Analytical Methods

PAPER

View Article Online
View Journal | View Issue

Open Access Article. Published on 11 August 2017. Downloaded on 10/23/2025 10:18:34 PM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

Check for updates

Cite this: *Anal. Methods*, 2017, **9**, 5310

Multi-residue analysis of selected pharmaceuticals in wastewater samples by stir-bar sorptive extraction followed by liquid desorption and liquid chromatography-mass spectrometry

A. Klančar, M. Zakotnik, R. Roškar and J. Trontelj *

New organic pollutants such as pharmaceuticals have been recently classified as emerging pollutants. These are chemically active compounds and may pose a risk to environmental organisms as well as to humans. Nowadays, separation and analytical techniques enable detection and quantification at extremely low concentrations. We aimed to develop and optimise a new method that would minimise (organic) solvent consumption and miniaturise and simplify sample preparation through the use of stir-bar sorptive extraction of selected pharmaceuticals, followed by liquid desorption and liquid chromatography coupled to mass spectrometry. Several conditions and parameters that affect the extraction procedure (extraction time, sample pH, sample volume, extraction temperature, and addition of salt and organic modifier) were evaluated for suitable extraction efficiency. The optimised method (sample volume, 20 mL; sample pH, 9; extraction time, 150 min; and desorption time, 15 min; in an acetonitrile and methanol [50 : 50 v/v] mixture at 50 °C, without any added modifier) was validated and provided recoveries above 80% for 12 analytes, and for 6 analytes they were between 45% and 65%, while for another 6 analytes the recoveries were below 25%. The method provided a wide linear range (mainly 1.25–1250 ng L^{-1}, R^2 > 0.99) with very low limits of quantification for all analytes (1.25–5.0 ng L^{-1}). The method was used to test 15 wastewater samples and 22 out of 24 monitored pharmaceuticals were detected, generally in concentrations below 200 ng L^{-1}.

Received 24th May 2017
Accepted 10th August 2017

DOI: 10.1039/c7ay01310h

rsc.li/methods

1. Introduction

In recent decades environmental analysis has become of extensive interest to scientists, as new organic pollutants such as pharmaceuticals, personal care products, pesticides and polycyclic aromatic hydrocarbons are classified as emerging pollutants. Many of these are chemically active compounds that may pose a risk to environmental organisms as well as to humans.[1,2]

Today, it is well recognized that the increased use of the above-mentioned compounds inevitably leads to their occurrence in the environment. However, because of the large dilution factor when they enter water bodies, their concentrations mostly reach only trace levels and their determination usually requires a pre-concentration step before separation and detection. In parallel with analytical progress, modern guidelines on green analytical chemistry have been established regarding minimising (organic) solvent consumption, miniaturization and simplification.[3] As a result, in the 1990s new sample preparation techniques such as solid-phase micro-extraction (SPME) and stir-bar sorptive extraction (SBSE) became of increasing interest as alternatives to more solvent-consuming techniques such as liquid and solid-phase extractions.[4]

The technique of SPME established the basis for further development of equilibrium partitioning or sorptive extraction. In 1999, Baltussen *et al.* published the theory of SBSE.[5] Briefly, the efficiency of analyte partitioning into the solid phase (polydimethylsiloxane [PDMS] is the widest commercially available) can be compared to the distribution characterised by the octanol–water partition coefficient ($K_{PDMS/W} \sim K_{O/W}$). Thus, the polarity of the organic analyte roughly predicts the extraction efficiency. Based on experimental data, an equation was set for the theoretically predicted extraction efficiency of the analyte at equilibrium (eqn (1), where EE is the extraction efficiency). According to the equation, it is assumed that successful extractions are limited to analytes with higher $K_{O/W}$ (log $K_{O/W}$ > 2.7). In addition to the $K_{O/W}$, the phase ratio β (ratio of water sample volume, V_w) and volume of PDMS (V_{PDMS} coated on stir bar) also play key roles in the extraction efficiency (eqn (2)).

$$\mathrm{EE}\ (\%) = (K_{O/W})/(K_{O/W} + \beta) \quad (1)$$

University of Ljubljana, Faculty of Pharmacy, Department of Biopharmaceutics and Pharmacokinetics, Askerceva 7, 1000 Ljubljana, Slovenia. E-mail: anita.klancar@ffa.uni-lj.si; maticzakotnik@gmail.com; robert.roskar@ffa.uni-lj.si; jurij.trontelj@ffa.uni-lj.si; Fax: +386 1 425 80 31; Tel: +386 1 4769 649; +386 1 4769 655; +386 1 4769 547

This journal is © The Royal Society of Chemistry 2017

Open Access Article. Published on 11 August 2017. Downloaded on 10/23/2025 10:18:34 PM. This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

$$\beta = V_w/V_{PDMS} \quad (2)$$

Following the publication of the advantages of higher recoveries in SBSE than in SPME, several research groups have been developing SBSE for various analyses.[3,6–8] The primary concept of SBSE was first designed for connection with a thermal desorption system followed by gas chromatography coupled to mass spectrometry detection.[5,9] Although several methods have been developed, only a minority use the alternative approach of solvent back-extraction or liquid desorption coupled to mass spectrometry detection.[10] The latter method allows cost-effective determination of analytes and also reduces solvent consumption. The SBSE method in general reduces the amount of sample needed for the analysis. In addition, the loaded stir bars can be conveniently sent to the laboratory and can be stored for a week without any significant loss of analytes.[11] Moreover, the convenience of sampling with stir bars enables extended research in large environments.

In recent years, the SBSE method has been used in environmental and food analysis and also in biomedical and life science applications (reviewed by Camino-Sánchez *et al.*).[7] Focusing solely on pharmaceuticals, this sampling technique has been used mainly on biomedical fluids such as urine, serum, plasma and saliva.[3,9,12,13] However, for the purpose of environmental monitoring of pharmaceuticals, the methodology using the generally available stationary phases for SBSE is still lacking, particularly for water bodies. Apart from the reports of hormone analytics,[13–17] there have been only a few reports on SBSE determination of pharmaceuticals in the environment and all have dealt with only a small number of pharmaceuticals, mostly using a combination of thermal desorption and gas chromatography.[6,9,18–21] However, there are some reports on the new in-house prepared stir-bar coatings[6,19,22–24] and two new commercial polar coatings (ethylene glycol modified silicone (EG Silicone Twister®) and polyacrylate (Acrylate Twister®)).[21,25]

In response to the lack of methodology for routine SBSE monitoring of pharmaceuticals in wastewater samples, the aim of our research was to develop and optimise SBSE for selected pharmaceuticals, followed by liquid desorption and liquid chromatographic separation coupled to mass spectrometry detection. In comparison with existing methodologies, SBSE is suitable for less well equipped laboratories as thermal desorption is not required. Moreover, this approach does not expose analytes to extremely high temperatures and enables analysis in replicates when needed. Linearity, repeatability and limits of quantification were evaluated before testing wastewater samples. Primarily, the concentrations of selected pharmaceuticals in ten effluent wastewater samples from wastewater treatment plants (WWTPs) were determined. In cases where influent samples were available, removal efficiencies for five WWTPs were also calculated.

2. Experimental protocol

2.1. Standards and reagents

Selected standards of amitriptyline hydrochloride, azithromycin, bromazepam, carbamazepine, clomipramine hydrochloride, clonazepam, desipramine hydrochloride, diazepam, donepezil hydrochloride monohydrate, escitalopram oxalate, fluoxetine hydrochloride, haloperidol, loperamide hydrochloride, loratadine, metoprolol tartrate, promethazine hydrochloride, propranolol hydrochloride, raloxifene hydrochloride, selegiline hydrochloride, sertraline hydrochloride, tramadol hydrochloride, triclosan, venlafaxine hydrochloride, verapamil hydrochloride and ziprasidone hydrochloride monohydrate were purchased from Sigma-Aldrich (Germany); imatinib mesylate and risperidone were purchased from Sequoia Research Products (UK). All pharmaceutical standards were of high-purity grade (>98%).

Reagents for preparation of standards and samples included acetonitrile (ACN), dichloromethane, formic acid (98–100%), methanol (MeOH), 2-propanol and potassium dihydrogen phosphate from Merck (Germany); ammonium formate and sodium chloride were from Sigma-Aldrich. Ultra-pure water was produced in a Millipore Milli-Q water purification system A10 Advantage (Millipore Corporation, USA). Solvents for liquid chromatography-mass spectrometry (LC-MS/MS) analyses were LC-MS grade acetonitrile ChromasolV® (Sigma-Aldrich), Milli-Q water and formic acid (98–100%) Suprapur® (Merck).

GERSTEL Twister® stir bars (GERSTEL GmbH & Co., Germany) were used in SBSE procedures. The bars were 10 mm in length and externally coated with a layer of PDMS 1 mm thick. The dimension of 10 × 1 mm corresponds approximately to 63 μL of PDMS per stir bar.

2.2. Standard solutions

Standard stock solutions of each analyte were prepared by dissolving 5 mg of accurately weighed standard in methanol to yield 1 mg mL^{-1} final concentration of the active ingredient. With further dilution in methanol, a multicomponent solution of 27 analytes at 10 μg L^{-1} final concentration was obtained (working standard solution). All calibration standards were prepared in methanol by serial dilution of working standard solution, in concentrations from 1.25 ng L^{-1} to 1250 ng L^{-1}. All working standard solutions were stored at −20 °C and renewed daily.

2.3. Extraction procedure

The extraction procedure was optimised using 20 mL aliquots of working standard solution (10 μg L^{-1}). Sample pH was adjusted to a selected value (pH 3–9) with 50 mM KH_2PO_4 buffer. In preliminary experiments, factors influencing the extraction efficiency (time, temperature, pH, and addition of NaCl and MeOH) and desorption efficiency (time and organic solvents in combination with stirring or ultrasonic treatment) were evaluated. Before use, all stir bars were stirred in 2 mL MeOH for 30 min at 990 rpm and room temperature; the bars were then dried with lint-free tissue and placed in 25 mL glass vials each containing 20 mL of sample. For extraction under optimal conditions, samples were preheated to 50 °C and adjusted to pH 9 without extra addition of NaCl or MeOH; the extraction time was 150 min and the stirring speed was 990 rpm. The bars were then removed from samples with a magnetic rod, rinsed in Milli-Q water, dried with lint-free tissue and placed in glass vials with 2 mL of a mixture of organic solvents ACN : MeOH

Open Access Article. Published on 11 August 2017. Downloaded on 10/23/2025 10:18:34 PM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

(50 : 50 v/v) for single liquid desorption (stirring) for 15 min at 50 °C and a stirring speed of 990 rpm. Aliquots of 500 μL were taken for LC-MS/MS analysis.

After use, the stir bars were reconditioned in a mixture of dichloromethane : MeOH (50 : 50 v/v) for 30 min, then in pure MeOH for 30 min, at room temperature with a stirring speed of 990 rpm. The bars were dried and stored for further extraction procedures.

The optimised method was evaluated by determining the linear range, determination coefficients, limits of quantification, imprecision and extraction efficiency (Table 3). For quantification, fluoxetine-d_5 was used as the internal calibration standard. The linear range was determined in 20 mL aliquots of ultra pure water spiked at eight concentration levels from 1.25 ng L^{-1} to 1250 ng L^{-1}, each in duplicate. Back calculation of the concentration and bias from nominal values was determined for each calibration point. Limits of quantification were set as the lowest standard on the calibration curve that exhibited acceptable accuracy (deviation from the nominal value ±20%) and precision (expressed as relative standard deviation%) while having the signal to noise ratio >30 : 1.

2.4. LC-MS/MS analysis

The obtained pharmaceutical samples were analysed in an Agilent 1290 Infinity LC coupled to an Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies, USA). Portions (1 μL) of samples were injected onto a 100 × 3.0 mm, 2.7 μm Poroshell EC-C18 column (Agilent) at 50 °C and eluted with mobile phases A (0.1% formic acid in water) and B (ACN) using the following linear gradient (time [min]; % B; flow-rate [mL min^{-1}]): (0; 8; 0.5), (0; 5; 15; 0.5), (1; 15; 0.65), (2; 33; 0.65), (3; 35; 0.65), (4.8; 40; 0.65), (5.1; 50; 0.65), (5.3; 70; 0.65), (6.5; 95; 0.65), (6.7; 95; 0.65), (6.9; 8; 0.65), (8.2; 8; 0.5). The run time was 8.2 min. After each injection the sampling needle was washed with solvent MeOH : H_2O (80 : 20 v/v). A JetStream® electrospray source was used for MS. The instrumental parameters were as follows: drying gas temperature, 275 °C; drying gas flow, 5 L min^{-1}; nebulizer pressure, 45 psi; sheath gas temperature, 320 °C; sheath gas flow, 11 L min^{-1}; capillary entrance voltage, 4000 V; and nozzle voltage, 1000 V. Quadrupoles Q_1 and Q_3 were set at wide (1.2 amu) or widest (2.5 amu) mass resolution. MassHunter Workstation software (Agilent) was used for instrument control and data acquisition and quantification. In order to achieve optimum results for MS detection, the Optimizer software (MassHunter, Agilent Technologies) automatically optimized fragmentor voltage, collision energy and multiple reaction monitoring (MRM) transitions for each target compound. MRM transitions and other quantification settings for analytes using dMRM mode are shown in Table 1.

Table 1 Multiple reaction monitoring quantification settings for all analytes determined; analytes included in the method optimisation are indicated in bold[a]

Analyte	Retention time (min)	MRM (*m/z*)			CE (eV)	Frag (V)	P	Q1 resolution	Q3 resolution
Amitriptyline	**4.43**	**278.2**	**>**	**91.0**	**24**	**103**	**+**	**Widest**	**Widest**
Azithromycin	2.63	749.5	>	83.0	69	200	+	Widest	Widest
Bromazepam	3.56	316.0	>	182.0	40	160	+	Widest	Widest
Carbamazepine	4.13	237.1	>	193.9	12	103	+	Widest	Widest
Clomipramine	**5.15**	**315.2**	**>**	**86.1**	**16**	**106**	**+**	**Widest**	**Widest**
Clonazepam	4.81	316.1	>	270.1	21	121	+	Widest	Widest
Desipramine	**4.02**	**267.2**	**>**	**72.1**	**12**	**98**	**+**	**Widest**	**Wide**
Diazepam	6.14	285.1	>	193.0	32	159	+	Wide	Wide
Donepezil	**3.11**	**380.2**	**>**	**65.0**	**97**	**86**	**+**	**Wide**	**Wide**
Escitalopram	**3.40**	**325.2**	**>**	**109.0**	**29**	**81**	**+**	**Widest**	**Widest**
Fluoxetine	**4.76**	**310.1**	**>**	**148.1**	**1**	**100**	**+**	**Widest**	**Widest**
Haloperidol	**3.66**	**376.2**	**>**	**165.0**	**21**	**126**	**+**	**Widest**	**Wide**
Imatinib	2.80	494.3	>	395.2	25	200	+	Widest	Widest
Loperamide	**5.84**	**477.2**	**>**	**266.1**	**24**	**159**	**+**	**Widest**	**Wide**
Loratadine	**5.96**	**383.2**	**>**	**337.1**	**21**	**121**	**+**	**Widest**	**Widest**
Metoprolol	2.58	268.2	>	116.0	12	96	+	Widest	Wide
Promethazine	**3.74**	**285.1**	**>**	**86.1**	**12**	**55**	**+**	**Widest**	**Wide**
Propranolol	**3.11**	**260.2**	**>**	**56.1**	**25**	**126**	**+**	**Widest**	**Widest**
Raloxifene	3.28	474.2	>	112.0	32	200	+	Widest	Widest
Risperidone	2.88	411.2	>	191.1	28	159	+	Widest	Wide
Selegiline	**2.43**	**188.1**	**>**	**91.0**	**21**	**81**	**+**	**Widest**	**Widest**
Sertraline	**4.89**	**306.1**	**>**	**158.9**	**24**	**60**	**+**	**Widest**	**Widest**
Tramadol	2.58	264.2	>	58.1	17	81	+	Wide	Wide
Triclosan	**6.80**	**286.9**	**>**	**35.1**	**4**	**60**	**−**	**Widest**	**Widest**
Venlafaxine	**2.91**	**278.2**	**>**	**58.1**	**17**	**81**	**+**	**Widest**	**Widest**
Verapamil	**4.39**	**455.3**	**>**	**150.1**	**41**	**184**	**+**	**Widest**	**Widest**
Ziprasidone	**3.15**	**413.1**	**>**	**194.0**	**25**	**161**	**+**	**Widest**	**Widest**
Fluoxetine-d_5	**4.77**	**315.2**	**>**	**153.2**	**1**	**100**	**+**	**Widest**	**Widest**

[a] MRM, multiple reaction monitoring; CE, collision energy; Frag, fragmentor voltage; P, polarity; Q, quadrupole; wide = 1.2 amu; widest = 2.5 amu.

 This journal is © The Royal Society of Chemistry 2017

Open Access Article. Published on 11 August 2017. Downloaded on 10/23/2025 10:18:34 PM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

2.5. Wastewater sample collection

Samples were collected from ten WWTPs located in different regions in Slovenia. Effluent wastewater samples were readily available but influent samples were accessible only in half of the treatment plants. Data obtained were divided into two parts: the first set comprised WWTPs A, B, C, D and E where only the effluent samples were available and this set was used only for the initial screening of the presence of target pharmaceuticals; the second set comprised WWTPs I, II, III, IV and V where both influent and effluent samples were available. The determined concentrations of pharmaceuticals were used for the calculation of removal efficiency (eqn (3)).

$$\text{Removal efficiency (\%)} = \frac{(\text{influent conc.} - \text{effluent conc.})}{\text{influent conc.}} \times 100\% \quad (3)$$

All WWTPs were municipal plants and received both domestic and industrial sewage. The capacities of plants A–E were 1000, 100 000, 68 000, 6500 and 15 500 population equivalents, respectively. Plants A and B used a secondary wastewater treatment, plants C and D a tertiary wastewater treatment and plant E a primary treatment. The second set of WWTPs I–V is described in detail in Section 3.4.2, where removal efficiency is discussed. All wastewater samples were collected in the spring of 2016 and were 24 h composite samples (time-proportional) collected in clean glass bottles, and then stored at 4 °C and analysed within 24 h.

3. Results and discussion

3.1. Optimisation of the extraction procedure

First, a set of 27 environmentally relevant pharmaceuticals were selected. For the optimisation process, 17 of these compounds were chosen on the basis of their log $K_{O/W}$ value (between 2.7 and 5.5) where good extraction recovery with stir bars could be expected.

Several conditions and parameters may affect stir-bar extraction, the most important being extraction time, sample pH, extraction temperature and the addition of NaCl and MeOH. All these parameters were evaluated and optimised to achieve acceptable extraction efficiency. Since the number of varied parameters was relatively low, one-variable-at-a-time methodology was used. Following the preliminary development and optimisation experiments, some parameters such as analyte concentration, 10 μg L^{-1}; sample volume, 20 mL; stirring speed, 990 rpm; and stir bar dimensions, 1 × 10 mm were fixed for the optimisation tests. The extraction time was fixed at 60 min for all experiments (except for those testing the influence of the extraction time) and the temperature was fixed at 50 °C (except for testing the influence of the temperature). To facilitate comparison between parameters during the extraction processes, conditions for the desorption process were fixed as follows: desorption solvent, 2 mL ACN : MeOH (50 : 50 v/v); stirring for 15 min at 990 rpm; and temperature, 50 °C.

The influence of sample pH on SBSE efficiency was evaluated within the pH range of 3–9, proposed as harmless to the PDMS phase. The regulation of sample pH was assessed at four levels: pH 3, 5, 7 and 9. The relative extraction efficiency for each analyte was calculated with reference to the highest extraction efficiency (100%). As shown in Fig. 1, samples at pH 9 provided the best results for almost all analytes, except for propranolol and ziprasidone, where recovery was highest at pH 7; triclosan reached maximum at pH 5. This observation is closely in line with pH partition principles, *i.e.* the theory of distribution of the un-ionized form of analyte and the relationship between pK_a and pH values.

After the pH was set at 9, the effect of NaCl addition was tested from 0 to 2000 mg (0–10% w/v). An increase of salt gradually decreased almost all extraction efficiencies (Fig. 2A). Such a decrease could be the result of increased viscosity with the salt addition, as this could slow down the extraction kinetics of these analytes or affect the extraction polymer sorbent. Similar results have been reported.[6,26] The addition of MeOH to the water samples was also investigated, with the aim of minimising adsorption of analytes to the vial surface. MeOH was added at five levels from 1% to 30% (v/v). In accordance with the general sample preparation procedure, 1% MeOH was

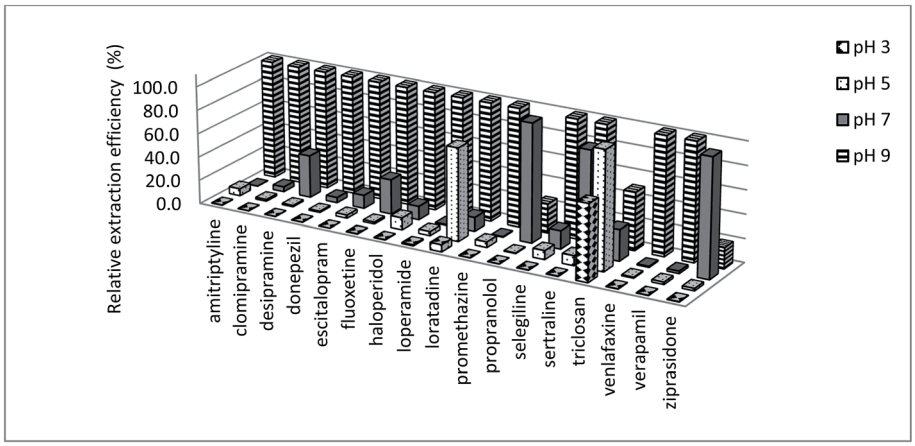

Fig. 1 Relative extraction efficiency (%) at various sample pH values for selected analytes.

Open Access Article. Published on 11 August 2017. Downloaded on 10/23/2025 10:18:34 PM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

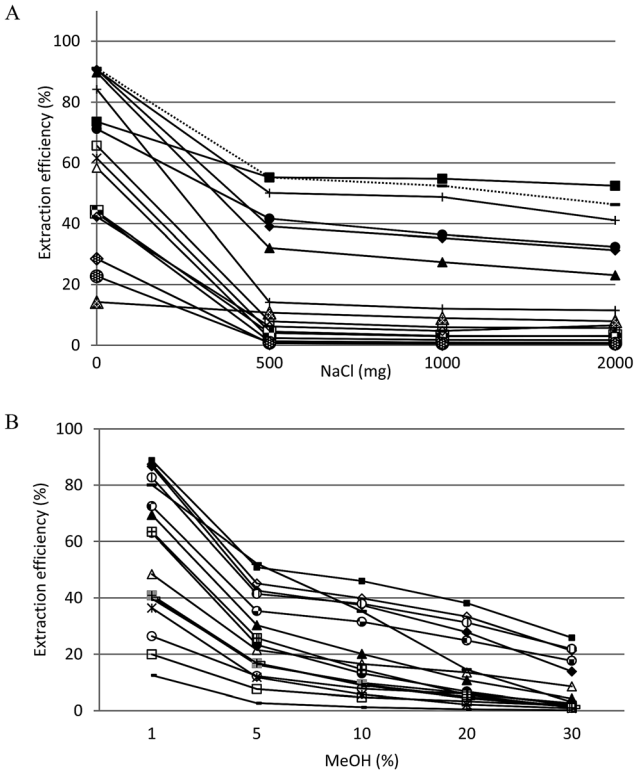

Fig. 2 Influence of addition of salt NaCl (0–10% (w/v)) (A) and organic solvent MeOH (B) on extraction efficiency for selected analytes (analytes are indicated in bold in Table 1).

considered as the reference value. As shown in Fig. 2B, the extraction efficiencies of analytes decreased proportionally with increasing addition of the organic solvent, and evidently any extra addition of MeOH was ineffective.

As the extraction efficiency was not enhanced by the addition of NaCl or MeOH, all subsequent SBSE experiments were performed only in the presence of 1% MeOH, which was inevitable as the consequence of spiking water blanks with standards dissolved in methanol during the preparation of test samples.

The influences of extraction time and temperature on extraction efficiency were then tested. Taking into account the initial optimised extraction conditions, samples were extracted at four temperature levels between room temperature (25 °C) and 50 °C. Elevated temperatures were achieved using a multi-position digital magnetic hotplate stirrer (Ika Werke, Germany). The relative increase or decrease in extraction efficiency for each analyte was calculated in relation to its extraction efficiency at room temperature (Fig. 3). The results showed that heating the samples improved the extraction efficiency and the highest temperature tested (50 °C) provided the best results for 12 of the tested analytes or at least comparable results to other tested temperatures; the exception was triclosan, where the extraction efficiency was lowest at 50 °C. Overall, extraction efficiencies were up to 6.5-fold higher at the highest temperature tested. In line with evidence on the stability of the analytes at higher temperatures (data not shown), the temperature of 50 °C was selected as the final optimised temperature for SBSE.

In the next step, the influence of the extraction time on extraction efficiency was evaluated by measuring sample response at regular intervals up to 180 min under predetermined conditions (Fig. 4). Since the maximal extraction efficiency for all analytes was reached after 150 min or less, any further prolongation of extraction time was not considered justified and 150 min was adopted as the most efficient compromise between method sensitivity and time consumption.

Lastly, to determine whether increasing the sample volume improved the method sensitivity, the extraction efficiency with sample volumes between 20 mL and 100 mL was measured (Fig. 5). The increase in the response for each analyte was calculated in relation to the response in 20 mL. Responses obtained by increasing the sample volume did not increase proportionally: using 100 mL of sample during the extraction process increased the relative response no more than 2.5-fold instead of an estimated 5-fold. As the extraction time was set at 1 hour, a complete equilibrium may not have been reached in all samples – especially the ones with higher volumes leading to apparent poor correlation between the sample volume and analyte responses. The fact that a higher sample volume would demand inconveniently long extraction times to reach equilibrium[23] and the above-mentioned poor correlation between the sample volume and extraction efficiency in initial experiments,

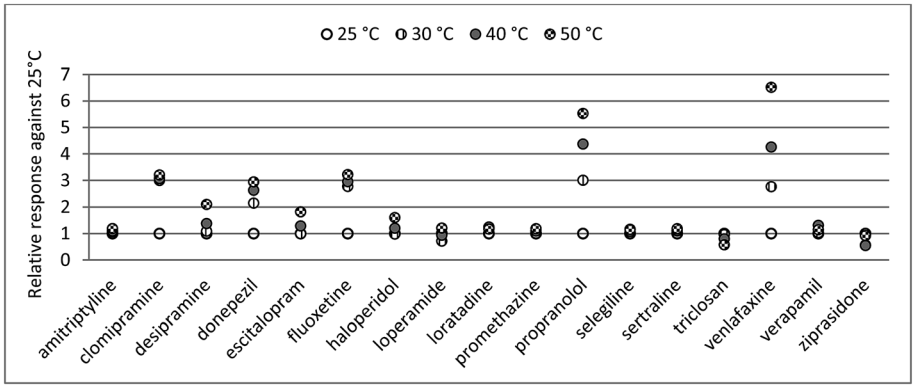

Fig. 3 Influence of the sample temperature on extraction efficiency for the selected analytes (compared with the reference temperature of 25 °C).

Open Access Article. Published on 11 August 2017. Downloaded on 10/23/2025 10:18:34 PM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

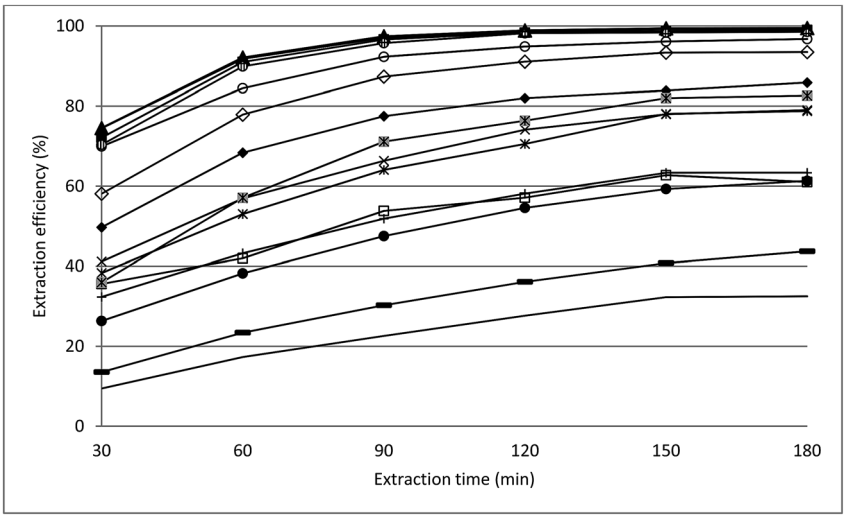

Fig. 4 Extraction efficiency as a function of extraction time for selected analytes (analytes are indicated in bold in Table 1).

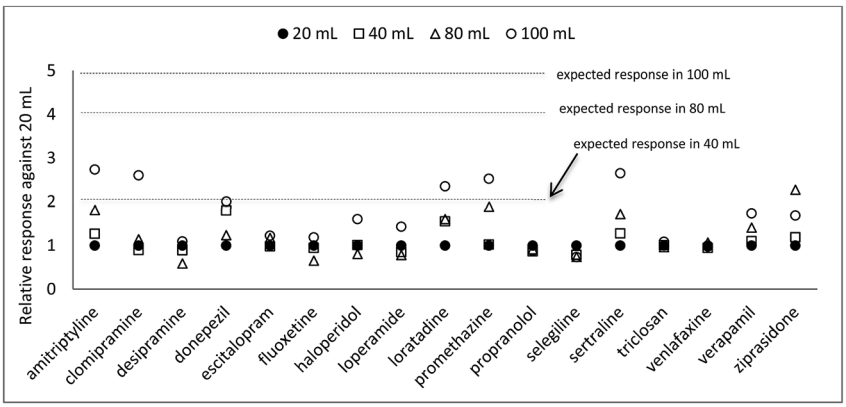

Fig. 5 Influence of the sample volume on analyte response (compared with responses in the reference volume of 20 mL).

a volume of 20 mL was selected as the optimum sample volume for further optimisation experiments.

3.2. Optimisation of the desorption procedure

Desorption parameters were evaluated under optimised extraction conditions of 20 mL sample at 10 μg L^{-1}, sample pH of 9, 50 °C and 990 rpm. Some parameters such as a desorption solvent volume of 2 mL, a stirring speed of 990 rpm, an extraction time of 60 min prior to desorption, and stir bar dimensions of 1 × 10 mm were fixed during the desorption process.

In order to enhance desorption, a few desorption solvents were examined (Fig. 6). Desorption conditions were set at 15 min and 50 °C. The tested organic solvents all contained acetonitrile, either solely or in combination with MeOH (50 : 50 v/v), or with

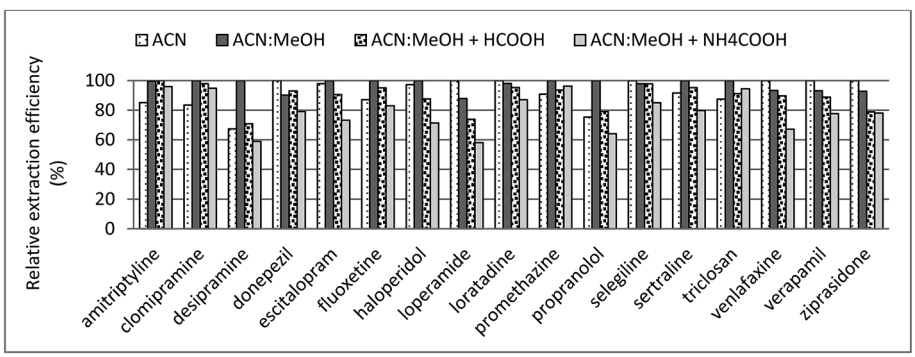

Fig. 6 Influence of the desorption solvent on extraction efficiency for selected analytes.

Open Access Article. Published on 11 August 2017. Downloaded on 10/23/2025 10:18:34 PM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

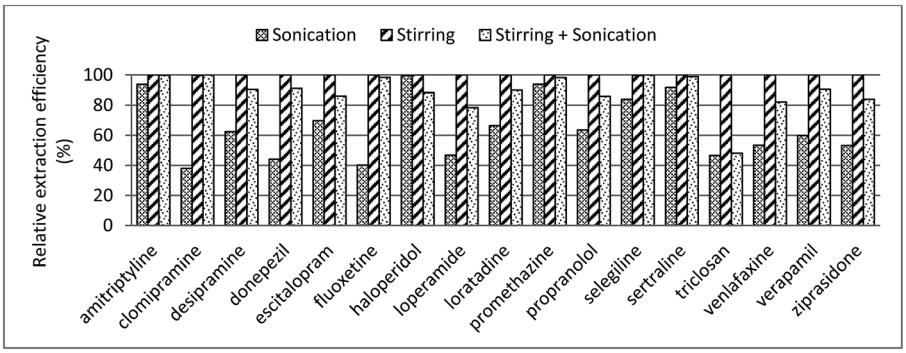

Fig. 7 Influence of desorption on extraction efficiency for selected analytes.

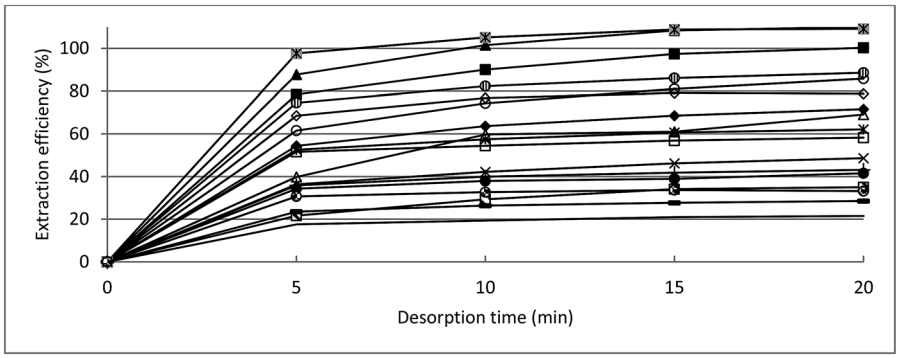

Fig. 8 Extraction efficiency as a function of desorption time for selected analytes (analytes are indicated in bold in Table 1).

the addition of formic acid (0.2% final concentration) or ammonium formate (2.5 mmol final concentration). All the solvents showed good and sufficient extraction efficiency (calculated as relative extraction efficiency) but the most promising (the best for 10 of 17 tested analytes) was a mixture of ACN and MeOH (50 : 50 v/v). This was selected as the optimal desorption solvent.

Following determination of the most suitable solvent for desorption, the best overall extraction efficiency was examined. Desorption was performed by stirring or sonication or by their combination (Fig. 7); the desorption time was 15 min with stirring at 50 °C and/or 15 min of sonication at room temperature. There was no solvent replacement as the combination of stirring and sonication was used. The resulting extraction efficiencies showed the superiority of stirring over sonication (calculated as the relative extraction efficiency), and their combination improved the extraction efficiency when compared with sonication alone. In some cases the improvement gained by additional sonication was negligible; therefore, stirring only was selected as the standard liquid desorption protocol for further experiments.

Next, extraction efficiency as a function of desorption time (*i.e.* desorption profiles) was evaluated between 5 and 20 min at 50 °C. The amount of analyte desorbed significantly increased during the first 5 min, followed by a slight increase between 5 and 15 min; further stirring did not result in any extra increase of analyte desorbed (Fig. 8). Even though the difference in the desorption efficiency between 5 and 15 min of stirring appears negligible, at 15 min the repeatability was improved and therefore 15 min of stirring was selected as optimal for further experiments.

Table 2 Influence of four consecutive desorption processes on extraction efficiency for selected analytes

	Extraction efficiency (%)							
Analyte	1st	2nd[a]		3rd[a]		4th[a]		Total
Amitriptyline	89.0	4.0	(4.5)	2.0	(2.2)	1.5	(1.6)	96.5
Clomipramine	41.2	2.4	(5.8)	1.2	(2.9)	0.9	(2.2)	45.7
Desipramine	64.1	1.4	(2.2)	0.5	(0.8)	0.3	(0.5)	66.4
Donepezil	34.4	1.1	(3.3)	0.6	(1.7)	0.4	(1.3)	36.5
Escitalopram	67.5	3.5	(5.2)	2.0	(2.9)	1.4	(2.0)	74.3
Fluoxetine	38.3	1.3	(3.4)	0.6	(1.7)	0.5	(1.3)	40.7
Haloperidol	92.6	4.3	(4.6)	2.1	(2.3)	1.6	(1.7)	100.6
Loperamide	33.3	0.8	(2.4)	0.4	(1.1)	0.3	(0.8)	34.7
Loratadine	79.6	1.7	(2.2)	1.0	(1.2)	0.7	(0.8)	82.9
Promethazine	71.8	0.3	(0.5)	0.2	(0.3)	0.3	(0.4)	72.6
Propranolol	17.9	0.8	(4.2)	0.4	(2.3)	0.3	(1.8)	19.4
Selegiline	69.9	3.5	(5.0)	1.5	(2.2)	0.7	(1.0)	75.5
Sertraline	85.2	5.4	(6.3)	2.6	(3.0)	2.0	(2.3)	95.1
Triclosan	43.8	6.0	(13.6)	3.0	(6.8)	1.8	(4.1)	54.5
Venlafaxine	22.7	0.8	(3.4)	0.4	(1.7)	0.3	(1.3)	24.2
Verapamil	62.6	1.1	(1.7)	0.5	(0.8)	0.4	(0.6)	64.6
Ziprasidone	11.5	0.2	(1.9)	0.0	(0.4)	0.1	(0.6)	11.9

[a] Relative enhancement compared with first desorption efficiency is shown in parentheses.

 This journal is © The Royal Society of Chemistry 2017

Open Access Article. Published on 11 August 2017. Downloaded on 10/23/2025 10:18:34 PM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

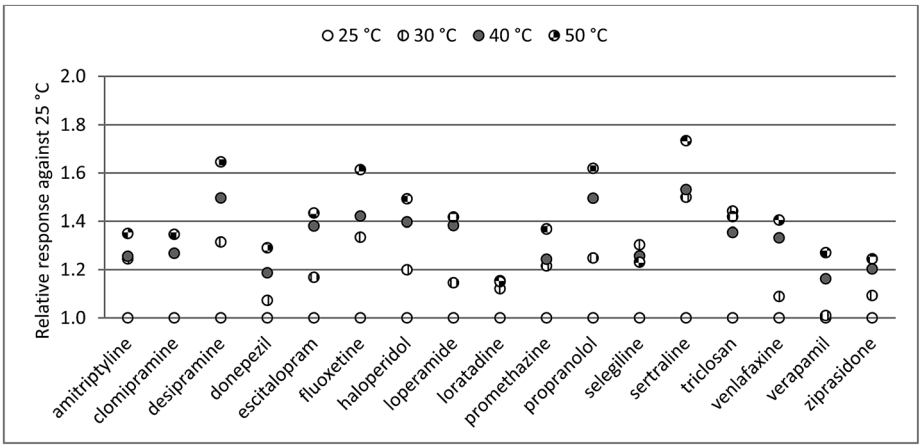

Fig. 9 Influence of the sample heating on extraction efficiency during the desorption process for selected analytes (compared with responses at the reference temperature of 25 °C).

Afterwards, stir bars were subjected to four consecutive desorption processes, each time with a fresh desorption solvent. The contribution of consecutive desorptions was calculated as the absolute or a relative enhancement compared with the extraction efficiency during the first desorption process. The individual relative enhancements were mostly below 5%, cumulatively up to 10% (Table 2); however, such desorption processes are time consuming and therefore additional desorption steps were omitted.

Lastly, the temperature of the desorption process was tested in a range from room temperature (25 °C) to 50 °C, maintaining the previously set optimal parameters (Fig. 9). For each analyte, the relative increase in the overall extraction efficiency was compared with the extraction efficiency at room temperature. As

Table 3 Compound-specific predicted properties and validation parameters

Analyte	log $K_{O/W}$[a]	pK_a[a]	Linear range (ng L^{-1})	R^2	LOQ (ng L^{-1})	Bias[b] (%)	RSD (%) $n = 6$	EE[c] (%)	Theoretical EE[d] (%)
Amitriptyline	5.10	9.76	1.25–1250	0.9996	1.25	−1.0	3.3	113	100
Azithromycin	3.03	9.57	1.25–500	0.9425	1.25	−18.0	20.2	24	73
Desipramine	4.02	10.02	1.25–500	0.9994	1.25	−3.7	1.5	101	96
Diazepam	2.63	2.92	1.25–500	0.9990	1.25	0.0	4.3	53	52
Donepezil	4.14	8.62	1.25–1250	0.9939	1.25	−17.8	0.9	92	97
Escitalopram	3.58	9.78	1.25–1250	0.9950	1.25	−6.4	1.7	81	90
Fluoxetine	4.09	9.80	1.25–1250	0.9995	1.25	−7.1	1.3	122	97
Haloperidol	3.70	8.05	1.25–1250	0.9953	1.25	−6.3	2.3	83	93
Imatinib	3.47	8.27	5.00–1250	0.9678	5.00	−7.0	13.2	7	88
Clomipramine	5.04	9.20	1.25–1250	0.9992	1.25	−0.3	2.9	116	100
Loperamide	4.44	9.41	1.25–500	0.9960	1.25	−2.8	4.3	65	99
Loratadine	4.80	4.33	1.25–1250	0.9993	1.25	−6.8	3.2	100	99
Metoprolol	1.80	9.67	1.25–500	0.9914	1.25	−18.7	3.3	7	14
Promethazine	4.52	9.05	1.25–1250	0.9990	1.25	−1.5	2.4	108	99
Propranolol	3.03	9.67	1.25–500	0.9958	1.25	−9.6	2.1	55	73
Raloxifene	5.45	7.95	5.00–1250	0.9959	5.00	−7.0	13.3	8	100
Risperidone	3.27	8.76	1.25–500	0.9981	1.25	−5.0	5.8	14	82
Selegiline	3.08	8.67	1.25–1250	0.9996	1.25	−6.0	2.1	90	75
Sertraline	5.06	9.85	1.25–1250	0.9996	1.25	−7.4	2.4	130	100
Tramadol	2.71	9.23	1.25–500	0.9971	1.25	−8.5	4.9	49	56
Triclosan	5.53	7.68	1.25–1250	0.9988	1.25	−18.7	11.6	45	100
Venlafaxine	2.69	8.91	1.25–500	0.9939	1.25	−0.9	3.6	60	55
Verapamil	5.23	9.68	1.25–1250	0.9996	1.25	−1.0	1.8	115	100
Ziprasidone	4.42	7.22	1.25–500	0.9967	1.25	−7.2	5.3	18	100
Bromazepam[e]	2.09	2.68	5.00–1250	0.6603	5.00	−9.2	8.2	2	24
Carbamazepine[e]	2.10	−3.80	5.00–500	0.9976	5.00	−2.3	2.7	2	24
Clonazepam[e]	2.76	1.86	5.00–500	0.9948	5.00	−4.2	2.0	1	59

[a] Predicted by ALOGPS 2.1 online software. [b] Data shown at LOQ. [c] Data shown at 250 ng L^{-1}. [d] Calculated using eqn (1); log $K_{O/W}$, partition coefficient (octanol/water); R^2, determination coefficient; LOQ, limit of quantification; RSD, relative standard deviation; EE, extraction efficiency. [e] Analytes in italics were excluded due to their too-low extraction efficiency.

Open Access Article. Published on 11 August 2017. Downloaded on 10/23/2025 10:18:34 PM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

expected, similarly to the results of testing the temperature effects during the extraction process, the higher the desorption temperature the better the extraction efficiency. For example, the relative extraction efficiencies were from 1.2-fold to 1.7-fold higher at 50 °C than at room temperature. Accordingly, a temperature of 50 °C was selected for further experiments.

3.3. Performance of the SBSE method for selected analytes

After optimisation of the entire process for selected analytes we then expanded the range of analytes (Table 1) and evaluated the linear range, determination coefficients, limits of quantification, imprecision and extraction efficiency (Table 3). The determined limits of quantification were between 1.25 and 5.00 ng L^{-1}. Determination coefficients were mainly higher than 0.99, with the exception of azithromycin, imatinib and bromazepam. Extraction efficiencies were calculated by comparison of the peak area obtained from the extracted and un-extracted samples at the corresponding concentration. The imprecision was expressed as the relative standard deviation of six replicates. If the extraction efficiency of an analyte was below the arbitrary defined 5% threshold, that analyte was defined as unsuitable for determination by SBSE and was excluded from the list of target analytes (italics in Table 3 for bromazepam, carbamazepine and clonazepam). Comparison of the theoretically predicted extraction efficiencies with those experimentally obtained confirmed good agreement for more than half of analytes, while for some analytes, there was a notable discrepancy; for instance, azithromycin, imatinib and risperidone with log $K_{O/W}$ values of 3.03, 3.47 and 3.27, respectively, near the lowest values of $K_{O/W}$ (2.7) that should result in successful extractions. In contrast, raloxifene and triclosan, with higher log $K_{O/W}$ values of 5.45 and 5.53, respectively, might be highly adsorbed on the surface of equipment or permanently onto the stir-bar stationery phase, which could explain the unexpectedly low extraction efficiencies (8% and 45%, respectively). In the case of ziprasidone, it is clearly evident how the pH of the wastewater sample can affect the extraction efficiency; the extraction efficiency is higher when measured at pH 7 (Fig. 1), as is the correlation between the theoretical and experimental values. The example of ziprasidone demonstrates the necessity for alteration of sample pH to provide high amounts of un-ionized analyte.

3.4. Performance of the SBSE method in wastewater samples

3.4.1 Application to wastewater effluent samples. The extraction efficiency of SBSE can be affected by the complex composition of the matrix.[27] A high level of organic matter in wastewater samples may impede the sorption process and, most importantly, may affect the ionization process at the LC-MS interface, producing a matrix effect. A standard addition method was chosen for simultaneous achievement of two important goals: (1) calibration of the method in each of the real sample matrices assuring constant ionization and response conditions in all samples (also tested in ultra-pure water) and (2) achievement of very low limits of quantification by extrapolating the calibration curves to zero standard addition. Analysis of effluent samples from WWTPs A–E demonstrated the presence of several pharmaceutical compounds. Analytes that were detected at least once are shown in Table 4. A total of 21 out of 24 analytes were detected; 12 were present in every wastewater sample tested. Particularly high concentrations of tramadol

Table 4 Concentrations of detected analytes in WWTP effluent samples A–E[a]

		Concentration (ng L^{-1})				
Analyte	Frequency (%)	A	B	C	D	E
Amitriptyline	100	29.0	17.5	7.8	22.0	9.8
Azithromycin	100	5290.0	320.2	188.1	5271.0	508.3
Desipramine	40	nd	nd	5.5	6.0	nd
Diazepam	60	nd	37.8	24.0	12.2	nd
Donepezil	40	nd	nd	20.0	17.5	nd
Escitalopram	100	54.1	186.5	89.2	92.7	72.4
Fluoxetine	40	nd	46.7	21.8	nd	nd
Haloperidol	100	1.3	11.1	10.1	4.2	5.0
Loperamide	60	nd	4.7	4.4	3.6	nd
Loratadine	100	7.2	21.7	10.6	8.6	6.1
Metoprolol	100	50.8	68.2	27.1	31.3	80.8
Promethazine	20	nd	nd	52.2	nd	nd
Propranolol	100	55.7	62.8	18.9	13.9	32.8
Raloxifene	80	<LOQ	nd	<LOQ	<LOQ	<LOQ
Risperidone	80	1.3	2.1	1.8	3.3	nd
Selegiline	40	nd	6.2	2.4	nd	nd
Sertraline	100	6.1	2.9	1.7	174.0	2.7
Tramadol	100	26 106.8	25 964.0	6123.2	8605.5	10 560.2
Triclosan	100	53.1	126.8	71.1	280.0	39.1
Venlafaxine	100	111.2	324.5	157.8	186.5	169.7
Verapamil	100	15.8	29.5	13.4	23.8	12.2

[a] nd, not detected; LOQ, limit of quantification.

 This journal is © The Royal Society of Chemistry 2017

Open Access Article. Published on 11 August 2017. Downloaded on 10/23/2025 10:18:34 PM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

Table 5 Concentrations of influent and effluent wastewater samples from five wastewater treatment plants and calculated removal efficiencies (%)[a]

	Concentration (ng L^{-1})														
	WWTP I			WWTP II			WWTP III			WWTP IV			WWTP V		
Analyte	In	Eff	Removal (%)	In	Eff	Removal (%)	In	Eff	Removal (%)	In	Eff	Removal (%)	In	Eff	Removal (%)
Amitriptyline	17.3	15.4	10.6	6.3	5.6	11.5	17.9	8.7	51.6	18.0	5.4	70.1	6.9	7.6	−10.1
Azithromycin	146.3	nd	100.0	434.8	276.8	36.3	3029.9	103.9	96.6	156.0	137.6	11.8	171.0	153.4	10.3
Desipramine	<LOQ	nd	100.0	3.2	<LOQ	—	1598.1	3.8	99.8	4.9	4.3	12.4	3.0	2.3	20.6
Diazepam	53.4	12.9	75.8	20.3	17.3	14.6	8.2	2.7	67.1	25.1	16.0	36.1	22.1	22.3	−1.0
Donepezil	10.7	8.9	17.0	18.3	11.5	37.1	10.9	7.1	34.7	16.9	14.7	13.2	11.1	10.1	8.4
Escitalopram	110.7	96.7	12.6	148.1	143.5	3.0	64.3	44.1	31.5	71.6	61.4	14.2	71.0	69.1	2.6
Fluoxetine	14.5	8.1	44.0	nd	nd	—	7.6	nd	—	nd	nd	—	17.6	nd	100.0
Haloperidol	13.5	8.1	40.3	57.5	4.6	92.0	19.4	nd	—	5.9	4.5	23.3	11.5	6.7	41.2
Clomipramine	<LOQ	nd	—	nd	nd	—	0.1	nd	—	nd	nd	—	<LOQ	1.7	—
Loperamide	11.0	<LOQ	—	<LOQ	<LOQ	—	nd	nd	—	3.6	<LOQ	—	<LOQ	2.1	—
Loratadine	2.6	3.3	−23.5	9.7	7.5	22.6	3.2	2.2	30.5	10.2	8.4	17.2	6.3	6.5	−2.3
Metoprolol	80.7	62.5	22.6	45.4	25.2	44.5	85.3	23.2	72.7	24.5	13.0	46.8	48.3	44.6	7.7
Propranolol	64.2	47.1	26.6	40.3	39.3	2.4	42.6	11.0	74.2	45.5	19.8	56.4	45.5	42.3	6.9
Promethazine	nd	nd	—	nd	nd	—	nd	nd	—	nd	nd	—	12.6	nd	—
Raloxifene	<LOQ	<LOQ	—	nd	nd	—	nd	nd	—	<LOQ	<LOQ	—	<LOQ	<LOQ	—
Risperidone	2.7	2.6	4.8	5.1	<LOQ	—	3.5	nd	100.0	2.7	<LOQ	—	6.7	nd	—
Selegiline	5.6	4.7	17.5	nd	nd	—	3.0	<LOQ	—	nd	nd	—	nd	nd	—
Sertraline	509.0	160.0	68.6	169.7	166.4	1.9	2.5	1.7	30.9	4.6	1.9	58.1	2.9	2.5	15.4
Tramadol	9488.8	13 910.4	−46.6	18 737.2	43 860.2	−134.1	6599.6	15 239.6	−130.9	11 392.0	6359.9	44.2	3230.9	8618.1	−166.7
Triclosan	325.3	61.9	81.0	47.1	11.7	75.2	106.8	38.8	63.7	3483.0	45.0	98.7	137.7	133.8	2.8
Venlafaxine	345.2	354.0	−2.5	274.3	550.6	−100.7	254.3	144.8	43.1	232.0	202.3	12.8	85.0	109.0	−28.3
Verapamil	29.4	26.3	10.5	64.1	49.1	23.4	13.7	1.6	88.5	12.2	12.0	1.9	21.3	20.9	1.7

[a] nd, not detected; —, not applicable; In, influent sample; Eff, effluent sample; WWTP, wastewater treatment plant; LOQ, limit of quantification.

Open Access Article. Published on 11 August 2017. Downloaded on 10/23/2025 10:18:34 PM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

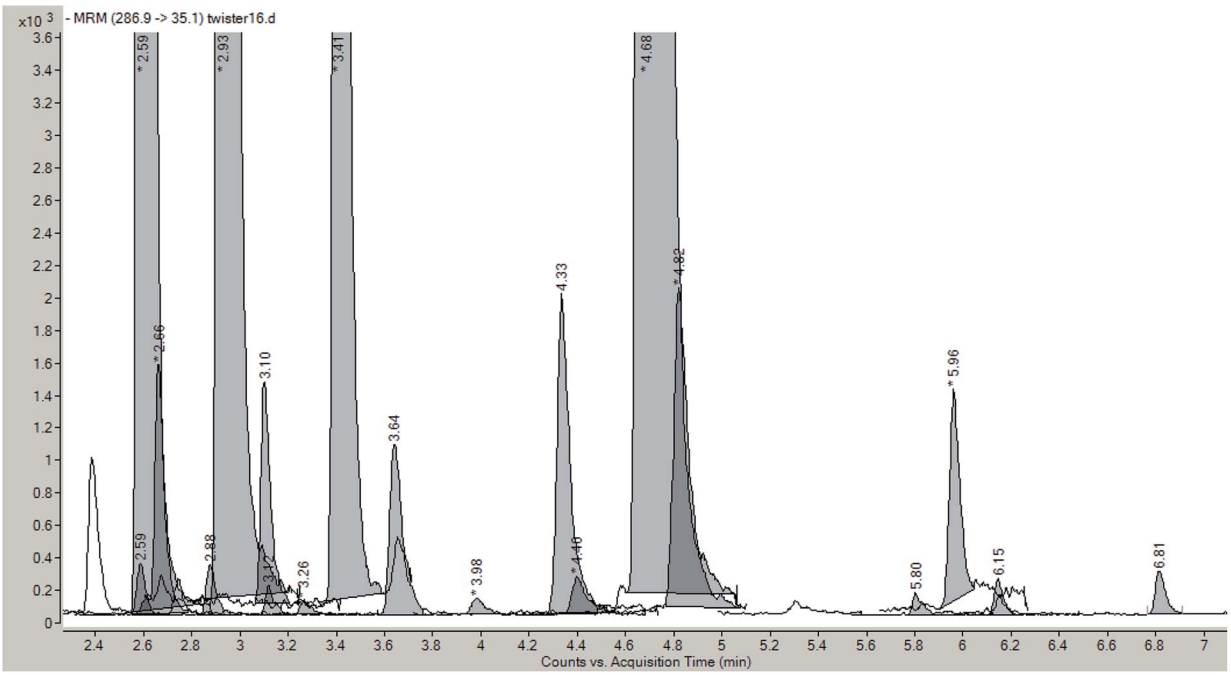

Fig. 10 Chromatogram of effluent wastewater sample from WWTP IV. Chromatographic peaks ranked according to their ascending retention times (min); tramadol (*2.59), metoprolol (2.59), azithromycin (2.66), risperidone (2.88), venlafaxine (2.93), propranolol (3.10), donepezil (3.11), raloxifene (3.26), escitalopram (3.41), haloperidol (3.64), desipramine (3.98), verapamil (4.33), amitriptyline (4.40), fluoxetine-d_5 (4.68), sertraline (4.82), loperamide (5.80), loratadine (5.96), diazepam (6.15) and triclosan (6.81).

Table 6 Characteristics of five WWTPs used for calculation of removal efficiency

WWTP	Population served (PE)	Flow (×1000 m^3 per year)	Technology	Removal efficiency[a] (%) average; median; range
I	4000	237	Secondary	42.1; 26.6; 4.8–100
II	4000	585	Secondary	30.4; 23.0; 1.9–75
III	55 000	1790	Tertiary	63.2; 65.4; 30.5–100
IV	50 000	7093	Tertiary	34.5; 23.3; 1.9–98.7
V	60 000	4702	Tertiary	19.8; 8.4; 1.7–100

[a] Data indicating induction of particular analyte during removal process (negative removal rate) were excluded; PE, population equivalent.

(>6 μg L^{-1}) were found in every sample; concentrations of azithromycin varied greatly from low to high levels. Overall, the majority of measured concentrations were below 200 ng L^{-1}.

3.4.2 Removal efficiency of selected WWTPs using the new SBSE method. The removal efficiencies of particular WWTPs were evaluated by analysing influent and effluent wastewater samples. Both types of sample were accessible in WWTPs I–V (Table 5). A representative chromatogram of effluent from WWTP IV is shown in Fig. 10. Removal efficiency was calculated using eqn (3).

The main focus of our paper is not to discuss the removal efficiency results but rather to emphasize the applicability of the method to a variety of purposes. According to the data, tramadol was measured in the highest concentrations and showed extreme resistance to the removal processes. Its effluent concentrations in four out of five WWTP samples actually increased when compared with influent concentrations. Similar results were obtained for venlafaxine. However, it should be noted that analyte concentrations may fluctuate significantly over time and that results may be highly dependent on the sampling technique. Numerous factors can significantly affect pollutant concentrations; for example, precipitations (rain) and hydraulic retention time of wastewater in a WWTP.

Such irregularities are minimised as far as possible in 24 h composite sampling, where concentrations measured reflect the best estimations of actual conditions, when on-line measurements are not available. Overall, removal efficiencies varied from 2% to complete (100%) elimination of the monitored pharmaceuticals present in the influent samples (Table 5).

A brief overlook found that the best removal efficiency was achieved in WWTP III, which is not surprising as it employs a moving-bed biofilm reactor (membrane bioreactor), whereas the other sampled WWTPs used fixed-bed treatments (conventional active sludge technology). For the pharmaceutical

Open Access Article. Published on 11 August 2017. Downloaded on 10/23/2025 10:18:34 PM.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

pollutants that were monitored, it could be claimed that biological treatment, either conventional or advanced, has a key role in terms of removal efficiency, since there was no significant difference between secondary (biological nutrient removal) and tertiary treatment (disinfection, mainly UV light) (Table 6).

4. Conclusion

The present work describes the development of the stir-bar sorptive extraction process and the new rapid analytical LC-MS/MS method for determination of target pharmaceutical compounds. The developed method was critically evaluated in a set of 27 representative pharmaceuticals from a wide range of therapeutic classes, based on their occurrence in WWTP effluents and their suitability for SBSE (log $K_{O/W}$ between 2.7 and 5.5). The optimised SBSE method provided recoveries above 80% for 12 analytes, and for 6 analytes they were between 45% and 65%, while for another 6 analytes the recoveries were below 25%. The method provided a wide linear range (mainly 1.25–1250 ng L^{-1}, $R^2 > 0.99$) with very low limits of quantification for all analytes (1.25–5.00 ng L^{-1}). The validated method was applied to wastewater samples to determine the presence of target analytes, and half of these were detected in all samples. In general, our results show that contamination of tested Slovene wastewaters by pharmaceutical pollutants is substantial and is readily measured using the newly developed technique. A further useful application of the method is in estimation of WWTP removal rates. Overall, the proposed method permits rapid and simple extraction of samples, which results in high throughput and a low workload per sample. The stir-bar technique represents a simple and efficient alternative to classical extraction methods for monitoring aquatic contamination, which is crucial for adequate protection of the environment against emerging pharmaceutical contaminants. Thus, the method is highly suitable for use in routine monitoring of certain pharmaceuticals in a variety of environmental water samples.

Conflicts of interest

No competing financial interests exist.

Acknowledgements

The research formed part of the LIFE+ project: LIFE13 ENV/SI/000466 LIFE PharmDegrade supported by the financial mechanism LIFE Environment of the European Commission.

References

1 W. C. Li, *Environ. Pollut.*, 2014, **187**, 193–201.
2 L. Cizmas, V. K. Sharma, C. M. Gray and T. J. McDonald, *Environ. Chem. Lett.*, 2015, **13**, 381–394.
3 F. David and P. Sandra, *J. Chromatogr. A*, 2007, **1152**, 54–69.
4 E. Baltussen, C. A. Cramers and P. J. Sandra, *Anal. Bioanal. Chem.*, 2002, **373**, 3–22.
5 H. A. Baltussen, P. J. F. Sandra, F. David and C. Cramers, *J. Microcolumn Sep.*, 1999, **11**, 737–747.
6 A. Prieto, O. Basauri, R. Rodil, A. Usobiaga, L. A. Fernandez, N. Etxebarria and O. Zuloaga, *J. Chromatogr. A*, 2010, **1217**, 2642–2666.
7 F. J. Camino-Sánchez, R. Rodríguez-Gómez, A. Zafra-Gómez, A. Santos-Fandila and J. L. Vílchez, *Talanta*, 2014, **130**, 388–399.
8 C. Blasco, G. Font and Y. Pico, *J. Chromatogr. A*, 2002, **970**, 201–212.
9 M. Kawaguchi, R. Ito, K. Saito and H. Nakazawa, *J. Pharm. Biomed. Anal.*, 2006, **40**, 500–508.
10 M. He, B. Chen and B. Hu, *Anal. Bioanal. Chem.*, 2014, **406**, 2001–2026.
11 D. Benanou, F. Acobas, M. R. Deroubin, F. David and P. Sandra, *Anal. Bioanal. Chem.*, 2003, **376**, 69–77.
12 C. Nazyropoulou and V. Samanidou, *Bioanalysis*, 2015, **7**, 2241–2250.
13 C. Almeida and J. M. Nogueira, *J. Pharm. Biomed. Anal.*, 2006, **41**, 1303–1311.
14 M. Kawaguchi, Y. Ishii, N. Sakui, N. Okanouchi, R. Ito, K. Inoue, K. Saito and H. Nakazawa, *J. Chromatogr. A*, 2004, **1049**, 1–8.
15 Y. Hu, Y. Zheng, F. Zhu and G. Li, *J. Chromatogr. A*, 2007, **1148**, 16–22.
16 C. Hu, M. He, B. Chen, C. Zhong and B. Hu, *J. Chromatogr. A*, 2013, **1310**, 21–30.
17 X. Huang, J. Lin, D. Yuan and R. Hu, *J. Chromatogr. A*, 2009, **1216**, 3508–3511.
18 E. Van Hoeck, F. Canale, C. Cordero, S. Compernolle, C. Bicchi and P. Sandra, *Anal. Bioanal. Chem.*, 2009, **393**, 907–919.
19 N. Gilart, N. Miralles, R. M. Marce, F. Borrull and N. Fontanals, *Anal. Chim. Acta*, 2013, **774**, 51–60.
20 A. R. Silva, F. C. Portugal and J. M. Nogueira, *J. Chromatogr. A*, 2008, **1209**, 10–16.
21 I. Aparicio, J. Martín, J. L. Santos, J. L. Malvar and E. Alonso, *J. Chromatogr. A*, 2017, **1500**, 43–52.
22 N. Gilart, R. M. Marcé, F. Borrull and N. Fontanals, *TrAC, Trends Anal. Chem.*, 2014, **54**, 11–23.
23 D. Bratkowska, R. M. Marcé, P. A. G. Cormack, F. Borrull and N. Fontanals, *Anal. Chim. Acta*, 2011, **706**, 135–142.
24 S. S. Lakade, F. Borrull, K. G. Furton, A. Kabir, N. Fontanals and R. M. Marce, *Talanta*, 2015, **144**, 1342–1351.
25 N. Gilart, P. A. Cormack, R. M. Marce, F. Borrull and N. Fontanals, *J. Chromatogr. A*, 2013, **1295**, 42–47.
26 A. Prieto, O. Zuloaga, A. Usobiaga, N. Etxebarria and L. A. Fernández, *Anal. Bioanal. Chem.*, 2008, **390**, 739–748.
27 J. B. Quintana, R. Rodil, S. Muniategui-Lorenzo, P. López-Mahía and D. Prada-Rodríguez, *J. Chromatogr. A*, 2007, **1174**, 27–39.