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Development and validation of an analytical procedure to detect spatio-temporal differences in antidepressant use through a wastewater-based approach

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Abstract

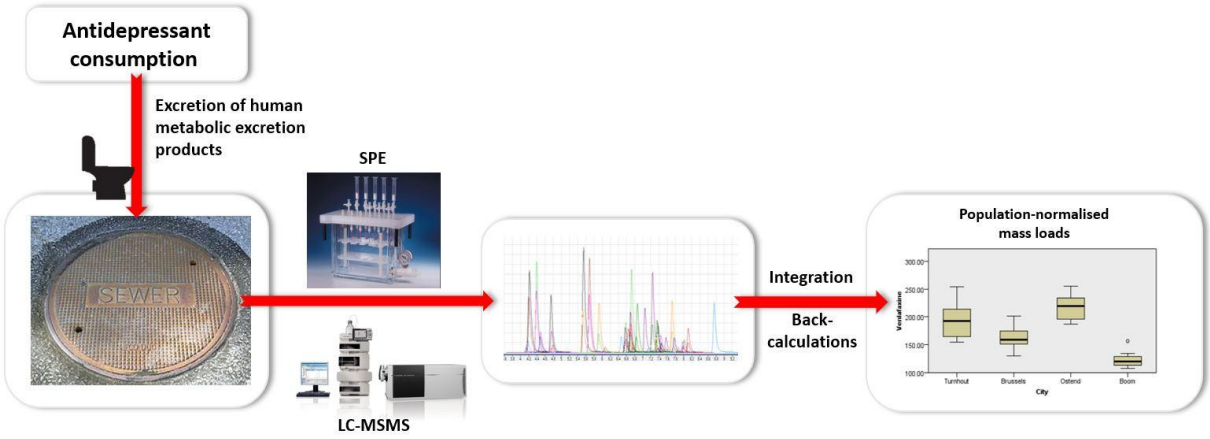
Wastewater-based epidemiology applies the analysis of human metabolic excretion products of xenobiotics in wastewater to estimate the community-wide use of these compounds. A new bioanalytical method was developed, optimised and validated for the analysis of a broad range of antidepressants and their metabolites at trace concentrations in influent wastewater. The assay was based on solid-phase extraction and liquid chromatography coupled to tandem mass spectrometry. For most compounds, Oasis® HLB cartridges were used for sample preparation. Oasis® MCX cartridges were used for extraction of normirtazapine, moclobemide, sertraline, and melitracen in particular. The Kinetex XBC18 column with a gradient elution resulted in appropriate separation for the analytes under investigation. Validation was done according to the European Medicines Agency guidelines on bioanalytical method validation. For 27 compounds, the performance criteria met the requirements for method validation. For these analytes, the lower limit of quantification (LLOQ) ranged between 1 and 25 ng/L. Furthermore, all targeted biomarkers showed high in-sample stability during 24 h, with the exception of mianserin. The validated assay was applied to influent wastewater samples collected from four wastewater treatment plants in Belgium. Among these four locations, a total of 18 out of 27 biomarkers for antidepressant use were present in the samples in concentrations above the LLOQ. Additionally, the proposed methodology proved capable of analysing high resolution spatio-temporal trends. Mann-Kendall trend analyses showed that antidepressant use is stable throughout the week, except for trazodone which increased throughout the week.

Declarations of interest

None

AcN, acetonitrile; dMRM, Dynamic multiple reaction monitoring; DRI, Dopamine reuptake inhibitor; EMA, European Medicines Agency; ESI, Electrospray ionisation; HIS, Health interview survey; HLB, Hydrophilic-lipophilic balance; IWW, Influent wastewater; LLOQ, Lower limit of quantification; MAO-I, Monoamine oxidase inhibitors; MCX, Mixed cation exchange; MeOH, Methanol; NARI, Selective noradrenaline reuptake inhibitors; NaSSA, Noradrenergic and specific serotonergic antidepressants; Q/q ratio, quantifier/qualifier ratio; QC, Quality control; NIHDI, National institute for health and disability insurance; RPLC-MS/MS, Reversed-phase liquid chromatography coupled to tandem mass-spectrometry; RSD, Relative standard deviation; S/N, Signal-to-noise; SARI, Serotonin antagonists and reuptake inhibitors; SNRI, Serotonin and noradrenaline reuptake inhibitors; SPE, Solid-phase extraction; SSRE, Selective serotonin reuptake enhancer; SSRI, Selective serotonin reuptake inhibitors; TCA, Tricyclic antidepressants; UHPLC, Ultra high performance liquid chromatography; WBE, Wastewater-based epidemiology; WWTP, Wastewater treatment plant

Graphical abstract



Introduction

Mental disorders (e.g. depressive disorders) are characterised by a combination of abnormal perceptions, thoughts, behaviour, emotions and relationships with others (1). The burden of mental disorders continues to grow worldwide leading to a significant impact on health and to major social and economic consequences (1,2). As a result, the global burden of mental illness accounts for 32% of years lived with disability and 13% of disability-adjusted life-years (2). In order to enhance the quality of life and well-being of individuals and communities, and thus to increase the resilience of society as a whole, there is a need for comprehensive mental health policies and plans to reduce mental disorders and their consequences worldwide (1,3). Depression is the most costly and burdensome mental health condition, especially when persistent and of moderate or severe intensity. Globally, more than 300 million people suffer from depression (4). Additionally, depression is the leading cause of disability worldwide and can lead to suicide. Especially in 15-29-year-olds, self-harm is among the leading causes of death (4,5).

Effective pharmacological and non-pharmacological treatments for moderate and severe depression are available. Overall, antidepressant drugs are used more frequently than non-pharmacological interventions (5). Antidepressants work in a variety of ways by affecting the different neurotransmitters, which are involved in regulating mood (6). These compounds are subdivided based on their chemical structure and/or working mechanism. Classes of antidepressant (see Figure S.1.) include selective serotonin reuptake inhibitors (SSRI), tricyclic antidepressants (TCA), monoamine oxidase inhibitors (MAO-I), serotonin and noradrenaline reuptake inhibitors (SNRI), selective noradrenaline reuptake inhibitors (NARI), serotonin antagonists and reuptake inhibitors (SARI), noradrenergic and specific serotonergic antidepressants (NaSSA), selective serotonin reuptake enhancer (SSRE), dopamine reuptake inhibitor (DRI) and antidepressants with a direct effect on neuroreceptors (6,7).

Currently, monitoring the use of antidepressants is predominantly based on general population surveys (GPS) and on reimbursement, prescription and sales data of prescription pharmaceuticals (8).

While GPS provide particularly valuable data about patterns in well-being and medicinal use in the general population, they also have some inherent limitations and challenges. Objective measurements of a highly stigmatised and hidden behaviour, such as depression, are problematic, resulting in various biases being introduced in the collected figures. In particular, concealment and reporting bias are commonly encountered with self-reported surveys. Furthermore, data recording is not performed on a yearly basis and its processing is laborious, resulting in the interpretation of the data in a lag-behind timeframe. Data on the reimbursement of pharmaceuticals, on the other hand, do not give information about the actual amount of medicine used in the population. Additionally, these figures do not deliver information on the amounts of pharmaceuticals used in hospital settings. Furthermore, for Belgium, data are only collected for pharmaceuticals that are refunded by the National Institute for Health and Disability Insurance (NIHDI) (9). Finally, prescription and sales data are also inherently linked to multiple uncertainties, such as sales without prescription, incorrect and illicit use of pharmaceuticals and household disposal (10). If pharmaceuticals are subject to illegal trade or clandestine manufacturing, purely relying on prescription and sales data would lead to underestimation of the consumption of pharmaceuticals (11). In this light, a new complementary method is needed to provide objective and real-time information on the legal and illegal use of antidepressants.

Wastewater-based epidemiology (WBE) is such an innovative approach that is able to offer objective and complementary information about exposure to xenobiotics in defined population groups through the analysis of human metabolic excretion products (biomarkers) in wastewater. The WBE approach takes into account that excreted human biomarkers resulting from exposure to or consumption of xenobiotics are collected and pooled by the urban sewage system, providing valuable evidence about the amount and type of substances used by a population. Since its first application in 2008 (12), WBE quickly showed its potential in estimating illicit drug consumption at the population level in near real-time, with frequent intervals (daily basis), and high spatial resolution (13–16). In this light, WBE has been able to provide objective, direct, anonymous and complementary information on the use of these compounds.

WBE can also be used to assess community health. For example, it is applied to monitor alcohol and tobacco use, to measure endogenous substances of disease or health and to estimate the exposure to emerging contaminants such as pesticides or flame retardants (17–21). It should be noted that WBE applications for pharmaceuticals are far more limited in comparison to illicit drugs. However, they can add valuable insights such as area-based health assessments, using pharmaceutical consumption as a proxy for disease prevalence. In addition, monitoring the consumption of pharmaceuticals can be used as an indicator for potential misuse, in near real-time (22).

Published methods to date that measure antidepressants in wastewater consist mostly of a preconcentration and purification step based on solid-phase extraction (SPE), followed by analysis of the resulting extracts by reversed-phase liquid chromatography coupled to tandem mass-spectrometry (RPLC-MS/MS). However, these bioanalytical assays focus on the determination of only a limited number of antidepressants and metabolites in influent wastewater (IWW), mainly the SSRIs (e.g. citalopram, fluvoxamine, fluoxetine, paroxetine and sertraline), a few TCAs (e.g. amitriptyline and nortriptyline), venlafaxine or bupropion (23–26). Currently, a bioanalytical method to simultaneously measure a broad range of antidepressants and metabolites in IWW is not available.

The goal of this study was to develop a multi-analyte bioanalytical assay capable of measuring a broad range of antidepressants and their metabolites in the low ng/L concentration range in influent wastewater in order to evaluate spatial and temporal trends in the community-wide use of these compounds. The method was applied to influent wastewater samples collected from wastewater

treatment plants (WWTPs) within different locations in Belgium, to obtain an overview on the use of antidepressants in the areas under investigation and to determine if the developed WBE approach is sensitive enough to pick up the use of these psychoactive compounds. The proposed WBE methodology can be used as an early-warning information system to quickly identify changes and patterns in the use of antidepressants. In the end, this may lead to a more complete picture of the use and misuse of these substances.

Materials and Methods

Reagents and Materials

Reference standards and deuterated internal standards for the investigated analytes were purchased from the Cerilliant Corporation (Round Rock, Texas, US), Duphar (Weesp, NL), H. Lundbeck A/S (Kopenhagen, DK), LGC Standards (Teddington, UK), Novartis (Basel, CH), Organon International (Oss, NL), Pfizer Inc. (New York, US), Pharmacia & Upjohn (Kalamazoo, Michigan, US), Hoffman-La Roche (Basel, CH), Sigma Aldrich International GmbH (St. Gallen, CH), Toronto Research Chemicals (Toronto, CA). Reference standards and deuterated internal reference standards were of analytical grade (purity > 98%) and purchased as chemical powders or as solutions at respective concentrations of 1 mg/mL or 100 µg/mL in methanol (MeOH) or acetonitrile (AcN). AcN and MeOH were purchased from Merck (Darmstadt, DE). Milli-Q ultrapure water was obtained through an Elga LabWater Purelab Flex system (Veolia Water Solutions & Technologies Belgium, Tienen, BE). Oasis HLB (60 mg, 3 mL) and Oasis MCX (60 mg, 3 mL) SPE cartridges were purchased from Waters (New Bedford, Massachusetts, US). A Supelco Visiprep SPE Vacuum Manifold 24-port model with a self-cleaning dry vacuum system Welch 2023 was used for the loading of the sample on the cartridges and the drying of the cartridges. Statistical analysis was done with the SPSS Statistics 22 software (IBM, Armonk, US). An overview of all compounds under investigation is given in Table S.1.

Samples and sample treatment

As illustrated in Table 1, IWW samples were collected from four Belgian WWTPs covering approximately 1.2 million inhabitants: Brussels, Ostend, Turnhout, and Boom. Daily 24-h composite wastewater samples were collected time- or flow-proportionally in order to obtain samples that were representative for an entire day. For each WWTP, at least seven daily samples covering one week of sampling were collected, i.e. a total of 28 samples. For each sample, a volume of 500 mL was aliquoted and stored at -20 °C after collection.

Method development

Liquid chromatography tandem mass spectrometry

An Agilent 1290 Infinity Ultra High Performance LC (UHPLC) was used, equipped with a degasser, a thermostatic column compartment, a binary high-pressure gradient pump and an autosampler module. The LC separation was based on an existing in-house method for the analysis of antidepressants in serum, with some adjustments (27). For LC separation, a Kinetex® XBC18 100 Å (150 mm x 2.1 mm, 2.6 µm) column was tested for the chromatographic separation of the compounds of interest. The best separation was achieved with this column by using a mobile phase A composed of 0.1% v/v formic acid in ultrapure water and mobile phase B composed of 10/90 ultrapure water/acetonitrile + 0.1% formic acid v/v, at a flow rate of 0.3 mL/min. The gradient was optimised to achieve maximal separation combined with a reasonable run time: 0.0-0.5 min: 95% A; 0.5-15.0 min 95-0% A; 15.0-17.0 min 0% A; 17.0-17.1 min 0-95% A. The total run time including

column equilibration was 20.0 min. An injection volume of 2 µL was used based on the peak shape of the analytes of interest.

An Agilent 6460 triple quadrupole mass spectrometer (MS) with an electrospray interface (ESI) operating in positive ionisation mode was used for the detection and quantification of the analytes of interest. The following source parameters were applied: gas temperature 300 °C, gas flow 5 L/min, nebulizer 45 psi and capillary voltage 3500 V. Dynamic multiple reaction monitoring (dMRM) was used since this approach addresses the limitations of classical MRM methods for a large number of analytes by replacing group segmentation with individual time windows for every compound transition and by minimising the amount of individual MRM transitions that is monitored during each cycle. The inevitability of co-eluting peaks is of lesser concern with this method as long as the individual ion transitions are unique. Optimised compound-dependent MS/MS parameters, such as fragmentor voltage, collision energy and MRM transitions, were optimised in the previously mentioned serum method (27) and further adapted to the new analytical conditions for each compound individually in order to acquire two MRM transitions (qualifier and quantifier) for the compounds of interest and one for the IS.

Analytes were positively identified if their retention time did not differ more than ± 0.4 min with that of the reference standard (23,28). In addition, the quantifier/qualifier (Q/q) ratio in the extracted samples was compared with the average ratio of reference standards to provide a second conformation criterion besides retention time. A tolerance level of 30% relative standard deviation (RSD) was set for compound identification (23,29).

Sample preparation

Prior to SPE, 50 mL IWW was centrifuged for 30 min at *2465 g* to remove solid particles and was spiked with 50 µL of an internal standard (IS) mix yielding final concentrations of 100 ng/L. For Oasis MCX, samples were first acidified to pH 2 with a 6 M HCl solution before IS addition and centrifugation.

In order to acquire a high and reproducible recovery of analytes, optimisation of a suitable SPE procedure with different sorbent materials was required. Based on different extraction protocols found in the literature (23–26), different SPE sorbents (Oasis HLB and MCX) were tested using varying sorbent sizes, elution solvents, washing solvents, solvent volumes and pH conditions in order to obtain maximum recoveries for the compounds of interest.

Oasis® HLB cartridges consist of a copolymeric sorbent with hydrophilic and lipophilic properties, which can be used for the attainment of a wide range of compounds (23). Oasis® MCX cartridges contain a sorbent with strong cation-exchange sulfonic acid groups, which can be applied in sample preparation for compounds with amino functional groups (23). The peak areas of blank tap water with all targeted analytes spiked at 200 ng/L pre- and post-SPE were compared to estimate the recovery in the different experiments. A design of experiment was conducted with varying extraction protocols in order to obtain the most suitable cartridge for the extraction of the biomarkers for antidepressant use from the wastewater matrix, as illustrated in Table 2.

After extraction, samples were evaporated to dryness at 37 °C under a gentle stream of nitrogen and reconstituted in 150 µL of the starting mobile phase, consisting of 95.5% ultrapure water 4.5% ACN + 0.1% formic acid v/v. Extracts were transferred to a centrifugal filter (0.45 µm) and centrifuged for 5 min at *10000 g*. No significant loss in absolute recoveries was observed due to adhesion onto the centrifugal filter membrane. This was tested by standard addition of 200 ng/L standard mixture pre-

and post-centrifugation to 150 µL MeOH. The resulting filtrate was transferred to an autosampler vial ready to be analysed with LC-MS/MS.

Method validation

Method validation was based on the Bioanalytical Method Validation guidelines provided by the European Medicines Agency (EMA) (30). Tap water was used as a matrix for method validation. This approach had to be adopted as IWW always contains measurable amounts of the investigated compounds and thus would not be suitable as a blank matrix for validation. Performance features, such as precision, accuracy, selectivity/specificity, linearity, calibration range, matrix effects, carry-over, recovery, sensitivity, lower limit of quantification (LLOQ) and stability, were evaluated during method validation.

Multi-component calibration curves consisting of six calibration levels were prepared by spiking 50 mL tap water with 50 µL of a suitable reference standard mixture and the IS mixture prior to SPE. The IS should be chemically similar to the compounds under investigation and should be able to correct for matrix effects. The assignment of an IS to an analyte is shown in Table S.1. A double blank (tap water without the addition of standards or internal standards), a blank (tap water without the addition of standards) and four quality control (QC) samples at LLOQ, low, medium and high levels in the calibration range were included.

Calibration curves were $1/x$ or $1/x^2$ weighted based on measured concentrations of the analytes of interest in IWW and were compiled by plotting the ratio between the peak areas of the standard and the assigned internal standard against the theoretically spiked concentration. Whereas $1/x$ was considered the most suitable weighting when high concentrations of biomarkers are measured in wastewater, $1/x^2$ was considered more suitable for concentrations in the lower ranges of the calibration curve. In addition, for high variance situations, $1/x^2$ weighing proved to be the most rugged and appropriate at the lower range of the calibration curve. The LLOQ was defined as the concentration of the lowest calibration standard (30). The analyte signal of the LLOQ sample should be at least five times higher than the signal present in the blank sample.

Carry-over was addressed by injecting a blank sample (tap water) after injection of the highest calibration level in the instrumental worklist. Carry-over should not be higher than 20% of the peak area of the LLOQ and 5% of the IS peak (30). Specificity of the method was validated by comparing blank tap water samples (n = 3) with tap water samples spiked at the LLOQ concentration (n=3). No peaks were observed in the blank samples at the retention times of the compounds of interest (30). Interference between the signal of the reference standard and the IS was evaluated as well by: (i) separately injecting the standard at 200 ng/L and monitoring the response of the IS and (ii) by injecting a sample only with the IS at 100 ng/L and monitoring the response of the reference standard at the sensitivity required for monitoring.

The within- and between-day accuracy and precision of the method were determined by analysing a minimum of 4 replicates of 4 quality control levels (LLOQ, QC low, medium and high) in the calibration range over a 3-day period. These performance features were compared to the acceptance criteria of < 15% bias and < 15% RSD. At the LLOQ, limits for accuracy and precision were set respectively at < 20% bias and < 20% RSD (30).

The extraction efficiency of the optimised method was assessed by comparing the peak areas of the compounds in the chromatograms of tap water samples spiked at 200 ng/L pre-SPE with those spiked post-SPE. Comparability between the recoveries in IWW and tap water was assessed by evaluating the extraction recovery in IWW by standard addition tests. The peak areas of 6 different IWW

samples (different sources of IWW) spiked pre- and post-SPE were compared after subtraction of the areas of the native analyte in the sample (28).

In many WBE applications, matrix effects are validated according to the recommendations of Matuszewski et al (23,28,31). However, finding blank wastewater that can be used for these experiments is virtually impossible. In this study, a different set-up was used as it is of more importance to evaluate whether the sensitivity of the method is sufficient to distinguish the analytes of interests from matrix interferences and to assess if the IS corrects for potential matrix effects. Therefore, a set-up based on standard addition was used to assess the matrix effects in IWW samples. IWW was pooled from two different sources and spiked with high concentrations (1-5 µg/L) of reference standards. In addition, the native concentration was determined by analysing different non-spiked IWW samples originating from the same IWW pool. After subtracting the native concentration for the analytes under investigations, measured concentrations were assessed for matrix effects. The accuracy criteria were used to assess whether the assigned IS corrects for occurring matrix effects. For a suitable biomarker, measured concentrations should be within ± 20% of the spiked concentration. It is important to note that calibration curves are not influenced by matrix effects because they are constructed by using spiked tap water.

Stability experiments

The set-up of the stability experiments was done according to McCall et al., Baker et al. and van Nuijs et al. (32–34). A combined wastewater pool, containing IWW from different sources, was divided into three aliquots of 500 mL: a non-spiked 'control' IWW sample and two spiked IWW samples. Aliquots were placed on a magnetic stirrer to simulate sewer currents and were spiked with a standard mixture containing concentrations of each investigated compound ranging from 1 µg/L – 5 µg/L. Spiked concentrations were substantially higher than the native concentration of the targeted biomarkers in wastewater samples. Fifty mL of wastewater obtained from each aliquot was extracted immediately after spiking and then after 2, 6, 10 and 24 h. After the extraction, cartridges were stored at -20 °C and eluted all together within 7 days.

A non-spiked 'control' was analysed in parallel to measure the native concentrations of the analytes of interest. Prior to the calculation of a mean concentration for each time point, the native concentrations were subtracted from the measured concentration in the spiked aliquots. Subsequently, mean concentrations at each time point were expressed as a relative percentage to the mean concentration at t = 0 h. This residual percentage was used for each compound separately to assess the in-sample stability based on McCall et al. (2016): high stability (< 20% transformation), medium stability (20-60% transformation), low stability (60-100% transformation) and variable stability during a 24 h-period (32). Biomarkers that show medium to low stability are to be excluded from the proposed bioanalytical assay as they are not suitable to perform back-calculations in WBE.

In addition, long-term storage of the targeted biomarkers on to SPE sorbents was assessed. Pooled wastewater samples spiked at concentrations ranging from 1-5 µg/L together with two non-spiked 'control' sample were extracted on SPE-cartridges, washed and dried under vacuum. Samples were equally divided into two sets of cartridges. For one set of samples, elution and subsequent analysis were performed immediately after drying the cartridge. For the other set, cartridges were stored at -20 °C in an air-tight freezer bag after drying. Elution and subsequent analysis of these cartridges were performed after 1.5 months of storage. The results of this experiment were normalised as a residual percentage against time point 0. This residual was used to assess the long-term on-cartridge stability during storage at -20 °C (33).

Results and Discussion

Method Optimisation

Selection of a suitable SPE procedure

Equations used for calculating absolute and relative recoveries are given in the Supplementary Information (Equations S.1 and S.2). Higher absolute recoveries (see Table S.3 and S.4) were obtained using 60 mg sorbent size compared to larger sorbent sizes, and this was observed for both Oasis MCX and HLB. The use of methanol containing 2% v/v formic acid and methanol containing 5% v/v ammonia as elution solvent resulted in the highest absolute recoveries for the HLB and MCX procedures, respectively.

Subsequently, the effect of different washing solvents was tested. Three experiments with different washing solvents and the addition of the internal standard mixture were conducted in triplicate. Relative recoveries were calculated to test for reproducibility, as summarised in Table S.5.

Washing of the MCX cartridges with 3 mL MeOH containing 2% v/v formic acid proved to have a positive effect on the peak shape of the investigated compounds in IWW. This observation was prominent for sertraline-D_3 and melitracen-D_6 in particular. Therefore, sertraline and melitracen were included in the MCX procedure. However, a decrease in relative recoveries was observed for normianserin, mianserin, duloxetine, norclomipramine, imipramine, desipramine, trimipramine, nortrimipramine, melitracen and clomipramine by using MeOH containing 2% v/v formic acid as a washing solvent for MCX. In addition, high relative recoveries could not be obtained with MCX for agomelatine, dosulepine, nordosulepine, paroxetine, opipramol and phenelzine, as shown in figure 1. Therefore, these compounds were included in the HLB procedure and, as a result, multiple SPE procedures were needed for the extraction of the analytes of interest, because of their broad range of physicochemical properties. No loss in relative recoveries was observed with HLB with 5% v/v MeOH in ultrapure water as a washing solvent. In addition, the most reproducible relative recoveries were obtained with this washing solvent and washing of the cartridges with 5% v/v MeOH in ultrapure water proved to have a minor positive effect on the peak shape of the analytes of interest in wastewater. Both washing solvents were used because of the positive effect on signal intensity.

Liquid Chromatography-tandem mass spectrometry

The Kinetex XBC18 column with a gradient elution gave good retention for the broad range of analytes. All compounds of interest were well retained on this column and the separation capacity was sufficient. Figure 2 shows a chromatogram of the MRM quantifier transitions in a tap water sample spiked at 200 ng/L. All compounds elute within 9 minutes with the first compound eluting at 4.14 minutes.

Table S.2. summarises the optimised dMRM conditions and retention times for all analytes and deuterated internal standards. For each analyte, the most abundant transition with regards to absolute abundance and signal-to-noise ratio (S/N) was used for quantification (quantifier), whilst the second transition was used for confirmation (qualifier). The effect of the matrix on the peak intensity was taken into consideration when choosing the most appropriate quantifier and qualifier. This is important because matrix-induced suppression or enhancement could result in reduced or improved chromatographic peak intensities respectively. Where necessary, other transitions were chosen for quantification and confirmation.

Method validation

For 27 compounds, the performance criteria met the requirements for method validation provided by the EMA guidelines, which were summarised in the 'Methods and Materials' section. Phenelzine

and opipramol were excluded from the bioanalytical assay due to stability issues encountered in tap water and due to bad chromatographic behaviour. Furthermore, it is important to point out that these substances are used rather exceptionally and are not part of a standard treatment for depression in Belgium, making this exclusion of less concern (7). Table S.1. summarises the assignment of the IS to the compounds under investigation. Agomelatine, dosulepin, nordosulepin, norfluoxetine, paroxetine and nortrimipramine did unfortunately not measure up to the validation requirements due to the poor sensitivity of the IS peaks. Ion suppression due to matrix effects was observed for the IS of these compounds. An alternative IS capable of correcting for the matrix effects, could not be identified for these analytes. In addition, signal suppression due to matrix effects resulted in poor repeatability of the paroxetine peaks.

The validated method proved to be selective for target biomarkers for antidepressants use in tap water. No peaks with areas above 10% of the peak area of the LLOQ and 5% of the peak area of the IS were observed in blank tap water samples at the retention times of the analytes of interest, which complies with the EMA guidelines. Additionally, injection of IS did not result in interferences on the analytes and vice versa.

A linear calibration curve ranging from low ng/L to low µg/L concentrations was obtained for 32 compounds in tap water, as illustrated in Table 3. However, due to matrix effects in IWW samples, only 27 out of these 32 compounds could be accurately quantified. During method development, the LLOQ was optimised in order to achieve the lowest possible concentration that the LC-MS/MS was capable of quantifying in terms of accuracy and precision (28). For some analytes, a lower LLOQ was obtained with one SPE procedure compared to the other. Therefore, the procedure offering the lowest LLOQ was considered for the final bioanalytical assay. A finalised overview of all compounds is given in Table 3.

The within-run and between-run accuracy and precision measured at the LLOQ level were within the set criteria. Intra- and interday accuracy and precision results at three different spiking levels, including QC low, QC mid and QC high were within the range of < 15% bias and < 15% RSD, respectively (Table 3). Peak areas of blank samples analysed after the highest calibration level did not exceed 20% of the peak area of the LLOQ of the standard or 5% of the peak area of the IS which is compatible with the EMA guidelines related to carry-over.

Matrix effects

Standard addition experiments were used to assess the matrix effects in IWW samples. This is especially important for compounds where no deuterated analogue was available to evaluate whether the IS robustly compensates for potential matrix effects. This was assessed by standard addition of two different pools of IWW samples, one spiked with 200 ng/L and the other with 1 µg/L. Each pool of IWW samples consisted of IWW from different sources. To compare matrix effects between these two sets of experiments, relative responses (as a percentage of the spiked concentration) were calculated for each compound separately for each SPE procedure. Figure 3 summarises the outcomes of the recovery experiments in IWW samples. All measured concentrations for the HLB procedure were within ± 20% of the spiked concentration except for normirtazapine, moclobemide, imipramine, reboxetine, atomoxetine, duloxetine, and tianeptine. For the MCX procedure, all measured concentrations were within the acceptable range of 80%-120%. Therefore, normirtazapine and moclobemide were measured using the MCX procedure.

The observed deviations in recoveries originated from differences in response between the analyte and the corresponding IS in wastewater due to different signal suppression or enhancement. The IS did not robustly correct for matrix effects for 5 compounds (i.e. imipramine, duloxetine, tianeptine,

reboxetine and atomoxetine). For the remaining 27 compounds, the IS did correct for matrix effects. Quantification of atomoxetine, imipramine, duloxetine, tianeptine and reboxetine was not possible when using their deuterated IS and these were excluded from the bioanalytical assay. Selection of an alternative IS did not result in acceptable accuracies due to different matrix effects. This was an unexpected observation, and further highlights the need for a thorough assessment of matrix effects during method validation, even when deuterated analogues are used.

Stability experiments

The in-sample stability of the targeted antidepressant biomarkers was assessed, as in-sample conditions can result in the transformation of biomarkers and subsequently the over- or underestimations of pharmaceutical consumption when applying WBE. The assessment of the in-sewer stability was not included in this study and the omission of in-sewer transformation of biomarkers could result in an unknown degree of uncertainty (31).

Table 4 gives the residual percentage of the targeted biomarkers at five different time points (0 h, 2 h, 6 h, 10 h and 24 h). An overview of the residual percentage over time for the in-sample stability experiments is given in Table 4.

All targeted biomarkers showed high in-sample stability for 24 h. However, for mianserin a substantial decrease (>20%) was observed after 24 h. Therefore, its metabolite, normianserin, should be considered as the more appropriate biomarker to monitor the use of mianserin because of its high in-sample stability in contrast to the medium in-sample stability of the parent compound.

All compounds under investigation showed high on cartridge-stability (< 20% transformation) after 1.5 months of storage at -20 °C in an air-tight freezer bag, as shown in Table 4. This allows to elute the compounds from the cartridges within 1.5 months after loading the sample. This method of storage could be more convenient than freezing large volumes of wastewater samples. In addition, processing of high amounts of samples could be done in a more convenient way by eluting more cartridges in total.

Application of the bioanalytical assay to wastewater samples

The applicability of the validated bioanalytical assay was assessed by analysing influent 24 h composite wastewater samples from four different WWTPs in Belgium (Boom, Brussels, Turnhout and Ostend). Among these four locations, a total of 18 out of 27 biomarkers for antidepressant use were present in concentrations above the LLOQ. Calculated population-normalised loads of the analytes under investigation are shown in Table S.6. Highest population-normalised loads were found for venlafaxine, citalopram, trazodone and their metabolites, which is in line with the reimbursement rates from the NIHDI. Considerable amounts of moclobemide were measured in the wastewater in three out of four locations. Scientific data suggest that < 0.5% of moclobemide is excreted in the urine as a parent compound (35). For this reason, high concentrations of moclobemide would not be expected. However, Q/q-ratios and retention times of the peak compared to the IS peak suggest that the observed peak matches with moclobemide. Additionally, moclobemide is not expected to appear in wastewater as a metabolite of other compounds to our knowledge (35). Additional samples from different locations would need to be collected and analysed to confirm this finding.

Dosulepin, duloxetine and paroxetine are prescribed in a relatively high number of patients suffering from a depression in Belgium. Unfortunately, dosulepin, nordosulepin, duloxetine and paroxetine could not be included in the developed bioanalytical assay, since validation requirements were not reached for these analytes. The possibility to use dosulepin sulfoxide or nordosulepin sulfoxide as

potential biomarkers needs to be assessed in the future as only 0.3 and 0.1% of the dose is excreted as dosulepin and nordosulepin (36). Similarly, less than 2% of a dose of paroxetine is found in the urine as the unchanged parent drug (37). The metabolites proposed by Haddock et al. can possibly serve as biomarkers to monitor the use of paroxetine in the future (37).

For four substances, the parent compound and a metabolite were included in the bioanalytical assay. Table 5 shows the expected and observed metabolite (M)/parent compound (PC) ratios for bupropion, citalopram, mirtazapine and venlafaxine, based on metabolism and excretion rates found in literature. To our knowledge, this is the first time that a M/PC ratio was calculated for bupropion/bupropion-OH in IWW. For citalopram, mirtazapine and venlafaxine, the results were in line with observed M/PC ratios in other studies. Balancing human excretion of pharmaceuticals and their metabolites to the concentrations found in IWW remains difficult because of the scarcity of data on the pharmacokinetic fate of pharmaceuticals in humans, as discussed by Gurke et al. (38). In addition, IWW cannot be 100% regarded as diluted urine. This explains why expected and measured ratios may not match exactly, yet have the same order of magnitude. It is worth noting that differences between expected and observed M/PC ratios do not appear to be related to the in-sample stability of these compounds, since all abovementioned compounds were shown to have high in-sample stability. For trazodone/mCPP, a M/PC ratio could not be calculated since metabolic excretion rates for mCPP could not be found in the literature. In this study, a mean M/PC ratio of 0.4 was found for trazodone/mCPP.

Spatial differences were observed for all compounds. For instance, the highest population-normalised mass loads for venlafaxine were obtained in Ostend. Fluvoxamine was only measured during the sampling period in Turnhout and Boom. Moclobemide, on the other hand, was detected in Brussels, Ostend and Turnhout. A Shapiro-Wilk normality test combined with the low sample size for each location indicated that non-parametric tests should be used to analyse spatio-temporal differences in antidepressant use. In this light, a Kruskal-Wallis test indicated that there were geographical differences in the use of all antidepressants. The results from the Kruskal-Wallis with Dunn-Bonferroni post hoc tests are visualised in Figure S.2 and S.3.

To evaluate temporal variations in antidepressant use, the calculated mass loads (in mg/day/1000 inhabitants) were scaled to daily proportions by dividing the mass loads by the sum of the mass loads within seven days of sampling. Samples were collected within the same sampling period to minimise daily differences. A Mann-Kendall trend analysis was used to detect a monotonic increase or decrease in the antidepressants used within the 7-day sampling period. The Mann-Kendall trend analysis test results showed that only trazodone followed a monotonic trend over time. A positive S-value indicates that the proportion of trazodone increased during the sampling week, as illustrated in Figure S.4. For all other antidepressants, proportions were distributed independently during the sampling period. The use of these antidepressants seems stable throughout the week.

Conclusions

A sensitive bioanalytical assay was developed and validated for the simultaneous detection of 27 biomarkers for antidepressant use in influent wastewater based on SPE and LC-MS/MS. The performance criteria of these compounds met the acceptance criteria set by the EMA. For five compounds, the deuterated analogue used as IS did not correct for matrix effects and highlights the need of a thorough assessment of matrix effects during method validation even if deuterated analogues are used as IS. All compounds, with the exception of mianserin, showed a high in-sample stability. The proposed bioanalytical assay also proved to be applicable when retrieving

epidemiological data on the spatial and temporal consumption patterns of antidepressants. Calculated mass loads were in line with prescription data of these pharmaceuticals.

Figures

Figure 1 Relative recoveries (in % ± SD, n=3) in tap water for all compounds per protocol.

Figure 2 Chromatogram of the MRM quantifier transitions in tap water spiked at 200 ng/L.

Figure 3 Standard addition experiment (in % recovery ± RSD) in IWW ($n_{pool\ 1}$ =6, $n_{pool\ 2}$=2).

Tables

Table 1 Summary of the sampling locations and periods.

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Table 3 Method validation criteria: intra- and interday accuracy and precision at four concentration levels: Lower limit of quantification, LLOQ; Quality control low, QCL; Quality control mid, QCM and Quality control high, QCH.

*Table 4 Stability of antidepressants in influent wastewater samples at room temperature (in-sample stability) and on SPE cartridges stored at -20 °C (on-cartridge stability).(a): TCA, (b): SSRIs, (c): NARIs, (d) DRIs, (e): SNRIs, (f): NaSSA, (g): MAO-I and (h): SARIs. *: Only one measurement available.*

Table 5 Comparison between expected and measured metabolite (M)/parent compound (PC) ratios in influent wastewater. Expected ratios were calculated based on the metabolism and excretion rates found in literature or retrieved from Gurke et al. (38).

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Table 1 Summary of the sampling locations and periods.

Location	WWTP	Sampling period	Inhabitants covered by WWTP	Sampling mode
Brussels	Brussel-Noord	23OCT'17 – 29OCT'17	954,200	Daily 24-h composite samples collected time- or flow-proportionally
Ostend	Oostende	14OCT'17 – 20OCT'17	160,000	
Turnhout	Turnhout	21OCT'17 – 27OCT'17	43,200	
Boom	Boom	23OCT'17 – 29OCT'17	30,000	

Table 2 Optimising a suitable SPE procedure: all variables.

Condition	Oasis HLB	Oasis MCX
Sorbent size	60 mg, 150 mg	60 mg, 150 mg
Condition solvent	Methanol (4 mL), followed by ultrapure water (4 mL)	Methanol (4 mL), followed by ultrapure water at pH 2 (4 mL)
Washing solvents (3 mL)	None Ultrapure water 5% v/v Methanol solution 10% v/v Methanol solution 20% v/v Methanol solution Methanol	None Ultrapure water Ultrapure water (2% v/v formic acid) Ultrapure water, followed by methanol Ultrapure water (2% v/v formic acid), followed by methanol 5% v/v methanol solution 10% v/v methanol solution 20% v/v methanol solution Methanol Methanol (2% v/v formic acid)
Elution solvents	Methanol Methanol (2% v/v acetic acid) Methanol (5% v/v acetic acid) Methanol (2% v/v formic acid) Methanol (5% v/v formic acid)	Methanol (5% v/v ammonia) Methanol, followed by methanol (5% v/v ammonia)
Elution solvent volumes	4 mL, 6 mL, 8 mL, 10 mL	

Table 3 Method validation criteria: intra- and interday accuracy and precision at four concentration levels: Lower limit of quantification, LLOQ; Quality control low, QCL; Quality control mid, QCM and Quality control high, QCH.

Compound	SPE-procedure	Linear range (ng/L)	Accuracy (%)								Precision (%RSD)							
			Intraday (n=4)				Interday (n=12)				Intraday (n=4)				Interday (n=12)			
			LLOQ	QCL	QCM	QCH	LLOQ	QCL	QCM	QCH	LLOQ	QCL	QCM	QCH	LLOQ	QCL	QCM	QCH
Normirtazapine	MCX	3-750	100.9	98.0	101.7	111.0	95.7	96.9	102.3	109.4	7.7	7.5	4.7	2.2	9.0	4.8	6.7	3.1
Moclobemide	MCX	2.5-400	86.4	93.6	102.6	108.8	93.5	95.7	103.9	109.7	2.5	4.4	3.3	1.0	7.5	5.7	3.5	1.9
Venlafaxine, O-desmethyl	MCX	5-1500	97.2	95.3	103.0	111.5	99.9	93.7	102.9	111.0	2.2	1.6	4.4	2.0	3.9	5.9	3.2	2.1
Mirtazapine	MCX	3-750	115.5	93.4	109.0	107.8	108.5	93.9	103.7	105.4	5.5	1.8	4.6	3.7	9.5	4.8	8.3	4.7
Bupropion-OH	MCX	1-400	88.8	96.8	101.5	112.0	100.7	98.6	102.3	111.0	8.5	4.4	4.3	2.9	11.8	7.6	3.2	3.2
mCPP	HLB	7.5-750	114.2	94.2	110.0	110.1	110.9	96.3	96.2	106.1	1.0	4.0	3.7	1.9	3.1	6.0	11.3	4.9
Bupropion	MCX	2.5-400	108.8	95.1	106.1	102.7	110.8	96.7	106.4	103.7	1.1	2.3	2.9	2.9	5.9	5.2	4.1	4.3
Venlafaxine	MCX	5-1500	99.2	87.4	112.9	112.1	99.3	90.0	108.6	108.4	2.6	3.0	2.3	0.8	2.7	6.2	5.1	4.6
Trazodone	MCX	5-750	104.1	94.7	98.8	100.8	108.4	96.2	104.3	104.3	2.2	4.7	3.3	2.6	4.5	6.3	5.3	4.7
Normianserin	HLB	7.5-750	106.2	104.6	105.0	107.3	108.6	99.5	106.3	107.2	5.0	4.5	5.6	5.6	8.5	9.5	4.9	5.1
Mianserin	HLB	7.5-400	96.3	106.6	103.5	109.6	100.5	102.1	102.6	110.4	8.6	3.8	4.2	3.5	9.2	4.9	3.8	2.7
Norcitalopram	HLB	2.5-1500	91.8	93.4	108.8	113.4	98.0	91.9	107.1	109.9	5.8	4.2	4.1	1.2	8.6	5.9	4.9	4.2
Nordoxepin	MCX	7.5-400	113.2	110.1	105.7	112.2	105.2	108.7	104.2	110.1	1.2	2.5	2.2	1.6	8.5	5.7	3.4	2.9
Citalopram	MCX	7.5-1500	92.8	98.5	105.9	112.4	99.3	100.5	104.1	108.5	5.2	3.6	1.4	2.1	7.2	5.3	7.4	5.7
Reboxetine	HLB	2.5-400	103.0	95.7	105.5	108.8	97.9	93.9	102.0	108.7	1.8	8.7	6.9	2.7	7.5	6.7	6.0	4.4
Doxepin	HLB	7.5-750	112.7	102.6	91.2	112.8	108.5	105.4	92.1	111.1	7.4	4.2	4.8	1.7	7.6	5.9	3.9	1.8
Tianeptine	HLB	2.5-400	108.7	90.3	90.1	104.0	102.7	95.6	93.0	107.4	9.5	5.8	2.5	1.9	10.0	9.7	6.6	3.7
Atomoxetine	HLB	10-750	103.2	101.8	109.0	110.7	102.5	96.0	103.2	107.7	3.2	3.1	4.5	0.7	5.3	10.0	7.4	4.9
Desipramine	HLB	5-400	106.7	98.6	100.1	106.2	101.7	97.4	97.6	104.8	6.2	9.8	4.5	2.2	7.8	8.6	5.5	5.0
Normaprotiline	HLB	25-750	106.2	96.9	90.5	101.5	106.9	97.8	94.2	105.7	9.4	11.3	10.2	5.4	7.0	11.8	8.5	6.5
Fluvoxamine	HLB	5-400	110.8	87.9	93.8	100.4	111.8	97.8	101.5	105.2	5.8	1.1	10.4	3.7	5.8	10.8	8.3	5.7
Imipramine	HLB	2.5-400	90.0	97.9	100.7	104.9	93.3	98.1	102.3	101.9	8.6	7.9	3.9	4.2	9.8	10.0	6.5	8.6
Nortriptyline	HLB	10-750	103.6	93.4	107.3	111.3	96.4	96.7	108.5	109.0	6.8	3.3	3.2	1.8	8.6	8.4	4.3	2.7
Duloxetine	HLB	10-750	112.5	101.3	101.3	105.7	108.1	102.7	100.9	108.1	5.4	1.0	4.4	2.5	7.8	5.5	5.7	3.9
Maprotiline	HLB	10-750	96.1	101.4	94.0	97.3	97.8	101.0	98.3	99.4	6.4	6.0	6.6	6.2	11.0	6.4	7.6	6.5
Amitriptyline	HLB	15-750	91.5	102.9	99.5	96.7	91.2	102.9	97.9	96.8	6.0	6.0	4.4	4.4	7.6	7.1	6.7	6.4
Trimipramine	HLB	2.5-750	99.1	101.2	110.4	106.7	91.8	100.8	103.4	101.1	8.0	9.1	3.0	1.6	7.9	8.0	8.1	7.2
Fluoxetine	HLB	7.5-750	102.7	93.4	90.4	103.9	98.1	93.0	93.9	106.8	4.5	4.5	9.7	4.0	6.9	5.3	7.5	5.5
Sertraline	MCX	15-750	97.6	108.4	94.4	99.6	103.6	102.2	97.7	106.6	2.7	5.1	3.4	3.4	10.0	8.1	7.5	6.4
Norclomipramine	HLB	5-400	95.7	98.3	97.5	109.1	91.7	101.7	96.2	103.3	8.8	3.7	4.7	4.0	11.0	8.4	5.9	6.2

Melitracen	MCX	10-400	106.8	98.9	91.3	92.7	110.4	100.5	98.4	102.6	12.3	3.5	3.7	1.3	9.1	5.2	7.1	8.4
Clomipramine	HLB	2.5-400	87.6	92.6	95.4	105.7	87.7	98.0	94.0	103.0	2.0	7.5	3.2	4.0	4.5	9.4	4.3	6.0

*Table 4 Stability of antidepressants in influent wastewater samples at room temperature (in-sample stability) and on SPE cartridges stored at -20 °C (on-cartridge stability).(a): TCA, (b): SSRIs, (c): NARIs, (d) DRIs, (e): SNRIs, (f): NaSSA, (g): MAO-I and (h): SARIs. *: Only one measurement available.*

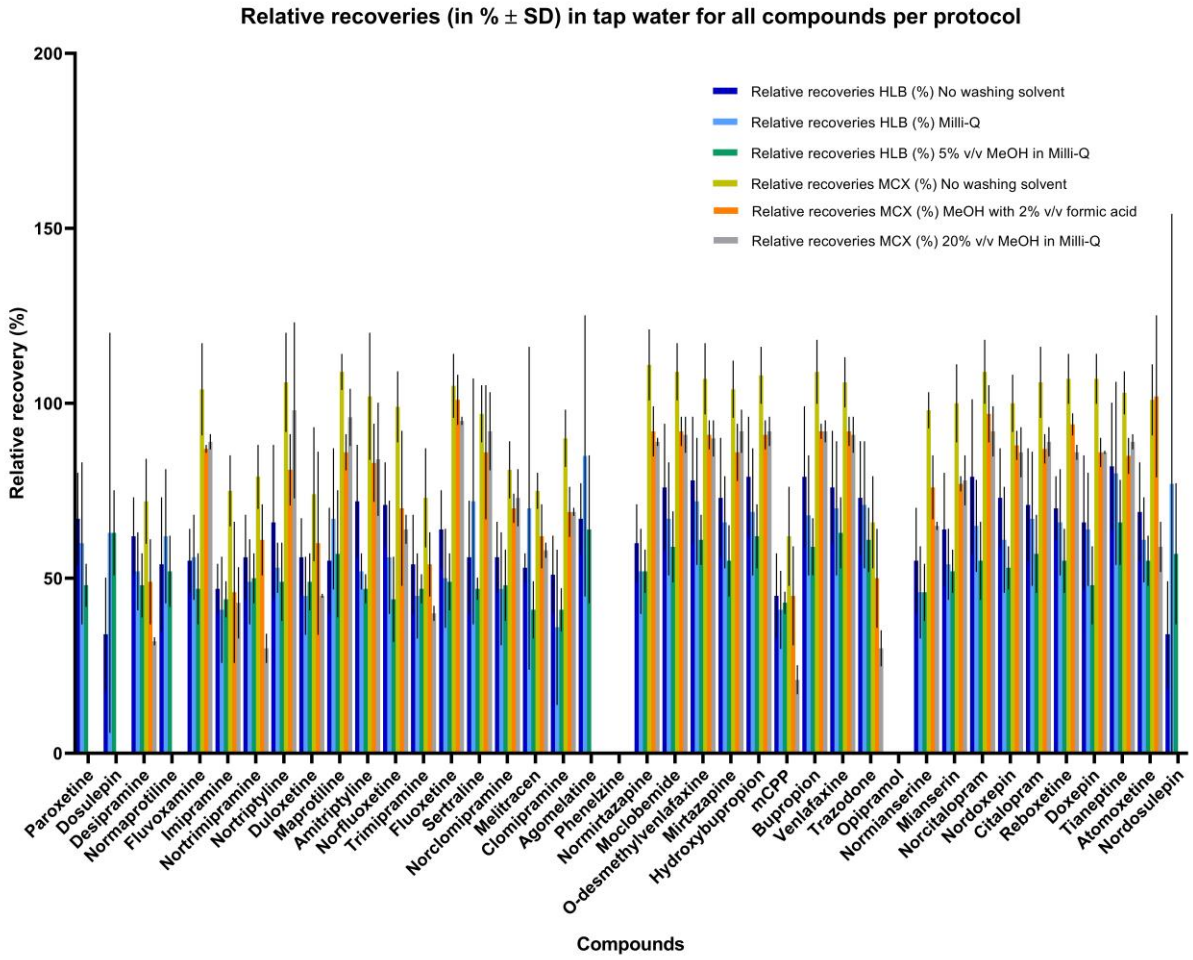
	In-sample stability				**On-cartridge stability**
	Residual percentage (± %RSD) (n=2)				**Residual percentage (± %RSD) (n=3)**
Compound	2 h	6 h	10 h	24 h	1.5 month
Amitryptiline[a]	101.1 ± 13.8	109.1 ± 9.9	100.2 ± 8.6	81.7 ± 3.4	94.4 ± 19.5
Nortriptyline[a]	114.8 ± 17.7	107.4 ± 1.3	93.2 ± 10.0	81.7 ± 14.1	95.0 ± 7.4
Clomipramine[a]	127.5 ± 11.6	113.3 ± 6.1	116.8 ± 8.9	90.9 ± 11.9	101.0 ± 4.4
Norclomipramine[a]	90.2 ± 5.3	100.5 ± 19.6	107.0 ± 15.8	102.7 ± 16.6	105.6 ± 18.6
Doxepin[a]	108.5 ± 0.1	102.5 ± 4.6	106.4 ± 3.8	106.3 ± 5.1	104.6 ± 18.0
Nordoxepin[a]	101.1 ± 3.0	102.5 ± 6.4	94.0 ± 3.1	91.7 ± 5.9	100.2 ± 11.3
Imipramine[a]	98.1 ± 3.2	94.7 ± 7.4	99.9 ± 6.5	93.6 ± 7.4	82.4 ± 7.9
Desipramine[a]	89.9 ± 17.7	102.1 ± 0.8	101.0 ± 26.3	85.8 ± 2.4	99.2 ± 8.9
Normaprotiline[a]	112.2 ± 21.0	110.2 ± 10.7	91.5 ± 30.4	82.7 ± 14.9	93.2 ± 14.3
Maprotiline[a]	113.7 ± 19.0	111.7 ± 16.8	116.8 ± 9.9	96.7 ± 7.9	95.6 ± 8.8
Melitracen[a]	96.7 ± 0.2	92.7 ± 0.9	90.4 ± 5.7	83.6 ± 1.4	97.5 ± 5.6
Trimipramine[a]	104.6 ± 3.6	104.6 ± 1.9	105.9 ± 4.6	98.8 ± 2.8	96.5 ± 6.6
Norcitalopram[b]	113.4 ± 8.3	108.6 ± 10.5	107.8 ± 7.2	101.7 ± 3.1	116.2 ± 7.3
Citalopram[b]	102.0 ± 1.0	98.9 ± 0.5	96.8 ± 2.9	99.3 ± 2.1	96.6 ± 5.7
Fluvoxamine[b]	104.5 ± 6.7	102.3 ± 8.1	92.4 ± 11.9	96.2 ± 3.5	98.0 ± 8.9
Fluoxetine[b]	114.0 ± 5.6	119.3 ± 4.6	119.2 ± 14.1	116.6*	88.8 ± 16.4
Sertraline[b]	101.3 ± 1.9	100.4 ± 5.0	96.0 ± 4.9	94.1 ± 1.6	94.0 ± 4.1
Reboxetine[c]	105.4 ± 3.0	104.9 ± 3.8	102.5 ± 5.1	97.7 ± 5.8	95.2 ± 1.7
Bupropion[d]	100.1 ± 4.6	99.1 ± 1.3	97.3 ± 1.8	99.6 ± 2.7	98.1 ± 7.6
Bupropion-OH[d]	100.4 ± 4.7	101.0 ± 1.3	99.7 ± 3.5	101.0 ± 1.6	99.7 ± 2.5
Venlafaxine[e]	103.8 ± 0.6	101.9 ± 3.9	100.0 ± 1.9	98.4 ± 2.2	102.0 ± 1.7
O-desmethyl-venlafaxine[e]	97.8 ± 1.6	101.3 ± 1.9	100.7 ± 2.2	104.5 ± 3.5	98.7 ± 5.1
Mianserin[f]	94.8 ± 3.9	87.0 ± 0.7	95.6 ± 9.5	76.5 ± 9.1	101.2 ± 12.1
Normianserin[f]	101.2 ± 7.9	106.8 ± 9.5	95.3 ± 10.4	83.4 ± 19.5	89.4 ± 16.7
Mirtazapine[f]	96.1 ± 11.9	91.4 ± 6.5	90.5 ± 4.1	91.1 ± 0.7	98.5 ± 7.4
Normirtazapine[f]	105.2 ± 0.1	104.9 ± 1.0	96.3 ± 1.9	103.3 ± 4.4	97.5 ± 2.4
Moclobemide[g]	103.4 ± 0.2	100.6 ± 1.0	101.1 ± 0.3	102.7 ± 0.5	96.9 ± 6.6
Trazodone[h]	111.8 ± 12.8	97.4 ± 6.7	102.1 ± 3.1	92.9 ± 1.9	93.5 ± 4.7
m-chlorophenyl-piperazine[h]	104.4 ± 1.8	106.4 ± 8.6	100.4 ± 1.4	99.2 ± 6.6	99.2 ± 6.7

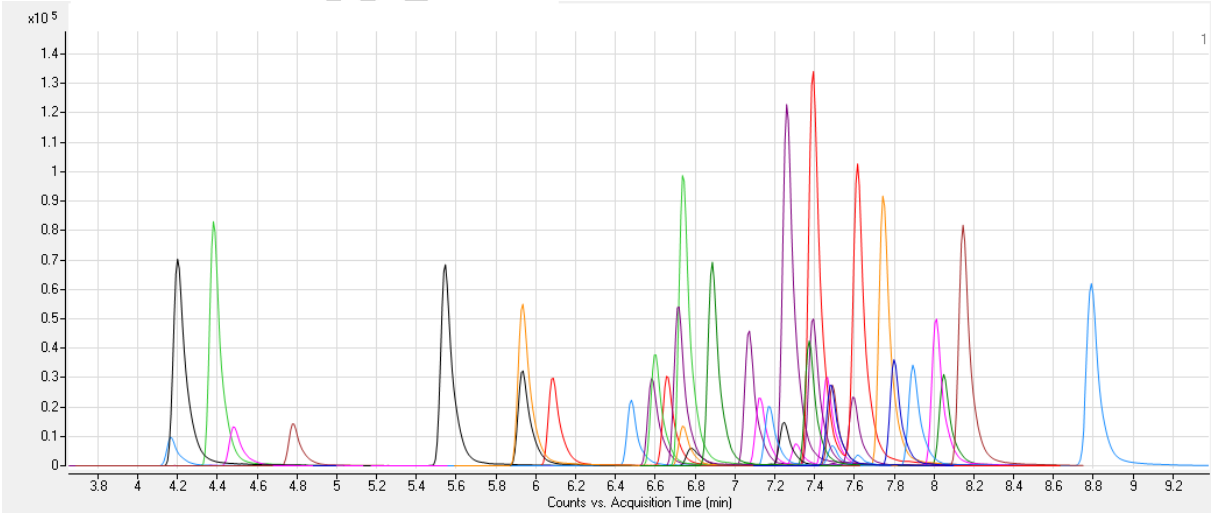
Table 5 Comparison between expected and measured metabolite (M)/parent compound (PC) ratios in influent wastewater. Expected ratios were calculated based on the metabolism and excretion rates found in literature or retrieved from Gurke et al. (37)*.*

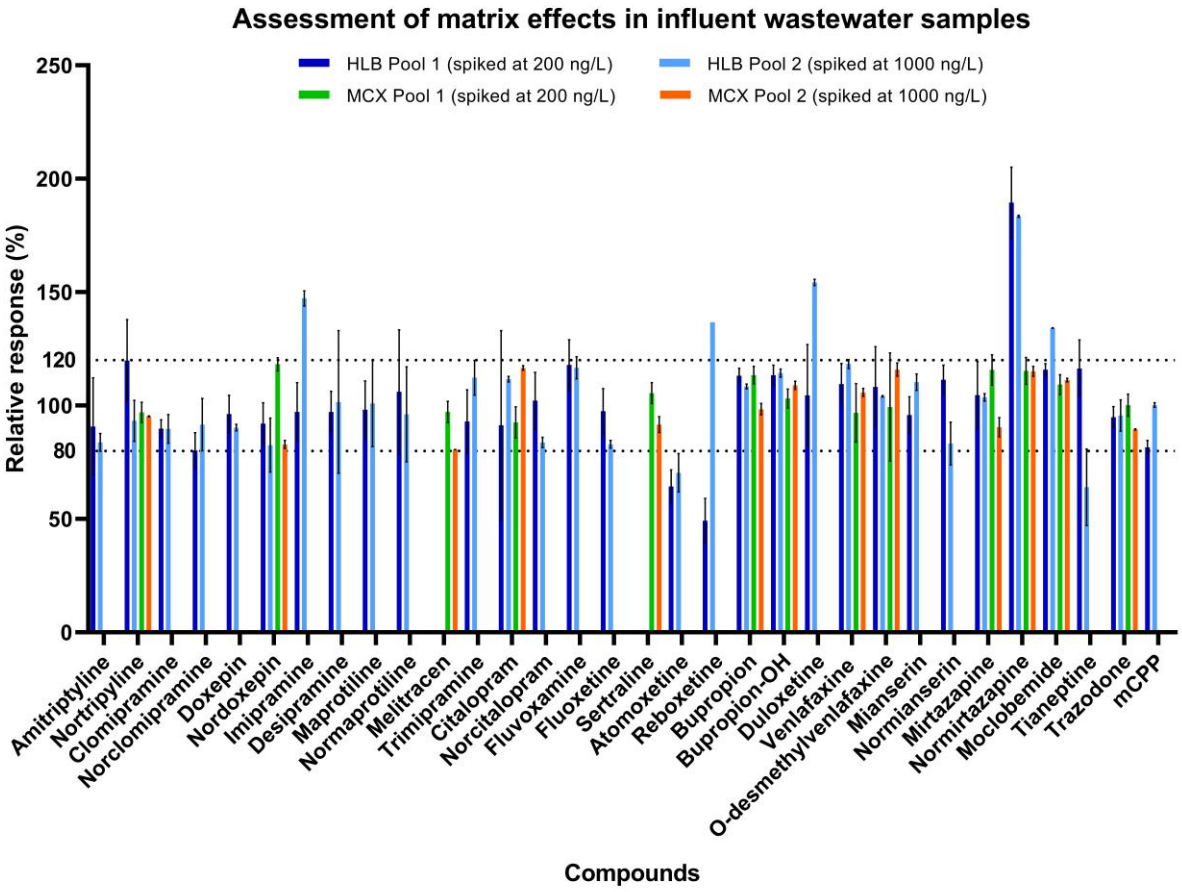
Parent compound/ metabolite	***Excretion rate***		***M/PC ratio in IWW***		
	PC	M	Expected	Measured (this study)	Measured (other studies)
Bupropion/bupropion-OH	0.5%	4%	8.0	4.5	-
Citalopram/norcitalopram	12%	12%	1.0	0.5	0.6-0.8 (23,37,38)
Mirtazapine/normirtazapine	4-29%	25-35%	0.9–8.8	0.3	0.3-0.5 (37,39)
Venlafaxine/O-desmethylvenlafaxine	5%	29-48%	5.8–9.6	2.4	2.0-5.9 (23,37–40)

Highlights

- Fully validated method for 27 compounds
- Different matrix effects for 5 compounds vs deuterated standard
- High in-sample stability for all targeted biomarkers
- Successfully applied to influent wastewater samples


Relative recoveries (in % ± SD) in tap water for all compounds per protocol
Relative recoveries HLB (%) No washing solvent
Relative recoveries HLB (%) Milli-Q
Relative recoveries HLB (%) 5% v/v MeOH in Milli-Q
Relative recoveries MCX (%) No washing solvent
Relative recoveries MCX (%) MeOH with 2% v/v formic acid
Relative recoveries MCX (%) 20% v/v MeOH in Milli-Q
Relative recovery (%)
200
150
100
50
0
Paroxetine
Dosulepin
Desipramine
Normaprotiline
Fluvoxamine
Imipramine
Nortrimipramine
Nortriptyline
Duloxetine
Maprotiline
Amitriptyline
Norfluoxetine
Trimipramine
Fluoxetine
Sertraline
Norclomipramine
Melitracen
Clomipramine
Agomelatine
Phenelzine
Normirtazapine
Moclobemide
O-desmethylvenlafaxine
Mirtazapine
Hydroxybupropion
mCPP
Bupropion
Venlafaxine
Trazodone
Opipramol
Normianserine
Mianserin
Norcitalopram
Nordoxepin
Citalopram
Reboxetine
Doxepin
Tianeptine
Atomoxetine
Nordosulepin
Compounds


x10 5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
3.8
4
4.2
4.4
4.6
4.8
5
5.2
5.4
5.6
5.8
6
6.2
6.4
6.6
6.8
7
7.2
7.4
7.6
7.8
8
8.2
8.4
8.6
8.8
9
9.2
Counts vs. Acquisition Time (min)


Assessment of matrix effects in influent wastewater samples
HLB Pool 1 (spiked at 200 ng/L)
HLB Pool 2 (spiked at 1000 ng/L)
MCX Pool 1 (spiked at 200 ng/L)
MCX Pool 2 (spiked at 1000 ng/L)
Relative response (%)
250
200
150
120
100
80
50
0
Amitriptyline
Nortripyline
Clomipramine
Norclomipramine
Doxepin
Nordoxepin
Imipramine
Desipramine
Maprotiline
Normaprotiline
Melitracen
Trimipramine
Citalopram
Norcitalopram
Fluvoxamine
Fluoxetine
Sertraline
Atomoxetine
Reboxetine
Bupropion
Bupropion-OH
Duloxetine
Venlafaxine
O-desmethylvenlafaxine
Mianserin
Normianserin
Mirtazapine
Normirtazapine
Moclobemide
Tianeptine
Trazodone
mCPP
Compounds