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Abstract

Water pollution from pharmaceutical drugs is becoming an environmental issue of increasing concern, making water quality monitoring a crucial priority to safeguard public health. In particular, the presence of antidepressants, benzodiazepines, antiepileptics, and antipsychotics require specific attention as they are known to be harmful to aquatic biota. In this study, a multi-class comprehensive method for the detection of 105 pharmaceutical residues in small (30 mL) water samples was developed according to fit-for-purpose criteria and then applied to provide wide screening of samples obtained from four Wastewater Treatment Plants (WWTPs) in northern Italy. The filtered samples (0.22 µm filters) were extracted by SPE, and then eluted. 5 µL of the concentrated samples were analyzed by a UHPLC-QTOF-HRMS method validated for screening purposes. Adequate sensitivity was recorded for all target analytes, with limits of detection below 5 ng/L for 76 out of 105 analytes. A total of 23 out of the 105 targeted pharmaceutical drugs was detected in all samples. Several further compounds were detected over wide concentration intervals, ranging from ng/L to µg/L. In addition, the retrospective analysis of full-scan QTOF-HRMS data was exploited to carry out an untargeted screening of some drugs' metabolites. As a proof of concept, it was investigated the presence of the carbamazepine metabolites, which is among the most frequently detected contaminants of emerging concern in wastewater. Thanks to this approach, 10,11-dihydro-10-hydroxycarbamazepine, 10,11-dihydro-10,11-dihydroxycarbamazepine and carbamazepine-10,11-epoxide were identified, the latter requiring particular attention, since it exhibits antiepileptic properties similar to carbamazepine and potential neurotoxic effects in living organism.

Keywords: environmental monitoring; pharmaceutical drugs; UHPLC-QTOF-HRMS; waste-based epidemiology; contaminants of emerging concern

1. Introduction

Contaminants of emerging concern (CECs) are chemical substances from anthropogenic origin present in the environment at trace and ultra-trace levels [1,2]. CECs usually refer to a wide range of substances classified as pesticides, pharmaceuticals, personal care products, flame retardants, hormones, *etc*., with pharmaceuticals and pesticides being most frequently detected due to their widespread use [3]. Among the pharmaceutical prescriptions, non-steroidal anti-inflammatory, cardiovascular, anti-depressant, and antipsychotic drugs are the most represented. Several active pharmaceutical ingredients and some of their metabolites are known to substantially or partially survive the conventional processes of wastewater treatment because they are bio-recalcitrant to the most common microorganisms used in the civil waste water treatment plants (WWTPs). Furthermore, the hydrophobicity of these drugs also prevents their efficient partition on the solid phase used for water purification in the plant, or their incorporation into the spent bacterial sludge [4], increasing their spreading into the aquatic environments. Monitoring the water quality is therefore crucial to safeguard the human health and protect the environmental biota.

In recent years, the persistence of xenobiotics in wastewater has also led to the development of wastewater-based epidemiology (WBE), which emerged as an essential complementary methodology for the evaluation of pharmaceutical and illicit drugs prevalence in selected populations. Conventional methods to estimate the rate of drugs use in a community already exist and are based on self-reported surveys, overdose/toxicological reports, and drug-related crime statistics [5–7]. Traditional approaches such as self-reported surveys are commonly affected by high cost, delayed outcome, limited coverage, and biases from nonresponse and unbalanced selection of sampled populations, with higher prevalence of drug abusers. For these reasons, sewage epidemiology established itself as a comprehensive, real-time, and cost-effective approach to reliably measure the average drug consumption within a community. The quantification of either the parent drugs or their human-specific metabolites in wastewater [8,9] is increasingly adopted to complement other conventional methods for the estimation of drugs use/abuse in large communities.

While the existing analytical procedures are generally addressed to the determination of specific classes of pharmaceutical products, the present study is aimed to create an unprecedented method for the simultaneous determination of a panel of hazardous pharmaceutical drugs in wastewater. It combines an effective solid phase extraction (SPE) of the analytes from the matrix followed by their detection by ultra-high-pressure liquid-chromatography (UHPLC) and time-of-flight high resolution mass spectrometry (TOF-HRMS). This analytical method achieved the semi-quantitative determination of 105 pharmaceutical drugs and provided a qualitative identification of their main metabolites. The targeted

analytes included 11 antidepressants, 15 antipsychotics, 5 antiepileptics, 26 benzodiazepines, 3 barbiturates, 7 cardiovascular drugs, 3 non-steroidal anti-inflammatory drugs, 9 analgesics, and other 26 pharmaceutical drugs from different classes. The method was applied to real samples collected from wastewater influents in Northern Italy.

The selection of chemical compounds for this study was based on the literature information about the pharmaceutical residues most frequently found in wastewater [10–12] and those consistently identified in biological samples, as suggested by our own experience [17]. Our procedure was also designed to reduce the volume of sample analyzed, moving from the 100-250 mL typically reported in the literature [14–16] to only 30 mL of water.

2. Materials and methods

2.1 Reagents and standards

The 105 pure standards of the targeted drugs were purchased from either LGC Promochem SRL (Milan, Italy) or Sigma-Aldrich (Milan, Italy). Methanol, formic acid, and acetonitrile were provided by Sigma-Aldrich (Milan, Italy). Ultra-pure water was obtained using a Milli-Q® UF-Plus apparatus (Millipore, Bedford, MA, USA). Stock standard solutions were stored at −20 °C until used. Three compounds were used as the internal standards (IS), including two isotopically marked molecules (cocaine-D3, nitrazepam-D5, and coumachlor). A working solution mixture was prepared by dilution in methanol containing all 105 reference substances at the final concentration 1 μg/mL. Lastly, an internal standard mixture working solution containing the three selected IS was prepared in methanol at the final concentration of 1 μg/mL.

2.2 Real samples collection

The real samples were collected as 24 h composite samples of the inlet wastewater from four wastewater treatment plants located in the North West of Italy. The automatic sampler carries out a sampling cycle of 24-hourly aliquots, 350 mL of wastewater every 60 minutes, every day. The sampled water is collected in a refrigerated container and a 1 L aliquot of composite water is transferred into a glass container. Since the treatment plants involved in the present study asked to remain anonymous, they are identified as Sites 1, 2, 3, and 4. The aliquots, once taken from the sampler, are collected in refrigerated 1 L glass bottles and stored at −20°C until the moment of analysis.

2.3 Sample preparation

The spiked samples used in the method development and validation were prepared from ultra-pure water (Milli-Q® UF-Plus) fortified at five concentration levels (5, 10, 25, 100 and 1000 ng/L) with the working solution mixture. Wastewater samples (100 mL) were centrifuged at 4000 rpm for 5 min and vacuum-filtered through 0.22 μm filter device (Steriflip-GP 50mL, 22um, Merck Life Science Srl). Then, a 30 mL aliquot of filtered wastewater or standard solution was spiked with the internal standards mixture (final concentration 25 ng/L) and extracted using an Oasis HLB solid phase extraction (SPE) cartridge (200 mg, 6 cm^3, Waters, Milford, MA). SPE cartridges were conditioned with 5 mL methanol and 5 mL ultrapure water, loaded with the entire samples volume, left to dry under vacuum for 30 min and eluted with 10 mL methanol. The eluate was dried for 4 hours at 40°C using a vacuum concentrator (Thermo Scientific™ Savant™ SpeedVac™). The dry residue was reconstituted with 50 μL methanol, centrifuged for 5 min at 13,000 g, and 5 μL of the supernatant was injected into the UHPLC system.

2.4 UHPLC- QTOF-HRMS- analysis

UHPLC separation was performed with a Phenomenex Kinetex C18 column (100 × 2.1 mm, 1.7 μm) maintained at 45 °C on the SCIEX ExionLC™ AC system. The mobile phases consisted of water (A) and acetonitrile (B), both mixed with formic acid 5 mM. The LC flow rate was set at 0.5 mL/min and the mobile phase eluted under the following linear gradient conditions (A:B, v:v): isocratic elution at 95:5 for 0.5 min, from 95:5 to 5:95 in 7.5 min, isocratic elution at 5:95 for 0.5 min and final re-equilibration at the initial conditions for 2.5 min. The total run time was 11 min. All analyses were performed using a quadrupole/time-of-flight SCIEX X500R QTOF mass spectrometer (Sciex, Darmstadt, Germany) equipped with a Turbo VTM electrospray ion source operating in both positive- and negative-ion modes. Data acquisition involved the collection of a preliminary TOF high-resolution full scan mass spectrum, followed by a SWATH™ acquisition protocol which used a variable window setup (16 windows covering mass range from m/z 130.0 to 520.0 at 0.025 resolving power). The identification of the 105 target analytes was based on the coincidence of their retention times, precursor ion and characteristic fragment ion m/z values (mass error accepted ≤5 ppb) with those of the corresponding pure standards. Furthermore, the adoption of HRMS with SWATH™ acquisition mode enabled a retrospective investigation of the dataset files aimed to detect unexpected and untargeted compounds, for example the metabolites of contaminants identified in certain wastewater specimen. The full list of the target analytes is reported in Table 1. The internal standards were selected based on our previous experience [16].

2.5 Validation

Specificity, sensitivity, recovery, and calibration model for the analytical method described above were investigated. Specificity was ensured every time the compound signal was correctly extracted and identified by the instrument without interferences. In this HRMS study, specificity was verified when the chromatographic peak detected at the expected retention time was associated to a molecular ion affected by a m/z error lower than 5 ppb with respect to the theoretical exact mass. Sensitivity for each target analyte was expressed by its LOD value. LOD values were experimentally tested by spiking the aqueous matrix with the target analytes at increasing concentration levels (5, 10, 15 ng/L) and verifying the minimal concentration at which the observed instrumental signal-to-noise ratio (S/N) was higher than 3. The extraction recovery was determined by comparing the experimental results obtained from three water samples spiked at the concentration level of 20 ng/L, before and after the extraction step.

The large number of target analytes and the limited number of isotopically-labeled IS make the present method suitable for screening and semi-quantitative purposes. For the initial and approximate quantification of the pharmaceutical compounds detected in the real wastewater samples, ultra-pure water was fortified with the working solution mixture at five concentration levels (5, 10, 25, 100 and 1000 ng/L), using cocaine-d3, nitrazepam-d5 and coumachlor as internal standards (IS). From the resulting solutions, a calibration model was built for each analyte, in which each calibration point was obtained in triplicate, at the five selected concentration levels.

2.6 Untargeted investigation of hazardous metabolites

The presence of drugs and their metabolic products in wastewater is mainly due to the urinary excretion of the consuming subjects. Acquisition of full scan high resolution mass spectra provides the chance of carrying out delayed retrospective analyses to verify the presence of drug metabolites, not directly targeted in the initial screening. Carbamazepine was selected as a typical model compound for testing the HRMS and the SWATH^{TM} acquisition method potential in real samples, since it is one of the drugs most frequently detected in wastewater treatment plants and in water bodies with clear and demonstrated environmental toxicity [17–19]. The metabolic pattern of carbamazepine is well known [20] and it is shown in Figure 1. Biotransformation includes oxidation, hydroxylation and hydrolysis transformation. The expected metabolites were identified in the real water samples based on the fragmentation patterns and the exact m/z of both their precursor and the fragment ions.

3 Results and discussion

3.1 Method validation

The developed method proved adequate for the individual detection of 105 target analytes and 3 internal standards in only 30 mL sample. Treating a low sample volume, i.e. 30 mL instead of 100-250 mL typically used, allowed us to reduce the preliminary steps, the volume of extraction solvent, and the energy consumption, making the whole procedure more sustainable for the environment. Also, loading a smaller volume of samples may extend the usability of the SPE cartridges. The chromatographic run was completed in only 11 min, including the final re-equilibration time (2.5 min). The fast data acquisition for a large number of target compounds within a single run is in agreement with the efficiency requirement needed for routine application. As shown in Table 1, all compounds eluted in the first 5.10 min. The total elution and acquisition time was extended to 7.5 min to investigate the potential presence of unknown metabolites and/or contaminants in the retrospective data screening for untargeted analytes. The adoption of HRMS with SWATH^{TM} acquisition mode enables a retrospective investigation of the dataset files aimed to detect unexpected and untargeted compounds, for example the metabolites of contaminants positively identified in certain wastewater specimen. Any time a contaminant of emerging concern is repeatedly detected in wastewater, the retrospective investigation becomes an effective tool to reconsider the data without the need of repeating the analysis on stored samples, often no more available. This feature is of particular interest for the detection of metabolites. Furthermore, all coeluting substances could be quantified without interferences using the capability of high-resolution mass spectrometry that always provided significant differences in m/z values of precursor and characteristic fragment ion. In practice, all analytes were properly identified, with no interference in their signals and the specificity proved optimal, as each m/z peak showed a calculated mass error lower than 5 ppb.

The LOD, calibration, and recovery results obtained from the method validation experiments for the ultra-pure water samples fortified with 105 analytes and 3 internal standards are reported in the Tables S1 of the Supplementary Material. The LOD was verified by spiking pure water with decreasing concentrations (15, 10, 5 ng/L) until a response equivalent to three times the background noise was observed. This purely experimental process proved that for 76 analytes out of 105 (72%), a LOD lower than 5 ng/L was verified. For 13 analytes, the estimated LODs were between 5 and 10 ng/L, while the remaining 16 analytes (15%) showed LOD values between 10 and 15 ng/L. The estimated LODs are in agreement with the concentration ranges generally detected in wastewater and fully adequate for almost all the target analytes. In particular, the 5 ng/L limit represents the current lowest LOD value measured in several other studies [21–23].

The calibration curves were calculated from three replicates only, according to the semi-quantitative purpose of the present method and its application for screening a very large number of targeted and potentially untargeted analytes. For the same reason, application of the Mandel's test for non-linearity proved that the introduction of a quadratic term in the calibration curves, even when it improved the

data-fitness, was not justified by the fit-for-purpose criteria followed in the present validation. Therefore, linear calibration was set up for all target analytes, whose ranges and equations are reported in Table S1.

The overall analytes' recovery was judged satisfactory, taking into account that several pre-concentration steps were involved in the procedure, including SPE and solvent evaporation of the extract, both leading to a potential loss of analytes. Recoveries higher than 70% was obtained for 62 out of 105 analytes and a recovery lower than 50% for 8 analytes only (Figure 2 & Table S1).

3.2 Application to wastewater samples

The analytical method was applied to the analysis of inlet wastewater samples collected at four different urban wastewater treatment plants during year 2022. A total of 23 out of the 105 targeted pharmaceutical drugs was detected in almost all sites and limited differences were observed among several drugs arrays found at the different sampling sites. These homogeneous results suggest that similar drug prescriptions and consumption rates are uniformly distributed in northern Italy. Another possible reason for detecting some compounds rather than others may rely on their different physical and chemical properties (e.g., dissociation constants, partition coefficients, chemical stability) together with different metabolism and excretion kinetics.

Table 2 shows the average concentration of the pharmaceutical drugs found in the influent samples at the different WWTPs and Figure 3 shows an example of the extracted ion chromatogram (XIC) evidencing 10 of the pharmaceutical drugs found in a real sample of Site 4. The highest absolute concentration was detected for paracetamol (higher than 1 μg/L), which is currently consumed by a large percentage of general population. It is noteworthy that among the 20 best-selling active principle in Italy (according to the Federfarma 2021 report), several were identified, for example bisoprolol (concentration range 25-80 ng/L), nebivolol (found only in the site 1 at concentration higher than 60 ng/L) and ramipril (concentration range 10-25 ng/L), all belonging to the class of cardiovascular drugs. Among these, also atenolol, propafenone, and telmisartan were consistently identified.

The classes of antidepressants, benzodiazepines, antiepileptics, and antipsychotic require particular attention as they are known to be harmful to the aquatic environments; for example, the effects of bioaccumulation of these active ingredients in fish include endocrine effects, developmental alteration and behavioral changes [24,25]. Among these, citalopram (concentration range 50-200 ng/L), lorazepam (concentration range 20-160 ng/L), trazodone (concentration range 5-20 ng/L) and carbamazepine (concentration range 100-600 ng/L) were detected in almost all samples. Particularly alarming is the case of tramadol (concentration range 40-215 ng/L) which is the active ingredient of several common opioid pain-relieving prescriptions. Recently in Italy, tramadol has also been classified among the illicit

drugs, suggesting that its use is not restricted to medical therapies, but also abused for recreational purposes or misused for *off label* treatments.

The drug concentrations detected in 24-h representative samples are comparable or even higher than those observed in similar studies [26,27]. It is deduced that the total amounts of the screened pharmaceutical drugs released in the water acceptor bodies can be worryingly high, to the extent that constant monitoring may be required, particularly when scarce removal in the WWTP is expected. For a plant with 100,000 inhabitants equivalent with a flow rate of about 24,000 m^3/day, it is possible to provide an estimate of the load (g/day) of a pharmaceutical drug arriving at a selected WWTP. Mass load of pharmaceutical drug residues can be determined using the following equation [28]:

$$Load\ (\frac{g}{day}) = concentration\ (\frac{ng}{L}) \times flow\ (\frac{\mathrm{L}}{\mathrm{day}}) \times \frac{100}{100 + stability(\%)} \times \frac{100}{100 - sorption\ (\%)} \times \frac{1}{10^9}$$

Taking tramadol and venlafaxine as model molecules and using the data reported in Table 3 (% of stability and sorption data were provided by the literature [29,30]), it is possible to calculate the mass of active ingredient present in the inlet wastewater entering the Site 3 treatment plant. The analytical results reported in Table 2 together with the water flow yield a mass load of about 10 g/day (≈ 4 Kg per year) for tramadol and about 20 g/day (≈ 7 Kg per year) for venlafaxine, respectively. These approximate calculations provide two important pieces of information: a) the total amounts of these drugs represent a significant threat to the survival and reproduction capabilities of living aquatic organisms [31,32] and b) highly efficient abatement procedures in the purification plants are needed to avoid significant release in the environment of pharmaceutical drugs.

3.3 Untargeted screening for metabolites

A subsidiary scope of the present study was to verify if the analytical method based on full scan HRMS acquisition prove capable of identifying drug metabolites by means of untargeted screening strategies. Carbamazepine was selected as a model compound, due to its high environmental concern. In particular, previous studies have pointed out that certain metabolites raise as much concern for the aquatic environments as the corresponding parent drug [39–41]. The acquired data files were cross-examined in search of the expected metabolites [36]. The presence of 10,11-dihydro-10-hydroxycarbamazepine, 10,11-dihydro-10,11-dihydroxycarbamazepine, and carbamazepine-10,11-epoxide was instrumentally revealed and structurally characterized by the fragmentation pattern and exact mass of both their precursor and fragment ions (Table S2). In Figure 3, an example of the HRMS fragmentation pattern of one of the carbamazepine metabolites is reported.

Great attention was paid to the carbamazepine-10,11-epoxide as it is not only a metabolic oxidation product of carbamazepine, but also proved to possess antiepileptic properties similar to carbamazepine, possibly producing neurotoxic effects and having its own activity and environmental eco-toxicity [15]. The approximate concentration ratio between carbamazepine-10,11-epoxide and carbamazepine is higher than 3 in all analyzed samples (the signals intensity ratio between metabolite and precursor are reported in Table S3), suggesting a higher concentration of the metabolite with respect to the parent drug in wastewater. It is concluded that wastewater monitoring should include the most environmentally relevant drug metabolites among the target analytes of acquisition and processing methods of analysis.

4. Conclusions

The developed analytical method based on solid phase extraction of samples followed by UHPLC-QTOF-HRMS detection allowed the simultaneous quantification of 105 pharmaceuticals drugs and their metabolites in wastewater samples. The application of QTOF-HRMS technique allowed the combination of high-resolution full scan untargeted screening and targeted analysis, thus representing an effective method for fast and convenient environmental screening of drugs.

The collected data on the real samples are consistent with those available in the literature and confirm that many of the investigated pharmaceutical drugs are present in wastewater at a level that pose a health issue to the biota, considering also the increased risk associated with long-term simultaneous exposure to a mix of a large number of pharmaceutical products and their metabolites. In conclusion, the wastewater surveillance is essential not only to identify the pharmaceutical drugs used in the area, but also to monitor the purity of waters and the possible health risks for the inhabitants. In the future, analyses will be carried out *i*) to study the variation in the substances found over time and in the different territories at both intra- and inter-regional levels, *ii*) to evaluate the percentage abatement for the detected compounds in traditional WWTPs and, as a consequence, *iii*) to evaluate the real input of these CECs into the water acceptor bodies in term of total amount of released pollutants.

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Conflict of interest: none

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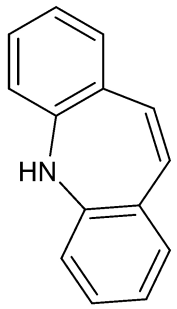
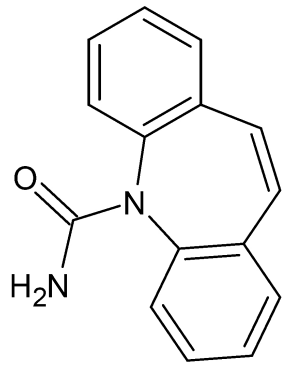
Iminostilbene



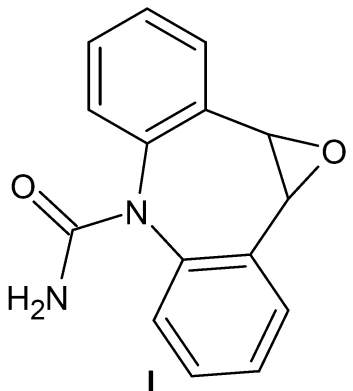
Carbamazepine (CBZ)



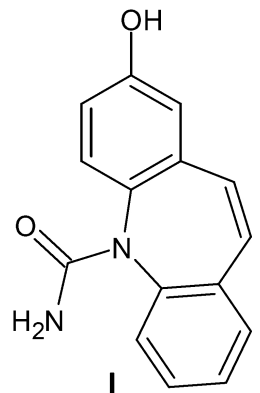
Oxidation

Hydroxylation

carbamazepine-10,11-epoxide



2-hydroxy- carbamazepine
3-hydroxy- carbamazepine

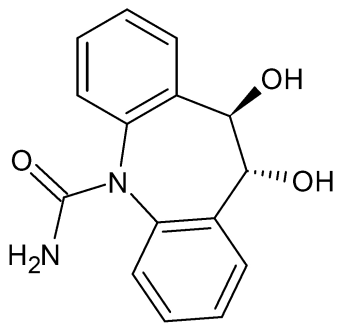


Hydrolysis

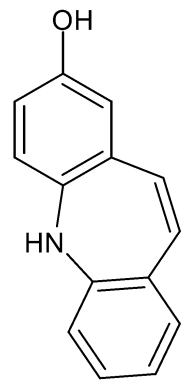
Epoxide hydrolase

Hydrolysis β- glucoronidase

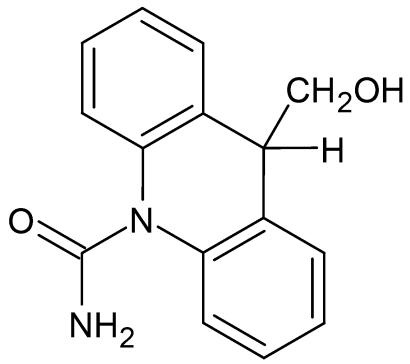
10,11-dihydro- 10,11, *trans*-dihydroxy-CBZ



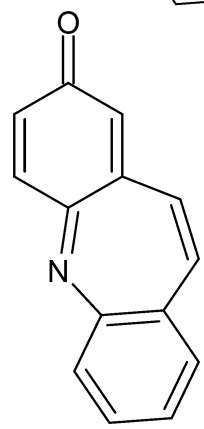
2-hydroxyiminostilbene



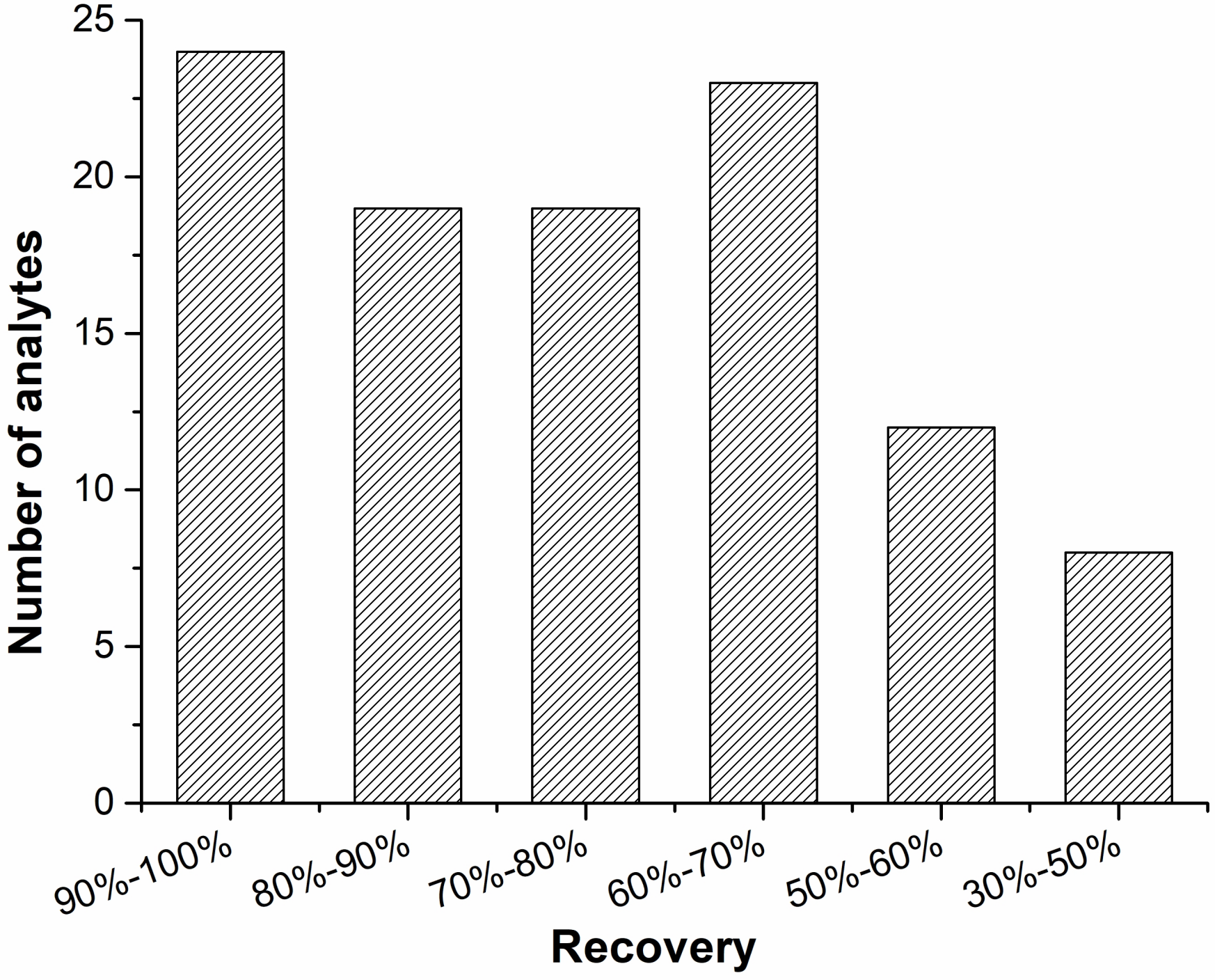
Oxidation

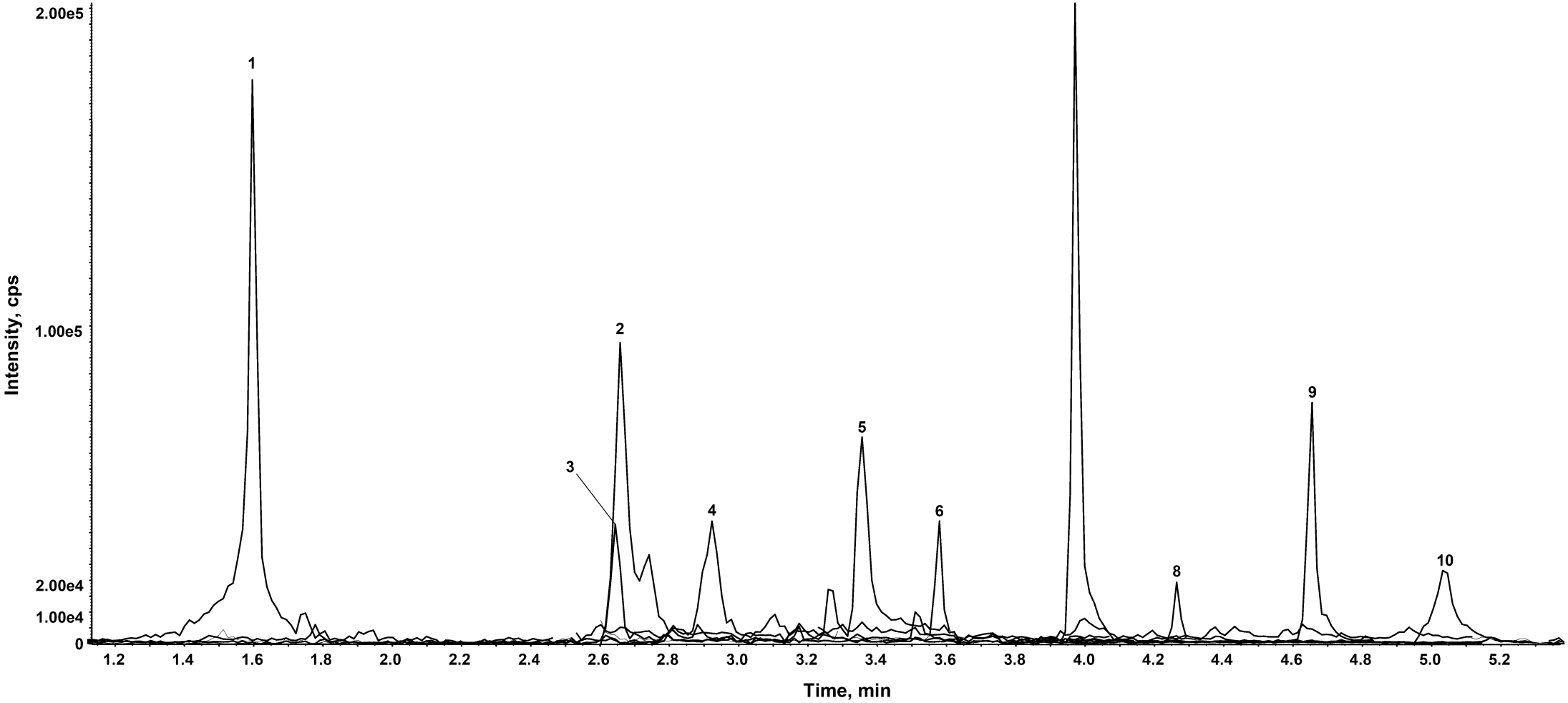


9-hydroxymethyl-10-carbamoyl acridan



Iminoquinone


25
20
15
10
5
0
Number of analytes
90%-100%
80%-90%
70%-80%
60%-70%
50%-60%
30%-50%
Recovery


2.00e5
1.00e5
2.00e4
1.00e4
0
Intensity, cps
1
2
3
4
5
6
7
8
9
10
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0
4.2
4.4
4.6
4.8
5.0
5.2
Time, min

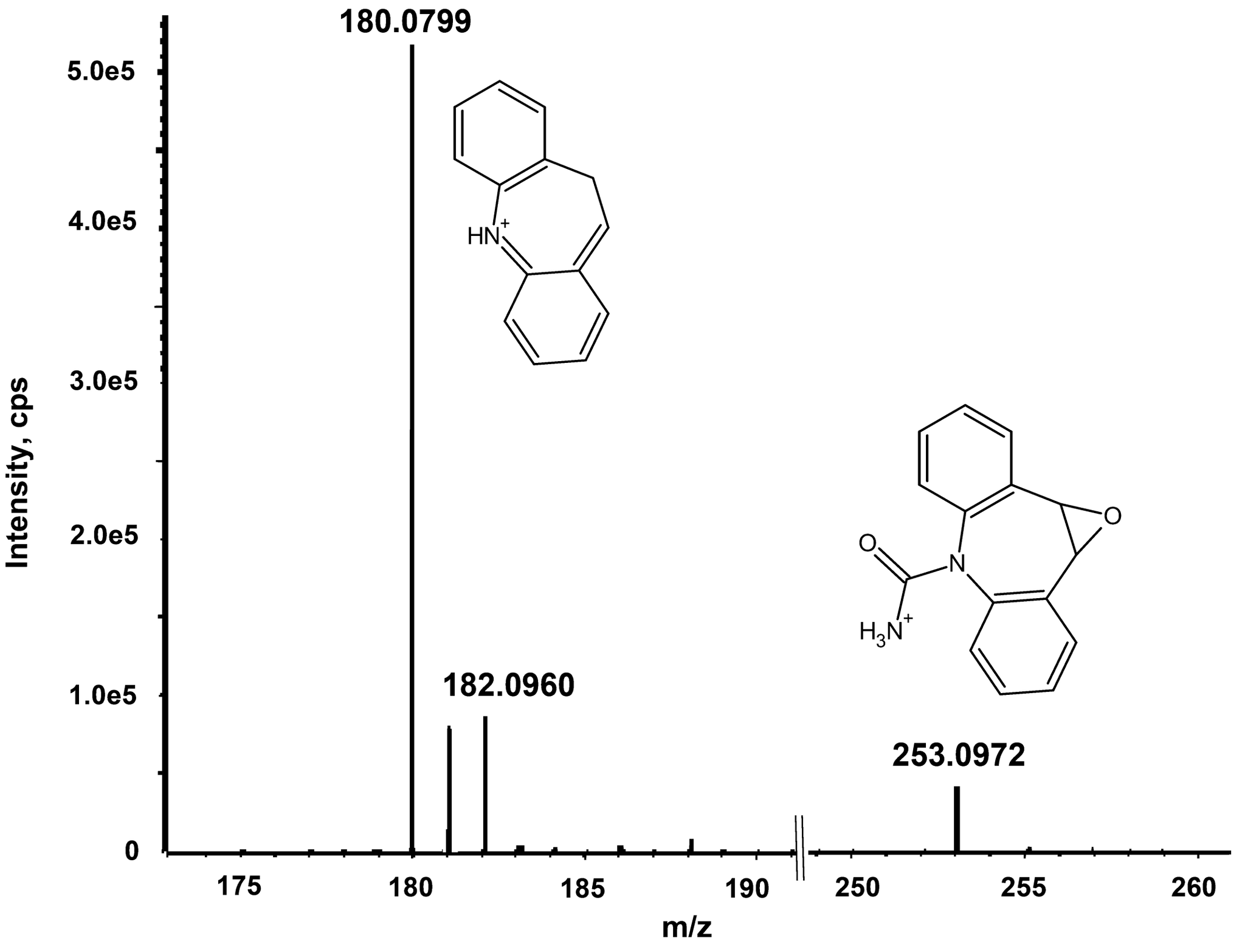

180.0799
182.0960
253.0972
5.0e5
4.0e5
3.0e5
2.0e5
1.0e5
0
Intensity, cps
175
180
185
190
250
255
260
m/z
HN+
O
N
O
H3N+

Figure 1 Metabolic pathway of carbamazepine [23]

Figure 2 Number of targeted analytes grouped by their percent recovery.

Figure 3 Chromatographic profile of the 10 pharmaceutical drugs found in the Site 4 within a 1.2–5.2 min retention time interval. Extracted ion chromatograms (XICs) resulting from the optimized data acquisition method, built by the Scheduled Algorithm Pro in SCIEX OS Software. The numbered peaks correspond to: 1) Atenolol, 2) Tramadol, 3) Lidocaine, 4) Tapentadol, 5) Bisoprolol, 6) Amisulpride, 7) Carbamazepine, 8) Lorazepam, 9) Ketoprofen, and 10) Propafenone

Figure 4 HRMS fragmentation pattern of carbamazepine 10,11-epoxide

Table 1: List of the 105 substances under study (target analytes).

Compound	Formula	Charge	Precursor theoretical m/z	Fragment theoretical m/z	Retention time, min	Internal Standard
Antidepressants						
Amitriptyline	$C_{20}H_{23}N$	$[M+H]^+$	278.1903	91.0545	4.22	Cocaine-D3
Bupropion	$C_{13}H_{18}ClNO$	$[M+H]^+$	240.1150	131.0721	3.19	Coumachlor
Citalopram	$C_{20}H_{21}FN_2O$	$[M+H]^+$	325.1711	109.0453	3.84	Cocaine-D3
Clonidine	$C_9H_9Cl_2N_3$	$[M+H]^+$	230.0246	212.9972	1.88	Cocaine-D3
Fluoxetine	$C_{17}H_{18}F_3NO$	$[M+H]^+$	310.1413	265.1630	4.45	Cocaine-D3
Fluvoxamine	$C_{15}H_{21}F_3N_2O_2$	$[M+H]^+$	319.1628	71.0509	4.10	Cocaine-D3
Mianserin	$C_{18}H_{20}N_2$	$[M+H]^+$	265.1699	208.1124	3.79	Nitrazepam-D5
Mirtazapine	$C_{17}H_{19}N_3$	$[M+H]^+$	266.1652	195.0915	2.90	Nitrazepam-D5
Paroxetine	$C_{19}H_{20}FNO_3$	$[M+H]^+$	330.1500	192.1187	3.99	Coumachlor
Sertraline	$C_{17}H_{17}Cl_2N$	$[M+H]^+$	306.0811	158.9765	4.44	Nitrazepam-D5
Trazodone	$C_{19}H_{22}ClN_5O$	$[M+H]^+$	372.1586	176.0804	3.30	Nitrazepam-D5
Benzodiazepines and analogues						
7-Aminoclonazepam	$C_{15}H_{12}ClN_3O$	$[M+H]^+$	286.0742	121.0757	2.77	Nitrazepam-D5
7-Aminoflunitrazepam	$C_{16}H_{14}FN_3O$	$[M+H]^+$	284.1194	135.0916	3.07	Nitrazepam-D5
7-Aminonitrazepam	$C_{15}H_{13}N_3O$	$[M+H]^+$	252.1131	121.0760	1.97	Nitrazepam-D5
Alprazolam	$C_{17}H_{13}ClN_4$	$[M+H]^+$	309.0902	281.0698	4.30	Nitrazepam-D5
Bromazepam	$C_{14}H_{10}BrN_3O$	$[M+H]^+$	316.0080	182.0836	3.68	Nitrazepam-D5
Brotizolam	$C_{15}H_{10}BrClN_4S$	$[M+H]^+$	392.9571	314.0395	4.51	Cocaine-D3
Chlordiazepoxide	$C_{16}H_{14}ClN_3O$	$[M+H]^+$	300.0898	227.0499	3.35	Nitrazepam-D5
Clobazam	$C_{16}H_{13}ClN_2O_2$	$[M+H]^+$	301.0738	259.0630	4.64	Nitrazepam-D5
Clonazepam	$C_{15}H_{10}ClN_3O_3$	$[M+H]^+$	316.0484	270.0562	4.23	Nitrazepam-D5
Clotiazepam	$C_{16}H_{15}ClN_2OS$	$[M+H]^+$	319.0666	278.0570	4.92	Nitrazepam-D5
Delorazepam	$C_{15}H_{10}Cl_2N_2O$	$[M+H]^+$	305.0243	140.0264	4.58	Nitrazepam-D5
Demoxepam	$C_{15}H_{11}ClN_2O_2$	$[M+H]^+$	287.0581	241.1100	4.19	Nitrazepam-D5
Desalchilflurazepam	$C_{17}H_{15}ClFN_3O$	$[M+H]^+$	332.0960	140.0257	4.94	Nitrazepam-D5
Diazepam	$C_{16}H_{13}ClN_2O$	$[M+H]^+$	285.0789	154.0413	4.84	Nitrazepam-D5
Diclazepam	$C_{16}H_{12}Cl_2N_2O$	$[M+H]^+$	319.0399	227.0502	5.10	Nitrazepam-D5
Diltiazem	$C_{22}H_{26}N_2O_4S$	$[M+H]^+$	415.1686	178.0305	3.82	Cocaine-D3

Flunitrazepam	$C_{16}H_{12}FN_3O_3$	$[M+H]^+$	314.0936	268.0991	4.44	Nitrazepam-D5
Flurazepam	$C_{21}H_{23}ClFN_3O$	$[M+H]^+$	388.1586	315.0672	3.71	Nitrazepam-D5
Lorazepam	$C_{15}H_{10}Cl_2N_2O_2$	$[M+H]^+$	321.0192	275.0144	4.20	Nitrazepam-D5
Lormetazepam	$C_{16}H_{12}Cl_2N_2O_2$	$[M+H]^+$	335.0349	289.0286	4.62	Nitrazepam-D5
Midazolam	$C_{18}H_{13}ClFN_3$	$[M+H]^+$	326.0855	291.1152	3.64	Nitrazepam-D5
Nordiazepam	$C_{15}H_{11}ClN_2O$	$[M+H]^+$	271.0633	140.0256	4.37	Nitrazepam-D5
Oxazepam	$C_{15}H_{11}ClN_2O_2$	$[M+H]^+$	287.0582	241.0528	4.09	Nitrazepam-D5
Temazepam	$C_{16}H_{13}ClN_2O_2$	$[M+H]^+$	301.0738	255.0679	4.47	Nitrazepam-D5
Triazolam	$C_{17}H_{12}Cl_2N_4$	$[M+H]^+$	343.0512	308.0822	4.37	Nitrazepam-D5
Zolpidem	$C_{19}H_{21}N_3O$	$[M+H]^+$	308.1757	236.1287	3.18	Nitrazepam-D5
Zopiclone	$C_{17}H_{17}ClN_6O_3$	$[M+H]^+$	389.1123	245.0225	2.78	Nitrazepam-D5
Barbiturates						
Amobarbital	$C_{11}H_{18}N_2O_3$	$[M-H]^-$	225.1245	41.9986	3.89	Nitrazepam-D5
Barbital	$C_8H_{12}N_2O_3$	$[M-H]^-$	183.0775	68.9012	2.25	Nitrazepam-D5
Secobarbital	$C_{12}H_{18}N_2O_3$	$[M-H]^-$	237.1245	41.9985	4.09	Nitrazepam-D5
Antipsychotic						
Amisulpride	$C_{17}H_{27}N_3O_4S$	$[M+H]^+$	370.1795	242.0477	2.55	Cocaine-D3
Aripiprazole	$C_{23}H_{27}Cl_2N_3O_2$	$[M+H]^+$	448.1553	285.0899	4.23	Coumachlor
Carbamazepine	$C_{15}H_{12}N_2O$	$[M+H]^+$	237.1022	194.0949	3.90	Nitrazepam-D5
Chlorpromazine	$C_{17}H_{19}ClN_2S$	$[M+H]^+$	319.1030	86.0962	4.42	Coumachlor
Clozapine	$C_{18}H_{19}ClN_4$	$[M+H]^+$	327.1371	270.0794	3.52	Nitrazepam-D5
Haloperidol	$C_{21}H_{23}ClFNO_2$	$[M+H]^+$	376.1474	165.0697	3.95	Coumachlor
Levomepromazine	$C_{19}H_{24}N_2OS$	$[M+H]^+$	329.1682	100.1121	4.23	Cocaine-D3
Olanzapine	$C_{17}H_{20}N_4S$	$[M+H]^+$	313.1481	256.0893	2.19	Nitrazepam-D5
Periciazine	$C_{21}H_{23}N_3OS$	$[M+H]^+$	366.1635	142.1223	3.89	Nitrazepam-D5
Promazine	$C_{17}H_{20}N_2S$	$[M+H]^+$	285.1420	86.0975	3.93	Cocaine-D3
Quetiapine	$C_{21}H_{25}N_3O_2S$	$[M+H]^+$	384.1740	253.0795	3.63	Nitrazepam-D5
Risperidone	$C_{23}H_{27}FN_4O_2$	$[M+H]^+$	411.2191	191.1174	3.32	Nitrazepam-D5
Tiapride	$C_{15}H_{24}N_2O_4S$	$[M+H]^+$	329.1530	256.0615	1.98	Cocaine-D3
Venlafaxine	$C_{17}H_{27}NO_2$	$[M+H]^+$	278.2115	58.0656	3.25	Cocaine-D3
Ziprasidone	$C_{21}H_{21}ClN_4OS$	$[M+H]^+$	413.1197	194.0373	3.65	Cocaine-D3
Zuclopenthixol	$C_{22}H_{25}ClN_2OS$	$[M+H]^+$	401.1449	271.0339	4.61	Nitrazepam-D5

Antiepileptic						
Lamotrigine	$C_9H_7Cl_2N_5$	$[M+H]^+$	256.0151	210.9820	2.73	Nitrazepam-D5
Oxcarbazepine	$C_{15}H_{12}N_2O_2$	$[M+H]^+$	253.0972	180.0810	3.58	Nitrazepam-D5
Pregabalin	$C_8H_{17}NO_2$	$[M+H]^+$	160.1332	55.0547	1.78	Nitrazepam-D5
Valproic acid	$C_8H_{16}O_2$	$[M-H]^-$	143.1078	98,7310	4.63	Nitrazepam-D5
Cardiovascular Drugs						
Atenolol	$C_{14}H_{22}N_2O_3$	$[M+H]^+$	267.1703	145.0638	1.60	Cocaine-D3
Bisoprolol	$C_{18}H_{31}NO_4$	$[M+H]^+$	326.2326	116.1068	3.38	Nitrazepam-D5
Nebivolol	$C_{22}H_{25}F_2NO_4$	$[M+H]^+$	406.1824	151.0561	4.24	Nitrazepam-D5
Propafenone	$C_{21}H_{27}NO_3$	$[M+H]^+$	342.2064	116.1067	4.12	Nitrazepam-D5
Ramipril	$C_{23}H_{32}N_2O_5$	$[M+H]^+$	417.2384	234.1497	3.97	Cocaine-D3
Telmisartan	$C_{33}H_{30}N_4O_2$	$[M+H]^+$	515.2442	497.2324	4.54	Nitrazepam-D5
Verapamil	$C_{27}H_{38}N_2O_4$	$[M+H]^+$	455.2904	165.0906	4.23	Nitrazepam-D5
Non-steroidal anti-inflammatory Drugs						
Ibuprofen	$C_{13}H_{18}O_2$	$[M-H]^-$	205.1234	161.1330	5.41	Coumachlor
Ketoprofen	$C_{16}H_{14}O_3$	$[M+H]^+$	255.1016	105.0328	4.58	Coumachlor
Ketorolac	$C_{15}H_{13}NO_3$	$[M+H]^+$	256.0968	105.0334	4.11	Coumachlor
Analgesics / opioids						
Buprenorphine	$C_{29}H_{41}NO_4$	$[M+H]^+$	468.3108	414.2637	3.85	Cocaine-D3
Dihydrocodeine	$C18H23NO_3$	$[M+H]^+$	302.1751	199.0756	1.84	Cocaine-D3
Embutramide	$C_{17}H_{27}NO_3$	$[M+H]^+$	294.2064	121.0644	4.30	Coumachlor
Hydromorphone	$C_{17}H_{19}NO_3$	$[M+H]^+$	286.1438	185.0588	1.48	Nitrazepam-D5
Methadone	$C_{21}H_{27}NO$	$[M+H]^+$	310.2165	105.0328	4.26	Cocaine-D3
Oxycodone	$C_{18}H_{21}NO_4$	$[M+H]^+$	316.1543	241.1062	2.07	Cocaine-D3
Paracetamol	$C_8H_9NO_2$	$[M+H]^+$	152.0706	110.0604	1.53	Coumachlor
Phenacetin	$C_{10}H_{13}NO_2$	$[M+H]^+$	180.1019	110.0606	3.29	Cocaine-D3
Tapentadol	$C_{14}H_{23}NO$	$[M+H]^+$	222.1852	107.0488	2.90	Cocaine-D3
Tramadol	$C_{16}H_{25}NO_2$	$[M+H]^+$	264.1958	58.0656	2.91	Cocaine-D3
Others						
Atropine	$C_{17}H_{23}NO_3$	$[M+H]^+$	290.1751	124.1124	2.52	Cocaine-D3
Biperiden	$C_{21}H_{29}NO$	$[M+H]^+$	312.2322	98.0965	4.33	Coumachlor
Dextromethorphan	$C_{18}H_{25}NO$	$[M+H]^+$	272.2009	215.1416	3.59	Cocaine-D3

Diphenhydramine	$C_{17}H_{21}NO$	$[M+H]^+$	256.1696	167.0840	3.68	Cocaine-D3
Diphenidine	$C_{19}H_{23}N$	$[M+H]^+$	266.1903	181.0996	3.71	Cocaine-D3
Disulfiram	$C_{10}H_{20}N_2S_4$	$[M+H]^+$	297.0582	116.0526	5.90	Cocaine-D3
Glibenclamide	$C_{23}H_{28}ClN_3O_5S$	$[M+H]^+$	494.1511	369.0270	5.45	Nitrazepam-D5
Gliclazide	$C_{15}H_{21}N_3O_3S$	$[M+H]^+$	324.1376	127.1225	4.86	Cocaine-D3
Levamisole	$C_{11}H_{12}N_2S$	$[M+H]^+$	205.0794	178.0687	2.00	Nitrazepam-D5
Lidocaine	$C_{14}H_{22}N_2O$	$[M+H]^+$	235.1805	86.0965	2.49	Cocaine-D3
Loperamide	$C_{29}H_{33}ClN_2O_2$	$[M+H]^+$	477.2303	266.1544	4.82	Coumachlor
Metformin	$C_4H_{11}N_5$	$[M+H]^+$	130.1087	71.0602	0.61	Cocaine-D3
Methylphenidate	$C_{14}H_{19}NO_2$	$[M+H]^+$	234.1489	84.0808	2.86	Cocaine-D3
Metoclopramide	$C_{14}H_{22}ClN_3O_2$	$[M+H]^+$	300.1473	227.0586	2.72	Cocaine-D3
Naloxone	$C_{19}H_{21}NO_4$	$[M+H]^+$	328.1543	310.1432	1.87	Cocaine-D3
Oxybutynin	$C_{22}H_{31}NO_3$	$[M+H]^+$	358.2377	142.1232	4.54	Nitrazepam-D5
Phendimetrazine	$C_{12}H_{17}NO$	$[M+H]^+$	192.1383	146.0960	2.09	Cocaine-D3
Promethazine	$C_{17}H_{20}N_2S$	$[M+H]^+$	285.1420	86.0960	3.93	Cocaine-D3
Scopolamine	$C_{17}H_{21}NO_4$	$[M+H]^+$	304.1543	138.0901	2.03	Cocaine-D3
Sildenafil	$C_{22}H_{30}N_6O_4S$	$[M+H]^+$	475.2122	58.0648	3.74	Nitrazepam-D5
Tadalafil	$C_{22}H_{19}N_3O_4$	$[M+H]^+$	390.1448	268.1082	4.36	Nitrazepam-D5
Ticlopidine	$C_{14}H_{14}ClNS$	$[M+H]^+$	264.0608	125.0144	3.17	Cocaine-D3
Vardenafil	$C_{23}H_{32}N_6O_4S$	$[M+H]^+$	489.2279	299.1100	3.55	Nitrazepam-D5
Warfarin	$C_{19}H_{16}O_4$	$[M+H]^+$	309.1121	250.1561	3.95	Cocaine-D3

Table 2 Average concentration (ng/L) of the drugs found in the influent samples to the different WWTPs. The number of analyzed samples for each site is 2, 3, 4, and 6, for Site 1, 2, 3 and for 4, respectively.

n.d. = not detected.

Compound	**Site 1**	**Site 2**	**Site 3**	**Site 4**
Antidepressants				
Bupropion	n.d	n.d	n.d	n.d
Citalopram	220	54	56	n.d
Mirtazapine	13	23	17	n.d
Trazodone	5	19	13	5

Benzodiazepine				
Lorazepam	29	76	160	24
Lormetazepam	9	75	160	9
Oxazepam	n.d	19	36	7
Temazepam	n.d	7	8	n.d
Antipsychotic				
Amisulpride	n.d	120	71	18
Carbamazepine	100	450	600	530
Quetiapine	n.d	39	22	11
Tiapride	n,d	n.d	5	n.d
Venlafaxine	n.d	> 1000	630	n.d
Antiepileptic				
Lamotrigine	n.d	350	860	n.d
Oxcarbazepine	n.d	380	200	n.d
Pregabalin	> 1000	n.d	n.d	n.d
Cardiovascular Drugs				
Atenolol	n.d	n.d	n.d	500
Bisoprolol	25	62	73	77
Nebivolol	68	n.d	n.d	n.d
Propafenone	220	95	44	30
Ramipril	17	26	n.d	n.d
Telmisartan	350	190	120	n.d
Non-steroidal anti-inflammatory Drugs				
Ketoprofen	320	48	420	900
Ketorolac	n.d	n.d	n.d	n.d
Analgesic/opioids				
Paracetamol	> 1000	n.d	≥ 1000	≥ 1000
Tapentadol	44	240	380	100
Tramadol	41	80	170	215
Others				
Dextromethorphan	260	n.d	n.d	n.d
Gliclazide	32	18	180	n.d

Lidocaine	43	270	> 1000	82
Metoclopramide	n.d	18	19	n.d

Table 3 Overview of parameters used in the sewage epidemiology calculations for each compound

Compound	**Concentration (ng/L)**	**Flow (L/day)**	**Stability (%)** [a]	**Sorption (%)** [b]
Tramadol	380	2.40E+07	-11	1
Venlafaxine	630	2.40E+07	-20	0.4

[a] Stability change in raw wastewater at 19 °C after 12 h

[b] Average sorption to soil or sludge in collected wastewater samples

Highlights

- Wastewater-based epidemiology as an essential complementary monitoring methodology
- Method validation for pharmaceutical drugs detection in 30 mL wastewater
- Simultaneous detection of 105 pharmaceutical drugs and some metabolites
- High-Resolution Mass Spectrometry allows targeted and untargeted analysis
- Limits of detection in the 5-15 ng/L range are achieved

Comprehensive wastewater surveillance of pharmaceutical drugs and metabolites by means of UHPLC-QTOF-HRMS

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Supplementary materials

Table S1 Results of LOD verified and Recovery (RE%)

Compounds	LOD Verified (S/N>3) (ng/L)	Linear range tested (ng/L)	Equation	RE%
Antidepressants				
Amitriptyline	5	5-1000	$y = 2.27*10^{-2} x +6.36*10^{-1}$	100
Bupropion	10	10-1000	$y = 6.12*10^{-3} x +2,58*10^{-1}$	51
Citalopram	5	5-1000	$y = 5.22*10^{-4} x +1.16*10^{-3}$	101
Clonidine	5	5-1000	$y = 5.04*10^{-3} x +5.59*10^{-1}$	72
Fluoxetine	5	5-1000	$y =7.79 *10^{-3} x +1.63*10^{-2}$	64
Fluvoxamine	15	25-1000	$y = 8.54*10^{-5} x +8.10*10^{-3}$	58
Mianserin	15	25-1000	$y = 9.60*10^{-4} x +1.00*10^{-2}$	86
Mirtazapine	5	5-1000	$y = 1.73*10^{-3} x +3.18*10^{-3}$	63
Paroxetine	5	5-1000	$y = 5.79*10^{-4} x +8.21*10^{-3}$	48
Sertraline	5	5-1000	$y = 2.00*10^{-3} x +2.48*10^{-2}$	92
Trazodone	5	5-1000	$y = 1.94*10^{-3} x +8.38*10^{-3}$	74
Benzodiazepines and analogues				
7-Aminoclonazepam	15	25-1000	$y = 4.24*10^{-4} x +6.69*10^{-3}$	52
7-Aminoflunitrazepam	5	5-1000	$y = 8.39*10^{-4} x +4.27*10^{-3}$	83

7-Aminonitrazepam	15	25-1000	$y = 7.55*10^{-4} x +3.32*10^{-3}$	54
Alprazolam	5	5-1000	$y = 9.55*10^{-4} x +1.55*10^{-4}$	94
Bromazepam	15	25-1000	$y = 1.16*10^{-3} x +2.06*10^{-3}$	82
Brotizolam	5	5-1000	$y = 4.79*10^{-3} x +1.82*10^{-2}$	74
Chlordiazepoxide	5	5-1000	$y = 1.93*10^{-3} x +1.37*10^{-2}$	93
Clobazam	5	5-1000	$y = 1.35*10^{-3} x +6.68*10^{-3}$	95
Clonazepam	5	5-1000	$y = 3.52*10^{-4} x -3.36*10^{-3}$	94
Clotiazepam	10	10-1000	$y = 1.55*10^{-1} x -2.15*10^{-3}$	78
Delorazepam	5	5-1000	$y = 4.65*10^{-4} x -5.60*10^{-3}$	78
Demoxepam	5	5-1000	$y = 1.21*10^{-2} x +6.04*10^{-3}$	70
Desalchilflurazepam	15	25-1000	$y = 1.55*10^{-4} x +9.34*10^{-3}$	93
Diazepam	5	5-1000	$y = 5.38*10^{-4} x -5.40*10^{-4}$	93
Diclazepam	5	5-1000	$y = 2.84*10^{-4} x -2.63*10^{-4}$	77
Diltiazem	5	5-1000	$y = 5.68*10^{-4} x +6.91*10^{-3}$	70
Flunitrazepam	5	5-1000	$y = 5.12*10^{-4} x -6.81*10^{-3}$	100
Flurazepam	5	5-1000	$y = 5.03*10^{-3} x +3.33*10^{-3}$	85
Lorazepam	5	5-1000	$y = 1.11*10^{-3} x +2.49*10^{-4}$	91
Lormetazepam	5	5-1000	$y = 1.56*10^{-3} x +1.21*10^{-2}$	82
Midazolam	5	5-1000	$y = 1.32*10^{-3} x -2.19*10^{-3}$	94
Nordiazepam	5	5-1000	$y = 6.85*10^{-4} x -8.25*10^{-3}$	83
Oxazepam	5	5-1000	$y = 2.18*10^{-3} x -1.79*10^{-2}$	96
Temazepam	5	5-1000	$y = 2.81*10^{-3} x -8.10*10^{-3}$	90
Triazolam	5	5-1000	$y = 1.01*10^{-3} x -1.49*10^{-3}$	81
Zolpidem	5	5-1000	$y = 2.65*10^{-3} x -3.38*10^{-3}$	90
Zopiclone	10	10-1000	$y = 1.63*10^{-4} x +8.12*10^{-4}$	41
Barbiturates				
Amobarbital	15	25-1000	$y = 4.01*10^{-3} x -2.70*10^{-2}$	63
Barbital	15	25-1000	$y = 9.29*10^{-4} x -2.54*10^{-3}$	55
Secobarbital	10	10-1000	$y = 2.76*10^{-3} x +4.43*10^{-3}$	64
Antipsychotic				
Amisulpride	5	5-1000	$y = 5.50*10^{-4} x +9.46*10^{-3}$	67
Aripiprazole	15	25-1000	$y = 2.61*10^{-4} x -8.29*10^{-3}$	55

Carbamazepine	5	5-1000	$y = 3.61*10^{-3} x +2.74*10^{-2}$	82
Chlorpromazine	10	10-1000	$y = 1.38*10^{-1} x -7.01*10^{-2}$	54
Clozapine	5	5-1000	$y = 1.27*10^{-3} x -4.44*10^{-3}$	63
Haloperidol	5	5-1000	$y = 4.82*10^{-3} x +9.36*10^{-3}$	81
Levomepromazine	15	25-1000	$y = 1.48*10^{-4} x +3.51*10^{-4}$	86
Olanzapine	10	10-1000	$y = 7.48*10^{-4} x +2.16*10^{-4}$	33
Periciazine	5	5-1000	$y = 1.28*10^{-3} x +8.61*10^{-3}$	64
Promazine	10	10-1000	$y = 3.34*10^{-2} x -1.02*10^{-2}$	61
Quetiapine	5	5-1000	$y = 2.96*10^{-3} x +3.66*10^{-4}$	70
Risperidone	5	5-1000	$y = 2.65*10^{-3} x +2.65*10^{-4}$	62
Tiapride	5	5-1000	$y = 6.61*10^{-3} x +5.21*10^{-1}$	97
Venlafaxine	5	5-1000	$y = 8.91*10^{-3} x +4.64*10^{-2}$	67
Ziprasidone	5	5-1000	$y = 7.62*10^{-4} x -5.12*10^{-4}$	100
Zuclopenthixol	5	5-1000	$y = 3.69*10^{-2} x -1.41*10^{-2}$	50
Antiepileptics				
Lamotrigine	15	25-1000	$y = 3.62*10^{-5} x +1.71*10^{-3}$	69
Oxcarbazepine	5	5-1000	$y = 1.17*10^{-3} x -6.17*10^{-3}$	91
Tramadol	15	25-1000	$y = 2.22*10^{-2} x +1.40*10^{-2}$	33
Valproic acid	5	5-1000	$y = 2.86*10^{-4} x +1.83*10^{-3}$	77
Cardiovascular Drugs				
Atenolol	5	5-1000	$y = 3.62*10^{-5} x +3.31*10^{-3}$	46
Bisoprolol	5	5-1000	$y = 1.13*10^{-1} x -2.11*10^{-2}$	57
Nebivolol	5	5-1000	$y = 4.92*10^{-2} x -1.54*10^{-1}$	56
Propafenone	5	5-1000	$y = 1.47*10^{-3} x -5.94*10^{-3}$	77
Ramipril	5	5-1000	$y = 1.70*10^{-2} x +6.75*10^{-1}$	67
Telmisartan	5	5-1000	$y = 6.22*10^{-5} x -5.69*10^{-4}$	86
Verapamil	5	5-1000	$y = 1.59*10^{-3} x -5.89*10^{-3}$	88
Non-steroidal anti-inflammatory Drugs				
Ibuprofen	10	10-1000	$y = 1.18*10^{-4} x +2.18*10^{-3}$	65
Ketoprofen	5	5-1000	$y = 7.93*10^{-4} x -9.58*10^{-3}$	76
Ketorolac	5	5-1000	$y = 2.11*10^{-3} x +2.19*10^{-3}$	87

Analgesics / opioids				
Buprenorphine	5	5-1000	$y = 8.48*10^{-4} x -7.13*10^{-3}$	66
Dihydrocodeine	5	5-1000	$y = 2.50*10^{-4} x +9.34*10^{-4}$	77
Embutramide	5	5-1000	$y = 3.19*10^{-3} x +2.26*10^{-2}$	90
Hydromorphone	15	25-1000	$y = 8.14*10^{-4} x -5,47*10^{-3}$	69
Methadone	5	5-1000	$y = 3.18*10^{-4} x +5.74*10^{-3}$	101
Oxycodone	5	5-1000	$y = 4.84*10^{-3} x +2.79*10^{-1}$	46
Paracetamol	10	10-1000	$y = 1.31*10^{-1} x +5.91*10^{-1}$	80
Phenacetin	5	5-1000	$y = 2.67*10^{-4} x +1.67*10^{-3}$	67
Tapentadol	5	5-1000	$y = 1.02*10^{-3} x +8.77*10^{-3}$	90
Others				
Atropine	5	5-1000	$y = 3.84*10^{-4} x +1.18*10^{-2}$	90
Biperiden	5	5-1000	$y = 2.64*10^{-1} x -5.36*10^{-1}$	99
Dextromethorphan	5	5-1000	$y = 2.25*10^{-2} x +7.42*10^{-1}$	83
Diphenhydramine	5	5-1000	$y = 1.01*10^{-3} x +6.04*10^{-2}$	89
Diphenidine	5	5-1000	$y = 8.12*10^{-4} x -6.76*10^{-3}$	74
Disulfiram	15	25-1000	$y = 1.04*10^{-4} x +3.06*10^{-4}$	60
Glibenclamide	5	5-1000	$y = 1.67*10^{-2} x -4.48*10^{-2}$	62
Gliclazide	5	5-1000	$y = 1.55*10^{-4} x +1.78*10^{-2}$	83
Levamisole	10	10-1000	$y = 1.98*10^{-3} x -1.77*10^{-3}$	70
Lidocaine	5	5-1000	$y = 2.38*10^{-2} x -9.25*10^{-2}$	77
Loperamide	5	5-1000	$y = 4.76*10^{-3} x +1.19*10^{-1}$	96
Metformin	15	25-1000	$y = 3.31*10^{-4} x +9.36*10^{-2}$	56
Methylphenidate	5	5-1000	$y = 1.05*10^{-3} x -9.88*10^{-3}$	86
Metoclopramide	5	5-1000	$y = 6.56*10^{-4} x +8.94*10^{-2}$	75
Naloxone	5	5-1000	$y = 2.13*10^{-4} x +1.50*10^{-2}$	36
Oxybutynin	5	5-1000	$y = 1.30*10^{-1} x -1.26*10^{-1}$	85
Phendimetrazine	10	10-1000	$y = 1.10*10^{-4} x +1.26*10^{-2}$	50
Promethazine	10	10-1000	$y = 3.29*10^{-2} x +4.07*10^{-1}$	61
Scopolamine	5	5-1000	$y = 6.21*10^{-3} x +2.76*10^{-1}$	39
Sildenafil	10	10-1000	$y = 7.63*10^{-2} x -7.81*10^{-2}$	76
Tadalafil	5	5-1000	$y = 4.20*10^{-4} x +4.60*10^{-4}$	63

Ticlopidine	15	25-1000	$y = 3.72*10^{-4} x -5.31*10^{-3}$	61
Vardenafil	5	5-1000	$y = 5.93*10^{-3} x -3.60*10^{-2}$	65
Warfarin	5	5-1000	$y = 1.05*10^{-2} x +4.58*10^{-1}$	68

Table S2 List of the metabolites of carbamazepine found in the samples and the details using for the qualitative identification

Metabolite	**Formula**	**Charge**	**Precursor theoretical m/z**	**Fragment theoretical m/z**	**Rt, min**
10,11-Dihydro-10-hydroxycarbamazepine	$C_{15}H_{14}N_2O_2$	$[M+H]^+$	255.1128	194.0959	3.18
Carbamazepine 10,11-epoxide	$C_{15}H_{12}N2O_2$	$[M+H]^+$	253.0972	180.0806	2.93
10,11-Dihydro-10,11-dihydroxycarbamazepine	$C_{15}H_{14}N_2O_3$	$[M+H]^+$	271.1077	210.093	2.92

Table S3 The ratio of the intensity of the signals detected between metabolite and precursor

Sample origin	Ratio carbamazepine 10,11-epoxide / carbamazepine
Site 1	5
Site 2	4.4
Site 3	3.6
Site 4	3.4