Laboratory of Analytical Chemistry
Department of Chemistry
University of Athens

Target screening of 2327 emerging pollutants in environmental samples by RP-LC-QTOF-HR-MS/MS with an accurate-mass database

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CEST
International Conference on Environmental Science and Technology

Emerging Pollutants (EPs)

- Pesticides
- Pharmaceuticals
- Illicit drugs
- Personal care products
- Endocrine disruptive compounds (EDCs)
- Flame retardants
- Food additives
- Disinfection by-products

+

Metabolites & Transformation Products (TPs)

Wastewater:

Potentially tens of thousands of substances

database of 2327 EPs

> 700 pesticides

> 200 pharmaceuticals, illicit, DoA

~ 300 steroids & doping compounds

~ 100 compounds like industrial chemicals, food additives, dies and natural occurring compounds (aminoacids)

~ 300 metabolites & TPs

Workflow

Sample

Sample Extraction

LC-QTOF

UHPLC + Bruker QTOF
Chromatographic separation
with accurate mass
bbCID data acquisition

Data Processing

Screener bbCID Database
Accurate mass database:
Qualifier Ions and RT

Identification
Confirmation

Report

Sampling & Sample preparation

Location: **WWTP of Athens, Greece**

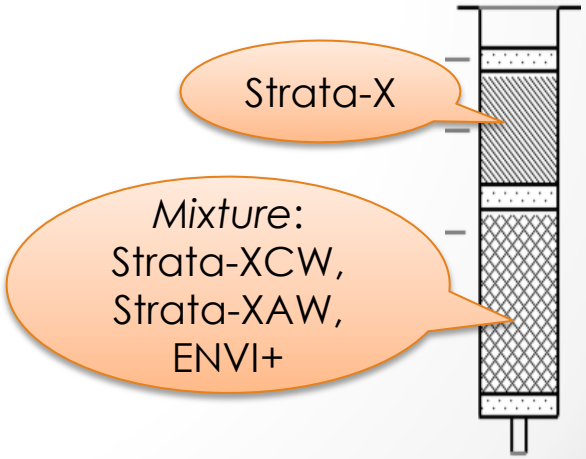
Period: March 2014

Samples: 24-h composite flow-proportional samples of influent wastewaters & effluent wastewaters over a week

Sample Preparation:

- ✓ **100 mL** wastewater (GFF filtration)
- ✓ **IS** spiking (100 ng/L)
- ✓ SPE *Mixed-bed cartridges*
- ✓ Extraction: **Neutral, Basic & Acidic Compounds**

→ 100 times preconcentration



*Kern et al. EST (2009) 43(18):7039

Instrumentation

RP-UHPLC

Column: Acclaim RSLC 120 C18 2.2 µm, 2.1 × 100 mm

Pre-column: VanGuard (Waters): Acquity UPLC BEH C18 1.7 µm, 2.1 × 5 mm

- 20 min chromatogram
  - Gradient elution program in the M.P.
  - Gradient elution program in the flow rate

MS

MaXis Impact

Ultra High Resolution

Time-of-Flight Mass Spectrometer

- Positive & Negative ESI
  - bbCID mode

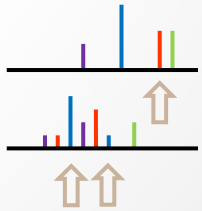
(MS & MS/MS simultaneously)

bbCID mode

Low CE (4 ev) *(pass all)* → MS spectra

High CE (25 ev) *(fragment all)* → MS/MS spectra

Rapidly alternating MS & MS/MS acquisitions



MS

✓ Detection

bbCID MS/MS

✓ Confirmation

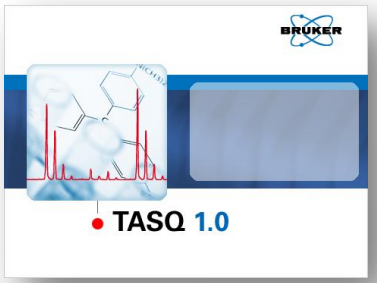
Database

2224 compounds in (+) ESI

580 compounds in (-) ESI

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	m/z (POS)	RT POS	sum formula	name	CAS	comment	comment	relativeRe	minimum	indivSigm	indivMass	QI 1	QI 2	QI 3	QI 1 min	QI 1
2	184.0191768	3.69	C4H10NO3PS	Acephate	(30560-19-1)							142.9926				
3	142.9926277	3.69	C2H8O3PS^1+	Acephate Fragm 143	(30560-19-1)							94.9893	110.9664	124.9821		
4	270.1255331	11.34	C14H20ClNO2	Acetochlor	(34256-82-1)							224.0837				
5	224.0836682	11.41	C12H15ClNO^1+	Acetochlor Fragm 224	(34256-82-1)							148.1121	133.0886	224.0837		
6	379.03031	10.21	C14H7ClF3NO5NH4^1+	Acifluorfen (NH4)	(50594-66-6)											
7	265.0374463	11.98	C12H9ClN2O3	Aclonifen	(74070-46-5)							248.0347	218.0367	194.0475		
8	287.019391	11.98	C12H9ClN2O3Na^1+	Aclonifen (Na)	(74070-46-5)							248.0347	218.0367	194.0475		
9	181.0859207	11.26	C10H13O3^1+	Acrinathrin Fragm. 181	(101007-06-1)							181.0859	213.1121	230.1387		
10	270.1255331	11.4	C14H20ClNO2	Alachlor	(15972-60-8)							162.1277	147.1043	132.0808		
11	162.1277259	11.4	C11H16N^1+	Alachlor Fragm 162	(15972-60-8)							162.1277	147.1043	132.0808		
12	238.0993183	11.4	C13H17ClNO^1+	Alachlor Fragm 238	(15972-60-8)							162.1277	147.1043	132.0808		
13	191.0848748	7.36	C7H14N2O2S	Aldicarb	(116-06-3)							89.0419				
14	208.1114239	7.36	C7H14N2O2SNH4^1+	Aldicarb (NH4)	(116-06-3)							89.0419				
15	116.0528464	7.36	C5H10NS^1+	Aldicarb Fragm 116	(116-06-3)							89.0419				
16	89.0419474	7.36	C4H9S^1+	Aldicarb Fragm 89	(116-06-3)							89.0419				

- Retention time
- Molecular Formula
- Adducts
- In-source fragments
- bbCID fragments
- Ion Ratios



TargetAnalysis

DataAnalysis

Optimization-Validation

validation dataset

- 195 compounds
- 10% of the compounds of the total database
- Representative retention time
- Representative physicochemical properties
- Representative ionization behavior
- Compounds from every class of EPs

Screening method:

The method used to identify the non-compliant samples (true positive) with a β-error less than 5%

Optimization of the evaluation method

Successful Identification Rate >95%

Find	Area	1000 (+)/ 600 (-)	
	Intensity	250(+)/ 150 (-)	
Scoring		min	max
	ret. Time (min)	0.1	0.4
	accuracy (ppm)	2.5	5
	mSigma threshold	100	200

C spiked samples (μg/L)	% 'missed compounds due to Area / Intensity threshold	False Negative results (compounds)	Successful Identification Rate (%)
1	0	1	99
0.5	0	2	99
0.25	1.3	2	97
0.05	3.3	3	95
0.025	5.2	5	92

Validation

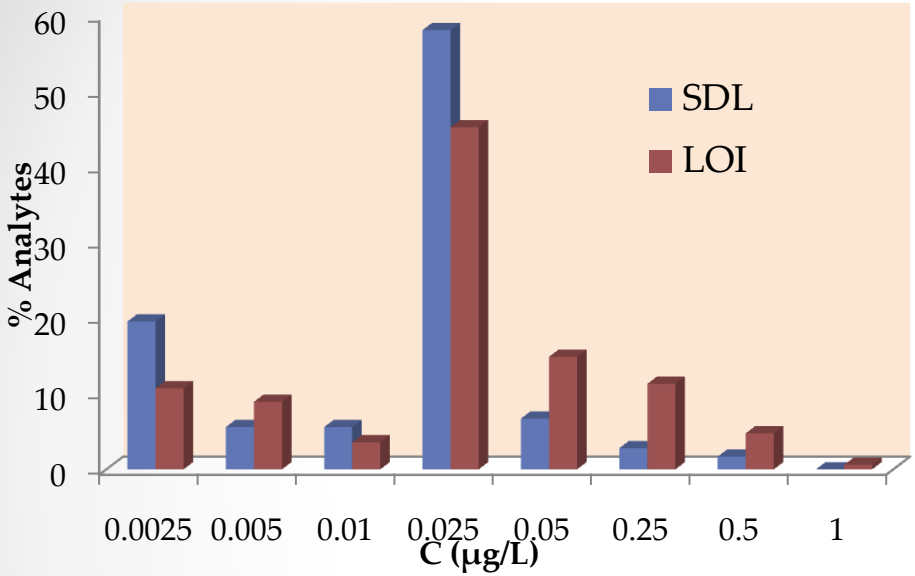
Propose an HRMS oriented validation scheme,
according to 2002/657/EC, SANCO 12571/2013 & literature

- **Calibration curves** (**solvent**, **matrix** & **spiked** samples) (8 levels of concentration)
- **Repeatability**, **recoveries** and **matrix effect**
- The **screening detection limit (SDL)** and the **limit of identification (LOI)**:
  - **SDL:** the lowest concentration level tested for which a compound was detected in all samples; **2 Identification Points (IPs)**
  - **LOI:** the lowest concentration tested for which a compound was satisfactorily identified in all spiked samples; **4 Identification Points (IPs)**
- Decision limit (**CCα**) & Detection capability (**CCβ**)

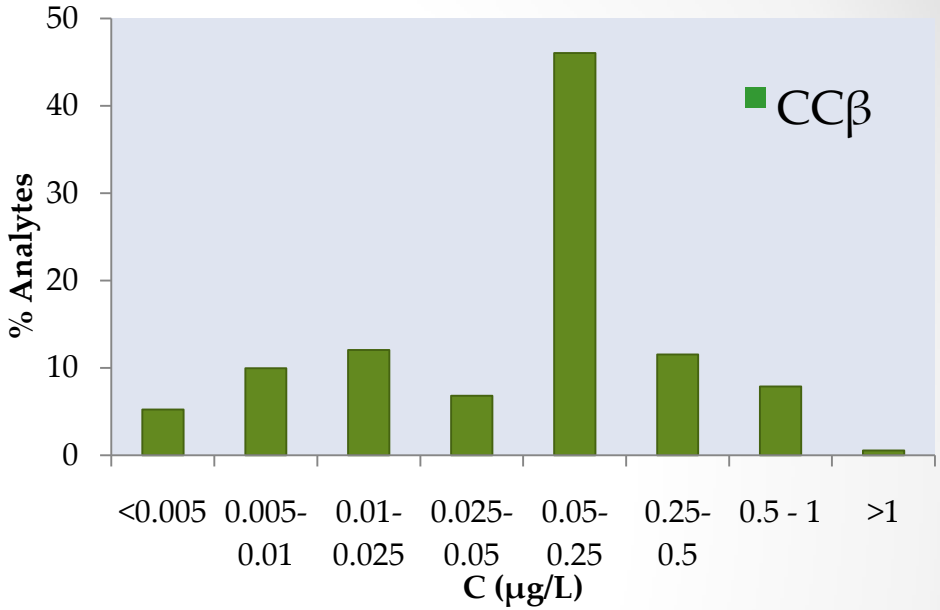
Parameter	Thresholds	Identification Points (IP)
Precursor ion (accuracy) +t_R	2 ppm	**2.5**
Isotopic fit (ratio of M+1, M+2)	200 mSigma	**0.5**
Fragments (accuracy)	5 ppm	**2.5**

Validation results

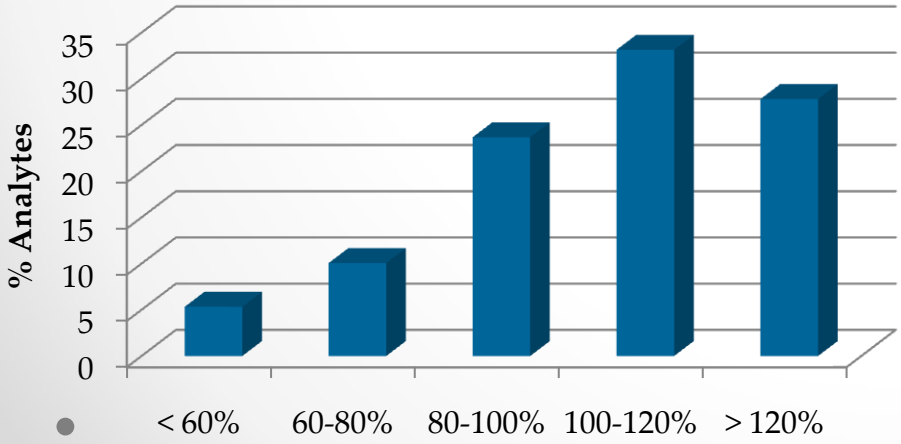
Screening Detection Limits (SDL) – Limits of Identification (LOI)



Detection capability CCβ



% Recoveries



% Matrix Effect

91.7 % analytes → signal ***suppression***

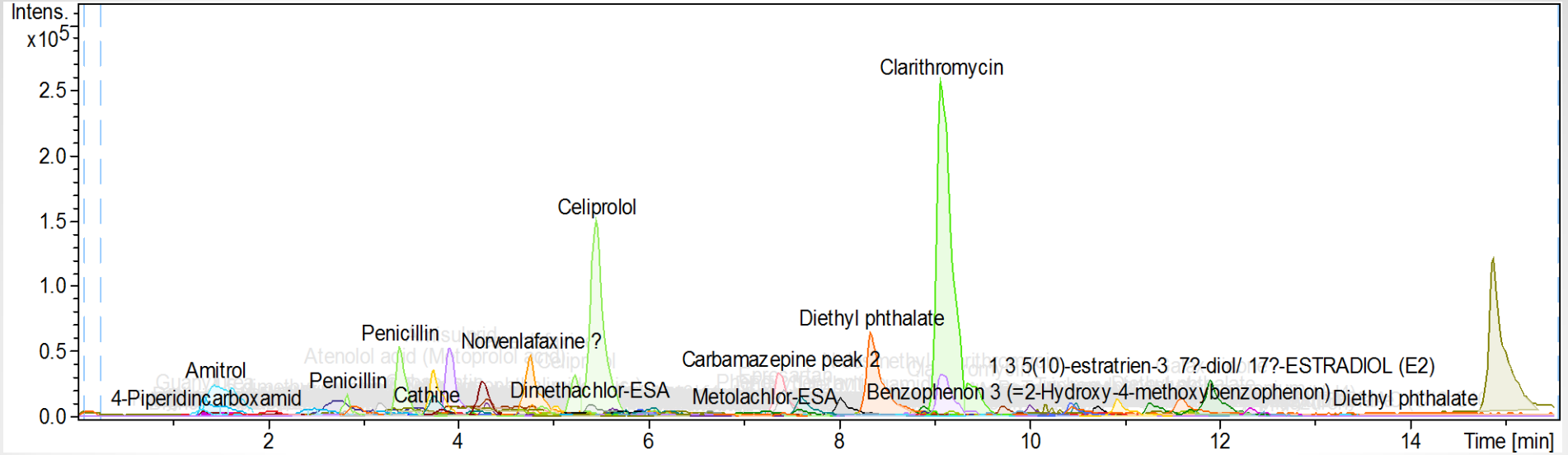
8.3 % analytes → signal ***enhancement***

% Repeatability (*n*=6)

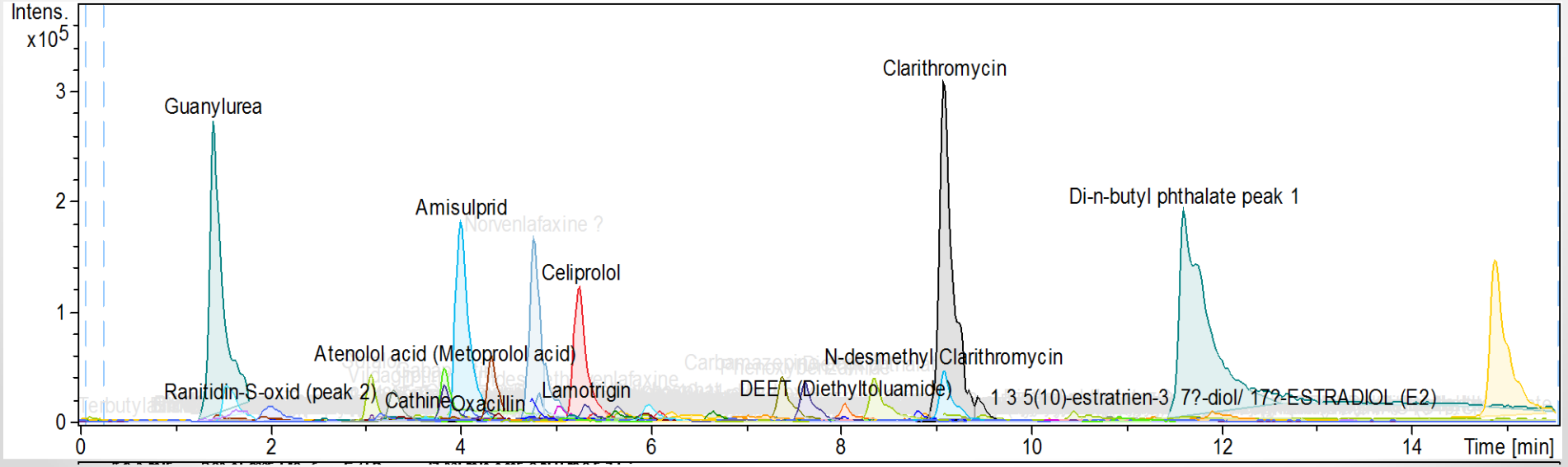
94.5 % analytes → <20% RSD

Results

Influent wastewater



Effluent wastewater



Results

371 compounds detected

Influent wastewaters: **338** compounds

Effluent wastewaters: **301** compounds

219	**Screened compounds**	192
188	**Identified compounds**	109

Screening: **≥2IPs** (t_R + precursor ion)

Identification: **≥4IPs** (t_R + precursor ion + fragment ion)

- 61 pesticides
- 205 licit & illicit drugs
- 4 sweeteners
- 10 PFCs
- 8 Aminoacids
- 47 TPs

- 51 pesticides
- 191 licit & illicit drugs
- 4 sweeteners
- 11 PFCs
- 4 Aminoacids
- 49 TPs

Results

Screening of 1-OH-BTR

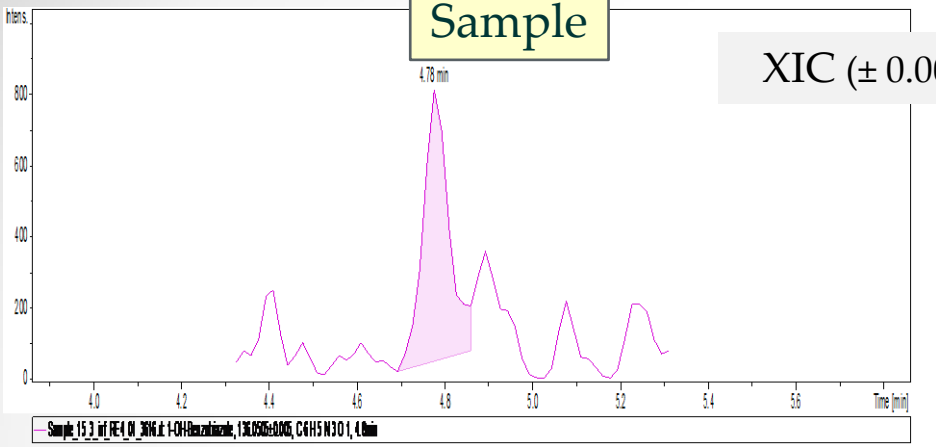
2.5 IPs

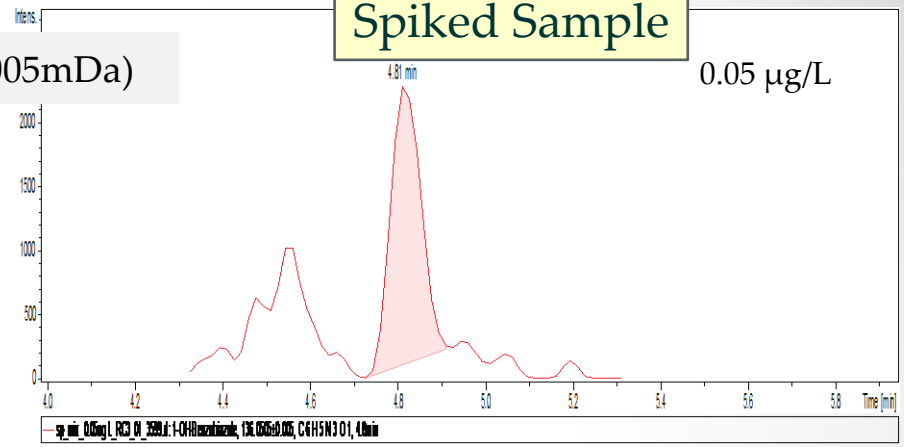
Sample

Spiked Sample

XIC (± 0.005mDa)

0.05 µg/L

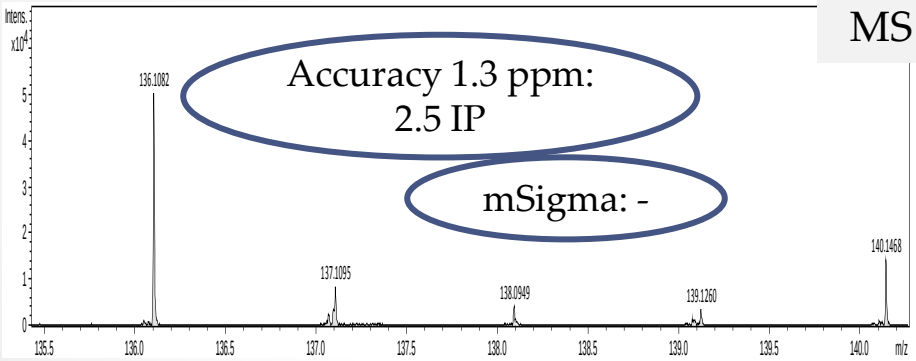


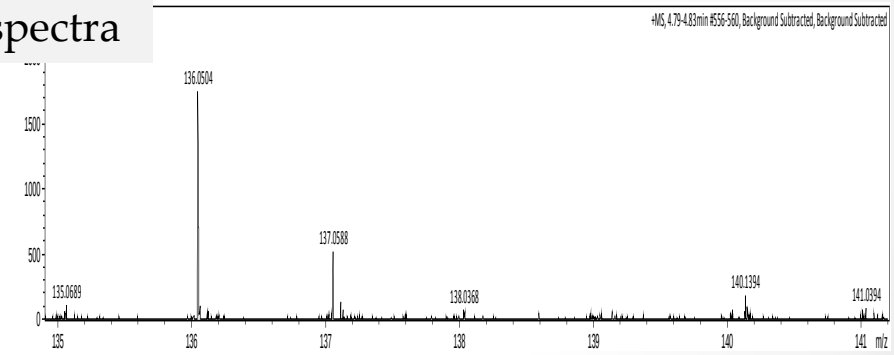


MS spectra

Accuracy 1.3 ppm:
2.5 IP

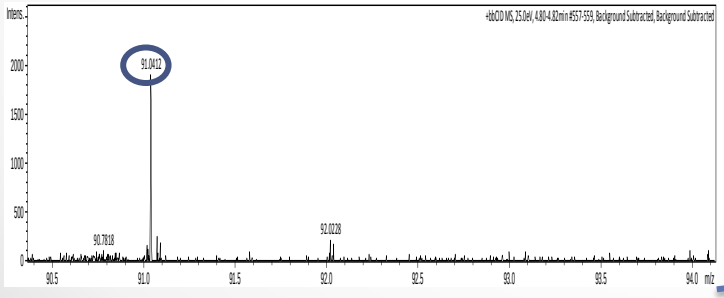
mSigma: -

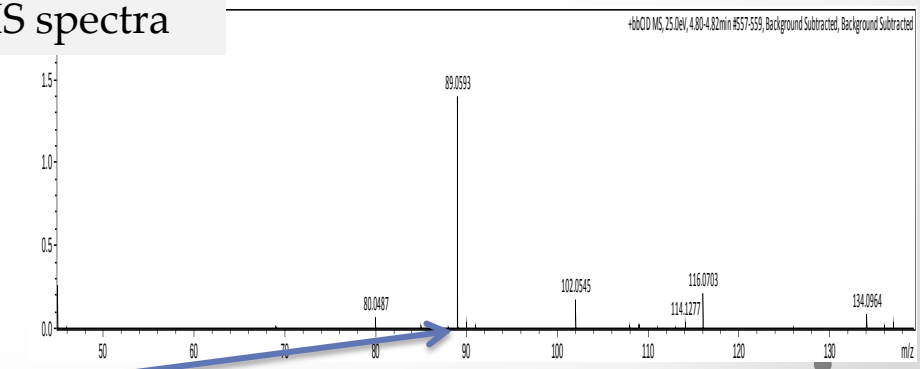




no MS/MS spectra for the sample

MS/MS spectra





Results

8 IPs

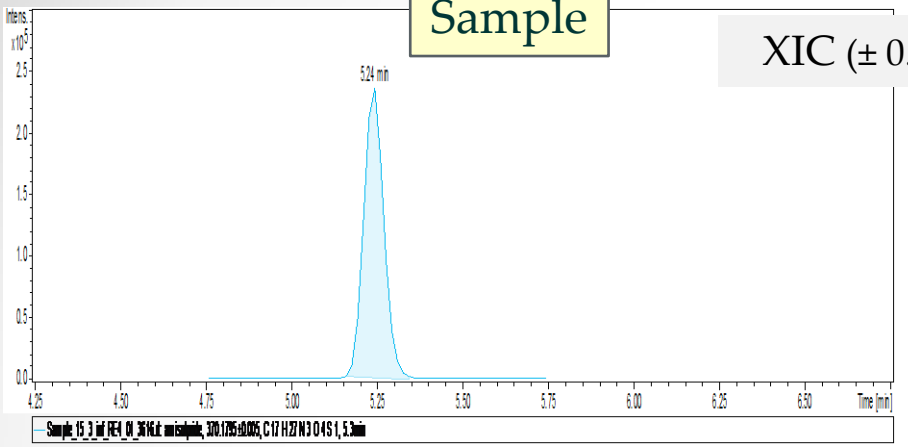
Identification of Amisulpride

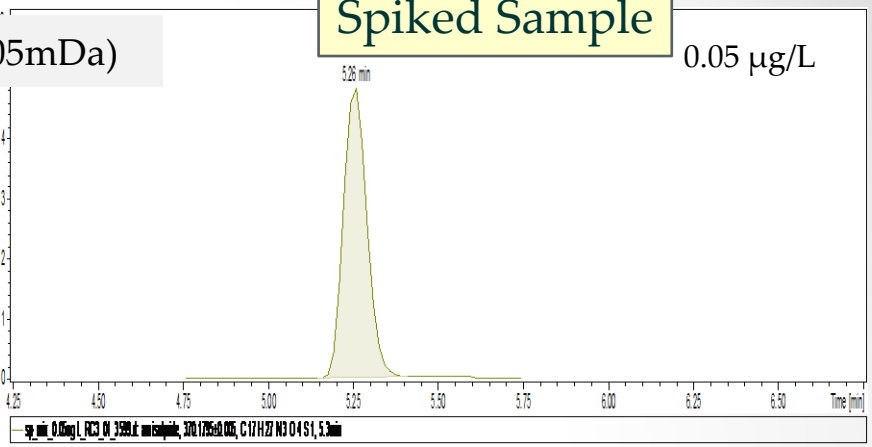
Sample

Spiked Sample

XIC (± 0.005mDa)

0.05 µg/L

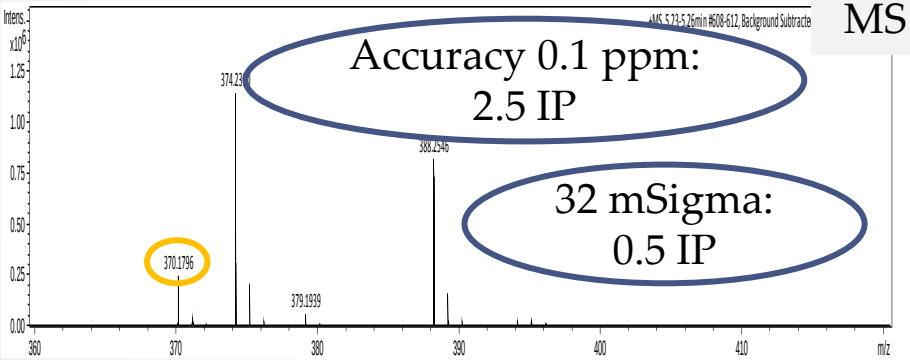


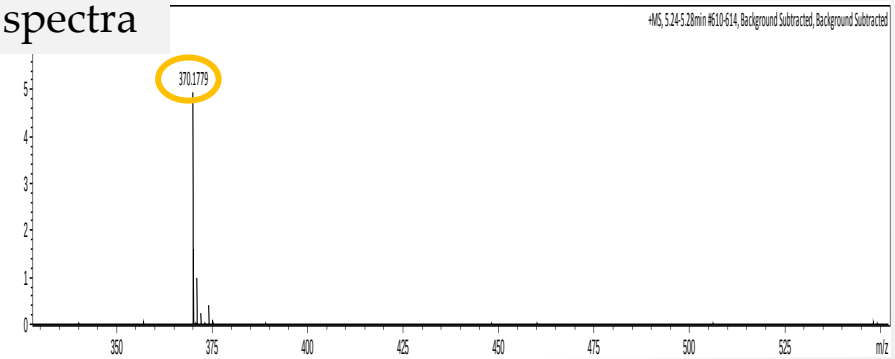


MS spectra

Accuracy 0.1 ppm:
2.5 IP

32 mSigma:
0.5 IP

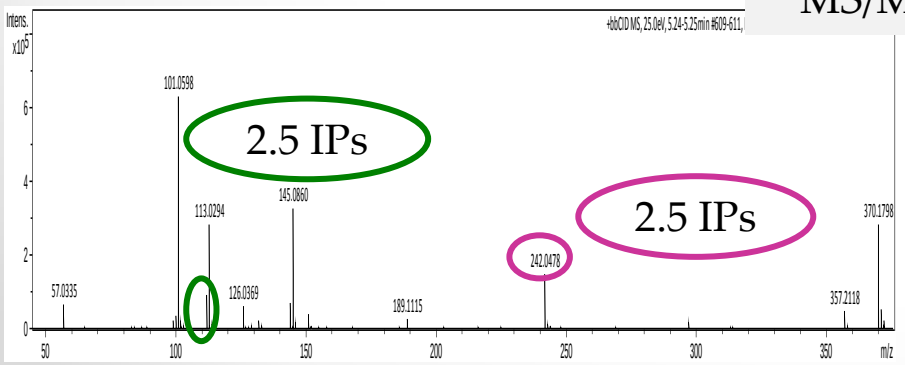


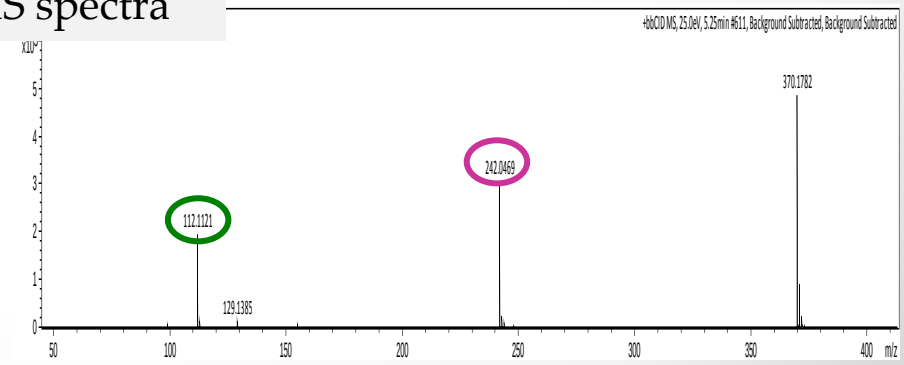


MS/MS spectra

2.5 IPs

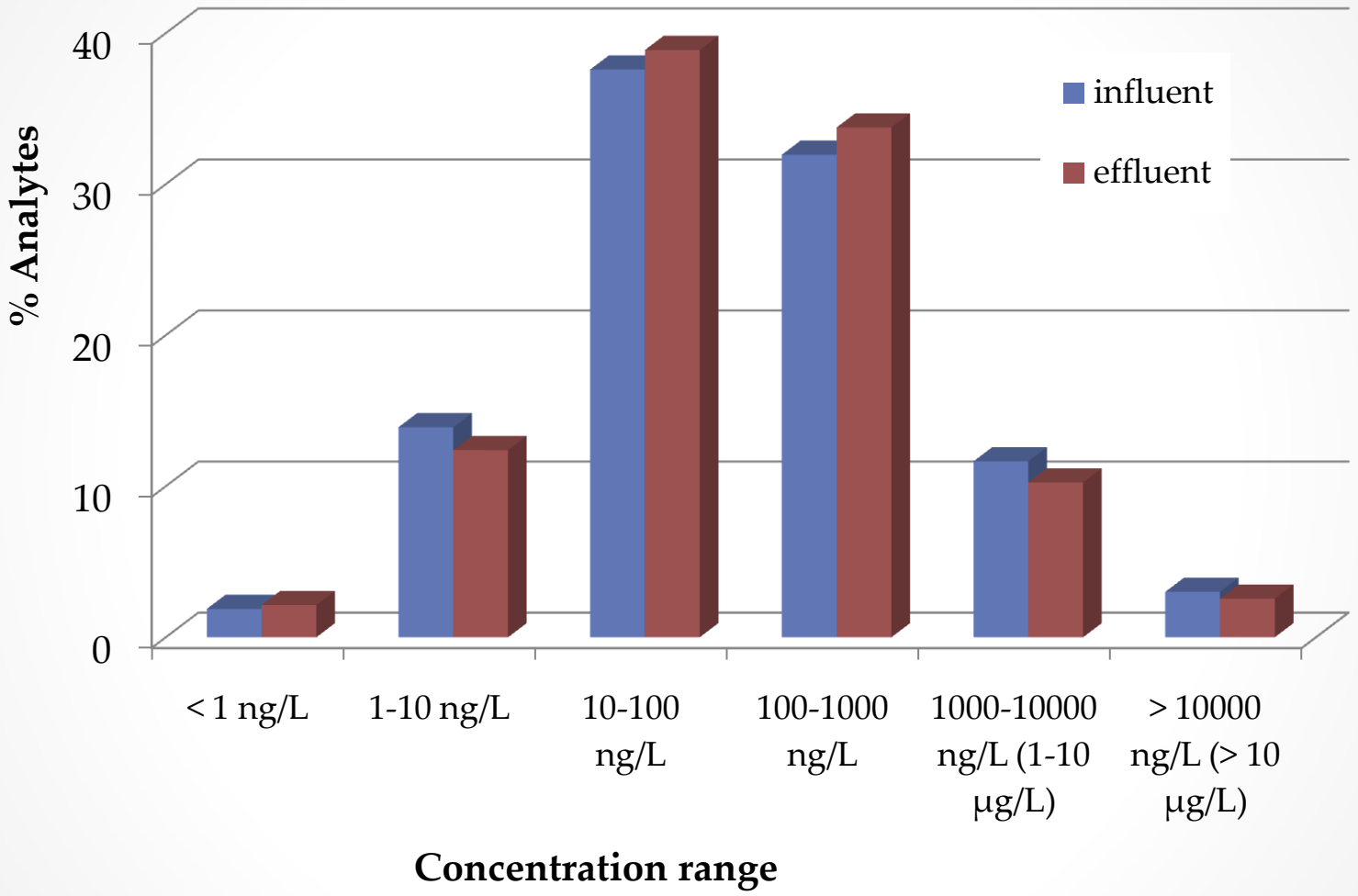
2.5 IPs





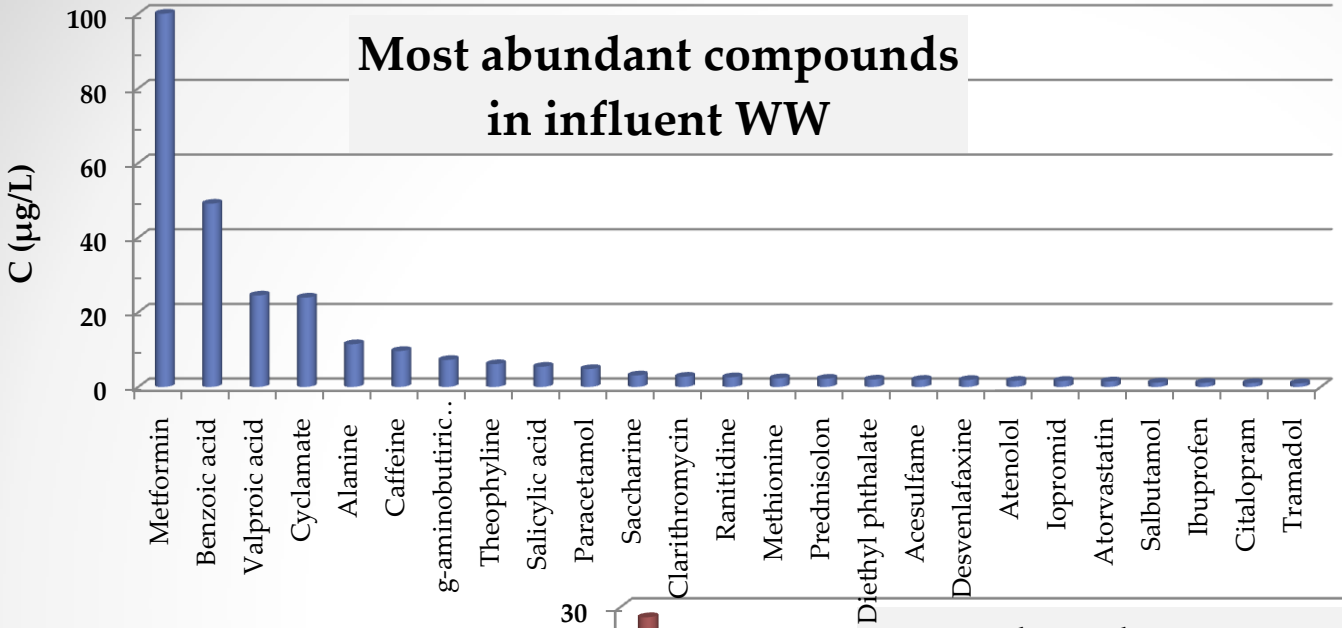
Results

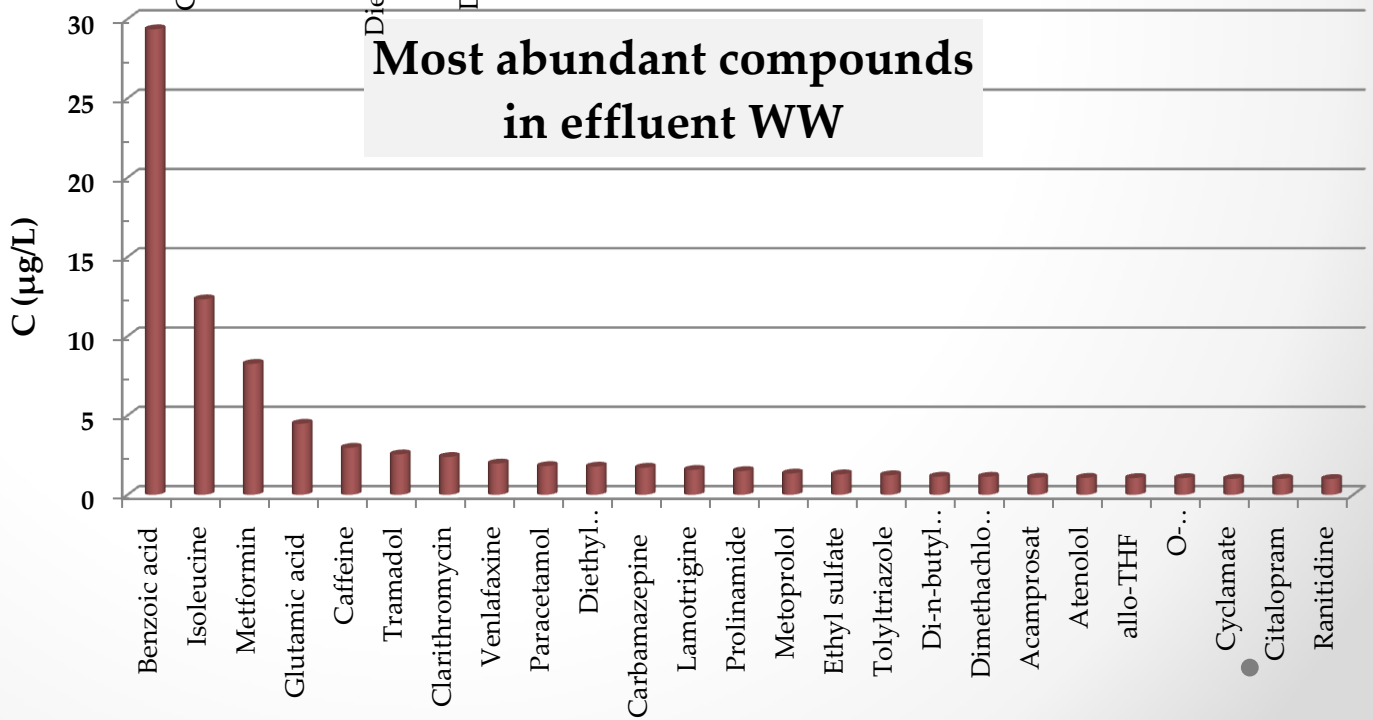
Quantitation



Results

Quantitation





Results

Quantitation

- **Drugs**

Metformin →most abundant
Haloperidol → less abundant
Influent: 0.14 ng/L - 93 µg/L
Effluent: 0.5 ng/L - 35 µg/L

- **Pesticides**

Fluometuron, Azoxystrobin & Dimethachlor met.
Influent: 0.3 ng/L- 2.04 µg/L
Effluent: 0.2 ng/L- 13.6 µg/L

- **Sweeteners**

Influent: 0.6 µg/L sucralose - 24 µg/L cyclamate
Effluent: removal >60 %

- **Other chemicals**

Benzoic acid: 49 µg/L (influent) - 29 µg/L (effluent)
Ethyl sulfate: 3.6 µg/L (influent) – 1.3 µg/L (effluent)

- **Aminoacids**

Influent: Conc. >2 µg/L (8 AAs) Valine: most abundant
Effluent: significant removal rates (4 AAs), Val & GABA > 10 µg/L

Conclusions

- In-house database with information for 2327 compounds
- Mixed-bed SPE covers a wide range of compounds
- Optimization & validation of the target screening method
- HR-MS oriented validation scheme & IPs system
- Application in influent & effluent wastewater samples
- Screening, Identification & Quantification of the analytes

✓ Continuously increasing database with new reference standards
→ *Retrospective* target screening

Special thanks to…

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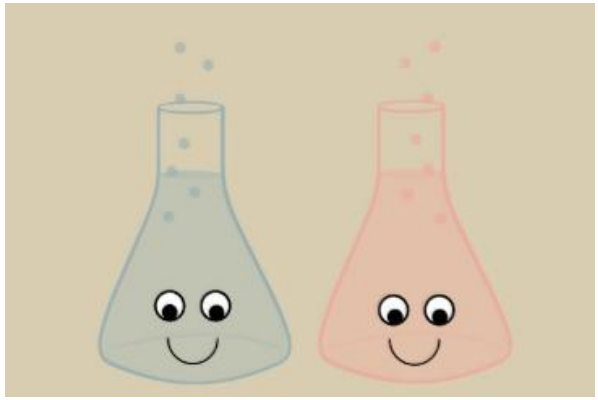
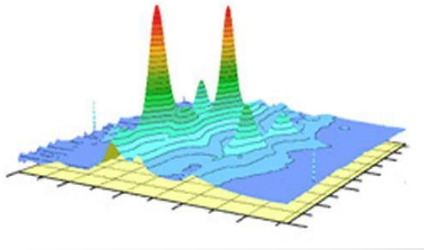
eawag aquatic research

Thank you for your attention! ! !

TrAMS Trace Analysis and Mass Spectrometry Group





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European Union
European Social Fund

OPERATIONAL PROGRAMME
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programme for development
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