Multi-residue determination of psychoactive pharmaceuticals, illicit drugs and related metabolites in wastewater by Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry

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Abstract

This study presents a new multi-residue analytical method for the simultaneous determination of 38 psychoactive drugs (including benzodiazepines, antidepressants and drugs of abuse) and related metabolites in raw wastewater. Potential analyte losses during sample filtration and stability in wastewater were evaluated. Analyte losses, especially for 12 compounds, were observed during filtration, indicating a strong sorption onto the filter material. In order to overcome this effect, filtered water samples were combined with methanolic washes of the corresponding filters and the resulting

solutions were solid-phase extracted on mixed-mode (reverse-phase plus cation-exchange) sorbents. Extracts were analyzed by ultra-high performance liquid chromatography-tandem mass spectrometry. Quantification was performed by the internal standard method with isotopic labeled analogs. Recovery percentages varied between 65% and 137%; method quantification limits ranged between 0.2 and 22 ng/L in ultrapure water and between 0.3 and 30 ng/L in wastewater for all the analytes but three (for which they were ~60-80 ng/L). The analysis of 24 h-composite samples collected during one week in the city of Santiago de Compostela demonstrated the ubiquity of 31 analytes, which were positively quantified in all samples. The highest concentrations were found for some of the antidepressants, with mean and maximum levels exceeding, in some cases, the levels previously reported in literature. This fact could be related to the additional washing step of the filters using methanol, which allowed to desorb retained analytes highlighting the importance of this step during the sample preparation protocol.

Keywords: Benzodiazepines; Antidepressants; Substances of abuse; Sewage analysis; Solid-phase extraction; Ultra-High Performance Liquid Chromatography

1. Introduction

Psychoactive pharmaceuticals, drugs of abuse and their metabolites are widely known to be present in urban wastewaters due to their high rates of production and consumption [1-9]. According to the Health Indicators of the Organization for Economic Co-operation and Development (OECD) [10], the use of antidepressants has increased considerably in most OECD countries since the year 2000, with selective serotonin reuptake inhibitors (SSRIs, e.g. fluoxetine, paroxetine, sertraline, citalopram) being the most popular in Spain [11]. Anxiolytics and hypnotics, particularly benzodiazepines (lorazepam, diazepam, alprazolam), are another group of medicines with remarkable rates of prescription [12]. And, among drugs of abuse, cannabis accounts for the largest estimate of abuse in the European Union, followed by cocaine, ecstasy, other amphetamine-derived compounds and opioids [13]. Following this widespread consumption, residues of licit and illicit psychoactive substances enter sewage systems continuously [1-9], a fact that may imply environmental consequences (if they end in surface waters [9, 14, 15]) and act as a measurable indicator of their use in different communities [2, 5, 16, 17].

Most of the analytical methodologies developed for the determination of psychoactive substances in wastewater remove suspended particles by filtration or centrifugation prior to solid-phase extracting the aqueous phase. However, organic molecules may get adsorbed onto solids following a process that depends on the properties of both the substance (pK_a, K_{ow}, etc.), the suspended particle matter (SPM) and the water itself (pH, Total Organic Carbon - TOC) and the filtering materials. Therefore, sorption is very hard to predict and, if a proper evaluation of the portion of substance adsorbed is not performed, it is possible to underestimate its levels in real samples [18-24]. In the case of drugs and pharmaceuticals, Baker et al. [18] assessed the

sorption onto wastewater SPM for 16 out of the 38 analytes included in our study and concluded that it was >10% for methadone and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), and >30% (up to 89% in one of the samples) for some antidepressants like fluoxetine and norfluoxetine. This result highlighted the need to take sorption processes into consideration for the development of future sample preparation protocols for these analytes.

In this line, this study presents a novel sample preparation strategy for the solid-phase extraction (SPE) of 38 psychoactive drugs and metabolites in wastewater. Particular attention was paid to pretreatment steps, including water filtration, washing of the filters and in-sample stability, in order to avoid the under-reporting of concentrations in real wastewater. The analytes were carefully selected to be the most frequently prescribed psychoactive pharmaceuticals, the most frequently abused drugs in Spain and their most relevant metabolites: i) seven benzodiazepines and two of their metabolites; ii) methylphenidate - a psycho-stimulant drug used in the treatment of attention deficit hyperactivity disorder - and its main metabolite ritalinic acid; iii) eight antidepressants and five of their metabolites; and iv) eight illicit drugs, five metabolites and levamisole, the most common adulterant of cocaine. Analytes were separated and detected by ultra-high performance liquid chromatography (UHPLC) coupled to tandem mass spectrometry (MS/MS). Parameters affecting UHPLC separation and MS/MS detection were carefully optimized and the final method was validated in terms of trueness, precision and quantification limits. Finally, it was applied to the analysis of 24 h-composite raw wastewater samples collected during one week in the city of Santiago de Compostela (NW of Spain).

2. Experimental

2.1. Reagents and materials

Analyte standards were supplied by Cerilliant (Round Rock, TX, USA) as individual solutions of 100 μg/mL of norsertraline and O-desmethylvenlafaxine, or 1000 μg/mL in methanol (MeOH) of alprazolam, α-hydroxyalprazolam, diazepam, nordiazepam, oxazepam, temazepam, lorazepam, lormetazepam, chlordiazepoxide, methylphenidate, ritalinic acid, citalopram, N-desmethylcitalopram, fluoxetine, norfluoxetine, sertraline, venlafaxine, mirtazapine, N-desmethylmirtazapine, duloxetine, paroxetine, trazodone, amphetamine, methamphetamine, 3,4-methylendioxymethamphetamine (MDMA), cocaine, benzoylecgonine, cocaethylene, levamisole, 11-hydroxy-Δ9-THC (THC-OH), 11-nor-9-carboxy-Δ9-THC (THC-COOH), meta-chlorophenylpiperazine (mCPP), mephedrone, ketamine, methadone and EDDP. Isotopic labeled analogs (α-hydroxyalprazolam-D5, alprazolam-D5, diazepam-D5, nordiazepam-D5, oxazepam-D5, temazepam-D5, lorazepam-D4, methylphenidate-D9, ritalinic acid-D10, citalopram-D6, N-desmethylcitalopram-D3, fluoxetine-D6, norfluoxetine-D6, sertraline-D3, norsertraline-^{13}C6, venlafaxine-D6, O-desmethylvenlafaxine-D6, duloxetine-D3, paroxetine-D6, trazodone-D6, amphetamine-D6, methamphetamine-D5, MDMA-D5, cocaine-D3, benzoylecgonine-D3, cocaethylene-D3, THC-OH-D3, THC-COOH-D3, mCPP-D8, mephedrone-D3, ketamine-D4, methadone-D3 and EDDP-D3) were also supplied by Cerilliant as 100 μg/mL solutions in MeOH and used as internal standards (IS). Mixed stock solutions containing all the analytes (10 μg/mL) or all the IS (2 μg/mL) were prepared in MeOH and stored in the dark at -20°C until use.

HPLC-grade MeOH, acetonitrile (ACN), acetic acid (100%) and ammonia solution in ultrapure water (25%) were supplied by Merck (Darmstadt, Germany). Formic acid (95-97%) and NH_3 solution in MeOH (7M) were supplied by Sigma-Aldrich (San Luis, Mi, USA). Ultrapure water was obtained in the laboratory by

purifying demineralized water in a Milli-Q Gradient A-10 system (Merck-Millipore, Bedford, MA, USA).

2.2. Filtration tests

Potential sorption of analytes onto different filter materials was assessed by vacuum filtering 100 mL aliquots of ultrapure water, spiked with 5 ng/mL of all the analytes, through different types of filters: 0.7 µm glass microfiber filters GF/A (Whatman, Kent, U.K.), 0.45 µm mixed cellulose membranes (Millipore, Bedford, MA, USA), 0.45 µm hydrophilic nylon membranes (Millipore) and 0.45 µm hydrophilic PVDF membranes (Millipore). IS were added after filtration and samples (n=3 in every case) solid-phase extracted as detailed in section 2.4. Losses were calculated as:

$$Filtration\ loss\ (\%) = \left(1 - \left(\frac{Response}{Average\left(Response_{No\ filtr}\right)}\right)\right) \times 100$$

Where $Response$ is the IS-corrected response in a filtered sample and $Average\left(Response_{No\ filtr}\right)$ is the average of the IS-corrected responses in non-filtered samples. One-way ANOVA (α=0.05) were performed to compare the mean losses of every analyte with the four types of filters.

For the filters providing the best performance (GF), experiments were repeated with raw wastewater in order to assess the combined sorption onto the filter and the SPM. Aliquots (100 mL, n=3) were spiked with 5 ng/mL of all the analytes before and after being filtered, IS added after filtration and losses calculated as:

$$Filtration\ loss\ (\%) = \left(1 - \left(\frac{Average\ (Response_{Before})}{Average\left(Response_{After}\right)}\right)\right) \times 100$$

Where $Average(Response_{Before})$ is the average of the IS-corrected responses in samples spiked before filtration; and $Average\left(Response_{After}\right)$ is the average of the IS-

corrected responses in samples spiked after filtration. Individual Student's t-tests (α=0.05) were run for all the analytes to assess whether there were statistically significant differences between their mean filtration losses in ultrapure water *versus* in raw wastewater, i.e., whether there were differences between their sorption onto filters (exclusively) or their combined sorption onto filters and SPM.

Finally, potential recovery of the analytes adsorbed by means of a methanolic wash of the filter was assessed with raw wastewater samples spiked with 2 ng/mL of all the analytes before and after filtration (n=3). Filters were washed with 2×5 mL of MeOH. Washes were collected, spiked with IS and made to a final volume of 1 mL for instrumental analysis.

2.3. Antidepressant biodegradation tests

Biodegradation of benzodiazepines and drugs of abuse was not assessed since it had been already reported in literature [25, 26].

Potential biodegradation of antidepressants was evaluated by spiking 10 mL of raw wastewater (n=3) with 500 ng/mL of these analytes and collecting 0.7 mL aliquots at the beginning of the experiment and at different times up to 48 h. Each aliquot was passed through a 0.22 µm GHP membrane syringe filter (Pall laboratory, NY, USA). Subsequently, 0.7 mL of MeOH were used to wash the filter and collected over the water fraction. The resulting solutions were spiked with 100 ng/mL of IS and kept at -20°C until analysis (by direct injection into the UHPLC-MS/MS system). Signals were compared to the response of a standard in ultrapure water:MeOH 1:1 containing 250 ng/mL and 100 ng/mL of analytes and IS, respectively.

2.4. Sampling and sample treatment

Raw wastewater samples were collected at the wastewater treatment plant (WWTP) of Santiago de Compostela (NW of Spain), which treats mostly domestic wastewater and serves a population of ~136,500 inhabitants. Composite samples of 24 h were taken in April 2016 for seven consecutive days, from 10.00 a.m. to 10.00 a.m. of the following day. A Sigma SD900 portable sampler from Hach (Loveland, CO, USA) worked in time proportional mode collecting 120 mL of water every 10 min. Composite samples were transferred to the laboratory and extracted within 8 h after the end of the sampling.

The sample preparation protocol was adapted from two previously published works [26, 27]. Under final working conditions, 100.0 mL aliquots were spiked with 20 ng of IS and vacuum-filtered through 0.7 µm glass microfiber filters GF/A. Filters were washed with 2×5.0 mL of MeOH, which were collected together with the filtered aqueous sample. Resulting solutions were solid-phase extracted onto mixed reverse phase-cation exchange cartridges (Oasis MCX-150 mg, Waters Corp., Milford, MA, USA), previously conditioned with 5.0 mL of MeOH containing 5% of NH_3 followed by 5.0 mL of ultrapure water. Sorbents were washed with 10.0 mL of ultrapure water and dried under nitrogen for 30 min. Analytes were recovered with 10.0 mL of 5% NH_3 in MeOH. Eluates were evaporated to dryness under nitrogen (99.999%) using both a Turbo-Vap II (Zymark, Hopkinton, MA USA) and a Mini-Vap concentrator (Sigma-Aldrich). They were redissolved in 100 µL of MeOH for instrumental analysis. Every sample was processed in triplicate.

2.5. UHPLC-MS/MS analysis

Samples (2 µL) were injected into a Waters Acquity UPLC® H class system (Milford, MA, USA) equipped with a sample manager, a quaternary solvent pump and a

column oven. Chromatographic separation was carried out at 50°C on a Kinetex® EVO C18 100 Å column (50 × 2.1 mm I.D., particle size 1.7 μm) from Phenomenex (Torrance, CA, USA), protected with a C18 pre-column (4 × 2 mm I.D), also from Phenomenex. A dual eluent system consisting of (A) 5 mM of NH_3 in ultrapure water and (B) 5 mM of NH_3 in MeOH was employed at a flow rate of 0.5 mL/min. The gradient elution started with 30% B, increasing to 60% B in 4 min and then to 100% B in 0.01 min. 100% B was held for 2 min. Return to initial conditions (30% B) was performed in 0.01 min and held for 2 min for reconditioning.

The UPLC® system was coupled to a triple quadrupole mass spectrometer Xevo TQD (Waters Corp., Milford, MA, USA) equipped with an electrospray ionization (ESI) source. Nitrogen, used as desolvation and cone gas, was provided by a nitrogen generator (Peak Scientific Spain, Barcelona, Spain). Argon, for the collision induced dissociation, was purchased from Praxair (Madrid, Spain). Ionization was performed in positive mode using the following parameters: 4 kV (capillary voltage), 150°C (source temperature), 500°C (desolvation temperature), 1000 L/h (desolvation gas flow, N_2) and 50 L/h (cone gas flow, N_2). Collision energy (CE) and cone voltage (CV) values were adjusted individually for every compound. MS analyses were done in Selected Reaction Monitoring (SRM) mode recording one (IS) or two (analytes) precursor/product ion transitions per compound. Selected transitions, together with their corresponding CE and CV values, retention times (RT) and labeled compounds used as IS are listed in the Supplementary Material, Table S1.

2.6. Method validation

The method was validated in terms of linearity, instrumental repeatability, instrumental and method quantification limits (IQLs and MQLs), trueness and

precision. Analytes were quantified using the corresponding isotopic labeled analog as IS. In those (five) cases where no labeled analog was available, the labeled compound providing the best results in terms of trueness was selected (Table S1).

Calibration was performed using a 13-point calibration curve ranging from individual IQLs to 1500 ng/mL. For sertraline, fluoxetine and lormetazepam, it ranged from IQL to 500 ng/mL; IS level in all cases: 200 ng/mL. IQLs were calculated as the concentration of a standard providing a signal-to-noise ratio (S/N) of 10. MQLs were assessed from measured concentrations in ultrapure water and wastewater samples containing (or spiked with) low concentrations of all the analytes, downscaling the levels for which the signal-to-noise ratio is 10. Instrumental repeatability was assessed as the relative standard deviation (%RSD) of six consecutive injections of two different standards (containing 5 and 50 ng/mL of all the analytes and 200 ng/mL of IS).

Trueness and precision of the whole method were estimated from recovery experiments performed in ultrapure water spiked at two concentration levels (20 and 100 ng/L of all the analytes, 200 ng/L of all IS) and in raw wastewater spiked with 500 ng/L of all the analytes and 200 ng/L of all IS. In the latter case, IS only-spiked aliquots were analyzed simultaneously in order to correct for the levels of analytes in sewage. Responses (analyte area/IS area) in ultrapure water or differences between responses of analyte-spiked and non-spiked aliquots of wastewater were compared with calibration curves in MeOH.

3. Results and discussion

3.1. UHPLC-MS/MS optimization

MS/MS conditions (transitions, CE and CV values) were optimized by direct infusion of individual standard solutions (10 µg/mL) in MeOH. Ionization was

performed in positive mode. Two SRM transitions (one quantifier, one qualifier) were acquired per analyte and one transition per IS. CV and CE values providing the highest intensities were selected individually (Table S1).

Chromatographic separation was carried out on a Kinetex® EVO C18 column stable throughout the whole pH range (1-12). Several modifiers giving different pH values were considered for the mobile phase (consisting of ultrapure water – mobile phase A, and MeOH – mobile phase B): formic acid 0.1% (pH 2.7); ammonium acetate 5 mM (pH 7.0); and NH_3 5 mM (pH 10.5). Figure 1 displays the chromatograms of nine analytes representative of the different behaviours observed in the three different scenarios. Since most substances have basic groups, a basic pH ensures their neutralization, increasing their retention on the C18 phase and improving peaks shape. As an example, amphetamine derivatives split in two in acidic medium, so formic acid was discarded for them. This splitting was also observed for other basic compounds such as mephedrone, ketamine or methylphenidate, whose height was between 1.5 and 3 times higher with NH_3 than with ammonium acetate. Higher peaks were also obtained in basic medium for other basic, less polar species (e.g. duloxetine, sertraline) and for amphoteric compounds (e.g. benzoylecgonine, ritalinic acid), what demonstrates the higher sensitivity of the proposed method when a basic eluent system was used. On the contrary, THC-COOH and THC-OH peaks were higher with formic acid but, since this was a minor behaviour, NH_3 was added at a concentration of 5 mM to both the aqueous and the organic phase. The use of MeOH or ACN was also considered, but no significant differences were observed neither on peak shapes nor on analyte intensities (data not shown), so MeOH was selected due to its lower price.

The consecutive injection of 1, 2, 3, 4 and 5 µL of a standard showed that the greater the volume injected, the higher the intensity. As it is shown in Figure S1 of the

Supplementary Material, a reasonable peak width was maintained in all cases (e.g. trazodone) excepting ritalinic acid and benzoylecgonine, for which peaks were split. Although a certain percentage of water in the solvent used to prepare standards and reconstitute extracts could have avoided the split, it was discarded due to the already known poor stability of some of the investigated species in water (e.g. cocaine [28]). Alternatively, a compromise injection volume of 2 µL, which provided high signal intensity avoiding significant peak widening for benzoylecgonine and ritalinic acid, was adopted. As an example of the chromatographic performance, Figure 2 shows the extracted ion chromatogram (EIC) for the first transition of all the analytes in a 100 ng/mL standard.

3.2. Assessment of losses during sample filtration

In first instance, sorption of analytes onto filters (exclusively) was assessed by comparing different filter materials: GF, cellulose membranes, hydrophilic nylon and hydrophilic PVDF (see section 2.2). Losses were lower than 30%, independently of the material used, for all analytes but methadone, its metabolite EDDP, the two cannabinoids and most of the antidepressants (Figure 3). An ANOVA statistical test allowed to conclude that differences between mean losses observed with the four types of filters were statistically significant, at the 95% of confidence level, for thirteen compounds: citalopram, N-desmethylcitalopram, fluoxetine, norfluoxetine, sertraline, mirtazapine, duloxetine, paroxetine, trazodone, THC-OH, THC-COOH, methadone and EDDP. For ten of them, higher losses were observed with cellulose membranes followed by PVDF filters. For mirtazapine and EDDP, there was barely no difference between these two materials. Conversely, lower adsorption occurred on GF and hydrophilic nylon, which were regarded as the best filtering materials. However, THC-

298 OH and THC-COOH disappeared completely after being filtered through nylon, what
299 prevented the selection of this material in favour of the use of GF.

300 Combined sorption onto both SPM and GF filters was further assessed with raw
301 wastewater and compared to the (exclusive) sorption on GF filters occurring with
302 ultrapure water (Figure 4). At the 95% of confidence level, there were no statistically
303 significant differences between the mean losses observed in these two matrices,
304 indicating a strong sorption onto the filter material barely affected by the content of
305 SPM. Only O-desmethylvenlafaxine, mirtazapine and EDDP underwent a significantly
306 higher loss in ultrapure water. This may be attributed to the fact that dissolved organic
307 matter partially prevents sorption to the filter unit.

308 Desorption of analytes from filters by washing them with 2×10 mL of MeOH was
309 evaluated with raw wastewater samples as explained in section 2.2. For most of the
310 compounds, recoveries in the water extract were above 80% (data not shown),
311 demonstrating again that sorption has not a great impact on them. For citalopram, N-
312 desmethylcitalopram, methadone and EDDP, recoveries varied between 60% and 80%,
313 and for some compounds with high K_{ow} (i.e. fluoxetine, norfluoxetine, sertraline,
314 norsertraline, duloxetine, paroxetine, THC-OH and THC-COOH) they were below 50%
315 (Supplementary Material, Figure S2). For these analytes, recoveries in filter washes
316 reached values above 20% of the total addition in the water sample. For citalopram, N-
317 desmethylcitalopram, methadone and EDDP they varied between 9 and 24% (Figure
318 S2). For the remaining compounds, filter washes recoveries were below 10% in all
319 cases.

320 Therefore, the combination of the filtered water sample and the methanolic filter
321 washes was further extracted and analysed, as explained in sections 2.4 and 2.5, in order
322 to improve the accuracy and the sensitivity of the method. Moreover, the addition of IS

before filtration allowed us to compensate for the uncertainty associated to the sorption occurring during filtration, and also to avoid a potential underestimation of the concentrations found in wastewater.

3.3. Stability of antidepressants in wastewater

Stability experiments were performed as explained in section 2.3 for antidepressants and their metabolites. After sample filtration, recovery experiments showed that 0.7 mL MeOH were necessary to sweep the analytes from the filter, especially fluoxetine, norfluoxetine, sertraline, norsertraline, duloxetine and paroxetine. A second MeOH wash was not necessary, since less than 5% of the analytes were eluted in this fraction (data not shown). Figure S3 of the Supplementary Material compiles the biodegradation profiles for all the antidepressants along 48 h at room temperature. Since no significant degradation was observed in any case (relative responses >80%), no degradation is expected during the 24 h sampling.

3.4. Method performance

UHPLC-MS/MS performance parameters (linearity, instrumental repeatability and IQLs) are displayed in Table 1. The representation of the ratio analyte area/IS area versus analyte concentration fitted a linear model with determination coefficients (R^2) between 0.9928 and 0.9987. The linear range was IQL-1500 ng/mL for all the analytes but sertraline, fluoxetine and lormetazepam, for which it was IQL-500 ng/mL. %RSD values for six repeated injections of a standard varied between 0.6 and 6.9% at 5 ng/mL and between 1.4 and 13% at 50 ng/mL. IQLs were between 0.1 and 13 ng/mL.

The combined SPE-UHPLC-MS/MS method was validated in terms of trueness, precision and MQLs. Percentages of recovery (%R) for quatriplicate analyses of

ultrapure water samples spiked with 20 ng/L of all the analytes and 200 ng/L of IS varied between 71% for lormetazepam and 132% for norsertraline (Table 1). THC-COOH could not be recovered in this case since its MQL in ultrapure water was higher than the spiked level (22 ng/L). %RSD were between 1% and 10%. At 100 ng/L in ultrapure water, %R varied from 72% for N-desmethylmirtazapine to137% for mCPP .%RSD were between 3% and 27%. In raw wastewater experiments (spiking level: 500 ng/L of all the analytes, 200 ng/L of IS), %R varied from 65% for duloxetine to 134% for N-desmethylmirtazapine, with %RSD between 1% and 14%. Finally, MQLs ranged from 0.2 ng/L to 22 ng/L in ultrapure water and from 0.3 ng/L to 30 ng/L in wastewater for all the analytes but mCPP (82 ng/L), amphetamine (64 ng/L) and mephedrone (83 ng/L).

Table S2 offers a comparative of the performance of the proposed method *versus* other multi-residue analytical methods for the determination of psychoactive substances in wastewater. MQLs were in the same order of magnitude than MQLs reached by other methodologies [4, 6, 7, 26, 29-31]. Trueness was similar or even better, with IS corrected %R in the 65-134% range *versus* 39-226% [29], 32-125% [30] or 51-130% [4]. It must be noticed, however, that both MQL and %R values can be estimated in different ways and, therefore, performance figures offered by different researchers might not be readily comparable. In terms of analysis time, the optimized SPE protocol is as long as other off-line SPE protocols, being, of course, slower than the in-line SPE-LC-MS/MS method developed by Fedorova et al. [31], or the Auto-SPE on HLB discs optimized by Baz-Lomba et al. [30]. The chromatographic separation is, conversely, the fastest (8 min in total).

3.5. Occurrence in 24 h-composite wastewater samples

The optimized and validated method was used to analyze 24 h-composite raw wastewater samples collected during one week in April 2016 at the WWTP of Santiago de Compostela (Spain).

Out of the 38 analytes, 31 could be positively quantified in all samples and two (α-hydroxyalprazolam and EDDP) in five and four samples, respectively (Table 2). Amphetamine, methamphetamine, mCPP, mephedrone and ketamine were always <MDL. This may be related to their relatively high MDL values in the method proposed (10-83 ng/L). Moreover, the consumption figures of these particular substances in Spain are usually very low [13], resulting in very low concentrations in wastewater.

Among drugs of abuse, benzoylecgonine (196-489 ng/L) was the metabolite quantified at the highest levels, followed by its precursor cocaine (82-259 ng/L), the cocaine adulterant levamisole (41-129 ng/L) and the THC metabolites THC-COOH (26-70 ng/L) and THC-OH (19-62 ng/L). These values reflect the pattern of consumption of illicit drugs in Spain [13], with cocaine and cannabis being the most abused substances and, therefore, the ones found in higher amounts in wastewater [32]. Other compounds such as MDMA (2-13 ng/L), methadone (3-13 ng/L) and its metabolite EDDP (<MDL-14 ng/L) were quantified at lower levels.

Benzodiazepine-related compounds were quantified at mean levels <100 ng/L, in the same order of magnitude than in other European studies [6, 9, 26, 29, 33, 34]. Lorazepam (44-182 ng/L) and oxazepam (9-100 ng/L) were the most abundant species, but they were exceeded, in any case, by ritalinic acid (metabolite of methylphenidate, 63-195 ng/L).

Among all the analytes, the highest concentrations were found for the antidepressant metabolite O-desmethylvenlafaxine (1066-1231 ng/L), followed by its precursor venlafaxine (459-1063 ng/L). These values are in good agreement with the

values reported for venlafaxine in the Slovakian city of Trencín (391-947 ng/L) [7], but they are considerably higher than the levels observed in other Slovakian [7], Greek [6], German [9] and British [29, 33] cities. Sertraline (176-455 ng/L) and its metabolite norsertraline (209-531 ng/L) were also quantified at very high concentrations when compared to other European studies [6, 9, 34, 35], a fact that could be associated to their desorption from filtered particles in the present work. These substances are highly retained onto SPM and common sample preparation methodologies (separating aqueous and solid phases and adding IS after filtration) may underestimate their real levels in wastewater. However, several factors may affect the occurrence of antidepressants in sewage from different countries (i.e. different rates of prescription) and any association with sample preparation/methodological issues may be considered cautiously. Fluoxetine, norfluoxetine and paroxetine concentrations were in line with or lower than the concentrations reported in other countries [6, 9, 26, 29, 34-36]. Citalopram (110-183 ng/L), N-desmethylcitalopram (79-136 ng/L) and mirtazapine (27-81 ng/L) had already been found at similar/higher levels in other cities [6, 9, 34, 36], whereas N-desmethylmirtazapine (14-22 ng/L), duloxetine (17-60 ng/L) and trazodone (35-545 ng/L) are usually not detected or detected at lower concentrations.

Regarding weekly concentrations profiles, and in accordance with the expected pattern of constant consumption, no clear trend could be observed for any of the investigated pharmaceuticals, neither for antidepressants nor for benzodiazepines. Conversely, higher levels were detected for all drugs/related compounds in the weekend samples (Saturday, Sunday, Monday), reflecting the recreational use of these substances in the city of Santiago de Compostela.

4. Conclusions

A new SPE-UHPLC-MS/MS method has been developed for the multi-residue determination of 38 psychoactive drugs (covering the most consumed psychoactive pharmaceuticals and drugs of abuse in Spain) in wastewater. Sample filtration proved to be a critical step in the loss of the most hydrophobic analytes (methadone, EDDP, THC-COOH, THC-OH and eight antidepressants). This led us to include a washing of the filters (and its subsequent extraction together with the filtered water) in the final protocol. This step allowed to reach higher absolute recoveries and lower limits of detection for these compounds, sometimes not detected, or detected at low –potentially underestimated– concentrations in real wastewater by other methodologies that simply extract the filtered aqueous phase (in the best scenario, adding IS before filtration). The analysis of seven 24 h-composite raw wastewater samples demonstrated the ubiquity of most of the analytes, with some of the antidepressants quantified at very high levels when compared to other European studies.

Appendix A. Supplementary material

Declarations of interest: none

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594

Figure Captions

Figure 1. Chromatograms of some representative analytes using a water/MeOH mobile phase at pH 2.7 (purple line), pH 7.0 (green line) and pH 10.5 (red line).

Figure 2. Extracted Ion Chromatogram (EIC) for the first transition (Q1) of all the analytes in a 100 ng/mL standard mixture.

Figure 3. Analyte losses (%) during ultrapure water filtration through glass microfiber filters (GF), cellulose membranes, hydrophilic nylon and hydrophilic PVDF filters. Error bars represent the standard deviation. Numbers above the bars are p-values for ANOVA statistical tests assessing the presence/absence of statistically significant differences between the mean losses observed with the four types of filters ($\alpha = 0.05$).

Figure 4. Analyte losses (%) observed with glass microfiber filters (GF) in ultrapure water and in raw wastewater. Error bars represent the standard deviation. Numbers above the bars are p-values for Student's t-tests assessing the presence/absence of statistically significant differences between the mean losses observed in the two matrices ($\alpha = 0.05$).

Table 1. Method performance parameters: determination coefficients (R^2), relative standard deviations (%RSD), instrumental quantification limits (IQL), recovery values (%R) and limits of quantification of the whole method (MQL).

	Analyte	Linearity (R^2) [a]	Repeatability (% RSD, n=6)		IQL (ng/mL)	Trueness and precisión (%R (%RSD), n=4)			MQL (ng/L)	
			5 ng/mL[b]	50 ng/mL		Ultrapure (20 ng/L)	Ultrapure (100 ng/L)	Wastewater (500 ng/L)	Ultrapure	Wastewater
Benzodiazepines and methylphenidate	Alprazolam	0.9982	6.9	2.2	0.4	106 (8)	104 (3)	107 (3)	0.9	1.4
	α-hydroxyalprazolam	0.9961	4.3	1.4	1.1	102 (3)	119 (27)	100 (3)	2.8	4.5
	Diazepam	0.9975	1.5	2.4	0.2	98 (6)	114 (12)	106 (1)	0.5	1.2
	Nordiazepam	0.9973	1.6	1.8	1.7	89 (4)	108 (10)	91 (2)	2.8	3.0
	Oxazepam	0.9984	4.8	1.8	1.1	101 (3)	100 (4)	100 (6)	1.7	8.0
	Temazepam	0.9960	1.5	2.6	0.7	106 (6)	100 (5)	91 (3)	1.7	3.3
	Lorazepam	0.9948	6.1	3.5	0.5	113 (10)	101 (12)	76 (11)	0.9	21
	Lormetazepam	0.9968	1.5	1.8	1.4	71 (10)	99 (3)	99 (12)	3.4	3.7
	Chlordiazepoxide	0.9978	1.4	2.5	1.1	72 (6)	98 (6)	71 (3)	2.3	4.5
	Methylphenidate	0.9985	2.8	2.5	1.0	100 (5)	110 (8)	75 (1)	1.1	1.6
	Ritalinic acid	0.9982	1.5	3.1	0.4	104 (5)	114 (8)	83 (4)	0.4	0.7
Antidepressants	Citalopram	0.9943	1.5	2.5	0.9	104 (4)	105 (9)	99 (2)	0.9	5.5
	N-desmethylcitalopram	0.9969	3.0	1.4	0.5	104 (1)	103 (9)	107 (3)	1.2	6.3
	Fluoxetine	0.9955	1.8	1.6	0.1	92 (7)	106 (8)	82 (2)	1.0	1.8
	Norfluoxetine	0.9986	2.2	2.7	0.1	106 (5)	110 (9)	94 (12)	0.5	3.3
	Sertraline	0.9981	2.9	3.0	0.3	107 (5)	107 (5)	93 (2)	0.8	4.4
	Norsertraline	0.9948	NA	13	10	132 (9)	92 (16)	114 (2)	10	30
	Venlafaxine	0.9966	3.1	2.9	0.1	104 (4)	104 (7)	98 (3)	0.7	0.9
	O-desmethylvenlafaxine	0.9985	2.4	1.7	0.4	103 (6)	108 (11)	91 (11)	1.1	7.7
	Mirtazapine	0.9976	0.8	2.3	0.1	99 (4)	103 (13)	102 (4)	0.2	1.1
	N-desmethylmirtazapine	0.9928	3.6	3.1	0.1	105 (6)	72 (8)	134 (6)	0.4	6.8
	Duloxetine	0.9987	4.0	3.8	0.1	106 (8)	119 (11)	65 (2)	0.3	8.6
	Paroxetine	0.9953	2.3	1.4	0.4	106 (8)	102 (9)	108 (11)	1.1	6.9
	Trazodone	0.9979	0.6	2.5	0.1	101 (6)	105 (7)	101 (2)	0.4	1.8

Illicit drugs	**Amphetamine**	0.9932	NA	3.1	6.6	90 (2)	103 (10)	106 (14)	9.0	64
	Methamphetamine	0.9964	2.0	2.9	1.1	89 (5)	103 (9)	102 (2)	1.7	27
	MDMA	0.9971	1.4	2.6	1.1	101 (4)	123 (16)	100 (6)	1.5	1.2
	Cocaine	0.9975	2.0	2.1	0.1	106 (4)	110 (5)	100 (3)	1.1	3.2
	Benzoylecgonine	0.9968	2.0	3.6	0.2	105 (5)	105 (4)	95 (7)	0.3	3.3
	Cocaethylene	0.9977	1.9	2.3	0.1	105 (2)	107 (7)	96 (2)	0.2	0.3
	Levamisole	0.9930	0.8	3.8	0.9	97(6)	92 (9)	79 (2)	1.2	5.8
	THC-OH	0.9985	NA	2.3	7.0	102 (2)	106 (4)	88 (3)	8.2	9.3
	THC-COOH	0.9946	NA	4.0	13	NA[c]	117 (7)	121 (8)	22	25
	mCPP	0.9967	6.9	2.4	5.0	101 (7)	137 (18)	102 (4)	5.5	82
	Mephedrone	0.9978	1.1	1.8	1.0	106 (4)	101 (10)	114 (3)	1.3	83
	Ketamine	0.9965	0.6	2.3	0.1	105 (7)	103 (9)	116 (3)	0.7	10
	Methadone	0.9971	1.4	2.0	0.2	104 (7)	97 (6)	95 (2)	1.1	1.7
	EDDP	0.9972	3.7	2.6	2.7	87 (6)	98 (11)	79 (3)	3.2	4.8

[a] Linear range: IQL - 1500 ng/mL for all the analytes excepting sertraline, fluoxetine and lormetazepam (IQL - 500 ng/mL)

[b] Repeatability at 5 ng/mL was not calculated for norsertraline, amphetamine, THC-OH and THC-COOH since their IQL > 5 ng/mL

[c] Recovery at 20 ng/L was not calculated for THC-COOH since its MQL > 20 ng/L

Table 2. Analyte concentration (mean in ng/L and %RSD in brackets) in 24 h-composite raw wastewater samples collected during one week in Santiago de Compostela. <MDL: not detected. <MQL: detected, but below the MQL.

	Analyte	Mo	Tu	W	Th	Fr	Sa	Su
Benzodiazepines and methylphenidate	Alprazolam	5 (24)	2 (28)	4 (11)	6 (29)	3 (12)	3 (5)	4 (29)
	α-hydroxyalprazolam	9 (22)	<MQL	<MQL	14 (15)	7 (19)	6 (19)	9 (21)
	Diazepam	3 (35)	2 (1)	2 (22)	4 (7)	2 (26)	2 (12)	3 (1)
	Nordiazepam	13 (6)	8 (14)	10 (9)	17 (2)	11 (33)	8 (9)	13 (1)
	Oxazepam	25 (8)	10 (25)	27 (16)	100 (20)	32 (3)	9 (25)	57 (19)
	Temazepam	12 (2)	8 (25)	12 (20)	15 (20)	11 (7)	12 (16)	11 (6)
	Lorazepam	71 (8)	68 (4)	60 (9)	182 (24)	44 (17)	78 (38)	72 (15)
	Lormetazepam	11 (5)	12 (15)	13 (23)	10 (29)	6 (8)	7 (20)	8 (18)
	Chlordiazepoxide	12 (9)	8 (21)	12 (18)	16 (11)	9 (27)	7 (3)	10 (23)
	Methylphenidate	8 (25)	8 (16)	10 (6)	13 (19)	11 (8)	6 (11)	8 (12)
	Ritalinic acid	99 (10)	75 (4)	97 (8)	195 (1)	145 (8)	63 (7)	115 (11)
Antidepressants	Citalopram	140 (4)	119 (11)	161 (2)	110 (5)	123 (5)	129 (7)	183 (4)
	N-desmethylcitalopram	136 (3)	79 (16)	113 (3)	92 (10)	79 (12)	88 (9)	110 (4)
	Fluoxetine	38 (13)	29 (4)	49 (9)	23 (8)	36 (1)	36 (6)	64 (7)
	Norfluoxetine	32 (29)	16 (12)	32 (3)	19 (6)	23 (8)	26 (29)	43 (5)
	Sertraline	258 (7)	227 (3)	396 (2)	176 (5)	254 (9)	232 (8)	455 (4)
	Norsertraline	233 (4)	230 (17)	471 (13)	209 (9)	309 (24)	256 (9)	531 (31)
	Venlafaxine	551 (6)	459 (2)	1063 (6)	661 (1)	466 (3)	478 (1)	549 (1)
	O-desmethylvenlafaxine	1231 (5)	1102 (15)	1066 (13)	1124 (6)	1152 (5)	1175 (2)	1213 (6)
	Mirtazapine	81 (8)	31 (16)	33 (7)	27 (3)	30 (2)	33 (13)	36 (19)
	N-desmethylmirtazapine	20 (11)	17 (7)	21 (8)	14 (14)	16 (13)	16 (26)	22 (19)
	Duloxetine	60 (22)	21 (8)	45 (18)	17 (19)	23 (6)	24 (16)	46 (21)
	Paroxetine	24 (10)	21 (10)	26 (8)	13 (8)	18 (10)	17 (1)	35 (8)
	Trazodone	62 (12)	45 (9)	45 (4)	35 (9)	545 (8)	92 (7)	99 (2)

Illicit drugs	**Amphetamine**	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	Methamphetamine	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	MDMA	6 (6)	2 (26)	2 (6)	6 (13)	8 (11)	11 (12)	13 (10)
	Cocaine	169 (25)	82 (24)	165 (1)	230 (5)	215 (1)	259 (4)	240 (9)
	Benzoylecgonine	398 (6)	196 (31)	289 (11)	331 (7)	371 (9)	464 (3)	489 (24)
	Cocaethylene	4 (11)	1 (36)	4 (9)	7 (3)	7 (11)	11 (11)	13 (5)
	Levamisole	69 (15)	41 (16)	47 (4)	63 (2)	76 (14)	129 (23)	129 (23)
	THC-OH	58 (14)	31 (23)	19 (8)	54 (12)	34 (18)	28 (6)	62 (11)
	THC-COOH	53 (18)	26 (4)	33 (20)	70 (9)	29 (4)	47 (18)	56 (8)
	mCPP	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	Mephedrone	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	Ketamine	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	Methadone	8 (30)	3 (19)	6 (34)	13 (10)	4 (13)	4 (24)	7 (1)
	EDDP	12 (16)	<MDL	<MQL	14 (12)	<MDL	5 (9)	10 (16)

Figure 1

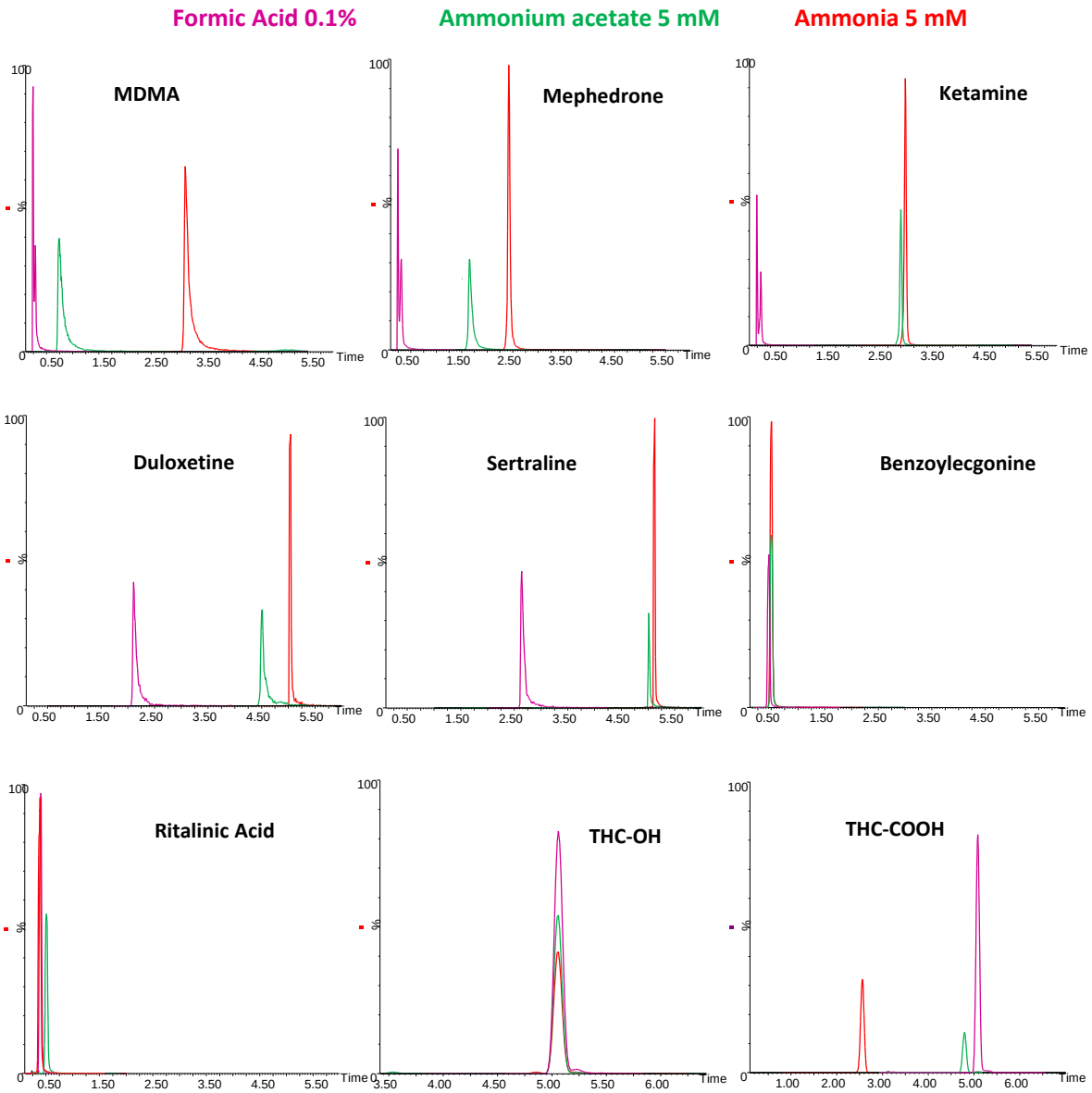

Formic Acid 0.1%
Ammonium acetate 5 mM
Ammonia 5 mM
MDMA
Mephedrone
Ketamine
Duloxetine
Sertraline
Benzoylecgonine
Ritalinic Acid
THC-OH
THC-COOH
Time

Figure 2

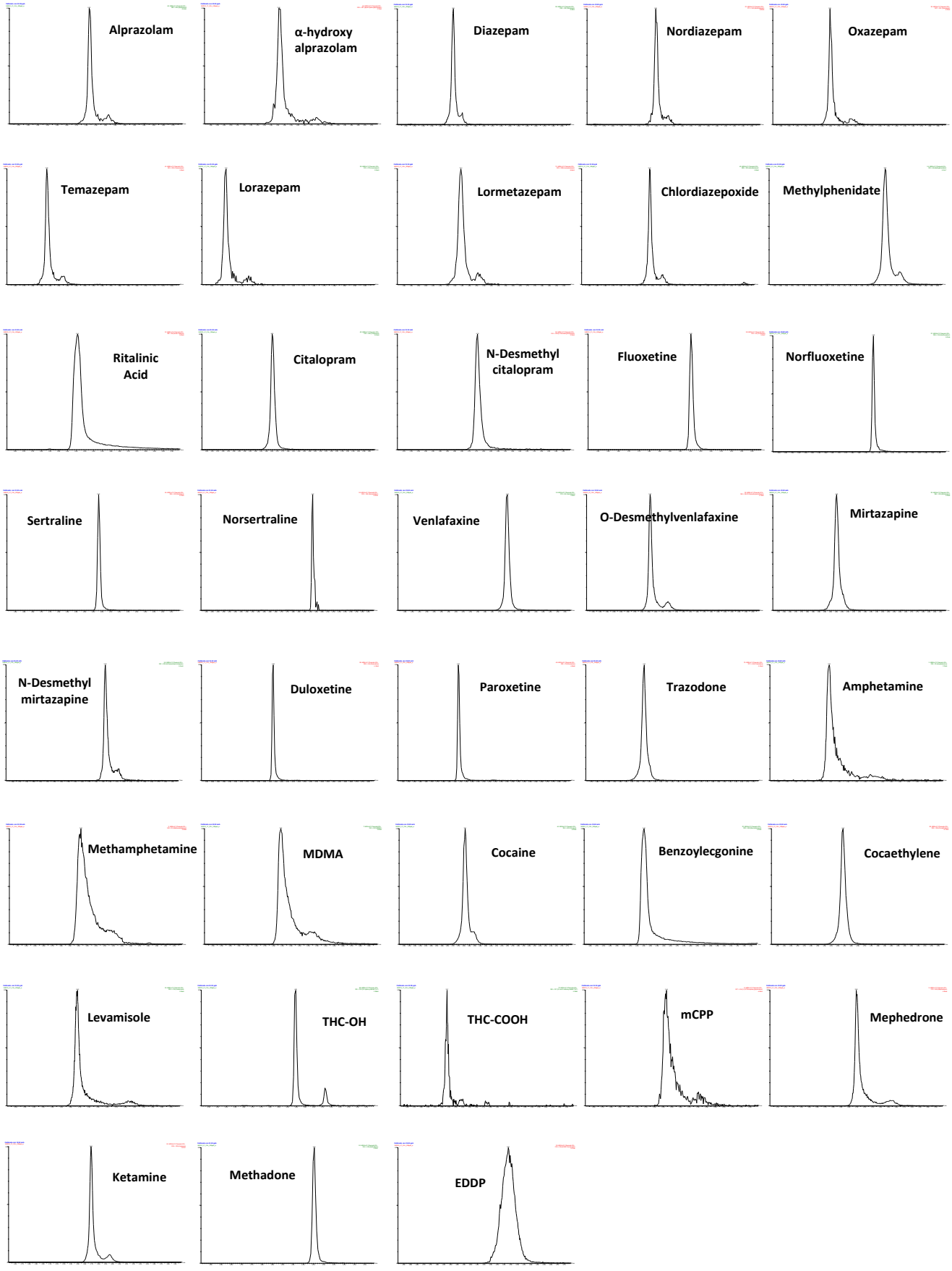

Alprazolam
α-hydroxy alprazolam
Diazepam
Nordiazepam
Oxazepam
Temazepam
Lorazepam
Lormetazepam
Chlordiazepoxide
Methylphenidate
Ritalinic Acid
Citalopram
N-Desmethyl citalopram
Fluoxetine
Norfluoxetine
Sertraline
Norsertraline
Venlafaxine
O-Desmethylvenlafaxine
Mirtazapine
N-Desmethyl mirtazapine
Duloxetine
Paroxetine
Trazodone
Amphetamine
Methamphetamine
MDMA
Cocaine
Benzoylecgonine
Cocaethylene
Levamisole
THC-OH
THC-COOH
mCPP
Mephedrone
Ketamine
Methadone
EDDP

Figure 3

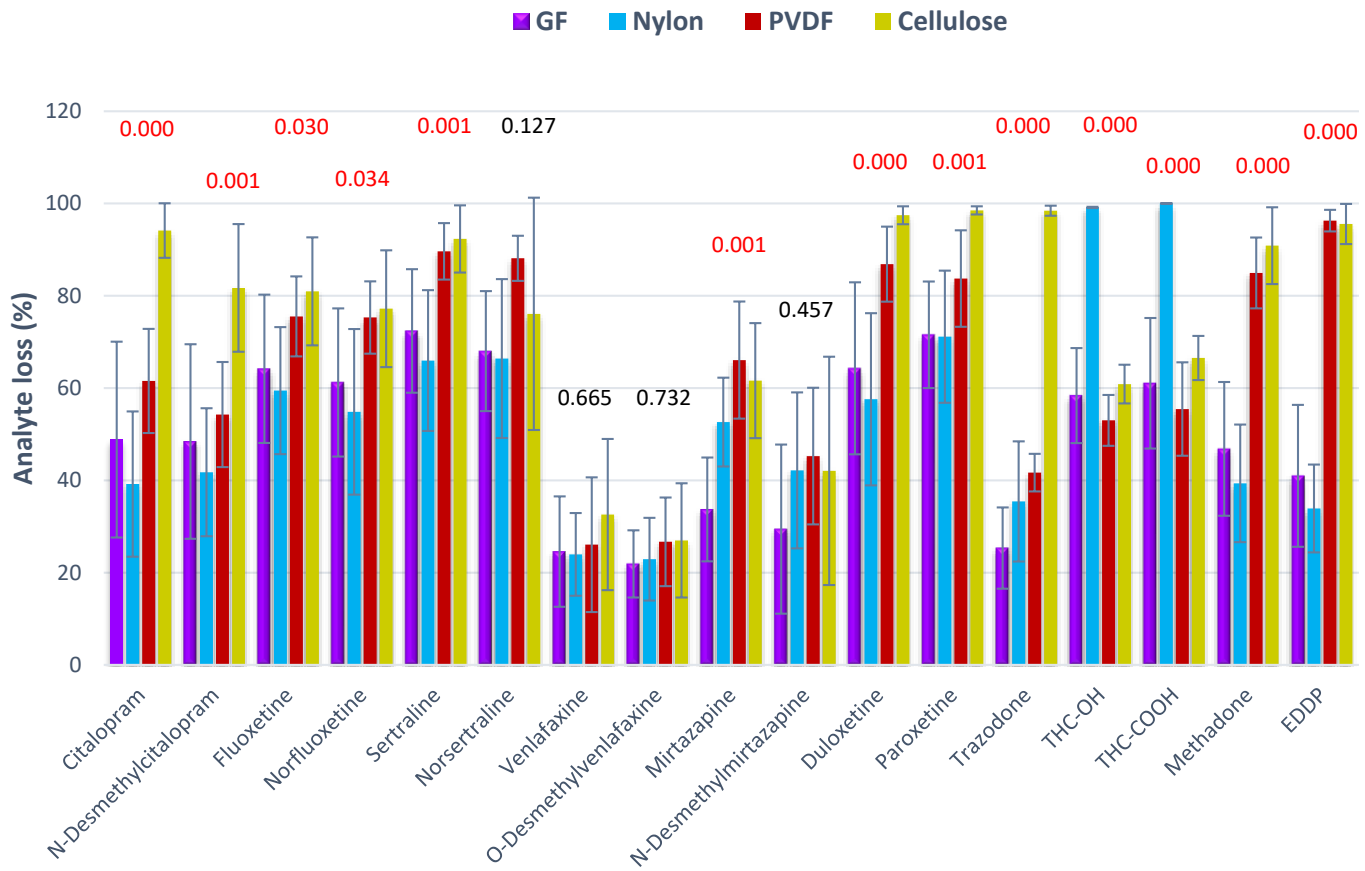
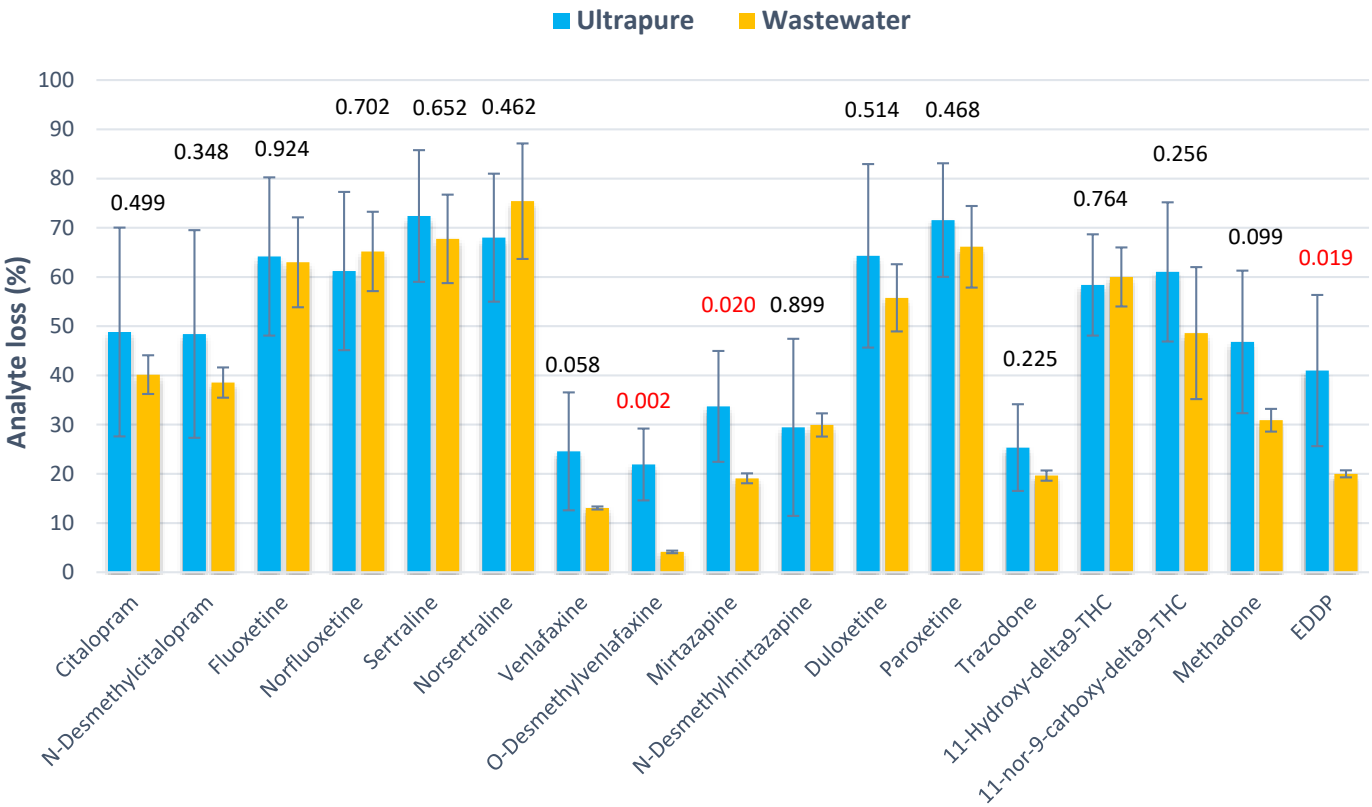

GF
Nylon
PVDF
Cellulose
Analyte loss (%)
120
100
80
60
40
20
0
0.000
0.001
0.030
0.034
0.001
0.127
0.665
0.732
0.001
0.457
0.000
0.001
0.000
0.000
0.000
0.000
0.000
Citalopram
N-Desmethylcitalopram
Fluoxetine
Norfluoxetine
Sertraline
Norsertraline
Venlafaxine
O-Desmethylvenlafaxine
Mirtazapine
N-Desmethylmirtazapine
Duloxetine
Paroxetine
Trazodone
THC-OH
THC-COOH
Methadone
EDDP

Figure 4


Ultrapure
Wastewater
Analyte loss (%)
100
90
80
70
60
50
40
30
20
10
0
0.499
0.348
0.924
0.702
0.652
0.462
0.058
0.002
0.020
0.899
0.514
0.468
0.225
0.764
0.256
0.099
0.019
Citalopram
N-Desmethylcitalopram
Fluoxetine
Norfluoxetine
Sertraline
Norsertraline
Venlafaxine
O-Desmethylvenlafaxine
Mirtazapine
N-Desmethylmirtazapine
Duloxetine
Paroxetine
Trazodone
11-Hydroxy-delta9-THC
11-nor-9-carboxy-delta9-THC
Methadone
EDDP

Appendix A. Supplementary material to:

Multi-residue determination of psychoactive pharmaceuticals, illicit drugs and related metabolites in wastewater by Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry

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Table S1. Analytes, labeled compounds used as IS, retention time (RT), cone voltage (CV), quantifier (Q1) and qualifier (Q2) *m/z* transition (precursor > product ion), and collision energy (CE) selected in every case.

	Analyte	IS	RT (min)	CV (V)	Q1	CE (V)	Q2	CE (V)
Benzodiazepines and methylphenidate	**Alprazolam**	Alprazolam-D5	2.7	60	309>205	46	309>281	34
	α-hydroxyalprazolam	α-hydroxyalprazolam-D5	2.5	64	325>297	24	325>216	38
	Diazepam	Diazepam-D5	3.7	60	285>193	32	285>89	62
	Nordiazepam	Nordiazepam-D5	3.3	60	271>140	30	271>165	30
	Oxazepam	Oxazepam-D5	2.6	38	287>241	22	287>269	14
	Temazepam	Temazepam-D5	3.0	42	301>255	20	301>283	14
	Lorazepam	Lorazepam-D4	2.7	45	321>275	20	321>303	14
	Lormetazepam	Alprazolam-D5	3.2	40	335>289	22	335>317	14
	Chlordiazepoxide	Alprazolam-D5	3.2	34	300>283	14	300>227	24
	Methylphenidate	Methylphenidate-D9	3.5	6	234>84	20	234>91	40
	Ritalinic acid	Ritalinic Acid-D10	0.4	22	220>84	20	220>56	38
Antidepressants	**Citalopram**	Citalopram-D6	4.7	50	325>109	30	325>262	22
	N-desmethylcitalopram	N-desmethylcitalopram-D3	4.8	44	311>109	26	311>262	16
	Fluoxetine	Fluoxetine-D6	5.0	26	310>44	14	310>148	8
	Norfluoxetine	Norfluoxetine-D6	5.0	18	296>134	8	296>30	10
	Sertraline	Sertraline-D3	5.0	24	306>159	24	306>275	12
	Norsertraline	Norsertraline-13C6	5.0	14	292>159	20	292>275	8
	Venlafaxine	Venlafaxine-D6	4.8	38	278>58	20	278>260	12
	O-desmethylvenlafaxine	O-desmethylvenlafaxine-D6	2.9	32	264>58	18	264>246	12
	Mirtazapine	Trazodone-D6	4.1	46	266>195	28	266>72	24
	N-desmethylmirtazapine	Cocaine-D3	2.9	46	252>195	24	252>209	24
	Duloxetine	Duloxetine-D3	5.0	16	298>154	6	298>44	46
	Paroxetine	Paroxetine-D6	4.9	48	330>70	30	330>44	22

	Analyte	IS	RT (min)	CV (V)	Q1	CE (V)	Q2	CE (V)
	Trazodone	Trazodone-D6	4.2	52	372>148	38	372>176	26
Illicit drugs	**Amphetamine**	Amphetamine-D6	2.2	24	136>91	16	136>119	10
	Methamphetamine	Methamphetamine-D5	3.1	28	150>91	16	150>119	10
	MDMA	MDMA-D5	2.9	28	194>163	12	194>105	26
	Cocaine	Cocaine-D3	3.8	30	304>182	22	304>82	32
	Benzoylecgonine	Benzoylecgonine-D3	0.5	44	290>168	20	290>105	32
	Cocaethylene	Cocaethylene-D3	4.5	32	318>196	22	318>82	32
	Levamisole	Cocaine-D3	1.7	48	205>178	22	205>91	36
	THC-OH	THC-OH-D3	5.0	32	331>313	14	331>193	26
	THC-COOH	THC-COOH-D3	2.2	46	345>327	18	345>299	22
	mCPP	mCPP-D8	2.4	44	197>154	18	197>44	22
	Mephedrone	Mephedrone-D3	2.1	30	178>160	12	178>145	20
	Ketamine	Ketamine-D4	2.7	36	238>125	24	238>207	16
	Methadone	Methadone-D3	5.1	50	310>105	32	310>265	16
	EDDP	EDDP-D3	5.3	28	278>234	34	278>249	26
Internal Standards	Alprazolam-D5	-	2.7	60	314>286	34	-	-
	α-hydroxyalprazolam-D5	-	2.5	64	330>302	24	-	-
	Diazepam-D5	-	3.7	60	290>198	32	-	-
	Nordiazepam-D5	-	3.3	60	276>140	30	-	-
	Oxazepam-D5	-	2.6	38	292>246	22	-	-
	Temazepam-D5	-	3.0	42	306>260	20	-	-
	Lorazepam-D4	-	2.7	45	325>279	20	-	-
	Methylphenidate-D9	-	3.5	6	243>93	20	-	-
	Ritalinic Acid-D10	-	0.4	22	230>93	20	-	-
	Citalopram-D6	-	4.7	50	331>109	30	-	-
	N-desmethylcitalopram-D3	-	4.8	44	314>109	26	-	-
	Fluoxetine-D6	-	5.0	26	316>44	14	-	-

Analyte	IS	RT (min)	CV (V)	Q1	CE (V)	Q2	CE (V)
Norfluoxetine-D6	-	5.0	18	302>30	10	-	-
Sertraline-D3	-	5.0	24	309>275	12	-	-
Norsertraline-^{13}C6	-	5.0	14	298>159	20	-	-
Venlafaxine-D6	-	4.8	38	284>64	20	-	-
O-desmethylvenlafaxine-D6	-	2.9	32	270>64	18	-	-
Duloxetine-D3	-	5.0	16	301>157	6	-	-
Paroxetine-D6	-	4.9	48	336>74	30	-	-
Trazodone-D6	-	4.2	52	378>150	38	-	-
Amphetamine-D6	-	2.2	24	142>125	10	-	-
Methamphetamine-D5	-	3.1	28	155>92	16	-	-
MDMA-D5	-	2.9	28	199>165	12	-	-
Cocaine-D3	-	3.8	30	307>185	22	-	-
Benzoylecgonine-D3	-	0.5	44	293>171	20	-	-
Cocaethylene-D3	-	4.5	32	321>199	22	-	-
THC-OH-D3	-	5.0	32	334>316	14	-	-
THC-COOH-D3	-	2.2	46	348>46	40	-	-
mCPP-D8	-	2.4	44	205>158	18	-	-
Mephedrone-D3	-	2.1	30	181>148	20	-	-
Ketamine-D4	-	2.7	36	242>129	24	-	-
Methadone-D3	-	5.1	50	313>105	32	-	-
EDDP-D3	-	5.3	28	281>234	34	-	-

Table S2. Comparison of the performance of the proposed method with that of other multi-residue analytical methods for the determination of psychoactive substances in wastewater. Abbreviations: percentage of recovery (%R); method quantification limit (MQL); Glass fiber (GF); Polytetrafluoroethylene (PTFE); Triple quadrupole (QqQ); Quadrupole-time-of-flight (QTOF); Multiple reaction monitoring (MRM); Product ion scan (PIS); Internal standards (IS); Methanol (MeOH); Acetonitrile (ACN); Acetic acid (AA); Formic acid (FA); Positive ionization (PI); Negative ionization (NI).

Analytes	Sample preparation		Separation and detection		%R	MQL (ng/L)	Ref.
	Pretreatment	Extraction	LC-MS	Run time			
65 stimulants, including illicit drugs, benzodiazepines, antidepressants and selected metabolites	100 mL of sample Filtration through GF 2.7 µm filters + GF 0.7 µm filters Acidification to pH 1.8-1.9 Addition of IS	SPE on Oasis MCX 60 mg Filtration of the extract through 0.2 µm PTFE filters	UPLC-(ESI+)-MS/MS on QqQ (MRM) Acquity UPLC BEH C18 column (150 × 1 mm, 1.7 µm) Mobile phase: 0.3% AA in water:MeOH 80:20 and 0.3% AA in MeOH	34 min	(IS corrected %R) 39-226% > 60% for most analytes	0.5-140	[30]
68 psychoactive substances, including illicit drugs, benzodiazepines, antidepressants and selected metabolites	50 mL of sample Filtration through GF 0.7 µm filters Acidification to pH 2.5 Addition of IS	SPE on Strata XC 200 mg Filtration of the extract through 0.2 µm RC filters	UPLC-(ESI+ and ESI-)-MS/MS on QqQ (MRM) Kinetex PFP column (50 × 2.1 mm, 1.7 µm) Mobile phase: 0.05% FA in water and 0.05% FA in MeOH	65 min (PI) 35 min (NI)	(Absolute %R) 22-142% 80-120% for > 80% analytes	0.3-558	[6]
23 psychoactive pharmaceuticals, including benzodiazepines, selected metabolites and related pharmaceuticals	100 mL of sample Addition of IS Filtration through GF 0.7 µm filters + membrane 0.45 µm filters	SPE on Oasis MCX 60 mg	HPLC-(ESI+)-MS/MS on QqQ (MRM) Synergi Fusion column (100 × 2.0 mm, 4 µm) Mobile phase: 0.1% AA in water and 0.1% AA in MeOH	29 min	(IS corrected %R) 84-109%	0.1-18	[26]

51 psychoactive substances including illicit drugs, benzodiazepines, antidepressants and selected metabolites	100 mL of sample Addition of IS	Auto SPE-DEX HLB discs (47 mm i.d.)	UPLC-(ESI+)-MS on QTOF (MS^e) Acquity UPLC HSS C18 column (150 × 2.1 mm, 1.8 µm) Mobile phase: 5 mM Ammonium Formate in water, 0.1% FA in ACN	15 min	(IS corrected %R) 32-125% > 60% for most analytes	0.4-187	[31]
23 psychoactive pharmaceuticals including illicit drugs, benzodiazepines, antidepressants and selected metabolites	1 mL of sample Addition of IS Filtration through cellulose 0.45 µm syringe filters	In-line SPE Hyperil Gold (20 × 2.1, 12 µm)	HPLC-(ESI+)-MS/MS on QqQ (MRM) HPLC-MS on Q-Orbitrap (Full Scan and Product Ion Scan) Cogen bidentate column (50 × 2.1 mm, 3 µm) Mobile phase: water and ACN	15 min (extraction + LC-MS)	(matrix-matched %R) 34-139% (MRM) 93-146% (PIS)	1.3-15 (MRM) 1.7-11 (PIS)	[7], [32]
27 psychoactive pharmaceuticals including antidepressants and selected metabolites	100 mL of sample Centrifugation Filtration through GF 1 µm filter Addition of IS	SPE on Oasis HLB 200 mg	HPLC-(ESI+)-MS/MS on QqQ (MRM) Hypersil Gold column (150 × 2.1 mm, 3 µm) Mobile phase: 0.1% FA in water and 0.1% FA in MeOH	41 min	(IS corrected %R) 51-130%	0.1-20	[4]
38 psychoactive substances including illicit drugs, benzodiazepines, antidepressants and selected metabolites	100 mL of sample Addition of IS Filtration through GF 0.7 µm filter Wash of the filters with 2 × 5 mL MeOH	SPE on Oasis MCX 150 mg	UPLC-(ESI+)-MS/MS on QqQ (MRM) Kinetex EVO C18 column (50 × 2.1, 1.7 µm) Mobile phase: 5 mM NH_3 in water and 5 mM NH_3 in MeOH	8 min	(IS corrected %R) 65-134%	0.3-83	This study

Figure S1. Effect of the injection volume on the peak shape of trazodone, ritalinic acid and benzoylecgonine.

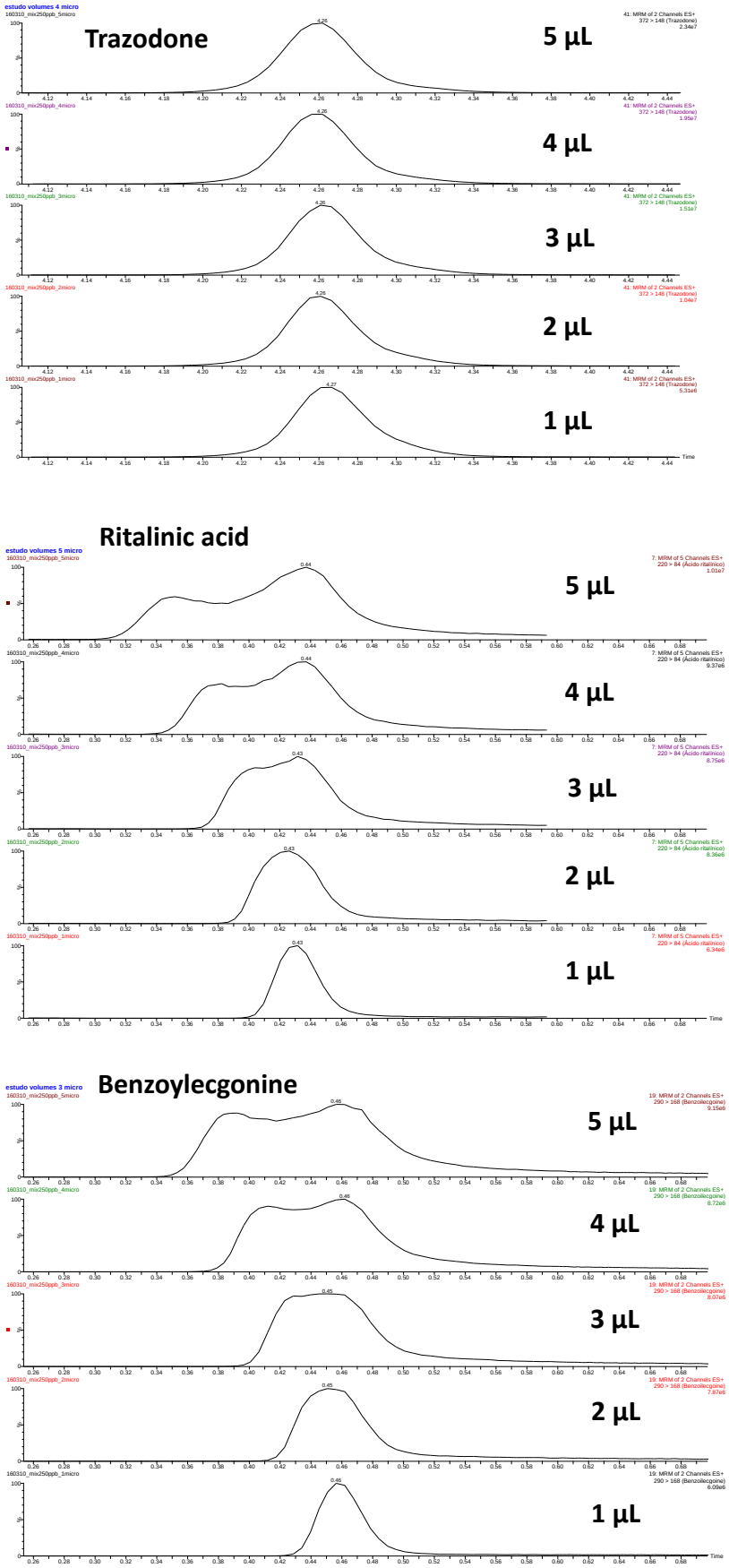

Figure S2. Analyte recovery (%, relative to analyte response when spiked after filtration) in wastewater samples and in the methanolic washes of the filters used for their filtration.

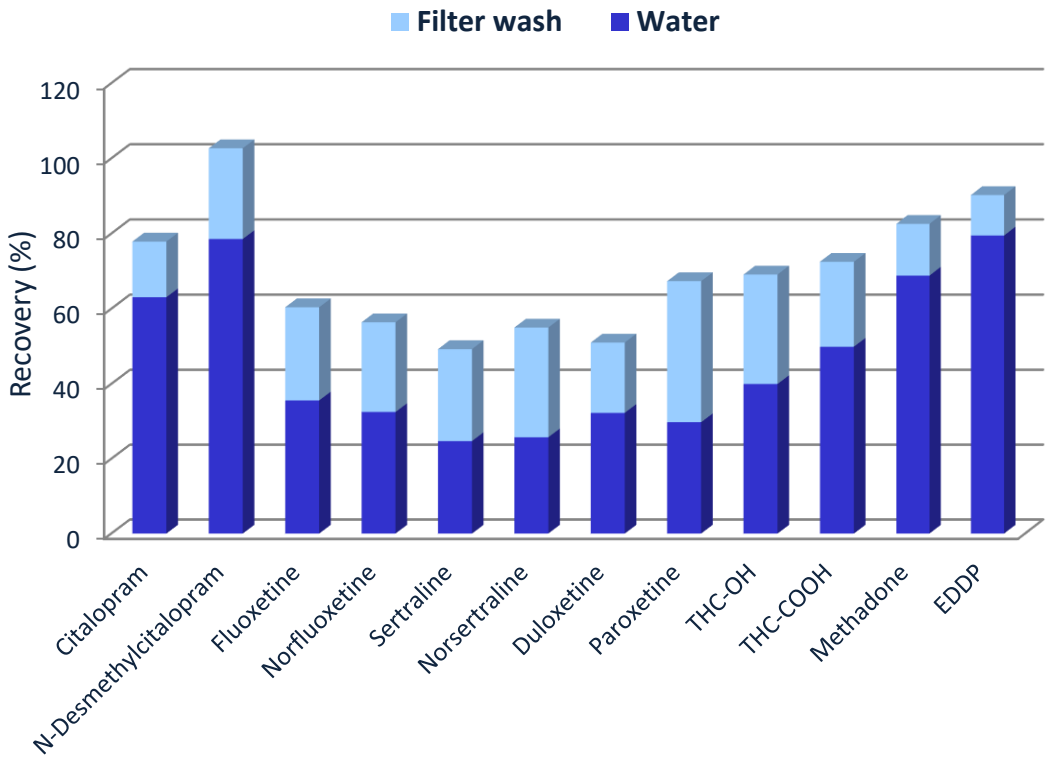

Figure S3. Antidepressant biodegradation profiles in wastewater along 48 h at room temperature.