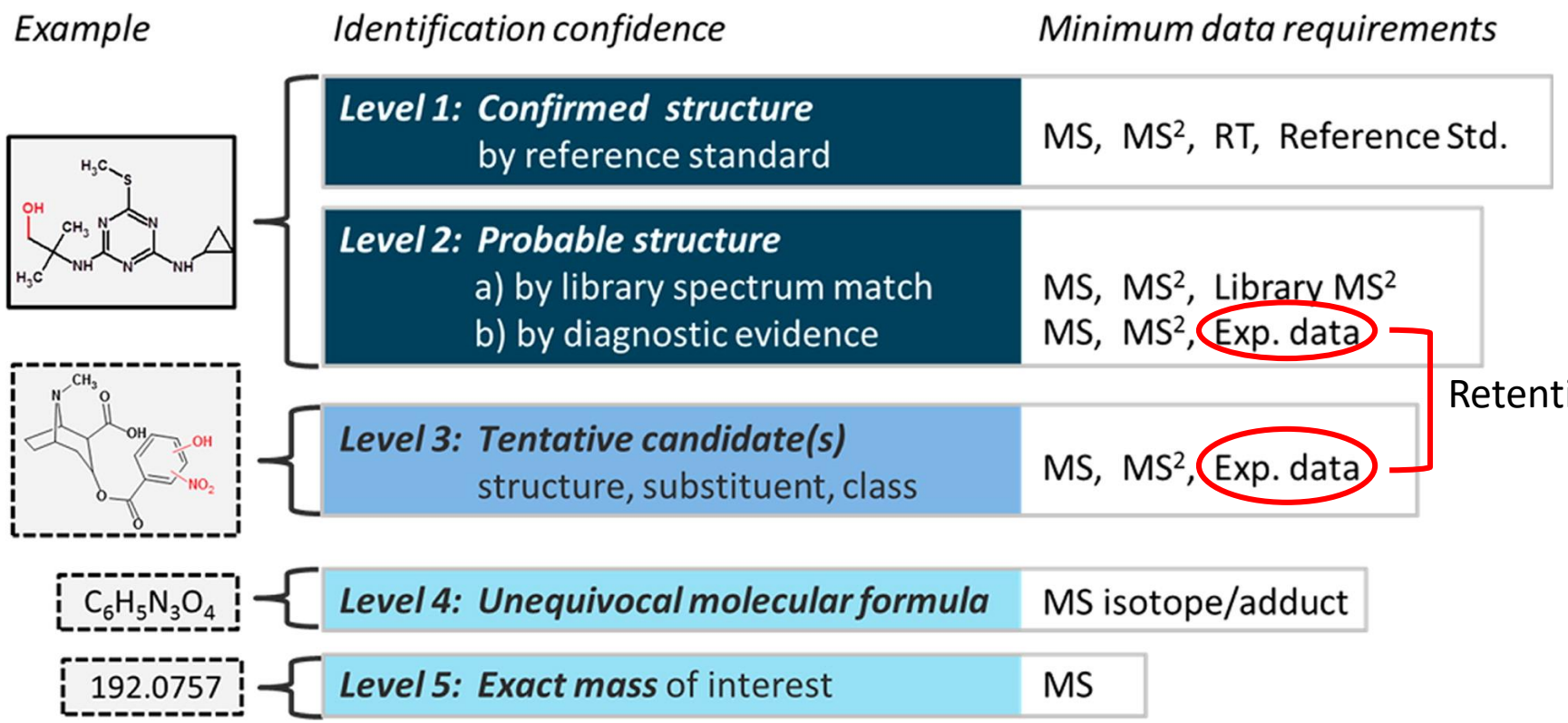
http://trams.chem.uoa.gr/
TrAMS
Trace Analysis and Mass Spectrometry Group

Development and Prediction of Liquid Chromatographic Retention Time Indices (RTI) to facilitate non-target identification

Presenter:

Reza Aalizadeh

Identification confidence in HRMS



Proposed identification confidence levels in high resolution mass spectrometric analysis. Note: MS2 is intended to also represent any form of MS fragmentation (e.g., MSe, MSn).

Published in: Emma L. Schymanski; Junho Jeon; Rebekka Gulde; Kathrin Fenner; Matthias Ruff; Heinz P. Singer; Juliane Hollender; *Environ. Sci. Technol.* **2014,** 48, 2097-2098.
DOI: 10.1021/es5002105
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Dataset development

- RPLC: Two extensive datasets consist of **1863** and **308 compounds** were developed for **(+)** and **(-) ESI-LC-HRMS**, respectively

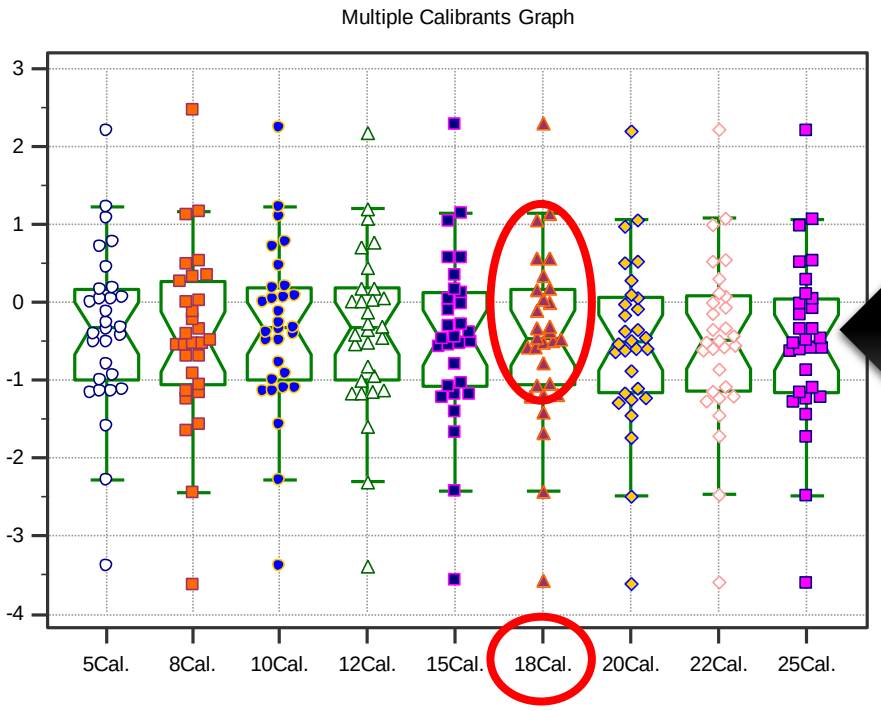
  LC conditions with column (**Acclaim C18**)

  A: H_2O/MeOH 90:10 with 5 mM ammonium formate (for -ESI) & 0.01% formic acid (for +ESI)

  B: MeOH with 5 mM ammonium formate (for -ESI) & 0.01% formic acid (for +ESI)

RTI Calibrants for RPLC-(+)ESI-HRMS

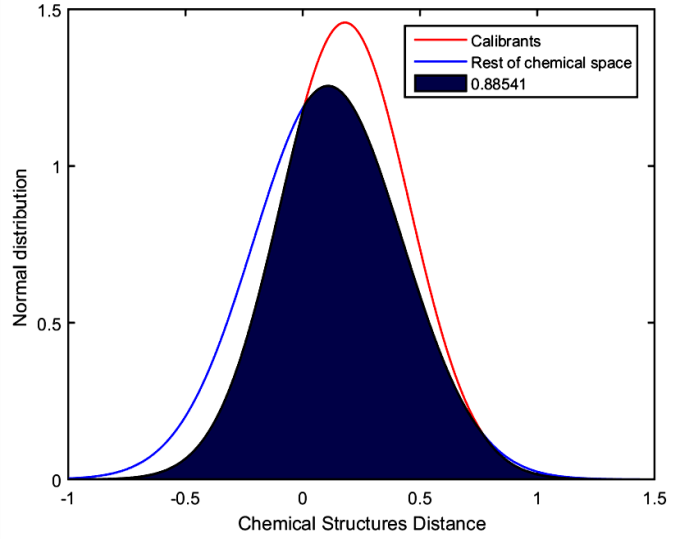
ACO-Similarity Indices
(in-house developed chemometric method for selection of calibrants)

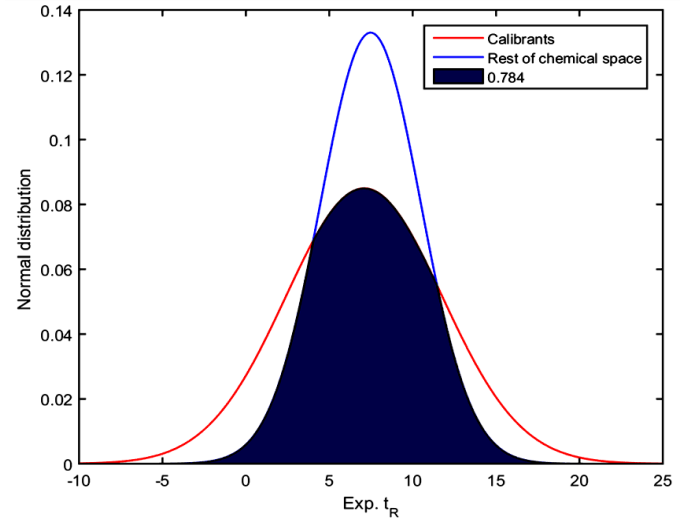


Calibrants	RT (Acclaim C18)
Guanylurea	1.31
Amitrole	1.39
Histamine	1.58
Chlormequat	1.67
Methamidophos	2.76
Vancomycin	3.26
Cefoperazone	4.36
Trichlorfon	5.23
Butocarboxim	6.07
Dichlorvos	7.00
Tylosin	7.88
TCMTB	9.25
rifaximin	10.06
Spinosad A	11.34
Emamectin B1a	12.40
AvermectinB1a	13.64
Nigericin	13.94
Ivermectin B1a	14.40

The limit of selection of calibrants was set to 5, 8, 10, 12, 15, 18, 20, 22 and 25. Out of these calibrants, 18 were selected as optimum number due to the inclusion of high chemical features and overlap between the Rt observed

ACO-Similarity Indices
Calculation of molecular descriptors
PCA
Normalized mean distance
Increase overlap between normal distribution

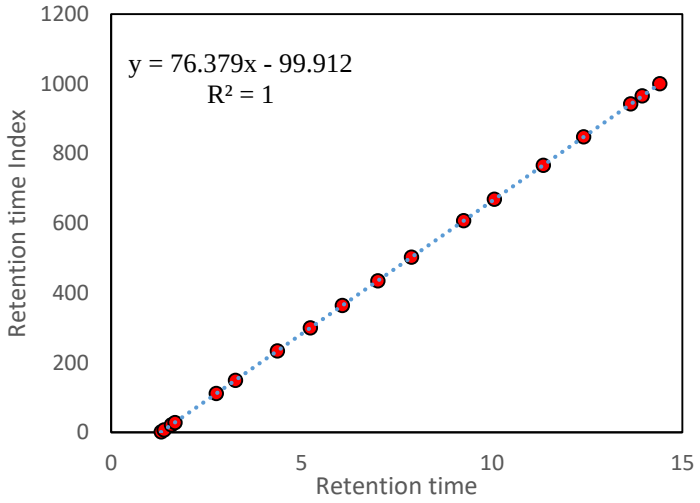




RTI for RPLC-(+)ESI-HRMS

ACO based QSRR models for RTI system using SMILES information

RTI = 76.379(RT) - 99.912



$$RTI = \frac{(RTx - RTmin)}{(RTmax - RTmin)} * 1000$$

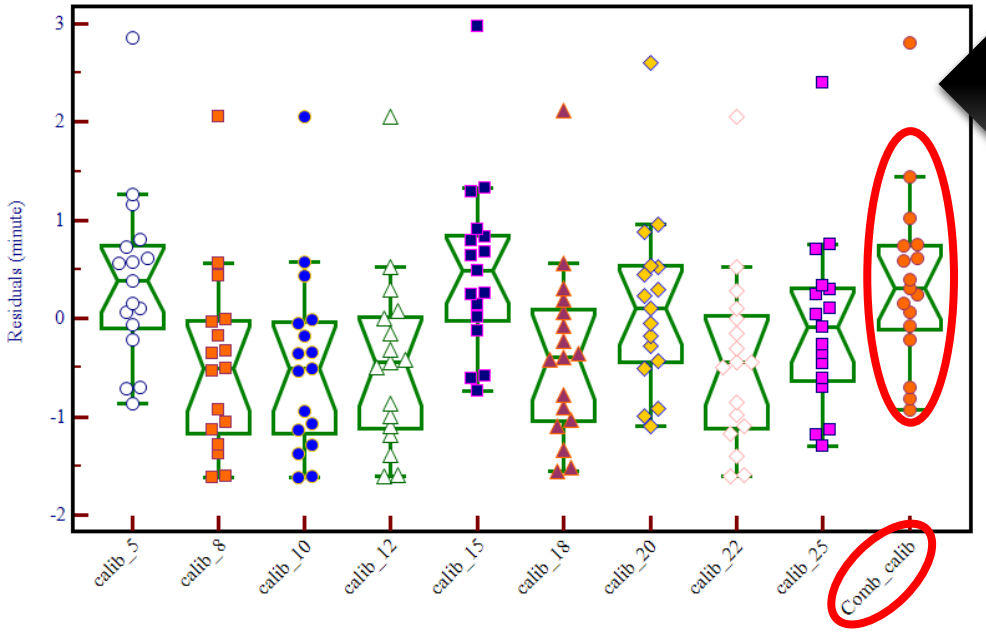
Calibrants	RT (Acclaim C18)	RTI
Guanylurea	1.31	1
Amitrole	1.39	6.111536
Histamine	1.58	20.62643
Chlormequat	1.67	27.50191
Methamidophos	2.76	110.7716
Vancomycin	3.26	148.9687
Cefoperazone	4.36	233.0023
Trichlorfon	5.23	299.4652
Butocarboxim	6.07	363.6364
Dichlorvos	7	434.683
Tylosin	7.88	501.9099
TCMTB	9.25	606.5699
rifaximin	10.06	668.4492
Spinosad A	11.34	766.2338
Emamectin B1a	12.4	847.2116
AvermectinB1a	13.64	941.9404
Nigericin	13.94	964.8587
Ivermectin B1a	14.4	1000

Predicted by QSRR model developed based on LC conditions with a C18 column (**Acclaim C18**)
A: H_2O/MeOH 90:10 with 5 mM ammonium formate & 0.01% formic acid .
B: MeOH with 5 mM ammonium formate & 0.01% formic acid
Gradient: 99/1

	Training			Test		
	R2	RMSE	F	R2	RMSE	F
MLR	0.835	92.575	1515.130	0.870	83.184	426.416
SVM	**0.861**	84.869	1838.745	**0.880**	80.029	467.038

RTI for LC-(-)ESI-HRMS

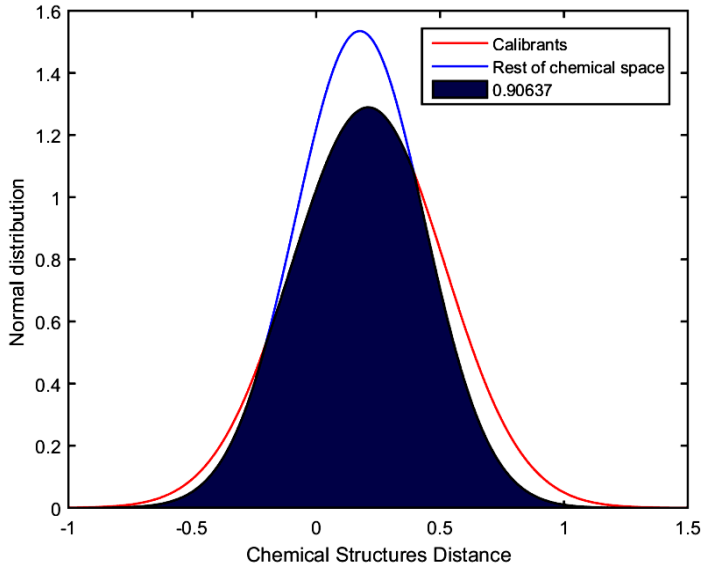
ACO-Similarity Indices
(in-house developed chemometric method for selection of calibrants)

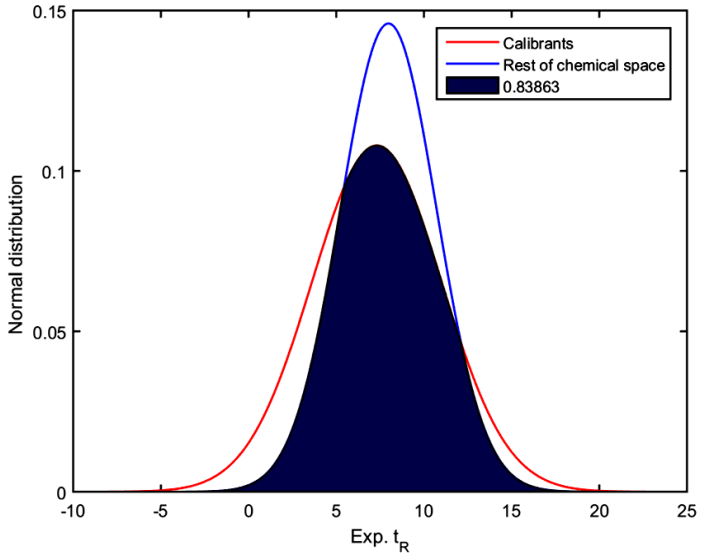


Calibrants	RT (Acclaim C18)
Amitrole	1.67
benzoic acid	2.88
Acephate	3.09
Salicylic acid	3.58
Simazine 2-Hydroxy	4.96
Tepraloxydim	5.26
Bromoxynil	5.35
MCPA	6.49
Valproic acid	7.04
Phenytoin	7.16
Flamprop	7.49
Benodanil	7.99
Dinoterb	8.13
Inabenfide	9.23
Coumaphos	10.98
triclosan	12.02
AvermectinB1a	13.64
salinomycin	14.67

The limit of selection of calibrants was set to 5, 8, 10, 12, 15, 18, 20, 22 and 25. Out of these calibrants, 18 were selected as optimum number due to the inclusion of high chemical features and overlap between the Rt observed

ACO-Similarity Indices



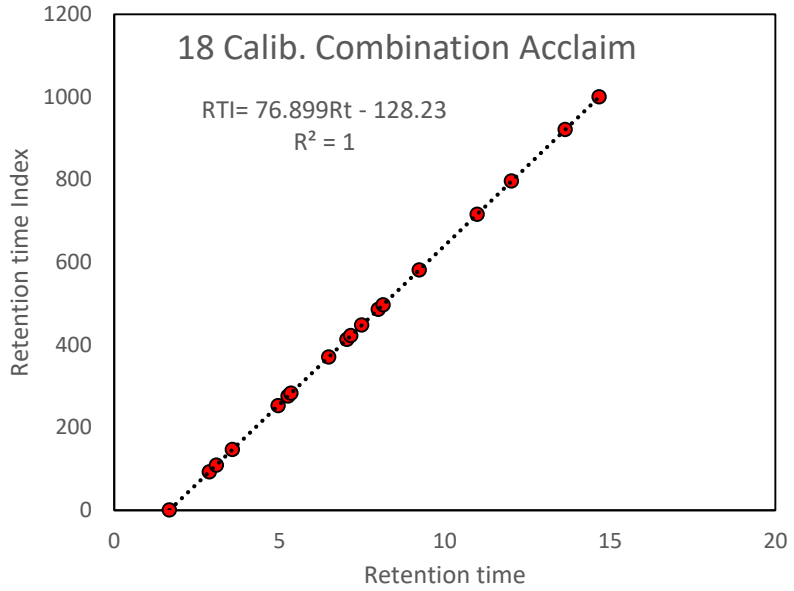


RTI for RPLC-(-)ESI-HRMS

ACO based QSRR models for RTI system using SMILES information

$$RTI = \frac{(RTx - RTmin)}{(RTmax - RTmin)} * 1000$$

RTI = 76.899(Rt) - 128.23

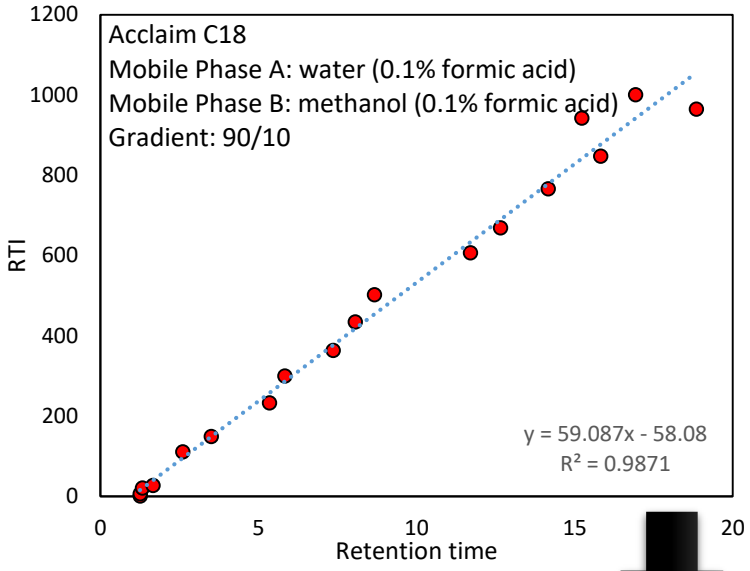


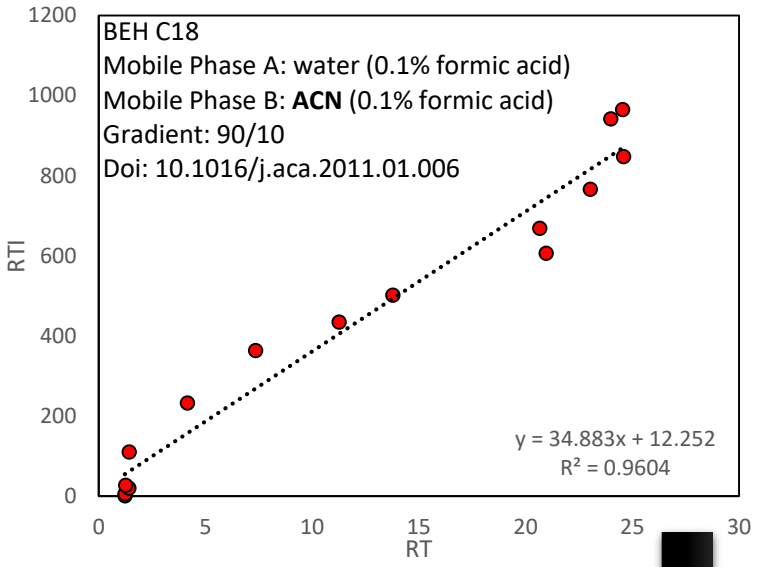
Calibrants	RT (Acclaim C18)	RTI
Amitrole	1.67	1
benzoic acid	2.88	93.07692
Acephate	3.09	109.2308
Salicylic acid	3.58	146.9231
Simazine 2-Hydroxy	4.96	253.0769
Tepraloxydim	5.26	276.1538
Bromoxynil	5.35	283.0769
MCPA	6.49	370.7692
Valproic acid	7.04	413.0769
Phenytoin	7.16	422.3077
Flamprop	7.49	447.6923
Benodanil	7.99	486.1538
Dinoterb	8.13	496.9231
Inabenfide	9.23	581.5385
Coumaphos	10.98	716.1538
triclosan	12.02	796.1538
AvermectinB1a	13.64	920.7692
salinomycin	14.67	1000

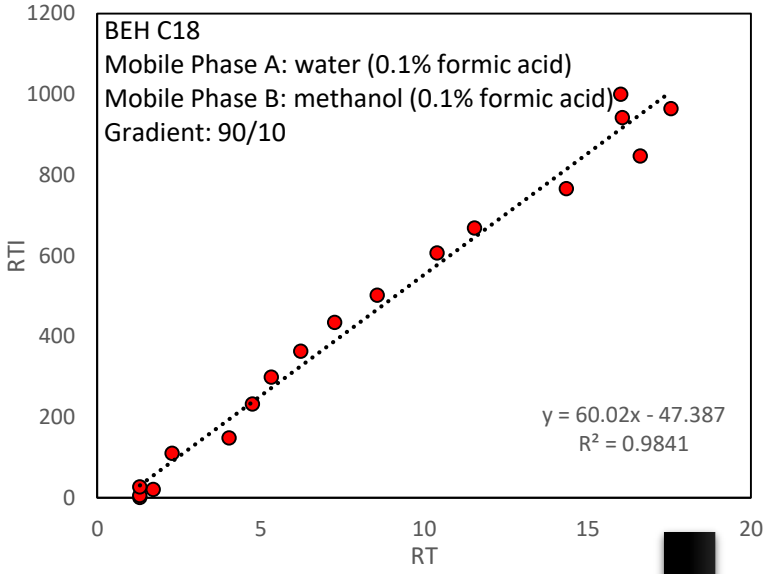
	Training				Test		
	R^2	RMSE	F	Q^2_{LOO}	R^2	RMSE	F
ACO-MLR	0.844	1.086	213.86	0.830	0.876	1.109	62.280
ACO-SVM	0.952	0.6231	648.22	0.832	0.884	1.040	54.590

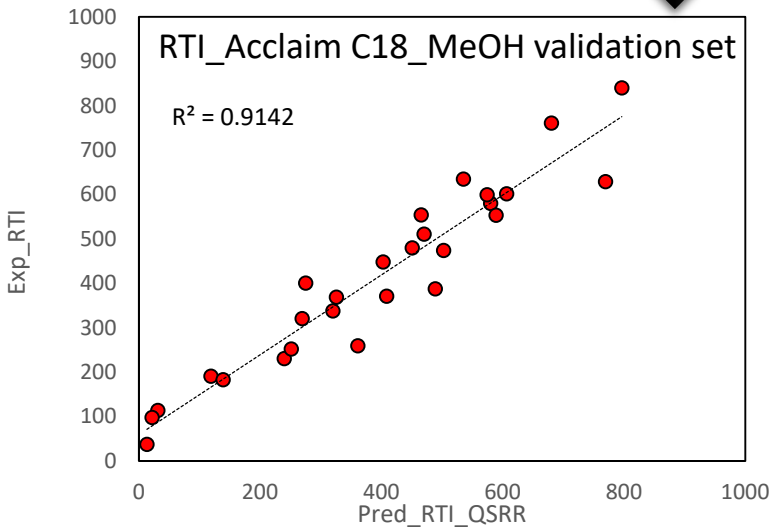
RTI(+)ESI-LC-HRMS

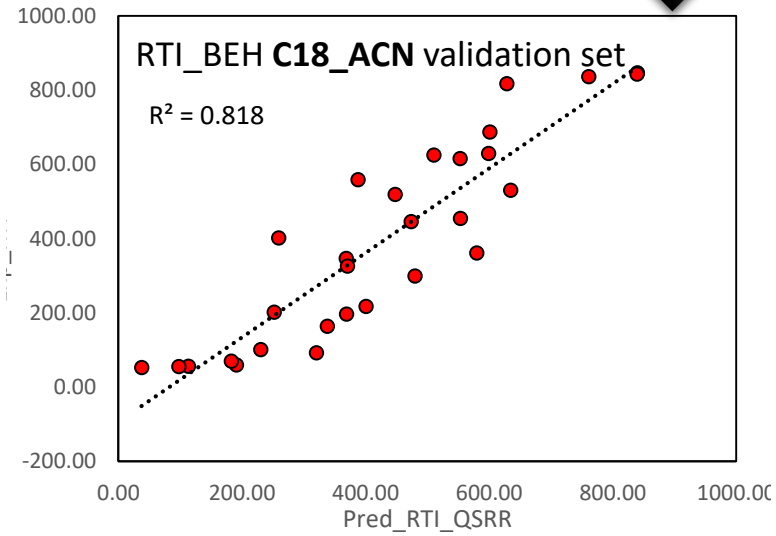
Different LC conditions and the external validation accuracy

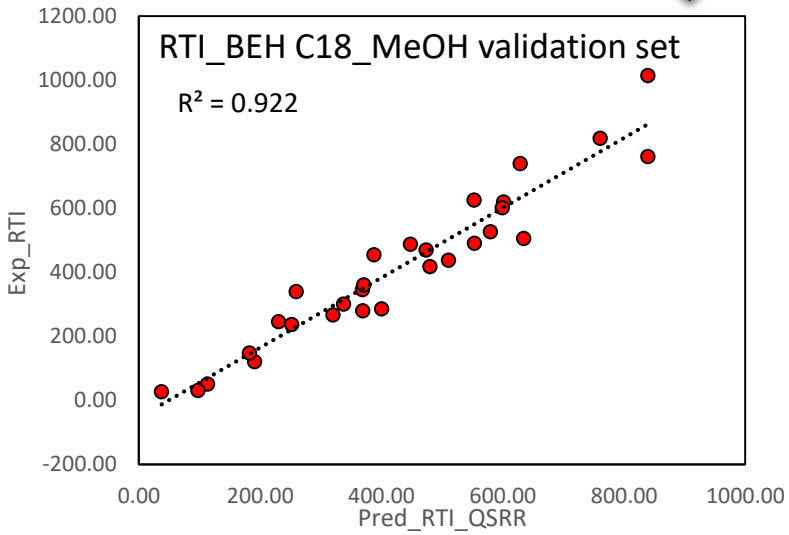






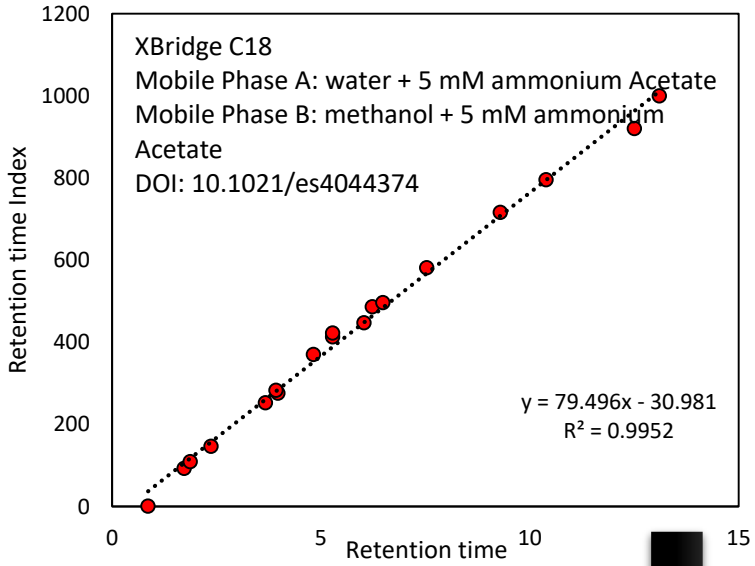


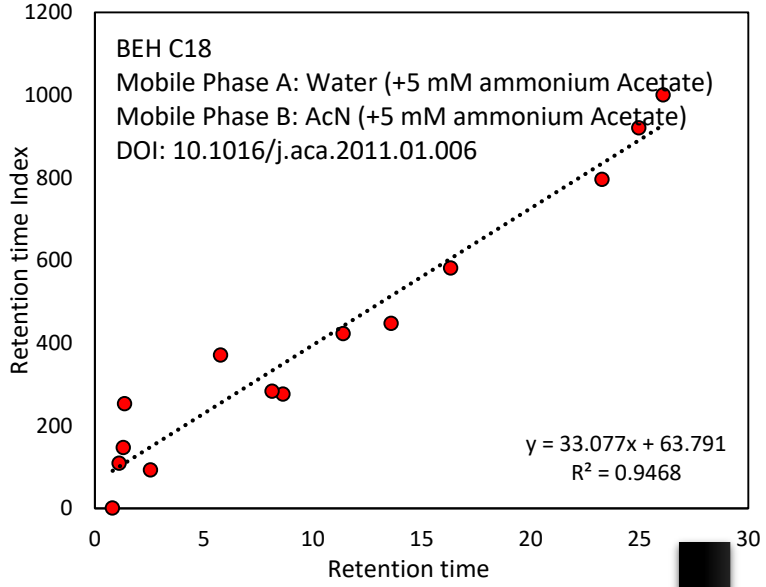


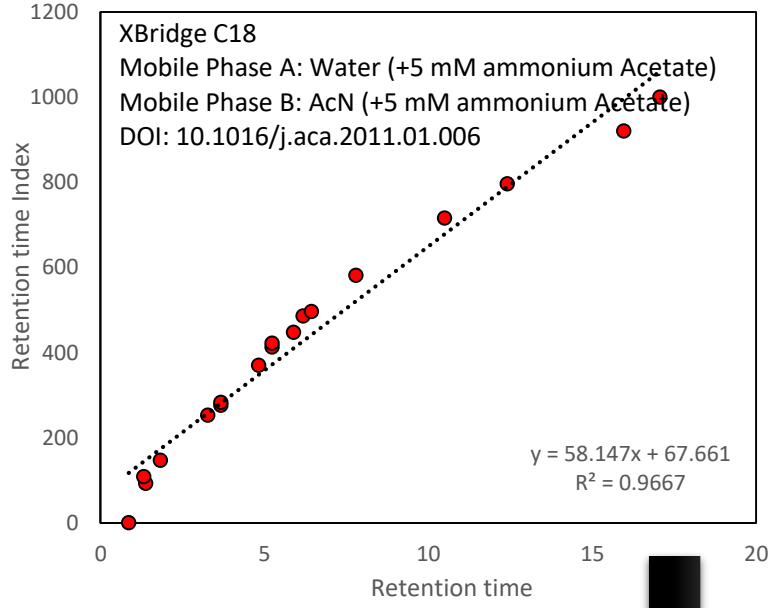


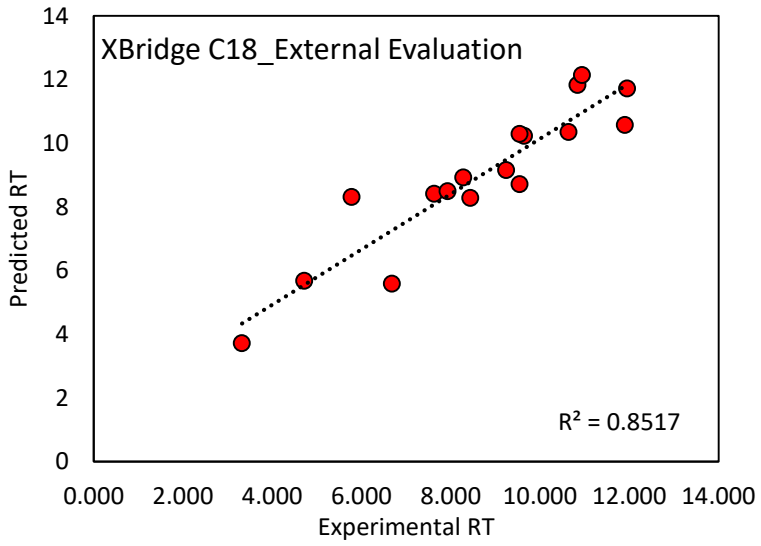
RTI(-)ESI-LC-HRMS

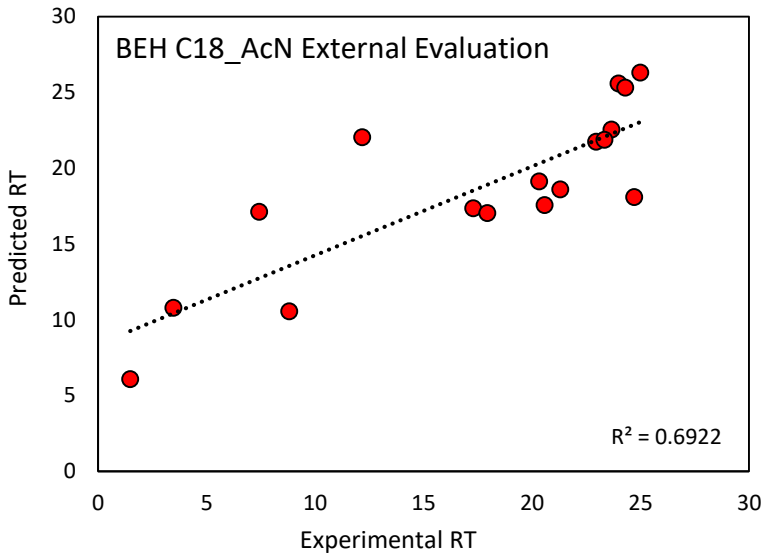
Different LC conditions and the external validation accuracy

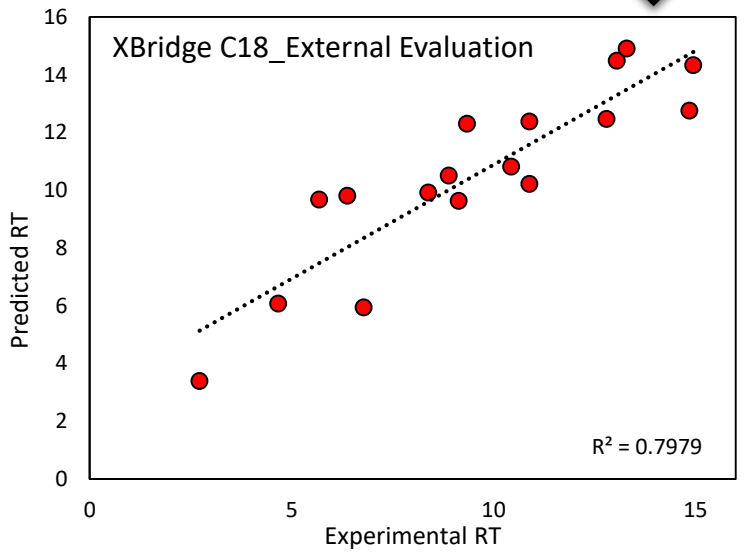








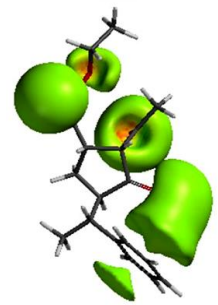




Prediction of RTI workflow

Affinity Propagation
(Training (80%) & Test (20%) set)
(doi:10.1126/science.1136800)

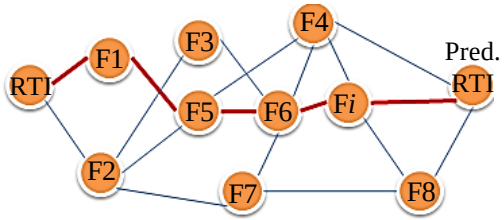
Molecular Descriptors (Molecule version 1.0)



Opt. by semi-empirical
Energy = -687.77 KJ/mol
PSA = 48.527 Å²

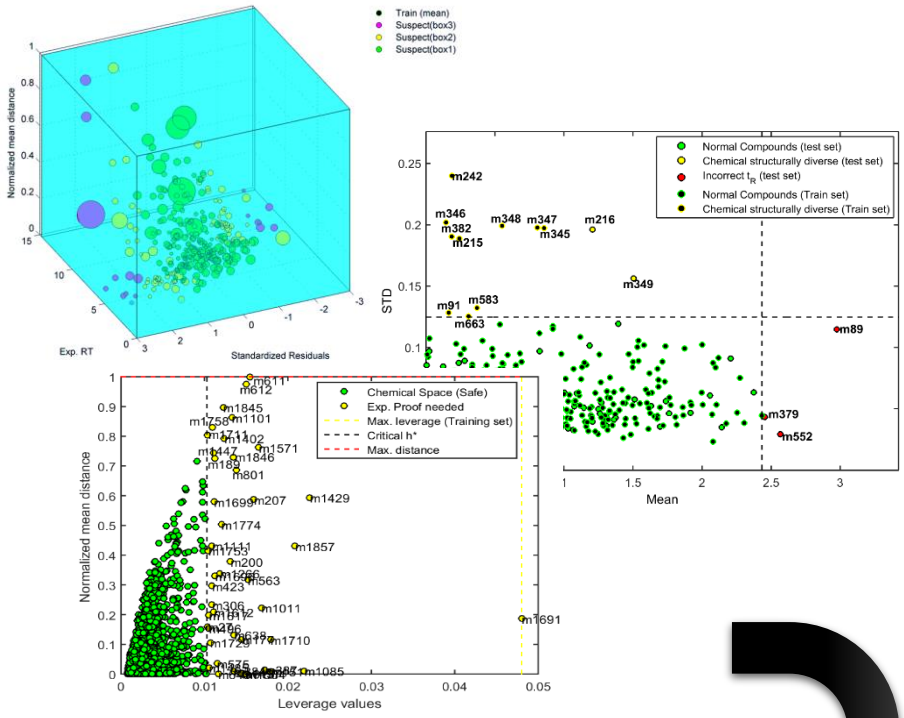
SMILES + Exp. RTI

Ant Colony Optimization (ACO)
(For selecting relevant molecular descriptor)
(doi:10.1016/j.chemolab.2009.05.005)11



Linear and Non-linear Regressions
(Multiple Linear Regressions (MLR) Support Vector Machine (SVM))

Evaluation of Internal and External Accuracy
(doi:10.1021/ci200211n)
(doi:10.1021/ci300084j)
(doi:10.1002/jcc.23231)



Application to Suspect List

Applicability Domain
(OTrAMS)
(Monte Carlo Simulation)
(DOI: 10.1002/jcc.21351)
(Chemical Space Failure)
(DOI: 10.1039/C6EM00679E)

RTI Predicted for Suspect List
(Rejected or Accepted)

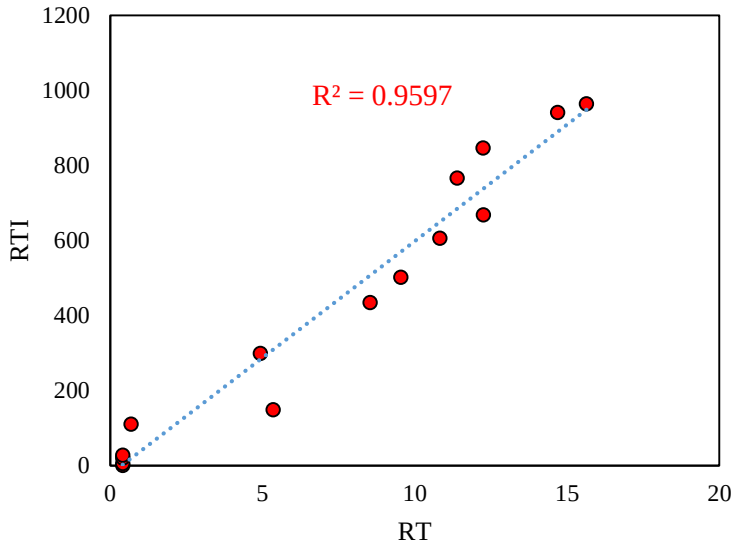
Evaluation of RTI (+ESI) system by

Department Effect-Directed Analysis,
Helmholtz Centre for Environmental Research - UFZ

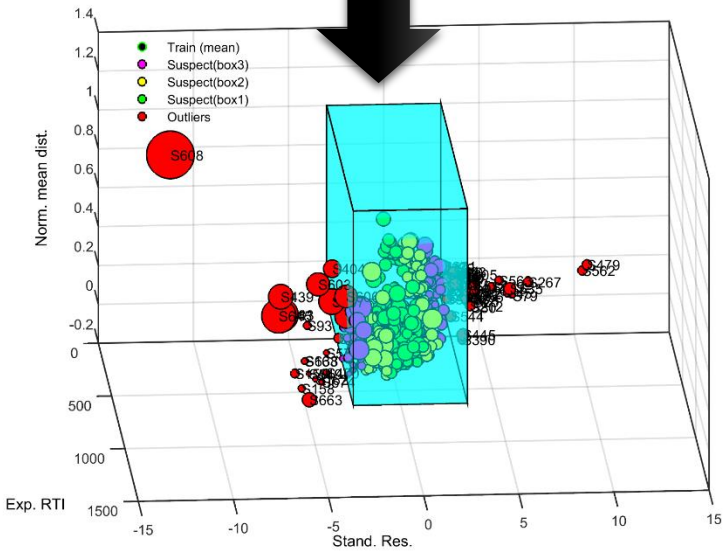
Different LC conditions and the external validation accuracy

LC conditions

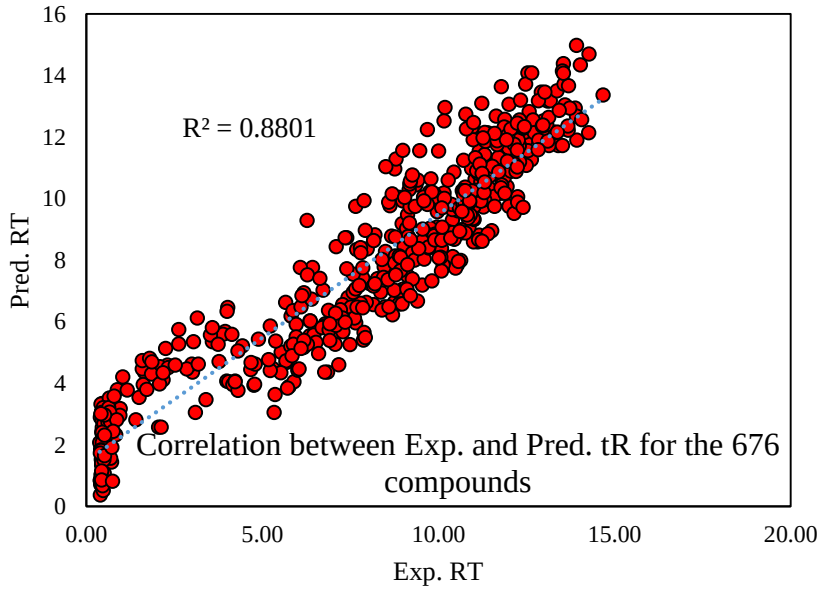
Column:	Phenomenex Kinetex C18 EVO 50x2.1 mm, 2.6 µm, precolumn 4x2.1 mm, 2.6 µm		
Molibe phase:	A	Water 0.1% formic acid	
	B	Methanol containing 0.1% formic acid	
Gradient:	time (min)	A	B
	0.00	95	5
	1.00	95	5
	13.00	0	100
	24.00	0	100



RTI = 62.17RT - 23.532

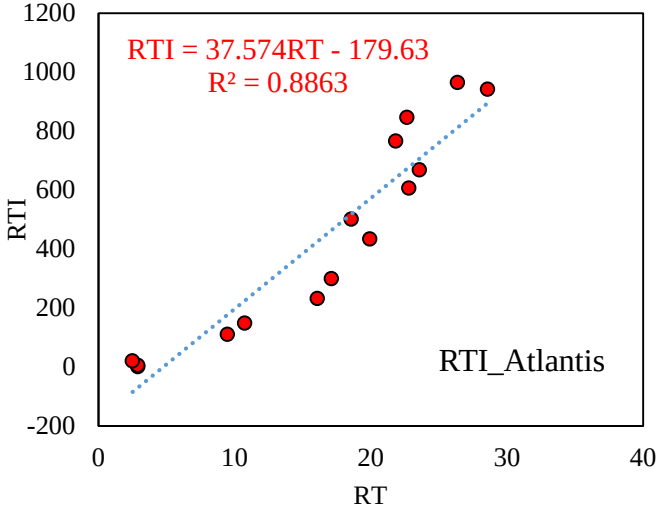


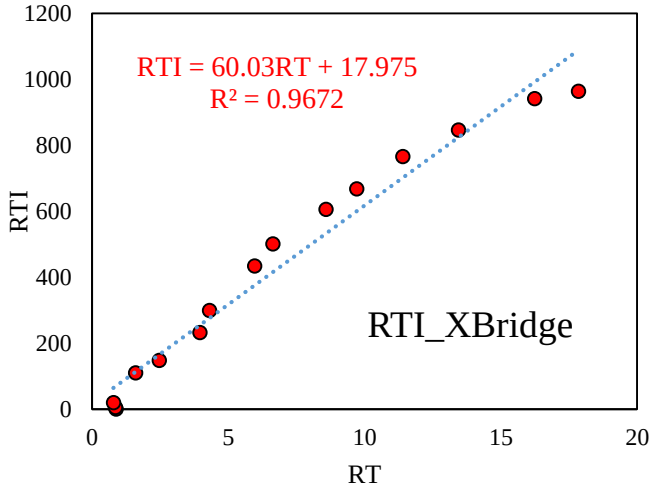
676 compounds (external set)
500 compounds ΔRT<2 min
176 (67) compounds ΔRT>2 (3) min



Correlation between Exp. and Pred. tR for the 676 compounds

Evaluation of RTI (+ESI) system by *Eawag*





LC conditions

Column: XBridge C18 3.5um, 2.1x50mm with pre-column, Waters

Molibe phase: A Nanopure water +0.1% Formic acid

B MeOH +0.1% Formic acid

Gradient: 90/10 at 0 min, 50/50 at 4 min, 5/95 at 17 min, 5/95 at 25 min, 90/10 at 25.1 min, 90/10 at 30 min

Column: Atlantis T3 3um, 3.0x150mm with pre-column, Waters

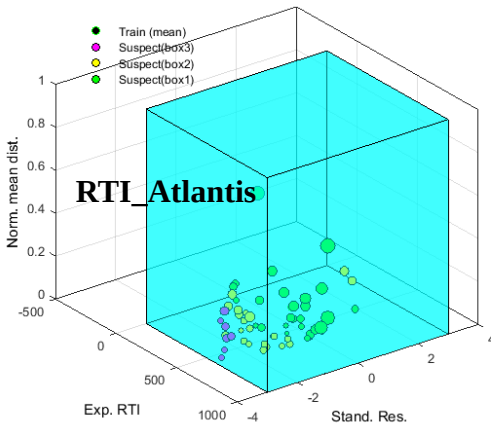
Molibe phase: A Nanopure water +0.1% Formic acid

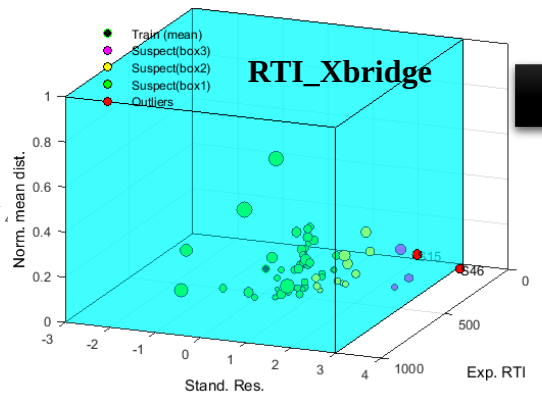
B MeOH +0.1% Formic acid

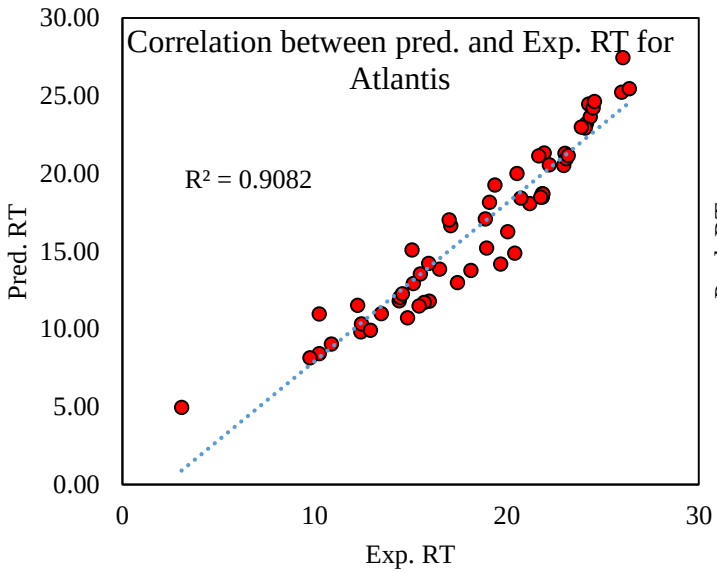
Gradient: 95/5 constant for 3 min, 5/95 at 22 min, 5/95 at 30 min, 95/5 at 30.1 min, 95/5 at 30 min

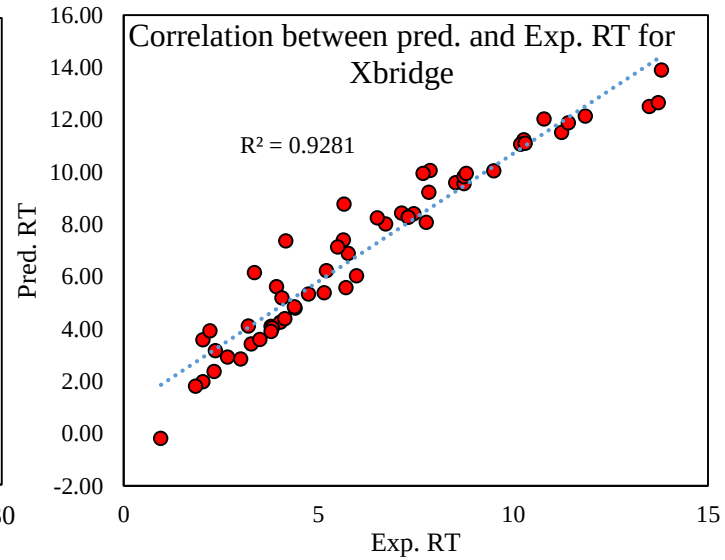
52 comp ΔRT<2 min
7 comp ΔRT>2 min

54 comp ΔRT<2 min
3 (2) comp ΔRT>2 (3) min

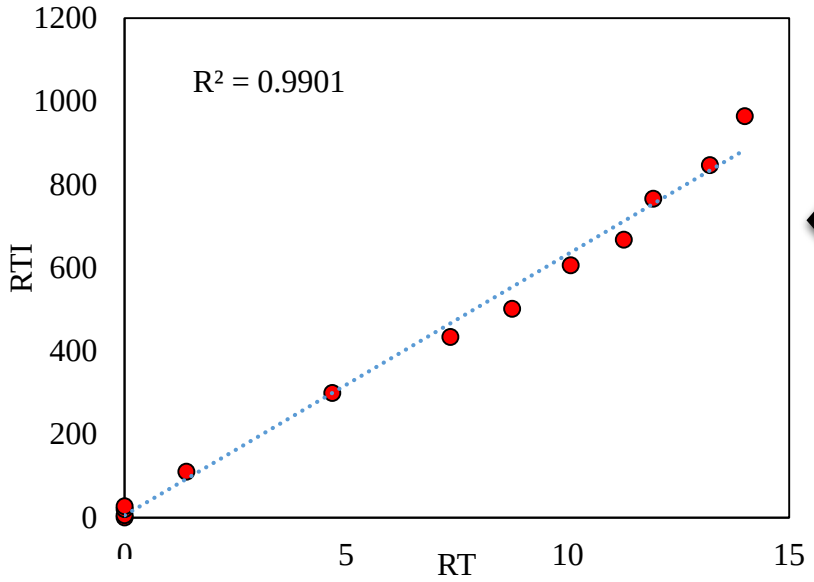








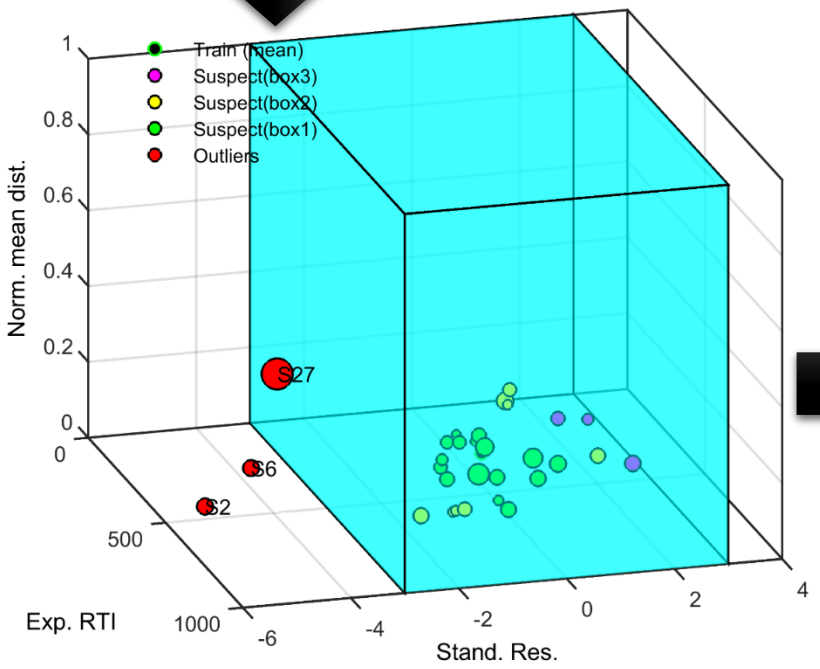
Evaluation of RTI (+ESI) system by *Universitat Jaume I*



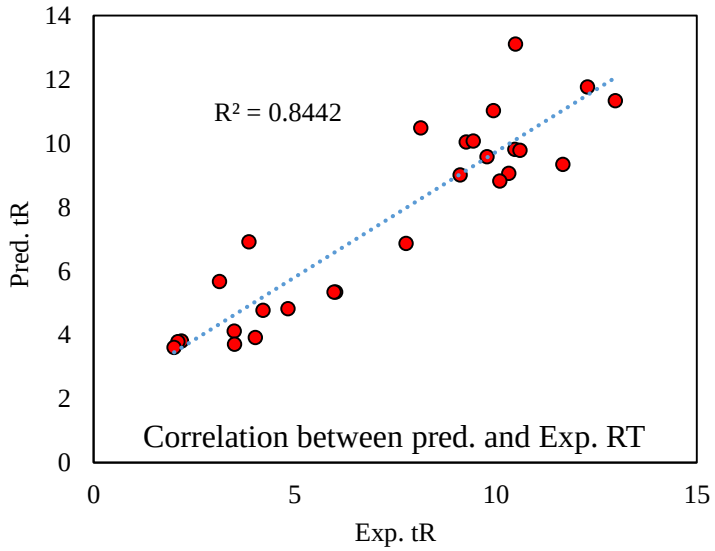
LC conditions

Column:		Waters Cortecs C18 2.1x100 mm, 2.7 µm
Molibe phase:	A	H2O 0.01% HCOOH
	B	MeOH 0.01% HCOOH
Gradient:		10 %B(0);10 %B-90% over 14min;90%B(2);90 %B-10% over 0.1min;10%B(2)

RTI = 62.705RT + 5.5564



24/30 comp ΔRT<2 min
3 (3) /30 comp ΔRT>2 (3) min

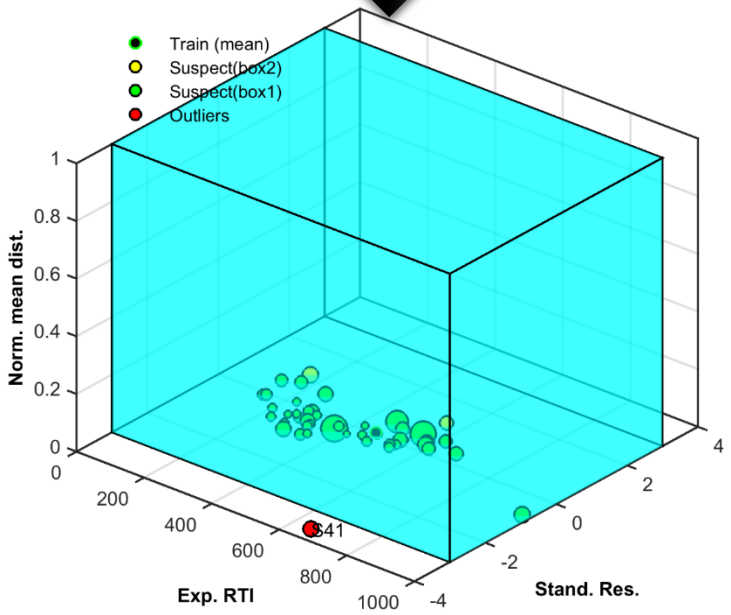


Evaluation of RTI (+ESI) system by *SLU*

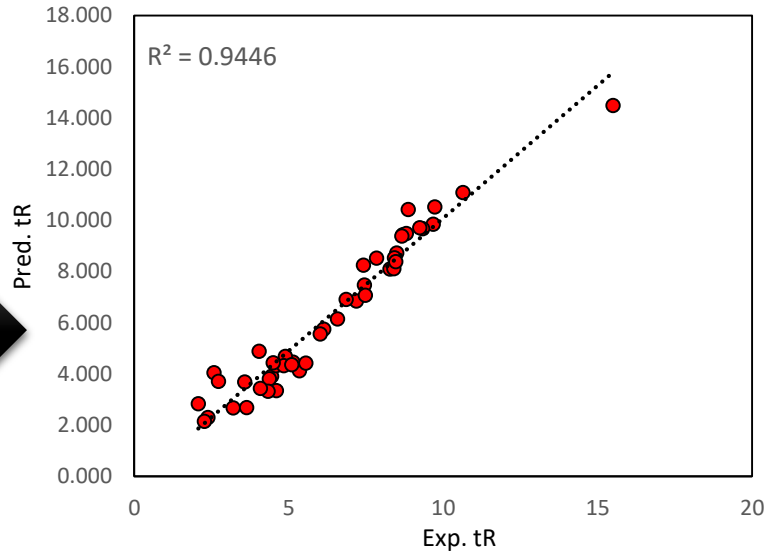
Column:		Waters, Acquity UPLC HSS T3 1,8 um; 2,1 x 100 mm
Mobile phase:	A	H2O, 0,01% formic acid, 5mM ammonium formate
	B	Acetonitrile. 0,01% formic acid
Gradient:	Time	%A
	0	95
	0.5	95
	16	5
	16.1	1
	19	1
	19.1	95
	21	95

(Prediction limits at 95% CI)
Excluded (high residuals):
Nigericin
%%%%%%%%%%%%%%%%%%%%
Not observed:
Amitrol
Vancomycin
Cefoperazone
%%%%%%%%%%%%%%%%%%%%
intercept: -1.4603
Slope: 63.1008
R^2: 0.9898
%%%%%%%%%%%%%%%%%%%%

RTI = 63.1008RT -1.4603



44/47 comp ΔRT<1 min
2/47 comp 1<ΔRT<2 min
1/47comp ΔRT>3 min



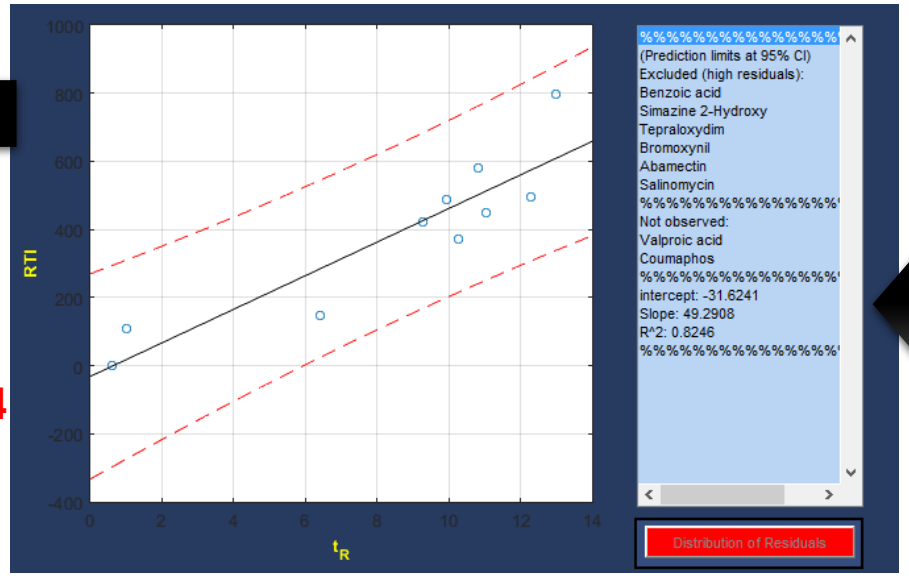
Evaluation of RTI (-ESI) system by

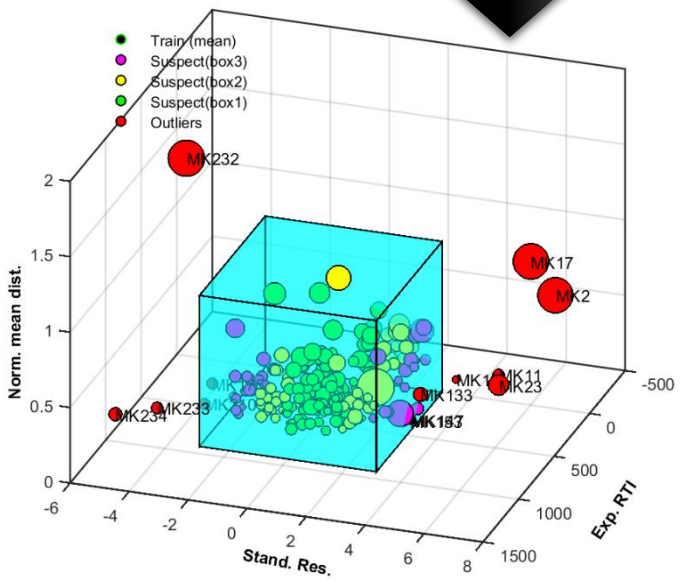
Department Effect-Directed Analysis,
Helmholtz Centre for Environmental Research - UFZ

Different LC conditions and the external validation accuracy

(Prediction limits at 95% CI)
Excluded (high residuals):
Benzoic acid
Simazine 2-Hydroxy
Tepraloxydim
Bromoxynil
Abamectin
Salinomycin
%%%%%%%%%%%%%%%%
Not observed:
Valproic acid
Coumaphos
%%%%%%%%%%%%%%%%
intercept: -31.6241
Slope: 49.2908
R^2: 0.8246
%%%%%%%%%%%%%%%%

RTI = 49.30RT – 31.624

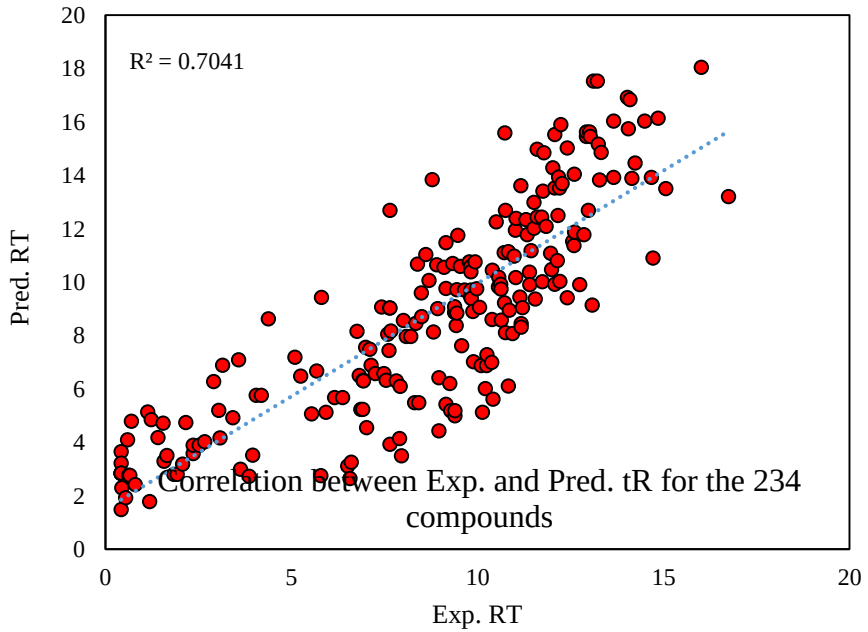




234 compounds (external set)
185 compounds ΔRT<2 min
36 (13) compounds ΔRT>2 (3) min

LC conditions

Column:	Phenomenex Kinetex C18 EVO 50x2.1 mm, 2.6 µm, precolumn 4x2.1 mm, 2.6 µm		
Mobile phase:	A	Water 0.1% formic acid	
	B	Methanol containing 0.1% formic acid	
Gradient:	time (min)	A	B
	0.00	95	5
	1.00	95	5
	13.00	0	100
	24.00	0	100

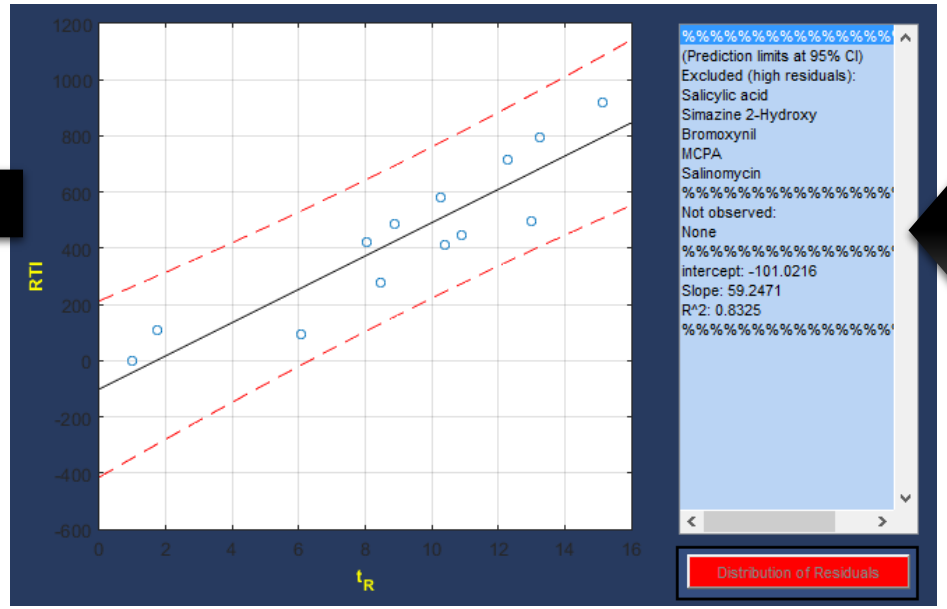


Correlation between Exp. and Pred. tR for the 234 compounds

Evaluation of RTI (-ESI) system by *Universitat Jaume I*

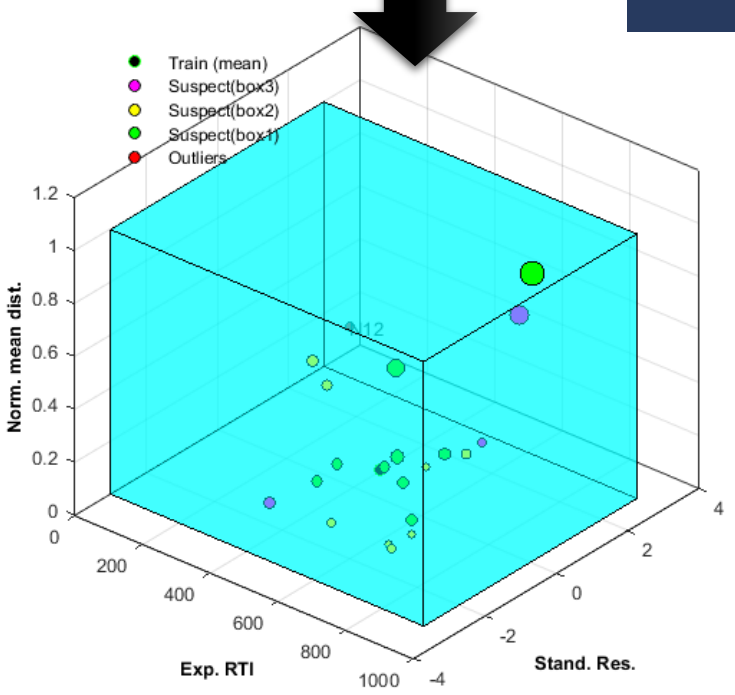
(Prediction limits at 95% CI)
Excluded (high residuals):
Salicylic acid
Simazine 2-Hydroxy
Bromoxynil
MCPA
Salinomycin
%%%%%%%%%%%%%%%%
Not observed:
None
%%%%%%%%%%%%%%%%
intercept: -101.0216
Slope: 59.2471
R^2: 0.8325
%%%%%%%%%%%%%%%%

RTI = 59.247(RT) -101.0216

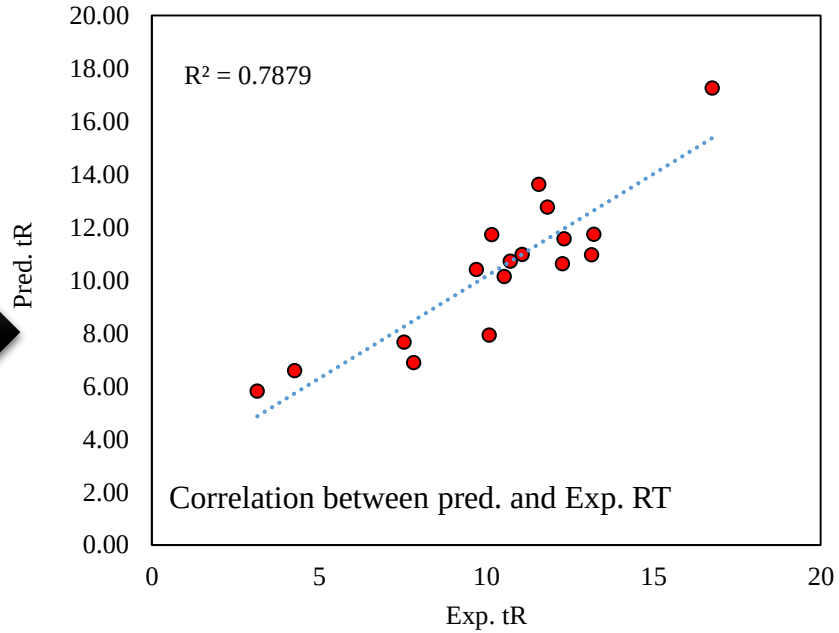


LC conditions

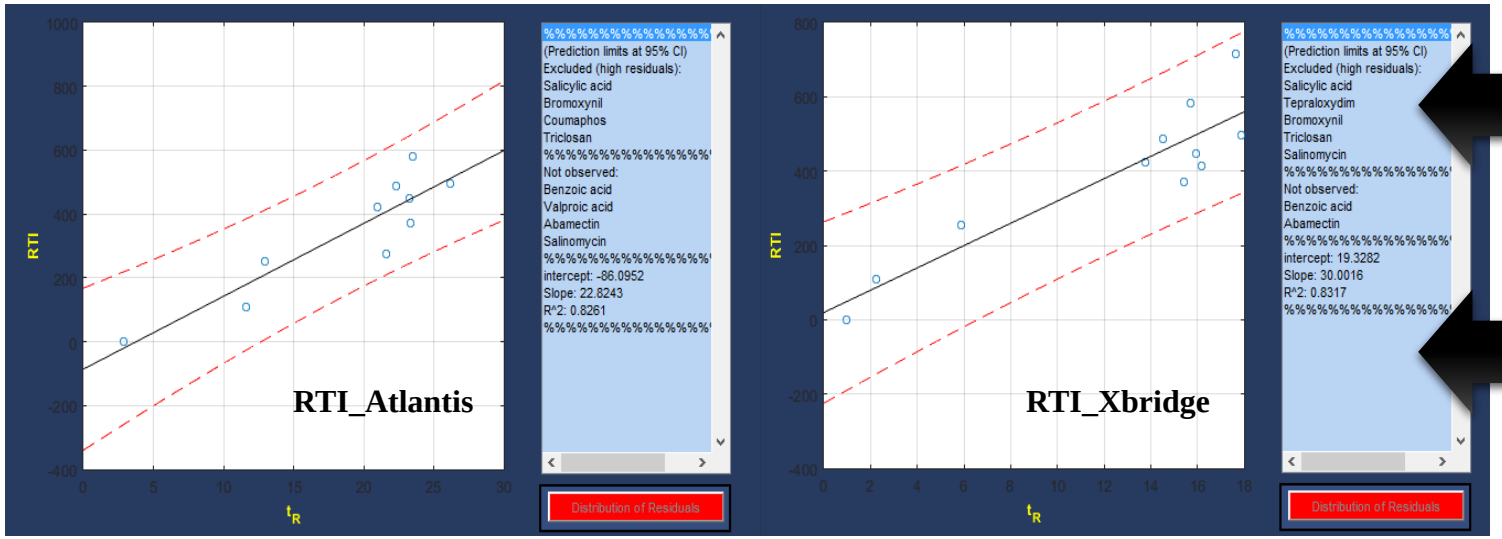
Column:		Waters Cortecs C18 2.1x100 mm, 2.7 µm
Mobile phase:	A	H2O 0.01% HCOOH
	B	MeOH 0.01% HCOOH
Gradient:		10 %B(0);10 %B-90% over 14min;90%B(2);90 %B-10% over 0.1min;10%B(2)



21 compounds (external set)
17/21 comp ΔRT<2 min
3 (1) /30 comp ΔRT>2 (3) min



Evaluation of RTI (-ESI) system by *Eawag*



LC conditions

Column: XBridge C18 3.5um, 2.1x50mm with pre-column, Waters

Mobile phase: A Nanopure water +0.1% Formic acid

B MeOH +0.1% Formic acid

Gradient: 90/10 at 0 min, 50/50 at 4 min, 5/95 at 17 min, 5/95 at 25 min, 90/10 at 25.1 min, 90/10 at 30 min

Column: Atlantis T3 3um, 3.0x150mm with pre-column, Waters

Mobile phase: A Nanopure water +0.1% Formic acid

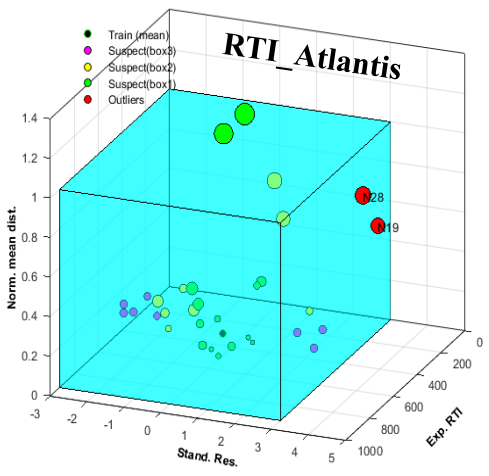
B MeOH +0.1% Formic acid

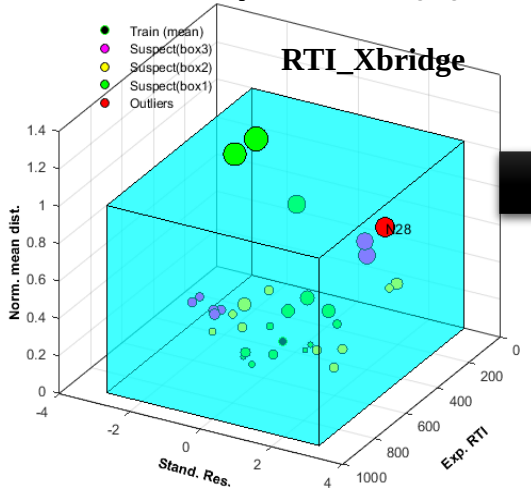
Gradient: 95/5 constant for 3 min, 5/95 at 22 min, 5/95 at 30 min, 95/5 at 30.1 min, 95/5 at 30 min

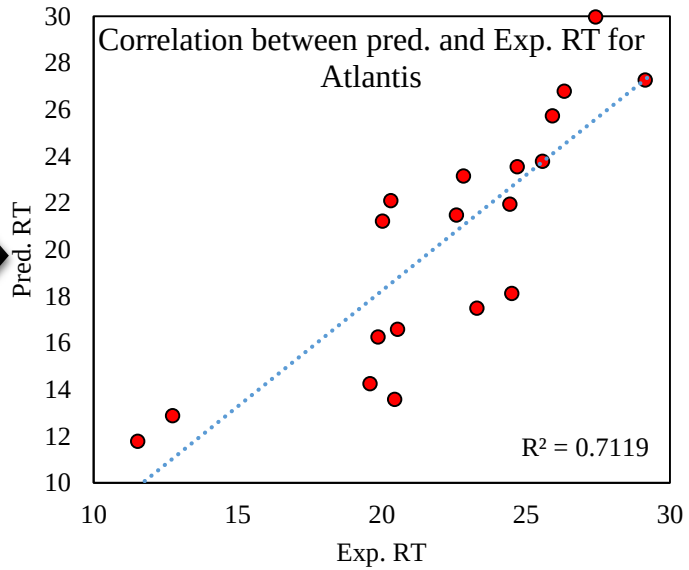
10 of compounds were Surfactant. 3 of them were belonging to –ESA class of compounds.

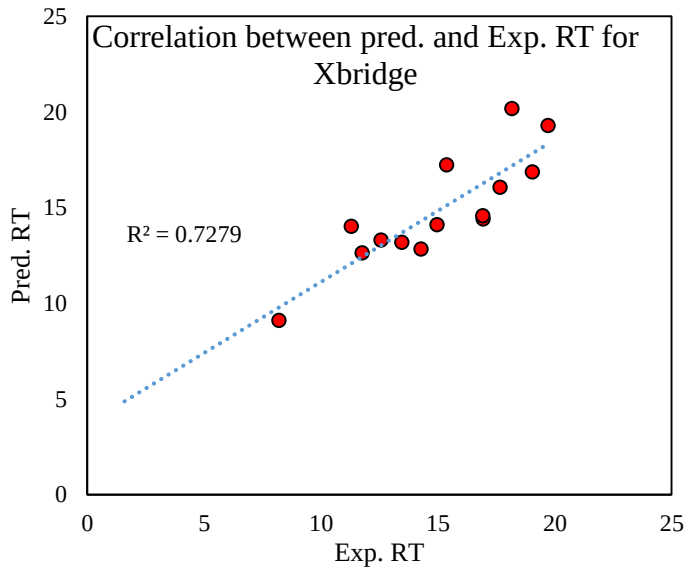
15 comp ΔRT<2 min
18 comp ΔRT>2 min

16 comp ΔRT<2 min
17 comp ΔRT>2 (3) min

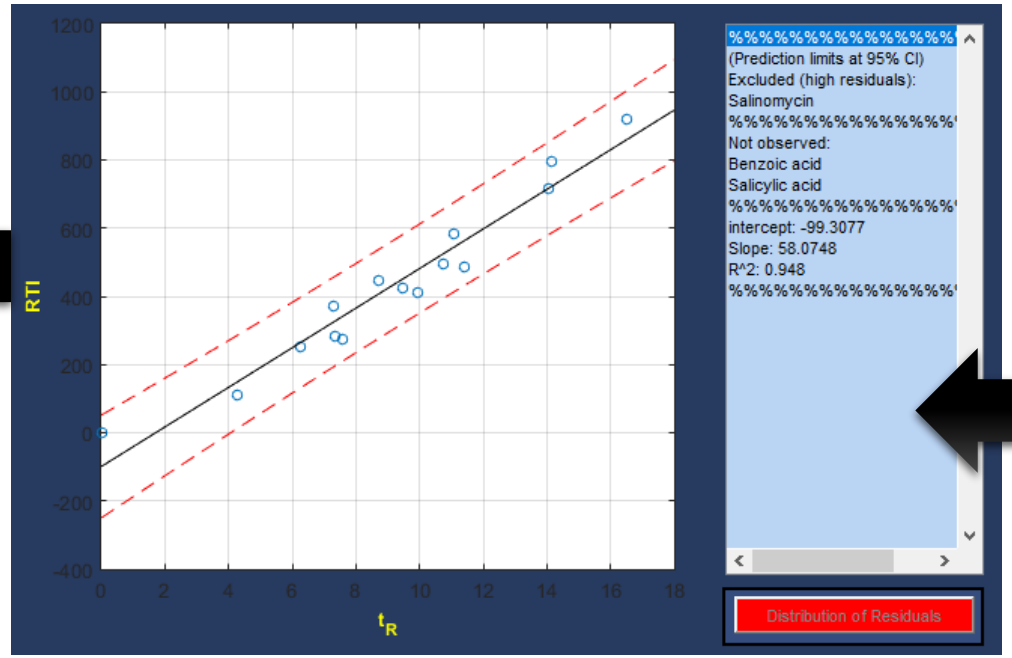






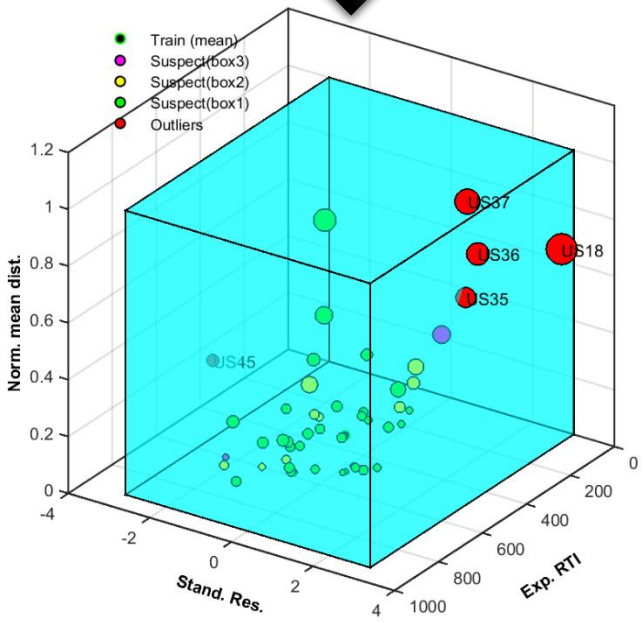


Evaluation of RTI (-ESI) system by *UC-Davis(indoor dust project)*

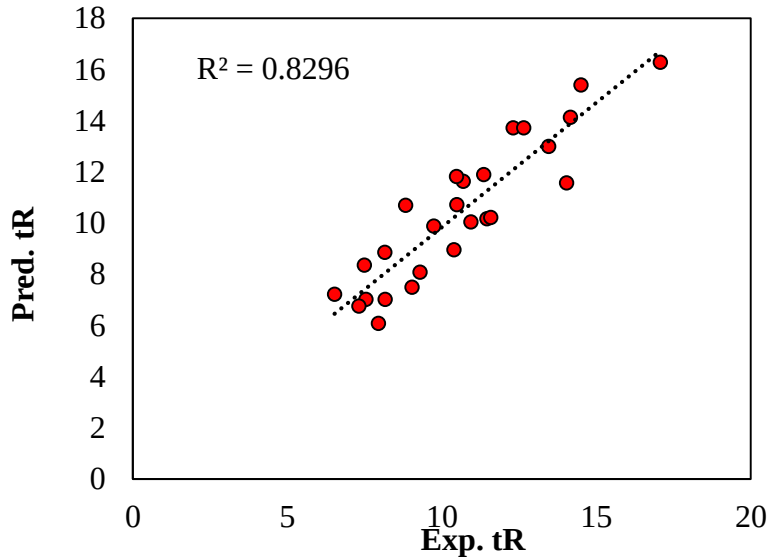


Column:	Zorbax Eclipse Plus (100 mm length, 2.5 mm ID, 1.8 µm particle size)	
Mobile phase:	A	MiliQ + 1 mM ammounium fluoride
	B	acetonitrile
Gradient:	Time	%B
	1.5	2
	15	100
	20	2
Flow rate		350 µl/min

RTI = 58.0748 RT -99.3077



33/49 comp ΔRT<1 min
9/49 comp 1<ΔRT<2 min
2(5) comp ΔRT>2 (3)min



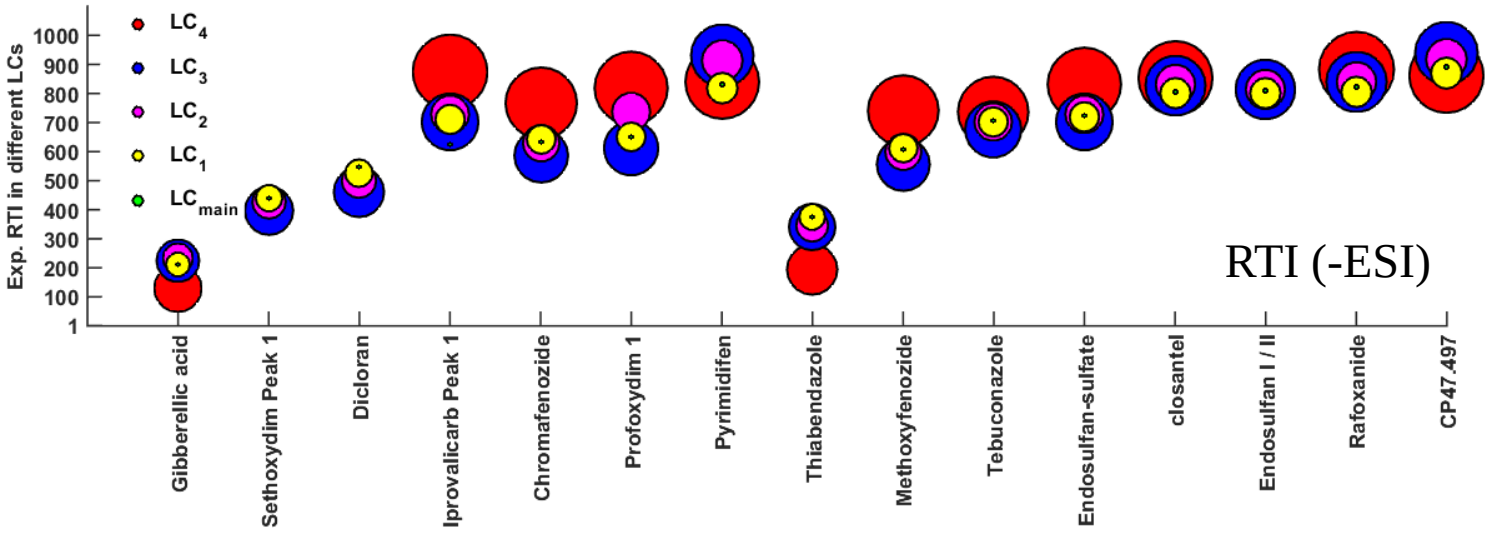
Harmonizing the Retention Time between Laboratories – Use of RTI in suspect screening and Retrospective Analysis

Name	tR_TUM	tR_UOA	tR_UFZ	RTI_UOA	RTI_TUM	RTI_UFZ
atrazine	27.69	8.16	9.88	523.34	506.15	590.71
benzophenone-3	31.16	10.83	11.89	727.27	668.91	715.67
Boscalid	29.84	9.4	11.23	618.05	606.99	674.64
carbamazepine	26.45	7.36	9.54	462.20	448.00	569.60
Carbetamide	25.81	6.58	6.60	402.70	418.00	386.80
Carbofuran	27.17	7.01	7.2	435.50	481.75	424.09
Carboxin	27.84	7.46	NA	469.88	513.18	NA
chlorbromuron	29.70	9.55	NA	629.51	600.42	NA
Chlorfenvinphos	31.15	10.97	12.48	737.97	668.44	752.35
chloridazon	24.49	5.34	6.53	307.95	356.04	382.44
Chlorotoluron	27.34	7.98	9.64	509.59	489.73	575.79
Chlorpropham	30.28	9.78	11.3	647.08	627.63	678.99

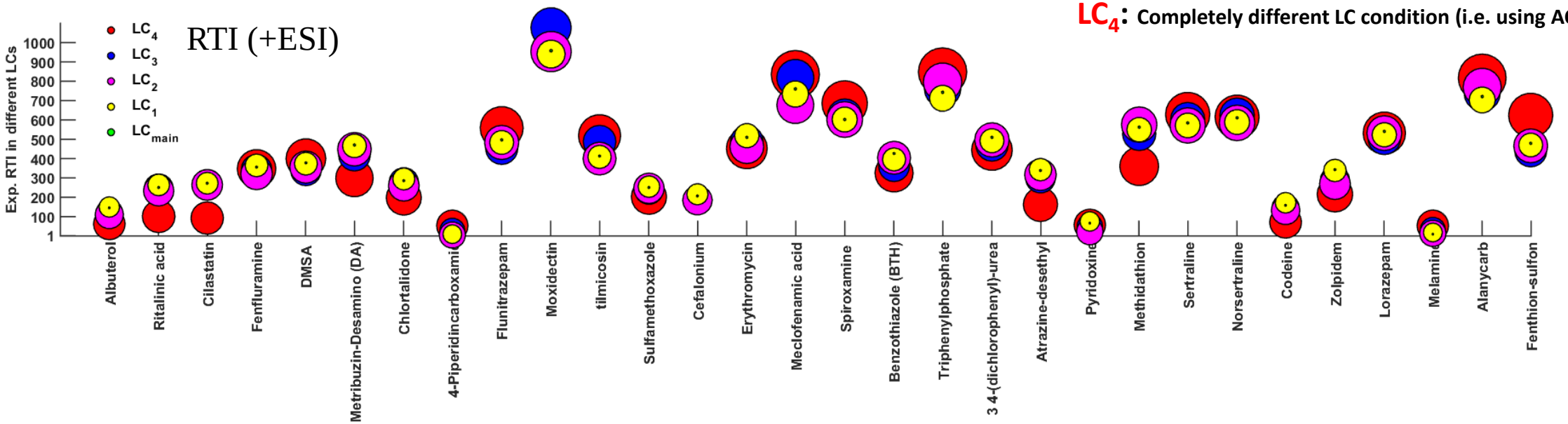
Name	tR_UOA	tR_Eawag	RTI_UOA	RTI_Eawag
Ranitidine	3.14	2.04	139.92	140.44
Atenolol	3.09	2.04	136.10	140.44
Gabapentin	3.78	3.01	188.80	198.67
Benzotriazol	4.76	3.79	263.65	245.49
Tramadol	4.88	4.03	272.82	259.90
Fenofibrate	12.55	13.81	858.64	846.99
Sotalol	2.96	1.85	126.17	129.03
Carbamazepin	5.81	6.52	343.85	409.37
Dimethenamid	9.26	8.74	607.36	542.64
Acetochlor	10.23	10.20	681.45	630.28
Fluoxetine	8.60	8.06	556.95	501.96

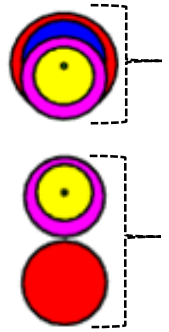
Compound Samples tR	tR_UJI	tR_UoA	RTI_UOA	RTI_UJI
BAYg5919	9.45	9.08	593.61	573.84
Clindamycinsulfoxide	6.10	5.18	295.73	363.78
14-Hydroxy-clarithromycin	8.65	7.32	459.18	523.68
Norcitalopram	7.87	6.63	406.48	474.77
α-Hydroxymidazolam	8.99	8.43	544.00	540.00
5-Hydroxy-propafenone	7.93	6.62	405.72	478.53
N-Desisopropyl-propafenone	7.2	5.96	355.31	432.76
Met D617 verapamil	7.38	6.09	365.24	444.04
N-Desmethyldiltiazem	8.69	5.83	345.38	526.19
O-Desmethyldiltiazem	7.01	7.30	457.66	420.84
N,N-Didesmethyldiltiazem	7.15	5.93	353.02	429.62
O-Deacetyldiltiazem	7.60	6.51	397.32	457.84
O-Deacetyl-O-desmethyl diltiazem	5.97	6.67	409.50	355.60
10-Hydroxycarbazepine	7.04	5.96	355.34	422.72
Norquetiapine	8.48	7.37	463.01	513.02
Quetiapine N-oxide	8.76	7.58	479.04	530.58
Met 590 clarithromycin	8.05	6.68	410.30	486.05
O-Desalkyl Quetiapine carboxylic acid	8.91	7.70	488.21	539.98

Cloud plot of unification rate for Exp. RTI (±ESI)



RTI (-ESI)





The larger it gets, the less accurate is the LC condition

RTI is reliable in all LC conditions except LC_4

LC_{main}: The main LC conditions used to develop dataset and RTI

LC_1: Same gradient elution program and mobile phase (MeOH:H2O), and different stationary phase

LC_2: Same mobile phase (MeOH:H2O), and different stationary phase and gradient elution program

LC_3: Different mobile phase (MeOH:H2O (different pH)) stationary phase and gradient elution program

LC_4: Completely different LC condition (i.e. using ACN)

For more details about the evaluation of UOA RTI system
and LC quality assessment as well as application of RTI
in non-target/suspect screening:

Thursday 22.6.2017 - NORMAN: Suspect and non-target screening with high resolution mass spectrometry– current status and new developments

10:45 Progress with the retention time prediction/index in liquid chromatography Prof. Nikolaos Thomaidis, UoA

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