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Targeted multi-analyte UHPLC-MS/MS methodology for emerging contaminants in septic tank wastewater, sludge and receiving surface water†

Maike Wilschnack, [a] Bess Homer, [b] Elise Cartmell, [b] Kyari Yates [a] and Bruce Petrie *[a]

Septic tanks treat wastewater of individual houses and small communities (up to 2000 people in Scotland) in rural and semi-urban areas and are understudied sources of surface water contamination. A multi-analyte methodology with solid phase extraction (SPE), ultra-sonic extraction, and direct injection sample preparation methods was developed to analyse a comprehensive range of emerging contaminants (ECs) including prescription and over-the-counter pharmaceuticals and related metabolites, natural and synthetic hormones, and other human wastewater marker compounds in septic tank influent and effluent, river water, suspended solids, and septic tank sludge by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS). The number of quantifiable compounds in each matrix varied from 68 in septic tank wastewater to 59 in sludge illustrating its applicability across a range of matrices. Method quantification limits were 2.9×10^{-5}–1.2 μg L^{-1} in septic tank influent, effluent and river water, with ≤0.01 μg L^{-1} achieved for 60% of ECs in all three water matrices, and 0.080–49 μg kg^{-1} in sludge. The developed method was applied to a septic tank (292 population equivalents) and the receiving river in the North-East of Scotland. Across all samples analysed, 43 of 68 ECs were detected in at least one matrix, demonstrating the method's sensitivity. The effluent concentrations suggest limited removal of ECs in septic tanks and a potential impact to river water quality for some ECs. However, further monitoring is required to better appreciate this. The developed methodology for a wide variety of ECs in a range of liquid and solid phases will allow, for the first time, a comprehensive assessment of ECs fate and removal in septic tanks, and their impact to surface water quality.

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Introduction

Over the past years, a large variety of emerging contaminants (ECs), such as prescription or over-the-counter pharmaceuticals and related metabolites, natural and synthetic hormones, and other human wastewater marker compounds (*e.g.*, caffeine), have been reported in various water sources worldwide in the ng to μg L^{-1} range.[1–5] Due to their incomplete removal in conventional (biological) wastewater treatment, and ubiquitous presence in influent, treated wastewater discharges are considered the main entry source of ECs into the environment.[6–8]

So far, research has focused on centralised wastewater treatment works (WWTWs) and their receiving surface waters.[1,7,9–11] However, it is conservatively estimated that 9% of the Scottish population, are served by a public or privately owned septic tank.[12–14] Septic tanks are typically located in rural and semi-urban areas and treat wastewater from individual houses and small communities (up to 2000 people in Scotland).[4,14] In a watertight underground tank, often designed as a series of rectangular chambers, heavy solids settle as sludge to the bottom, while oil, grease and lighter solids float to the top.[12] The sludge and scum need to be removed from the tank (typically every few months to every few years), and transported to a centralised WWTWs for further treatment.[15] The septic tank effluent might be further treated, for example through subsoil infiltration systems, before being released into the ground or a nearby water body.[12,16]

Septic tank effluents can contain ECs in higher concentrations than in centralised WWTWs.[13,17] For instance, Stanford and Weinberg[17] reported the active ingredient in hormonal contraceptives 17α-ethinylestradiol up to 0.4 μg L^{-1} in a septic tank effluent serving a boarding school for girls, which is 4- to

[a] School of Pharmacy and Life Sciences, Robert Gordon University, Aberdeen, AB10 7GJ, UK. E-mail: b.r.petrie@rgu.ac.uk; Tel: +44 (0)1224 262824

[b] Scottish Water, 55 Buckstone Terrace, Edinburgh EH10 6XH, UK

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400-times higher than centralised WWTWs influents.[18] In a septic tank, ECs can be removed through the physical separation of the sludge and scum, when they are bound to particles or oil, and *via* anaerobic biodegradation.[19] However, there is little information on the performance of septic tanks for the removal of ECs, and the effect of septic tank discharges to water quality. To this date, most studies focused on a few compounds only (maximum = 22),[13,20–24] and there is a lack of multi-analyte methods for the analysis of ECs in septic tanks.

Most commonly, ECs are analysed by reversed-phase liquid chromatography coupled to tandem mass spectrometry as a highly sensitive and selective detector (LC-MS/MS).[9–11] It is a suitable approach to determine low concentrations of ECs in the presence of other organics at comparatively high concentrations in complex environmental matrices, such as wastewater.[9,11,19,25] Typically, solid phase extraction (SPE) is used to enrich, isolate and/or purify the target ECs, with reversed-phase hydrophilic-lipophilic balanced (HLB) polymeric sorbents being the most common.[3,26,27] Although a wide range of compounds can be analysed with HLB sorbents, recoveries are low for very polar compounds such as the antidiabetic drug metformin.[28–30] Hence, for very polar ECs, direct injection is proposed as a second sample preparation method.[30] In wastewater, different ECs are present in a wide concentration range from low ng L^{-1} (*e.g.*, ciprofloxacin) to high μg L^{-1} (*e.g.*, metformin).[29,31,32] As septic tanks are used by fewer people than centralised WWTWs, the variations in concentration and detection of ECs in effluents can be higher.[13] The wide concentration range, poses a challenge for 'SPE-only' methods, as it requires the dilution and re-analysis of samples following data processing, when concentrations are above the calibration range.[13,33] At the same time, method detection limits for ECs present at lower concentrations might not be reached by direct injection. Analysing each sample by direct injection and after SPE, allows the determination of a comprehensive range of ECs of different polarities over a wide concentration range without the need for further sample processing (*e.g.*, dilution) and re-analysis.

Environmental samples are typically filtered prior to analysis to remove suspended solids. Due to the extra effort associated with analysing both matrices, most studies focus on the aqueous part of the sample only.[7,34] However, ECs can adsorb to solid particulate matter, and desorb again once in the environment.[1,35] Thus, analysing the aqueous part of the sample only leads to underestimation of the total concentration in the sample.[11] Furthermore, in wastewater treatment, ECs can also adsorb to sludge, and for instance enter the environment when sludge is applied in agriculture.[36] Most studies analysed ECs only in the liquid phase of septic tank effluent,[20,21] and the receiving water bodies.[13,22–24,37] Developing a multi-analyte method for the analysis of ECs in septic tank influent and effluent, including suspended solids, sludge, and the receiving surface water will allow a more accurate assessment of the performance of septic tanks for the removal of ECs and their effect to water quality. The most common methods for the extraction of ECs from solid environmental matrices, such as suspended solids or sludge, are microwave accelerated extraction (MAE), pressurised liquid extraction (PLE), and ultra-sonic extraction (USE).[1,11,30,38] There is little difference found in the performance and extraction efficiency of the three methods.[36,39,40] MAE and PLE are easier to automatise than USE. However, USE offers advantages due to low costs and easy operation for effective extraction of ECs from solid environmental samples.[1,36]

Therefore, the aim of the study was to develop a comprehensive multi-analyte methodology with SPE, USE, and direct injection as sample preparation methods to analyse a broad range of ECs in septic tank influent and effluent, river water, suspended solids, and septic tank sludge by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS). The developed method was applied to a septic tank and the receiving surface water in a rural area in the North-East of Scotland.

Materials and methods

Materials

A total of 68 ECs (prescription or over-the-counter pharmaceuticals and related metabolites, natural and synthetic hormones, and other human wastewater marker compounds) were selected for method development (S1: Table S1†). The selection included those identified in prioritisation schemes by the European Union (EU) and the United Kingdom (UK),[41–46] and those which posed the greatest threat to Scotland based on environmental risk assessment calculations (S2). Chemical names and properties of selected ECs and where they were obtained from are detailed in Tables S1 and S2.† Water was produced at ultra-pure quality in the laboratory (resistivity = 18.2 MΩ cm at 25 °C, PurA-Q18.2, LabPro, European Instruments, Oxford, UK), and methanol (HPLC grade, ≥99.9%) was purchased from Fisher Scientific (Loughborough, UK). Formic acid (≥99.0%, Fisher Scientific), ammonium formate (≥99.0%, Sigma Aldrich, Gillingham, UK), ammonium fluoride (NH_4F, ≥99.99%, Sigma Aldrich), and ammonium hydroxide (NH_4OH, 35%, Fison Instruments Ltd, Glasgow, UK) were used as mobile phase buffers and in ultrasonic extraction. Oasis HLB (60 mg, 3 mL; and 200 mg, 6 mL) SPE cartridges were purchased from Waters (Manchester, UK). Polytetrafluoroethylene (PTFE), cellulose acetate (CA), polyvinylidene fluoride hydrophilic (PVDF-HL), and polyvinylidene fluoride hydrophobic (PVDF) Q-Fil syringe filter (13 mm, 0.22 μm) from Greyhound (Birkenhead, UK) were received from Crawford Scientific Ltd (Strathaven, UK) and glass fibre filter (GF/F) discs (0.7 μm, 47 mm) were purchased from Fisher Scientific.

Liquid samples (1 L septic tank influent, septic tank effluent, and river water), used during method development and validation were collected in the North-East of Scotland in polypropylene bottles in summer 2021. Samples were transported to the laboratory and frozen within 1 h after collection. The septic tank sludge (0.5 L) was collected in November 2021 with a custom-made polyvinylchloride sludge sampler (Fig. S1†), and frozen until processing.

Sample preparation of liquid samples

In a preliminary study, four different syringe filters were tested to minimize loss of ECs during the filtration step. Wastewater samples spiked with 60 ECs (available at the time of the experiment) were filtered through PVDF-HL, PTFE, CA, and PVDF syringes to determine any losses.

The SPE method (Fig. 1) was developed based on a previous method for the analysis of septic tank effluent and river water.[13] Initially, the samples were filtered under vacuum with a GF/F filter. Oasis HLB cartridges (3 mL, 60 mg) were conditioned under gravity with 2 mL methanol and 2 mL water for equilibration at a flow rate of 1 mL min^{-1}. 50 mL wastewater, and 100 mL river water, were spiked with a 50 μL isotopic labelled surrogate working mix ($c = 100$ μg L^{-1}), mixed and loaded onto the cartridges using vacuum at a flow rate of 5 mL min^{-1} and then dried for 20 min. The samples were eluted under gravity with 4 mL methanol at a flow rate of 1 mL min^{-1}, and the solvent was evaporated at 40 °C under nitrogen.[13] The dried residue was then redissolved in 500 μL water/methanol (95/5, v/v), and filtered through a PVDF-HL syringe filter prior to UHPLC-MS/MS injection. For direct injection, environmental samples were filtered through a PVDF-HL syringe filter, before 450 μL of the sample was spiked with 50 μL isotopic labelled surrogates ($c = 100$ μg L^{-1}).

Extraction of solid matrices by ultra-sonic extraction

The sludge was frozen and freeze dried using a Heto Drywinner freeze dryer by Copley. The selected ECs were extracted from solid matrices with a Clifton Range ultra-sonic water bath (280 W, 50/60 Hz) using three extraction cycles similar to that described by Al-Khazrajy and Boxall.[25] Briefly, 0.1 g of freeze-dried sludge (dry weight) was weighed into a 10 mL polypropylene centrifuge tube, spiked with 50 μL isotopically labelled surrogates ($c = 100$ μg L^{-1}) and left overnight. In the first cycle, 2 mL of 2% NH_4OH in methanol was added. The suspension was vortexed, ultra-sonicated for 15 min at 50 °C, and centrifuged at 2260 g for 15 min. The supernatant was collected in a 50 mL Duran® glass bottle. The extraction was repeated using 2 mL of 2% formic acid in methanol and then 2 mL of methanol. The combined supernatants were filtered through a wet GF/F disc and diluted with water to 100 mL (methanol < 5%). The extracts were cleaned up by Oasis HLB SPE cartridges (6 mL, 200 mg) following the same procedure as described for the extraction of liquid samples. For sludge samples, the reconstituted extract was centrifuged for 10 min at 17 000*g* prior to filtration through a PVDF-HL syringe filter.

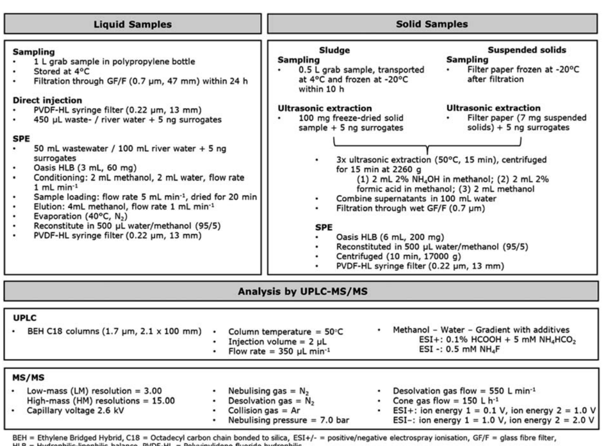

Fig. 1 Overview of analytical workflow from sample preparation to analysis, for liquid and solid samples by ESI+ and ESI− methods.

Liquid chromatography tandem mass spectrometry

Samples were analysed with UHPLC-MS/MS using an ACQUITY UPLC system from Waters (Waters Corporation, Milford, MA) with a Xevo TQ-XS Triple Quadrupole Mass Spectrometer. Electrospray ionisation (ESI) was performed in both positive and negative modes with a capillary voltage of 2.6 kV, 3.00 low-mass (LM) resolutions, and 15.00 high-mass (HM) resolutions. The nebulising and desolvation gas was nitrogen, and the collision gas was argon. The gas temperature was 400 °C with a desolvation gas flow of 550 L min^{-1}, and a nebulising pressure of 7.0 bar. The cone gas flow was 150 L h^{-1}. The optimised ion energies were ion energy $1 = 0.1$ V and ion energy $2 = 1.0$ V in positive ionisation mode, and ion energy $1 = 1.0$ V and ion energy $2 = 2.0$ V in negative ionisation mode, respectively.

Two different mobile phases were used for the analysis of basic and acidic compounds in positive and negative ionisation, respectively.[30] Different additives to the mobile phase were tested. If not otherwise stated, the parameters were identical in both methods. Chromatographic separation was performed using reversed-phase ACQUITY UPLC Ethylene Bridged Hybrid (BEH) C18 columns (1.7 μm, 2.1 × 100 mm, Waters). The column temperature was kept constant at 50 °C. The injection volume was 2 μL and the flow rate was 350 μL min^{-1}. A methanol–water-gradient along with additives was used as the mobile phase (S4: Table S3†). Additives were 5 mM ammonium formate and 0.1% formic acid in the positive ionisation method, and 0.5 mM NH_4F in the negative ionisation method.

Instrumental performance

The instrumental performance was validated in terms of detection and quantification limits, linearity, intra- and inter-day precision, and accuracy. All samples were spiked with isotopically labelled analytes as surrogate to correct for matrix effects and analyte loss during sample preparation ($c = 10$ μg L^{-1} at injection).[13]

The instrument detection (IDL) and quantification limits (IQL) for each analyte were determined by the lowest concentration with a signal-to-noise ratio (S/N) ≥ 3 or ≥ 10, respectively. Linearity was established through the injection of a range of standards between 0.05 and 100 μg L^{-1} (S8: eqn S3†).

Intra-day precision and accuracy were determined by injecting standards at concentrations of 1, 10, and 50 μg L^{-1} in triplicate within 24 h (S8: eqn S4 and S5†). This was repeated every 24 h over 3 days to establish inter-day precision and accuracy.

Method performance

The method performance was assessed for septic tank influent and effluent wastewater, river water, and sludge, for detection and quantification limits, matrix effects, absolute and relative recoveries, precision, and accuracy. Samples were prepared at three concentrations in triplicate. Spike concentrations were 1, 10, and 50 μg L^{-1} for direct injection of influent, effluent, and river water; 0.01, 0.1, and 0.5 μg L^{-1} for SPE of influent and effluent; 0.005, 0.05, and 0.25 μg L^{-1} for SPE of river water, and 50, 250, 500 μg kg^{-1} for sludge (S5: Table S4†). Prior to spiking with ECs, samples were spiked with isotopically labelled ECs only and analysed to determine the analyte concentrations in the environmental samples. Water samples were analysed by direct injection and SPE (S5: Table S4†).

Absolute (REC_{abs}) and relative recoveries (REC) were calculated following eqn (1) and (2) from peak areas (A) and area ratios (ar) of spiked and unspiked (US) samples and standards (std), respectively.

$$\mathrm{REC_{abs}} = \frac{(A_{\mathrm{spiked-}} - A_{\mathrm{US}})}{A_{\mathrm{std}}} \times 100\% \tag{1}$$

$$\mathrm{REC} = \frac{(\mathrm{ar}_{\mathrm{spiked-}} - \mathrm{ar}_{\mathrm{US}})}{\mathrm{ar}_{\mathrm{std}}} \times 100\% \tag{2}$$

The method detection (MDL) and quantification limits (MQL) were calculated for each analyte from the IDL and IQL, respectively, the recovery and concentration factor c_F using eqn (3) and (4).

$$\mathrm{MDL} = \frac{(\mathrm{IDL} \times 100)}{\mathrm{REC} \times c_{\mathrm{F}}} \tag{3}$$

$$\mathrm{MQL} = \frac{(\mathrm{IQL} \times 100)}{\mathrm{REC} \times c_{\mathrm{F}}} \tag{4}$$

REC and c_F were specific for each matrix and sample preparation method. c_F was 0.9 for direct injection, 100 for septic tank influent and effluent in the SPE method, and 200 for river water in the SPE method. For solid matrices, c_F is replaced with a conversion factor of 0.2 g mL^{-1}, based on the extraction of 0.1 g sludge.

The relative standard deviation of the replicates was calculated for method precision. Accuracies were determined from the percentage deviation of the concentrations added to the samples from the calculated concentrations.

To ensure instrumental and method performance, blanks and quality control standards with concentrations of 1, 10, and 50 μg L^{-1} were injected before and after every batch of samples.

Application to a septic tank and receiving river

A septic tank and the receiving surface water in a rural area in the North-East of Scotland was investigated. The septic tank serves 292 population equivalents, with no tourist impact and around 8% non-household contribution.[14] The nominal dilution of the septic tank discharge into the river was calculated (S6: eqn S1 and S2†). The receiving river mainly flows through agricultural land, with single houses and smaller villages along side. In the catchment area, 1% of land use is classified as urban.[47] The largest settlement in the catchment area with a population of 3140 (mid-2020 estimate)[48] is located roughly 7 km upstream of the studied septic tank. It is served by a secondary biological WWTW that discharges into the river.

Sampling was conducted on the 10th of November 2021. Grab samples (1 L) were collected in polypropylene bottles at the influent and effluent point of the septic tank, in the river upstream and downstream of the septic tank discharge point at a minimum distance of five river widths, and from the sludge. Samples were transported to the laboratory at 4 °C. Liquid samples were filtered through 0.7 μm GF/F membrane filters within 24 h, processed as described previously, and analysed within 48 h. The filter papers were frozen at −20 °C until processing. The solids were extracted by ultra-sonic extraction following the previous description. All samples were prepared in duplicate.

Results and discussion

Liquid chromatography tandem mass spectrometry

All ECs were analysed using multiple reaction monitoring (MRM) transitions. The protonated ($[M + H]^+$) or deprotonated molecular ion ($[M - H]^-$) was monitored in ESI− and ESI+ mode, respectively. Following EU guidelines,[49] two MRM transitions were monitored for most ECs (one in the case of isotopic labelled surrogates), using the fragment with the highest response for quantification and the fragment with the second highest response for confirmation. Ion ratios were monitored. In accordance with the literature, only one stable fragment was found for ibuprofen, gemfibrozil and lidocaine,[3,30,50] which is considered semi-quantitative (optimised MS/MS parameters in S7: Table S5†).

Following optimisation of MS/MS parameters for all compounds the chromatography methods were developed using a methanol–water-gradient with additives as the mobile phase and a reversed-phase BEH C18 column. Two different mobile phases were used, since basic and neutral compounds are best analysed in positive ionisation mode from acidic solutions, whereas acidic compounds are more efficiently analysed in negative ionisation mode from basic solutions.[51] Different additives were tested to optimise separation, peak shape, and sensitivity. In the positive ionisation mode for the analysis of basic ECs, the use of 5 mM ammonium acetate with 0.1% formic acid was compared to using 5 mM ammonium formate and 0.1% formic acid. While the choice of ammonium salt generally had little effect on the chromatography, the peak shape improved substantially with ammonium formate in the mobile phase for metformin, guanylurea, and paracetamol. The highly polar drug metformin and its aerobic bacterial metabolite guanylurea are more suited to analysis by hydrophilic interaction chromatography (HILIC) columns,[2,29] but satisfactory chromatography could be achieved under reversed phased conditions with ammonium formate as an additive.

In the negative ionisation mode, ammonium hydroxide (0.1%) and different concentrations of NH_4F (0.1, 0.5 and 1 mM) in a methanol–water-gradient were considered to enable the analysis of estrogens together with acidic drugs.[30,52] Overall, NH_4F resulted in greater peak areas and sharper peaks than ammonium hydroxide. Improved sensitivity with NH_4F might be due to the strong basicity of the fluoride anion, and hence increased deprotonation of ECs in the gas phase.[53] Lower NH_4F concentration increased the sensitivity for estrogens, with optimum concentrations being 0.1 mM. However, decreased sensitivity for ibuprofen was noted. Since estrogens are expected to be found in significantly lower concentrations in wastewater and river water compared to ibuprofen,[6] 0.1 mM NH_4F was considered for further method development. However, in wastewater a contamination was present in the 17α-ethinylestradiol MS/MS spectrum at the same retention time. This was resolved from the 17α-ethinylestradiol peak by increasing the NH_4F concentration to 0.5 mM. With the reversed-phase BEH C18 column, good separation, sensitivity, and peak shape was achieved for all compounds using a methanol–water-gradient along with 5 mM ammonium formate and 0.1% formic acid in the ESI+ method, and 0.5 mM NH_4F in the ESI− method (Fig. 2).

Instrument performance

The IDL and IQL were determined as the lowest concentration with a S/N ≥ 3 and ≥ 10 and ranged from 0.002 to 1 μg L^{-1}, and from 0.005 to 5 μg L^{-1}, respectively (S8: Table S6†). For the majority of compounds, IQL ≤ 0.5 μg L^{-1} was achieved. A wide range of IQLs is commonly observed in multi-analyte methods for compounds with a variety of physicochemical properties, and similar to what has been reported before.[3,27,28,30]

Linearity was established through the injection of standards at concentrations between 0.05 and 100 μg L^{-1} (500 μg L^{-1} for paracetamol, ibuprofen, and metformin due to their higher concentrations in wastewater). A linear regression model was fitted (S8: eqn S3†), and the R^2 was calculated. For the compounds without the isotopically labelled EC, a different deuterated surrogate was assigned (S8: Table S6†). The choice was based on retention time, structural similarity, and eventually linearity. The linear dependency was in range of $0.938 \leq R^2 \geq 1.000$ (S8: Table S6†). Approximately two thirds of the ECs, 52 compounds in the positive method and four compounds in the negative method, have R^2 values ≥ 0.997. Atorvastatin and miconazole were calibrated externally using peak area as there was no suitable deuterated surrogate. Calibrations with $R^2 \geq 0.991$ were sufficient for accurate quantification, as indicated by the other instrumental performance criteria. Published studies for multi-analyte analysis of pharmaceuticals in wastewater accept $R^2 \leq 0.990$.[11] Miconazole, clotrimazole, and climbazole, have $R^2 < 0.980$, most likely due to the absence of suitable deuterated surrogate, and were analysed semi-quantitatively. Most compounds were linear over the whole concentration range from 0 to 100 μg L^{-1}.

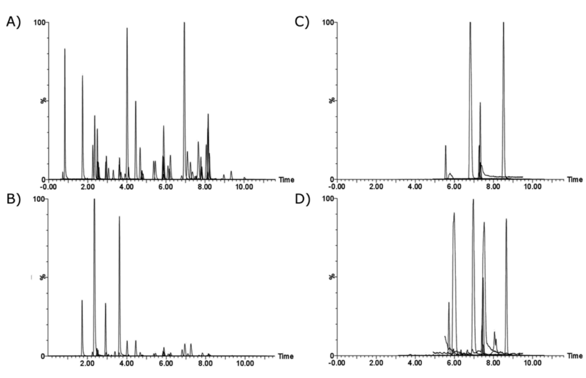

Fig. 2 Chromatograms (quantification MRM) of septic tank effluent spiked at $c = 62.5$ μg L^{-1} and analysed by direct injection (A and C), and at $c = 0.5$ μg L^{-1} and analysed by SPE (B and D) (details in S5: Table S4†), analysed with the ESI+ (A and B) and ESI− (C and D) method.

Intra- and inter-day accuracy and precision (S8: Table S7†) were determined by injecting three standards ($c = 1$ μg L^{-1}, 10 μg L^{-1}, and 50 μg L^{-1}) three times within 24 h, and repeatedly every 24 h over three days (S8: eqn S4 and S5†). In multi-analyte methods, accuracies are generally expected to be within an ideal range of 90–110%, or within the accepted range 80–120%.[10,28,54] A total of 63 compounds were accurate within the range of 90–110% in most samples above the IQL, with little or no difference between the intra- and inter-day accuracy ($p > 0.05$, S8: Table S7†). The remaining five compounds also have intra-day accuracies from 90% to 110% in most samples, but inter-day accuracies were 80% to 120% in most samples ($0.004 \geq p \leq 0.046$). As repeating the calibration every day is time-consuming, few ECs with inaccuracies are accepted in multi-analyte methods.[28] QC standards were therefore injected with every batch to ensure accuracies stay within the accepted range. Calibrations were repeated after the mass spectrometer was turned off for an extended period of time, at least once a year, or if the QC data fell out with the performance data.

In general, relative standard deviations ≤ 10% are expected in the instrumental performance. However, higher standard deviations ≥ 20% are accepted for few ECs in multi-analyte methods, as long as other validation parameters are suitable.[11,27] In the developed instrumental method, 50 ECs were very precise over all concentrations studied above the IQL with a relative standard deviation ≤ 10% except the occasional one concentration in the intra- and inter-day analysis. Of the remaining compounds, 15 had a relative standard deviation ≤ 20% over all three concentrations above the IQL. The remaining three ECS had relative standard deviation ≤ 10% in most samples. Overall, the method was very precise with relative standard deviations ≤ 10% for the majority of compounds.

The intra- and inter-day instrumental performance was high across the majority of ECs. In total, 94% of the compounds were precise and accurate with a suitable linear calibration using the area ratio. Atorvastatin was linear, precise and accurate using the peak area, and miconazole, clotrimazole and climbazole could be analysed on a semi-quantitative basis as they showed satisfactory accuracy and precision data.

Method performance

The most common syringe filter membrane used for ECs prior to UHPLC-MS/MS is PTFE.[11,30,31,54] However, low recoveries have been observed for some ECs including erythromycin and gemfibrozil.[55] Therefore, a range of syringe filters including PVDF-

HL, PTFE, CA, and PVDF were investigated to minimize loss of ECs during the filtration step.

Absolute recoveries were >75% for all four syringe filters for 49 ECs (S9: Table S8†). Similarly, Darwano *et al.*[1] reported high recoveries for most analytes with little variation between different syringe filters. However, for clarithromycin, erythromycin, chlorpheniramine, cetirizine and citalopram poorer recoveries were found with PVDF, which is in line with what has been reported before for antibiotics including clarithromycin.[56] For all five compounds recoveries were at least 20% higher in the other filters, with CA and PVDF-HL being more effective than PTFE. However, CA gave lower recoveries for amoxicillin, estrone, and 17β-estradiol than what was achieved with PTFE, PVDF, and PVDF-HL syringe filters (>80%). PVDF-HL syringe filters were the best compromise for the studied EC, giving recoveries >70% for the majority of ECs. The effective use of PVDF-HL syringe filters has, for example, also been reported by Wang *et al.*[57] Low recoveries of approximately 10% were only found for fluoxetine, miconazole, and clotrimazole, and this was observed for all four syringe filters. All samples were filtered through PVDF-HL syringe filters prior to UHPLC-MS/MS injection.

To determine method performance, septic tank influent and effluent, river water, and sludge samples were spiked at three concentrations (S5: Table S4†). Water samples were analysed by direct injection and SPE. Calculations were not practical for 29 ECs in at least one sample, when the environmental concentration exceeded the spike concentration, most common at lowest spike concentrations in effluent SPE samples.

In direct injection samples, absolute recoveries were 23–209% in septic tank influent, 19–192% in septic tank effluent, and 19–186% in river water (S9: Table S9†). Most ECs have absolute recoveries from 25 to 125% (Fig. 3). Recoveries over 100% were due to signal enhancement. This highlights the requirement of the use of deuterated surrogates to correct for matrix effects and variations in the instrumental and method performance.

For 41 ECs (63%), relative recoveries by direct injection were in the range of 90% to 110% in all three matrices, and in the range of 75% to 125% for a further 11 ECs (Fig. 4). The remaining 14 ECs have relative recoveries from 22 to 197%, most likely due to the absence of a suitable deuterated surrogate to account for matrix effects and analyte loss. Similar results have been reported by Oliveira *et al.*[58] who found relative recovery from 20 to 230%, with the majority recoveries being in the range of 70–150% in the analysis of ECs in wastewater influent and effluent by direct injection LC-MS/MS. The direct injection MDLs were 3.3×10^{-3}–$3.0\ \mu g\ L^{-1}$ in influent, were 4.1×10^{-3}–$3.7\ \mu g\ L^{-1}$ in effluent, and 3.6×10^{-3}–$3.4\ \mu g\ L^{-1}$ in river water. MQLs were 6.7×10^{-3}–$8.8\ \mu g\ L^{-1}$ in influent, 8.1×10^{-3}–$14\ \mu g\ L^{-1}$ in effluent, and 7.2×10^{-3}–$8.3\ \mu g\ L^{-1}$ in river water (S9: Table S10†). While these MQLs were sufficient for the determination of high use compounds, such as metformin or paracetamol,[29,31,32] hormones and antibiotics have predicted no-effect concentrations (PNEC) < $1\ \mu g\ L^{-1}$ and are reported in freshwater at $ng\ L^{-1}$. Hence, the use of a SPE method was

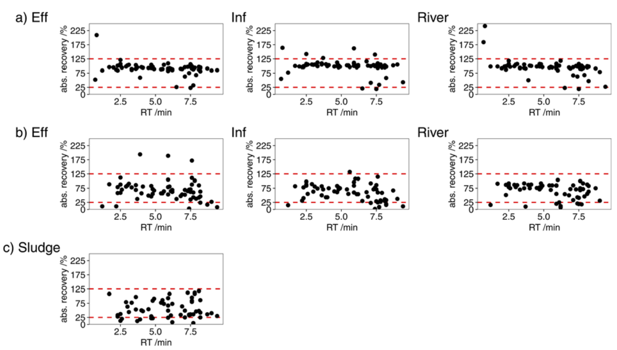

Fig. 3 Absolute recoveries (%) in influent, effluent and river water analysed by (a) direct injection and (b) SPE, and in (c) sludge.

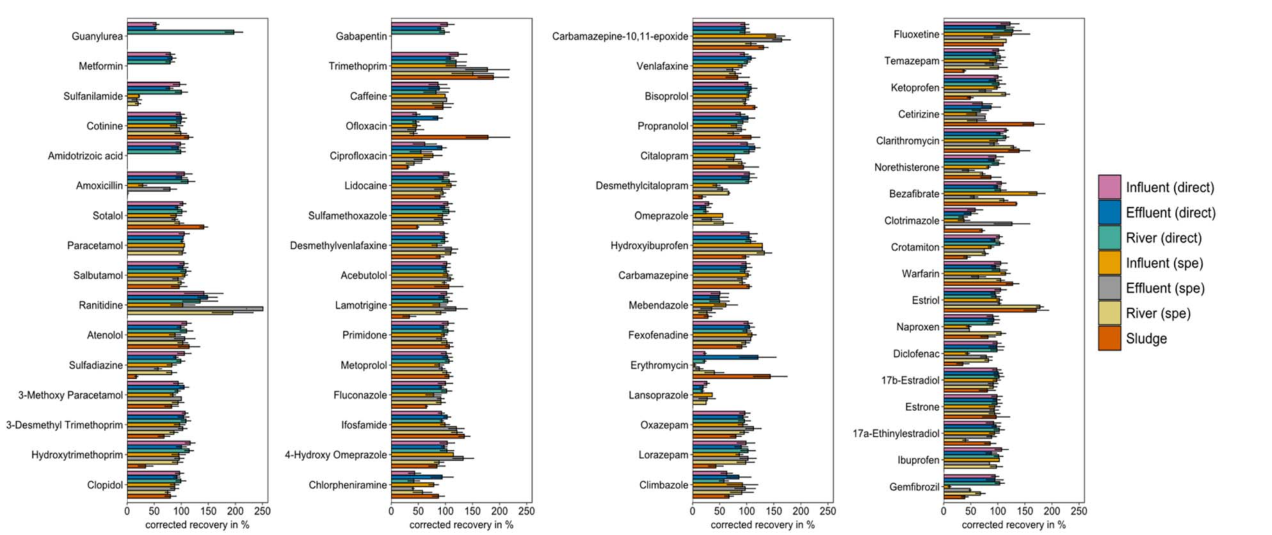

Fig. 4 Relative recoveries (%) in influent, effluent and river water analysed by direct injection and SPE, and in sludge, for the 66 ECs with assigned deuterated surrogate.

necessary to determine all ECs at the relevant concentrations. In direct injection, 29 ECs were very precise over all three concentrations with a relative standard deviation ≤ 10% in influent, effluent and river water (S9: Table S11†). Of the remaining compounds, 29 ECs were precise with a relative standard deviation ≤ 20% over all three concentrations in all matrices. The remaining ECs were precise for most spiked concentrations and matrices. Accuracies within the range of 75–125% were observed for the majority of 54 ECs. Most remaining ECs were accurate for most concentrations and matrices. This is similar to the results reported by Rapp-Wright *et al.*[31] for direct injection LC-MS/MS, and considering the complexitiy of matrices and the number of analytical steps involved, precision and accuracy were considered acceptable.

Absolute recoveries following SPE were 0–194% in septic tank influent, 1–200% in septic tank effluent, and 0–122% in river water (Fig. 3). The measured absolute recoveries were in the range of what has been previously reported using LC-MS/MS to determine multiple ECs in wastewater.[10,30,59] While the lack of selectivity of HLB allows the extractions of a wide range of analytes, matrix can be co-extracted and cause significant signal interference.[10] Signal interference is typically reported to be high in multi-residue LC-MS/MS methods using ESI as ionisation method and HLB columns in SPE due to lack of selectivity.[11,30,54] Lowest and no absolute recoveries from SPE were observed for the very polar compounds guanylurea, metformin, gabapentin, sulfanilamide, and amidotrizoic acid, and amoxicillin from river water (S9: Table S9†). HLB sorbents are known for their low recovery of very polar compounds,[27,28,30] *e.g.*, Klančar *et al.*[28] reported recoveries of 0.3% for metformin and 2.6% for gabapentin from river water. Due to the low absolute recoveries, guanylurea, metformin, gabapentin and amidotrizoic acid were determined by direct injection only. Relative recoveries for the remaining ECs analysed by SPE were 90–110% in all three matrices for 16 ECs and 75–125% for 17 ECs in all three matrices (Fig. 4). The remaining ECs had relative recoveries <75% or >125% in at least one water matrix. Similar relative recoveries have been reported by Anumol and Snyder in wastewater,[37] and the results used in the determination of concentrations to account for differences in the behaviour of the deuterated surrogate and analyte. The MDLs for SPE were 5.4×10^{-5}–0.073 μg L^{-1} in influent, 5.3×10^{-5}–0.033 μg L^{-1} in effluent, and 2.9×10^{-5}–0.40 μg L^{-1} in river water. MQLs were 1.5×10^{-4}–0.096 μg L^{-1} in influent, 1.6×10^{-4}–0.22 μg L^{-1} in effluent, and 6.6×10^{-5}–0.50 μg L^{-1} in river water (S9: Table S10†). Including SPE in the method preparation allows the determination of ECs at the relevant concentrations. The precision of 58 ECs was high over all three concentrations in influent, effluent and river water with relative standard deviations ≤ 20%. The remaining ten ECs were precise over most concentrations and matrices (S9: Table S12†). Similar precision were obtained by Ofrydopoulou *et al.*[27] The majority of ECs analysed by SPE had accuracies within the range of 75–125% for all concentrations above the MQL in influent, effluent and river water. Comparatively lower accuracies were found when the EC was present in the sample, *e.g.*, sulfanilamide in the effluent, trimethoprim at the smallest spike concentration in river water, and citalopram in influent. Lower accuracies were also found for amoxicillin in river water, with a low absolute recovery, and for warfarin at 1 μg L^{-1} close to the MQL (S9: Table S12†).

The USE method for the extraction of sediments described by Al-Khazrajy and Boxall[25] was modified to optimise extraction of the selected 68 ECs from sludge. To accommodate the higher concentrations of ECs in sludge compared to sediments,[60] a smaller mass of 0.1 g was used. Furthermore, the clean-up step was adjusted to keep it as similar as possible to the SPE of liquid samples. However, a larger SPE cartridge (200 mL for sludge) was chosen to avoid blocking of the cartridge during sample loading. Furthermore, an additional centrifuge step prior to filtration through a PVDF-HL syringe filter was necessary. The method was successfully applied for the extraction of 59 out 68 ECs from sludge (S9: Table S9†). Due to the complexity of the environmental matrices, a different number of analytes is often reported for different matrices in multi-analyte methods.[10,30] For example, the USE method is not suitable for very polar compounds, such as metformin, sulfanilamide and gabapentin with low absolute recoveries from SPE. Due to their high polarity they are more likely to stay in the water phase and less likely to be found in the sludge.[61] For the remaining compounds absolute recoveries from sludge were 12–112% (Fig. 3). The majority of ECs had relative recoveries of 75–125% from sludge (Fig. 4). Low relative recoveries below 50% (*e.g.*, diclofenac and sulfadiazine) and high relative recoveries over 150% (*e.g.*, trimethoprim and estriol) were found for ECs when the deuterated surrogate behaved differently than the analyte. MDLs and MQLs were 0.025–7.4 μg kg^{-1} and 0.080–49 μg kg^{-1}, respectively. However, only five ECs have MQLs > 10 μg kg^{-1} and only mebendazole has an MQL > 15 μg kg^{-1} (S9: Table S10†). Most ECs have accuracies within the range of 75–125% for all spike concentrations (S9: Table S12†). Lower accuracies were found for few ECs at one spike concentration, *e.g.*, for sulfadiazine at 50 μg kg^{-1} and for hydroxyibuprofen at 500 μg kg^{-1}. The precision of 53 ECs was high over all three spike concentrations with relative standard deviations ≤ 20%; the remaining six compounds have higher relative standard deviations at one concentration only.

The number of quantifiable compounds in each matrix varied from 68 in effluent to 59 in sludge, demonstrating the method's wide applicability.

Application to environmental matrices

The developed method was applied to samples collected from a septic tank in the North-East of Scotland at the influent and effluent point, from the sludge, and from the receiving river upstream and downstream of the septic tank's discharge point. Additionally, the suspended solids from the influent and effluent were analysed. At sampling time, the dilution factor of effluent into the river was 756.[62]

Across all samples analysed, 43 ECs were detected at least once (Table 1). Fifteen ECs from six different groups (analgesics, antibiotics, anticonvulsants, antihistamines, β-blockers, wastewater discharge marker) were found in all matrices.

Table 1 Concentrations detected in influent (Inf), effluent (Eff), river water upstream (Up) and downstream (Down) of the effluent discharge point, suspended solids (SuS), and sludge[a]

Class	EC	Inf ($\mu g\ L^{-1}$)	Eff ($\mu g\ L^{-1}$)	Up ($\mu g\ L^{-1}$)	Down ($\mu g\ L^{-1}$)	SuS Inf ($\mu g\ kg^{-1}$)	SuS Eff ($\mu g\ kg^{-1}$)	Sludge ($\mu g\ kg^{-1}$)
Anaesthetics	Lidocaine	0.025 ± 0.0056	0.043 ± 0.0020	nd	nd	$(2.6 \pm 0.14) \times 10^3$	$(1.1 \pm 0.084) \times 10^2$	nd
Analgesics	3-Methoxyparacetamol	8.2 ± 0.44	13 ± 0.85	$(2.3 \pm 0.10) \times 10^{-3}$	0.026 ± 0.0013	$(1.1 \pm 0.064) \times 10^2$	$(1.6 \pm 0.40) \times 10^2$	68 ± 8.3
	Diclofenac	2.4 ± 0.10	0.58 ± 0.0047	0.023 ± 0.0024	0.016 ± 0.00097	78 ± 2.6	47 ± 3.2	$(6.2 \pm 1.3) \times 10^2$
	Hydroxyibuprofen	2.7 ± 0.25	17 ± 0.56	$(2.9 \pm 0.41) \times 10^{-3}$	0.019 ± 0.0017	nd	nd	nd
	Ibuprofen	34 ± 1.8	26 ± 0.71	0.47 ± 0.083	0.63 ± 0.070	$(2.1 \pm 0.067) \times 10^4$	$(6.1 \pm 0.55) \times 10^3$	$(1.6 \pm 0.26) \times 10^3$
	Ketoprofen	nd	nd	nd	nd	nd	nd	nd
	Naproxen	3.7 ± 0.079	26 ± 0.26	$(3.2 \pm 0.12) \times 10^{-3}$	0.032 ± 0.0017	$(5.1 \pm 0.62) \times 10^2$	$(7.0 \pm 0.88) \times 10^2$	$(3.0 \pm 0.41) \times 10^2$
	Paracetamol	$(2.0 \pm 0.12) \times 10^2$	$(2.9 \pm 0.076) \times 10^2$	0.039 ± 0.0043	0.59 ± 0.053	$(1.4 \pm 0.45) \times 10^3$	$(1.3 \pm 0.18) \times 10^4$	$(3.6 \pm 0.045) \times 10^3$
Antibiotics	3-Desmethyl-trimethoprim	0.013 ± 0.0053	0.16 ± 0.014	$(1.8 \pm 0.23) \times 10^{-3}$	$(2.5 \pm 0.30) \times 10^{-3}$	78 ± 5.4	84 ± 10	4.8 ± 0.63
	Amoxicillin	nd	nd	nd	nd	—	—	—
	Ciprofloxacin	10 ± 0.61	2.4 ± 0.43	nd	nd	$(3.8 \pm 0.93) \times 10^3$	$(4.9 \pm 0.59) \times 10^2$	nd
	Clarithromycin	nd	nd	nd	nd	$(3.1 \pm 0.027) \times 10^3$	$(1.3 \pm 0.13) \times 10^3$	13 ± 2.0
	Erythromycin	0.077 ± 0.020	0.14 ± 0.016	nd	nd	nd	nd	—
	Ofloxacin	nd	nd	nd	nd	$(1.2 \pm 0.16) \times 10^2$	69 ± 15	—
	Sulfadiazine	nd	nd	nd	nd	nd	nd	nd
	Sulfamethoxazole	0.029 ± 0.0032	0.59 ± 0.029	$(3.1 \pm 0.55) \times 10^{-3}$	$(3.1 \pm 0.56) \times 10^{-3}$	nd	43 ± 3.7	nd
	Sulfanilamide	0.33 ± 0.031	0.13 ± 0.018	nd	nd	—	—	—
	Trimethoprim	0.014 ± 0.0047	0.25 ± 0.018	$(8.8 \pm 1.8) \times 10^{-4}$	$(1.1 \pm 0.14) \times 10^{-3}$	44 ± 3.0	63 ± 1.8	7.5 ± 0.67
	α-Hydroxytrimethoprim	nd	$(7.0 \pm 2.8) \times 10^{-3}$	nd	nd	nd	nd	nd
Anticoagulants	Warfarin	nd	nd	nd	nd	nd	nd	nd
Anticonvulsants	Carbamazepine	nd	$(7.2 \pm 0.43) \times 10^{-4}$	$(2.0 \pm 0.14) \times 10^{-4}$	$(1.4 \pm 0.097) \times 10^{-4}$	nd	nd	nd
	Carbamazepine-10,11-epoxide	nd	nd	nd	nd	nd	nd	nd
	Gabapentin	1.9 ± 0.30	7.4 ± 0.56	nd	nd	—	—	—
	Lamotrigine	0.97 ± 0.17	1.2 ± 0.055	$(3.0 \pm 0.26) \times 10^{-3}$	$(4.5 \pm 0.27) \times 10^{-3}$	$(4.3 \pm 0.68) \times 10^2$	$(1.3 \pm 0.15) \times 10^3$	70 ± 11
	Primidone	nd	nd	nd	nd	nd	nd	nd
Antidepressants	Citalopram	0.19 ± 0.014	0.14 ± 0.0091	nd	nd	$(7.2 \pm 1.5) \times 10^2$	$(1.3 \pm 0.26) \times 10^2$	$(1.4 \pm 0.088) \times 10^2$
	Desmethylcitalopram	0.14 ± 0.0084	0.079 ± 0.0030	nd	nd	$(1.0 \pm 0.22) \times 10^3$	$(1.2 \pm 0.26) \times 10^2$	$(1.5 \pm 0.067) \times 10^2$
	Desmethylvenlafaxine	0.028 ± 0.0015	0.21 ± 0.011	nd	$(6.3 \pm 1.7) \times 10^{-4}$	44 ± 7.2	47 ± 7.2	15 ± 3.3
	Fluoxetine	0.013 ± 0.0026	0.016 ± 0.0025	nd	nd	$(1.4 \pm 0.54) \times 10^2$	68 ± 12	nd
	Venlafaxine	0.081 ± 0.0025	0.14 ± 0.0084	nd	nd	$(2.0 \pm 0.12) \times 10^2$	66 ± 3.6	55 ± 10
Anti-diabetics	Guanylurea	nd	nd	nd	nd	—	—	—
	Metformin	$(2.2 \pm 0.082) \times 10^2$	$(1.6 \pm 0.067) \times 10^2$	0.85 ± 0.031	1.1 ± 0.038	—	—	—
Anti-fungals	Climbazole	nd	nd	nd	nd	nd	nd	nd
	Clotrimazole	nd	nd	nd	nd	$(5.1 \pm 0.64) \times 10^2$	$(4.8 \pm 0.89) \times 10^2$	$(1.7 \pm 0.11) \times 10^2$
	Fluconazole	nd	nd	nd	nd	nd	nd	nd
	Miconazole	nd	nd	nd	nd	nd	nd	78 ± 25

Table 1 (*Contd.*)

Class	EC	Inf ($\mu g\ L^{-1}$)	Eff ($\mu g\ L^{-1}$)	Up ($\mu g\ L^{-1}$)	Down ($\mu g\ L^{-1}$)	SuS Inf ($\mu g\ kg^{-1}$)	SuS Eff ($\mu g\ kg^{-1}$)	Sludge ($\mu g\ kg^{-1}$)
Anti-helmintics	Mebendazole	nd	nd	nd	nd	nd	nd	nd
Antihistamines	Cetirizine	1.2 ± 0.050	1.2 ± 0.046	$(3.4 \pm 0.32) \times 10^{-3}$	$(5.6 \pm 0.36) \times 10^{-3}$	$(1.0 \pm 0.18) \times 10^{2}$	$(1.2 \pm 0.14) \times 10^{2}$	$(1.1 \pm 0.060) \times 10^{2}$
	Chlorpheniramine	nd	$(1.8 \pm 0.034) \times 10^{-3}$	nd	nd	93 ± 4.0	39 ± 3.0	22 ± 6.0
	Fexofenadine	0.16 ± 0.0097	0.81 ± 0.018	0.013 ± 0.0014	0.012 ± 0.0016	$(2.6 \pm 0.049) \times 10^{2}$	$(1.1 \pm 0.088) \times 10^{2}$	$(3.4 \pm 0.67) \times 10^{3}$
Anti-pruritic	Crotamiton	0.74 ± 0.061	1.5 ± 0.13	nd	nd	$(2.2 \pm 0.14) \times 10^{2}$	$(2.3 \pm 0.51) \times 10^{2}$	89 ± 6.6
Antiulcer	Ranitidine	nd	nd	nd	nd	nd	nd	nd
	4-Hydroxyomeprazole	0.33 ± 0.023	0.43 ± 0.035	nd	nd	$(3.6 \pm 0.19) \times 10^{2}$	45 ± 4.5	$(1.1 \pm 0.027) \times 10^{2}$
	Lansoprazole	nd	nd	nd	nd	—	—	—
	Omeprazole	nd	nd	nd	nd	—	—	—
Benzodiazepines	Lorazepam	nd	nd	nd	nd	nd	nd	nd
	Oxazepam	nd	nd	nd	nd	nd	nd	nd
	Temazepam	nd	0.019 ± 0.0026	nd	nd	nd	nd	nd
Betablockers	Acebutolol	nd	nd	nd	nd	nd	nd	nd
	Atenolol	$(7.5 \pm 0.60) \times 10^{-3}$	0.088 ± 0.0067	$(3.8 \pm 0.27) \times 10^{-4}$	$(6.3 \pm 0.16) \times 10^{-4}$	59 ± 4.1	50 ± 12	53 ± 4.6
	Bisoprolol	0.23 ± 0.020	0.086 ± 0.0095	$(4.0 \pm 2.5) \times 10^{-4}$	$(3.6 \pm 0.42) \times 10^{-4}$	54 ± 10	41 ± 3.6	3.8 ± 0.36
	Metoprolol	nd	nd	nd	nd	nd	nd	nd
	Propranolol	0.43 ± 0.035	0.24 ± 0.029	nd	nd	$(6.2 \pm 0.98) \times 10^{2}$	$(3.7 \pm 0.77) \times 10^{2}$	46 ± 6.5
	Salbutamol	$(3.1 \pm 0.27) \times 10^{-3}$	0.011 ± 0.00030	nd	nd	11 ± 1.1	9.5 ± 1.0	5.7 ± 1.8
	Sotalol	nd	nd	nd	nd	nd	nd	nd
Chemotherapeutic	Ifosfamide	nd	nd	nd	nd	nd	nd	nd
Coccidiostat	Clopidol	nd	nd	nd	nd	nd	nd	nd
Hormones	17β-Estradiol	nd	nd	nd	nd	nd	nd	nd
	17α-Ethinylestradiol	nd	nd	nd	nd	nd	nd	nd
	Estriol	0.093 ± 0.0086	0.70 ± 0.013	nd	nd	nd	nd	16 ± 3.3
	Estrone	0.15 ± 0.0030	0.054 ± 0.0035	nd	nd	$(1.2 \pm 0.14) \times 10^{2}$	nd	15 ± 1.4
	Norethisterone	nd	nd	nd	nd	nd	nd	nd
Lipid regulators	Atorvastatin	2.1 ± 0.19	1.9 ± 0.090	nd	nd	nd	nd	$(3.9 \pm 2.3) \times 10^{2}$
	Bezafibrate	nd	nd	nd	nd	nd	nd	nd
	Gemfibrozil	nd	nd	nd	nd	nd	nd	nd
Wastewater discharge marker	Caffeine	$(1.4 \pm 0.12) \times 10^{2}$	41 ± 4.1	0.079 ± 0.025	0.19 ± 0.017	$(1.0 \pm 0.17) \times 10^{4}$	$(9.2 \pm 1.2) \times 10^{3}$	$(2.7 \pm 0.35) \times 10^{3}$
	Cotinine	1.1 ± 0.10	1.2 ± 0.086	$(1.1 \pm 0.055) \times 10^{-3}$	$(2.8 \pm 0.14) \times 10^{-3}$	85 ± 22	$(1.1 \pm 0.12) \times 10^{2}$	31 ± 4.3
X-ray contrast	Amidotrizoic acid	nd	nd	nd	nd	—	—	—

[a] nd, not detected; —, method not suitable.

In the influent, 34 ECs were detected at concentrations from $(7.5 \pm 0.60) \times 10^{-3}$ μg L^{-1} (atenolol) to $(2.2 \pm 0.082) \times 10^{2}$ μg L^{-1} (metformin). A wide concentration range is typically observed for different ECs in wastewater.[29,31,32]

The highest detection frequency was observed in the effluent, where 38 ECs could be quantified. ECs were found at concentrations lower (*e.g.*, ciprofloxacin), similar to (*e.g.*, venlafaxine) and higher (*e.g.*, gabapentin) than in the influent. Some determined effluent concentrations were in the range of what is typically reported in the influent of centralised WWTWs; for instance, both influent and effluent concentrations of metformin, were found to be $(2.2 \pm 0.082) \times 10^{2}$ μg L^{-1} and $(1.6 \pm 0.067) \times 10^{2}$ μg L^{-1}, respectively.[2] This suggests that in contrast to the high removal efficiency in centralised WWTWs of over 90% from the liquid phase,[2] metformin is not degraded in the septic tank. Furthermore, effluent concentrations of some compounds exceeded concentrations typically reported from centralised WWTWs. For example, the antipruitic drug crotamiton was present at (1.5 ± 0.13) μg L^{-1} in the effluent, higher than previously reported concentrations of 0.11–0.27 μg L^{-1} by Nakada *et al.*[26] in the UK. On the other hand, effluent concentrations of ECs such as fexofenadine, cetirizine, ciprofloxacin and lidocaine were similar to what has been reported in centralised WWTWs.[30,31] Further research is necessary to better understand the removal of different ECs in septic tanks.

In the river, 18 ECs were detected upstream and 19 downstream of the septic tank discharge point. The EC found at the highest concentration in the river, both upstream and downstream, was the anti-diabetic metformin at (0.85 ± 0.031) μg L^{-1} and (1.1 ± 0.038) μg L^{-1}, respectively. Metabolites can potentially have a significant effect on the total concentration of ECs in the environment, *e.g.*, both desmethylvenlafaxine and 3-desmethyltrimethoprim were detected at higher concentrations in the river than the parent compound. The contribution of the septic tank to the pharmaceutical concentrations in the river varied from no difference between upstream and downstream concentrations to a marked increase. The biggest contribution was found for paracetamol with an increase by a factor of 15 from (0.039 ± 0.0043) μg L^{-1} to (0.59 ± 0.053) μg L^{-1}. Other sources that contribute to ECs concentrations in the river are the secondary WWTWs and additional private septic tanks. Further work focussing on ECs in rural Scotland is needed to understand the impact of septic tank discharges on rivers.

With 30 detected ECs, detection frequencies in the suspended solids were similar to the wastewater. For most ECs, the liquid phase is the main contributor to the total concentrations in the septic tank discharge. However, clotrimazole, clarithromycin and ofloxacin that were not detected in the water, were found in the suspended solids at concentrations up to $(1.3 \pm 0.13) \times 10^{3}$ μg kg^{-1} for clarithromycin in the effluent. This stresses the importance of analysing the solids when assessing the impact of wastewater discharges to the environment. Most ECs had similar concentrations in the suspended solids of the influent and effluent, showing a potential for removal of ECs in the septic tank through sludge formation and consequent reduction of the total suspended solids in the effluent.

The 30 ECs that were determined in the sludge sample were found at concentrations from 4 (bisoprolol) to 3617 μg kg^{-1} (paracetamol). A wide concentration range of ECs in digested sludge from centralised WWTWs was also reported by Aydın *et al.*[63] at mean concentrations from 0.73 (sulfamethazine) to 147 μg kg^{-1} (clarithromycin), and a maximum concentration of 1496 μg kg^{-1} (clarithromycin). Higher levels of some ECs such as fexofenadine and diclofenac in the sludge *versus* the suspended solids may reflect an accumulation over time, whereas lower levels of other ECs such as caffeine, paracetamol and clarithromycin could be due to degradation in the sludge.[36] Future research on the distribution of ECs between the liquid and solid phase could increase the understanding of the removal of different ECs through sorption or degradation.

The contribution of the septic tank to the pharmaceutical concentrations detected in the river varies from no difference between upstream and downstream concentrations to an increase by the factor 15. The observed effluent concentrations of some pharmaceuticals suggest less removal in septic tanks than in centralised WWTWs. Finally, the detection of 30 ECs in the suspended solids in the effluent stresses the importance of including solid analysis when analysing environmental samples to avoid underestimation of the total concentration in the sample.

Conclusions

A new multi-analyte method was developed for the accurate determination of a broad range of ECs in liquid and solid environmental matrices of varying complexity. Analysing septic tank influent and effluent, including suspended solids, sludge, and the receiving surface water allows an accurate assessment of the performance of septic tanks for the removal of ECs and their effect on water quality. Including suspended solids in the analysis of environmental samples minimises underestimating the total concentration of ECs.

The reported effluent concentrations of some pharmaceuticals suggest less removal in septic tanks than in centralised WWTWs. Furthermore, the river sampling suggests that septic tanks have an impact on water quality for some ECs. Hence, a more robust sampling of septic tanks in Scotland is proposed to accurately determine their impact to the environment.

Author contributions

Maike Wilschnack: conceptualisation, data curation, formal analysis, investigation, methodology, validation, visualisation, writing – original draft; Bess Homer: conceptualisation, resources, writing – review & editing; Elise Cartmell: conceptualisation, resources, writing – review & editing; Kyari Yates: conceptualisation, supervision, writing – review & editing; Bruce Petrie: conceptualisation, funding acquisition, methodology, project administration, supervision, writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

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Electronic Supplementary Material 1

Targeted multi-analyte UHPLC-MS/MS methodology for emerging contaminants in septic tank wastewater and surface water

Maike Wilschnack[a], Bess Homer[b], Elise Cartmell[b], Kyari Yates[a], Bruce Petrie[a]

Contents

S1 General and chemical information

Table S1: General and chemical information (Substance Class, Chemical, Cas Number, Molecular Formula, Molecular Weight, Water solubility, Log K_{OW}, pKa, Supplier) of target analytes, ordered by substance class, alphabetically.

Class	Chemical	Cas No.	Mol. Formular	Mol. Weight (g mol^{-1})	Solubility (mg L^{-1})	Log K_{OW}	pKa (most acidic)	pKa (most basic)	Supplier
Anaesthetics	Lidocaine	137-58-6	$C_{14}H_{22}$N2O	234.34	4100 [a]	2.44 [a]	13.78 [e]	7.75 [e]	Sigma Aldrich
Analgesics	3-Methoxy-Paracetamol	3251-55-6	$C_9H_{11}NO_3$	181.19	-	0.09 [c]	-	-	LGC standards
	Diclofenac	15307-79-6	$C_{14}H_{11}Cl_2NO_2$	296.15	2.37 [a]	4.51 [a]	4 [e]	-2.1 [e]	Sigma Aldrich
	Hydroxyibuprofen	51146-55-5	$C_{13}H_{18}O_3$	222.28	-	2.29 [c]	4.63 [d]	-	Sigma Aldrich
	Ibuprofen	15687-27-1	$C_{13}H_{18}O_2$	206.29	21 [a]	3.97 [a]	4.85 [e]	-	Sigma Aldrich
	Ketoprofen	22071-15-4	$C_{16}H_{14}O_3$	254.29	51 [a]	3.13 [a]	3.88 [e]	-7.5 [e]	Sigma Aldrich
	Naproxen	22204-53-1	$C_{14}H_{14}O_3$	230.27	15.9 [a]	3.18 [a]	4.19 [e]	-4.8 [e]	Sigma Aldrich
	Paracetamol	103-90-2	$C_8H_9NO_2$	151.17	30400 [b]	0.91 [a]	9.46 [e]	-4.4 [e]	Sigma Aldrich
Antibiotics	3-Desmethyltrimethoprim	27653-69-6	$C_{13}H_{16}N_4O_3$	276.29	-	-	-	-	LGC standards
	α-Hydroxytrimethoprim	29606-06-2	$C1_4H_{18}N_4O_4$	306.32	-	-	-	-	LGC standards
	Amoxicillin	26787-78-0	$C_{16}H_{19}N_3O_5S$	365.4	3430 [b]	0.87 [a]	3.23 [e]	7.22 [e]	Sigma Aldrich
	Ciprofloxacin	85721-33-1	$C_{17}H_{18}FN_3O_3$	331.34	11500 [b]	0.28 [a]	5.56 [e]	8.77 [e]	Sigma Aldrich
	Clarithromycin	81103-11-9	$C_{38}H_{69}NO_{13}$	747.97	0.33 [a]	3.16 [a]	12.46 [e]	9 [e]	Sigma Aldrich
	Erythromycin	114-07-8	$C_{37}H_{67}NO_{13}$	733.93	0.52 [b]	2.6 [a]	12.45 [e]	9 [e]	Sigma Aldrich
	Ofloxacin	82419-36-1	$C_{18}H_{20}FN_3O_4$	361.37	28300 [a]	-0.39 [a]	5.35 [e]	6.72 [e]	Sigma Aldrich
	Sulfadiazine	68-35-9	$C_{10}H_{10}N_4O_2S$	250.28	77 [a]	-0.09 [a]	6.99 [e]	2.01 [e]	Sigma Aldrich
	Sulfamethoxazole	723-46-6	$C_{10}H_{11}N_3O_3S$	253.28	610 [a]	0.89 [a]	6.16 [e]	1.97 [e]	Sigma Aldrich
	Sulfanilamide	63-74-1	$C_6H_8N_2O_2S$	172.20	7500 [a]	-0.62 [a]	10.99 [e]	2.27 [e]	Sigma Aldrich
	Trimethoprim	738-70-5	$C_{14}H_{18}N_4O_3$	290.32	400 [a]	0.91 [a]	17.33 [e]	7.16 [e]	Sigma Aldrich
Anticoagulants	Warfarin	81-81-2	$C_{19}H_{16}O_4$	308.33	17 [a]	2.7 [a]	5.56 [e]	-6.9 [e]	Sigma Aldrich
Anticonvulsants	Carbamazepine	298-46-4	$C_{15}H_{12}N_2O$	236.28	17.7 [b]	2.77 [a]	15.96 [e]	-3.8 [e]	Sigma Aldrich

	Carbamazepine-10,11-epoxide	36507-30-9	$C_{15}H_{12}N_2O_2$	252.27	-	0.95 [c]	13.91 [b]	-0.50 [b]	LGC standards
	Gabapentin	60142-96-3	$C_9H_{17}NO_2$	171.24	34000 [c]	1.25 [a]	4.63 [e]	9.91 [e]	Sigma Aldrich
	Lamotrigine	84057-84-1	$C_9H_7Cl_2N_5$	256.09	170 [a]	1.93 [a]	14.98 [e]	5.58 [e]	Sigma Aldrich
	Primidone	125-33-7	$C_{12}H_{14}N_2O_2$	218.25	500 [a]	0.91 [a]	11.5 [e]	-6.2 [e]	Sigma Aldrich
Antidepressants	Citalopram	59729-32-7	$C_{20}H_{21}FN_2O$	324.40	31.1 [b]	3.76 [a]	-	9.78 [a]	Sigma Aldrich
	Desmethylcitalopram	144025-14-9	$C_{19}H_{19}FN_2O$	310.37	-	3.53 [c]	-	10.54 [d]	LGC standards
	Desmethylvenlafaxine	93413-62-8	$C_{16}H_{25}NO_2$	263.38	-	2.69 [d]	10.04 [b]	9.33 [b]	Sigma Aldrich
	Fluoxetine	56296-78-7	$C_{17}H_{18}F_3NO$	309.33	60.3 [b]	4.05 [a]	-	9.8 [e]	LGC standards
	Venlafaxine	99300-78-4	$C_{17}H_{27}N_1O_2$	277.41	267 [b]	3.28 [b]	14.42 [e]	8.91 [e]	Sigma Aldrich
Anti-diabetics	Guanylurea	207300-86-5	$C_2H_6N_4O$	102.10	-	-3.57 [c]	-	-	Sigma Aldrich
	Metformin	1115-70-4	$C_4H_{11}N5$	129.17	1000000 [b]	-2.6 [a]	-	12.4 [a]	Sigma Aldrich
Anti-fungals	Climbazole	38083-17-9	$C_{15}H_{17}ClN_2O_2$	292.76	-	3.76 [c]	18.87 [e]	6.49 [e]	TCI
	Clotrimazole	23593-75-1	$C_{22}H_{17}ClN_2$	344.84	0.49 [a]	6.1 [a]	-	6.26 [e]	Sigma Aldrich
	Fluconazole	86386-73-4	$C_{13}H_{12}F_2N_6O$	306.27	-	0.5 [a]	12.68 [e]	2.3 [e]	TCI
	Miconazole	22916-47-8	$C_{18}H_{14}Cl_4N_2O$	416.13	-	6.25 [c]	-	6.48 [e]	Sigma Aldrich
Anti-helmintics	Mebendazole	31431-39-7	$C_{16}H_{13}N_3O_3$	295.29	71.3 [a]	2.83 [a]	8.44 [e]	3.93 [e]	TCI
Antihistamines	Cetirizine	83881-52-1	$C_{21}H_{25}ClN_2O_3$	388.9	101 [a]	2.8 [a]	3.59 [e]	7.42 [b]	Sigma Aldrich
	Chlorpheniramine	113-92-8	$C_{16}H_{19}ClN_2$	274.79	5500 [a]	3.38 [a]	-	9.13 [a]	Sigma Aldrich
	Fexofenadine	153439-40-8	$C_{32}H_{39}NO_4$	501.67	0.02 [b]	2.94 [e]	4.04 [e]	9.01 [e]	Sigma Aldrich
Anti-pruritic	Crotamiton	483-63-6	$C_{13}H_{17}NO$	203.28	-	2.9 [d]	-	-0.6 [e]	Sigma Aldrich
Antiulcer	4-Hydroxyomeprazole	301669-82-9	$C_{16}H_{17}N_3O_3S$	331.40	-	1.93 [c]	9.68 [d]	3.93 [d]	LGC standards
	Lansoprazole	103577-45-3	$C_{16}H_{14}F3N_3O_2S$	369.36	0.97 [a]	3.68 [c]	9.35 [e]	4.16 [e]	TCI
	Omeprazole	73590-58-6	$C_{17}H_{19}N_3O_3S$	345.52	359 [a]	2.23 [a]	9.29 [e]	4.77 [e]	Sigma Aldrich
	Ranitidine	66357-59-3	$C_{13}H_{22}N_4O_3S$	314.41	24700 [b]	0.2 [a]	-	8.2 [a]	Sigma Aldrich
Benzodiazepines	Lorazepam	846-49-1	$C_{15}H_{10}Cl_2N_2O_2$	321.16	80 [a]	2.39 [a]	10.61 [e]	-2.2 [e]	Sigma Aldrich
	Oxazepam	604-75-1	$C_{15}H_{11}ClN_2O_2$	286.71	179 [b]	2.24 [a]	10.61 [e]	-1.5 [e]	Sigma Aldrich

	Temazepam	846-50-4	$C_{16}H_{13}ClN_2O_2$	300.75	164 [a]	2.19 [a]	10.68 [e]	-1.4 [e]	Sigma Aldrich
Betablockers	Acebutolol	34381-68-5	$C_{18}H_{28}N_2O_4$	336.43	259 [a]	1.71 [a]	13.91 [e]	9.65 [e]	Sigma Aldrich
	Atenolol	29122-68-7	$C_{14}H_{22}N_2O_3$	266.34	13300 [a]	0.16 [a]	14.08 [e]	9.67 [e]	Sigma Aldrich
	Bisoprolol	104344-23-2	$C_{18}H_{31}NO_4$	325.44	2240 [b]	2.2 [a]	14.09 [e]	9.67 [e]	Sigma Aldrich
	Metoprolol	56392-17-7	$C_{15}H_{25}NO_3$	267.37	4770 [b]	2.15 [a]	14.09 [e]	9.67 [e]	Sigma Aldrich
	Propranolol	318-98-9	$C_{16}H_{21}NO_2$	259.35	228 [e]	3.48 [a]	14.09 [e]	9.67 [e]	Sigma Aldrich
	Salbutamol	18559-94-9	$C_{13}H_{21}NO_3$	239.31	14100 [a]	1.4 [a]	10.12 [e]	9.4 [e]	Sigma Aldrich
	Sotalol	959-24-0	$C_{12}H_{20}N_2O_3S$	272.36	-	0.24 [c]	10.07 [e]	9.43 [e]	Sigma Aldrich
Chemotherapeutic	Ifosfamide	3778-73-2	$C_7H_{15}Cl_2N_2O_2P$	261.09	3780 [a]	0.86 [a]	14.64 [e]	-	Sigma Aldrich
Coccidiostat	Clopidol	2971-90-6	$C_7H_7Cl_2NO$	192.04	-	2.1 [c]	10.77 [d]	-	Sigma Aldrich
Hormones	17ß-Estradiol (E2)	50-28-2	$C_{18}H24O_2$	272.39	3.6 [a]	4.01 [a]	10.33 [e]	-0.88 [e]	Sigma Aldrich
	17α-Ethinylestradiol (EE2)	57-63-6	$C_{20}H24O_2$	296.41	11.3 [a]	3.67 [a]	10.33 [e]	-1.7 [e]	Sigma Aldrich
	Estriol (E3)	50-27-1	$C_{18}H_{24}O_3$	288.38	-	2.45 [a]	10.33 [e]	-3.2 [e]	Sigma Aldrich
	Estrone (E1)	53-16-7	$C_{18}H_{22}O_2$	270.37	0.76 [a]	2.6 [a]	10.33 [e]	-5.4 [e]	Sigma Aldrich
	Norethisterone	68-22-4	$C_{20}H_{26}O_2$	298.42	7.04 [c]	2.97 [c]	17.59 [e]	-1.7 [e]	Sigma Aldrich
Lipid regulators	Atorvastatin	344423-98-9	$C_{33}H_{35}FN_2O_5$	558.65	0.00112 [b]	6.36 [a]	4.31 [e]	-2.7 [e]	Sigma Aldrich
	Bezafibrate	41859-67-0	$C_{19}H_{20}ClNO_4$	361.83	1.2 [b]	4.25 [b]	3.83 [e]	-0.84 [e]	Sigma Aldrich
	Gemfibrozil	25812-30-0	$C_{15}H_{22}O_3$	250.33	4.96 [b]	4.39 [a]	4.42 [e]	-4.8 [e]	Sigma Aldrich
Wastewater discharge marker	Caffeine	58-05-02	$C_8H_{10}N_4O_2$	194.19	21700 [a]	0.16 [b]	-	0.52 [b]	Sigma Aldrich
	Cotinine	486-56-6	$C_{10}H_{12}N_2O$	176.22	999000 [b]	1.37 [d]	-	4.79 [d]	Sigma Aldrich
X-ray contrast	Amidotrizoic acid	117-96-4	$C_{11}H_9I_3N_2O_4$	613.91	-	3.3 [a]	2.17 [e]	-4.2 [ei]	Sigma Aldrich

[a] Drugbank [1], [b] Proctor et al., 2019 [2], [c] ChemSpider [3], [d] ChEMBL [4], [e] Drugbank using ChemAxon [1]

Table S2: CAS Number and supplier for deuterated pharmaceutical standards.

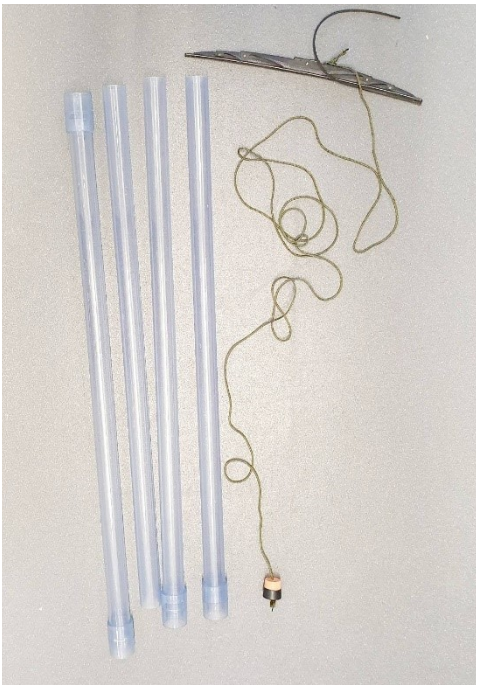
Compound	CAS	supplier
(±)-Acebutolol-d_5 hydrochloride	1189500-68-2	TRC
(±)-Atenolol-d_7	1202864-50-3	Analab
(±)-Bisoprolol-d_5	1189881-87-5	TRC
(±)-Chlorpheniramine-d_6 solution	129806-45-7	Sigma Aldrich
(±)-Citalopram-d_6 solution	1190003-26-9	Sigma Aldrich
(±)-Cotinine-d_3 solution	110952-70-0	Sigma Aldrich
(±)-Fluoxetine-d_6 solution	1173020-43-3	Sigma Aldrich
(±)-Ibuprofen-d_3	121662-14-4	Sigma Aldrich
(±)-Metoprolol-d_7 (+)-tartrate	2378803-75-7	Sigma Aldrich
(±)-Naproxen-d_3	958293-79-3	Sigma Aldrich
(±)-Propranolol-d_7 solution	1613439-56-7	Sigma Aldrich
(±)-Salbutamol-d_3	1219798-60-3	LGC standards
(±)-Sotalol-d_6 hydrochloride	1246820-85-8	LGC standards
(±)-Temazepan-d_5 solution	136765-51-0	Sigma Aldrich
(±)-Venlafaxine-D_6 solution	1062606-12-5	Sigma Aldrich
17β-Estradiol-d_4	66789-03-5	LGC standards
Acetaminophen-d_4	64315-36-2	Sigma Aldrich
Caffeine-^{13}C	202282-98-2	Sigma Aldrich
Carbamazepine-10,11-epoxide-d_{10}	1219804-16-6	LGC standards
Carbamazepine-d_{10} solution	132183-78-9	Sigma Aldrich
Clarithromycin-N-methyl-^{13}C,d_3	78088-19-4	LGC standards
Ciprofloxacin-d_8 Oxalate	1246819-94-2	TRC
Estrone-d_4	53866-34-5	Sigma Aldrich
Metformin-d_6 HCl	1185166-01-1	LGC standards
Ofloxacin-d_3	1173147-91-5	Sigma Aldrich
(±)-Oxazepam-d_5 solution	65854-78-6	Sigma Aldrich
Primidone-d_5	73738-06-4	Supelco

S2 Risk calculations

Risk calculations were performed for pharmaceuticals with annual prescription rates over 1,000,000 per item in Scotland in 2019 [5]. The risk quotient (RQ) is calculated by dividing the predicted and measured environmental concentrations in the UK by the predicted no effect concentration (PNEC). The PNEC was obtained from the lowest found value in the literature. A substance was included as a target analyte, if RQ > 1 [6,7].

S3 Sludge Sampler

Figure S1: Costume-made polyvinylchloride sludge sampler in its broken down form (a) and in use (b). For sampling, the sludge sampler was inserted into the septic tank until it reached the bottom, pulled up a few centimetres, and closed by pulling the cord up. It was then lifted up and the sludge was collected into a polypropylene bottle.

a) 

b) 

S4 LC Solvent gradient program

Table S3: LC solvent gradient program, mobile phase A: Water with additives, mobile phase B: methanol with additives. Additives were 5mM ammonium formate and 0.1% formic acid in the positive method, and 0.1mM ammonium fluoride in the negative method. The total run time was 14min for the positive, and 12min for the negative method.

Time /min	% A	
	positive	**negative**
0	95	95
0.5	95	95
8		20
9	20	20
9.1		95
11	20	95
11.1	95	95
12	95	95
14	95	

S5 Standard preparation

The standards were mainly purchased in solid form. Stock solutions were prepared by dissolving 10 mg of the accurately weighted standard in HPLC grade methanol (MeOH, Fisher Scientific) at a concentration of 1 mg mL^{-1}. The amoxicillin solution was prepared in water, due to their limited solubility, sulfadiazine was dissolved in acetonitrile (ACN, Fisher scientific), guanylurea sulphate was dissolved in MeOH/water (1/1, v/v), and mebendazole in

ACN/formic acid (9/1, v/v) [1]. The deuterated standards were mainly purchased as solutions. Otherwise, stock solutions were prepared as described for the standards. From the stock solutions, three separate mixtures of deuterated ECs (2 µg mL^{-1}), ECs except antibiotics (2 µg mL^{-1}), and antibiotics only (2 µg mL^{-1}) were prepared in MeOH. These were then further diluted to working solutions. Working solutions and antibiotic mixtures were prepared every 3 months. All solutions were stored in the dark at -20°C.

Table S4: Relevant concentrations in the method validation. 50 µL internal standard mixture (100 µg L^{-1}) and 50 µL standard working solutions of different concentrations were added to 0.4 mL direct injection sample, 50 mL influent and effluent, 100 mL river water, and 0.1 g sludge.

c (µg L^{-1}) standard working solution	**c (µg L^{-1}) in water for direct injection**	**c (µg L^{-1}) before SPE (effluent, influent)**	**c (µg L^{-1}) before SPE (river water)**	**c (ng g^{-1}) in sludge**	**c (µg L^{-1}) in vial after extraction and in direct injection**
0	0	0	0	0	0
10	1.25	0.01	0.005		1
100	12.5	0.1	0.05	50	10
500	62.5	0.5	0.25	250	50
1000				500	100

S6 Calculation of nominal dilution

The nominal dilution of the septic tank discharge into the river was calculated from the flow of the receiving river per day (f_{river}) and the calculated flow of the septic tank effluent per day (f_{ST}) following equation S1.

$$dilution = \frac{(f_{river} - f_{ST})}{f_{ST}} \qquad (S1)$$

The flow of the septic tank effluent per day was calculated by multiplying the population equivalents (PE) by the average daily discharge per person per day (0.7252 m^3/day) (equation S2).[8]

$$f_{ST} = PE \cdot 0.7252\ m^3 day^{-1} \qquad (S2)$$

S7 MS/MS detection parameters

Table S5: MS/MS detection parameters for studied compounds (precursor ion, cone voltage (CV), quantifier and qualifier ions with collision energies (CE)), sorted according to retention times (RT).

RT /min	Analyte	Precursor Ion /m/z	CV /V	Quantifier Ion	CE /eV	Qualifier Ion	CE /eV
Positive Ionisation							
0.7	Guanylurea	103.1	16	60.1	10	86.1	8
0.8	Metformin	130.2	27	60.1	12	71.2	17
0.8	Metformin-d_6	136.3	28	60.1	13	-	-
1.2	Sulfanilamide	173.1	27	92.1	16	108.1	14
1.7	Cotinine-d_3	180.2	13	80.1	22	-	-
1.7	Cotinine	177.1	34	80.1	19	98.1	21
2.0	Amidotrizoic acid	631.9	29	361.2	26	233.2	46
2.2	Amoxicillin	366.1	29	114.1	19	208.2	12
2.2	Sotalol-d_6	279.2	24	214.1	17	-	-
2.3	Sotalol	273.2	25	133.2	28	213.2	17
2.3	Paracetamol-d_4	156.1	23	114.1	16	-	-
2.4	Paracetamol	151.9	26	110.0	16	92.9	24
2.5	Salbutamol-d_3	243.0	21	151.2	21	-	-
2.5	Salbutamol	240.2	27	148.1	20	166.1	12
2.5	Ranitidine	315.1	31	176.1	16	130.1	25
2.5	Atenolol-d_7	274.2	23	145.1	24	-	-
2.5	Atenolol	267.3	38	145.1	30	190.1	16
2.6	Sulfadiazine	251.1	18	156.1	15	92.1	26
2.9	3-Methoxy Paracetamol	182.2	22	108.1	16	80.1	29
3.0	3-Desmethyl Trimethoprim	277.2	30	261.2	25	123.2	35
3.1	alpha-Hydroxy Trimethoprim	307.2	22	289.2	14	274.2	20
3.3	Clopidol	192.1	27	101.1	24	87.1	28
3.3	Gabapentin	172.2	23	154.2	12	137.2	15
3.6	Trimethoprim	291.2	23	230.1	15	261.2	23
3.6	Caffeine	195.1	16	138.1	17	110.1	23
3.6	Caffeine-^{13}C	198.1	31	140.1	19	-	-
3.7	Ofloxacin-d_8	365.1	30	261.2	27	-	-
3.7	Ofloxacin	362.2	30	318.2	18	261.2	25
3.9	Ciprofloxacin	332.1	17	314.2	21	288.2	17
4.0	Lidocaine	235.2	29	86.1	17	-	-
4.1	Sulfamethoxazole	254.1	32	156.1	16	92.2	28
4.2	Ciprofloxacin-d_8	340.1	24	322.2	21	-	-
4.5	Desmethylvenlafaxine	264.3	29	246.3	12	107.1	30
4.7	Acebutolol	337.3	20	116.2	18	319.3	16
4.7	Acebutolol-d_5	342.3	19	121.2	23	-	-
4.7	Lamotrigine	256.1	24	211.1	25	187.1	27
4.8	Primidone-d_5	227.1	14	164.2	12	-	-
4.8	Metoprolol	268.2	30	159.1	22	191.2	17
4.8	Metoprolol-d_7	275.3	29	123.2	18	-	-
4.8	Primidone	219.1	28	162.1	12	91.1	25
4.8	Fluconazole	307.1	29	238.2	15	220.1	18
5.4	Ifosfamide	261.1	15	92.1	23	154.0	18
5.5	4-Hydroxy Omeprazole	316.2	22	168.1	24	149.2	24
5.8	Carbamazepine-10,11-epoxide-d_{10}	263.1	26	190.2	22	-	-

5.8	Chlorpheniramine	275.2	30	230.1	18	167.1	43
5.8	Chlorpheniramine-d_6	281.1	26	230.1	16	-	-
5.8	Venlaflaxine-d_6	284.3	34	266.3	12	-	-
5.9	Bisoprolol-d_5	331.2	23	121.2	17	-	-
5.9	Carbamazepine-10,11-epoxide	253.1	20	180.1	20	210.2	14
5.9	Venlafaxine	278.3	36	260.3	10	215.2	16
5.9	Bisoprolol	326.3	20	116.2	16	222.2	10
6.0	Propranolol-d_7	267.1	22	189.2	18	-	-
6.1	Propranolol	260.2	50	116.1	16	183.1	18
6.2	Citalopram	325.2	24	262.2	20	116.1	25
6.2	Citalopram-d_6	331.2	24	109.1	31	-	-
6.2	Desmethylcitalopram	311.2	22	109.1	20	262.2	17
6.4	Omeprazole	346.2	21	198.1	11	180.1	23
6.8	Hydroxyibuprofen	240.2	25	205.2	12	163.2	16
6.9	Carbamazepine-d_{10}	247.1	33	204.2	20	-	-
6.9	Carbamazepine	237.2	33	194.2	18	179.2	32
7.1	Mebendazole	296.1	19	264.2	23	105.1	33
7.3	Fexofenadine	502.4	37	466.5	25	171.2	35
7.3	Lansoprazole	370.1	29	252.1	11	119.2	20
7.4	Erythromycin	734.5	37	158.2	30	576.4	19
7.5	Oxazepam-d_5	292.1	26	246.2	25	-	-
7.5	Oxazepam	287.1	26	241.1	25	269.1	17
7.6	Lorazepam	321.1	25	275.1	22	303.1	16
7.7	Fluoxetine-d_6	316.1	19	154.2	9	-	-
7.7	Climbazole	293.1	23	69.2	21	41.2	26
7.7	Fluoxetine	310.2	34	44.1	10	148.1	10
7.8	Temazepam-d_5	306.1	24	260.2	21	-	-
7.8	Temazepam	301.1	24	255.2	21	283.2	14
7.8	Ketoprofen	255.2	50	209.2	15	105.1	22
7.8	Cetirizine	389.2	30	201.2	22	166.1	40
8.1	Clarithromycin	748.5	29	158.2	32	558.4	24
8.1	Clarithromycin-^{13}C-d_3	752.6	25	162.2	29	-	-
8.1	N-Desmethylclarithromycin	734.6	28	144.2	30	576.5	18
8.1	Norethisterone	299.2	16	231.2	18	109.2	26
8.1	Bezafibrate	362.1	25	139.1	25	316.2	14
8.1	Clotrimazole	277.1	27	165.2	20	242.2	20
8.2	Crotamiton	204.2	27	69.1	22	136.2	17
8.2	Warfarin	309.1	32	163.1	14	251.2	19
9.0	Atorvastin-d_5	564.4	27	445.4	22	-	-
9.0	Atorvastatin	559.2	28	440.3	23	250.2	43
9.3	Miconazole	417.0	18	159.1	30	161.1	28
Negative ionisation							
5.7	Estriol	287.1	36	171.1	37	145.1	39
6.0	Naproxen	229.0	9	170.1	14	185.1	5
7.0	Diclofenac	294.1	21	250	10	178.1	29
7.4	E2-d_4	275.2	35	147.3	37	160.2	30
7.4	E2	271.2	25	145.1	40	183.2	40
7.5	Estrone	269.1	35	145.1	38	159.2	34
7.5	Estrone-d_4	273.2	39	147.1	36	160.1	36
7.5	EE2	295.1	20	159.1	36	145.1	38

7.6	Ibuprofen	205.1	12	161.3	12	-	-
7.6	Ibuprofen-d_3	208.1	13	164.2	8	-	-
8.7	Gemfibrozil	249.0	13	121.1	20	-	-

S8 Instrument performance

For each analyte, the ratios of the peak area against the peak area of the internal standard (area ratio, ar), were plotted against the standard concentrations (c) above the IQL. A linear regression model (equation S3) was fitted, where m was the slope of the calibration line and b was the intercept with the y-axis.

$$ar = m \cdot c + b \qquad \text{(S3)}$$

The coefficient of determination (R^2) was calculated.

Table S6: Instrument performance information for analytes with the selected internal standard (IS), correlation coefficient (R2), linear concentration range, and instrument detection (IDL) and quantification limits (IQL), ordered by method and retention time.

RT	**compound**	**IS**	**R^2**	**cali range (µg L^{-1})**	**IDL (µg L^{-1})**	**IQL (µg L^{-1})**
0.7	Guanylurea	Salbutamol-d_3	0.997	0.5 – 100	0.25	0.5
0.8	Metformin	Metformin-d_6	0.997	0.1 – 500	0.05	0.1
1.2	Sulfanilamide	Primidone-d_5	0.999	1 – 100	0.5	1
1.7	Cotinine	Cotinine-d_3	0.992	0.05 – 100	0.005	0.02
2.0	Amidotrizoic acid	Salbutamol-d_3	1.000	0.5 – 100	0.1	0.3
2.2	Amoxicillin	Paracetamol-d_4	0.998	1 – 100	0.8	1
2.3	Sotalol	Sotalol-d_6	1.000	0.05 – 100	0.005	0.04
2.4	Paracetamol	Paracetamol-d_4	0.998	1 – 500	0.1	0.2
2.5	Salbutamol	Salbutamol-d_3	1.000	0.05 – 100	0.005	0.04
2.5	Ranitidine	Paracetamol-d_4	0.998	0.1 – 100	0.05	0.1
2.5	Atenolol	Atenolol-d_7	1.000	0.1 – 100	0.03	0.1
2.6	Sulfadiazine	Caffeine-^{13}C	0.997	0.5 – 100	0.02	0.07
2.9	3-Methoxy Paracetamol	Cotinine-d_3	1.000	0.1 – 100	0.03	0.1
3.0	3-Desmethyl Trimethoprim	Cotinine-d^3	0.999	0.05 – 100	0.01	0.02
3.1	α-Hydroxy Trimethoprim	Caffeine-^{13}C	0.994	0.1 – 100	0.05	0.1
3.3	Clopidol	Caffeine-^{13}C	0.999	5 – 100	0.3	1.1
3.3	Gabapentin	Caffeine-^{13}C	0.997	1 – 100	0.5	1
3.6	Trimethoprim	Paracetamol-d_4	0.999	0.05 – 100	0.01	0.05
3.6	Caffeine	Caffeine-^{13}C	0.995	1 – 100	0.08	0.3

3.7	Ofloxacin	Ofloxacin-d_8	0.991	0.1 – 100	0.05	0.1
3.9	Ciprofloxacin	Ofloxacin-d_8	0.991	0.05 – 100	0.02	0.05
4.0	Lidocaine	Carbamazepine-d_{10}	0.999	0.05 – 100	0.002	0.005
4.1	Sulfamethoxazole	Caffeine-^{13}C	0.997	0.1 – 100	0.05	0.1
4.5	Desmethylvenlafaxine	Venlafaxine-d_6	1.000	0.05 – 100	0.005	0.01
4.7	Acebutolol	Acebutolol-d_5	1.000	0.05 – 100	0.005	0.01
4.7	Lamotrigine	Carbamazepine-d_{10}	1.000	0.05 – 100	0.002	0.005
4.8	Metoprolol	Metoprolol-d_7	0.998	0.5 – 100	0.1	0.5
4.8	Primidone	Primidone-d_5	0.999	0.5 – 100	0.1	0.2
4.9	Fluconazole	Caffeine-^{13}C	0.997	0.1 – 100	0.03	0.1
5.4	Ifosfamide	Venlafaxine-d_6	0.999	0.5 – 100	0.05	0.25
5.5	4-Hydroxy Omeprazole	Carbamazepine-d_{10}	1.000	0.1 – 100	0.01	0.1
5.9	Chlorpheniramine	Chlorpheniramine-d_6	0.999	0.1 – 100	0.05	0.1
5.9	Carbamazepine-10,11-epoxide	Carbamazepine-10,11-epoxide-d_{10}	0.998	0.5 – 100	0.03	0.1
5.9	Venlafaxine	Venlafaxine-d_6	1.000	0.5 – 100	0.1	0.25
5.9	Bisoprolol	Bisoprolol-d_5	1.000	0.05 – 100	0.01	0.03
6.1	Propranolol	Propranolol-d_7	1.000	0.5 – 100	blank	0.1
6.2	Citalopram	Citalopram-d_6	0.999	0.1 – 100	0.02	0.1
6.2	Desmethylcitalopram	Citalopram-d_6	0.998	0.1 – 100	0.03	0.1
6.5	Omeprazole	Caffeine-^{13}C	0.995	1 – 100	0.7	1
6.8	Hydroxyibuprofen	Paracetamol-d_4	0.999	1 – 100	0.5	1
6.9	Carbamazepine	Carbamazepine-d_{10}	0.999	0.05 – 100	0.005	0.02
7.1	Mebendazole	Carbamazepine-d_{10}	0.999	5 – 100	blank	5
7.3	Fexofenadine	Venlafaxine-d_6	1.000	0.5 – 100	0.03	0.11
7.4	Erythromycin	Clarithromycin-^{13}C-d_3	1.000	0.05 – 50	0.01	0.05
7.5	Lansoprazole	Citalopram-d_6	0.998	1 – 100	0.05	1
7.6	Oxazepam	Oxazepam-d_5	0.998	0.5 – 100	0.2	0.5
7.6	Lorazepam	Temazepam-d_5	0.999	0.5 – 100	0.1	0.5
7.7	Climbazole	Clarithromycin-^{13}C-d_3	0.942	0.5 – 100	0.1	0.25
7.7	Fluoxetine	Fluoxetine-d_6	0.999	0.1 – 100	0.03	0.1
7.8	Temazepam	Temazepam-d_5	1.000	0.1 – 100	0.04	0.1
7.8	Ketoprofen	Temazepam-d_5	0.999	0.5 – 100	0.15	0.5
7.9	Cetirizine	Metoprolol-d_7	0.998	0.5 – 100	0.06	0.2
8.1	Clarithromycin	Clarithromycin-^{13}C-d_3	0.999	0.05 – 100	0.005	0.01
8.1	Norethisterone	Carbamazepine-d_{10}	1.000	0.5 – 100	0.15	0.5
8.1	Bezafibrate	Carbamazepine-d_{10}	1.000	0.5 – 100	0.15	0.5
8.2	Crotamiton	Carbamazepine-d_{10}	0.999	0.05 – 100	0.002	0.005
8.2	Clotrimazole	Clarithromycin-^{13}C-d_3	0.978	0.5 – 100	0.05	0.1
8.2	Warfarin	Carbamazepine-d_{10}	1.000	0.1 – 100	0.05	0.1

9.0	Atorvastatin	peak area	1.000	0.05 – 100	0.002	0.005
9.4	Miconazole	peak area	0.938	0.5 – 100	blank	0.5
5.6	Estriol	Estrone-d_4	1.000	0.5 – 100	0.1	0.5
6.0	Naproxen	Ibuprofen-d_3	0.996	1 – 100	0.05	1
7.1	Diclofenac	Ibuprofen-d_3	0.994	0.5 – 100	blank	0.5
7.4	17β-Estradiol	17β-Estradiol-d_4	1.000	0.5 – 100	0.1	0.3
7.5	Estrone	Estrone-d_4	1.000	0.5 – 100	0.1	0.3
7.5	17α-Ethinylestradiol	17β-Estradiol-d_4	0.999	1 – 100	0.5	1
7.5	Ibuprofen	Ibuprofen-d_3	0.994	0.5 - 500	0.1	0.5
8.7	Gemfibrozil	Ibuprofen-d_3	0.995	0.1 – 100	0.05	0.1

For precision, the relative standard deviation of the replicates was calculated. Accuracies were determined from the percentage deviation of the standards from the calibration curve. Therefore, concentrations (c_{calc}) of the 1, 10, and 50 µg L^{-1} standards were calculated from the area ratios (ar) following subtraction of the calculated concentration (c_0) of the blank using equation S4.

$$c_{calc} = \frac{(ar - b)}{m} - c_0 \tag{S4}$$

Accuracy was then calculated from the ratio of the calculated and standard concentration (c_{std}) according to equation S5.

$$accuracy = \frac{c_{calc}}{c_{std}} \cdot 100\,\% \tag{S5}$$

Table S7: Intra- and Inter-day accuracy and precision (both in %) with p-values (two sample t-test with rstatix in R) for the repeated injection of 1, 10, and 50 µg L^{-1} standards.

RT	Analyte	Accuracy (/%)							Precision (/%)						
		Intra day			Inter day			p-value	Intra day			Inter day			p-value
		1 µg L^{-1}	10 µg L^{-1}	50 µg L^{-1}	1 µg L^{-1}	10 µg L^{-}	50 µg L^{-1}		1 µg L^{-1}	10 µg L^{-1}	50 µg L^{-1}	1 µg L^{-1}	10 µg L^{-}	50 µg L^{-1}	
0.7	Guanylurea	105	101	89	120	104	106	0.173	7	2	3	8	1	5	0.752
0.8	Metformin	90	98	106	90	100	108	0.891	9	1	4	3	1	5	0.532
1.2	Sulfanilamide	76	91	104	98	99	97	0.428	10	8	4	4	6	9	0.734
1.7	Cotinine	102	100	106	98	99	97	0.100	3	4	7	7	6	4	0.513
2.0	Amidotrizoic acid	100	102	99	94	100	105	0.794	13	7	6	5	11	11	0.858
2.2	Amoxicillin	99	110	100	118	100	113	0.336	9	10	4	20	15	10	0.117
2.3	Sotalol	100	100	98	108	90	99	0.961	7	2	2	2	8	3	0.843
2.4	Paracetamol	84	112	107	101	110	111	0.559	5	6	2	5	8	4	0.334
2.5	Salbutamol	100	101	96	97	96	91	0.160	9	1	4	9	5	7	0.461
2.5	Ranitidine	95	110	102	74	89	90	0.054	15	10	14	9	9	14	0.395
2.5	Atenolol	100	101	100	107	96	98	0.932	8	7	6	12	2	2	0.730
2.6	Sulfadiazine	106	101	90	93	92	84	0.199	7	4	3	7	4	6	0.491
2.9	3-Methoxy Paracetamol	122	98	100	124	97	110	0.722	6	6	2	4	8	5	0.755
3.0	3-Desmethyl Trimethoprim	101	96	94	116	101	109	0.098	7	4	5	4	11	5	0.602
3.1	α-Hydroxy Trimethoprim	107	101	92	111	108	97	0.441	3	8	5	16	7	1	0.609
3.3	Clopidol	[a]	104	94	[a]	108	96	0.711	[a]	6	8	[a]	6	7	0.674
3.3	Gabapentin	119	95	92	98	99	97	0.694	5	8	5	11	7	1	0.868
3.6	Trimethoprim	102	106	95	126	112	112	0.053	6	6	5	3	5	6	0.240
3.6	Caffeine	79	93	93	94	116	97	0.186	15	6	5	15	5	5	0.921
3.7	Ofloxacin	80	98	100	94	93	96	0.825	9	8	5	6	4	29	0.536
3.9	Ciprofloxacin	97	97	99	104	109	132	0.185	6	7	10	19	5	21	0.280

4.0	Lidocaine	100	100	101	95	93	93	0.004	6	1	4	16	10	4	0.206
4.1	Sulfamethoxazole	101	106	100	95	102	100	0.273	12	8	5	4	11	6	0.719
4.5	Desmethylvenlafaxine	85	99	100	97	100	94	0.650	6	3	5	10	6	2	0.632
4.7	Acebutolol	99	100	94	99	96	97	0.750	8	5	4	4	4	1	0.205
4.7	Lamotrigine	112	103	97	113	102	98	0.906	7	5	7	9	8	5	0.458
4.8	Primidone	102	99	104	104	108	106	0.100	6	4	8	3	5	3	0.225
4.8	Metoprolol	111	100	104	99	95	100	0.136	7	3	3	3	3	7	0.936
4.9	Fluconazole	102	100	94	98	94	94	0.367	6	7	2	7	5	2	0.954
5.4	Ifosfamide	90	99	93	95	93	88	0.615	9	1	6	2	8	7	0.843
5.5	4-Hydroxy Omeprazole	112	101	98	108	96	95	0.521	6	6	6	1	1	4	0.062
5.9	Chlorpheniramine	72	108	102	68	112	97	0.939	1	5	5	6	1	2	0.830
5.9	Carbamazepine-10,11-epoxide	106	101	95	96	101	96	0.513	12	6	7	10	1	6	0.488
5.9	Venlafaxine	97	102	101	94	103	98	0.590	7	6	4	5	5	5	0.625
5.9	Bisoprolol	100	101	100	100	100	103	0.426	2	4	6	6	5	4	0.423
6.1	Propranolol	100	100	98	101	111	94	0.626	7	10	5	4	7	3	0.185
6.2	Citalopram	98	98	96	94	101	101	0.634	5	4	5	7	3	3	0.907
6.2	Desmethylcitalopram	99	97	95	107	107	103	0.007	6	4	9	6	10	7	0.615
6.5	Omeprazole	[a]	99	103	[a]	106	98	0.773	18	21	16	[a]	13	21	0.752
6.8	Hydroxyibuprofen	98	99	94	97	105	108	0.178	23	5	7	18	5	4	0.722
6.9	Carbamazepine	102	100	99	100	96	99	0.288	4	6	5	4	3	5	0.132
7.1	Mebendazole	[a]	100	96	86	85	90	0.046	2	4	5	10	5	6	0.189
7.3	Fexofenadine	91	106	102	93	115	108	0.503	6	3	5	10	3	6	0.410
7.4	Erythromycin	110	99	[b]	105	87	[b]	0.523	8	9	2	2	2	9	0.498
7.5	Lansoprazole	125	100	101	136	121	84	0.794	28	11	7	36	14	30	0.287
7.6	Oxazepam	120	99	100	102	98	95	0.376	6	7	9	7	13	2	1.000

7.6	Lorazepam	108	104	112	103	108	101	0.233	9	10	13	5	1	8	0.085
7.7	Climbazole	[a]	82	110	[a]	116	106	0.451	10	1	7	4	1	5	0.479
7.7	Fluoxetine	96	107	93	80	105	92	0.514	5	5	4	2	4	5	0.520
7.8	Temazepam	107	103	102	104	106	103	0.639	6	4	8	9	5	6	0.717
7.8	Ketoprofen	93	102	104	84	97	91	0.169	5	5	10	12	11	1	0.786
7.9	Cetirizine	82	100	107	126	219	235	0.098	7	4	6	3	3	7	0.405
8.1	Clarithromycin	94	98	87	97	98	89	0.748	5	2	6	2	4	2	0.282
8.1	Norethisterone	116	101	99	105	105	106	0.967	15	4	3	16	9	12	0.299
8.1	Bezafibrate	115	100	98	97	94	92	0.194	7	3	6	2	7	3	0.541
8.2	Crotamiton	99	100	97	82	92	91	0.062	9	2	4	9	7	4	0.573
8.2	Clotrimazole	[a]	75	103	[a]	111	83	0.728	7	8	8	3	2	5	0.036
8.2	Warfarin	108	101	99	97	101	95	0.220	6	7	4	7	2	6	0.697
9.0	Atorvastatin	101	103	81	117	112	94	0.255	11	7	5	15	1	9	0.881
9.4	Miconazole	112	102	88	87	91	92	0.265	7	8	13	5	12	2	0.535
5.6	Estriol	101	102	98	76	83	85	0.012	14	3	4	12	6	3	0.947
6.0	Naproxen	115	96	104	106	106	111	0.703	4	8	6	5	2	15	0.798
7.1	Diclofenac	110	110	106	104	100	100	0.018	4	10	9	4	13	12	0.571
7.4	17β-Estradiol	101	105	102	105	101	102	0.846	15	6	6	17	13	12	0.222
7.5	Estrone	109	98	101	103	105	106	0.662	8	6	4	3	2	6	0.313
7.5	17α-Ethinylestradiol	112	109	99	115	101	108	0.834	4	3	5	2	13	5	0.570
7.5	Ibuprofen	[a]	106	98	[a]	112	92	0.981	9	2	11	10	11	10	0.829
8.7	Gemfibrozil	86	102	98	89	91	91	0.389	6	5	6	10	15	13	0.034

a) c ≤ IQL, b) c ≥ calibration range

S9 Method performance

Table S8: Absolute recoveries (REC) and relative standard deviation (sd) (both in %) from spiked wastewater (c = 10ng/mL) using polytetrafluoroethylene (PTFE), cellulose acetate (CA), polyvinylidene fluoride hydrophilic (PVDF-HL), and polyvinylidene fluoride hydrophobic (PVDF) syringe filters (n = 3). Sorted according to method and retention times.

RT /min	Analyte	PTFE REC	PTFE sd	CA REC	CA sd	PVDF REC	PVDF sd	PVDF-HL REC	PVDF-HL sd
positive									
0.7	Guanylurea	99	3	101	3	95	3	91	2
0.8	Metformin	97	4	97	4	88	4	88	2
1.2	Sulfanilamide	91	7	93	6	87	5	82	9
1.7	Cotinine	102	4	100	3	94	4	93	5
2.2	Amoxicillin	120	7	42	19	82	9	83	6
2.3	Sotalol	103	4	96	4	86	6	86	3
2.4	Paracetamol	100	3	98	2	104	2	101	3
2.5	Salbutamol	102	5	98	5	86	2	86	2
2.5	Ranitidine	99	4	99	7	85	4	83	4
2.5	Atenolol	96	5	95	7	85	6	83	3
2.6	Sulfadiazine	99	2	95	6	84	4	79	3
3.3	Gabapentin	103	5	100	6	88	2	88	5
3.6	Trimethoprim	102	3	97	3	88	3	87	4
3.6	Caffeine	95	9	91	8	88	10	89	11
3.7	Ofloxacin	100	4	97	7	79	3	83	1
3.9	Ciprofloxacin	99	2	93	7	84	3	79	3
4.0	Lidocaine	94	5	94	6	83	3	85	3
4.1	Sulfamethoxazole	98	4	97	7	88	3	87	3
4.5	Desmethylvenlafaxine	96	5	93	7	84	2	84	3
4.7	Acebutolol	92	5	91	6	73	3	84	4
4.7	Lamotrigine	100	6	94	5	87	4	85	4
4.8	Metoprolol	97	3	95	4	81	6	86	4
4.8	Primidone	106	4	100	4	91	4	87	6
4.8	Fluconazole	97	5	98	6	86	5	85	3
5.4	Ifosfamide	99	5	93	8	89	5	86	5
5.8	Chlorpheniramine	59	3	73	4	12	2	72	7
5.9	Carbamazepine-10,11-epoxide	101	4	96	6	88	3	88	4
5.9	Venlafaxine	76	10	87	11	59	3	79	3
5.9	Bisoprolol	88	7	90	6	67	4	83	3
6.1	Propranolol	55	3	52	5	42	4	57	7
6.2	Citalopram	27	47	39	3	6	15	49	6
6.2	Desmethylcitalopram	24	48	35	61	16	13	38	7
6.4	Omeprazole	98	3	91	6	87	2	87	4
6.8	Hydroxyibuprofen	100	3	95	6	91	2	90	3
6.9	Carbamazepine	100	3	94	6	87	2	87	3
7.1	Mebendazole	104	4	67	13	89	2	89	3

7.3	Fexofenadine	88	7	93	7	59	4	85	5
7.4	Erythromycin	56	34	82	16	18	12	79	5
7.5	Oxazepam	94	4	85	6	82	6	83	5
7.6	Lorazepam	96	3	79	6	81	5	82	3
7.7	Climbazole	81	6	58	15	69	4	68	5
7.7	Fluoxetine	5	13	26	13	7	22	11	12
7.8	Temazepam	96	3	88	4	80	2	84	4
7.8	Ketoprofen	106	4	103	4	95	2	91	4
7.8	Cetirizine	50	15	52	4	31	3	56	8
8.1	Clarithromycin	27	54	61	26	6	12	65	6
8.1	Norethisterone	102	3	76	5	85	4	84	3
8.1	Bezafibrate	101	9	98	4	89	15	89	22
8.1	Clotrimazole	28	39	13	59	9	14	10	31
8.2	Warfarin	100	7	96	4	93	11	90	12
9.0	Atorvastatin	88	4	65	6	71	3	75	2
9.3	Miconazole	8	28	11	22	4	7	11	28
negative									
5.7	Estriol	119	14	112	4	107	19	110	25
6.0	Naproxen	101	13	103	7	91	9	91	23
7.0	Diclofenac	96	9	98	5	91	15	101	16
7.4	E2	101	12	63	7	97	22	80	14
7.5	Estrone	95	12	37	6	84	17	71	9
7.6	Ibuprofen	98	11	99	5	91	12	92	19
8.7	Gemfibrozil	97	9	96	3	94	13	90	12

Table S9: Absolute recoveries (Rec) and relative standard deviation (sd) (both in %) for each analyte in influent, effluent, river water, and sludge (n = 3). If not otherwise stated all spiked samples were used in the calculation.

RT /min	**Analyte**	**Direct inject.**						**SPE**						**Sludge**	
		Influent		Effluent		River		Influent		Effluent		River			
		Rec	sd	Rec	sd	Rec	sd	Rec	sd	Rec	sd	Rec	sd	Rec	sd
0.7	Guanylurea	52	7	55	6	184	13	4	2	3[a]	4	2	1	[d]	-
0.8	Metformin	161	74[b]	192[a]	60[b]	186	86[b]	16	2	[b]	-	4[c]	3	[d]	-
0.8	Metformin-d$_6$	209	7	164	8	241	11	9	5	7	4	3	2	[d]	-
1.2	Sulfanilamide	84[a]	9	77	6	98	7	11[a]	2	15[a]	7	16	14	5[d]	5
1.7	Cotinine-d$_3$	96	8	100	6	98	8	89	11	82	4	91	12	108	30
1.7	Cotinine	94	3	103	9	95	5	80	6	[b]	-	90	10	100	14

2.0	Amidotrizoic acid	95	8	98	11	92	7	0	0	1	0	0	0	0[d]	-
2.2	Amoxicillin	87	7	96	9	98	11	11[a]	9	31[a]	6	1	1	0[d]	-
2.2	Sotalol-d_6	98	4	106	6	98	4	87	5	89	4	90	9	23	5
2.3	Sotalol	101	6	99	3	99	7	79	10	78	3	87	13	28	3
2.3	Paracetamol-d_4	84	5	96	3	87	7	58	2	40	2	51	12	33	2
2.4	Paracetamol	86[a]	5	[b]	-	90[a]	9	[b]	-	[b]	-	33[a]	11	[b]	-
2.5	Salbutamol-d_3	96	6	104	5	92	3	92	7	95	6	79	7	17	8
2.5	Salbutamol	101	4	107	6	101	6	112	18	88	4	79	7	13	3
2.5	Ranitidine	121	35	142	18	118	37	59	11	98	19	85	21	2[d]	1
2.5	Atenolol-d_7	97	5	103	6	98	7	100	4	93	6	91	7	31	9
2.5	Atenolol	107	7	103	6	107	12	88	11	84[a]	8	92	12	37	9
2.6	Sulfadiazine	94	3	105	14	97	9	70	4	55	11	75	4	20	9
2.9	3-Methoxy Paracetamol	98[a]	8	105[a]	12	99	11	81[c]	3	[b]	-	87	10	79	18
3.0	3-Desmethyl Trimethoprim	102	6	104	5	105	9	85	7	63	16	79	11	63	9
3.1	α-Hydroxy Trimethoprim	104	5	105	4	107	5	91	11	81	4	86	11	44	10
3.3	Clopidol	86[a]	6	99[a]	8	94[a]	13	78[a]	10	74[a]	5	70[a]	5	77	9
3.3	Gabapentin	89	8	112	18	92	12	7	5	[b]	-	2	2	7[d]	2
3.6	Trimethoprim	103	10	109	4	105	9	69	6	50[a]	7	75	10	53	19
3.6	Caffeine	59	29	99[c]	23	73	27	[b]	-	[b]	-	64[a]	20	101[e]	1
3.6	Caffeine-^{13}C	90	7	105	5	93	6	86	8	82	5	92	8	97	4
3.7	Ofloxacin	47	6	116	5	41	4	17	2	34	13	4	2	15	2
3.7	Ofloxacin-d_8	95	1	128	8	91	15	37	5	71	7	10	2	12	3
3.9	Ciprofloxacin	59	28	106	34	50	20	194	36	43	8	87	4	18	6
4.0	Lidocaine	96	3	99	5	101	6	57	2	43	5	75	10	50	16
4.1	Sulfamethoxazole	94	4	103	4	100	7	81	4	75	9	88	11	47	19
4.5	Desmethylvenlafaxine	93	3	98	6	98	6	49	3	64[a]	13	76	11	54	13

4.7	Acebutolol-d_5	102	8	106	7	105	11	80	8	76	3	79	7	22	5
4.7	Acebutolol	104	6	112	10	107	10	83	2	83	7	78	8	22	4
4.7	Lamotrigine	93	7	100	5	98	6	46	5	61	11	71	11	29[c]	10
4.8	Metoprolol-d_7	96	4	98	4	99	5	62	4	66	4	75	9	24	4
4.8	Metoprolol	97	10	102	8	106	8	55	8	71	13	77	8	22	4
4.8	Primidone-d_5	92	13	99	4	93	10	74	11	85	6	90	12	75	9
4.8	Primidone	96	8	95	3	98	8	72	8	78	5	92	10	84	9
4.9	Fluconazole	89	6	97	4	96	7	67	7	75	5	84	4	91	9
5.4	Ifosfamide	88	3	102	9	94	7	57	5	71	8	85	8	77	6
5.5	4-Hydroxy Omeprazole	89	6	102	2	97	9	57[a]	13	55[a]	2	69	9	86	26
5.8	Chlorpheniramine-d_6	175	12	164	25	209	16	111	10	96	7	44	13	72	12
5.9	Chlorpheniramine	82	23	162	9	97	25	189	11	[b]	-	20	11	67	38
5.8	Carbamazepine-10,11-epoxide-d_{10}	89	10	108	16	87	8	77	10	92	18	89	15	73	9
5.9	Carbamazepine-10,11-epoxide	87	5	100	4	91	7	79	8	84	9	84	7	93	9
5.8	Venlafaxine-d_6	95	6	99	7	99	7	58	4	60	4	70	5	55	8
5.9	Venlafaxine	91	5	108	11	101	8	53	7	[b]	-	55	12	49[e]	20
5.9	Bisoprolol-d_5	107	4	103	11	103	4	101	4	93	7	88	13	24	4
5.9	Bisoprolol	109	4	111	10	108	6	105	5	84[a]	15	85	12	24	7
6.0	Propranolol-d_7	94	9	102	12	106	11	80	6	71	8	33	10	[d]	-
6.1	Propranolol	90	6	95	12	101	9	65	5	54[a]	6	25	6	32	12
6.2	Citalopram-d_6	84	7	96	18	113	8	117	5	108	16	13	5	65	10
6.2	Citalopram	85	12	103	6	118	13	69	14	[b]	-	12	4	73[c]	10
6.2	Desmethylcitalopram	88	12	99	9	118	12	62	9	54[a]	7	7	6	8	3
6.5	Omeprazole	26[a]	6	21[a]	13	23[a]	3	37[a]	19	30[a]	6	54[a]	19	[d]	-
6.8	Hydroxyibuprofen	90[a]	9	116[c]	20	95[a]	5	[b]	-	[b]	-	50[a]	13	34	1
6.9	Carbamazepine-d_{10}	91	8	103	6	93	10	52	6	51	5	79	10	72	14

6.9	Carbamazepine	89	5	101	5	91	5	53	5	45	7	72	10	64	5
7.1	Mebendazole	63[a]	2	41[a]	26	67[a]	2	52[a]	1	23[a]	5	33[a]	1	25	9
7.3	Fexofenadine	97	3	103	11	100	6	65[a]	2	[b]	-	71	4	112	31
7.4	Erythromycin	75	59	140	19	63	45	3	1	2	1	21	18	33[e]	22
7.5	Lansoprazole	23[a]	5	19[a]	8	19[a]	4	35	20	29[a]	20	19[a]	11	[d]	-
7.5	Oxazepam-d_5	90	8	102	17	83	16	143	32	60	22	68	11	107	16
7.6	Oxazepam	91	5	93	8	90	4	111	26	[b]	-	68	12	80	17
7.6	Lorazepam	93	15	94	11	101	22	88[a]	7	25[a]	8	75	14	84	21
7.7	Climbazole	88	5	100	4	89	6	60	14	9	7	18	7	36	16
7.7	Fluoxetine-d_6	26	7	35	10	73	13	34	6	13	5	1[a]	0	10	29
7.7	Fluoxetine	32	11	34	4	101	23	43	13	8	3	1[a]	3	5	2
7.8	Temazepam-d_5	94	6	105	5	92	9	102	5	32	13	72	7	108	13
7.8	Temazepam	95	7	100	5	99	6	99	14	19	7	72	7	79	13
7.8	Ketoprofen	94	5	100	9	96	7	101	8	27	11	92	13	112	13
7.9	Cetirizine	68	19	95	17	68	16	38	14	[b]	-	46	19	46	17
8.1	Clarithromycin	166	30	137	26	154	38	67	10	16	4	27	9	23[e]	7
8.1	Clarithromycin-^{13}C-d_3	144	25	104	34	140	42	68	16	17	5	21	7	39	9
8.1	Norethisterone	87	10	95	7	95	10	42	6	30	12	55	10	48	10
8.1	Bezafibrate	97	6	102	6	99	6	85	12	36	13	88	12	118	10
8.2	Clotrimazole	81	15	58	11	47	16	24	4	19	3	1	0	25[e]	11
8.2	Crotamiton	93	4	97	4	98	7	46	6	16[c]	4	63	6	42	12
8.2	Warfarin	95	4	98	4	97	8	63	5	33	9	83	7	86	15
9.0	Atorvastatin	84	7	105	5	79	5	27	9	[b]	-	31	7	40	5
9.4	Miconazole	85	30	43	8	27	17	8	2	11	6	1[a]	6	30[e]	11
5.6	Estriol	104	23	96[a]	4	95	18	64	5	132[c]	7	85	11	73	15
6.0	Naproxen	92[a]	8	102[a]	11	84	6	81[a]	7	[b]	-	104[a]	7	108[c]	17

7.1	Diclofenac	96[a]	5	99[a]	4	96[a]	3	69[a]	4	92[a]	18	85[a]	5	49[e]	9
7.4	17β-Estradiol-d_4	98	7	96	6	95	3	73	5	81	3	46	5	39	11
7.4	17β-Estradiol	98	9	99	9	98	10	70	10	77	9	43	5	35	12
7.5	Estrone-d_4	99	4	102	7	99	6	72	3	79	4	43	5	33	7
7.5	Estrone	106	15	102	3	97	6	62	12	86[c]	5	41	6	36	11
7.5	17α-Ethinylestradiol	103	17	103[a]	7	107	18	71[a]	11	71[a]	11	19[a]	3	35	12
7.5	Ibuprofen	101[a]	5	89[a]	6	89[a]	5	[b]	-	[b]	-	77[a]	11	[b]	-
7.6	Ibuprofen-d_3	99	15	109	14	93	8	172	14	115	7	100	10	95	15
8.7	Gemfibrozil	91	10	102[a]	4	91	10	17	4	67 [a]	4	75	9	36	7

a) calculated from $c \geq 10$ µgL^{-1}, b) c too high in sample, c) calculated from $c \geq 50$ µgL^{-1}, d) method not suitable, e) calculated from $c \geq 100$ µgL^{-1}

If an EC concentration in the SPE sample was too high for the calculation of the absolute recovery ($REC_{\text{SPE,matrix 1}}$) to be practical (Table S9), a theoretical absolute recovery was calculated from the average ratios of the absolute recoveries in direct injection ($REC_{\text{di,matrix}}$) and SPE ($REC_{\text{SPE,matrix}}$) (equation S6) and used to determine *MDL* and *MQL* as described in equations S4 and S5, respectively. For ECs withisotopically labelled EC, the absolute recovery of the isotopically labelled EC was used.

$$REC_{SPE,\,matrix\,1} = REC_{di,\,matrix\,1} \cdot 0.5 \cdot \left(\frac{REC_{SPE,\,matrix\,2}}{REC_{di,\,matrix\,2}} + \frac{REC_{SPE,\,matrix\,3}}{REC_{di,\,matrix\,3}}\right) \qquad \text{(S6)}$$

Relative recoveries (Figure 4) were calculated accordingly.

Table S10: Method detection (*MDL*) and quantitation limits (*MQL*) in influent, effluent, river water and sludge analysed by direct injection and *SPE*

RT	**Analyte**	**Direct injection in µg L^{-1}**						**SPE in µg L^{-1}**						**Sludge in µg kg^{-1}**	
/min		**Influent**		**Effluent**		**River**		**Influent**		**Effluent**		**River**			
		MDL	MQL	MDL	MQL	MDL	MQL	MDL	MQL	MDL	MQL	MDL	MQL	MDL	MQL

0.7	Guanylurea	0.53	1.1	0.51	1.0	0.15	0.30	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
0.8	Metformin	0.035	0.069	0.029	0.058	0.030	0.060	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
1.2	Sulfanilamide	0.66	1.3	0.72	1.4	0.57	1.1	0.045	0.091	0.033	0.067	0.016	0.031	[a]	[a]
1.7	Cotinine	$5.9 \cdot 10^{-3}$	0.019	$5.4 \cdot 10^{-3}$	0.017	$5.9 \cdot 10^{-3}$	0.019	$6.3 \cdot 10^{-5}$	$2.0 \cdot 10^{-4}$	$6.1 \cdot 10^{-5}$	$2.0 \cdot 10^{-4}$	$2.9 \cdot 10^{-5}$	$8.9 \cdot 10^{-5}$	0.025	0.080
2.0	Amidotrizoic acid	0.12	0.35	0.11	0.34	0.10	0.36	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
2.2	Amoxicillin	1.0	1.3	0.93	1.2	0.91	1.1	0.073	0.091	0.026	0.032	0.40	0.50	[a]	[a]
2.3	Sotalol	$5.5 \cdot 10^{-3}$	0.044	$5.6 \cdot 10^{-3}$	0.045	$5.6 \cdot 10^{-3}$	0.045	$5.8 \cdot 10^{-5}$	$4.6 \cdot 10^{-4}$	$5.6 \cdot 10^{-5}$	$4.5 \cdot 10^{-4}$	$2.9 \cdot 10^{-5}$	$2.3 \cdot 10^{-4}$	0.090	0.72
2.4	Paracetamol	0.086	0.29	0.077	0.26	0.082	0.27	$1.1 \cdot 10^{-3}$	$3.8 \cdot 10^{-3}$	$1.7 \cdot 10^{-3}$	$5.6 \cdot 10^{-3}$	$6.5 \cdot 10^{-4}$	$2.2 \cdot 10^{-3}$	1.0	3.1
2.5	Salbutamol	$5.5 \cdot 10^{-3}$	0.044	$5.2 \cdot 10^{-3}$	0.042	$5.5 \cdot 10^{-3}$	0.044	$5.4 \cdot 10^{-5}$	$4.3 \cdot 10^{-4}$	$5.3 \cdot 10^{-5}$	$4.2 \cdot 10^{-4}$	$3.2 \cdot 10^{-5}$	$2.5 \cdot 10^{-4}$	0.19	1.6
2.5	Ranitidine	0.046	0.092	0.039	0.078	0.047	0.094	$8.5 \cdot 10^{-4}$	$1.7 \cdot 10^{-3}$	$5.1 \cdot 10^{-4}$	$1.0 \cdot 10^{-3}$	$2.9 \cdot 10^{-4}$	$5.9 \cdot 10^{-4}$	[a]	[a]
2.5	Atenolol	0.026	0.10	0.027	0.11	0.026	0.10	$2.8 \cdot 10^{-4}$	$1.1 \cdot 10^{-3}$	$3.0 \cdot 10^{-4}$	$1.2 \cdot 10^{-3}$	$1.4 \cdot 10^{-4}$	$5.4 \cdot 10^{-4}$	0.33	1.3
2.6	Sulfadiazine	0.024	0.079	0.021	0.071	0.023	0.076	$2.9 \cdot 10^{-4}$	$9.5 \cdot 10^{-4}$	$3.6 \cdot 10^{-4}$	$1.2 \cdot 10^{-3}$	$1.3 \cdot 10^{-4}$	$4.4 \cdot 10^{-4}$	0.49	1.6
2.9	3-Methoxy Paracetamol	0.034	0.11	0.032	0.11	0.034	0.11	$3.7 \cdot 10^{-4}$	$1.2 \cdot 10^{-3}$	$3.4 \cdot 10^{-4}$	$1.1 \cdot 10^{-3}$	$1.7 \cdot 10^{-4}$	$5.7 \cdot 10^{-4}$	0.19	0.63
3.0	3-Desmethyl Trimethoprim	$5.6 \cdot 10^{-3}$	0.019	0.055	0.018	0.055	0.018	$6.1 \cdot 10^{-5}$	$2.0 \cdot 10^{-4}$	$8.2 \cdot 10^{-5}$	$2.7 \cdot 10^{-4}$	$3.3 \cdot 10^{-5}$	$1.1 \cdot 10^{-4}$	0.041	0.14
3.1	α-Hydroxy Trimethoprim	0.053	0.11	0.053	0.11	0.052	0.10	$5.5 \cdot 10^{-4}$	$1.1 \cdot 10^{-3}$	$6.2 \cdot 10^{-4}$	$1.2 \cdot 10^{-3}$	$2.9 \cdot 10^{-4}$	$5.8 \cdot 10^{-4}$	0.57	1.1
3.3	Clopidol	0.41	1.4	0.35	1.2	0.37	1.2	$4.0 \cdot 10^{-3}$	0.013	$4.3 \cdot 10^{-3}$	0.014	$2.3 \cdot 10^{-3}$	$7.5 \cdot 10^{-3}$	2.1	6.9
3.3	Gabapentin	0.62	1.2	0.50	0.99	0.60	1.2	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
3.6	Trimethoprim	0.013	0.045	0.013	0.042	0.013	0.044	$1.8 \cdot 10^{-4}$	$6.0 \cdot 10^{-4}$	$2.5 \cdot 10^{-4}$	$8.3 \cdot 10^{-4}$	$8.3 \cdot 10^{-5}$	$2.8 \cdot 10^{-4}$	0.12	0.39
3.6	Caffeine	0.099	0.35	0.090	0.31	0.096	0.33	$9.3 \cdot 10^{-4}$	$3.3 \cdot 10^{-3}$	$9.8 \cdot 10^{-3}$	$3.4 \cdot 10^{-3}$	$4.3 \cdot 10^{-4}$	$1.5 \cdot 10^{-3}$	0.40	1.4

3.7	Ofloxacin	0.058	0.12	0.043	0.087	0.14	0.27	$2.9 \cdot 10^{-3}$	$5.9 \cdot 10^{-3}$	$1.5 \cdot 10^{-3}$	$2.9 \cdot 10^{-3}$	$6.3 \cdot 10^{-3}$	0.013	1.6	3.2
3.9	Ciprofloxacin	0.038	0.094	0.021	0.052	0.044	0.11	$1.0 \cdot 10^{-4}$	$2.6 \cdot 10^{-4}$	$4.7 \cdot 10^{-4}$	$1.2 \cdot 10^{-3}$	$1.1 \cdot 10^{-4}$	$2.9 \cdot 10^{-4}$	0.56	1.4
4.0	Lidocaine	$6.3 \cdot 10^{-3}$	0.021	$6.1 \cdot 10^{-3}$	0.020	$6.0 \cdot 10^{-3}$	0.020	$9.6 \cdot 10^{-5}$	$3.2 \cdot 10^{-4}$	$1.3 \cdot 10^{-4}$	$4.2 \cdot 10^{-4}$	$3.6 \cdot 10^{-5}$	$1.2 \cdot 10^{-4}$	0.054	0.18
4.1	Sulfamethoxazole	0.059	0.12	0.054	0.11	0.056	0.11	$6.2 \cdot 10^{-4}$	$1.2 \cdot 10^{-3}$	$6.7 \cdot 10^{-4}$	$1.3 \cdot 10^{-3}$	$2.8 \cdot 10^{-4}$	$5.7 \cdot 10^{-4}$	0.54	1.1
4.5	Desmethylvenlafaxine	$6.0 \cdot 10^{-3}$	0.012	$5.7 \cdot 10^{-3}$	0.011	$5.7 \cdot 10^{-3}$	0.011	$1.0 \cdot 10^{-4}$	$2.0 \cdot 10^{-4}$	$7.8 \cdot 10^{-5}$	$1.6 \cdot 10^{-4}$	$3.3 \cdot 10^{-5}$	$6.6 \cdot 10^{-5}$	0.046	0.093
4.7	Acebutolol	0.053	0.18	0.050	0.17	0.052	0.17	$6.0 \cdot 10^{-4}$	$2.0 \cdot 10^{-3}$	$6.0 \cdot 10^{-4}$	$2.0 \cdot 10^{-3}$	$3.2 \cdot 10^{-4}$	$1.1 \cdot 10^{-3}$	1.1	3.7
4.7	Lamotrigine	0.21	0.70	0.20	0.65	0.20	0.67	$3.8 \cdot 10^{-3}$	0.013	$2.9 \cdot 10^{-3}$	$9.6 \cdot 10^{-3}$	$1.2 \cdot 10^{-3}$	$4.1 \cdot 10^{-3}$	3.1	10
4.8	Primidone	0.071	0.24	0.072	0.24	0.069	0.24	$8.5 \cdot 10^{-4}$	$2.8 \cdot 10^{-3}$	$7.8 \cdot 10^{-4}$	$2.6 \cdot 10^{-3}$	$3.3 \cdot 10^{-4}$	$1.1 \cdot 10^{-3}$	0.36	1.2
4.8	Metoprolol	0.11	0.57	0.11	0.54	0.10	0.52	$1.8 \cdot 10^{-3}$	$9.1 \cdot 10^{-3}$	$1.4 \cdot 10^{-3}$	$7.0 \cdot 10^{-3}$	$6.5 \cdot 10^{-4}$	$3.2 \cdot 10^{-3}$	2.3	12
4.9	Fluconazole	0.037	0.12	0.034	0.11	0.035	0.12	$4.5 \cdot 10^{-4}$	$1.5 \cdot 10^{-3}$	$4.0 \cdot 10^{-4}$	$1.3 \cdot 10^{-3}$	$1.8 \cdot 10^{-4}$	$6.0 \cdot 10^{-4}$	0.17	0.55
5.4	Ifosfamide	0.063	0.32	0.054	0.27	0.059	0.30	$8.8 \cdot 10^{-4}$	$4.4 \cdot 10^{-3}$	$7.0 \cdot 10^{-4}$	$3.5 \cdot 10^{-3}$	$2.9 \cdot 10^{-4}$	$1.5 \cdot 10^{-3}$	0.32	1.6
5.5	4-Hydroxy Omeprazole	0.012	0.12	0.011	0.11	0.011	0.11	$1.8 \cdot 10^{-4}$	$1.8 \cdot 10^{-3}$	$1.8 \cdot 10^{-4}$	$1.8 \cdot 10^{-3}$	$7.2 \cdot 10^{-5}$	$7.2 \cdot 10^{-4}$	0.058	0.58
5.9	Chlorpheniramine	0.15	0.48	0.073	0.24	0.12	0.41	$5.7 \cdot 10^{-4}$	$1.9 \cdot 10^{-3}$	$1.1 \cdot 10^{-3}$	$3.7 \cdot 10^{-3}$	$2.7 \cdot 10^{-3}$	$8.9 \cdot 10^{-3}$	0.79	2.6
5.9	Carbamazepine-10,11-epoxide	0.038	0.13	0.033	0.11	0.037	0.12	$3.8 \cdot 10^{-4}$	$1.3 \cdot 10^{-3}$	$3.6 \cdot 10^{-4}$	$1.2 \cdot 10^{-3}$	$1.8 \cdot 10^{-4}$	$6.0 \cdot 10^{-4}$	0.16	0.54
5.9	Venlafaxine	0.12	0.31	0.10	0.26	0.11	0.28	$1.9 \cdot 10^{-3}$	$4.7 \cdot 10^{-3}$	$1.6 \cdot 10^{-3}$	$4.1 \cdot 10^{-3}$	$9.1 \cdot 10^{-4}$	$2.3 \cdot 10^{-3}$	1.0	2.5
5.9	Bisoprolol	0.010	0.031	0.010	0.030	0.010	0.031	$9.5 \cdot 10^{-5}$	$2.9 \cdot 10^{-4}$	$1.2 \cdot 10^{-4}$	$3.6 \cdot 10^{-4}$	$5.9 \cdot 10^{-5}$	$1.8 \cdot 10^{-4}$	0.21	0.62
6.1	Propranolol	[b]	0.12	[b]	0.12	[b]	0.11	[b]	$1.3 \cdot$	[b]	$1.4 \cdot$	[b]	$2.0 \cdot$	[b]	1.6

									10^{-3}		10^{-3}		10^{-3}		
6.2	Citalopram	0.026	0.13	0.022	0.11	0.019	0.094	$1.7 \cdot 10^{-4}$	$8.5 \cdot 10^{-4}$	$1.9 \cdot 10^{-4}$	$9.3 \cdot 10^{-4}$	$8.3 \cdot 10^{-4}$	$4.2 \cdot 10^{-3}$	0.14	0.68
6.2	Desmethylcitalopram	0.032	0.13	0.028	0.11	0.024	0.094	$4.0 \cdot 10^{-4}$	$1.6 \cdot 10^{-3}$	$4.6 \cdot 10^{-4}$	$1.9 \cdot 10^{-3}$	$1.8 \cdot 10^{-3}$	$7.1 \cdot 10^{-3}$	1.7	6.6
6.5	Omeprazole	3.0	4.3	3.7	5.3	3.4	4.8	0.019	0.027	0.023	0.033	$6.5 \cdot 10^{-3}$	$9.3 \cdot 10^{-3}$	[a]	[a]
6.8	Hydroxyibuprofen	0.62	1.2	0.48	0.96	0.58	1.2	0.011	0.021	$8.2 \cdot 10^{-3}$	0.016	$5.0 \cdot 10^{-3}$	0.010	7.4	15
6.9	Carbamazepine	[b]	0.62	[b]	0.55	[b]	0.61	[b]	$9.4 \cdot 10^{-3}$	[b]	0.011	[b]	$3.5 \cdot 10^{-3}$	[b]	3.9
7.1	Mebendazole	[b]	8.8	[b]	14	[b]	8.3	[b]	0.096	[b]	0.22	[b]	0.076	[b]	49
7.3	Fexofenadine	0.038	0.13	0.036	0.12	0.037	0.12	$5.1 \cdot 10^{-4}$	$1.7 \cdot 10^{-3}$	$4.7 \cdot 10^{-4}$	$1.6 \cdot 10^{-3}$	$2.3 \cdot 10^{-4}$	$7.8 \cdot 10^{-4}$	0.15	0.49
7.4	Erythromycin	0.015	0.074	$7.9 \cdot 10^{-3}$	0.040	0.018	0.088	$3.3 \cdot 10^{-3}$	0.017	$5.0 \cdot 10^{-3}$	0.025	$2.4 \cdot 10^{-4}$	$1.2 \cdot 10^{-3}$	0.15	0.76
7.5	Lansoprazole	0.24	4.8	0.29	5.8	0.29	5.8	$1.4 \cdot 10^{-3}$	0.028	$1.7 \cdot 10^{-3}$	0.034	$1.3 \cdot 10^{-3}$	0.026	[a]	[a]
7.6	Oxazepam	0.24	0.61	0.24	0.60	0.25	0.62	$1.8 \cdot 10^{-3}$	$4.5 \cdot 10^{-3}$	$3.3 \cdot 10^{-3}$	$8.3 \cdot 10^{-3}$	$1.5 \cdot 10^{-3}$	$3.7 \cdot 10^{-3}$	1.3	3.1
7.6	Lorazepam	0.12	0.60	0.12	0.59	0.11	0.55	$1.1 \cdot 10^{-3}$	$5.7 \cdot 10^{-3}$	$4.0 \cdot 10^{-3}$	0.020	$6.7 \cdot 10^{-4}$	$3.3 \cdot 10^{-3}$	0.60	3.0
7.7	Climbazole	0.13	0.32	0.11	0.28	0.12	0.31	$1.7 \cdot 10^{-3}$	$4.2 \cdot 10^{-3}$	0.011	0.028	$2.8 \cdot 10^{-3}$	$6.9 \cdot 10^{-3}$	1.4	3.5
7.7	Fluoxetine	0.087	0.35	0.082	0.33	0.028	0.11	$5.8 \cdot 10^{-4}$	$2.3 \cdot 10^{-3}$	$3.1 \cdot 10^{-3}$	0.013	0.013	0.050	2.5	10
7.8	Temazepam	0.018	0.058	0.017	0.056	0.017	0.056	$1.5 \cdot 10^{-4}$	$5.1 \cdot 10^{-4}$	$7.9 \cdot 10^{-4}$	$2.6 \cdot 10^{-3}$	$1.0 \cdot 10^{-4}$	$3.5 \cdot 10^{-4}$	0.095	0.32
7.8	Ketoprofen	0.18	0.59	0.17	0.56	0.17	0.58	$1.6 \cdot 10^{-3}$	$5.3 \cdot 10^{-3}$	$1.0 \cdot 10^{-3}$	$3.4 \cdot 10^{-3}$	$6.5 \cdot 10^{-4}$	$2.2 \cdot 10^{-3}$	0.67	2.2
7.9	Cetirizine	0.098	0.33	0.070	0.23	0.098	0.33	$1.5 \cdot 10^{-3}$	$5.0 \cdot 10^{-3}$	$5.6 \cdot 10^{-3}$	0.019	$8.2 \cdot 10^{-4}$	$2.7 \cdot 10^{-3}$	0.65	2.2
8.1	Clarithromycin	$3.3 \cdot$	$6.7 \cdot$	$4.1 \cdot$	$8.1 \cdot$	$3.6 \cdot$	$7.2 \cdot$	$7.5 \cdot$	$1.5 \cdot$	$3.1 \cdot$	$6.3 \cdot$	$9.3 \cdot$	$1.9 \cdot$	0.11	0.22

		10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-5}	10^{-4}	10^{-4}	10^{-4}	10^{-5}	10^{-4}		
8.1	Norethisterone	0.19	0.64	0.18	0.58	0.18	0.58	3.6 · 10^{-3}	0.012	5.0 · 10^{-3}	0.017	1.4 · 10^{-3}	4.5 · 10^{-3}	1.6	5.2
8.1	Bezafibrate	0.64	2.1	0.61	2.0	0.62	2.1	6.5 · 10^{-3}	0.022	0.015	0.051	3.2 · 10^{-3}	0.011	2.4	7.9
8.2	Crotamiton	0.038	0.13	0.036	0.12	0.036	0.12	6.9 · 10^{-4}	2.3 · 10^{-3}	2.0 · 10^{-3}	6.6 · 10^{-3}	2.5 · 10^{-4}	8.4 · 10^{-4}	0.38	1.3
8.2	Clotrimazole	0.069	0.14	0.096	0.19	0.12	0.24	2.1 · 10^{-3}	4.2 · 10^{-3}	2.6 · 10^{-3}	5.3 · 10^{-3}	0.025	0.050	0.98	2.0
8.2	Warfarin	0.44	1.2	0.43	1.1	0.43	1.1	6.0 · 10^{-3}	0.016	0.011	0.030	2.3 · 10^{-3}	6.0 · 10^{-3}	2.3	5.8
9.0	Atorvastatin	0.070	0.23	0.056	0.19	0.074	0.25	1.9 · 10^{-3}	6.5 · 10^{-3}	1.4 · 10^{-3}	4.7 · 10^{-3}	8.5 · 10^{-4}	2.8 · 10^{-3}	0.66	2.2
9.4	Miconazole	[b]	0.65	[b]	1.3	[b]	2.1	[b]	0.063	[b]	0.045	[b]	0.25	[b]	8.3
5.6	Estriol	0.11	0.53	0.12	0.58	0.12	0.59	1.6 · 10^{-3}	7.8 · 10^{-3}	7.6 · 10^{-4}	3.8 · 10^{-3}	5.9 · 10^{-4}	2.9 · 10^{-3}	0.68	3.4
6.0	Naproxen	0.060	1.2	0.054	1.1	0.066	1.3	6.2 · 10^{-4}	0.012	4.6 · 10^{-4}	9.2 · 10^{-3}	2.4 · 10^{-4}	4.8 · 10^{-3}	0.25	4.9
7.1	Diclofenac	[b]	0.58	[b]	0.56	[b]	0.58	[b]	7.2 · 10^{-3}	[b]	5.4 · 10^{-3}	[b]	2.9 · 10^{-3}	[b]	5.1
7.4	17β-Estradiol	0.11	1.1	0.11	1.1	0.11	1.1	1.4 · 10^{-3}	0.014	1.3 · 10^{-3}	0.013	1.2 · 10^{-3}	0.012	1.4	14
7.5	Estrone	0.11	0.31	0.11	0.33	0.12	0.34	1.6 · 10^{-3}	4.8 · 10^{-3}	1.2 · 10^{-3}	3.5 · 10^{-3}	1.2 · 10^{-3}	3.7 · 10^{-3}	1.4	4.2
7.5	17α-Ethinylestradiol	0.54	1.1	0.54	1.1	0.52	1.0	7.0 · 10^{-3}	0.014	7.0 · 10^{-3}	0.014	0.013	0.026	7.2	14
7.5	Ibuprofen	0.10	0.34	0.087	0.29	0.11	0.36	5.2 · 10^{-4}	1.7 · 10^{-3}	6.8 · 10^{-4}	2.3 · 10^{-3}	4.3 · 10^{-4}	1.4 · 10^{-3}	0.47	1.6
8.7	Gemfibrozil	0.061	0.12	0.054	0.11	0.061	0.12	2.9 · 10^{-3}	5.9 · 10^{-3}	7.5 · 10^{-4}	1.5 · 10^{-3}	3.3 · 10^{-4}	6.7 · 10^{-4}	0.70	1.4

a) method not suitable, b) blank that could be corrected for

Table S11: Method accuracy and precision for direct injection (both in %), samples spiked with 1, 10, and 50 μg L^{-1} (n = 3).

RT	Analyte	Influent						Effluent						River					
		accuracy			precision			accuracy			precision			accuracy			precision		
		1	10	50	1	10	50	1	10	50	1	10	50	1	10	50	1	10	50
0.7	Guanylurea	94	109	97	4	3	5	[a]	117	116	[a]	2	6	107	103	90	9	9	5
0.8	Metformin	95	105	95	6	5	2	[a]	[a]	105	[a]	[a]	3	94	105	95	7	5	5
1.2	Sulfanilamide	[b]	109	107	[b]	9	4	[b]	104	105	[b]	11	3	[b]	102	104	[b]	12	9
1.7	Cotinine	118	111	103	5	4	5	102	106	104	1	10	6	117	106	110	4	6	6
2.0	Amidotrizoic acid	85	101	95	14	2	5	89	113	107	9	3	6	84	96	101	14	8	5
2.2	Amoxicillin	116	109	110	[b]	1	5	93	115	103	11	7	5	[b]	96	102	[b]	11	3
2.3	Sotalol	106	107	97	2	2	1	112	113	109	2	2	4	103	109	98	2	4	5
2.4	Paracetamol	[b]	110	105	[b]	8	6	[a]	[a]	[a]	[a]	[a]	[a]	[b]	111	104	[b]	4	3
2.5	Salbutamol	99	104	94	7	5	8	106	109	100	4	3	5	105	98	95	1	11	1
2.5	Ranitidine	[b]	105	124	[b]	4	8	[b]	104	111	[b]	5	9	[b]	103	128	2	6	5
2.5	Atenolol	98	102	92	10	3	8	[a]	101	101	[a]	6	6	93	107	91	3	10	4
2.6	Sulfadiazine	93	96	94	5	2	19	104	106	105	8	5	5	106	97	93	17	5	4
2.9	3-Methoxy Paracetamol	107	101	94	9	4	4	[a]	84	89	4	4	3	108	97	98	12	3	4
3.0	3-Desmethyl Trimethoprim	88	94	88	5	1	8	85	86	91	7	5	6	88	91	90	11	5	8
3.1	α-Hydroxy Trimethoprim	91	91	84	6	0	12	101	98	97	5	5	5	91	92	84	10	7	7
3.3	Clopidol	[a]	100	98	[b]	1	12	[a]	107	107	[b]	5	8	[a]	104	93	[b]	7	11
3.3	Gabapentin	95	94	93	7	2	14	[a]	97	105	2	2	4	107	95	89	[b]	[b]	7
3.6	Trimethoprim	95	101	100	2	3	6	102	109	104	3	3	4	94	101	100	8	7	5
3.6	Caffeine	[a]	107	109	[a]	8	14	[a]	[a]	108	[a]	[a]	9	[b]	117	113	[b]	9	4
3.7	Ofloxacin	162	160	58	5	2	5	130	144	82	5	4	5	165	153	61	5	12	9
3.9	Ciprofloxacin	83	119	47	11	2	5	76	97	62	10	3	2	90	117	49	25	12	10

4.0	Lidocaine	90	95	86	3	6	8	103	106	97	3	4	3	91	98	82	5	8	7
4.1	Sulfamethoxazole	93	95	90	7	4	10	96	98	100	4	7	3	94	91	86	9	6	1
4.5	Desmethylvenlafaxine	108	112	101	1	4	3	114	110	107	0	2	4	110	109	103	6	6	3
4.7	Acebutolol	102	110	99	3	4	6	106	110	100	9	2	2	105	108	99	5	4	0
4.7	Lamotrigine	101	96	89	9	6	6	102	111	95	12	8	1	97	100	90	4	5	8
4.8	Primidone	107	104	95	7	3	1	103	111	106	10	8	5	107	101	97	9	3	4
4.8	Metoprolol	111	105	102	14	11	3	115	109	99	10	5	10	119	103	97	3	2	5
4.9	Fluconazole	95	95	97	4	6	15	100	96	103	4	1	4	102	96	90	8	5	3
5.4	Ifosfamide	119	117	106	5	3	7	105	103	101	3	1	4	124	112	107	5	4	3
5.5	4-Hydroxy Omeprazole	102	96	92	4	5	6	97	103	97	3	4	1	103	98	89	3	8	4
5.9	Chlorpheniramine	141	100	76	4	5	5	121	135	82	6	12	10	140	100	77	8	4	4
5.9	Carbamazepine-10,11-epoxide	116	105	98	8	3	5	102	105	97	1	5	0	115	107	96	10	7	3
5.9	Venlafaxine	106	115	107	10	5	6	105	108	97	6	2	2	109	113	106	5	3	4
5.9	Bisoprolol	100	108	97	5	5	6	98	104	96	16	5	3	102	104	99	1	7	1
6.1	Propranolol	114	110	104	9	2	4	97	110	96	6	5	4	115	109	104	10	7	3
6.2	Citalopram	90	118	104	2	4	4	102	105	97	23	13	1	94	116	102	1	2	3
6.2	Desmethylcitalopram	103	113	102	6	7	8	120	124	111	24	11	5	98	116	106	3	1	3
6.5	Omeprazole	[b]	131	97	[b]	20	31	[b]	109	103	[b]	49	19	[b]	102	109	[b]	11	30
6.8	Hydroxyibuprofen	[a]	108	101	[a]	2	5	[a]	106	93	[a]	2	5	[a]	105	104	[a]	8	7
6.9	Carbamazepine	107	107	101	3	8	8	103	104	96	2	7	1	112	105	99	8	8	6
7.1	Mebendazole	172	218	192	4	5	4	12	199	199	10	3	2	185	210	196	7	10	2
7.3	Fexofenadine	112	113	103	2	3	8	[a]	112	102	6	5	1	114	110	105	7	8	7
7.4	Erythromycin	224	46	[b]	5	4	4	107	78	[b]	32	28	8	228	45	[b]	3	5	4
7.5	Lansoprazole	152	152	75	105	49	27	[b]	107	53	[b]	27	33	[b]	[b]	96	[b]	[b]	18
7.6	Oxazepam	121	111	96	10	1	11	116	109	98	3	4	11	114	110	103	11	13	12

7.6	Lorazepam	122	102	94	14	10	6	111	111	102	19	11	9	120	106	93	19	12	16
7.7	Climbazole	170	169	149	1	2	6	161	141	133	3	3	6	213	156	121	15	9	7
7.7	Fluoxetine	97	88	90	8	6	9	91	99	100	29	11	19	98	91	86	6	5	1
7.8	Temazepam	122	105	96	8	12	6	118	104	103	5	1	10	112	108	102	10	7	12
7.8	Ketoprofen	118	105	96	5	6	9	116	108	103	12	6	13	117	102	100	15	5	15
7.9	Cetirizine	[a]	235	222	[a]	3	6	[a]	206	185	[a]	2	5	352	242	214	9	1	6
8.1	Clarithromycin	107	116	100	1	2	6	125	105	103	29	2	6	106	116	100	7	2	5
8.1	Norethisterone	103	111	104	11	11	7	106	106	99	5	5	6	125	110	98	12	8	8
8.1	Bezafibrate	110	100	94	5	8	8	101	102	98	8	5	2	114	102	89	10	7	5
8.2	Crotamiton	147	154	138	6	3	10	142	187	146	31	26	12	140	173	132	15	17	7
8.2	Clotrimazole	99	98	91	1	3	8	103	108	103	3	8	6	100	97	92	9	5	5
8.2	Warfarin	114	106	95	5	8	7	115	109	98	3	2	2	114	107	93	7	10	7
9.0	Atorvastatin	113	101	91	2	3	1	139	131	117	2	1	3	135	129	116	4	3	4
9.4	Miconazole	157	178	174	4	10	13	47	52	41	5	3	13	37	104	104	8	9	18
5.6	Estriol	120	109	91	16	1	4	127	114	92	8	5	5	116	110	95	6	1	5
6.0	Naproxen	[a]	107	109	[a]	12	12	[b]	122	87	[b]	5	8	[b]	121	96	[b]	11	3
7.1	Diclofenac	[b]	102	115	[b]	6	11	[b]	121	95	[b]	10	11	[b]	109	106	[b]	12	3
7.4	17β-Estradiol	109	100	96	6	9	7	115	106	96	22	7	6	111	103	89	7	3	5
7.5	Estrone	122	110	95	4	2	2	100	106	98	9	7	8	105	102	94	5	2	5
7.5	17α-Ethinylestradiol	115	115	99	4	9	5	83	104	107	[b]	8	8	117	104	93	1	4	3
7.5	Ibuprofen	[b]	[a]	106	[b]	9	6	[b]	124	98	[b]	2	14	[b]	121	110	[b]	9	6
8.7	Gemfibrozil	116	94	104	13	8	10	[a]	114	88	4	13	4	99	105	93	15	13	3

a) concentration in sample higher than spiked levels, b) concentration above or below MQL

Table S12: Method accuracy and precision (both in %) for liquid samples analysed after SPE and sludge, spiked at three concentrations. Concentrations were 0.01, 0.1 and 0.5 µg L^{-1} in influent and effluent, 0.005, 0.05, and 0.25 µg L^{-1} in river water, and 50, 250, and 500 ng g^{-1} in the sludge (n = 3).

RT	**Analyte**	**Influent**	**Effluent**	**River**	**Sludge**

/min		Accuracy			Precision			Accuracy			Precision			Accuracy			Precision			Accuracy			Precision		
		1	10	50	1	10	50	1	10	50	1	10	50	1	10	50	1	10	50	10	50	100	1	10	50
0.7	Guanylurea	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
0.8	Metformin	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
1.2	Sulfanilamide	[b]	98	108	[b]	5	5	[b]	73[b]	134[b]	[b]	19	17	99	82	85	[b]	8	4	[a]	[a]	[a]	[a]	[a]	[a]
1.7	Cotinine	118	97	98	6	3	2	[c]	100[b]	74[b]	[c]	6	1	113	96	105	4	6	8	100	102	88	6	1	4
2.0	Amidotrizoic acid	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
2.2	Amoxicillin	[b]	119	77	[b]	22	16	[b]	117	92	[b]	8	10	[b]	[b]	150	[b]	[b]	1	[a]	[a]	[a]	[a]	[a]	[a]
2.3	Sotalol	106	95	96	19	3	4	108	106	105	3	9	4	99	96	101	7	5	0.3	78	80	76	5	4	1
2.4	Paracetamol	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[b]	117	99	[b]	12	0.2	[c]	[c]	[c]	[c]	[c]	[c]
2.5	Salbutamol	93	94	90	9	1	4	101	98	93	7	1	5	91	92	94	8	3	4	107	97	87	14	10	5
2.5	Ranitidine	[b]	88	112	[b]	11	8	[c]	98	116	[c]	4	3	[b]	92	108	[b]	18	13	84	97	106	12	12	11
2.5	Atenolol	86	106	99	4	3	6	[c]	82	97	[c]	9	8	98	93	101	8	7	10	[a]	107	89	[a]	8	4
2.6	Sulfadiazine	101	96	99	9	9	5	[c]	110	111	[c]	13	14	107	97	93	11	3	7	66	100	111	14	29	10
2.9	3-Methoxy Paracetamol	[c]	98	97	1	1	4	[c]	[c]	123	[c]	[c]	5	93	89	94	6	6	7	84	86	94	4	9	5
3.0	3-Desmethyl Trimethoprim	114	104	103	4	2	7	[c]	124	104	[c]	5	1	102	113	107	9	13	4	112	98	104	7	12	18
3.1	α-Hydroxy Trimethoprim	135	94	94	5	16	9	107	108	115	2	3	10	96	99	93	18	2	2	127	107	86	5	11	16
3.3	Clopidol	[b]	100	97	[b]	17	5	[b]	106	109	[b]	5	12	[b]	96	97	[b]	8	2	120	113	105	9	14	4
3.3	Gabapentin	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
3.6	Trimethoprim	85	111	103	4	7	5	[c]	124	101	[c]	5	9	60	120	106	4	22	3	84	82	83	16	19	4
3.6	Caffeine	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	76	108	98	6	10	1	[c]	[c]	[c]	[c]	[c]	[c]
3.7	Ofloxacin	115	101	136	16	16	3	78	117	60	23	18	14	[b]	106	133	[b]	13	33	[a]	[a]	[a]	[a]	[a]	[a]
3.9	Ciprofloxacin	[b]	80	78	[b]	7	5	[b]	105	65	[b]	14	11	[b]	81	78	[b]	13	34	167	152	97	35	2	8
4.0	Lidocaine	93	98	91	2	0	4	[c]	99	117	[c]	6	16	88	94	102	5	5	9	102	97	61	5	8	12
4.1	Sulfamethoxazole	106	99	105	1	5	4	118	103	100	1	3	11	106	105	100	5	10	2	88	91	126	16	24	4
4.5	Desmethylvenlafaxine	99	105	106	5	2	10	[c]	119	98	[c]	5	5	101	104	104	9	3	2	96	102	112	9	3	5

4.7	Acebutolol	90	104	96	3	3	5	99	102	94	2	1	2	96	99	95	1	3	6	103	107	88	1	4	7
4.7	Lamotrigine	106	92	88	8	13	2	121	88	100	12	7	18	100	89	98	2	9	6	[c]	101	92	[c]	7	12
4.8	Metoprolol	99	105	105	12	1	5	153	106	101	13	7	7	117	95	98	3	4	0	89	93	91	1	8	13
4.8	Primidone	102	102	103	4	7	3	111	103	104	7	5	11	105	100	101	13	5	1	90	94	86	5	6	1
4.9	Fluconazole	100	92	106	16	8	4	100	105	107	7	3	9	105	100	95	5	4	1	110	112	110	1	4	3
5.4	Ifosfamide	98	99	102	9	0	1	99	87	84	4	5	6	112	94	94	13	2	3	112	122	146	7	5	15
5.5	4-Hydroxy Omeprazole	[c]	[c]	[c]	[c]	[c]	[c]	[b]	87	100	[b]	6	15	99	93	99	10	2	4	116	91	92	12	6	8
5.9	Chlorpheniramine	[c]	[c]	96	[c]	[c]	5	[c]	109	104	[c]	5	1	116	72	122	4	6	6	88	163	101	2	2	24
5.9	Carbamazepine-10,11-epoxide	118	100	95	20	3	2	108	92	100	9	8	23	116	97	100	11	5	10	75	83	83	4	6	5
5.9	Venlafaxine	119	103	107	8	5	5	[c]	104	107	[c]	2	4	92	113	123	7	1	4	[c]	119	104	[c]	4	1
5.9	Bisoprolol	101	103	102	2	6	4	[c]	104	98	[c]	3	3	103	103	100	3	5	1	92	91	91	1	2	2
6.1	Propranolol	116	112	105	6	2	2	98	112	98	10	11	7	[b]	111	106	[b]	20	8	[c]	81	88	[c]	3	5
6.2	Citalopram	[c]	[c]	70	[c]	[c]	2	[c]	91	76	[c]	7	1	91	102	100	14	7	10	[c]	118	101	[c]	3	5
6.2	Desmethylcitalopram	[c]	106	112	[c]	0	3	[c]	117	104	[c]	8	2	[b]	97	105	[b]	3	0	[c]	[c]	110	[c]	[c]	12
6.5	Omeprazole	[a]	[a]	[a]	[a]	[a]	[a]	[b]	141	127	[b]	12	13	[b]	127	83	[b]	19	18	[a]	[a]	[a]	[a]	[a]	[a]
6.8	Hydroxyibuprofen	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[b]	100	97	[b]	4	18	[c]	105	68	[c]	6	6
6.9	Carbamazepine	111	110	106	4	1	2	112	103	99	0.5	3	9	110	107	109	5	6	6	102	97	91	3	3	5
7.1	Mebendazole	[b]	172	200	[b]	6	4	[b]	170	246	[b]	1	5	[b]	193	223	[b]	2	11	[c]	115	108	[c]	8	4
7.3	Fexofenadine	[c]	105	106	[c]	2	6	[c]	[c]	[c]	[c]	[c]	[c]	99	108	102	19	8	2	[c]	[c]	[c]	[c]	[c]	[c]
7.4	Erythromycin	63	50	58	5	2	10	171	43	30	28	7	11	238	51	[b]	22	21	[b]	86	87	75	12	6	5
7.5	Lansoprazole	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
7.6	Oxazepam	178	104	103	7	6	9	[c]	111	113	[c]	10	10	130	99	90	12	11	13	118	93	87	3	5	13
7.6	Lorazepam	[b]	115	111	[b]	1	6	[b]	115	104	[b]	17	8	[b]	105	100	[b]	6	11	98	88	58	4	1	17
7.7	Climbazole	[b]	77	125	[b]	1	1	[b]	101	122	[b]	1	4	[b]	114	120	[b]	24	32	54	99	116	9	11	8
7.7	Fluoxetine	101	97	96	17	9	33	92	107	100	20	8	21	[a]	[a]	[a]	[a]	[a]	[a]	97	76	109	18	26	17
7.8	Temazepam	129	97	97	3	1	3	110	110	104	9	18	9	124	102	97	19	9	8	129	134	107	6	12	17

7.8	Ketoprofen	114	104	101	5	1	3	110	124	101	4	15	9	124	105	110	14	8	15	114	115	115	12	12	4
7.9	Cetirizine	237	162	164	5	3	5	[c]	[c]	[c]	[c]	[c]	[c]	225	195	193	12	8	4	[c]	119	86	[c]	15	8
8.1	Clarithromycin	86	93	101	5	10	9	89	98	91	2	10	4	94	103	86	4	4	1	81	110	74	12	8	3
8.1	Norethisterone	[b]	104	109	[b]	6	4	[b]	115	92	[b]	17	3	112	101	110	21	8	7	138	100	90	8	4	5
8.1	Bezafibrate	97	99	90	13	3	6	[b]	109	93	[b]	9	4	110	92	101	4	3	15	63	87	89	4	3	1
8.2	Clotrimazole	[b]	116	126	[b]	14	10	151	150	160	11	7	5	[b]	148	130	[b]	30	11	[b]	108	101	[b]	12	5
8.2	Crotamiton	92	99	99	2	4	7	[c]	[c]	[c]	[c]	[c]	[c]	120	95	93	13	11	7	83	115	122	10	18	11
8.2	Warfarin	129	106	103	6	1	7	142	99	82	3	9	7	112	98	104	3	7	8	106	122	125	12	7	7
9.0	Atorvastatin	74	135	138	16	5	6	[c]	[c]	[c]	[c]	[c]	[c]	90	144	143	20	3	1	[c]	126	120	[c]	33	6
9.4	Miconazole	89	59	59	8	4	28	96	115	159	18	20	26	[a]	[a]	[a]	5	6	27	[c]	125	98	[c]	16	26
5.6	Estriol	97	97	104	8	6	4	[c]	88	89	[c]	11	2	102	101	98	6	8	14	94	80	94	2	7	18
6.0	Naproxen	[b]	106	110	[b]	11	3	[b]	[c]	[c]	[b]	[c]	[c]	98	99	92	7	7	10	[c]	98	86	[c]	12	5
7.1	Diclofenac	[b]	94	109	[b]	7	3	[c]	[c]	98	[c]	[c]	16	94	123	104	13	11	14	[c]	[c]	92	[c]	[c]	16
7.4	17β-Estradiol	96	98	97	10	7	5	111	109	91	13	9	4	118	98	99	[b]	5	10	110	84	83	18	4	11
7.5	Estrone	75	94	100	7	1	4	[c]	100	87	[c]	2	1	102	101	98	11	12	10	104	84	71	6	5	4
7.5	17α-Ethinylestradiol	[b]	103	94	[b]	5	7	[b]	101	91	[b]	0	7	[b]	89	109	[b]	13	11	107	85	86	11	3	14
7.5	Ibuprofen	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[b]	75	101	[b]	11	10	[c]	[c]	[c]	[c]	[c]	[c]
8.7	Gemfibrozil	101	105	116	16	9	3	91	109	104	21	43	16	120	117	106	7	9	16	60	86	73	11	17	20

a) method not suitable, b) concentration above or below MQL, c) concentration in sample higher than spiked levels

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