

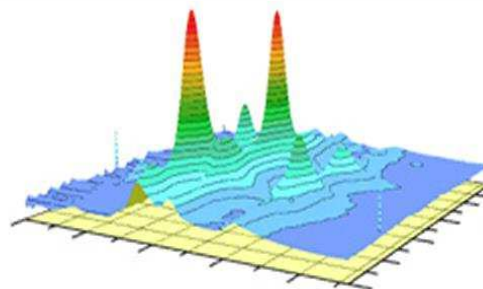


National and Kapodistrian University of Athens
School of Science, Department of Chemistry
Laboratory of Analytical Chemistry

Non-target Screening Workflows & Applications in Environmental Analysis

Prof. Nikolaos S. Thomaidis

University of Athens, Greece



Non-target Screening Instrumentation



- LC-ESI-QTOF
- GC-APCI-QTOF
- GC-EI-MS/MS



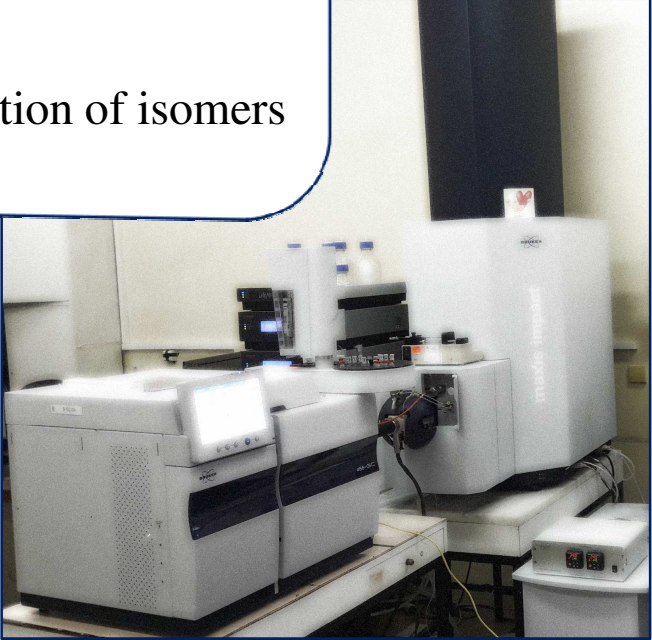
Multidimensional Chromatography–HRMS data



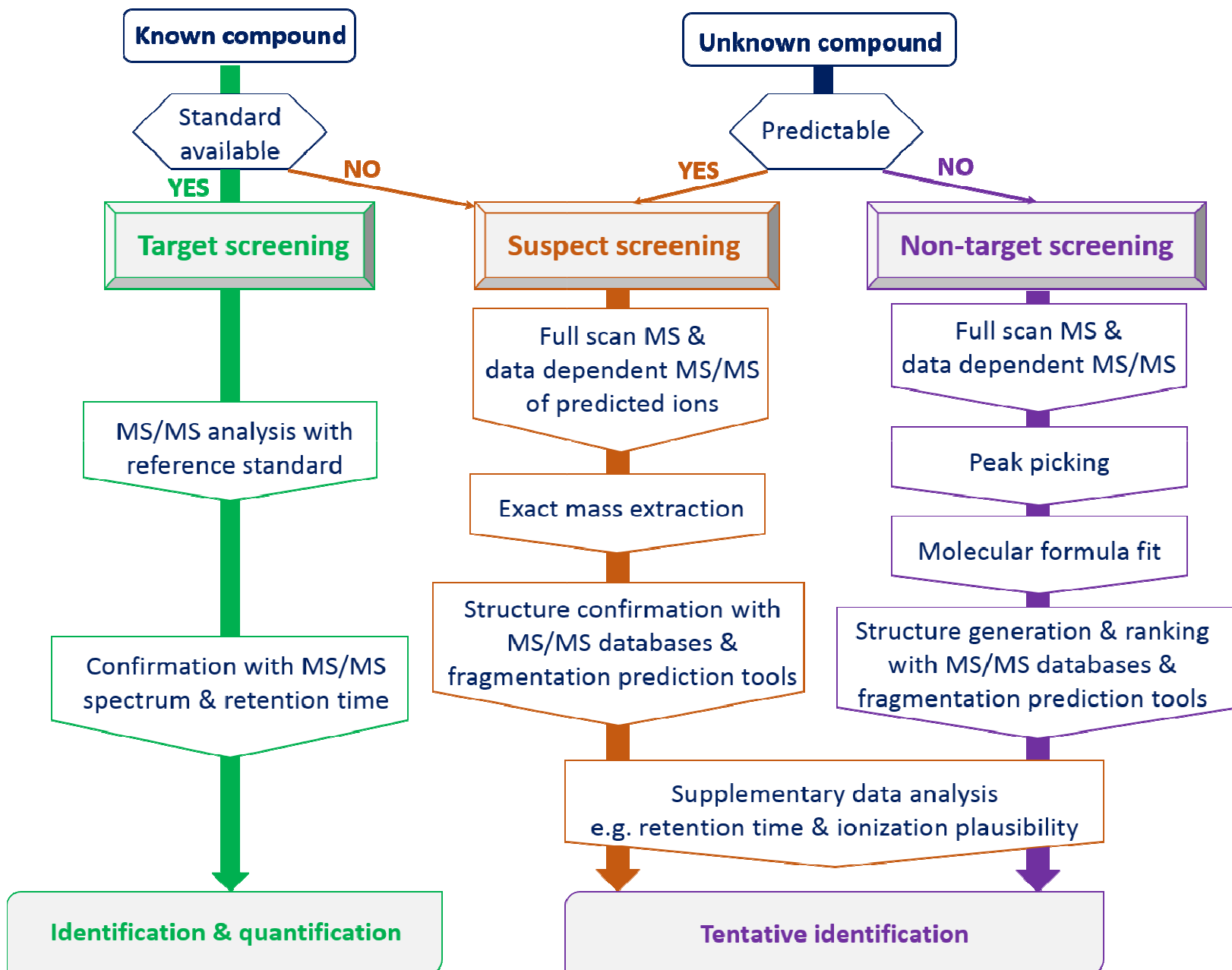
- polarity
- ionization type
- polymers
- thermostability
- separation of isomers

Coming soon...

- LC-TIMS-QTOFMS
- MALDI-TOFMS



Developed Workflows



Wide-scope target screening

Databases are continuously being updated

> **2,400** compounds databases for **LC-ESI-QTOFMS & GC-APCI-QTOFMS**

- Personal care products
- Steroids & hormones
- **Antibiotics (>50)**
- **Pharmaceuticals (>400)**
- **Illicit drugs**
- **New Psychoactive Substances (>450)**
- Industrial Chemicals (>90)
- Quaternary Ammonium Compounds
- Pesticides (>900)
- Stimulants
- Sweeteners
- Surfactants
- Biocides

Transformation Products/ Metabolites

- PAHs
- OCPs
- PCBs
- Pesticides
- PCNs
- PBDEs
- FAMES

Database Development

Precursor ion

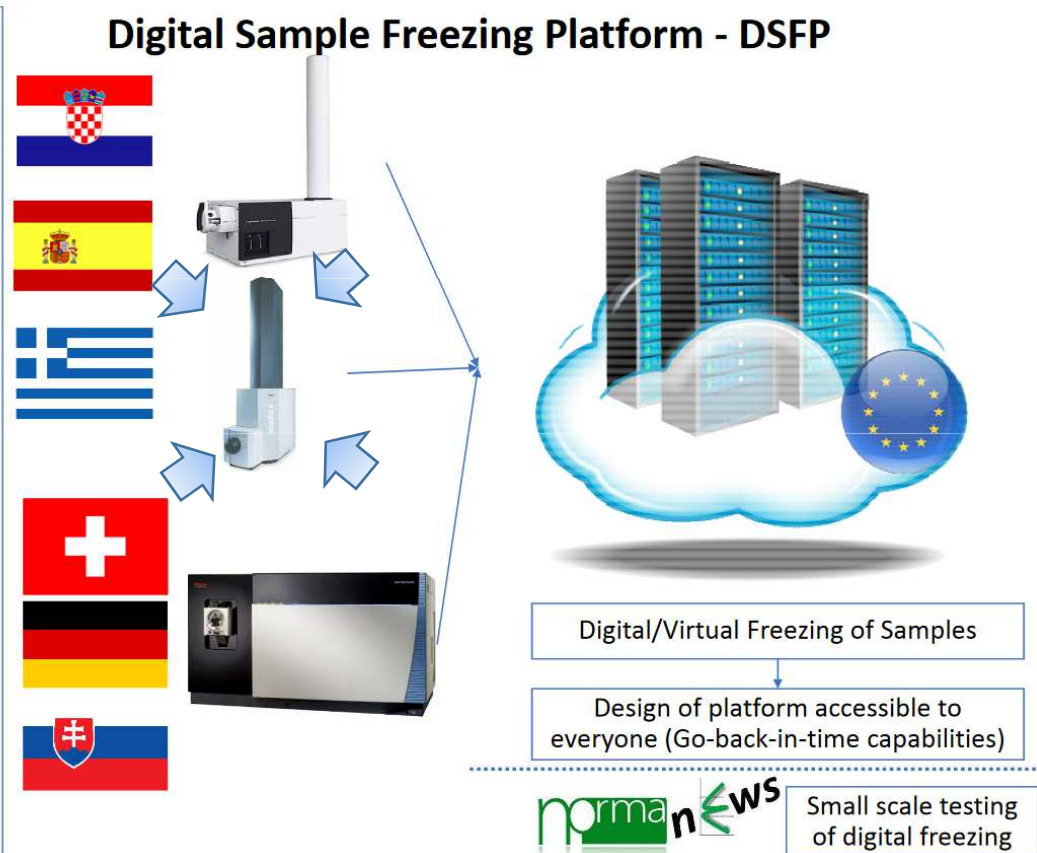
Adduct ions

Fragments

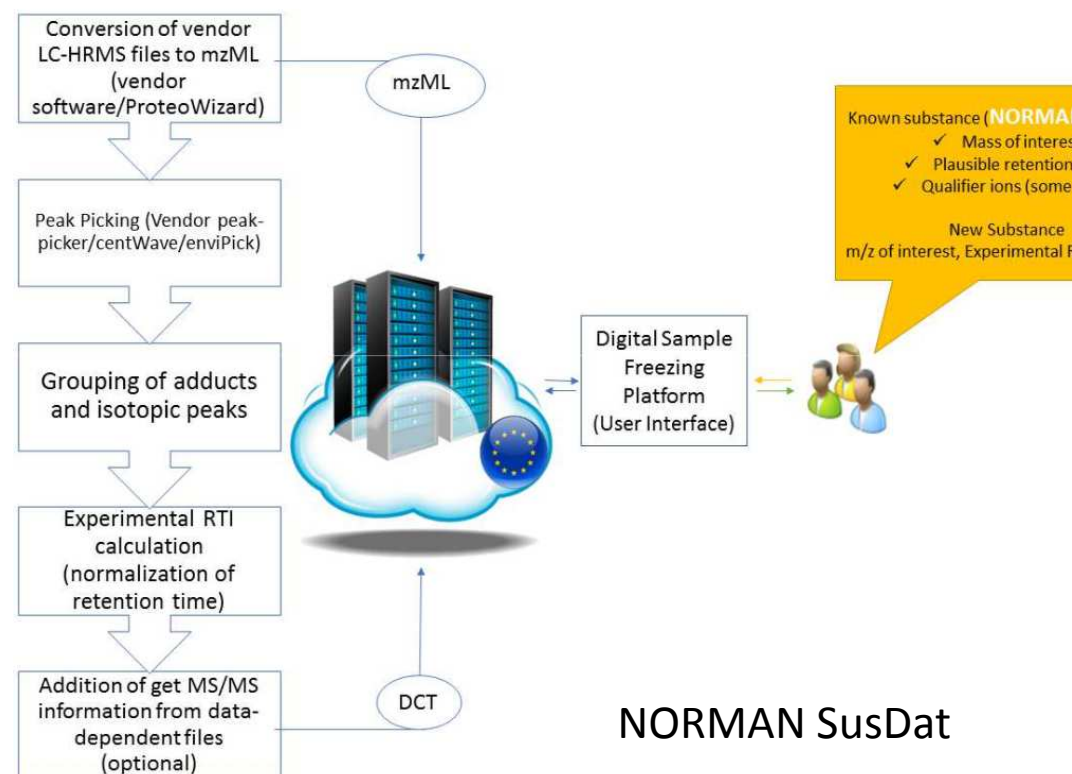
	A	B	C	D	E	F	G	H	I
1	m/z	RT	formula	name	Qual7 formula	Qual8 formula	Qual1	Qual2	Qual3
1880	584.2886	1.26	C21H41N7O12	Streptomycin-Dihydro					
1881	335.1754	4.16	C21H22N2O2	Strychnine			184.0757	264.1019	307.1441
1882	383.2493	12.21	C24H31F1N2O1	STS-135			135.1168	232.1132	206.134
1883	249.1022	13.61	C16H12N2O	Sudan I			156.0427	232.0982	128.0481
1884	277.1335	14.94	C18H16N2O	Sudan II					
1885	387.2101	7.31	C22H30N2O2S1	Sufentanil			238.126	111.0263	355.1839
1886	329.0245	5	C14H13ClO5S	Sulcotrione	C14H13ClO5SNH4 ⁺		139.0389	157.0492	111.0443
1887	277.0641	4.72	C13H12N2O3S	Sulfabenzamide		C13H12N2O3SNa ⁺	92.0495	108.0444	156.0114
1888	285.0208	4.56	C10H9ClN4O2S	Sulfachloropyridazine			120.0562	156.0118	108.0457
1889	313.0521	3.96	C12H13ClN4O2S	Sulfaclomide			158.048	108.0444	92.0495
1890	285.0208	5.45	C10H9ClN4O2S	Sulfaclozine			156.0118	108.0457	157.015
1891	251.0597	3.48	C10H10N4O2S	Sulfadiazine		C10H10N4O2SNa ⁺	92.0495	108.0444	96.0556
1892	255.0848	3.38	C10H6D4N4O2S	Sulfadiazine-d4					
1893	293.0703	4.11	C12H12N4O3S	Sulfadiazine-N4-Acetyl			136.0738	198.0203	293.0699
1894	315.106	5.46	C12H10D4N4O4S	Sulfadimethoxin-d4		C12H10D4N4O4SNa ⁺	308.176	263.1178	156.0763
1895	311.0809	5.6	C12H14N4O4S	Sulfadimethoxine			156.0762	218.0235	108.044
1896	353.0914	6.23	C14H16N4O5S	Sulfadimethoxine-N4-Acetyl					
1897	279.091	4.31	C12H14N4O2S	Sulfadimidine (Sulfamethazine)			122.0716	124.0872	126.0663
1898	283.1161	4.21	C12H10D4N4O2S	Sulfadimidine-d4					
1899	311.0809	4.75	C12H14N4O4S	Sulfadoxine		C12H14N4O4SNa ⁺	108.0444	92.0495	156.0114

Inspect screening workflows

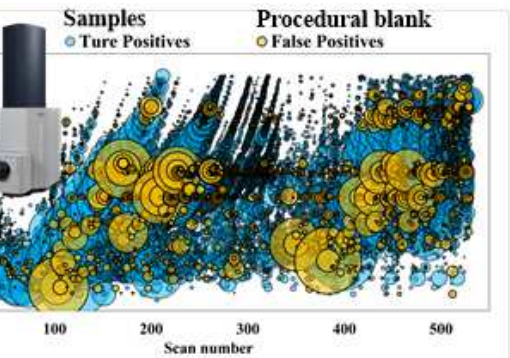
Digital Sample Freezing Platform (DSFP)



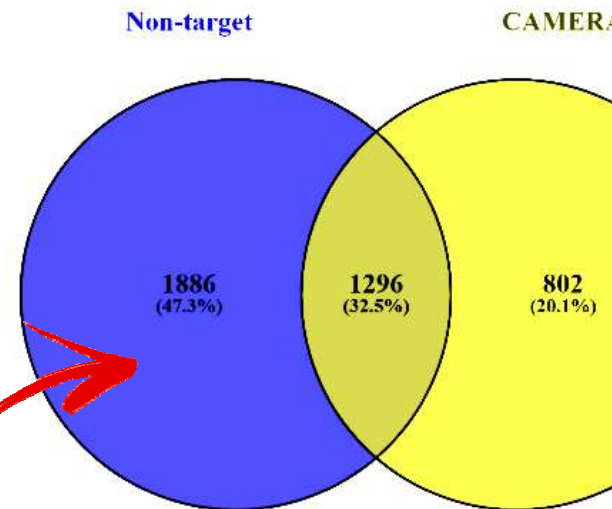
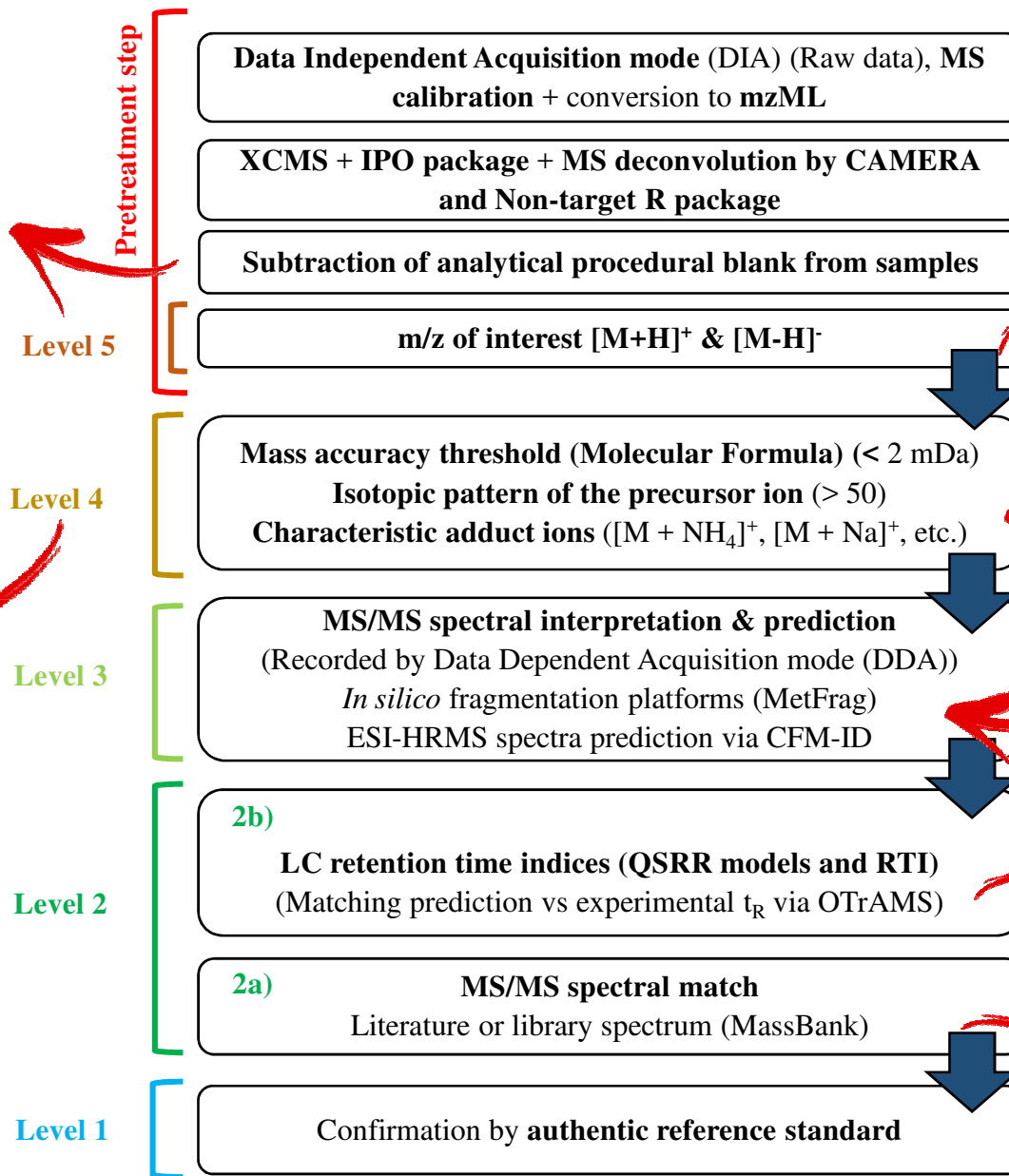
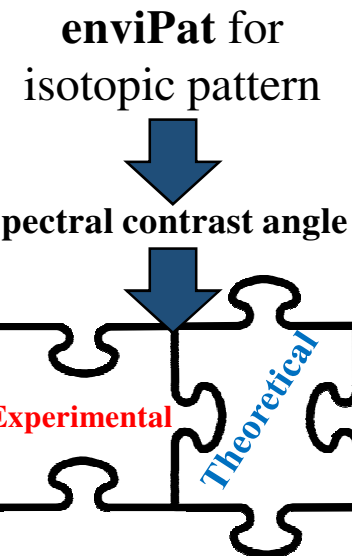
Digital Sample Freezing Methodology



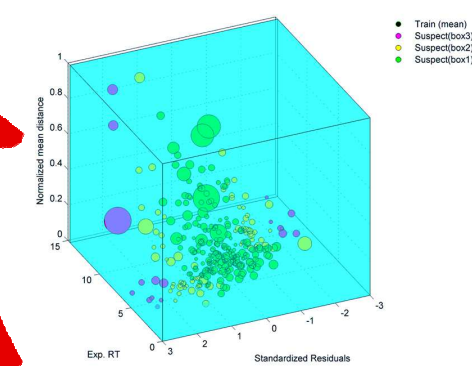
AutoSuspect



Deep Learning ANN for removal of procedural blank peaks



MS-ready Suspect list



Modified spectra similarity score

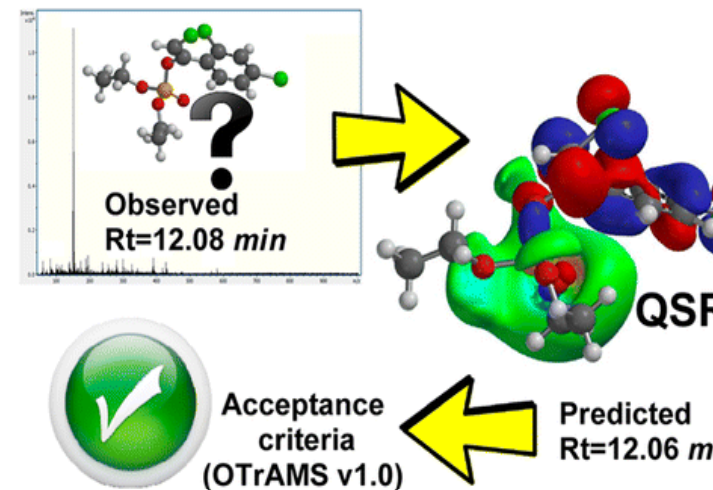
In-house developed retention time prediction tools supporting suspect and non-target screening

❖ Retention time prediction models

LC-ESI(+/-)-QTOFMS & HILIC-ESI(+/-)-QTOFMS

❖ Retention time index (RTI)

Harmonization of retention time among laboratories
(different instruments and/or chromatographic methods)



J. Chem. Inf. Model., 2016, 56, 1384–1398

Applications of retention time index prediction tools

NORMAN SusDat Database

EPA Chemistry Dashboard, Comp Tox *(to be applied)*

2 NORMAN Collaborative trials *(river water & indoor dust)* and ENTACT (EPA)

KWR Round Robin Test *(surface water, ground water and drinking water analysis via non-target screening)*

Numerous National & International Scientific Projects

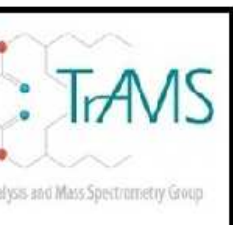
(EMBLAS II, Tremepol, WaterMicropol, Monitoring of Asopos river basin, HBM4EU, c.a.)

RTI Program for LC-(+/-)ESI-HRMS

UOA-RTI version 2.0.0

adeh, Emma L. Schymanski, Juliane Hollender, Martin Krauss, Maria
rtinez, Jaroslav Slobodnik, Nikolaos S. Thomaidis

atefully acknowledge the contributions of all those involved in the
of UOA-RTI system.



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was partially funded by NORMAN Joint Programme of Activities in 2016.

UOA-RTI

Select the ESI mode:
 +ESI -ESI

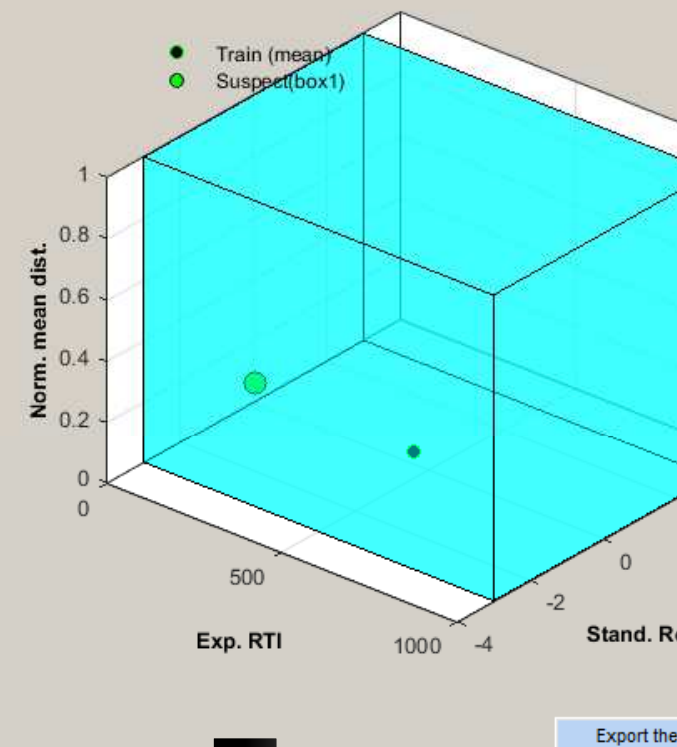
Select the RTI vs tR calibration mode:
 Automatic calibration mode
 Manual calibration mode

Single compound Batch mode

 Enter the tR value:

Select the Applicability domain method:
 Chemical space boundary OTrAMS

Selected ESI mode:
+ESI
 Calibration mode:
Automatic
 (Prediction limits at 95% CI)
 Excluded (high residuals):
EmamectinB1a
 Not observed:
Amitrol
Cefoperazone
Nigericin
 intercept: -86.431
 Slope: 62.3073
 R^2: 0.9593
 Compounds with Error:
None
 Name // Pred. RTI // Exp. tR// Pred. tR// AD
 x01: 6.4012 // 1.91 // 1.49 // Exp. & Pred. tR



	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Name	Name	SMILES	Pred. RTI	Exp. RT	Pred. RT	Box4	Side of AD	OTrAMS					
2	MK1	0477_Ben;OS(=O)(=C		148.3802	0.42	3.651883								Box2: tR is accepted in model developed in negative ESI:Th
3	MK2	0424_topa C[C@H](C		446.4338	0.42			9.698718816						Outside of applicability domain of model in negative ESI
4	MK3	2565_Mes NC1=CC(C		127.5231	0.42	3.22874								Box2: tR is accepted in model developed in negative ESI:Th
5	MK4	0053_2,4-(Nc1ccc(c(108.2699	0.42	2.838135								Box2: tR is accepted in model developed in negative ESI:Th
6	MK5	0423_5-FH FC1=CNC(108.2436	0.42	2.837602								Box2: tR is accepted in model developed in negative ESI:Th
7	MK6	2,4-diamii C1=CC(=C		108.2699	0.42	2.838135								Box2: tR is accepted in model developed in negative ESI:Th
8	MK7	2590_2-th O=C1NC(=		166.6703	0.42			4.02294709						Outside of applicability domain of model in negative ESI
9	MK8	Adenine NC1=C2N=		81.76027	0.44	2.300314								Box2: tR is accepted in model developed in negative ESI:Th
10	MK9	Dipicolini(O=C=O)C1		41.74257	0.42	1.488445								Box1: tR is accepted in model developed in negative ESI
11	MK10	Maleimidi O=C1NC(=		109.0083	0.42	2.853115								Box2: tR is accepted in model developed in negative ESI:Th
12	MK11	0919_Acyc COC(=O)c		403.1421	0.547216		8.820427							Box4: tR can not be accepted for the given structure in the
13	MK12	2595_Oxy O=C1NC(=		63.57966	0.547216	1.93147								Box1: tR is accepted in model developed in negative ESI

RTI website for LC-(+/-)ESI-HRMS (coming soon...)

Development and Prediction of Retention Time Indices for LC-HRMS (version 2.0.0)

National and Kapodistrian University of Athens

Select the target ESI:

- +ESI
- ESI

Select the RTI versus tR calibration mode:

- Prediction limits
- Auto-calibrate
- Manual

Upload RTI calibrants data...

Browse... Template_calibrants_(+ESI).csv

Upload complete

Default max. file size 1MB (*.csv file)

[Click here to build the calibration curve...](#)

[Click here to restart the current session...](#)

[Trace Analysis & Mass Spectrometry Group](#)

[NORMAN Suspect list Exchange website](#)

Retention Time Indices for LC-HRMS (version 2.0.0):

[About](#) [Chemical Conversion](#) [Calibrants](#) [Single compound](#) [Batch mode](#) [Comparison of Experimental RTIs](#)

Select the uncertainty measurement:

- OTrAMS
- Chemical space boundary

Enter the SMILES of a compound here:

NC(C)C(O)C1=CC=C(C)C=C1

Enter the tR for specific ESI mode

5.12

SMILES: NC(C)C(O)C1=CC=C(C)C=C1

Experimental tR: 5.12

The ESI selected: +ESI

Estimate RTI & its uncertainty

Experimental RTI: 233.5

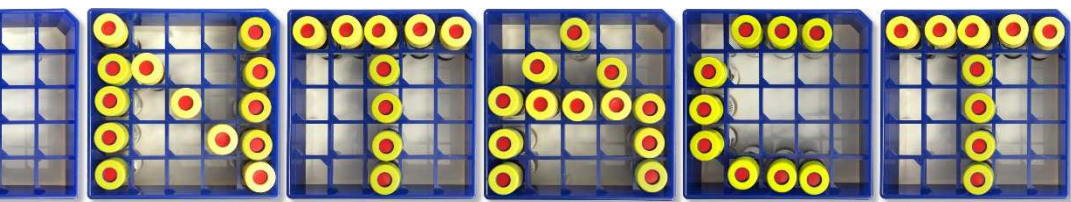
Predicted RTI: 157.43

Experimental tR: 5.12 min

Predicted tR: 3.91 min

Uncertainty: Exp. & Pred. tR are accepted for this candidate (box1)

RTI vs tR calibration curve: $RTI = 62.9984 \cdot tR - 89.0499$ >>> $(R^2 =) 0.969$

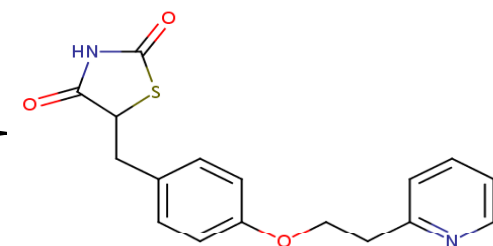
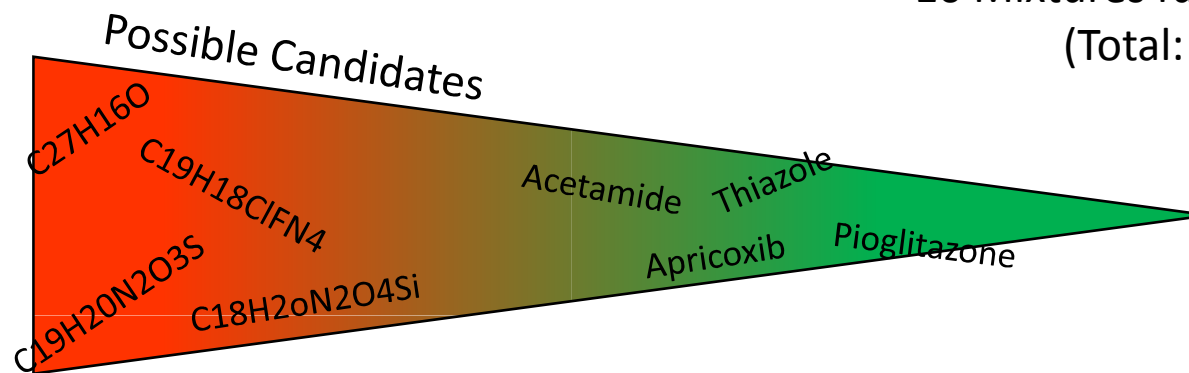
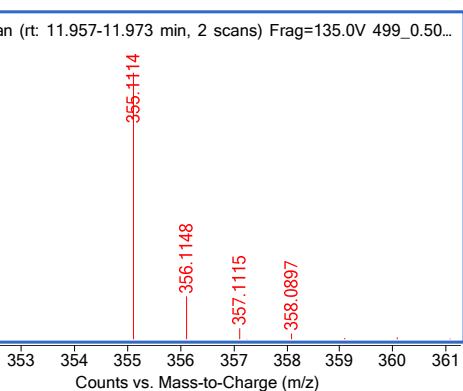


The Trial Samples:

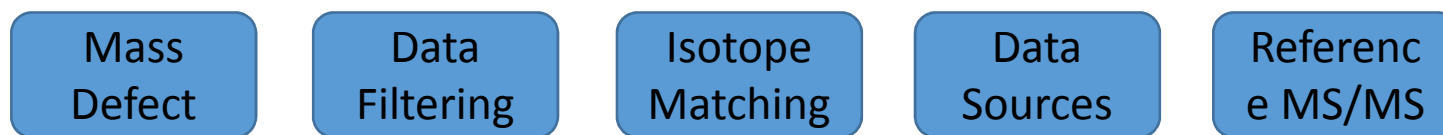


NTA Non-Targeted Analysis Collaborative Trial

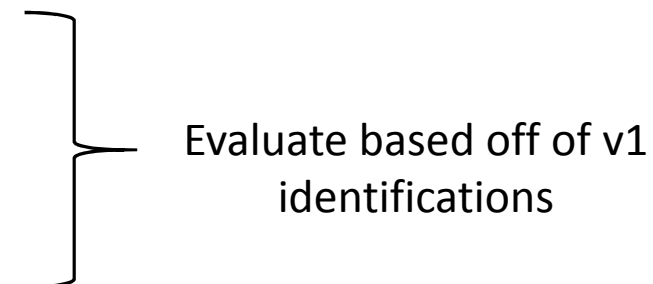
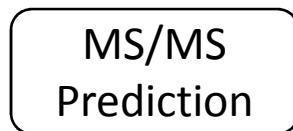
10 Mixtures ranging from 95 to 365 compounds
(Total: 1269 unique compounds)



Current NTA tools:
(EPA ENTACT v1)

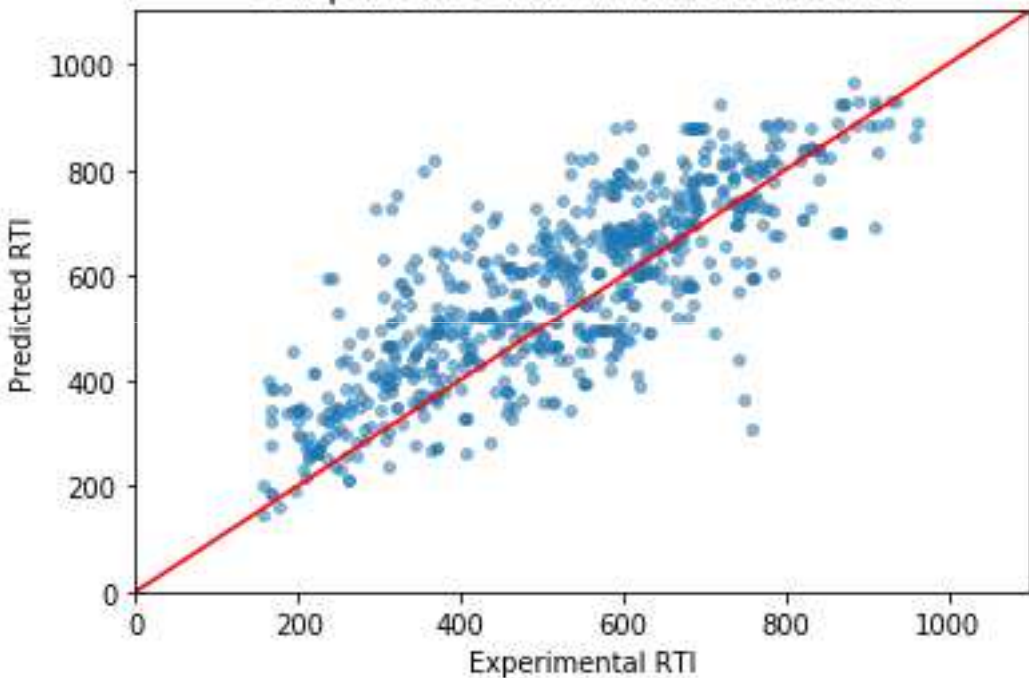


Potential NTA tools:

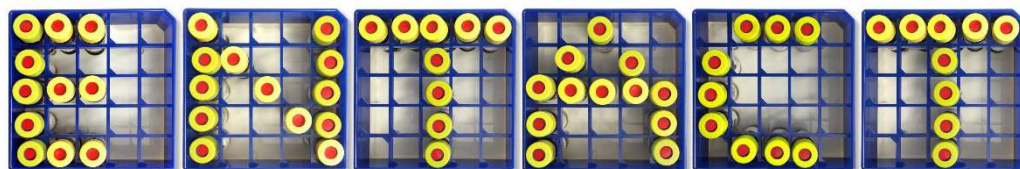
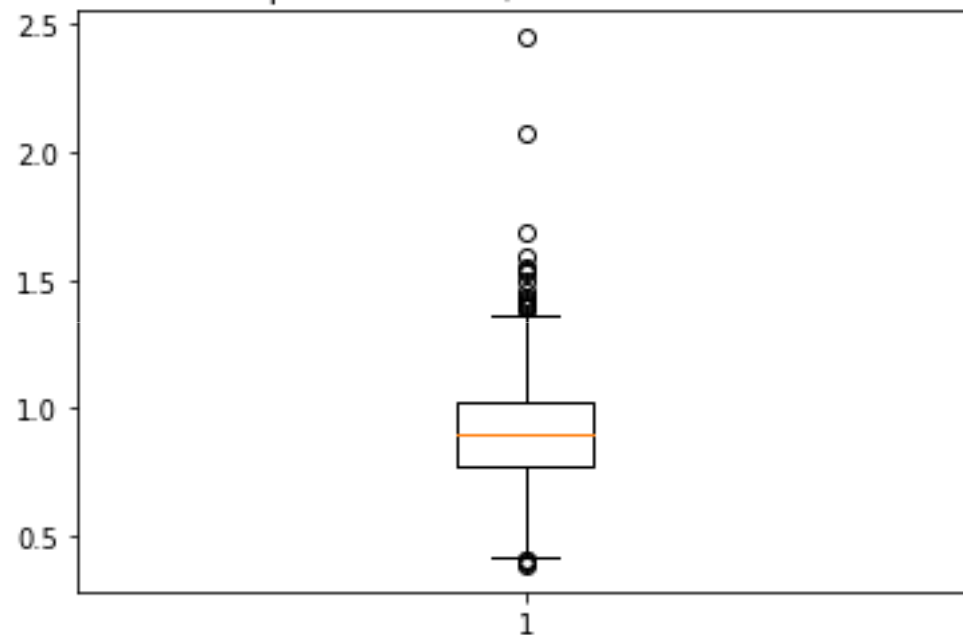


Original MeOH Data (positive calibrants, +ESI compounds)

Comparison of RTI for ENTACT v1 Passes



Experimental RTI/Predicted RTI Ratio

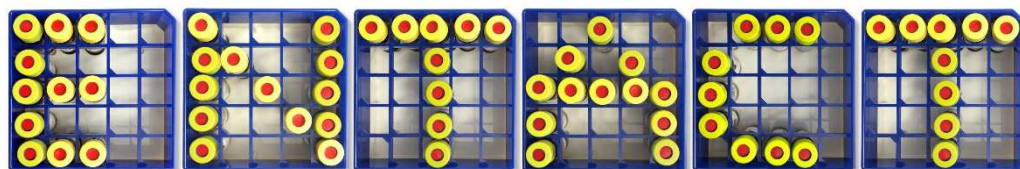
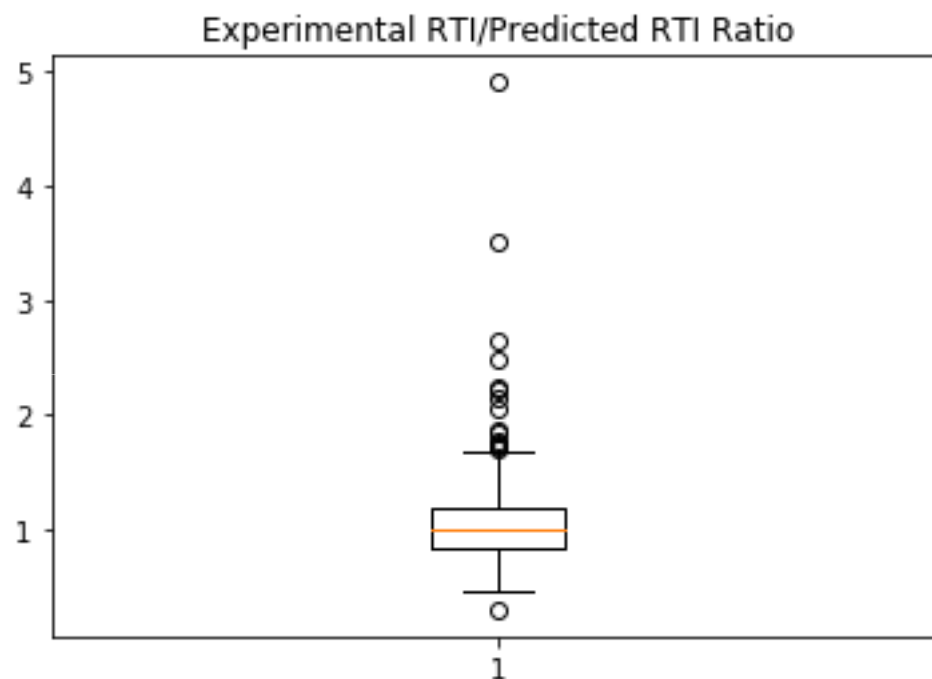
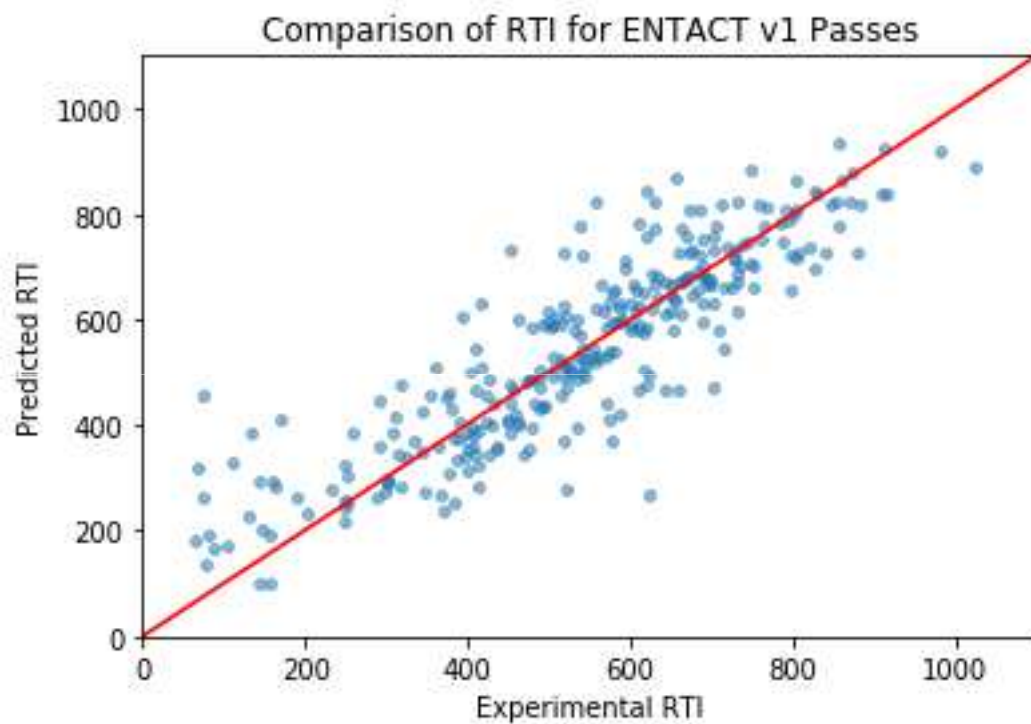


Median: 0.901

2.5 percentile: 0.535

97.5 percentile: 1.367

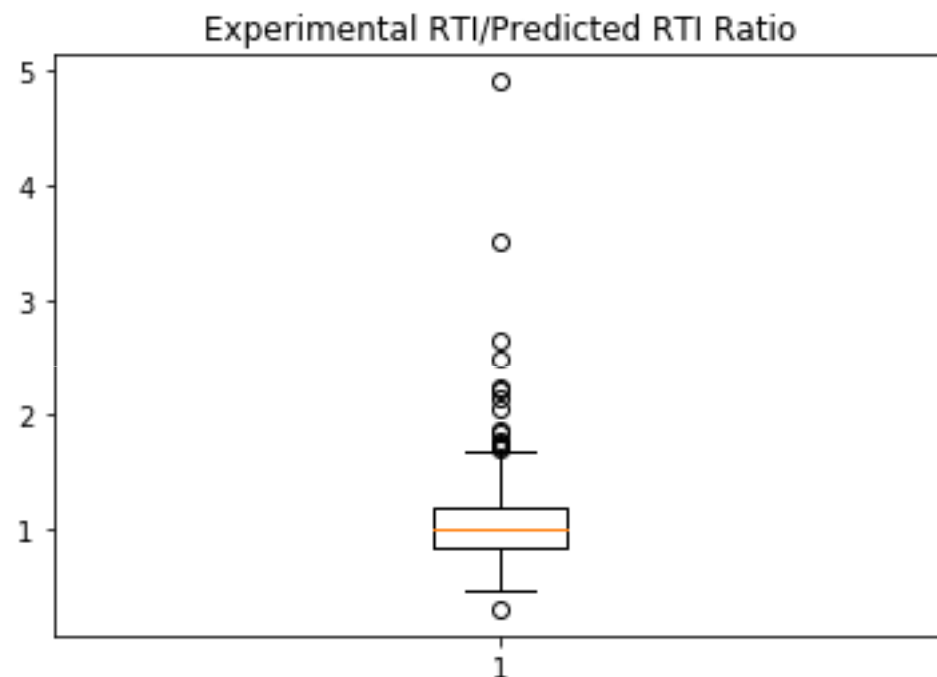
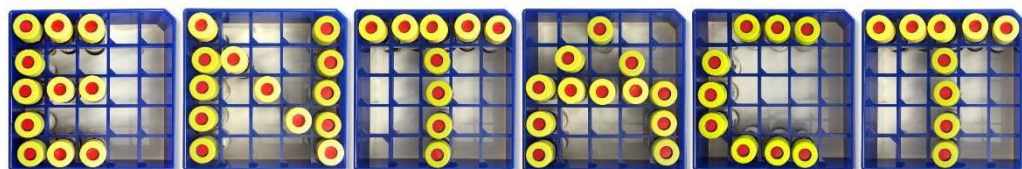
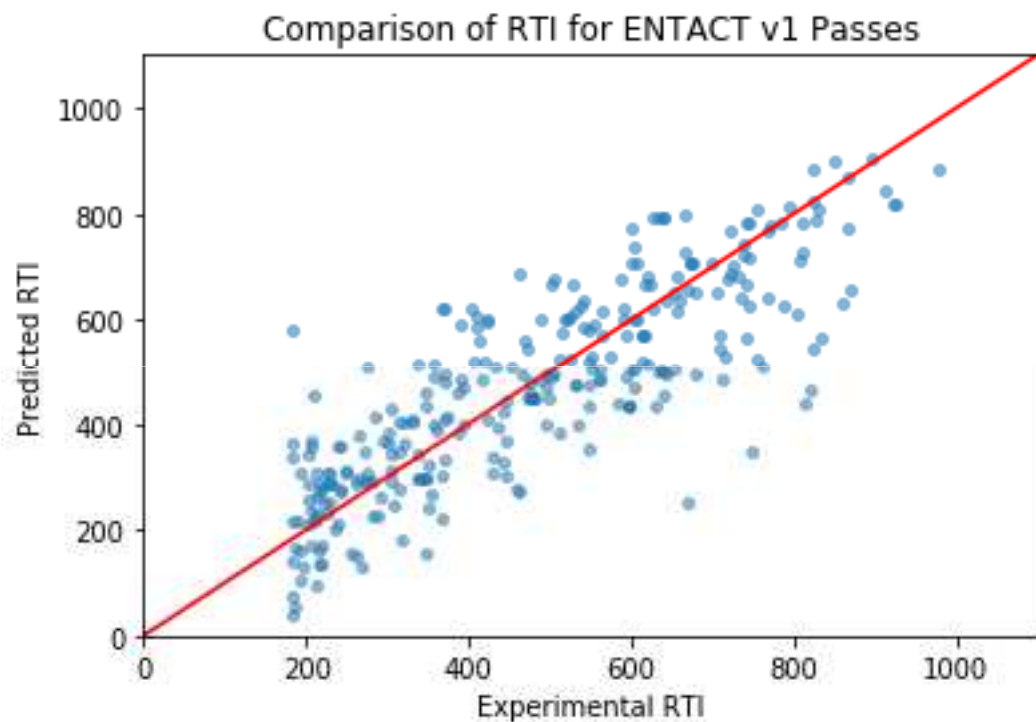
Updated ACN Data* (positive calibrants, all compounds)



Median: 0.997
2.5 percentile: 0.458
97.5 percentile: 1.450

Chromatography changed to Acetonitrile/Water with formic acid for positive mode analysis.

Original MeOH Data (negative calibrants, -ESI compounds)



Median: 1.000
2.5 percentile: 0.588
97.5 percentile: 2.007

Non-target screening workflow

Pretreatment step

Data Independent Acquisition mode (DIA) (Raw data), MS calibration + conversion to mzML

XCMS + IPO package + MS deconvolution by CAMERA and Non-target R package

Subtraction of analytical procedural blank from samples

Prioritization of the peak list by Chemometrics

m/z of interest

Mass accuracy threshold (Molecular Formula) (< 2 mDa)
Isotopic pattern of the precursor ion (> 50)
Characteristic adduct ions ($[M + NH_4]^+$, $[M + Na]^+$, etc.)

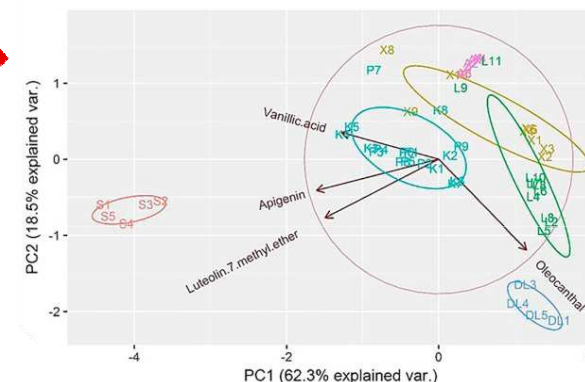
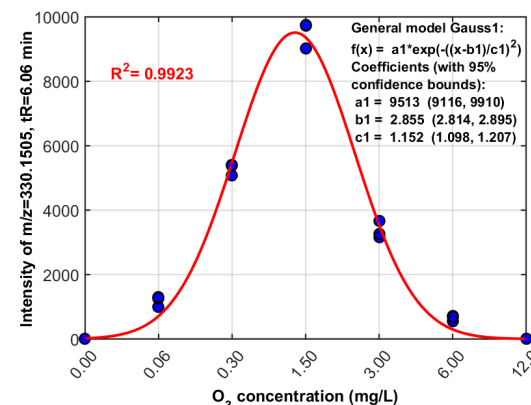
MS/MS spectral interpretation & prediction
(Recorded by Data Dependent Acquisition mode (DDA))
In silico fragmentation platforms (MetFrag)
ESI-HRMS spectra prediction via CFM-ID

2b) LC retention time indices (QSRR models and RTI)
(Matching prediction vs experimental t_R via OTrAMS)

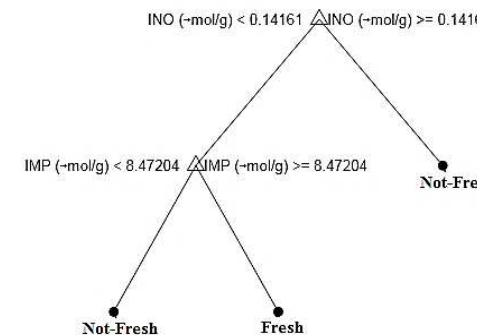
2a) MS/MS spectral match
library spectrum (MassBank, GNPS, MONA, mzClouds)

Confirmation by authentic reference standard

The screenshot shows the ChemoTrAMS software interface. It includes sections for 'Load MZ / Features' with options for supervised and unsupervised methods, 'Parameters For Features Selection' (Fitness, Iterations, Population, Number of MZ to be selected), 'Supervised Classification Methods' (Linear Discriminative Analysis, Random Forest, Support Vector Machine, Naive Bayes, Extreme Learning Machine, Artificial Neural Network, PLS-DA, Kohonen Self-Organizing Map), and 'Unsupervised Classification Methods' (Principal Components Analysis, Affinity Propagation). There are 'Export' and 'Plot' buttons at the bottom right, and 'Proceed', 'Save', 'Manual', 'About', 'Reset', and 'Exit' buttons at the bottom.



Logos for the Environmental Protection Agency (EPA), CompTox Dashboard, ChemSpider (The free chemical database), and PubChem.



Application of screening workflows

Samples

- Wastewater (influent & effluent)
- Sewage sludge
- Surface water
- Biota (fish, mussels)
- Sediment
- Soil



Risk Assessment

- Risk assessment of emerging contaminants using in-house acute toxicity model (ACO-SVM QSTR model)
- Incorporation of QSTR models in Ecotox module



Environmental Analysis – Projects completed



(2012-2015)

Transformation Products of Emerging Pollutants in the aquatic environment



(2012-2015)

Investigation of organic micropollutants' fate in wastewater treatment and study of their behavior during wastewater disposal to the aquatic environment



(2016-2017)

Environmental assessment of the use of restored Solid Waste Dump in Chios as shelter facility - UNHCR
EQS 2013/39/EC target screening (LC-QqQ/HRMS, GC-QqQ)

EMBLAS Environmental Monitoring in the Black Sea (UNDP, 2016-2018)

- I. EQS 2013/39/EC target screening (LC-QqQ, GC-QqQ)
- II. Wide-scope target screening of (>2,100 chemicals, LC-QToFMS)
- III. Non-target screening (LC-QToFMS, GC-APCI-QToFMS, GC-EI-MS)



seawater



sediment



biota

Environmental Analysis – NEW Projects

Monitoring of drinking water reservoirs:

Assessment of contamination of three lakes nearby Attica (**EYDAP**, 2018-2019)



Monitoring of Asopos river basin: Assessment of pollution/contamination and qualitative – quantitative investigation of contamination levels and possible sources of pollution (Region of Attica, 2018-2020)

- I. EQS 2013/39/EC target screening (**LC-QqQ, GC-QqQ**)
- I. Wide-scope target screening of (>2,300 chemicals, **LC-QToFMS, GC-APCI-QToFMS**)
- II. Non-target screening (**LC-QToFMS, GC-APCI-QToFMS, GC-EI-MS**)



river water



ground water



drinking water



sediment

Environmental Analysis – NEW Projects



“**LIFE APEX** - Systematic use of contaminant data from apex predators and their prey in chemicals management (2018-2022)”



Chemical contaminants in apex predators and prey samples

Prioritization of chemicals for monitoring activities

Time-trend analysis

**Wide scope target analysis, Suspect & Non-target screening
(LC-QToFMS & GC-APCI-QToFMS)**



Collaborative Trials (CTs)

2014

I. NORMAN CT - “Non-target Screening of organic substances in river water”

2016

II. NORMAN CT - “Non-target and suspect screening methods for organic substances in indoor dust”

- 25 institutes (EI, UFZ, EAWAG, EMEA, INIRIS, UoA etc.) from 16 countries



Analytical and Bioanalytical Chemistry

August 2015, Volume 407, Issue 21, pp 6237–6255 | Cite as

Non-target screening with high-resolution mass spectrometry: critical review using a collaborative trial of river water analysis

Authors

Authors and affiliations

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Peter Haglund, Thomas Letzel, Sylvia Grosse, **Nikolaos S. Thomaidis**, Anna Bletsou, Christian Zwiener, María Ibáñez,

Tania Portolés, [show 13 more](#)

Review

First Online: 15 May 2015

8

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4.4k

Downloads

104

Citations

Schymanski, E.L., Singer, H.P., Slobodnik, J. et al. Anal Bioanal Chem (2015) 407: 6237–6255

<https://doi.org/10.1007/s00216-015-8681-7>



NORMAN Collaborative Trial

Non-target and suspect screening methods for
organic substances in indoor dust

Background

This Collaborative Trial is organised by the NORMAN Association (www.norman-network.net) as part of its Joint Programme of Activities for the year 2015. The activity is a follow-up action to the discussion in the NORMAN General Assembly and a meeting of the Working Group on Emerging Substances in the Indoor Environment in Amsterdam (2014), where one of the recommendations was to organize a collaborative trial on **non-target and suspect screening methods for organic substances in indoor dust** in Europe.

Contributions in MS databases

➤ MassBank of North America

mona.fiehnlab.ucdavis.edu

Search MoNA

Search Spectra Browse Spectra Issue Tracker

Welcome to MoNA!

MassBank of North America (MoNA) is a metadata-centric, auto-curating repository designed for efficient storage and querying of mass spectral records. It intends to serve as the framework for a centralized, collaborative database of metabolite mass spectra, metadata and associated compounds. MoNA currently contains over 200,000 mass spectral records from experimental and in-silico libraries as well as from user contributions.

MoNA has recently been redesigned, with significant improvements to server-side architecture, query structure, and search speed. We are actively improving and adding features, so please be patient as functionality is added. If you notice any major issues, feel free to report them using the issue tracker linked below.

News

Fiehn HILIC Library

May 10, 2018
The Fiehn Lab HILIC MS-MS and retention time library has been uploaded, consisting of over 1,100 standards acquired on both Q Exactive and TripleTOF.

Improved Spectrum Browser

September 8, 2017
The spectrum browser has been updated with improved panel and table views. The infinite scroll has been replaced with pagination to make browsing spectra easier; the panel view now displays the most valuable metadata first, and the table view has fully customizable columns.

MSP Exports Updated

August 31, 2017
The MSP exports on the downloads page have been restructured to include as much metadata as possible while remaining fully compliant with NIST MS Search format, and is now compatible with MS-DIAL v2.70.

Submitter High Scores

Name	Avg. Score	Spectra
🏆 Q Tobias Schulze	★★★★★	2,867
🏆 Q Stephan Beisken	★★★★★	58
🏆 Q Nikolaos Thomaidis	★★★★★	1,492
4 Q Martin Krauss	★★★★★	622
5 Q Emma Schymanski	★★★★★	11,656
6 Q Alex Svatos	★★★★☆	691
7 Q Dejan Nikolic	★★★★☆	6
8 Q Kourosh Hoshmand	★★★★☆	20
9 Q Megan Showalter	★★★★☆	3,290
10 Q Ryo Taguchi	★★★★☆	2,563

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➤ MassBank EU

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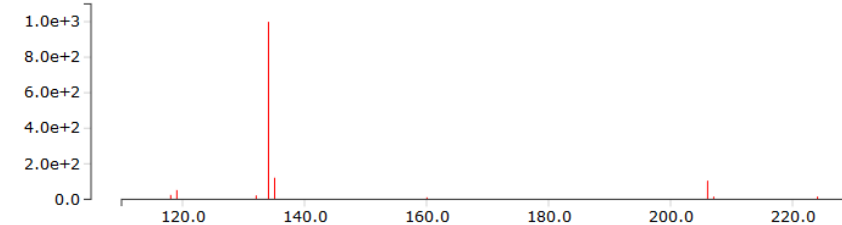
Contributor : [AAFC \(292\)](#) [Athens Univ. \(1,492\)](#)
[CASMI2016 \(622\)](#) [Chubu Univ. \(2,563\)](#)
[Eawag Additional Specs \(895\)](#) [Env Anal Chem, U Tuebingen \(119\)](#)

MassBank Record: AU206203

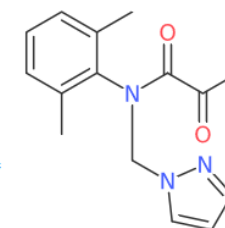
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Metazachlor-OXA; LC-ESI-QTOF; MS2; CE: 30 eV; R=35000; [M+H]⁺

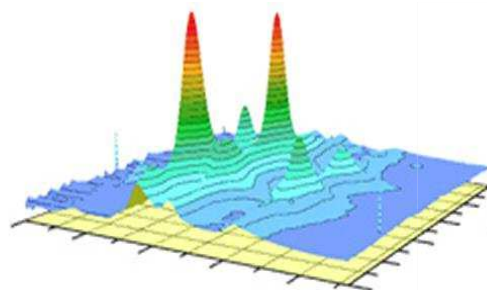
Mass Spectrum



Chemical Structure



*Thank you very much
for your attention!*



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