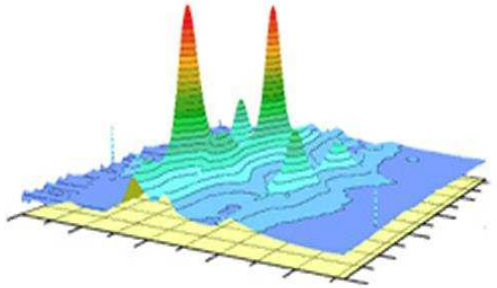
National and Kapodistrian University of Athens
School of Science, Department of Chemistry
Laboratory of Analytical Chemistry

Non-target Screening Workflows & Applications in Environmental Analysis

Prof. Nikolaos S. Thomaidis

University of Athens, Greece

TrAMS
Trace Analysis and Mass Spectrometry Group



Non-target Screening Instrumentation

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

Multidimensional Chromatography–HRMS data

polarity

thermostability

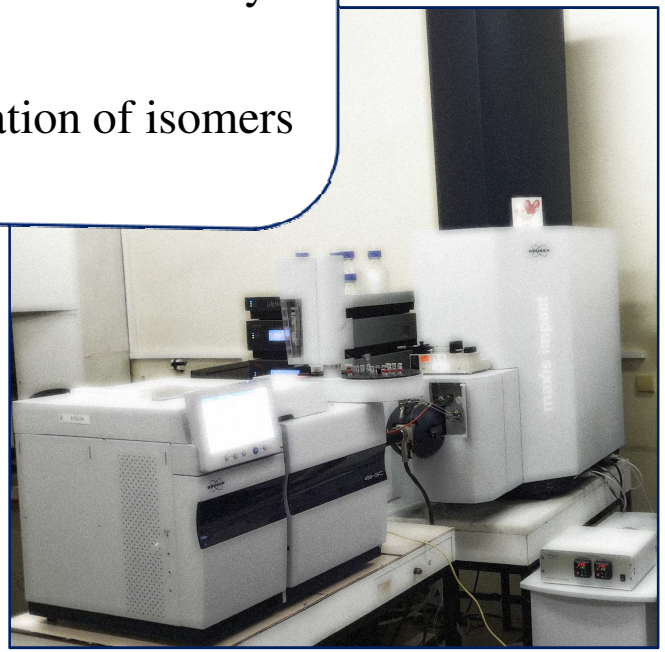
ionization type

separation of isomers

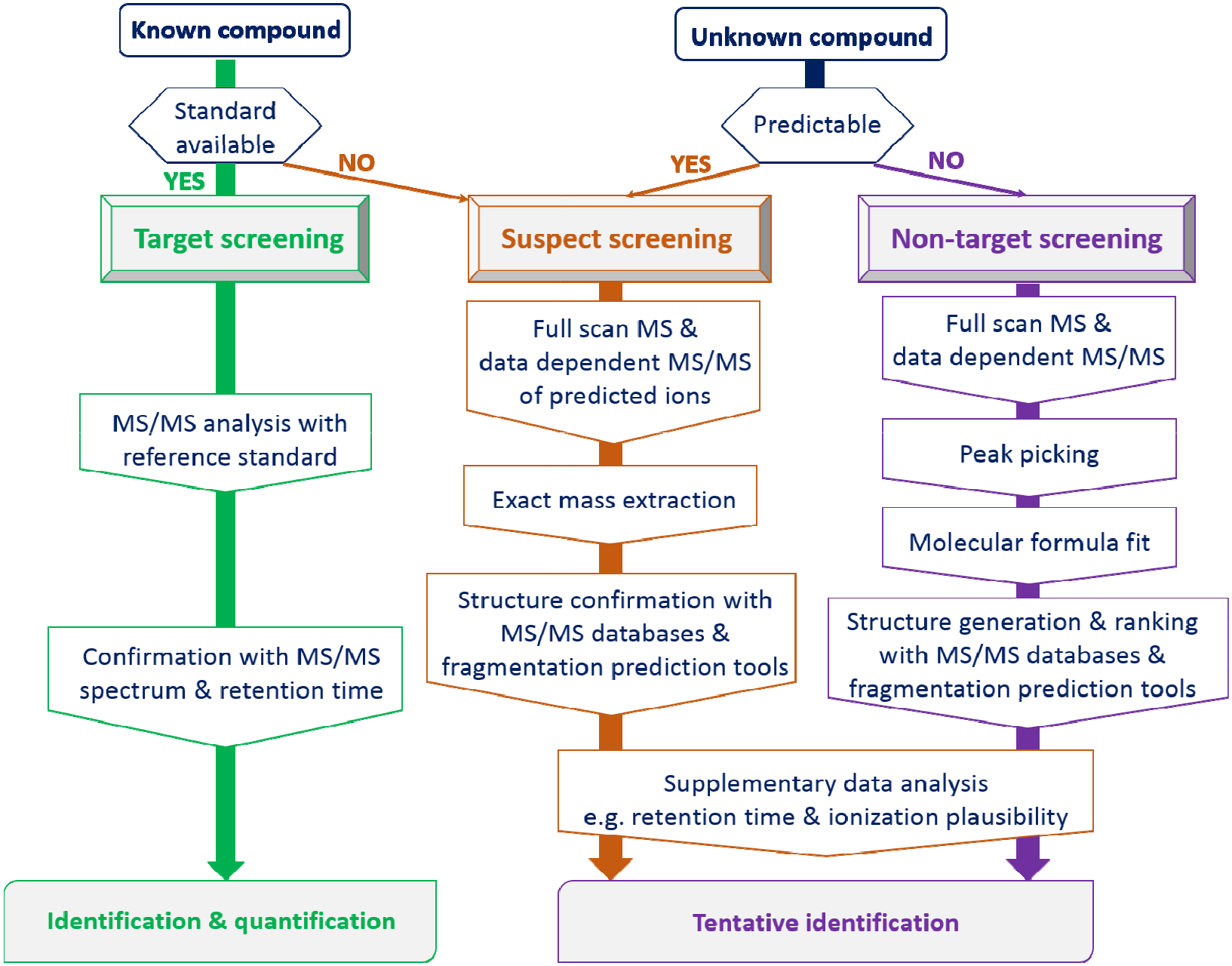
polymers

Coming soon…

- LC-TIMS-QTOFMS
- MALDI-TOFMS



eveloped Workflows



Wide-scope target screening

Databases are continuously being updat

> **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

atabase Development

Precursor ion

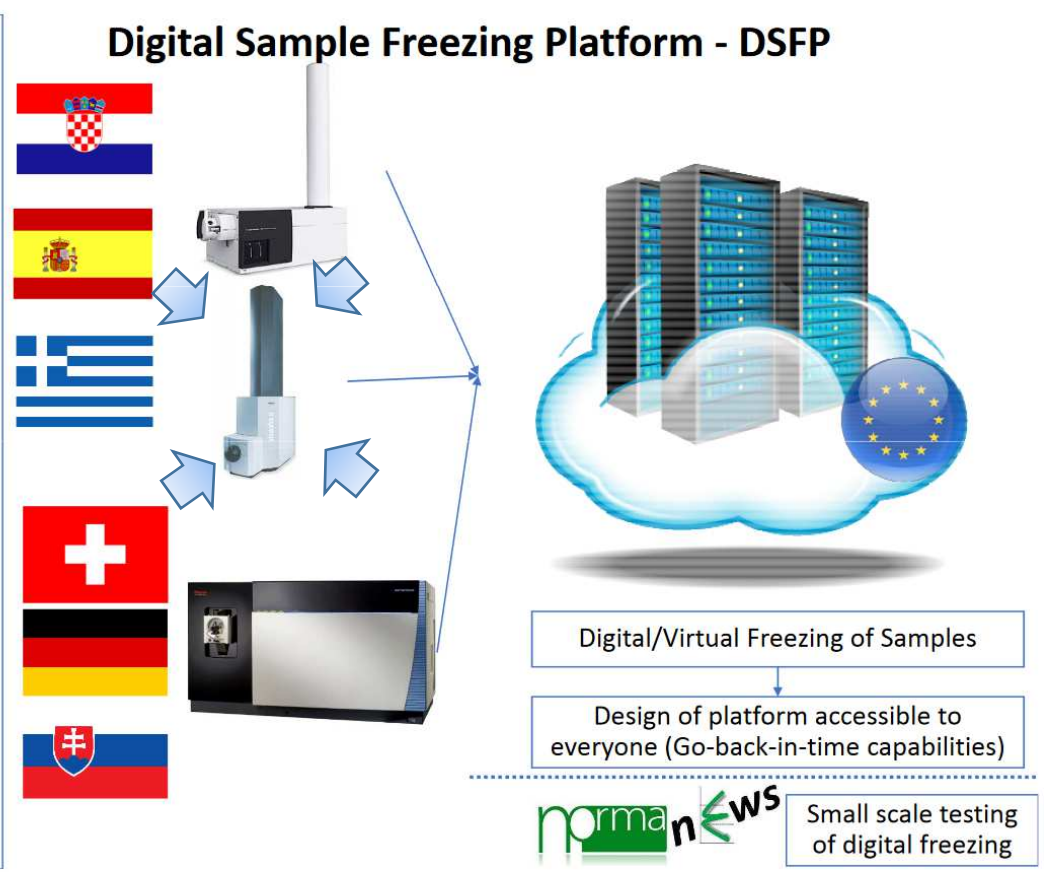
Adduct ions

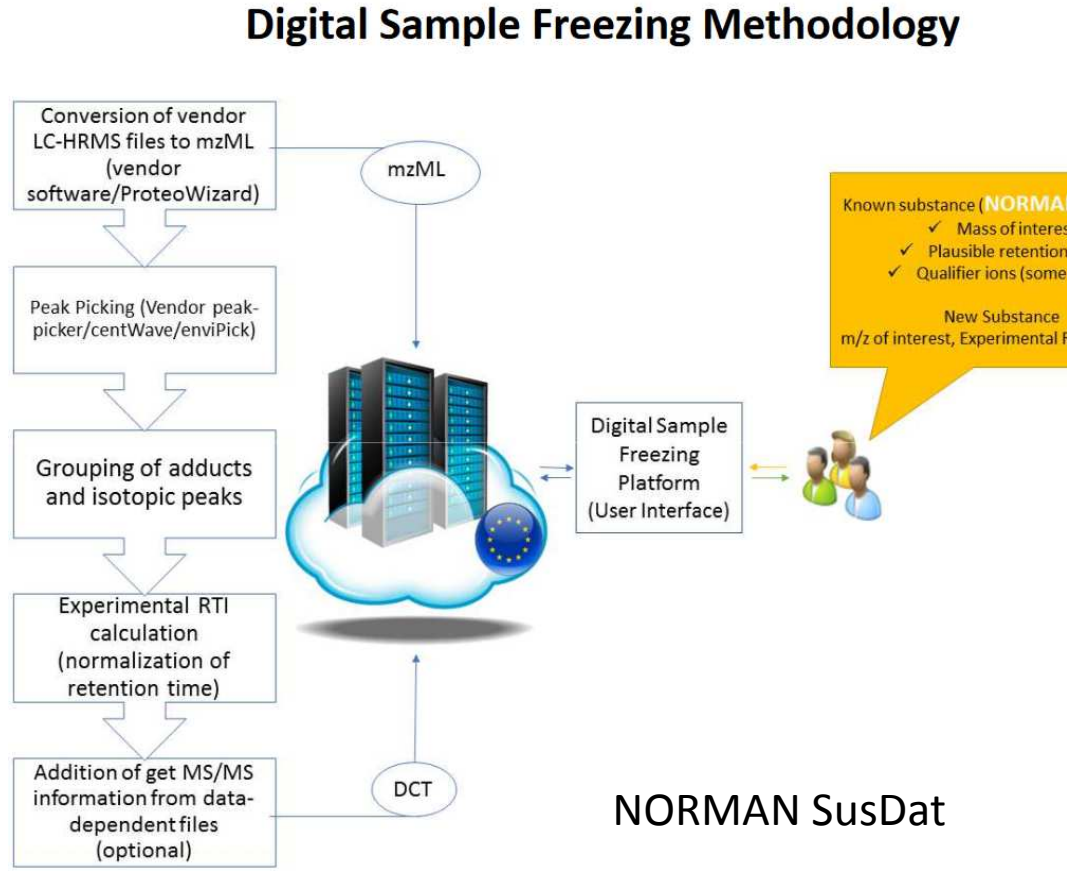
Fragments

	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	C14H13ClO5SNH4^1+		139.0389	157.0492	111.0443
1887	277.0641	4.72	C13H12N2O3S	Sulfabenzamide		C13H12N2O3SNa^1+	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^1+	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^1+	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^1+	108.0444	92.0495	156.0114

ıspect screening workflows

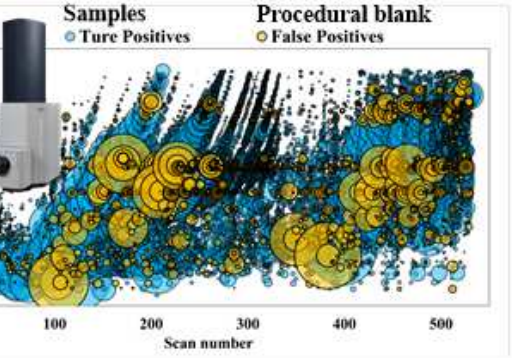
Digital Sample Freezing Platform (DSFP)





NORMAN SusDat

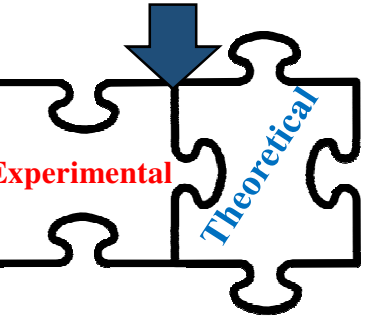
AutoSuspect



eep Learning ANN for removal of procedural blank peaks

enviPat for isotopic pattern

pectral contrast angle



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

Level 5 — **m/z of interest $[M+H]^+$ & $[M-H]^-$**

Level 4 — **Mass accuracy threshold (Molecular Formula)** (< 2 mDa)
Isotopic pattern of the precursor ion (> 50)
Characteristic adduct ions ($[M + NH_4]^+$, $[M + Na]^+$, etc.)

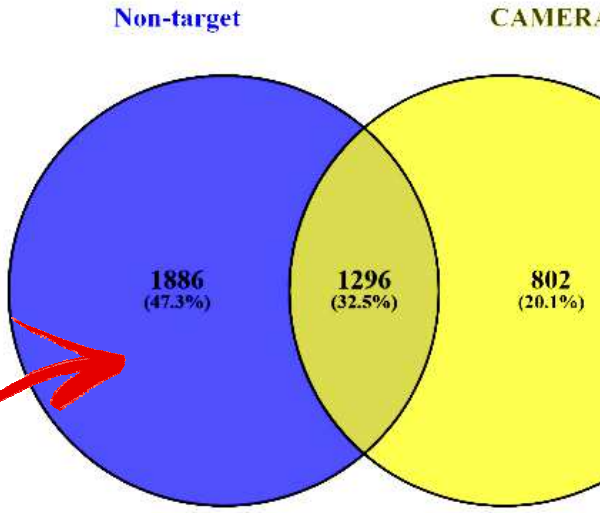
Level 3 — **MS/MS spectral interpretation & prediction**
(Recorded by Data Dependent Acquisition mode (DDA))
In silico fragmentation platforms (MetFrag)
ESI-HRMS spectra prediction via CFM-ID

Level 2

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

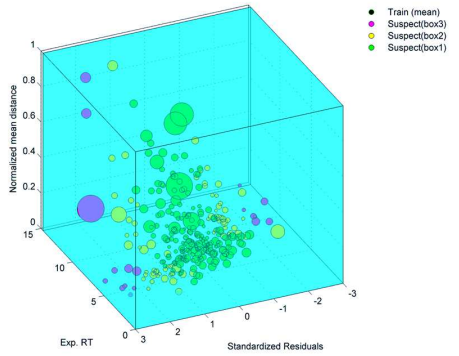
2a) **MS/MS spectral match**
Literature or library spectrum (MassBank)

Level 1 — Confirmation by **authentic reference standard**



MS-ready Suspect list

MetFrag CFM-ID



Modified spectra similarity score

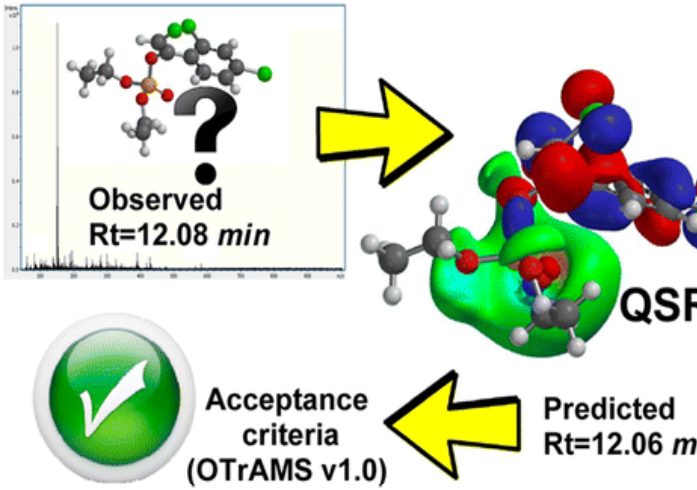
In-house developed retention time prediction tools supporting suspect and non-target screening

❖ 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 scre*
- Numerous National & International Scientific Projects
(EMBLAS II, Tremepol, WaterMicropol, Monitoring of Asopos river basin, HBM4EU, c.a.)

TI 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

ratefully acknolwedge the contributions of all those invloved in the
of UOA-RTI system.

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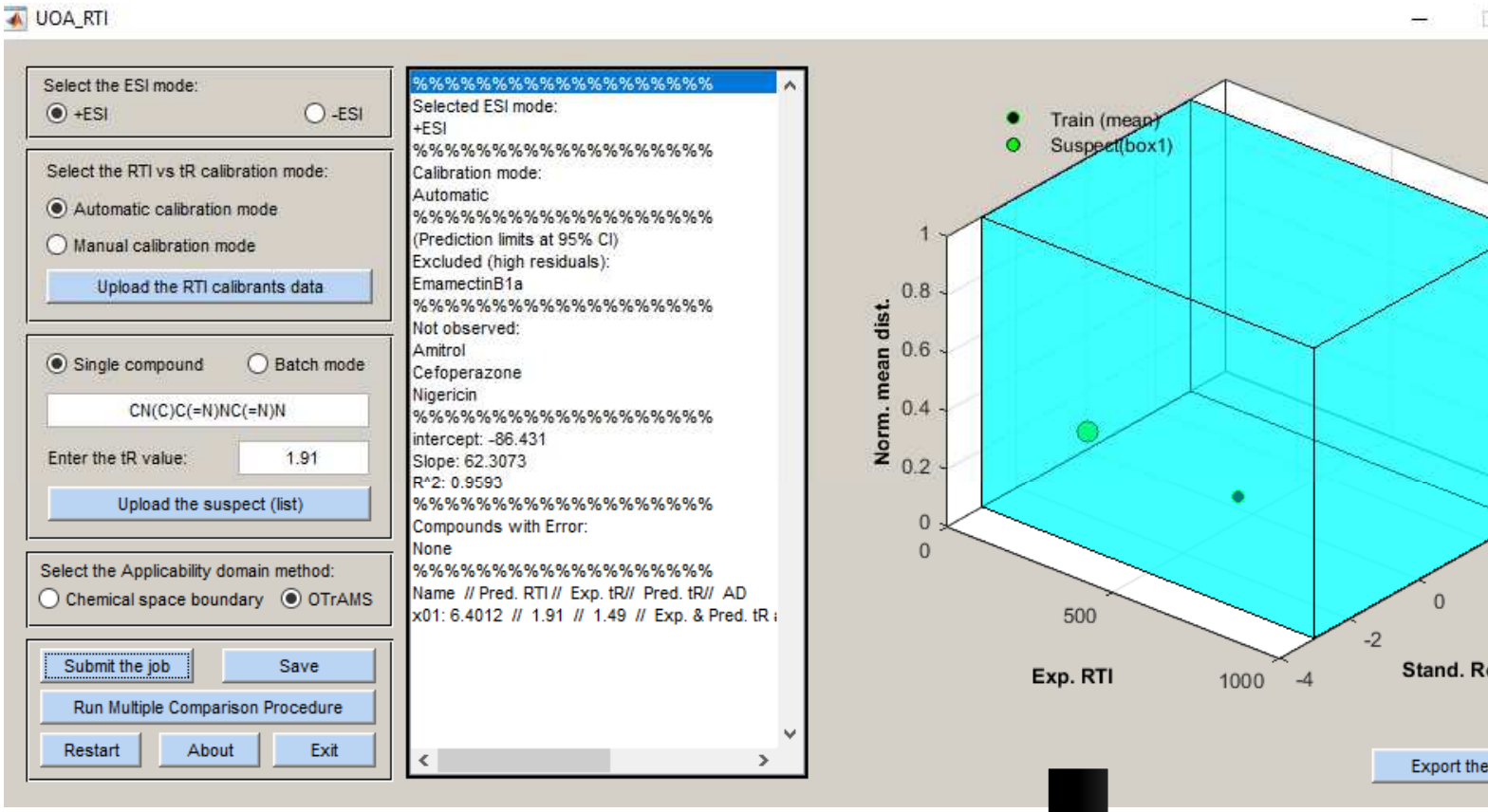
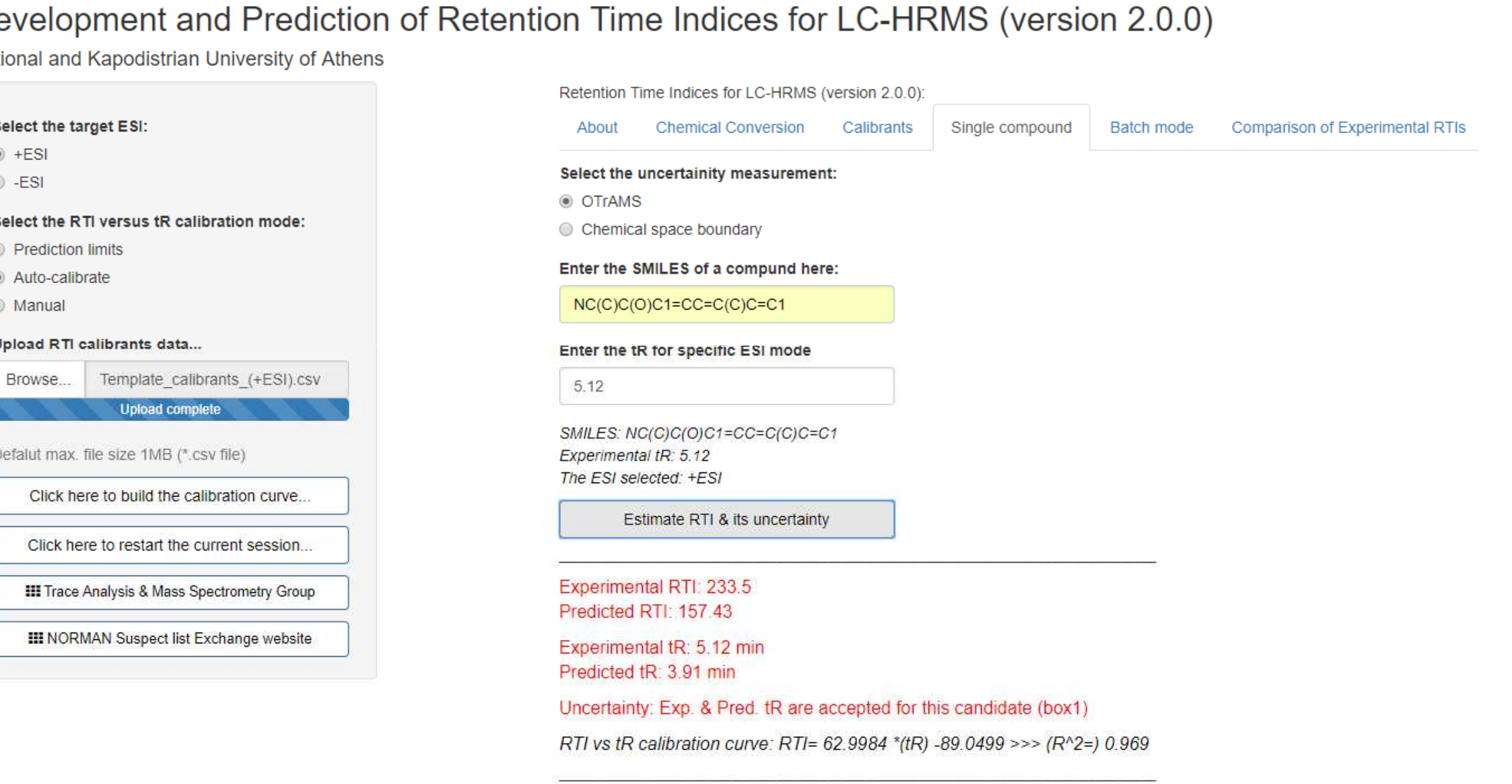


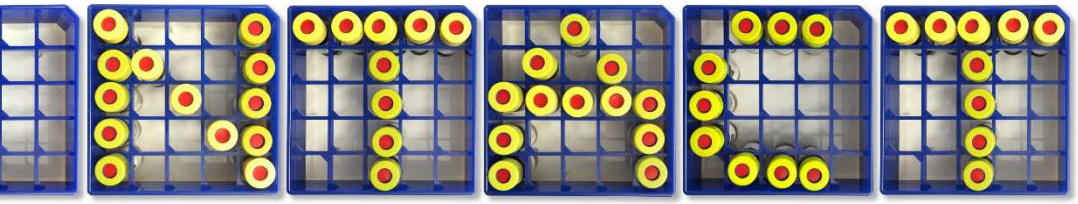
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Name	Name	SMILES	Pred. RTI	Exp. RT	Pred. RT	Box4	ide of AD	OTrAMS					
2	MK1	0477_Ben	OS(=O)(=C	148.3802	0.42	3.651883			Box2: tR is accepted in model developed in negative ESI:Th					
3	MK2	0424_Iopa	C[C@H](C	446.4338	0.42			9.698718816	Outside of applicability domain of 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(I	108.2699	0.42	2.838135			Box2: tR is accepted in model developed in negative ESI:Th					
6	MK5	0423_5-Fl	FC1=CNC(	108.2436	0.42	2.837602			Box2: tR is accepted in model developed in negative ESI:Th					
7	MK6	2,4-diami	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	OC(=O)C1	41.74257	0.42	1.488445			Box1: tR is accepted in model developed in negative ESI					
11	MK10	Maleimid	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					

External set | OTrAMS | internal fitting

READY

TI website for LC-(+/-)ESI-HRMS (coming soon…)

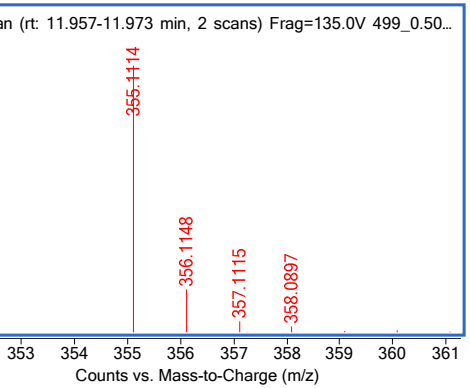


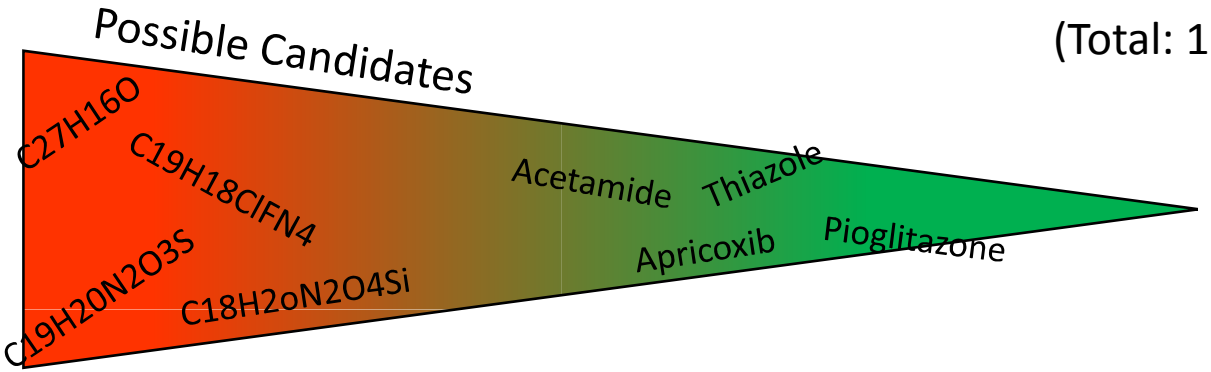


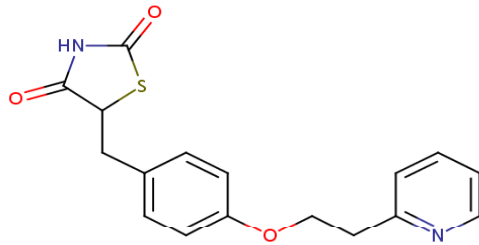
A'S Non-Targeted Analysis Collaborative Trial

The Trial Samples:

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







rrent NTA tools:
(EPA ENTACT v1)

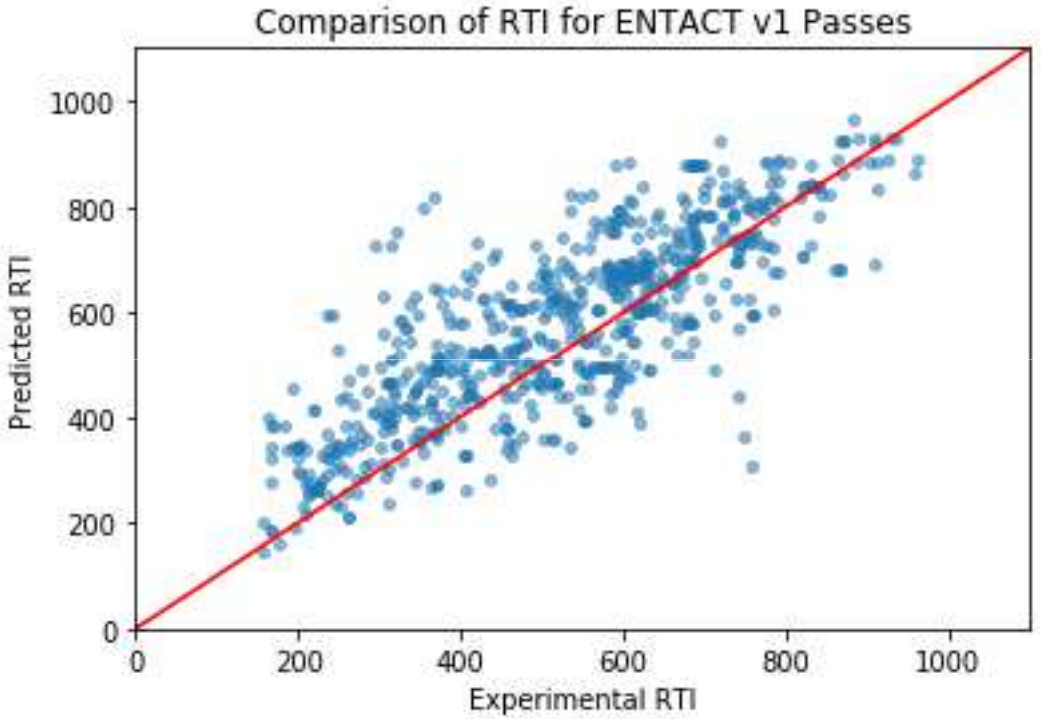
Mass Defect | Data Filtering | Isotope Matching | Data Sources | Referenc e MS/MS

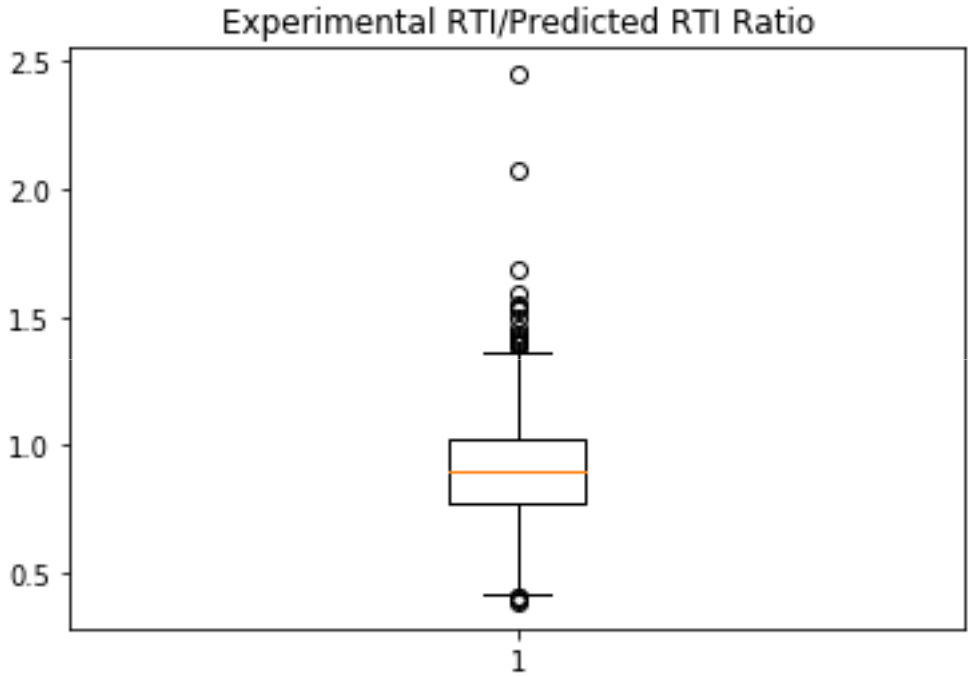
ential NTA tools:

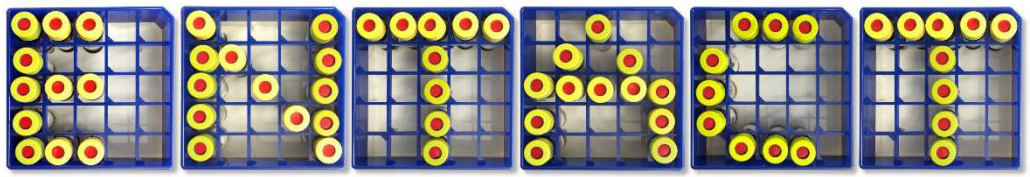
MS/MS Prediction | RT Prediction

Evaluate based off of v1 identifications

Original MeOH Data (positive calibrants, +ESI compounds)

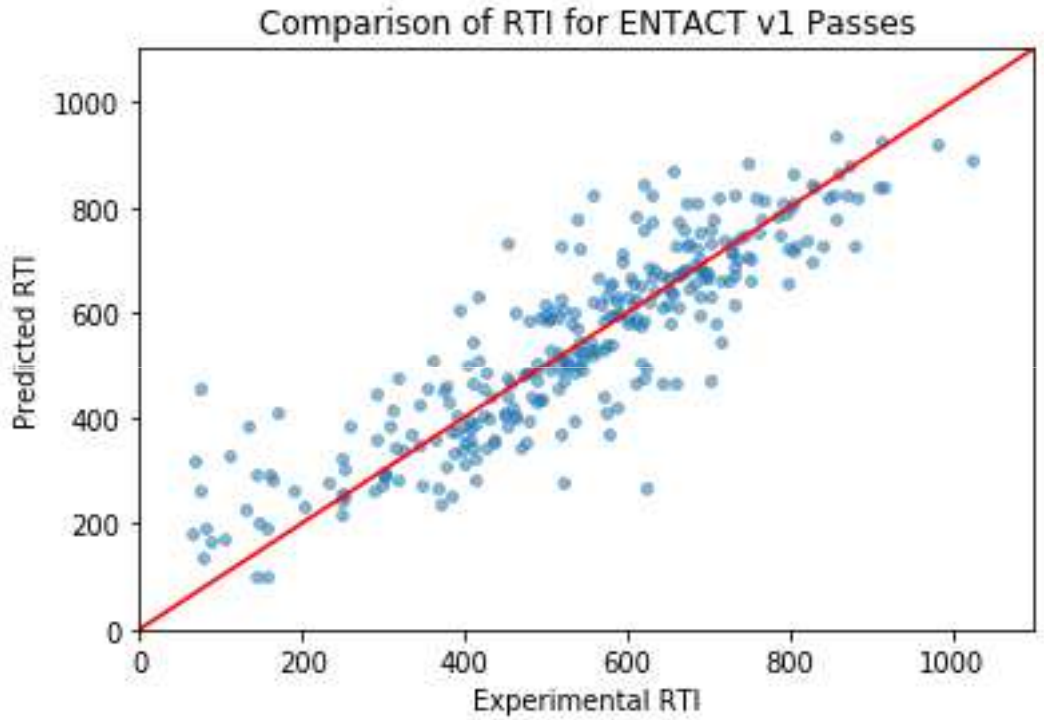


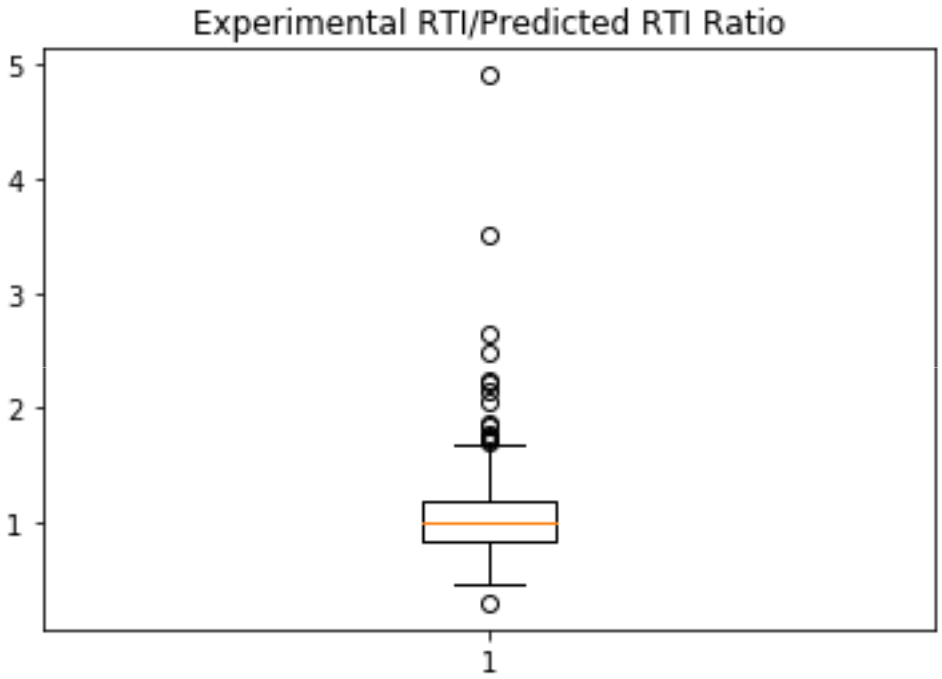


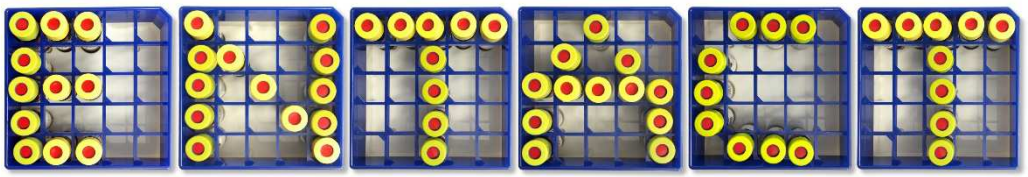


Median: 0.901
2.5 percentile: 0.535
97.5 percentile: 1.367

Updated ACN Data* (positive calibrants, all compounds)



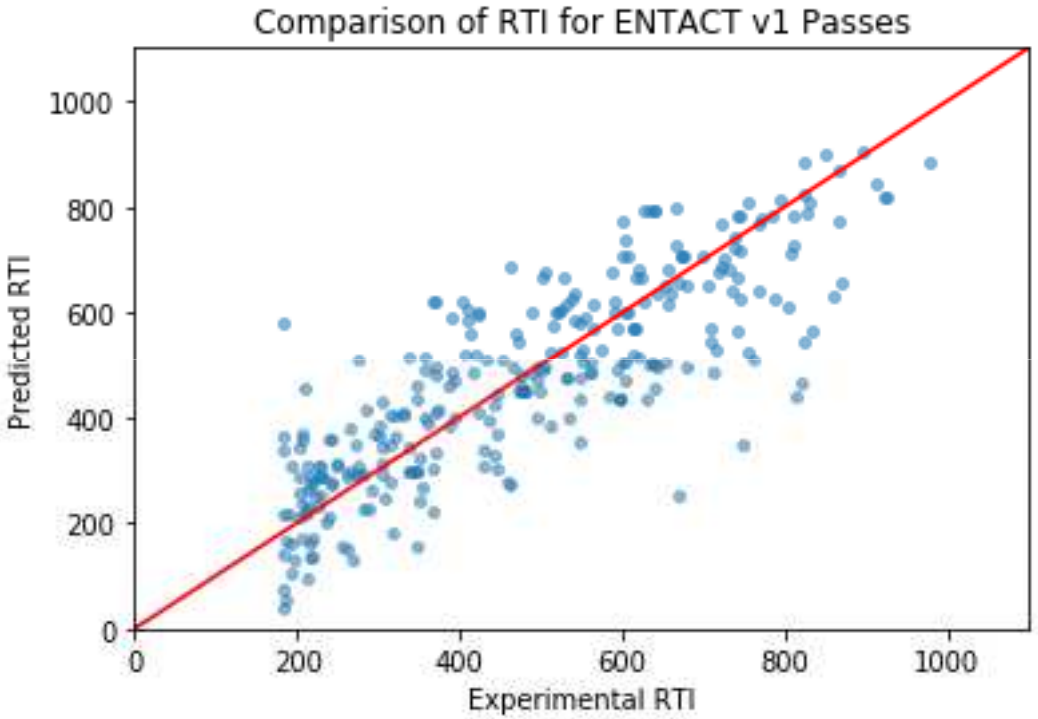


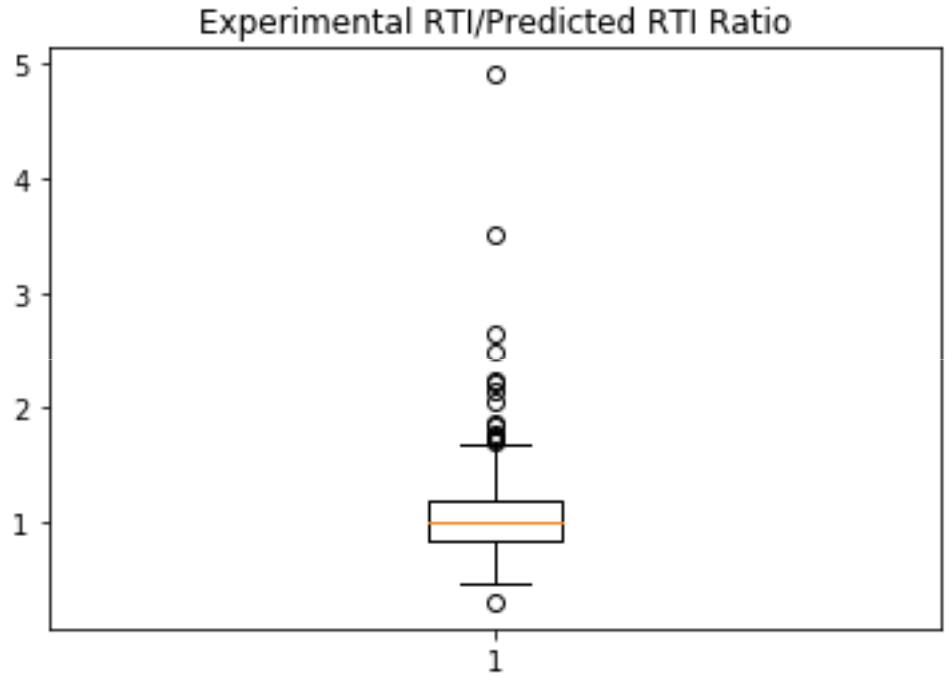


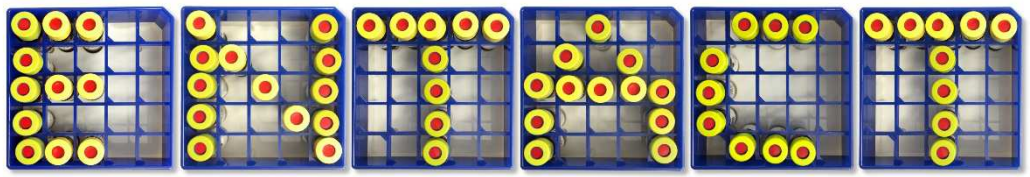
Median: 0.997
2.5 percentile: 0.458
97.5 percentile: 1.450

hromatography 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

n-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

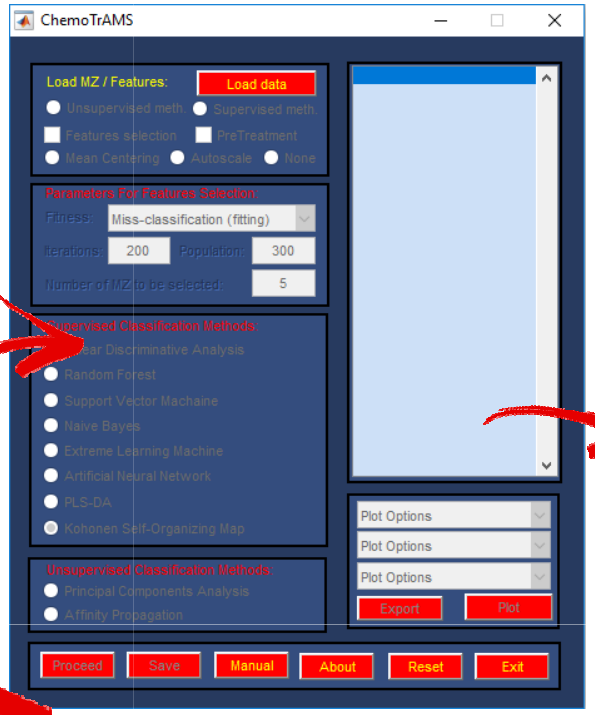
Mass accuracy threshold (Molecular Formula) (< 2 mDa)
Isotopic pattern of the precursor ion (> 50)
Characteristic adduct ions ([M + NH_4]$^+$, [M + Na]$^+$, etc.)

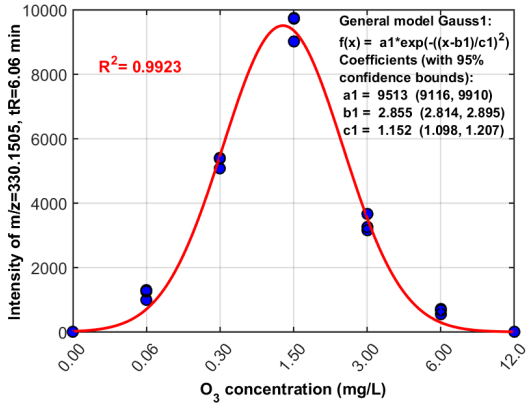
MS/MS spectral interpretation & prediction
(Recorded by Data Dependent Acquisition mode (DDA))
In silico fragmentation platforms (MetFrag)
ESI-HRMS spectra prediction via CFM-ID

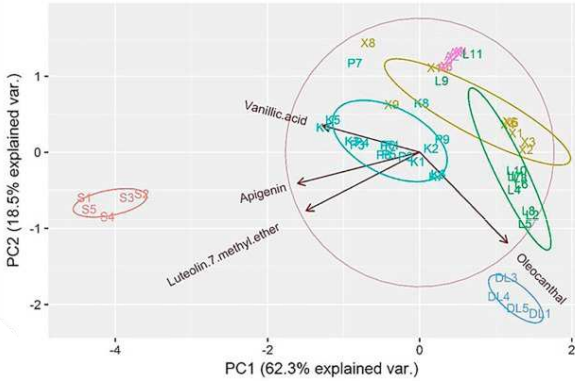
2b)
LC retention time indices (QSRR models and RTI)
(Matching prediction vs experimental t_R via OTrAMS)

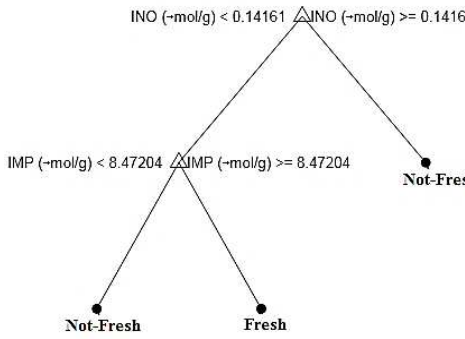
2a) **MS/MS spectral match**
library spectrum (MassBank, GNPS, MONA,mzClouds)

Confirmation by **authentic reference standard**









CompTox Dashboard
ChemSpider
The free chemical database
PubChem

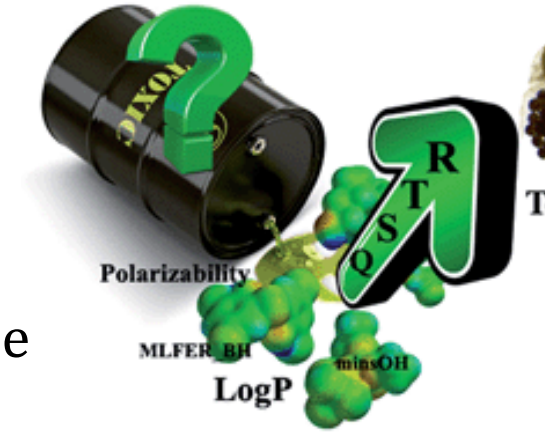
pplication of screening workflows

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



ENVIRON SCI-PROC IMP, 2017, 19,

nvironmental Analysis – Projects completed

in the

(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

UNHCR The UN Refugee Agency **(2016-2017)**

vironmental assessment of the use of restored Solid Waste Dump in Chios as shelter facility - UNHC
QS 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

nvironmental Analysis – NEW Projects

Monitoring of drinking water reservoirs: Assessment of contamination of three lakes nearby Attica (**EYDAP**, 2018-2019)

in the field

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)

. 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 wat

sediment

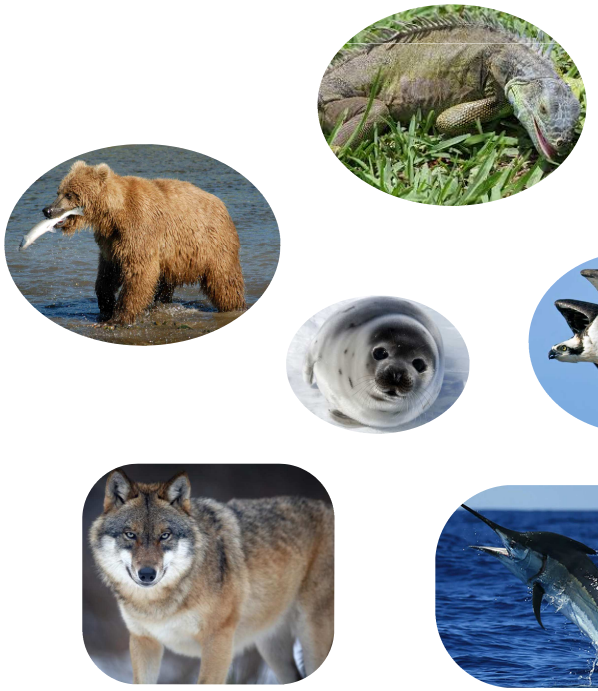
nvironmental Analysis – NEW Projects

"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

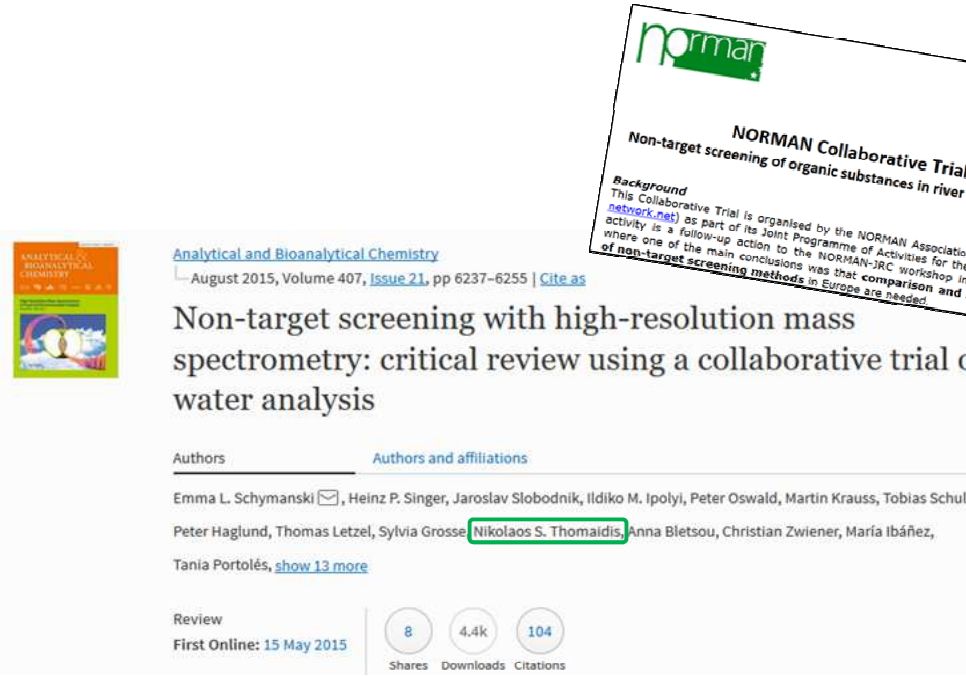


de scope target analysis, Suspect & Non-target screening
(**LC-QToFMS & GC-APCI-QToFMS**)

ollaborative Trials (CTs)

2014

I. **NORMAN** CT - "**Non-target** Screening of organic substances in **river water**"

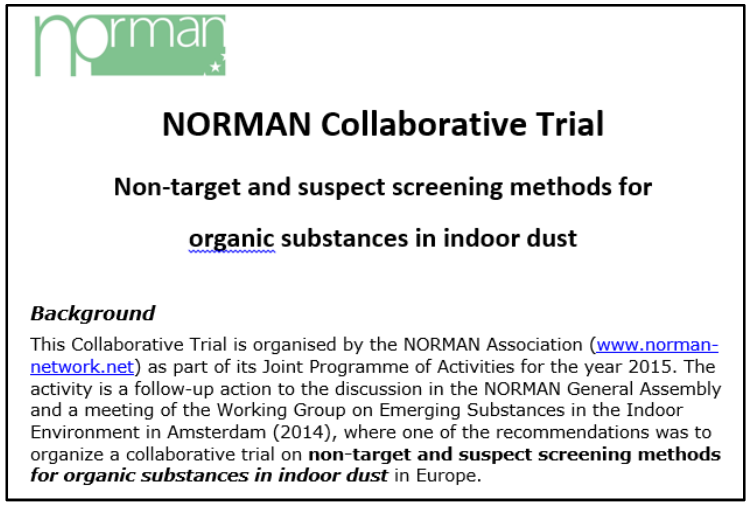


Schymanski, E.L., Singer, H.P., Slobodnik, J. et al. Anal Bioanal Chem (2015) 407: 623
https://doi.org/10.1007/s00216-015-8681-7

2016

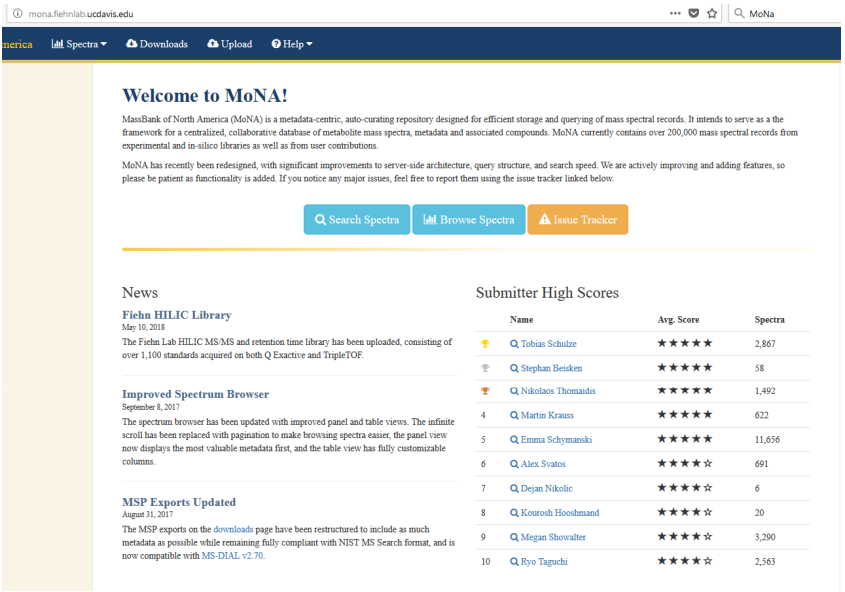
I. **NORMAN** CT - "**Non-target** and **suspect** screening methods for organic substances in **indoor dust**"

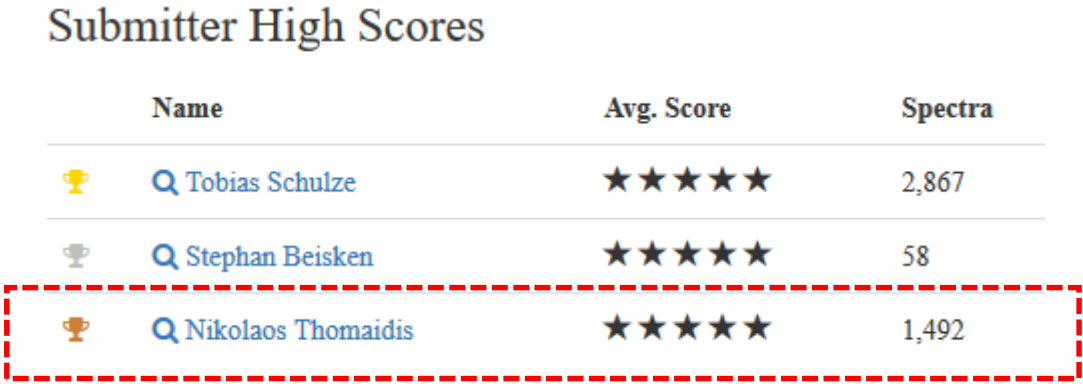
- **25 institutes** (EI, UFZ, EAWAG, EMEA, INIRIS, **UoA** etc.) from **16 countries**



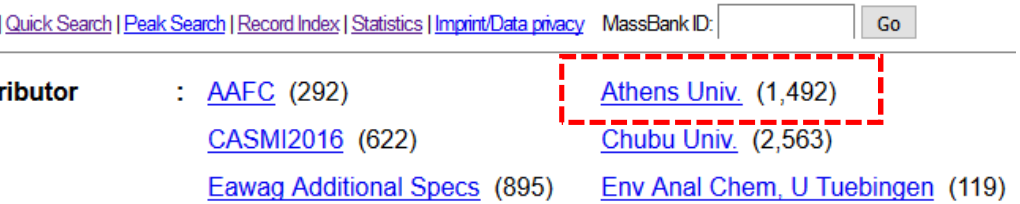
ontributions in MS databases

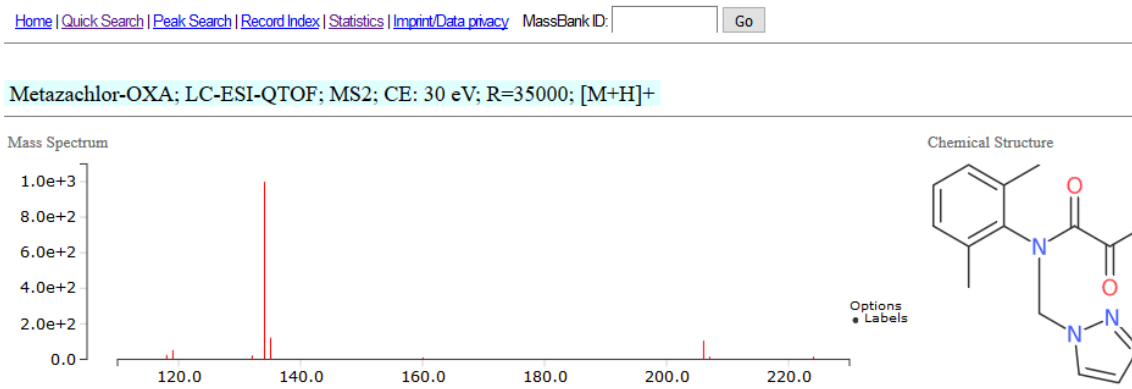
➢ MassBank of North America





➢ MassBank EU



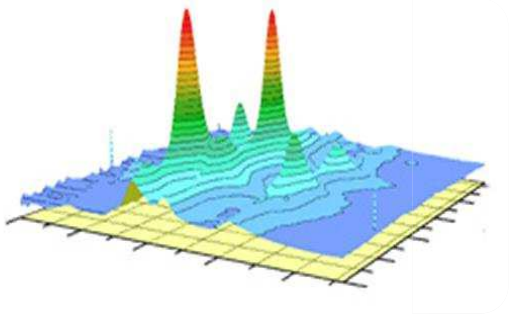


Thank you very much

for your attention!

TrAMS

Trace Analysis and Mass Spectrometry Group



NORMAN

Network of reference laboratories, research centres and organisations for monitoring of emerging environmental substances

http://trams.chem.uoa.gr/