Journal of Hazardous Materials 387 (2020) 121712

Contents lists available at ScienceDirect

Journal of Hazardous Materials

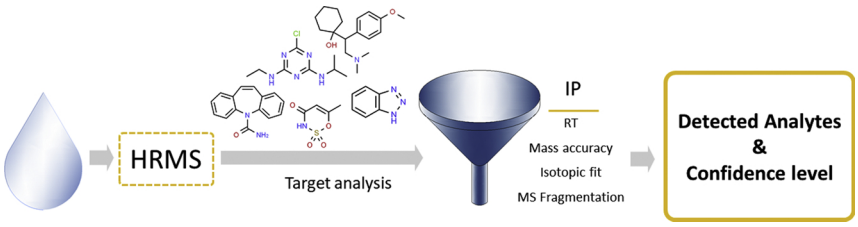
journal homepage: www.elsevier.com/locate/jhazmat

Wide-scope target screening of > 2000 emerging contaminants in wastewater samples with UPLC-Q-ToF-HRMS/MS and smart evaluation of its performance through the validation of 195 selected representative analytes

Pablo Gago-Ferrero[a,b,1], Anna A. Bletsou[a,1], Dimitrios E. Damalas[a], Reza Aalizadeh[a], Nikiforos A. Alygizakis[a], Heinz P. Singer[c], Juliane Hollender[c,d], Nikolaos S. Thomaidis[a,*]

[a] Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis Zographou, 15771 Athens, Greece
[b] Catalan Institute for Water Research (ICRA), Carrer Emili Grahit 101, 17003 Girona, Spain
[c] Eawag: Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133, 8600 Dübendorf, Switzerland
[d] Institute of Biogeochemistry and Pollutant Dynamics, ETH, 8092 Zürich, Switzerland

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: R Teresa

Keywords:
Wide-scope target screening
HRMS
Quantitation
Wastewater
Micropollutants
Identification points (IPs)

ABSTRACT

This study presents the development and validation of a comprehensive quantitative target methodology for the analysis of 2316 emerging pollutants in water based on Ultra-Performance Liquid Chromatography Quadrupole-Time-Of-Flight Mass Spectrometry (UPLC-Q-ToF-HRMS/MS). Target compounds include pesticides, pharmaceuticals, drugs of abuse, industrial chemicals, doping compounds, surfactants and transformation products, among others. The method was validated for 195 analytes, chosen to be representative of the chemical space of the target list, enabling the assessment of the performance of the method. The method involves a generic sample preparation based on mixed mode solid phase extraction, a UPLC-QTOF-MS/MS screening method using Data Independent Acquisition (DIA) mode, which provides MS and MS/MS spectra simultaneously and an elaborate strong post-acquisition evaluation of the data. The processing method was optimized to provide a successful identification rate > 95 % and to minimize the number of false positive results (< 5 %). Decision limit (CCα) and detection capability (CCβ) were also introduced in the validation scheme to provide more realistic metrics on the performance of a HRMS-based wide-scope screening method. A new system of identification points (IPs) based on the one described in the Commission Decision 2002/657/EC was applied to communicate the confidence level in the identification of the analytes. This system considers retention time, mass accuracy, isotopic fit and fragmentation; taking full advantage of the capacities of the HRMS instruments. Finally, 398 contaminants were detected and quantified in real wastewater.

* Corresponding author.
E-mail address: ntho@chem.uoa.gr (N.S. Thomaidis).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jhazmat.2019.121712
Received 24 July 2019; Received in revised form 16 November 2019; Accepted 16 November 2019
Available online 18 November 2019

1. Introduction

Emerging pollutants (EPs) are compounds that are increasingly being detected in the environment and are not currently included in routine environmental monitoring programs. These substances enter the aquatic environment mainly through wastewater treatment plant (WWTP) discharges due to their partial or inexistent removal during conventional wastewater treatments (Luo et al., 2014). There is a concern that these compounds may trigger unwanted ecological effects and thus they are candidates for future legislation due to their potential adverse effects and / or persistency (Eggen et al., 2014). The EPs encompass vast number of diverse substances but only a small portion has been sufficiently monitored in the water bodies (Loos et al., 2013).

The determination of EPs in environmental samples constitutes a great challenge and the most common choice has been the use of multi-residue methods that include a limited number of compounds (normally < 100). This automatically reveals a gap in environmental analysis concerning methods and techniques that can analyze simultaneously a higher number of EPs. Advances in the high resolving power mass analyzers (HRMS) have contributed towards the development of real wide-scope multi-residue screening methods (Hernandez et al., 2012) that also offer the potential of retrieving information about new analytes in post-acquisition approaches (retrospective analysis). In the literature there are few examples of wide-scope multi-residue target screening methodologies utilizing HRMS that analyze between 300 and 600 compounds in water samples using QTOF (Robles-Molina et al., 2014; Gomez et al., 2010) and Orbitrap (Ruff et al., 2015; Mechelke et al., 2019; Hollender et al., 2018) instruments. Apart from that, there are larger suspect-screening based methodologies (up to 1000 compounds) that used standards only for the substances that were firstly tentatively identified (Hernandez et al., 2015a; Moschet et al., 2014; Gago-Ferrero et al., 2018).

So far, one of the main deficiencies in wide-scope target screening analysis by HRMS methods is the lack of standardized criteria and harmonized guidance for the accurate identification and quantitation of the analytes (Kaufmann et al., 2015; Nielen et al., 2007; Diaz et al., 2013; Mol et al., 2012). For a comprehensive target analysis the use of reference standards is necessary in order to compare (I) retention times (RTs), (II) MS spectra profiles (precursor ion, adducts and in-source fragments) and (III) MS/MS spectra (fragment ions and ratios). To reduce the number of false negative and false positive findings, a careful optimization of the data processing parameters (mass accuracy, RT or signal thresholds) is essential. Since the size of HRMS data is enormous, automated solutions are required. Regarding the identification and quantitation, the resolving power of the mass analyzer is of great significance (Acena et al., 2015; Rajski et al., 2014). Therefore, the criteria defined in the Commission Decision 2002/657/EC (EC, 2002), SANCO 12571/2013 (SANCO, 2013) and the latest version SANTE 11813/2018 (SANTE, 2017) do not take full advantage of the available instrumentation capabilities and are still more low-resolution oriented. Also, the compounds used as a validation dataset should be selected upon well-defined criteria and follow a uniform protocol (Diaz et al., 2013; Leendert et al., 2015). For quantitative screening methodologies recovery values, screening detection limits (SDL) and limits of identification (LOI) are investigated as the main validation parameters to estimate the threshold concentration at which detection and identification become reliable, respectively (Diaz et al., 2013; Mol et al., 2012; Hernandez et al., 2015b; Boix et al., 2014; Vergeynst et al., 2014). It is noteworthy that in the majority of the studies in the literature up to now the HRMS methods are evaluated only for some analytes instead of for the whole list (Hernandez et al., 2015b; Moschet et al., 2013).

One objective of the present study was the development and validation of a comprehensive quantitative wide-scope multi-residue target method for the analysis of 2316 EPs in water including pesticides, pharmaceuticals, drugs of abuse, industrial chemicals, doping compounds, surfactants and several transformation products including metabolites. The method involves a generic sample preparation, UPLC-QTOF-MS/MS screening method and a strong post-acquisition evaluation of the data (where most of the developing efforts were devoted). To facilitate data evaluation, an in-house database was built with the RTs, MS and MS/MS ions information by injecting standards for all the target compounds. Additional objective of the study was the development of a validation approach for wide-scope quantitative HRMS screening methods, including decision limit (CCα) and detection capability (CCβ). The current evaluation system for the identifications of EPs (SANTE, 2017) was modified considering the new available opportunities brought by HRMS. In this regard, the optimization of the different screening parameters such as mass accuracy, isotopic and fragmentation pattern as well as peak score was carried out in order to minimize false negative results (less than 5 % as it is required for any screening method (SANCO, 2013)). Accurate identification criteria were set and a validation protocol is proposed in order to evaluate the performance criteria of the HRMS method including a new identification point (IP) system. Finally, the method was applied in real influent and an effluent wastewater from the WWTP of Athens (Greece).

2. Materials and methods

2.1. Reagents and chemicals

Information on the standards used on this study is provided in the Supporting Information (SI 1 and Table S1).

All the solvents used were UPLC-MS grade. Acetonitrile (ACN) and methanol (MeOH) were purchased from Merck (Darmstadt, Germany), whereas 2-propanol of LC–MS grade was purchased from Fisher Scientific (Geel, Belgium). Distilled water was provided by a Milli-Q purification apparatus (Millipore Direct-Q UV, Bedford, MA, USA). Sodium hydroxide monohydrate (NaOH) for trace analysis ≥99.9995 % and formic acid 99 % were purchased from Fluka (Buchs, Switzerland). For the sample preparation, the empty solid phase extraction polypropylene tubes (6 mL), as well as the cartridge sorbent materials Sepra ZT (Strata-X), Sepra ZT-WCX (Strata-X-CW) and ZT-WAX (Strata-X-AW) were obtained from Phenomenex (Torrance, USA). The Isolute ENV + sorbent material and the frits (20 µm, 6 mL) were from Biotage (Ystrad Mynach, UK). Glass fiber filters (GF/F, pore size 0.7 µm) used for wastewater filtration were obtained from Whatman International Ltd (Maidstone, England). Regenerated cellulose syringe filters (RC) of 15 mm diameter and 0.2 µm pore size were obtained from Phenomenex (Torrance, CA, USA).

2.2. Sample collection

Influent and effluent wastewater samples (24 -h composite flow proportional samples) were collected from the WWTP of Athens (Greece), on the 15th of March 2014 (Saturday) in triplicates. The WWTP of Athens is designed with primary sedimentation, activated sludge process with biological nitrogen and phosphorus removal and secondary sedimentation. The estimated sewage flow for the collected samples is 7,200,000 m^3 day^{-1}. The closest connected household is 0.5 km and the most remote 30 km from the WWTP. The residential population connected to the WWTP based on official census, excluding commuters, is 3,700,000 and the number of people estimated based on the number of house connections is 4,562,500. The WWTP is designed to serve a population equivalent of 5,200,000 and thus is by far the

largest in Greece and one of the largest in the world.

Wastewater was collected in pre-cleaned high-density polyethylene (HDPE) bottles (analyte sorption was checked and found to be negligible). Untreated and treated wastewaters were filtered with glass fiber filters (pore size 0.7 μm) immediately after arrival at the laboratory. Samples were stored in the dark at 4 °C until analysis.

2.3. Sample preparation

Sample extraction was carried out using the protocol described by Kern et al. and Gago-Ferrero at al. (Kern et al., 2009; Gago-Ferrero et al., 2015). Sample aliquots of 100 mL were adjusted to pH 6.5, and then spiked with an internal standard mix. Solid phase extraction (SPE) was conducted using four different SPE materials simultaneously in an in-house cartridge to achieve sufficient enrichment for a very broad range of compounds (200 mg Oasis HLB, 150 mg Isolute ENV+, 100 mg Strata-X-AW and 100 mg Strata-X-CV). The cartridges were preconditioned with methanol and water and the water samples were loaded, then there was a drying step under vacuum. The elution was conducted with 4 mL of methanol/ethyl acetate (v:v 50:50) containing 2 % ammonia, followed by 2 mL of methanol/ethyl acetate (v:v 50:50) containing 1.7 % formic acid. Extracts were evaporated under a gentle nitrogen stream to a volume of 100 μL and then reconstituted to 0.5 mL with a final proportion of MeOH/water (v:v 1:1). Finally, the extracts were filtered through a 0.2 μm RC filters.

2.4. UPLC- Q-TOF-MS/MS analysis

An ultrahigh-performance liquid chromatography (UHPLC) system, with a HPG-3400 pump (Dionex UltiMate 3000 RSLC, Thermo Fisher Scientific, Germany), interfaced to a QTOF mass spectrometer (Maxis Impact, Bruker Daltonics, Bremen, Germany) was used for the screening analysis. The chromatographic separation was performed on an Acclaim RSLC C18 column (2.1 × 100 mm, 2.2 μm) from Thermo Fisher Scientific (Dreieich, Germany) preceded by a guard column of the same packaging material, ACQUITY UPLC BEH C18 1.7 μm, VanGuard Pre-Column, Waters (Ireland), thermostated at 30 °C. The mobile phase composition in Positive Ionization mode (+ESI) consisted of (A) H_2O:MeOH (90:10) with 5 mM ammonium formate and 0.01 % formic acid and (B) MeOH with 5 mM ammonium formate and 0.01 % formic acid. For Negative Ionization mode (-ESI), the mobile phase consisted of (A) H_2O:MeOH (90:10) with 5 mM ammonium acetate and (B) MeOH with 5 mM ammonium acetate. The gradient elution program, which includes changes in the flow rate, was the same for both ionization modes. It is presented in detail in Table S2 (SI 2). The injection volume was set to 5 μL.

The operating parameters of the electrospray ionization interface (ESI) in positive mode were: capillary voltage, 2500 V; end plate offset, 500 V; nebulizer, 2 bar; drying gas, 8 L min^{-1}; dry temperature, 200 °C; and for negative mode: capillary voltage, 3500 V; end plate offset, 500 V; nebulizer, 2 bar; drying gas, 8 L min^{-1}; dry temperature, 200 °C. The QTOF MS system operates in broadband collision-induced dissociation (bbCID), a Data Independent Acquisition (DIA) mode, where two sequential full scan events are triggered. The first scan at low collision energy (4 eV) results in a MS full scan over the range *m/z* 50–1000. The second scan at high collision energy (25 eV) results in a MS/MS all ion fragment mode also in the range *m/z* 50–1000. The scan rate was 2 Hz per cycle.

A QTOF external calibration was daily performed with a sodium formate solution, and a segment (0.1-0.25 min) in every chromatogram was used for internal calibration, using a calibrant injection at the beginning of each run. The sodium formate calibration mixture consisted of a solution with sodium formate 10 mM in a mixture of water/isopropanol (1:1). The theoretical exact masses of the calibration ions with formulas $Na(NaCOOH)_{1-14}$ in the range of 50–1000 Da were used for calibration. The instrument provided a typical mass resolving power of 36,000–40,000 during calibration (39,274 at *m/z* 226.1593, 36,923 at *m/z* 430.9137, and 36,274 at *m/z* 702.8636). Post-acquisition data treatment was implemented with DataAnalysis 4.4 and TASQ 1.4 software (Bruker Daltonics, Bremen, Germany).

2.5. Development and validation of the method

2.5.1. Validation dataset selection strategy for wide-scope target screening methods

The individual evaluation of the performance and the proper analytical validation of 2316 particular substances is not feasible, since it would be an extremely time consuming task. Therefore, the selection of a validation dataset is necessary. In most of the studies this selection is carried out without following a systematic strategy with clear prioritization criteria. However, in the present work effort has been put in the compound selection criteria for the validation of wide-scope screening methodologies. Therefore, the validation dataset was selected by considering different criteria in order to guarantee its representativeness: (I) the RT (which is directly related to different physicochemical properties such as logP) and (II) the ionization mode. The validation set (Table S3) contained 195 compounds (approximately 10 % of the database) including pesticides, pharmaceuticals of different therapeutic families, illicit drugs, industrial chemicals and transformation products, representing a diverse set of chemical structures. In this way, a wide-scope validation of the sample preparation protocol was implemented.

Figure S1 (SI 3) compares the distribution of the RTs of the compounds included in the validation dataset with the RTs of the whole database, showing similar profiles in both cases. In positive ionization, the RTs of the selected compounds were in the range between 1.4 and 12.4 min, being the average RT (7.1 ± 2.8) min. This is comparable to the values obtained for the overall database (from 1.2 to 14.99 min. and average RT 7.6 ± 3.0 min). In the negative ionization mode the RTs in the validation dataset were in the range between 1.3 min and 13.7 min and the average t_R was 7.8 ± 2.9 min, also similar to the one obtained for the overall database (7.7 ± 3.0 min). Moreover, 30 % of the compounds in the validation dataset were ionized in ESI(-). This value is very close to the 25 % obtained for the overall database, providing a good representativeness in this regard.

2.5.2. HRMS data processing workflow - Optimization of the screening parameters to minimize false negative and false positive results

According to SANTE/11945/2017 (SANTE, 2017), a screening method is the one that can identify the non-compliant samples (true positive) with a β-error lower than 5 %. Thus, the aim of the optimization of the screening parameters was to accomplish this requirement. Standard solutions and spiked samples were used to define the thresholds of the screening parameters that provide successful identification rates (SIR) > 95 % and at the same time minimize the number of false positives. The screening parameters studied were: (1) peak area and intensity, (2) RT tolerance (dRT), (3) mass accuracy error (dMZ) and (4) isotopic fit score (dIF). Experiments were conducted using the validation dataset of the (+ESI) (153 compounds) and (-ESI) (42 compounds) at different concentrations (in the range 5−100 μg/L). Processing of the acquired data was carried out with TASQ 1.4 (Bruker Daltonics, Bremen, Germany). This software utilizes an algorithm that considers the molecular formula and the RT of each analyte in order to perform the screening, giving as a response a score for the detection of each compound. For each screening parameter (peak area and intensity, dRT, dMZ, dIF), two thresholds were set (after method processing optimization), a strict one and a wider one. Classification of the detected analytes was performed, according to their compliance to the predefined criteria. Additionally, a scoring system was used to visualize the compliance (or not) of the detected analytes with the aforementioned screening thresholds. In other words, these scores reflect the analytical evidence that support the screening/identification of each compound.

Consequently, the analytes were classified between "very good", "good" or "poor" scoring rate (Table 1). When "very good" score is obtained, the screening parameters are within very strict limits and no further evaluation is necessary. Compounds that get a "good" score rate, still meet the defined criteria for the screening, but manual inspection is recommended. In case of "poor" rate, the corresponding compound is beyond the criteria set and is discarded.

2.5.3. Method performance criteria

To evaluate the analytical performance of the method, linearity, accuracy, precision and matrix effects were considered, based on the wide-scope validation dataset. The linear dynamic range of the method (based on regression coefficients) was studied in standard solutions and in spiked effluent samples. The SDL and the LOI are measures to estimate the threshold concentration at which detection and identification become reliable, respectively. The SDL is established as the lowest concentration level tested for which a compound is detected in all spiked samples, at the expected RT and with a defined mass accuracy error of the precursor ion. The LOI is established as the lowest concentration tested for which a compound is satisfactorily identified. SDL was estimated as the concentration level at which the thresholds of (i) RT and (ii) mass accuracy of the precursor ion were satisfied, while for LOI the thresholds of (i) RT and mass accuracy of (ii) the precursor ion and (iii) fragment ion were satisfied, in a similar way than described by Boix et al. (Boix et al., 2014). According to the IPs system proposed in the current study, for SDL at least 2 IPs are required and for LOI at least 4 IPs. Additionally, decision limit (CCα) and detection capability (CCβ) values were calculated from the standard addition calibration curves, according to the equations (EC, 2002):

$$\boldsymbol{CC\alpha} = \frac{\boldsymbol{a}}{\boldsymbol{b}} + 2.33\frac{\boldsymbol{Sa}}{\boldsymbol{b}}(\text{at99\%confidencelevel})\text{and}\boldsymbol{CC\beta} = \boldsymbol{CC\alpha} + 1.64\frac{\boldsymbol{Sa}}{\boldsymbol{b}}(\text{at95\%confidencelevel}).$$

Where α is the y-intercept, b the slope of the calibration curve and S_α the standard deviation of the intercept. Verification of the CCβ concentration was afterwards performed through spiked samples.

Recovery tests were conducted with a pool of real effluent water. Method recovery rates at three concentration levels (0.5, 0.05 and 0.025 μg/L) were calculated by dividing the peak area of the spiked sample (before SPE) by the matrix-matched sample (spiked before instrumental analysis). As real samples may already contain target compounds, the signals corresponding to those substances were afterwards subtracted from the spiked samples. Matrix factor was estimated by dividing the peak area of matrix-matched standard solution by the peak area of the standard solution. As real samples might contain target compounds, wastewater samples were analyzed to determine their concentrations, which afterwards were subtracted from the spiked and the matrix-matched samples. Matrix effect was calculated by the equation: ME = (1-MF) × 100. Finally, precision was calculated in terms of repeatability and presented in % RSD.

2.6. Target compounds quantitation

Quantification of the detected analytes was based on peak areas and was performed by using standard addition calibration. Compounds detected in the first qualitative screening were afterwards spiked in different quantities into additional SPE experiments (using the same sample) and the extracts were reinjected to obtain three point calibration curves. Therefore, the method includes an analysis where all the dectected compounds are mixed together with the matrix to show that there are no direct co-compound quantitation effects arising from the mixture. The spiked quantity varied according to the estimated concentration of each analyte.

3. Results and discussion

3.1. Identification and Confirmation of the analytes

In this work we propose a system of identification points based on the Decision 2002/657/EC (EC, 2002), which takes full advantage of the capabilities of modern HRMS instruments (Table 4). In this system the exact mass of the precursor ion (most abundant ion in full scan MS spectrum) and the RT fit count together 2 IPs. The isotopic fit, which is a measure of the correlation between the theoretical and measured isotopic patterns of the peak, contain diagnostic information and earns 0.5 IP. Furthermore, fragment ions in MS/MS mode or in-source fragment ions in full scan MS earn 2.5 IPs. Practical advantages of the current identification point system are discussed in the section *Application of the method*.

3.2. Optimization of HRMS screening parameters

As a first step of screening parameters optimization, peak area and intensity thresholds were defined in order to obtain a 100 % SIR in standard solutions. The thresholds were set at 800 and 200 counts for area and intensity, respectively. Smoothing of the extracted ion chromatograms was also applied as part of the automatic filtering. After 100 % SIR was achieved, the scoring parameters were optimized to maximize the number of "very good" results within a reasonable range of acceptance taking into account the instrument performance for different parameters. Indicatively, Table 2 presents the results of the screening parameters for which better scores were achieved.

The criteria for the successful identification of a compound were set as follows: RT tolerance: RT_S / RT_W = < 0.1 / < 0.4 min; mass accuracy: MZ_S / MZ_W = < 2.5 / < 5 m Da; and isotopic fitting: IF_S / IF_W = < 100 / < 200 mSigma. These parameters were afterwards applied in the evaluation of standard solutions and spiked samples at different concentration levels obtaining in all cases successful identification rates (SIR) above 96 %, as it can be observed in Table 3. Interestingly, the wastewater matrix did not lead to a reduction of the SIR.

In the literature, the accuracy threshold is usually applied based on the instrument capabilities and the experience of the researcher, without further evaluation (Díaz et al., 2012). In contrast, in the present study a consistent investigation of the accuracy threshold value was performed.

3.3. Method performance criteria

3.3.1. SDL/LOI and CCα/CCβ

According to the guidelines for the validation of screening methods for residues of veterinary medicines (CRLs 2010 (CRLs, 2010), Commission Decision 2002/657/EC (EC, 2002)) the SDL of a qualitative screening method is the lowest level at which an analyte has been

Table 1
Individual ion score ratings for retention time, mass accuracy and isotopic fitting.

Factor	Requirement	Score rating	Characterization
Retention time (RT score)	dRT < RT_S	+ +	Very good
	RT_S < dRT < RT_W	+	Good
	RT_W < dRt	−	Poor
Mass accuracy (MZ score)	dMZ < MZ_S	+ +	Very good
	MZ_S < dMZ < MZ_W	+	Good
	MZ_W < dMZ	−	Poor
Isotopic fit (IF score)	dIF < IF_S	+ +	Very good
	IF_S < dIF < IF_W	+	Good
	IF_w < dIF	−	Poor

*$_S$ = strict, $_W$ = wide.

Table 2
Optimization of the screening parameters.

	Strict / Wide range*		
Ret. time tolerance (min)	0.05 / 0.4	0.1 / 0.4	0.1 / 0.4
Mass Accuracy	5 / 10 m Da	5 / 10 m Da	2.5 / 5 m Da
mSigma threshold (isotopic fit)	50 / 100	100 / 200	100 / 200
% *"Very Good"* Score	63.8	74.7	100
% *"Good"* Score	36.1	25.3	0

*Strict range corresponds to the threshold for which "very good" results are obtained - Wide range corresponds to the threshold for which "good" results are obtained.

Table 3
Successful identification rate of standard solutions and spiked samples at different concentration levels.

C standard solutions (μg/L)	**% Successful Identification rate (% SIR)**	C spiked samples (μg/L)[a]	**% Successful Identification rate (% SIR)**
200	100	1	99.3
100	100	0.5	98.7
50	99.3	0.25	98.7
10	97.2	0.05	98.0
5	96.5	0.025	96.4

[a] Concentration without considering the SPE enrichment factor (x200).

Table 4
Proposed Identification Point (IPs) system in HRMS analysis[a].

Requirements	**Identification Points earned**
Retention time + Precursor ion (accurate mass)	2.0
Isotopic fitting* (Abundance and accuracy of M + 1, M + 2,…)	0.5
Fragment ions (mass accuracy)	2.5

[a] Accuracy < 2 m Da / 5 ppm and resolution < 7500 units is required.
* At least one isotope.

detected with an acceptable false-negative rate of 5 % or lower. Indeed, CCβ is the smallest content of the analyte that may be detected, identified and/or quantified in a sample with an error probability of β (i.e. false compliant rate), which for screening tests should be < 5 %. Moreover, according to Directive 2002/657/EC, for screening methods the estimation of the detection capability CCβ is mandatory. The decision limit (CCα) and the detection capability (CCβ) values of all the compounds present in the validation dataset were calculated and the results are summarized in Table S4. In that table are also presented the corresponding IPs earned for each compound calculated for a concentration close to CCβ. Calculated values of CCα were in the range between 0.0019 and 0.98 μg/L (average 0.13 μg/L and median 0.070 μg/L) and values of CCβ were between 0.003 and 1.04 μg/L (average 0.16 μg/L and median 0.090 μg/L). In every case, at least 4 IPs should be earned at the CCβ concentration (which is a statistical measure) to confirm the detection of the compound. This was achieved for 184 out of the 195 compounds showing a very good performance of the method. For nortriptyline and ibuprofen 2.5 IPs were considered enough due to the practical impossibility of getting more. Nortriptyline presented low fragmentation under the conditions applied and no clear MS/MS spectrum was available and ibuprofen showed very low sensitivity. Apart from that, 9 compounds showed no fragmentation at all under those conditions, mostly in negative ionization mode. Therefore, no CCβ values are available for those substances.

It is important to note that although the SDL and LOI are thought sometimes to be equivalent to CCβ, it is not the same. LOI is a level of concentration, preselected, at which an analyte can be identified, while CCβ represent a statistical evaluation of the concentration at which an analyte can be identified with a beta-error 5 %. However, in the literature the most widely used validation parameters are SDLs and LOIs (Diaz et al., 2013; Mol et al., 2012; Hernandez et al., 2015b). CCβ values have been calculated only in a very limited number of studies like the one carried out by Leon et al., which investigated 87 banned veterinary drugs in biological samples using a wide-range HRMS screening method (Leon et al., 2012). In this regard, the work of Vergeynst et al. is particularly interesting as they proposed the use of CCα and CCβ as a realistic methodology to calculate decision limits in HRMS measurements (Vergeynst et al., 2013).

3.3.2. Validation results

Linearity was studied in solvent, in matrix extracts (pooled effluent wastewater) and in spiked samples for the compounds of the validation dataset. The linear dynamic range was studied in standard solution at 8 concentration levels, ranging from 0.5 μg/L to 200 μg/L. In spiked samples, linearity was studied at 9 concentration levels, from 0.0025 μg/L to 1 μg/L. The good linearity obtained in standards is demonstrated by the fact that 165 out of the 195 compounds were linear in the range 0.025–1 μg/L (47 of them also were linear between 0.0025 and 1 μg/L) with R^2 coefficients always above 0.98. In spiked samples 155 out of the 195 compounds showed good linearity at least between 0.5 μg/L and 100 μg/L. The specific linear range for each compound, the slope (b) and the correlation coefficients (r^2) of the calibration curve and the standard addition curve are presented in Table S5 (Supplementary Material).

Method precision data were estimated by the determination of repeatability values, in terms of %RSD and was estimated from the analysis of spiked samples, where six replicates were analyzed at three (3) fortification levels (1, 0.01, 0.005 μg/L). RSD values were below 20 % in all cases, except for bromohexine and methacriphos, which showed low sensitivity and for amphetamine at the lowest concentration level (0.005 μg/L). Fortified samples, standards in solvent, and standards in matrix were analyzed in order to evaluate the effectiveness of the extraction procedure and the matrix effects. Recovery rate and matrix effect experiments were performed at four fortification levels (0.5, 0.25, 0.05 and 0.025 μg/L). Acceptable absolute recovery rates (in the range 57–120 %) were observed for the vast majority of the studied compounds (> 75 % of the total). Results were very satisfactory taking into account the wide range of physicochemical properties of the studied substances. This is most probably due to the combination of mixed-mode materials which allows different interactions. The specific values of repeatability, recovery and matrix effect for each compound of the validation set are presented in Table S6, in Supplementary Material.

3.4. Application of the method

3.4.1. Screening of real influent & effluent wastewater

The optimized and validated method was applied in real influent and effluent wastewater samples collected on the same day from the WWTP of Athens. Confirmation of the detected analytes was performed based on the IPs system described in Table 2. In total, 398 compounds were detected in the samples; 367 in influent wastewater and 315 in effluent wastewater. Table 5 summarizes all the detected analytes in the different studied matrices accompanied by the IPs earned in each sample. In influent wastewater 217 compounds were detected earning ≥ 2 < 4 IPs and 150 extra substances (41 % of the total) were identified with additional MS/MS information (≥ 4 IPs). In effluent wastewater, 190 compounds were detected earning ≥2 < 4 IPs and 121 additional substances (38 % of the total) were identified earning ≥ 4 IPs. The detected substances included 66 pesticides, 215 drugs including 29 stimulants (most of them being amphetamine derivatives) and 9 sympathomimetics (ephedrine derivatives), 4 sweeteners, 10 perfluorinated compounds (PFCs), 8 amino acids and 31 industrial chemicals, among others. It is worth mentioning that a slightly higher number of transformation products (TPs) were detected in effluent

Table 5
Quantitative results of wastewater samples.

	Compound Name	CAS number	influent wastewater		effluent wastewater	
			IPs	**C (µg/L)**	**IPs**	**C (µg/L)**
	Pesticides					
1	Acetochlor	34256-82-1	2.5	0.003	2.5	0.0004
2	Amitrole	61-82-5	2.5	0.011	2	0.04
3	Atrazine	1912-24-9	2	0.03	< SDL	
4	Atrazine-desisopropyl	1007-28-9	2.5	0.016	2.5	0.004
5	Azoxystrobin	131860-33-8	≥5	0.06	≥5	0.03
6	Azoxystrobin acid	1185255-09-7	5	0.04	≥5	0.06
7	Carbendazim	10605-21-7	2	0.02	2	0.01
8	Carbofuran-3-hydroxy	16655-82-6	5	0.02	2.5	0.007
9	Carboxin	5234-68-4	2.5	0.0009	< SDL	
10	Chlormequat	7003-89-6	2.5	0.02	4.5	0.007
11	Climbazole	38083-17-9	5	0.15	≥5	0.14
12	Cycloheximide	66-81-9	2.5	0.011	< SDL	
13	Cyproconazole	94361-06-5	< SDL		2	0.002
14	Cyprodinil	121552-61-2	< SDL		2	0.002
15	Dalapon	75-99-0	2.5	0.01	< SDL	
16	Dazomet	533-74-4	5	0.05	2.5	0.035
17	DEET (Diethyltoluamide)	134-62-3	5	0.07	5	0.02
18	Difenoconazole	119446-68-3	2	0.01	< SDL	
19	Difenoxuron	14214-32-5	2.5	0.02	2.5	0.02
20	Diflubenzuron	35367-38-5	2.5	0.005	< SDL	
21	Dikegulac	18467-77-1	2	0.0009	< SDL	
22	Dimethachlor	50563-36-5	2	0.07	< SDL	
23	Dimethachlor-ESA	–	2.5	0.025	2.5	0.01
24	Dimethachlor-OXA	1086384-49-7	2.5	0.025	2	0.007
25	Dimethoate	60-51-5	2	0.03	2	0.025
26	Dinoterb	1420-07-1	5	0.03	2.5	0.01
27	Dioxacarb	6988-21-2	2	0.004	< SDL	
28	Diuron	330-54-1	≥5	0.031	≥5	0.02
29	Famoxadone	131807-57-3	2	0.0006	< SDL	
30	Fenuron	101-42-8	< SDL		2	0.018
31	Fipronil	120068-37-3	≥5	0.02	≥5	0.01
32	Fipronil sulfone	120068-36-2	2.5	0.0012	2.5	0.002
33	Fluazifop	69335-91-7	2.5	0.02	2.5	0.02
34	Fluconazole	86386-73-4	≥5	0.09	≥5	0.07
35	Fludioxonil	131341-86-1	2.5	0.005	2	0.004
36	Fluometuron	2164-17-2	≥5	0.041	≥5	0.025
37	Flutolanil	66332-96-5	2	0.01	2	0.01
38	Imazapyr	81334-34-1	2	0.01	2.5	0.01
39	Imidacloprid	138261-41-3	2	0.025	5	0.04
40	Iprovalicarb	140923-17-7	< SDL		2	0.02
41	Isoprocarb	2631-40-5	2.5	0.003	< SDL	
42	Metalaxyl	57837-19-1	≥5	0.003	≥5	0.008
43	Methiocarb (Mercaptodimethur)	2032-65-7	2	0.01	< SDL	
44	Methoxyfenozide	161050-58-4	4.5	0.051	≥5	0.034
45	Metobromuron	3060-89-7	2	0.004	< SDL	
46	Metolachlor	51218-45-2	5	0.002	5	0.007
47	Metolachlor-ESA	171118-09-5	2.5	0.003	2.5	0.005
48	Monocrotophos	6923-22-4	2	0.01	< SDL	
49	N-2,4-Dimethylphenylformamide (DMF. Metabolite Amitraz)	60397-77-5	2.5	0.008	2	0.0004
50	Napropamide	15299-99-7	2	0.007	2	0.02
51	Naptalam (N-1-Naphthylphthalamicacid)	132-66-1	< SDL		2.5	0.06
52	Oxycarboxin	5259-88-1	2.5	0.01	2	0.011
53	Penconazole	66246-88-6	2	0.03	≥5	0.04
54	Picaridin (Icaridin)	119515-38-7	2	0.03	2	0.03
55	Piperonyl butoxide	51-03-6	≥5	0.11	2	0.003
56	Pirimiphos-methyl	29232-93-7	2	0.01	4.5	0.02
57	Prometryn (Caparol)	7287-19-6	2	0.03	2	0.03
58	Propamocarb	24579-73-5	2.5	0.01	2.5	0.004
59	Propham	122-42-9	2.5	0.05	2.5	0.04
60	Propoxur	114-26-1	≥5	0.003	≥5	0.003
61	Simazine	122-34-9	2	0.05	2.5	0.04
62	Temephos	3383-96-8	2.5	0.04	< SDL	
63	Terbutryn	886-50-0	2	0.003	2.5	0.001
64	Thiabendazole	148-79-8	4.5	0.01	4.5	0.01
65	Thiamethoxam	153719-23-4	2.5	0.0006	5	0.001
66	Thiodicarb	59669-26-0	2	0.009	2.5	0.004
	Opiates, opioids					
67	Codeine (COD)	76-57-3	5	0.185	5	0.07
68	EDDP	30223-73-5	2	0.026	2	0.006
69	Hydrocodone	125-29-1	< SDL		2.5	0.02
70	Methadone (METH)	76-99-33	2	0.028	2.5	0.02

(*continued on next page*)

Table 5 (*continued*)

	Compound Name	CAS number	influent wastewater		effluent wastewater	
			IPs	**C (μg/L)**	**IPs**	**C (μg/L)**
71	Morphine	57-27-2	≥5	0.34	< SDL	
72	Norcodeine	467-15-2	< SDL		2.5	0.06
73	Normorphine	466-97-7	2.5	0.02	< SDL	
	Stimulants- Amphetamins					
74	2 C-D (2,5-dimethoxy-4-methylphenethylamine)	24333-19-5	2	0.004	2.5	0.003
75	3,4-DMA (dimethoxyamphtamine)	120-26-3	2	0.012	2	0.007
76	3,4-methylenedioxy-amphetamine (MDA)	4764-17-4	≥5	0.03	< SDL	
77	3,4-methylenedioxy-N-methylamphetamine (MDMA)	42542-10-9	2	0.02	< SDL	
78	4-methyl-2-hexanamine	105-41-9	2	0.05	2	0.01
79	4-Methyl-pyrrolidino-propiophenone (MPPP)	28117-80-8	2	0.01	2	0.01
80	Aminorex	2207-50-3	2	0.043	2	0.023
81	Amphetamine	300-62-9	≥5	0.042	< SDL	
82	Benzoylecgonine (BECG)	519-09-5	≥5	0.3	≥5	0.05
83	Cathine/ norpseudoephedrine	492-39-7	2.5	0.012	2.5	0.005
84	Cocaine (COC)	50-36-2	5	0.11	< SDL	
85	Dimefline	1165-48-6	< SDL		2.5	0.005
86	Dimethylamphetamine	1009-69-4	2.5	0.08	2.5	0.05
87	Ecgonine methyl ester (EME)	7143-09-01	2	0.11	< SDL	
88	Ethamivan	304-84-7	< SDL		≥5	0.028
89	Ethylamphetamine	457-87-4	2	0.06	2	0.03
90	Heptaminol	372-66-7	2.5	0.049	2.5	0.024
91	Mephentermine	100-92-5	2	0.074	2.5	0.097
92	Metaraminol (3,β-dihydroxyamphetamine)	337376-15-5	2.5	0.022	< SDL	
93	Methamphetamine (MA)	537-46-2	2.5	0.037	< SDL	
94	Midodrine	133163-28-7	2	0.03	2.5	0.018
95	Nikethamide	59-26-7	< SDL		2.5	0.01
96	Pemoline	2152-34-3	2	0.05	< SDL	
97	Phendimetrazine	17140-98-6	2	0.046	2	0.026
98	Phenelzine	51-71-8	2	0.036	2.5	0.011
99	Pholedrine (p-hydroxy-methylamphetamine)	6114-26-7	< SDL		2.5	0.005
100	PMMA (para-Methoxy-N-methylamphetamine)	3398-68-3	5	0.015	5	0.019
101	Pyrovalerone	3563-49-3	< SDL		2.5	0.031
102	TMA (trimethoxyamphtamine)	1082-23-1	2	0.016	2	0.016
	Sympathomimetics					
103	Apophedrin (Phenylethanolamine)	7568-93-6	5	0.07	5	0.03
104	Ephedrine	299-42-3	≥5	0.012	≥5	0.003
105	Etafedrine	48141-64-6	2.5	0.12	2.5	0.05
106	Isoetharine	7279-75-6	2.5	0.02	2.5	0.02
107	Metanephrine	5001-33-2	2.5	0.005	< SDL	
108	Methoxamine	337376-15-5	2	0.02	2.5	0.02
109	Norephedrine	492-41-1	2.5	0.043	≥5	0.02
110	Nylidrin	447-41-6	2.5	0.01	2	0.01
111	Phenylephrine	1416-03-1	< SDL		2	0.01
	Hallucinogenic (cannabinoids)					
112	Δ9-Tetrahydrocannabinol (THC)	1972-08-3	2	0.03	2	0.01
	Benzodiazepines tranquilizers					
113	7-amino-flunitrazepam	34084-50-9	2	0.03	2	0.02
114	Alprazolam	92623-85-3	2	0.03	≥5	0.01
115	Clobazam	22316-47-8	2	0.009	2	0.002
116	Diazepam	439-14-5	2.5	0.02	2.5	0.01
117	Lorazepam	846-49-1	< SDL		2.5	0.02
118	Midazolam	59467-70-8	< SDL		2	0.003
119	Nordiazepam	1088-11-5	2.5	0.005	2.5	0.002
120	Oxazepam	604-75-1	2.5	0.03	2.5	0.02
121	Temazepam	846-50-4	≥5	0.03	5	0.02
	Barbiturates					
122	Bemegride	64-65-3	2.5	0.08	2	0.01
123	Phenobarbital	50-06-6	2	0.03	2.5	0.02
124	Primidone	125-33-7	2.5	0.04	2.5	0.1
	Antipsychotics					
125	Amisulpride	71675-85-9	≥5	0.07	≥5	0.07
126	Amisulpride-N-Oxide	71675-85-9	2	0.004	5	0.01
127	Buspirone	36505-84-7	≥5	0.02	< SDL	
128	Clozapine	5786-21-0	2	0.15	2	0.08
129	Haloperidol	52-86-8	2.5	0.0001	≥5	0.0005
130	Levomepromazine sulfoxide	1881949	2	0.09	2.5	0.09
131	Paliperidone (9-OH-Risperidone)	147687-18-1	< SDL		2	0.01
132	Quetiapine	111974-69-7	≥5	0.02	2	0.01
133	Risperidone	106266-06-2	< SDL		2	0.005
134	Sulpiride	15676-16-1	≥5	0.04	≥5	0.04
	Antiepileptic					
135	10-Hydroxycarbamazepine	29331-92-8	5	0.05	5	0.06
136	Carbamazepine	298-46-4	5	1.61	≥5	1.7

(*continued on next page*)

Table 5 (*continued*)

	Compound Name	CAS number	influent wastewater		effluent wastewater	
			IPs	**C (μg/L)**	**IPs**	**C (μg/L)**
137	Carbamazepine-10,11-epoxid	36507-30-9	5	0.09	4.5	0.05
138	Lamotrigine	84057-84-1	≥5	1.1	5	1.6
139	Levetiracetam	102767-28-2	≥5	1.59	2.5	0.94
140	Oxcarbazepine	28721-07-5	5	0.04	5	0.05
141	Phenytoin	57-41-0	< SDL		2	0.03
142	Topiramate	97240-79-4	5	0.4	≥5	0.21
143	Valproic acid	99-66-1	2.5	1.55	2.5	0.6
	Antidepressants					
144	8-OH-Mirtazapine	–	2.5	0.07	< SDL	
145	Amitriptyline	50-48-6	≥5	0.23	5	0.11
146	Desmethyl mirtazapine	61337-68-6	2	0.03	2	0.04
147	Doxepine	1668-19-5	2	0.05	2	0.04
148	Maprotiline	10262-69-8	2	0.01	< SDL	
149	Mirtazapine	61337-67-5	≥5	0.33	≥5	0.19
150	Nortriptyline	894-71-3	2	0.004	2	0.01
	SSRIs (serotonin replacing inhibitors)					
151	Citalopram	59729-33-8	≥5	0.7	≥5	0.5
152	Fluoxetine	54910-89-3	2.5	0.1	2.5	0.07
153	Norcitalopram	144025-14-9	≥5	0.05	≥5	0.032
154	Sertraline	79617-96-2	2	0.09	2.5	0.03
	SNRIs (serotonin-norepinephrine reuptake inhibitors)					
155	Duloxetine	116539-59-4	< SDL		2.5	0.005
156	N,O- bisdesmethylvenlafaxine	135308-74-6	5	0.12	5	0.16
157	N-Desmethylvenlafaxine	149289-30-5	4.5	1.1	5	1.5
158	O-desmethylvenlafaxine	93413-62-8	≥5	0.89	≥5	1.1
159	Venlafaxine	93413-69-5	≥5	0.92	≥5	1
160	Venlafaxine-N-oxide	1094598-37-4	2	0.01	2.5	0.04
	Anesthetics					
161	Benzocaine	94-09-7	2	0.01	< SDL	
162	Bupivacaine	38396-39-3	2	0.004	< SDL	
163	Lidocaine	137-58-6	2.5	0.17	2.5	0.69
164	Mepivacaine	96-88-8	2.5	0.07	< SDL	
165	Norfentanyl	1609-66-1	< SDL		2.5	0.003
166	Para-fluorofentanyl	90736-23-5	2	0.002	2.5	0.004
167	Prilocaine	721-50-6	2	0.005	2.5	0.006
168	Procaine	59-46-1	2.5	0.002	2.5	0.006
	Antiviral drugs					
169	Amantadine	768-94-5	5	0.06	5	0.04
170	Atazanavir	198904-31-3	2.5	0.02	2.5	0.02
171	Darunavir	206361-99-1	5	0.15	5	0.1
172	Emtricitabine	143491-57-0	5	0.33	5	0.15
173	Ritonavir	155213-67-5	≥5	0.03	2.5	0.025
174	Tenofovir	147127-20-6	2.5	0.15	< SDL	
	Hypertension- diuretic drug					
175	Acetazolamide	59-66-5	5	0.03	5	0.004
176	Aliskiren	173334-57-1	≥5	0.27	≥5	0.25
177	Amiloride	2016-88-8	2	0.05	2.5	0.03
178	Bendroflumethiazide	73-48-3	2.5	0.01	< SDL	
179	Candesartan	139481-59-7	2	0.29	2	0.12
180	Chlorthalidone	77-36-1	< SDL		2	0.008
181	D617 (met. of verapamil)	34245-14-2	5	0.07	5	0.1
182	Deacetyldiltiazem	42399-40-6	5	0.063	5	0.08
183	Diltiazem	42399-41-7	5	0.1	5	0.07
184	Eprosartan	133040-01-4	5	0.84	2.5	0.22
185	Furosemide	54-31-9	≥5	0.9	2.5	0.93
186	Hydrochlorothiazide	58-93-5	≥5	0.68	≥5	0.72
187	Irbesartan	138402-11-6	2	0.2	2	0.006
188	Nordiltiazem	–	≥5	0.03	2.5	0.02
189	Phenoxybenzamine	59-96-1	2.5	0.41	2.5	0.45
190	Telmisartan	144701-48-4	2.5	0.22	5	0.18
191	Valsartan	137862-53-4	≥5	1.66	5	0.92
192	Verapamil	52-53-9	2.5	0.02	2	0.02
	Antidiabetic drugs					
193	Guanylurea (met.of metformin)	926-72-7	5	0.74	≥5	5
194	Lacosamide	175481-36-4	4.5	0.02	5	0.04
195	Metformin	657-24-9	≥5	93	≥5	35
196	Nateglinide	105816-04-4	2	0.005	< SDL	
197	Pioglitazone	111025-46-8	2	0.02	2	0.02
198	Sitagliptin	486460-32-6	≥5	0.48	< SDL	
199	Vildagliptin	274901-16-5	5	0.29	5	0.51
	Antihistamine					
200	Cetirizine	83881-52-1	≥5	0.14	5	0.18
201	Chlorpheniramine	132-22-9	2.5	0.01	2.5	0.008

(*continued on next page*)

Table 5 (*continued*)

	Compound Name	CAS number	influent wastewater		effluent wastewater	
			IPs	**C (μg/L)**	**IPs**	**C (μg/L)**
202	Crotamiton	483-63-6	2.5	0.01	5	0.01
203	Diphenhydramine	58-73-1	≥5	0.04	≥5	0.04
204	Hydroxyzine	68-88-2	2.5	0.004	< SDL	
205	Nororphenadrine (Tofenacin, Elamol)	15301-93-6	2	0.007	2.5	0.01
206	Orphenadrine	83-98-7	≥5	0.05	≥5	0.04
	Antiulcer					
207	Cimetidine	51481-61-9	2	0.07	2.5	0.5
208	Ranitidine	66357-35-5	≥5	2.6	≥5	1
209	Ranitidine-S-oxide	73851-70-4	2	0.17	2.5	0.11
	Cardiovascular diseases-intravascular					
210	Atorvastatin	134523-00-5	≥5	1.5	< SDL	
211	Clopidogrel Carboxylic acid	144457-28-3	≥5	0.6	≥5	0.56
212	Fenofibric acid	49562-28-9	≥5	0.61	2	0.3
213	Gemfibrozil	25812-30-0	≥5	0.24	2	0.05
214	Iopromide	73334-07-3	≥5	0.6	≥5	0.24
215	Propafenone	54063-53-5	≥5	0.56	≥5	0.53
216	Rosuvastatin	287714-41-4	2.5	0.17	2	0.13
	CNS stimulants					
217	Caffeine	58-08-2	≥5	9.6	5	3
218	Hydroxycotinine	34834-67-8	≥5	11.8	2.5	0.07
219	Cotinine	486-56-6	5	9.1	5	0.54
220	Paraxanthin (1,7-dimethylxanthine)	611-59-6	≥5	5.9	≥5	0.92
221	Nicotine	54-11-5	≥5	13	≥5	0.93
222	Theophylline (1,3-dimethylxanthine)	58-55-9	≥5	2	< SDL	
223	Pentoxyfylline	*6493-05-06	5	0.64	< SDL	
	Analgesics-NSAIDs					
224	4-Acetamidoantipyrine	83-15-8	5	0.07	5	0.09
225	4-Formylaminoantipyrine	1672-58-8	2.5	0.02	≥5	0.03
226	Antipyrine /Phenazone	60-80-0	< SDL		2	0.05
227	Diclofenac	15307-86-5	2.5	0.8	2.5	0.7
228	Fenbufen	36330-85-5	≥5	0.7	5	0.19
229	Fenoprofen	29679-58-1	2.5	0.9	2.5	0.2
230	Flufenamic acid	530-78-9	2.5	0.02	2.5	0.03
231	Flurbiprofen	51543-39-6	2	0.48	< SDL	
232	Ibuprofen	15687-27-1	≥5	1.1	< SDL	
233	Indoprofen	31842-01-0	2	0.22	< SDL	
234	Isopyrin (4-Isopropyl-aminoantipyrine)	3615-24-5	< SDL		2.5	0.02
235	Ketoprofen	22071-15-4	2.5	0.11	< SDL	
236	Meclofenamic Acid	644-62-2	2.5	0.02	< SDL	
237	Mefenamic acid	61-68-7	5	0.51	5	0.05
238	Meptazinol	54340-58-8	< SDL		2.5	0.003
239	Naproxen	22204-53-1	5	0.93	2	0.05
240	N-desmethyltramadol	75377-45-6	2.5	0.01	2.5	0.01
241	Niflumic acid	4394-00-7	≥5	0.34	≥5	0.27
242	Nimesulide	51803-78-2	< SDL		2.5	0.098
243	O-desmethyltramadol	73986-53-5	2.5	0.03	5	0.01
244	O-N-bisdesmethyltramadol	–	2.5	0.02	2.5	0.02
245	Oxaprozin	21256-18-8	2.5	0.44	< SDL	
246	Paracetamol	103-90-2	≥5	4.8	2.5	0.14
247	Pethidine	57-42-1	2	0.007	2.5	0.003
248	Salicylamide	65-45-2	2	0.01	2.5	0.11
249	Salicylic acid	69-72-7	5	5.4	≥5	0.14
250	Sulindac	38194-50-2	2	0.002	< SDL	
251	Tramadol-N-oxide	147441-56-3	2	0.1	2.5	0.12
	beta-blockers					
252	Albuterol	18559-94-9	2	0.005	2.5	0.02
253	Atenolol	29122-68-7	≥5	1.65	≥5	1.07
254	Atenolol acid (Metoprolol acid)	63659-18-7	5	0.47	≥5	0.12
255	Betaxolol	63659-18-7	2	0.008	< SDL	
256	Bisoprolol	66722-44-9	≥5	0.03	≥5	0.07
257	Carteolol	51781-06-7	2.5	0.002	< SDL	
258	Celiprolol	56980-93-9	≥5	0.42	≥5	0.33
259	Esmolol	103598-03-4	2.5	0.002	2.5	0.01
260	Metoprolol	37350-58-6	5	0.81	≥5	1.3
261	Pindolol	13523-86-9	2	0.001	< SDL	
262	Propranolol	525-66-6	5	0.13	2.5	0.21
263	Salbutamol	18559-94-9	2.5	1.2	2.5	0.72
264	Sotalol	3930-20-9	5	0.43	5	0.55
	Antibiotics					
265	Azithromycin	83905-01-5	2.5	0.03	≥5	0.06
266	Clarithromycin	81103-11-9	≥5	2.7	≥5	2.4
267	Levamisole	14769-73-4	2	0.06	≥5	0.1
268	Linezolid	165800-03-3	2	0.03	2.5	0.06

(*continued on next page*)

Table 5 (*continued*)

	Compound Name	CAS number	influent wastewater		effluent wastewater	
			IPs	**C (μg/L)**	**IPs**	**C (μg/L)**
269	Metronidazole	443-48-1	2.5	0.17	5	0.13
270	N4-Acetylsulfadiazine	127-74-2	2.5	0.07	2.5	0.46
271	N4-Acetylsulfamethazine (N4-Acetylsulfadimidine)	100-90-3	2	0.03	2.5	0.08
272	N4-Acetylsulfamethoxazole	21312-10-7	2.5	0.03	2	0.02
273	N-desmethyl Clarithromycin	101666-68-6	≥5	0.72	5	0.93
274	Nigericin	28380-24-7	2	0.41	2.5	0.84
275	Roxithromycin	80214-83-1	5	0.02	2	0.03
276	Sulfadiazine	68-35-9	5	0.04	5	0.03
277	Sulfadimidine	57-68-1	2	0.003	5	0.003
278	Sulfamethoxazole	723-46-6	5	0.19	≥5	0.16
279	Sulfapyridine	144-83-2	≥5	0.06	≥5	0.03
280	Ternidazol	1077-93-6	2	0.03	< SDL	
281	Trimethoprim	738-70-5	5	0.16	≥5	0.11
	Antibacterials - veterinary drugs					
282	Decoquinate	18507-89-6	< SDL		2	0.1
283	Enrofloxacin	93106-60-6	2.5	0.02	< SDL	
284	Marbofloxacin	115550-35-1	2	0.05	< SDL	
285	Triclocarban	101-20-2	2	0.11	2.5	0.35
286	Triclosan	3380-34-5	2	0.28	2.5	0.25
	Anticonvulsant					
287	Gabapentin	60142-96-3	≥5	0.29	≥5	0.14
288	Pregabalin	148553-50-8	5	0.38	5	0.25
289	Warfarin	81-81-2	2.5	0.12	< SDL	
	Chemotherapeutic-anti-cancer drugs					
290	Cyclophosphamide	50-18-0	2.5	0.009	2.5	0.03
291	Cytarabin	147-94-4	2	0.09	< SDL	
292	Ifosfamide	3778-73-2	2.5	0.1	2.5	0.28
	Other drugs					
293	Acamprosate	77337-76-9	2	0.52	2	0.84
294	Benserazide	14919-77-8	2	0.48	2	0.47
295	Benzamidine	618-39-3	2.5	0.7	2	0.65
296	dextromethorphan	125-71-3	< SDL		2	0.002
297	Fluocinolone acetonide	67-73-2	< SDL		2.5	0.01
298	Guaifenesin	93-14-1	2.5	0.56	< SDL	
299	Memantine	19982-08-2	2.5	0.04	2.5	0.06
300	Piracetam	7491-74-9	2.5	0.03	2.5	0.33
301	Vigabatrin	60643-86-9	2	0.18	< SDL	
	Steroids					
302	17β-Estradiol (E2)	50-28-2	2.5	0.09	2.5	0.03
303	19-Norandrosterone	1225-01-0	< SDL		2.5	0.24
304	allo-THF (Allotetrahydrocortisol)	302-91-0	2.5	0.17	< SDL	
305	Drostanolone metabolite	–	2.5	0.02	2.5	0.03
306	Mesterolone metabolite	–	2.5	0.07	2.5	0.13
307	Prednisolone	50-24-8	2.5	0.2	< SDL	
308	Progesterone	57-83-0	2.5	0.71	< SDL	
309	THE (Tetrahydrocortisone)	200-161-9	≥5	0.18	< SDL	
310	THF (Tetrahydrocortisol)	53-02-1	2.5	0.8	< SDL	
	PFCs					
311	PFBuS	375-73-5	2.5	0.007	5	0.006
312	PFDeA	335-76-2	5	0.05	2	0.05
313	PFHpA	375-85-9	2	0.006	2	0.006
314	PFHps	335-77-3	2.5	0.0007	2	0.0005
315	PFHxA	307-24-4	2	0.002	5	0.004
316	PFHxS	355-46-4	2.5	0.005	2	0.004
317	PFNA	375-95-1	2	0.01	2	0.01
318	PFOA	2395-00-8	≥5	0.008	5	0.006
319	PFOS	1763-23-1	2.5	0.03	2.5	0.004
320	PFPeA	2706-90-3	2	0.002	4.5	0.002
321	PFUnA	2058-94-8	< SDL		2	0.0003
	Sweeteners					
322	Acesulfame	33665-90-6	≥5	1.9	≥5	0.64
323	Cyclamate	139-05-9	5	14	2.5	0.35
324	Saccharine	81-07-2	2.5	3.1	2.5	0.011
325	Sucralose	56038-13-2	2.5	6	2.5	1.98
	Industrial Chemicals					
326	1-Hydroxy-Benzotriazole	2592-95-2	2	0.17	2.5	0.16
327	2-Aminobenzimidazole	934-32-7	< SDL		2.5	0.073
328	2-Amino-Benzothiazole	136-95-8	≥5	0.07	≥5	0.09
329	2-Aminoheptane	123-82-0	5	0.64	2	0.16
330	2-Me-S-Benzothiazole	615-22-5	≥5	0.09	≥5	0.06
331	2-OH-Benzothiazole	934-34-9	2	0.15	2.5	0.21
332	4-Hydroxy-Benzotriazole	26725-51-9	2	0.54	2.5	0.64
333	4-Me-Benzotriazole/5-Me Benzotriazole	29385-43-1	2	0.1	≥5	0.2

(*continued on next page*)

Table 5 (*continued*)

	Compound Name	CAS number	influent wastewater		effluent wastewater	
			IPs	**C (μg/L)**	**IPs**	**C (μg/L)**
334	4-Nonylphenol (4-NP)	104-40-5	≥5	0.07	2	0.03
335	4-Nonylphenol-mono-ethoxylate	104-35-8	2	0.01	2	0.004
336	4-Piperidin carboxamide	39546-32-2	2	1	2	0.95
337	4-Tert-octylphenol (4-t-OP)	27193-28-8	≥5	1.2	≥5	0.51
338	Benzenesulfonamide	98-10-2	2.5	0.12	2	0.07
339	Benzoic acid	65-85-0	2.5	22	2.5	14
340	Benzophenon 3 (2-Hydroxy-4-methoxybenzophenon)	131-57-7	2.5	0.5	< SDL	0.2
341	Benzothiazole (BTH)	95-16-9	2.5	0.6	2.5	0.7
342	Benzotriazole (BTR)	95-14-7	2	0.6	5	0.49
343	Benzyl butyl phthalate	85-68-7	≥5	0.05	≥5	0.08
344	Bisphenol A	80-05-7	5	0.03	< SDL	
345	Diethyl phthalate	84-66-2	5	2	5	1.8
346	Dimethyl phthalate	131-11-3	2.5	0.02	2.5	0.02
347	Di-n-butyl phthalate	84-74-2	5	0.33	5	1.1
348	Ethyl sulfate	540-82-9	2.5	3.6	2	1.3
349	Galaxolidone	–	2.5	0.59	2.5	0.6
350	Melamine	108-78-1	2	0.65	2	0.66
351	o-Toluenesulfonamide	88-19-7	2	0.13	2	0.09
352	Prolinamide	51-06-9	2	4.1	2	1.5
353	Tributylamine	102-82-9	2.5	0.008	2.5	0.01
354	Triethylphosphate	78-40-0	2.5	0.05	5	0.05
355	Triphenyl phosphate (TPP)	115-86-6	5	0.05	5	0.12
	Surfactants					
356	AES-C12, n = 0 (Dodecyl hydrogen sulfate)	151-41-7	≥5	26	2	0.23
357	AES-C12, n = 1 (2-(Dodecyloxy)ethyl hydrogen sulfate)	50602-06-7	≥5	33	5	0.72
358	AES-C12, n = 2 (2-(2-(Dodecyloxy)ethoxy)ethyl hydrogen sulfate)	7577-59-5	≥5	38	5	0.49
359	AES-C12, n = 3 (2-(2-(2-(Dodecyloxy)ethoxy) ethoxy)ethyl hydrogen sulfate)	–	≥5	44	2	0.36
360	AES-C12, n = 4 (3,6,9,12-Tetraoxatetracosyl hydrogen sulfate)	–	≥5	32	2	0.26
361	AES-C12, n = 5 (3,6,9,12,15-Pentaoxaheptacosyl hydrogen sulfate)	–	≥5	30	2	0.25
362	AES-C12, n = 6 (3,6,9,12,15,18-Hexaoxatriacontyl hydrogen sulfate)	–	≥5	32	< SDL	
363	AES-C12, n = 7 (3,6,9,12,15,18,21-Heptaoxatritriacontyl hydrogen sulfate)	–	≥5	34	< SDL	
364	AES-C12, n = 8 (3,6,9,12,15,18,21,24-Octaoxahexatriacontyl hydrogen sulfate)	–	≥5	36	< SDL	
365	AES-C12, n = 9 (3,6,9,12,15,18,21,24,27-Monaoxanonatriacontyl hydrogen sulfate)	–	≥5	28	< SDL	
366	AES-C14, n = 0 (Tetradecyl hydrogen sulfate)	4754-44-3	5	0.8	5	0.45
367	AES-C14, n = 1 (2-(Tetradecyloxy)ethyl hydrogen sulfate)	–	≥5	5.1	5	0.33
368	AES-C14, n = 2 (2-(2-(Tetradecyloxy)ethoxy)ethyl hydrogen sulfate)	–	≥5	5.5	5	0.22
369	AES-C14, n = 3 (2-(2-(2-(Tetradecyloxy)ethoxy)ethoxy)ethyl hydrogen sulfate)	–	≥5	6.1	2	0.15
370	AES-C14, n = 4 (3,6,9,12-tetraoxahexacosyl hydrogen sulfate)	–	≥5	5.0	2	0.10
371	AES-C14, n = 5 (3,6,9,12,15-pentaoxanonacosyl hydrogen sulfate)	–	≥5	4.7	< SDL	
372	AES-C14, n = 6 (3,6,9,12,15,18-hexaoxadotriacontyl hydrogen sulfate)	–	≥5	5.1	< SDL	
373	AES-C14, n = 7 (3,6,9,12,15,18,21-heptaoxapentatriacontyl hydrogen sulfate)	–	≥5	4.9	< SDL	
374	AES-C14, n = 8 (3,6,9,12,15,18,21,24-octaoxaoctatriacontyl hydrogen sulfate)	–	≥5	4.8	< SDL	
375	AES-C14, n = 9 (3,6,9,12,15,18,21,24,27-nonaoxahentetracontyl hydrogen sulfate)	–	≥5	2.6	< SDL	
376	Didecyldimethylammonium (DADMAC (C10:C10))	2390-68-3	2	0.02	2	0.01
377	Benzyl-dimethyl-dodecylammonium	139-07-1	2	0.07	< SDL	
378	C9-LAS	–	≥5	11	< SDL	
379	C10-LAS	–	≥5	413	≥5	2.0
380	C11-LAS	–	≥5	431	≥5	16
381	C12-LAS	–	≥5	266	≥5	17
382	C13-LAS	–	≥5	48	≥5	8.3
383	C14-LAS	–	≥5	1.0	< SDL	
384	C15-LAS	–	5	0.3	< SDL	
	Amino acids - Naturally occurring compounds					
385	1,4-butanediol (1,4 BD)	110-63-4	2	0.006	< SDL	
386	2-Phenethylamine	64-04-0	2.5	0.09	2	0.12
387	2-Phenylphenol	90-43-7	2.5	0.09	< SDL	
388	Adenosine	58-61-7	≥5	0.61	2.5	0.58
389	Alanine (Ala)	56-41-7	2.5	11	< SDL	
390	Dimethylaniline	95-68-1	2.5	0.02	< SDL	
391	g-Aminobutiric acid (GABA)	56-12-2	2.5	7.2	2.5	13
392	Glutamic acid (Glu)	56-86-0	2	13	2	4.5
393	Leucine (Leu)	328-39-2	2.5	12	< SDL	
394	Methionine (Met)	63-68-3	2.5	2.3	2	0.06
395	Proline (Pro)	147-85-3	2	7.8	< SDL	
396	Resveratrol	501-36-0	2.5	0.11	2.5	0.12
397	Serine (Ser)	56-45-1	2	2.8	< SDL	
398	Valine (Val)	72-18-4	≥5	59	2	48

wastewater (49 TPs) than in influent (47 TPs), probably because they are formed during wastewater treatment processes.

From the *well-known* target compounds that are reported usually in the literature, tramadol was not detected in the analyzed samples. Instead, O-desmethyl-venlafaxine was detected in all the samples. They are isobaric substances, with the same molecular formula and very close RTs. Therefore, errors in their identification can easily occur in similar methodologies if results are not carefully evaluated. MS/MS

fragmentation and experiments with spiked samples containing both compounds led to the conclusion that O-desmethyl-venlafaxine is the analyte eluted, as it is shown in Figure S2, in the Supplementary information. In Table S1, all the isobaric analytes considered in this work with similar retention time (< 1 min) are marked so that readers are aware of potential mistakes.

In Figure S3, a chromatogram of influent and effluent wastewater (in both ESI polarities) is presented, showing all the detected analytes. Note that in the chromatogram corresponding to influent wastewater (positive ionization mode) metformin was deselected intentionally since its intensity was one magnitude higher than the rest of the compounds. It can be observed that a much higher number of compounds were detected in positive ionization mode in comparison with the negative one. This can be explained by the fact that a higher number of compounds ionized better in positive and in general this mode also provides higher sensitivity (in most cases at least one order of magnitude).The presence of many N containing compounds (generally with a very good ionization in positive mode) is one of the main causes of this fact (Robles-Molina et al., 2014; Gomez et al., 2010; Ruff et al., 2015; Mechelke et al., 2019; Hollender et al., 2018; Hernandez et al., 2015a).

Fig. 1 shows in detail the identification of ephedrine in influent wastewater as an example of a case with consistent MS/MS evidences. Apart from a very high mass accuracy (0.2 ppm, 0.0 m Da) and good isotopic fitting (16 mSigma), 3 bbCID fragments ($C_{10}H_{14}N^+$, $C_{10}H_{14}N^+$ and $C_{19}H_9^+$) were observed in the MS/MS spectrum (Fig. 1 b1, b2) with the same ion ratios as in the spiked sample. It can be observed that the extracted ion chromatograms of ephedrine and the ones corresponding to the 3 identified fragment ions showed the same chromatographic picture in both the spiked sample and the original sample (Fig. 1 a1, a2). According to the proposed IPs, the identification of ephedrine earned 10 IPs in total. On the contrary, Fig. 2 shows the identification for clozapine in effluent wastewater, where it can be observed that no fragments were detected in the bbCID spectrum (Fig. 2 b1, b2). Mass accuracy was good (1.1 ppm/ 0.5 m Da) but no information on the isotopes was available due to the low intensity of the analyte peak. Therefore this identification earned only 2 IPs. It is evident that the two described identifications have different levels of confidence and the proposed IPs system helps at clearly communicating it.

To the authors' knowledge, this is the first study reporting the presence of such a high number of EPs (398) belonging to very different categories as described above. The number of studies in the literature reporting the identification of a high number of compounds in water using HRMS based analysis is limited. Mechelke et al. reported recently 157 compounds in effluent wastewater, 146 in influent wastewater and 121 in surface waters using a target method for 590 substances (Mechelke et al., 2019). Also in wastewater, Shymanski et al. provided quantitative values for 155 substances out the 376 studied target compounds (Schymanski et al., 2014). There are other studies dealing with the identification of large lists of compounds in surface water but following suspect strategies and confirming with standards the positive matches. This is the case of the study carried out by Moschet et al., reporting 141 compounds (mainly pesticides) in 8 river waters (Moschet et al., 2014) using a suspect screening approach from a list of 2188 potential suspects. Other studies following similar strategies confirmed up to 100 compounds with reference standard in different surface water sites (Hernandez et al., 2015b; Wode et al., 2015). A large collaborative trial organized by NORMAN Association that involved European 18 laboratories reported results on the presence of EPs in river water. 347 target compounds were identified by summing the

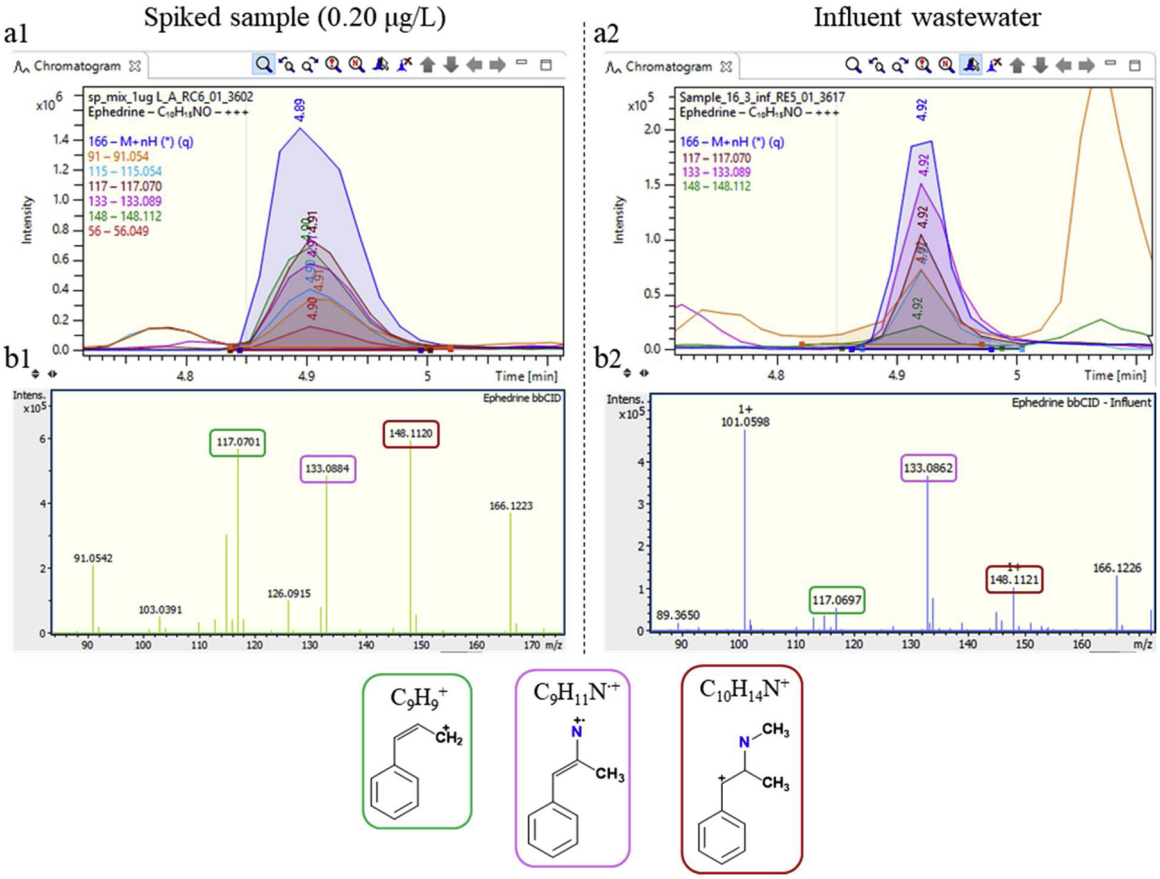


Fig. 1. Identification of ephedrine. (a1, a2) Extracted Ion Chromatograms (EICs) of ephedrine and its qualifier ions in spiked and non-spiked real influent wastewater samples, respectively. (b1, b2) bbCID MS/MS spectra of ephedrine in the aforementioned samples. 10 IPs were earned in the identification of ephedrine according to dRt, dm/z, dS and the 3 qualifier ions detected.

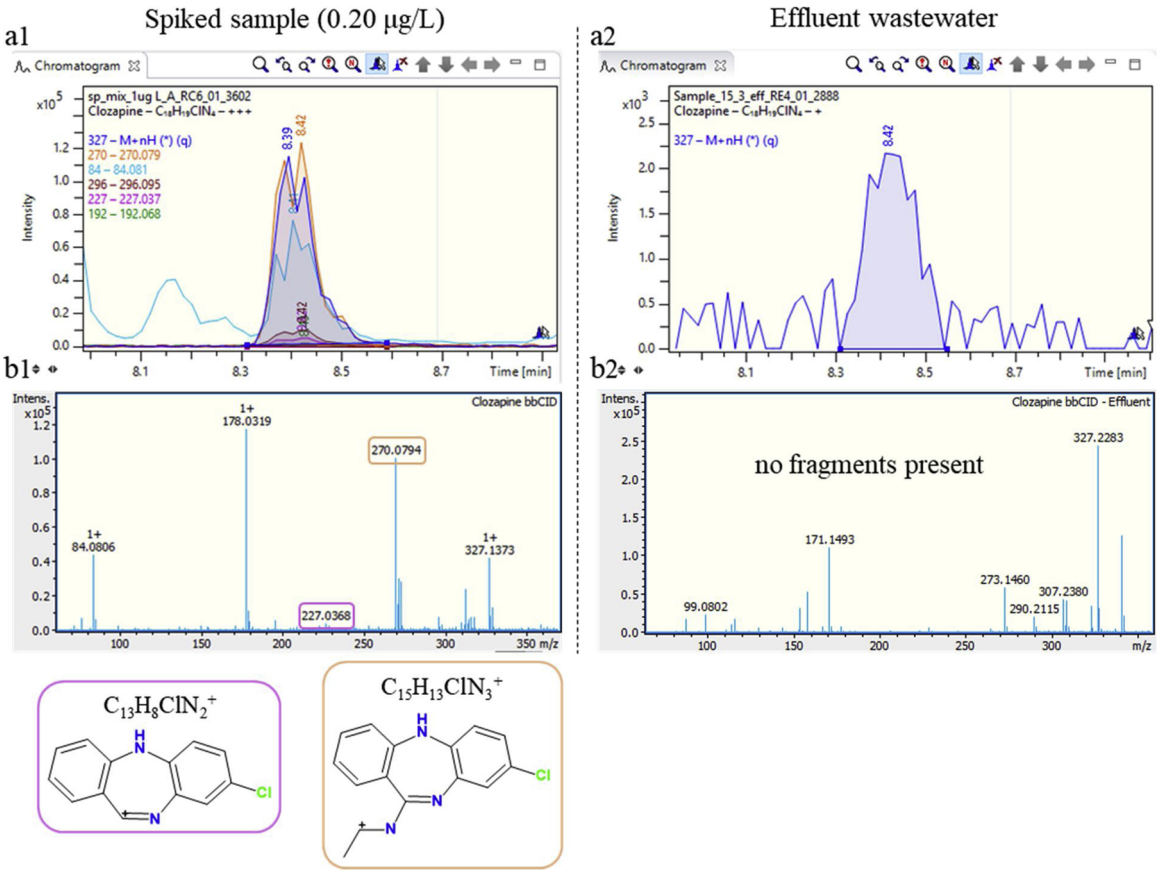


Fig. 2. Screening of clozapine. (a1, a2) Extracted Ion Chromatograms (EICs) of clozapine and its qualifier ions in spiked and non-spiked real effluent wastewater samples, respectively. (b1, b2) bbCID MS/MS spectra of clozapine in the aforementioned samples. 2 IPs were earned in the screening of clozapine according to dRt, dm/z. No isotopes and bbCID fragments were detected.

contribution of all the participants, employing LC and GC based techniques (Schymanski et al., 2015). The review of the literature makes evident the difficulty of identifying such a large number of EPs with a single method and shows the powerfulness of the method described in the present work.

3.4.2. *Quantitation of analytes*

As shown in Table 4, the concentrations of the analytes range in influent from 431 µg/L (C11- linear alkylbenzene sulfonate (LAS)) to 0.14 ng/L (haloperidol) and in effluent from 48 µg/L (valine) to 0.42 ng/L (acetochlor). In Fig. 3, the distribution of the concentrations of the analytes from the sub-ng level until some µg per L is presented. Apart from C11-LAS, other LAS homologues, alkyl ethoxysulfates surfactants (AES), metformin, valproic acid and caffeine were the most abundant substances in influent wastewater. In effluent, LAS surfactants, metformin and its metabolite guanylurea, and N-desmethylvenlafaxine were present at the highest concentrations. Regarding pesticides, the most abundant compounds were fluometuron, azoxystrobin and a metabolite of dimethachlor (dimethachlor ESA, (ethanesulfonic acid)), both in influent and effluent wastewaters. This confirms that WWTPs

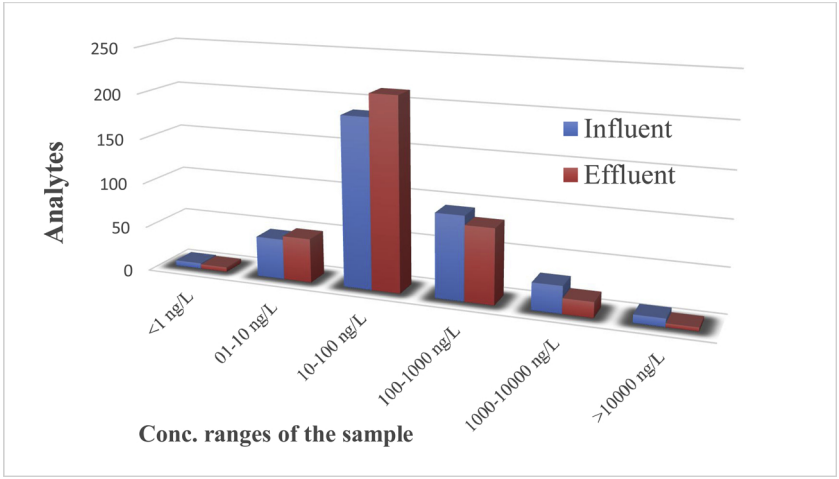


Fig. 3. Distribution of the concentrations of the analytes detected in the analyzed samples.

also release pesticides as shown in the past (Munz et al., 2017). Sweeteners were also present at high concentrations in the influent samples (from 6 μg/L sucralose to 14 μg/L cyclamate). Benzoic acid had the highest abundance among the rest of chemicals, at concentrations of 22 μg/L and 14 μg/L in influent and effluent wastewater, respectively. This compound is mainly used as conservative and in the production of other chemicals. Ethyl sulfate, which is a minor human metabolite after alcohol consumption (Rodriguez-Alvarez et al., 2014) was also present at relevant concentrations in the samples (up to 3.6 μg/L). Amino acids were present in wastewater at very high concentrations. In influent, the concentrations were higher than 2 μg/L for all the detected amino acids and in effluent, valine and g-aminobutiric acid (GABA) were present at concentrations over 10 μg/L despite their significant removal. So far there are no studies in the literature presenting quantitative data for such a large number of EPs in any matrix.

4. Conclusions

The analysis of EPs in water is of paramount environmental importance. There is a need of methodologies that allow the simultaneous analysis of a large number of compounds. In this regard, the recent advances in HRMS have opened new opportunities. In the present work a comprehensive quantitative target method for the analysis of 2316 EPs in water based in LC-HRMS/MS was developed. Target analytes included substances with a wide range of physicochemical properties including pesticides, pharmaceuticals, drugs of abuse, industrial chemicals, doping compounds and several TPs. It is the widest method developed so far in terms of number of compounds. It is worth mentioning that for the development of the described method an in-house database was built with the RTs, MS and MS/MS ions information by injecting standards for all the target compounds.

CCα and CCβ were evaluated in the validation stage in order to highlight the "fit for purpose" of a HRMS wide-scope target screening method. Data processing was optimized so that it provided a SIR > 95 % while the number of false positive results was minimized. In order to validate the methodology a representative group of compounds was carefully selected. Unlike most studies where the selection of validation set do not follow consistent criteria, in the present study the RTs (directly related to different physicochemical properties) and the ionization mode were considered. Therefore we obtained a representative validation set containing 195 compounds (approximately the 10 % of the database) of all the evaluated families. The described approach need the standards to be added after the first screening for quantification and no isotopically labelled compounds can be used as there would be some interference for the fragments in DIA.

In order to communicate the confidence level in the identifications of the analytes in an accurate way this work proposes a system of IPs based on the Decision 2002/657/EC (EC, 2002), which takes full advantage of the capabilities of modern HRMS instruments. It takes into account RT, mass accuracy, isotopic fit and fragmentation.

The method was applied with real influent and effluent wastewater from the WWTP of Athens and 398 EPs were detected and quantified (367 in influent wastewater and 315 in effluent wastewater). Concentrations were varied from the 0.14 ng/L detected for haloperidol to the 431 μg/L detected for C11-LAS.

Author contributions section

P.G. wrote the manuscript with support from A.B., N.A., D.D. and R.A. Analysis of the reference standards and sample extracts was carried out by A.B. and D.D., whereas N.A., A.B. and P.G. collected the influent and effluent wastewater samples, performed sample preparation and instrumental analysis. R.A. supported the generation of the target list database and preparation of the supplementary material. N.A. generated and uploaded the spectra of the reference compounds in MassBank. A.B., D.D. and N.A. performed data analysis and quantitation of target analytes. J.H. and H.S. participated in the planning of the experiments, provided guidance on the performance of the experiments, revised the manuscript and provided analytical reference standards. N.T. conceived the original ideas presented in the study, contributed to the experimental design, supervised the findings of this work and proof-read the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Declaration of Competing Interest

All the authors declare no conflict of interest

Acknowledgements

This project was implemented under the Operational Program "Education and Lifelong Learning" and funded by the European Union (European Social Fund) and Greek National Resources – ARISTEIA 624 (TREMEPOL project). Pablo Gago-Ferrero acknowledges the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement Smart-Workflow No 747698. Authors acknowledge Emma Schymanski for the useful discussion during the preparation of this paper and the script for presentation of extracted ion chromatograms.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2019.121712.

References

Acena, J., Stampachiacchiere, S., Perez, S., Barcelo, D., 2015. Advances in liquid chromatography-high-resolution mass spectrometry for quantitative and qualitative environmental analysis. Anal. Bioanal. Chem. 407 (21), 6289–6299.

Boix, C., Ibanez, M., Sancho, J.V., Leon, N., Yusa, V., Hernandez, F., 2014. Qualitative screening of 116 veterinary drugs in feed by liquid chromatography-high resolution mass spectrometry: potential application to quantitative analysis. Food Chem. 160, 313–320.

Guidelines for the validation of screening methods for residues of veterinary medicides. Community Reference Laboratories residues (CRLs) 20/1/2010 2010.

Diaz, R., Ibanez, M., Sancho, J.V., Hernandez, F., 2013. Qualitative validation of a liquid chromatography-quadrupole-time of flight mass spectrometry screening method for organic pollutants in waters. J. Chromatogr. A 1276, 47–57.

Díaz, R., Ibáñez, M., Sancho, J.V., Hernández, F., 2012. Target and non-target screening strategies for organic contaminants, residues and illicit substances in food, environmental and human biological samples by UHPLC-QTOF-MS. Anal Method 4 (1), 196–209.

Commission Decision of 12 August (2002/657/EC). Official Journal of the European Communities 2002; L221/8.

Eggen, R.I.L., Hollender, J., Joss, A., Schärer, M., Stamm, C., 2014. Reducing the discharge of micropollutants in the aquatic environment: the benefits of upgrading wastewater treatment plants. Environ. Sci. Technol. 48 (14), 7683–7689.

Gago-Ferrero, P., Schymanski, E.L., Bletsou, A.A., Aalizadeh, R., Hollender, J., Thomaidis, N.S., 2015. Extended suspect and non-target strategies to characterize emerging polar organic contaminants in raw wastewater with LC-HRMS/MS. Environ. Sci. Technol. 49 (20), 12333–12341.

Gago-Ferrero, P., Krettek, A., Fischer, S., Wiberg, K., 2018. Ahrens. Suspect screening and regulatory databases: a powerful combination to identify emerging micropollutants. Environ. Sci. Technol. 52 (12), 6881–6894.

Gomez, M.J., Gomez-Ramos, M.M., Malato, O., Mezcua, M., Fernandez-Alba, A.R., 2010. Rapid automated screening, identification and quantification of organic micro-contaminants and their main transformation products in wastewater and river waters using liquid chromatography-quadrupole-time-of-flight mass spectrometry with an accurate-mass database. J. Chromatogr. A 1217 (45), 7038–7054.

Hernandez, F., Sancho, J.V., Ibanez, M., Abad, E., Portoles, T., Mattioli, L., 2012. Current use of high-resolution mass spectrometry in the environmental sciences. Anal. Bioanal. Chem. 403 (5), 1251–1264.

Hernandez, F., Ibanez, M., Botero-Coy, A.M., Bade, R., Bustos-Lopez, M.C., Rincon, J., Moncayo, A., Bijlsma, L., 2015a. LC-QTOF MS screening of more than 1,000 licit and illicit drugs and their metabolites in wastewater and surface waters from the area of Bogota, Colombia. Anal. Bioanal. Chem. 407 (21), 6405–6416.

Hernandez, F., Ibanez, M., Portoles, T., Cervera, M.I., Sancho, J.V., Lopez, F.J., 2015b. Advancing towards universal screening for organic pollutants in waters. J. Hazard. Mater. 282, 86–95.

Hollender, J., Rothardt, J., Radny, D., Loos, M., Epting, J., Huggenberger, P., Borer, P., Singer, H.P., 2018. Comprehensive micropollutant screening using LC-HRMS/MS at

three riverbank filtration sites to assess natural attenuation and potential implications for human health. Water Res. X 1, 100007.
Kaufmann, A., Butcher, P., Maden, K., Walker, S., Widmer, M., 2015. Reliability of veterinary drug residue confirmation: high resolution mass spectrometry versus tandem mass spectrometry. Anal. Chim. Acta 856, 54–67.
Kern, S., Fenner, K., Singer, H., Schwarzenbach, R.P., Hollender, J., 2009. Identification of transformation products of organic contaminants in natural waters by computer-aided prediction and high-resolution mass spectrometry. Environ. Sci. Technol. 43, 7039–7046.
Leendert, V., Van Langenhove, H., Demeestere, K., 2015. Trends in liquid chromatography coupled to high-resolution mass spectrometry for multi-residue analysis of organic micropollutants in aquatic environments. TrAC Trend Anal. Chem. 67, 192–208.
Leon, N., Roca, M., Igualada, C., Martins, C.P., Pastor, A., Yusa, V., 2012. Wide-range screening of banned veterinary drugs in urine by ultra high liquid chromatography coupled to high-resolution mass spectrometry. J. Chromatogr. A 1258, 55–65.
Loos, R., Carvalho, R., Antonio, D.C., Comero, S., Locoro, G., Tavazzi, S., Paracchini, B., Ghiani, M., Lettieri, T., Blaha, L., Jarosova, B., Voorspoels, S., Servaes, K., Haglund, P., Fick, J., Lindberg, R.H., Schwesig, D., Gawlik, B.M., 2013. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. Water Res. 47 (17), 6475–6487.
Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S., Wang, X.C., 2014. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci. Total Environ. 473–474, 619–641.
Mechelke, J., Longrée, P., Singer, H., Hollender, J., 2019. Vacuum-assisted evaporative concentration combined with LC-HRMS/MS for ultra-trace-level screening of organic micropollutants in environmental water samples. Anal. Bioanal. Chem. 1–13.
Mol, H.G., Zomer, P., de Koning, M., 2012. Qualitative aspects and validation of a screening method for pesticides in vegetables and fruits based on liquid chromatography coupled to full scan high resolution (Orbitrap) mass spectrometry. Anal. Bioanal. Chem. 403 (10), 2891–2908.
Moschet, C., Piazzoli, A., Singer, H., Hollender, J., 2013. Alleviating the reference standard dilemma using a systematic exact mass suspect screening approach with liquid chromatography-high resolution mass spectrometry. Anal. Chem. 85 (21), 10312–10320.
Moschet, C., Wittmer, I., Simovic, J., Junghans, M., Piazzoli, A., Singer, H., Stamm, C., Leu, C., Hollender, J., 2014. How a complete pesticide screening changes the assessment of surface water quality. Environ. Sci. Technol. 48 (10), 5423–5432.
Munz, N.A., Burdon, F.J., de Zwart, D., Junghans, M., Melo, L., Reyes, M., Schönenberger, U., Singer, H.P., Spycher, B., Hollender, J., Stamm, C., 2017. Pesticides drive risk of micropollutants in wastewater-impacted streams during low flow conditions. Water Res. 110, 366–377.
Nielen, M.W., van Engelen, M.C., Zuiderent, R., Ramaker, R., 2007. Screening and confirmation criteria for hormone residue analysis using liquid chromatography accurate mass time-of-flight, Fourier transform ion cyclotron resonance and orbitrap mass spectrometry techniques. Anal. Chim. Acta 586 (1–2), 122–129.
Rajski, L., Gomez-Ramos, M., Fernandez-Alba, A.R., 2014. Large pesticide multiresidue screening method by liquid chromatography-Orbitrap mass spectrometry in full scan mode applied to fruit and vegetables. J. Chromatogr. A 1360, 119–127.
Robles-Molina, J., Lara-Ortega, F.J., Gilbert-Lopez, B., Garcia-Reyes, J.F., Molina-Diaz, A., 2014. Multi-residue method for the determination of over 400 priority and emerging pollutants in water and wastewater by solid-phase extraction and liquid chromatography-time-of-flight mass spectrometry. J. Chromatogr. A 1350, 30–43.
Rodriguez-Alvarez, T., Rodil, R., Cela, R., Quintana, J.B., 2014. Ion-pair reversed-phase liquid chromatography-quadrupole-time-of-flight and triple-quadrupole-mass spectrometry determination of ethyl sulfate in wastewater for alcohol consumption tracing. J. Chromatogr. A 1328, 35–42.
Ruff, M., Mueller, M.S., Loos, M., Singer, H., 2015. Quantitative target and systematic non-target analysis of polar organic micro-pollutants along the river Rhine using high-resolution mass-spectrometry - Identification of unknown sources and compounds. Water Res. 87, 145–154.
Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed, SANCO/12571/2013. European Commission, Health & Consumer protection directorate-general 2013.
Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed, SANTE/11813/2017. European Commission, Directorate general for health and food safety 2017.
Schymanski, E.L., Singer, H.P., Longree, P., Loos, M., Ruff, M., Stravs, M.A., Ripolles-Vidal, C., Hollender, J., 2014. Strategies to characterize polar organic contamination in wastewater: exploring the capability of high resolution mass spectrometry. Environ. Sci. Technol. 48 (3), 1811–1819.
Schymanski, E.L., Singer, H., Slobodnik, J., Ipolyi, I.M., Oswald, P., Krauss, M., Schulze, T., Haglund, P., Letzel, T., Grosse, S., Thomaidis, N.S., Bletsou, A., Zwiener, C., Ibáñez, M., Portolés, T., De Boer, R., Reid, M.J., Onghena, M., Kunkel, U., Schulz, W., Guillon, A., Noyon, N., Leroy, G., Bados, P., Bogialli, S., Stipaničev, D., Rostkowski, P., Hollender, J., 2015. Non-target screening with high-resolution mass spectrometry: critical review using a collaborative trial on water analysis. Anal. Bioanal. Chem. 407 (21), 6237–6255.
Vergeynst, L., Van Langenhove, H., Joos, P., Demeestere, K., 2013. Accurate mass determination, quantification and determination of detection limits in liquid chromatography-high-resolution time-of-flight mass spectrometry: challenges and practical solutions. Anal. Chim. Acta 789, 74–82.
Vergeynst, L., Van Langenhove, H., Joos, P., Demeestere, K., 2014. Suspect screening and target quantification of multi-class pharmaceuticals in surface water based on large-volume injection liquid chromatography and time-of-flight mass spectrometry. Anal. Bioanal. Chem. 406 (11), 2533–2547.
Wode, F., van Baar, P., Dunnbier, U., Hecht, F., Taute, T., Jekel, M., Reemtsma, T., 2015. Search for over 2000 current and legacy micropollutants on a wastewater infiltration site with a UPLC-high resolution MS target screening method. Water Res. 69, 274–283.