Targeted determination of more than 1500 micropollutants and transformation products in wastewater samples by liquid chromatography quadrupole-time-of-flight mass spectrometry with an accurate-mass database

Anna A. Bletsou, Aikaterini K. Psoma, Pablo Gago Ferrero, Nikolaos S. Thomaidis

Laboratory of Analytical Chemistry, Department of Chemistry, University of Athens, Panepistimiopolis Zographou, 15771 Athens, Greece

e-mail: ntho@chem.uoa.gr

Abstract

High resolution mass spectrometry has dramatically improved the possibilities of the environmental analysis. The present study describes the development of an analytical method, based on liquid chromatography quadrupole-time-of-flight mass spectrometry (***LC–QToF-MS***) for the target determination of more than 1500 ***contaminants of emerging concern*** (CECs) and ***transformation products (TPs)*** including, among others, pharmaceuticals, illicit drugs, personal care products, pesticides, industrial chemicals, and sweeteners in wastewater. Analytes were extracted from ***wastewater samples*** by ***mixed mode solid-phase extraction***, and data were acquired through broad-band Collision Induced Dissociation (***bbCID***) mode, providing MS and MS/MS spectra, simultaneously, in both positive and negative ionization mode (two separate runs). The in-house mass spectral database was built by injection of standard solution of the analytes and it includes information of the retention time, parent ions and adducts, as well as fragment ions. The raw data were analyzed with Bruker Target Analysis 1.3 software.

Retention time, ***accurate mass*** of the precursor ion and adducts, ***isotopic pattern***, in combination with absence of the peak in the procedural blank were the parameters used for confirmation of the target compounds. Experimental ***fragment ions*** were also considered, along with the ion ratio, intensity and isotopic pattern. Furthermore, semi-quantitation of these contaminants was possible.

The method herein presented, in addition of providing accurate information about the presence of a large number of relevant substances, has the advantage that the data generated can be further processed for suspect and non-target screening, expanding the information on the samples. An important advantage of this method is that ***retrospective investigation*** of the data is available to look for the presence of additional CECs and their TPs, which were not considered at the time of the analysis.

Sampling-Sample Preparation

Mixed-bed SPE cartridges

Oasis HLB

Mixture: Strata-XCW, Strata-XAW, ENVI+

24-h composite flow-proportional samples of influent wastewaters & effluent wastewaters *(March 2014)*

WWTP of Athens, Greece

Elution

↳ MeOH: ethyl acetate (1.7 % Formic acid)

↳ MeOH: ethyl acetate (2% Ammonia)

in-house database

✓ *more* than **700 pesticides**

✓ *more* than **800 EPs & TPs**

1500 compounds + ESI screening

500 compounds - ESI screening

~200 common compounds

Analysis

Acclaim RSLC 120 C18 2.2µm 120Å 2.1 × 100 mm

Gradient elution: H2O/MeOH

+ESI : 5 mM amm. formate 0.01% formic acid

-ESI : 5 mM amm. acetate

Flow rate: 200-480 µL/min

Chromatogram: 20 min

HPLC-HRMS -QTOF-MS/MS

*** bb-CID ***

+ ESI **- ESI**

Collision Energy

MS: 4 eV ***MS/MS***: 25 eV

Scan: 50-1000 m/z

Spectra rate: 2 Hz

Resolution ≥ 30,000

MS & MS/MS data in a single run

TargetAnalysis

#	Id	Cmpd.Name	Formula	PMI	m/z calc.	m/z me...	Err [ppm]	Err [mDa]	mSigma	RT exp...	RT meas...	deltaRT ...	I	Area
18	++++	2-Amino-Benzothiazole	C 7 H 6 N 2 S 1	[M+H]+	151.0324	151.0322	1.6	0.2	4.4	6.94	6.94	-0.00	8210	37623
33	++++	Metolachlor	C 15 H 22 Cl 1 N 1 O 2	[M+H]+	284.1412	284.1410	0.5	0.1	46.9	11.36	11.36	-0.00	6026	29646
9	++++	tramadol	C 16 H 25 N 1 O 2	[M+H]+	264.1958	264.1957	0.5	0.1	11.6	6.09	6.09	-0.00	43288	209223
22	++++	venlafaxine	C 17 H 27 N 1 O 2	[M+H]+	278.2115	278.2113	0.7	0.2	13.6	7.28	7.28	0.00	10963	51782
7	++++	Benzotriazole (BTR)	C 6 H 5 N 3	[M+H]+	120.0556	120.0555	-0.6	-0.1	10.3	5.91	5.91	-0.00	246820	1073...
2	++++	Metformin	C 4 H 11 N 5	[M+H]+	130.1087	130.1087	-0.2	-0.0	13.1	1.38	1.34	0.04	136314	667219
19	++++	Me-Benzotriazole	C 7 H 7 N 3	[M+H]+	134.0713	134.0713	-0.0	-0.0	6.8	7.03	7.01	0.02	160974	1016...
1	++++	melamine	C 3 H 6 N 6	[M+H]+	127.0727	127.0725	1.2	0.2	33.5	1.33	1.34	-0.01	17657	99037
13	++++	Lamotrigine	C 9 H 7 Cl 2 N 5	[M+H]+	256.0151	256.0146	-1.9	-0.5	17.2	6.49	6.49	-0.00	29431	132265
11	++++	Metoprolol tratrate	C 15 H 25 N 1 O 3	[M+H]+	268.1907	268.1904	-1.4	-0.4	16.8	6.14	6.14	-0.00	6722	31658
10	++++	Norvenlafaxine	C 16 H 25 N 1 O 2	[M+H]+	264.1958	264.1957	0.5	0.1	11.6	6.09	6.09	-0.00	43288	209223
16	+++	Schradan	C 8 H 24 N 4 O 3 P 2	[M+H]+	287.1396	287.1395	0.4	0.1	62.9	6.54	6.54	-0.00	2819	12706
14	+++	chloridazon	C 10 H 8 Cl 1 N 3 O 1	[M+H]+	222.0429	222.0427	0.9	0.2	66.5	6.53	6.51	0.02	2434	9597
32	++	Prometryn	C 10 H 19 N 5 S 1	[M+H]+	242.1434	242.1428	2.4	0.6	4.5	11.18	11.28	-0.10	5038	42026
31	++	terbutryn	C 10 H 19 N 5 S 1	[M+H]+	242.1434	242.1428	2.4	0.6	4.5	11.28	11.28	0.00	9028	42065

Criteria

- ***deltaRT*** ≤ 0.05 min
- ***Accuracy***: Error ≤ 5 ppm
- ***Isotopic fit***: ≤ 20 mSigma
- ***MS/MS fragments***, ion ratio
- ***Ion Intensity*** > 500 (+ESI) / 200 (-ESI)
- ***Area*** > 2000 (+ESI) / 800 (-ESI)

Validation

200 target compound over the whole range of the databases

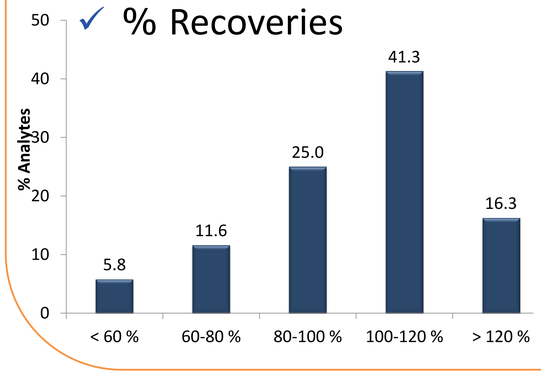
- 170 + ESI
- 50 - ESI

✓ Linearity in stds, spiked samples & matrix-matched samples

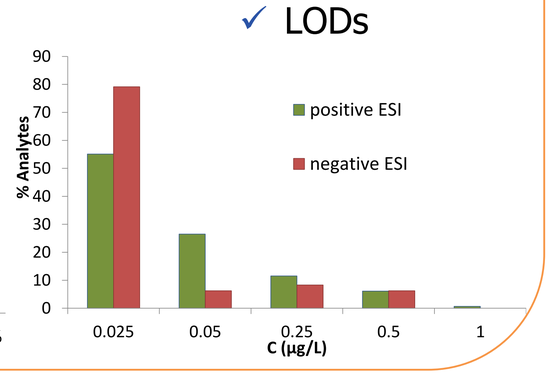
R^2> 0.92- 0.9999

✓ Repeatability: %RSD <20% (for 82.7% of analytes)

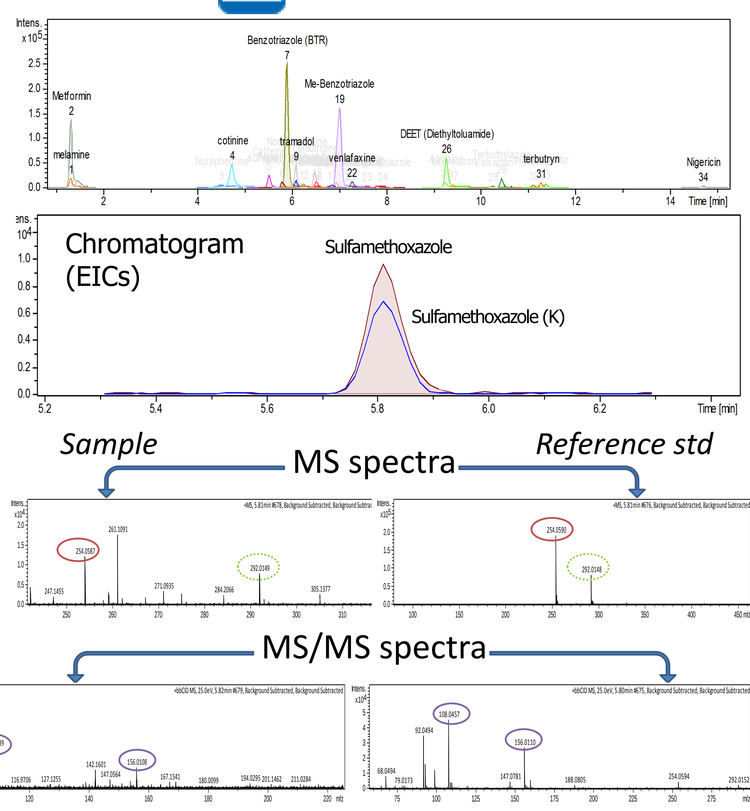
✓ % Recoveries



✓ LODs



DataAnalysis



Results

Concentration of target analytes ranged from **ng-mg/L**

1.9 **ng/L** (Benzotriazole)

0.5 **mg/L** (Metformin)

26.1 **µg/L** (Caffeine)

effluent		influent
123	**Compounds detected**	**176**
75	pharmaceuticals & drugs of abuse	103
23	pesticides	39
6	PFCs	6
4	sweeteners	4
10	Disinfection by-products & PCP	19
5	Aminoacids	5

Conclusions

- ✓ HR-MS & MS/MS data in a single run, with Resolution ≥ 30,000.
- ✓ Formation of a database of over 1500 EPs, including t_R, adducts and qualifier ions.
- ✓ Generic SPE, covering a wide range of analytes.
- ✓ Validation of the method, with good repeatability and recoveries.
- ✓ Screening of wastewater samples and quantification of analytes.

European Union European Social Fund — Co- financed by Greece and the European Union

OPERATIONAL PROGRAMME EDUCATION AND LIFELONG LEARNING investing in knowledge society — MINISTRY OF EDUCATION, LIFELONG LEARNING AND RELIGIOUS AFFAIRS — MANAGING AUTHORITY

NSRF 2007-2013 programme for development — EUROPEAN SOCIAL FUND

This research has been co-financed by the European Union and Greek national funds through the OperationalProgram "Education and Lifelong Learning" of the National StrategicReference Framework (NSRF)– ARISTEIA 624 (TREMEPOL project).

TrAMS Trace Analysis and Mass Spectrometry Group