



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

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Abstract

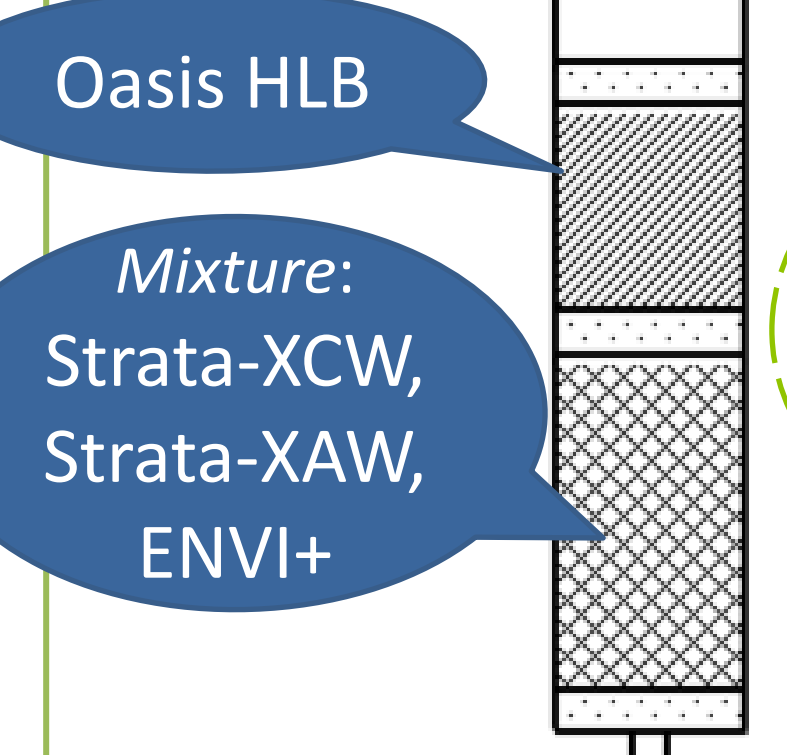
High resolution mass spectrometry has dramatically improved the possibilities of the environmental analysis. The present study describes the development of an analytical method, based on liquid chromatography quadrupole-time-of-flight mass spectrometry (**LC-QToF-MS**) for the target determination of 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



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

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



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

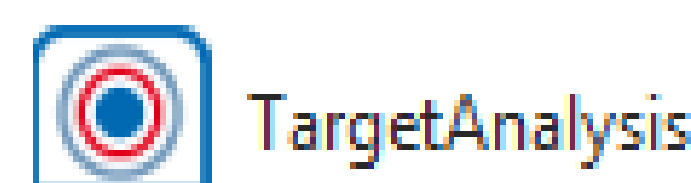
Analysis

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



Validation

200 target compound over the whole range of the databases

- ▶ 170 + ESI
- ▶ 50 - ESI

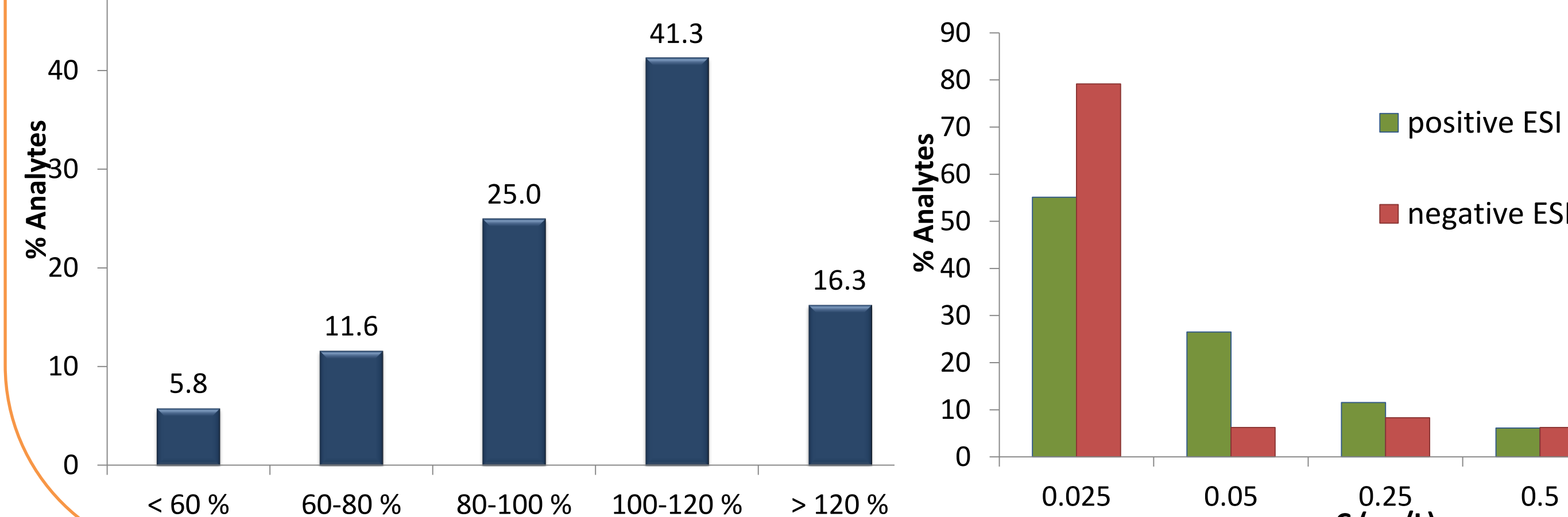
✓ Linearity in stds, spiked samples & matrix-matched samples

$R^2 > 0.92 - 0.9999$

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

✓ % Recoveries

✓ LODs



Criteria

- $\Delta RT \leq 0.05$ 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)

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

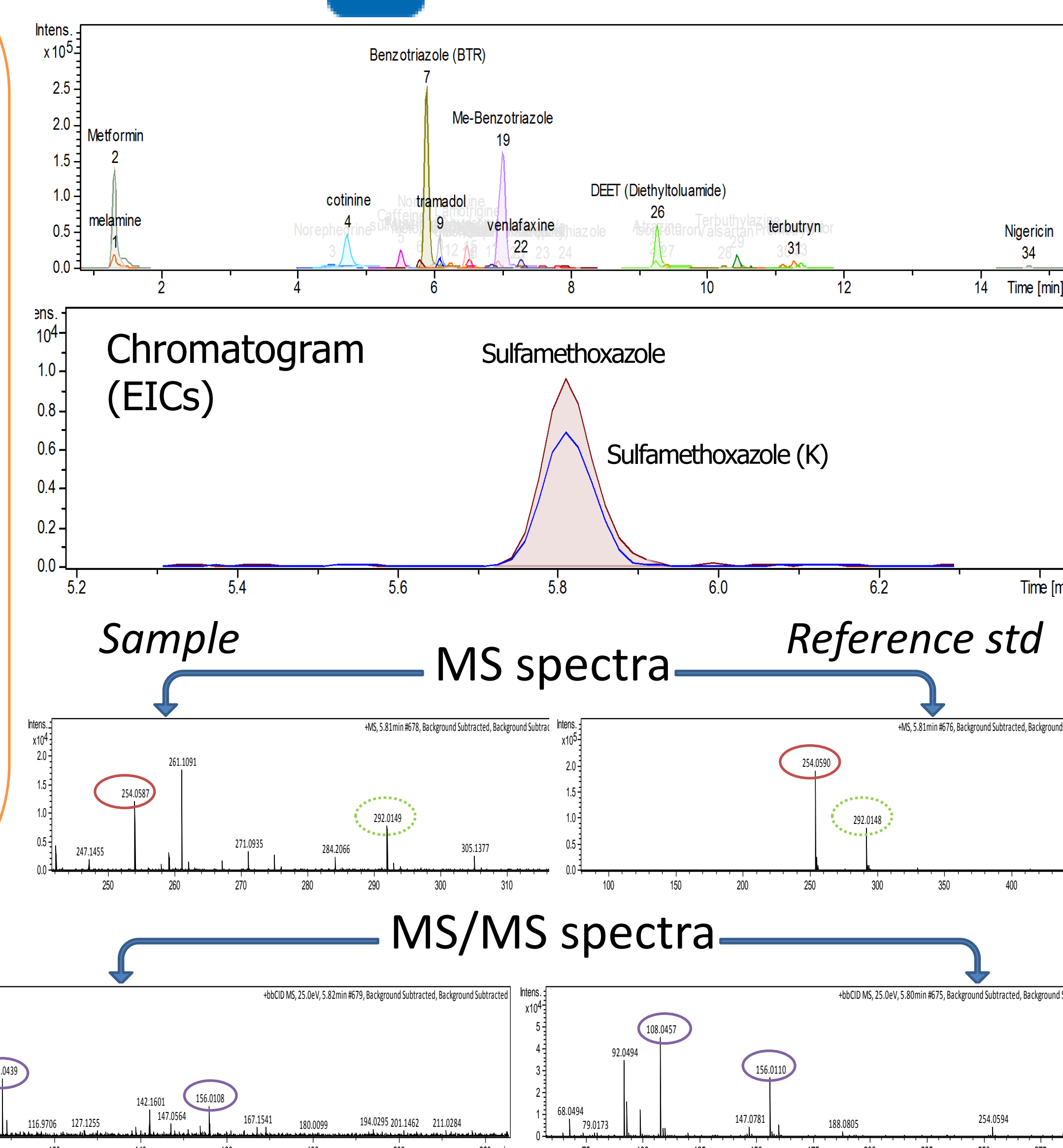
Results

1.9 ng/L
(Benzotriazole)

0.5 mg/L
(Metformin)

26.1 µg/L
(Caffeine)

effluent	Compounds detected	influent
123		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 $\geq 30,000$.
- ✓ 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.

