PAPER

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High-throughput evaluation of organic contaminant removal efficiency in a wastewater treatment plant using direct injection UHPLC-Orbitrap-MS/MS†

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The removal efficiency (RE) of organic contaminants in wastewater treatment plants (WWTPs) is a major determinant of the environmental impact of these contaminants. However, RE data are available for only a few chemicals due to the time and cost required for conventional target analysis. In the present study, we applied non-target screening analysis to evaluate the RE of polar contaminants, by analyzing influent and effluent samples from a Swedish WWTP with direct injection UHPLC-Orbitrap-MS/MS. Matrix effects were evaluated by spiking the samples with isotope-labeled standards of 40 polar contaminants. For 85% of the compounds, the matrix effects in the influent and effluent were not significantly different. Approximately 10 000 compounds were detected in the wastewater, of which 319 were identified by using the online database mzCloud. Level 1 identification confidence was achieved for 31 compounds for which we had reference standards, and level 2 was achieved for the remainder. RE was calculated from the ratio of the peak areas in the influent and the effluent from the non-target analysis. Good agreement was found with RE determined from the target analysis of the target compounds. The method generated reliable estimates of RE for large numbers of contaminants with comparatively low effort and is foreseen to be particularly useful in applications where information on a large number of chemicals is needed.

Environmental significance

Organic contaminants are constantly discharged from wastewater treatment plants into surface waters. The removal efficiency of organic contaminants in wastewater treatment plants is a major determinant of the environmental impact of these contaminants. Our manuscript shows that non-target analysis, combining a direct injection method and state-of-the-art high-resolution mass spectrometry, can be used to efficiently and reliably estimate removal efficiency for a large number of polar organic contaminants with comparatively low effort. Our work illustrates an easy and simple concept for overcoming the data limitations that have hampered our efforts to understand contaminant behavior. For instance, the method creates exciting new research opportunities to generate QSARs for predicting removal efficiency and new possibilities for regulators to prioritize contaminants for up-stream control of emissions.

1. Introduction

During the past two decades, the detection of polar organic contaminants (*e.g.*, from pharmaceuticals and personal care products) in surface waters in the ng–μg L^{-1} concentration range has raised concerns about their potential harmful effects on aquatic ecosystems.[1–5] Polar contaminants often reach recipient waters due to their incomplete elimination from wastewater streams by conventional treatment technologies.[6]

The removal efficiency (RE) in wastewater treatment plants (WWTPs) is therefore a major determinant of the environmental impact of these contaminants. The RE can be estimated by quantifying selected contaminants in wastewater prior to and after wastewater treatment.[7–10] However, due to the limits inherent to conventional target analysis, such measurements are available for only a few contaminants.[11]
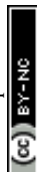
State-of-the-art high-resolution mass spectrometry (HRMS) techniques using full scan mode have opened new possibilities in environmental analysis. One of them is to screen for both known and unknown/unexpected compounds within one run.[12–14] Liquid chromatography tandem mass spectrometry (LC-MS/MS) has been widely applied to characterize emerging contaminants in wastewater and its impacted systems.[15–18] HRMS-based screening approaches are able to provide extensive information on the components present in a sample. The combination of a highly accurate exact mass calculation with MS/MS information

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c7em00552k

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is often sufficient for assigning a chemical identity to a peak. Therefore a reference standard is not always required as in target analysis.[19–21] Since unbiased non-target screening generates enormous datasets and the data processing can be time- and resource-consuming, dedicated approaches for data reduction and compound prioritization are encouraged.[22] One strategic approach is to study the changes in the chemical composition of an environmental matrix by assessing the differences between samples collected at different points in space or time. This approach can be employed to study chemical fate processes in the environment. It has been used to identify contaminant transformation products,[13,14,23–25] but there are few reports of this strategy being employed to investigate other processes.[18,26–29]

In the present study, we further explored the process-oriented applications of non-target analysis by using this technique to evaluate the overall RE of organic contaminants in WWTPs. Combining a direct injection method and LC-Orbitrap-HRMS, we acquired full scan datasets of sampled wastewater influent and effluent from a municipal WWTP. We carried out a robust matrix effect test and assessed the potential of taking the difference in the signal strengths of the identified chemicals to estimate their RE. Complementary target analysis was carried out for 42 target compounds in order to: (1) evaluate the ability of the non-target screening approach to identify relevant contaminants in these matrices and (2) compare the non-target RE results obtained from peak area ratios with the quantitative data.

2. Experimental methods

2.1. Chemicals and reagents

All non-isotope-labeled standards (purity >98%) were purchased from Sigma-Aldrich (Steinheim, Germany) or Toronto Research Chemicals Inc. (North York, Canada) and were stored under recommended conditions until use. The isotope-labeled standards were purchased from Toronto Research Chemicals Inc. and CDN Isotopes (Pointe-Claire Quebec, Canada). Details of these standards are provided in Tables S1 and S2 in the ESI.† Stock solutions of the standards were prepared in methanol and stored in amber CERTAN® capillary bottles in the dark at −20 °C. LC/MS-grade formic acid and sulfuric acid were purchased from Sigma-Aldrich. LC/MS-grade acetonitrile, methanol, and sodium hydroxide were purchased from VWR (Stockholm, Sweden). Milli-Q water was produced by using a Milli-Q Integral Water Purification System (Merck Millipore, Stockholm, Sweden). A non-labeled standard mixture containing 42 standards of a broad spectrum of polar organic contaminants (Table S1†) and an internal standard mixture containing 40 isotope-labeled standards (Table S2†) were prepared in methanol at a concentration of 5 µg mL^{-1}; both standard solution mixtures were stored in the dark at −20 °C until use.

2.2. Sampling and sample preparation

Flow-proportional influent and effluent samples (24 h) were collected in parallel from the Henriksdal municipal WWTP in Stockholm, Sweden, where wastewater is subjected to mechanical, chemical and biological treatment, with sand filtration as the final treatment step. The samples were collected during dry weather on June 15, 2016, filled into 1 L HDPE containers and stored in the dark at −20 °C until preparation for analysis.

Two sample preparation methods, direct injection and solid phase extraction (SPE), were employed to explore differences in the non-target screening results arising from sample enrichment. For direct injection, triplicated influent and effluent samples (1 mL) were spiked with the isotope-labeled standard mixture (50 ng absolute amount for each compound) before the pH was adjusted to neutral using a sulfuric acid solution (0.3 M) and a sodium hydroxide solution (1 M). Each sample was then filtered directly into a glass LC-vial using a 0.45 µm PTFE syringe filter.

The SPE method was based on Kern et al. (2009), a method which has been well documented and widely applied in suspect and non-target screening analysis.[21,26–29] Briefly, both influent and effluent samples (100 mL) were spiked with the isotope-labeled standard mixture (50 ng absolute amount for each compound) before filtration through glass fiber filters (GF/F; 0.47 µm; Whatman, Brentford, UK). To enrich compounds with a broad range of physical–chemical properties, self-packed two-layer cartridges were used containing 200 mg Oasis HLB (Waters, Milford MA, USA) as a top layer and a mixture of 150 mg Isolute ENV+ (Biotage AB, Uppsala, Sweden), 100 mg Strata-X-CW cation exchanger and 100 mg Strata-X-AW anion exchanger material (Phenomenex, Torrance, CA, USA) as a bottom layer. The two layers were separated by a PE frit (Supelco, Bellefonte, PA, USA). The conditioning of the cartridges was performed using 5 mL methanol followed by 10 mL Milli-Q water. The samples were extracted at a flow rate of approximately 10 mL min^{-1}. The cartridges were then completely dried under vacuum for 1 h before they were eluted first with 6 mL of a freshly prepared basic solution of ethyl acetate : methanol (50 : 50) containing 0.5% ammonia, and then with 3 mL of an acidic solution of ethyl acetate : methanol (50 : 50) containing 1.7% formic acid. The final pH of the extract was neutral. The sample extracts were evaporated under a gentle nitrogen stream at a temperature of 35 °C to 100 µL (after rinsing the glass wall twice with 200 µL of methanol). The 100 µL extract was reconstituted with 900 µL Milli-Q water, vortexed and then filtered through a 0.45 µm PTFE syringe filter into a glass LC-vial. All prepared samples were stored frozen until analysis.

2.3. Matrix effect tests

We tested for matrix effects using the wastewater influent and effluent samples treated with SPE and five additional types of wastewater prepared by combining different proportions of influent and effluent (i.e., 0 : 100 v/v, 25 : 75 v/v, 50 : 50 v/v, 75 : 25 v/v, and 100 : 0 v/v; influent : effluent) that were prepared for and analyzed by direct injection as described above. Three types of blanks were prepared: methanol, and Milli-Q water with and without the isotope-labeled standard mixture (50 ng for each compound). All samples were prepared in triplicate and stored frozen until analysis.

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2.4. UHPLC-Orbitrap-MS/MS analysis

All samples were analyzed with ultrahigh performance liquid chromatography coupled to a Q Exactive™ HF Hybrid Quadrupole-Orbitrap™ mass spectrometer (UHPLC-Orbitrap-MS/MS, Thermo Fisher Scientific, San Jose, USA) using electrospray ionization (ESI). The samples were injected twice, once each under ESI positive mode and ESI negative mode. For both ESI positive and negative modes, a reversed-phase Hypersil GOLD™ aQ C18 polar-endcapped column (2.1 mm × 100 mm; particle size of 1.9 μm; Thermo Fisher Scientific, San Jose, USA) was used with a binary mobile phase gradient consisting of (A) water and (B) acetonitrile, both containing 0.1% formic acid. First the gradient was linearly ramped from 95% A to 95% B within 10.0 min, and maintained for 5.0 min, followed by a linear gradient to 95% A within 0.1 min, which was then maintained for another 2.9 min. The injection volume was 10 μL. Throughout the whole separation, the flow rate was 0.4 mL min^{-1}, the sampler compartment temperature was 10 °C, and the column temperature was 40 °C. The Orbitrap-MS/MS was operated in the data-dependent acquisition (top N) mode. Mass accuracy calibration of the high-resolution Orbitrap MS/MS was performed every two weeks in both positive and negative ionization modes. Detailed information about the instrumental analysis is provided in the ESI.† The samples were analyzed in triplicate and each sample was injected three times, as multiple injections have been proven useful in reducing false positive results and in partially correcting for false negatives generated during the peak picking step in the non-target analysis protocol.[30,31]

2.5. HRMS data post-processing workflow

All HRMS data were processed using Compound Discoverer 2.1 (Thermo Scientific). The processing procedure consisted of peak picking and integration, retention time alignment, unknown compound detection, isotope and adduct peak grouping, unknown compound grouping, blank subtraction (using both the blank samples prepared in Milli-Q water and methanol), and database searching (see the ESI† for relevant parameters). Only the compounds detected in all three injections of all the triplicate samples and for which the coefficient of variation of the signal intensity was <30% were retained for later steps. Compound online-searching was enabled by an integrated function in Compound Discoverer using the database mzCloud, which features a searchable collection of high-resolution and accurate MS/MS databases. At the time of the study (September 2017), the database contained approximately 7000 compounds with a large variety of substances such as pharmaceuticals, personal care products, pesticides, and industrial chemicals.

2.6. Quantitative target analysis

Quantitative target analysis was applied to the MS output of both the direct injection and the SPE-enriched samples. All steps, including establishing the calibration curve, peak integration, and quantification, were carried out in Xcalibur 3.1

(Thermo Scientific). The target analytes were quantified using the internal standard method. The five analytes for which a corresponding isotope-labeled internal standard was not available were quantified using those isotope-labeled compounds that were most similar in terms of retention time and molecular structure (see Table S1†). The influent samples were analyzed both undiluted and after dilution with Milli-Q water by a factor of 5 to ensure that the injected concentrations of all target compounds were within the linear dynamic range of the instrument. Calibration curves were obtained by a weighted ($1/x$) linear least-squares regression of a series of 11 calibration standards with analyte concentrations ranging from 0.05 to 100 μg L^{-1}, where R^2 values were >0.99 for all compounds but glimepiride, for which the R^2 value was 0.92. A weighted linear least-squares regression was used to compensate for the unequal weighting of the calibration points when doing a linear regression to determine a calibration curve. The calibration series was measured at the beginning and end of each sequence. Four of the calibration standards and blanks were measured every 15 samples for quality control purposes. The limit of detection (LOD) and limit of quantification (LOQ) for each analyte were determined on the basis of the lowest calibration standard in which the ion used for quantification had a signal-to-noise ratio of >3 : 1 and >10 : 1, respectively.

2.7. Wastewater treatment efficiency determination

RE (%) was calculated as the change in concentration between influent and effluent:

$$\text{RE } (\%) = \frac{C_{\text{IN}} - C_{\text{EFF}}}{C_{\text{IN}}} \times 100\% \qquad (1)$$

where C_{EFF} and C_{IN} are the concentrations of a compound in effluent and influent, respectively. Positive values of RE indicate a decreased concentration after wastewater treatment, while negative values indicate an increased concentration.

Since quantitative determination is not possible within the context of non-target analysis for unknown chemicals and for chemicals without reference standards, we tested the possibility of using the peak area as an indicator of chemical abundance by conducting the matrix effect tests as described above. If the influence of the matrix on the signal of a chemical is similar between influent and effluent, eqn (1) can then be simplified to:

$$\text{RE } (\%) = \frac{A_{\text{IN}} - A_{\text{EFF}}}{A_{\text{IN}}} \times 100\% \qquad (2)$$

where A_{EFF} and A_{IN} are the measured peak areas of a compound in effluent and influent.

The non-target workflow was simultaneously applied to WWTP influent and effluent samples. For all the identified compounds, eqn (2) was applied to estimate RE as a non-target approach. Additionally, for the 42 compounds for which we had reference compounds, we also quantitatively determined their RE with eqn (1) using the concentrations from the target analysis. When the concentration of an analyte in effluent was <LOQ, this concentration was set as the respective LOQ. Uncertainty analysis was performed and is presented in the ESI.†

2.8. Multivariate explorative analysis of the chemical space

Principal component analysis (PCA) was performed to explore the chemical space of the studied compounds using Software for Chemometric Analysis (SCAN) (Minitab Inc., USA, 1995). To this end, 195 descriptors were calculated *in silico* using PaDEL Descriptors ver. 2.21 software. Calculations were performed starting from SMILES, which represented the molecular structure of the compounds investigated in this study.

3. Results and discussion

3.1. Performance of the non-target screening approach

In the calibration standard samples, all of the standard compounds, *i.e.*, 42 native standards (Table S1†) and 40 isotope-labeled standards (Table S2†), including monoisotopic peaks and their diagnostic isotopic peaks, were detected and unambiguously identified using mzCloud with a match score of >88, supported by the correct retention time and MS/MS spectra matching with their corresponding reference standards. This demonstrates the validity and reliability of the parameterization of the non-target screening workflow. The identification of each standard compound in the calibration series is summarized in Table S3.† The non-target LOD (NLOD) was introduced in this study and defined as the lowest calibration standard concentration where the compound was unequivocally identified (*i.e.*, mzCloud match score >70, peak area >3000, and S/N > 5) by the applied non-target screening approach. For 33 of the 42 target compounds, the NLOD was equal to the LOD for target analysis or the next highest calibration standard (Table S3†), suggesting that the effectiveness of the non-target screening approach in identifying contaminants is comparable to that using target analysis of the data for many substances. Fig. 1 illustrates the identification results for bezafibrate, showing the extracted ion chromatograms (Fig. 1A) and the match of its detected MS/MS against the library (Fig. 1B). The documentation of the identification of the remaining target compounds is provided in Fig. S1–S39.†

3.2. Matrix effects

LC-ESI-MS analysis is frequently influenced by matrix effects. To explore how the matrix would influence the estimation of RE in the context of non-target analysis, the mixture of 40 isotope-labeled standards was injected in triplicate into seven matrices, *i.e.*, SPE-enriched influent water, SPE-enriched effluent water, filtered influent water, filtered effluent water, and 3 different mixtures thereof. The matrix effect for each standard was then assessed by calculating its response in the matrix samples relative to the response in the blank sample
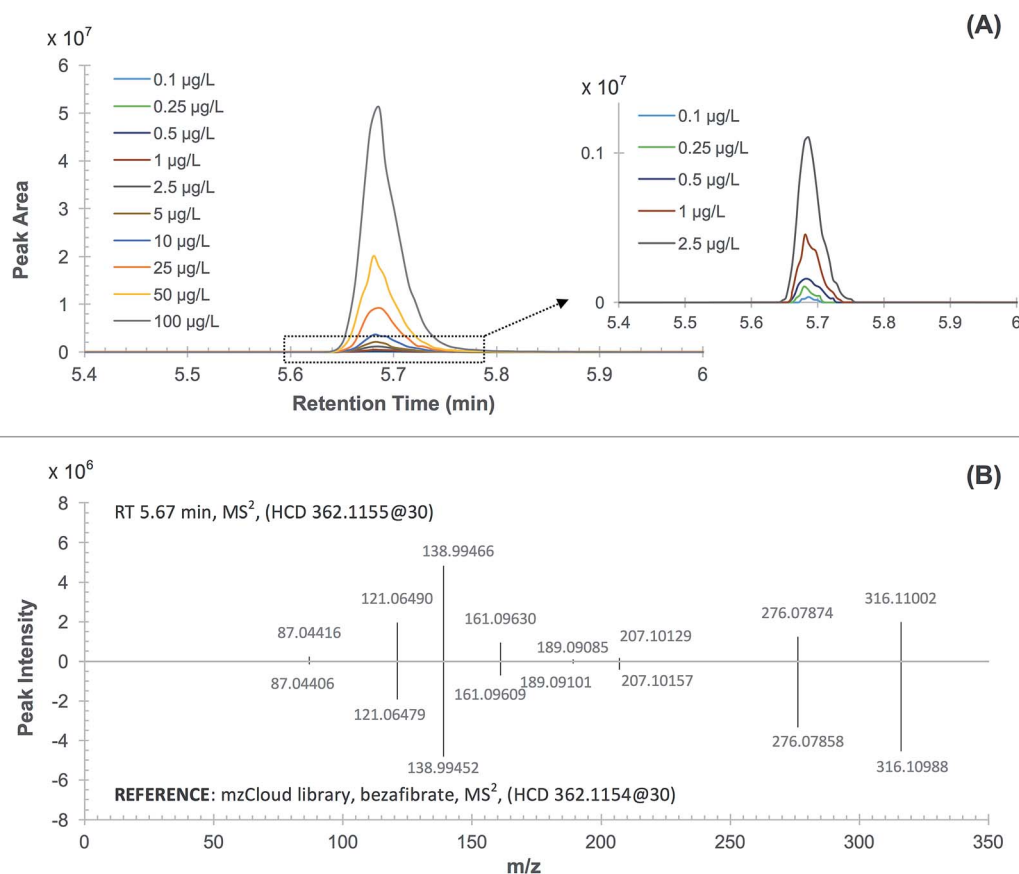

Fig. 1 Identification of bezafibrate (mzCloud match score: 99.0) in the calibration series standards using the non-target analysis protocol. (A) Extracted ion chromatograms identified in the calibration standards. (B) The matching of the measured MS/MS spectrum of the compound (top) with the library record (bottom).

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containing the isotope-labeled standard mixture in Milli-Q water. Generally, relative responses from the SPE-enriched samples were lower than those from the direct-injection samples. Since the SPE method showed good recovery of all target compounds when spiked in deionized water at 10 μg L^{-1} (see the ESI†), we attribute the higher matrix effect in the SPE-enriched samples to higher concentrations of interfering compounds.

For all standards in all five matrices without enrichment, the relative standard deviation of the peak area ($n = 9$) was <21% (see Fig. 2). For 33 out of the 40 standards, the five matrices analyzed by direct injection had similar responses to Milli-Q water, with the relative response factors ranging from 0.75 to 1.25. The remaining 7 standards (atorvastatin-d_5, bicalutamide-d_4, climbazole-d_4, fluoxetine-d_5, glimepiride-d_5, guanyl urea-$^{15}N_4$, and triclosan-d_3) had a response that was markedly suppressed (by up to 85%) in the matrix samples as compared to the Milli-Q water. Ion suppression was observed in both influent and effluent, yet to different extents for four of the compounds (atorvastatin-d_5, bicalutamide-d_4, glimepiride-d_5, and triclosan-d_3). The 7 standards showing matrix effects did not group around specific retention times, so it was not possible to predict from retention time which compound would be affected by matrix suppression.

One-way ANOVA was performed to test whether the differences between the mean response in direct-injection influent
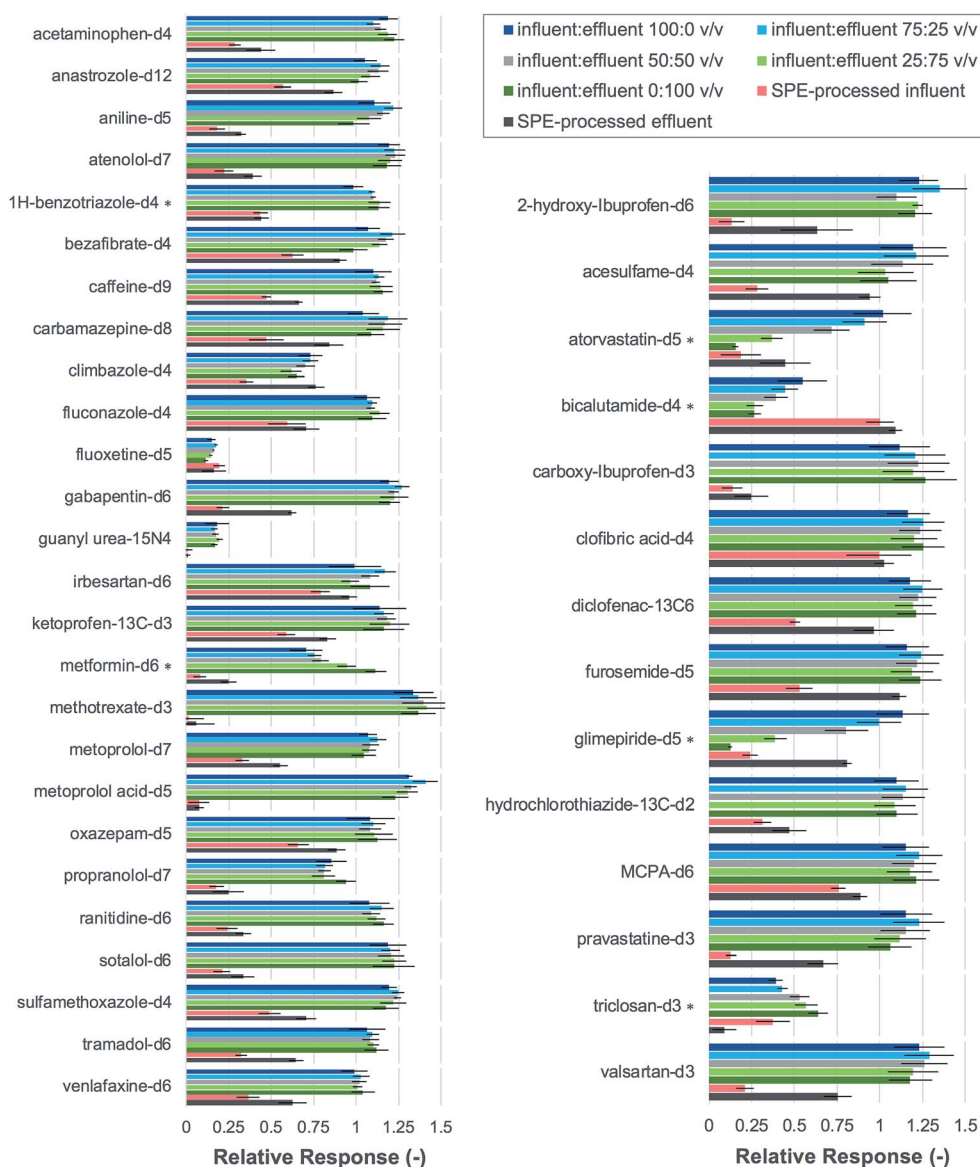

Fig. 2 Results from matrix effect tests with the 40 isotope-labeled standards. The response of the standards in SPE-enriched wastewater samples (bottom 2 bars) and filtered wastewater samples (top 5 bars) consisting of different proportions of influent and effluent is shown relative to the response in Milli-Q water. Error bars represent standard deviation ($n = 9$). The left column shows the results for standards detected under positive ionization mode; the right column under negative ionization mode. Compounds marked with * indicate that matrix effects differed significantly at a confidence level of 95% between 100% influent (filtered) and 100% effluent (filtered).

and effluent were statistically significant for the tested compounds. The results indicate that for 34 out of the 40 compounds no statistically significant difference was found at a confidence level of 95%, which indicated that the matrix effects in influent and effluent were comparable for a large majority of the compounds. The six compounds (marked with an asterisk in Fig. 2) for which matrix effects differed significantly are 1H-benzotriazole-d_4 and metformin-d_6 under the ESI positive mode, and atorvastatine-d_5, bicalutamide-d_4, glimepiride-d_5, and triclosan-d_3 under the ESI negative mode. The ion suppression increased with increasing proportion of influent in the influent/effluent mixtures for 1H-benzotriazole-d_4, metformin-d_6, and triclosan-d_3, while it decreased for atorvastatin-d_5, bicalutamide-d_4, and glimepiride-d_5.

In contrast, for 28 compounds statistically significant differences were observed between the influent and effluent water that had been pre-concentrated on SPE columns (Fig. 2). Generally, most of the isotope-labeled standards had higher ion suppression in the SPE-enriched influent than in the effluent. We hypothesize this to be attributed to higher concentrations of interfering compounds in the SPE extracts. The results show that the data from direct injection are much more suitable for the estimation of RE from peak areas (eqn (2)) than the data from the samples that had been pre-concentrated on SPE columns.

3.3. Estimating contaminant removal efficiency

3.3.1. Non-target screening results from direct injection. In total, 6547 and 5798 features (referred to as potential compounds) were detected under ESI positive and negative modes, respectively, in both the direct-injection influent and effluent samples (see Fig. S40† for the peak area distributions). Of these compounds, a total of 217 compounds detected in ESI positive mode and 118 compounds detected in ESI negative mode (16 were common to both) were identified by the online database mzCloud with a match score ≥70 (other relevant

parameters of the non-target screening protocol can be found in the ESI†).

RE was calculated from the direct-injection samples using the simplified method (eqn (2)) for all 319 compounds. Fig. 3 compares the RE of some of these compounds to the RE determined from the results of target analyses conducted at the same WWTP in the context of the Swedish screening programs for chemical contaminants.[32] The Swedish screening program provided data for up to 40 influent samples and 89 effluent samples (24 h flow-proportional) collected and analyzed during the period of 2005–2009. For all of the compounds common to both datasets, the uncertainty ranges (defined as mean ± standard deviation) of the RE estimated from this study and the RE calculated from the Swedish screening program overlap. This is evidence of the reliability of the non-target approach to estimating the RE of organic contaminants.

The non-target screening results also show consistencies with literature data. For instance, the two carbamazepine metabolites (carbamazepine-10,11-epoxide and 10,11-dihydro-10,11-dihydroxy-carbamazepine) were found in both influent and effluent; they have been widely reported in human blood samples, wastewater samples, and surface water systems.[33–36] In addition, coupled parent-product occurrence was observed. One example is that the 50% removal of valsartan was accompanied by the formation of valsartan acid that was detected only in effluent. Other compounds present only in effluent that hence can be classified as newly formed, e.g., 2-methoxyestradiol, carboxyl-clopidogrel, and penicillic acid, are expected from the degradation of known precursors via reactions that occur during wastewater treatment such as methylation, carboxylation, and hydroxylation.

In total, out of the 319 identified compounds, 67 had negative RE values, corresponding to an increased concentration after wastewater treatment. This can result from the formation of transformation products during the wastewater treatment and/or the back-transformation of native compounds from the
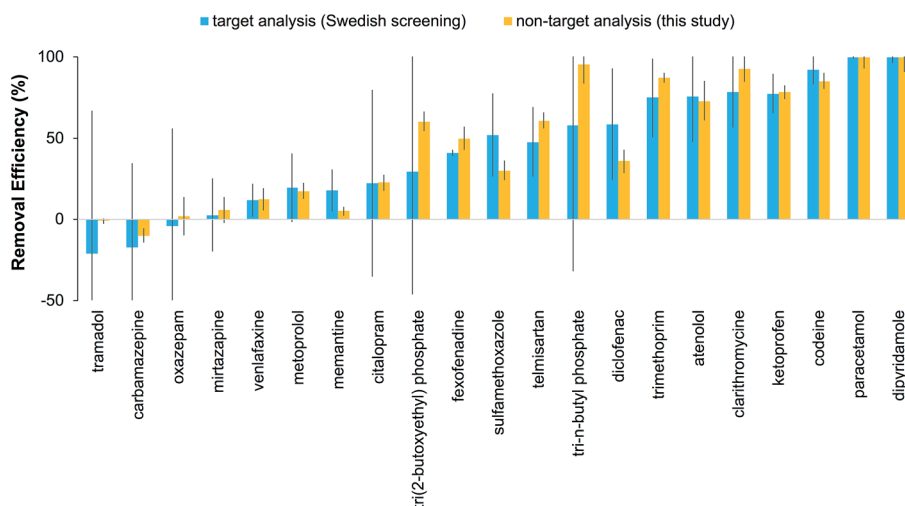

Fig. 3 Comparison of removal efficiency (RE, %) from this study using the direct injection/non-target analysis approach to the RE calculated from target analysis data from a Swedish screening project for the same WWTP.

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degradation of their conjugate metabolites. Two examples are given in the following section.

3.3.2. Comparison with the target analysis results. To further evaluate our non-target approach for measuring RE, we carried out target analysis for both the direct-injection samples and the SPE-enriched samples for the 42 compounds for which we had reference standards, and we used these concentrations to determine RE according to eqn (1). Seven compounds (anastrozole, chlorthalidone, clofibric acid, fluoxetine, glimepiride, MCPA, and methotrexate, see Table 1 and Fig. 4) were not quantified in the matrix from either direct-injection samples or SPE-enriched samples, despite a sample preconcentration factor of 100 in the latter (see Table 1). Of the remaining 35 compounds that were detected in either influent or effluent, six (atorvastatin, bezafibrate, chlorothiazide, climbazole, irbesartan, and ranitidine, see Table 1 and Fig. 4) were only captured in the SPE-enriched samples due to their relatively low concentrations (<LOD; Table S3†).

In general, there was good consistency in the detection of the compounds between the target and the non-target analysis. The six compounds that were not detected in the target analysis using direct injection were also not detected by the non-target approach using direct injection. However, three compounds (2-chlorobenzoic acid, pravastatin, and triclosan, see Table 1 and Fig. 4) were detected with target analysis but not with non-target analysis. These compounds can be considered false negatives of the non-target approach. They were present at concentration close to the LOD for target analysis, and the NLOD for non-target analysis was higher than the LOD for target analysis (Table S3†). The remaining compounds were all unequivocally identified using the non-target screening approach.

Table 1 Comparison of the detection of the target compounds using the four types of analysis[a]

| Target compound[b] | Target analysis | | Non-target analysis | |
	Direct injection	SPE	Direct injection	SPE
2-Chlorobenzoic acid	+	+	−	−
Anastrozole	−	−	−	−
Atorvastatin	−	+	−	−
Bezafibrate	−	+	−	+
Chlorothiazide	−	+	−	+
Chlorthalidone	−	−	−	−
Climbazole	−	+	−	+
Clofibric acid	−	−	−	−
Fluoxetine	−	−	−	−
Glimepiride	−	−	−	−
Irbesartan	−	+	−	+
MCPA	−	−	−	−
Methotrexate	−	−	−	−
Pravastatin	+	+	−	−
Ranitidine	−	+	−	+
Triclosan	+	+	−	−

[a] A plus sign indicates that the compound was detected/identified, while a minus sign indicates that the compound was not detected/identified. [b] Out of the 42 target compounds, 26 were detected in all cases and are not shown in the table.

The concentrations of the detected compounds ranged from 0.062 μg L^{-1} for ranitidine to 270 μg L^{-1} for caffeine in the influent water, and from 0.056 μg L^{-1} for chlorothiazide to 4.7 μg L^{-1} for gabapentin in the effluent water (Table S4†). They agree well with the concentration ranges reported for a large variety of pharmaceuticals in influent and effluent from Swedish WWTPs.[37–39] The concentrations of most of the detected compounds were higher in the influent water than in the effluent water, with two exceptions: irbesartan and chlorothiazide. For irbesartan the increase in concentration during treatment can be attributed to the decomposition of its conjugate metabolites. Chlorothiazide, on the other hand, may have been formed as the product of the transformation of another parent chemical. It has been shown that chlorothiazide can be generated from both hydrolysis and microbial degradation of the diuretic drug hydrochlorothiazide.[13,40,41]

RE was determined from the results of the target analysis for both the direct-injection samples and the SPE-enriched samples using eqn (1). The results are compared with the RE determined from non-target analysis using eqn (2) (Fig. 4 and S41†). For the target analysis, RE ranges from close to 0% for carbamazepine and oxazepam to 100% for acetaminophen and caffeine, which is in line with previous findings on the removal of these compounds in conventional WWTPs.[37] For the 29 compounds that were detected by both direct injection and the SPE method, the average difference for RE between the two sample processing methods is 14% and the median difference is 4%.

Comparing the RE of the tested chemicals from the target and non-target analyses, there is good agreement between the two sets of data obtained using the direct injection method, with an average RE difference of 10% and a median difference of 3%. For all compounds but 1H-benzotriazole, no statistically significant difference was observed at a confidence level of 95%. The non-target RE of 1H-benzotriazole is statistically lower than the target analysis value by 10%. This can be attributed to the more pronounced matrix effect on 1H-benzotriazole in the influent than in the effluent (see Fig. 2), leading to an underestimation of RE by eqn (2). While a significant difference in the response factor was also observed for another detected compound, metformin, the RE difference between target and non-target analyses is only 3%. This can be explained by the high RE of metformin, as a consequence of which the difference in matrix effects between influent and effluent was less influential on RE.

In contrast to the good agreement for RE obtained from the target and non-target analysis data using the direct injection method (Fig. S41†), poor agreement was found for the data using the SPE method, with an average RE difference of 46% and a median difference of 38% (Fig. 4). A significant difference was found at a confidence level of 95% for 29 out of the 35 tested compounds. Negative RE values were obtained for nearly one third of the compounds using the SPE/non-target method. The underestimation of RE by this method is consistent with the matrix effect results which showed large differences in the response factors between influent and effluent for SPE-enriched samples. Hence the simplification of eqn (1) to eqn (2), which was used in the non-target method, was not valid for the data

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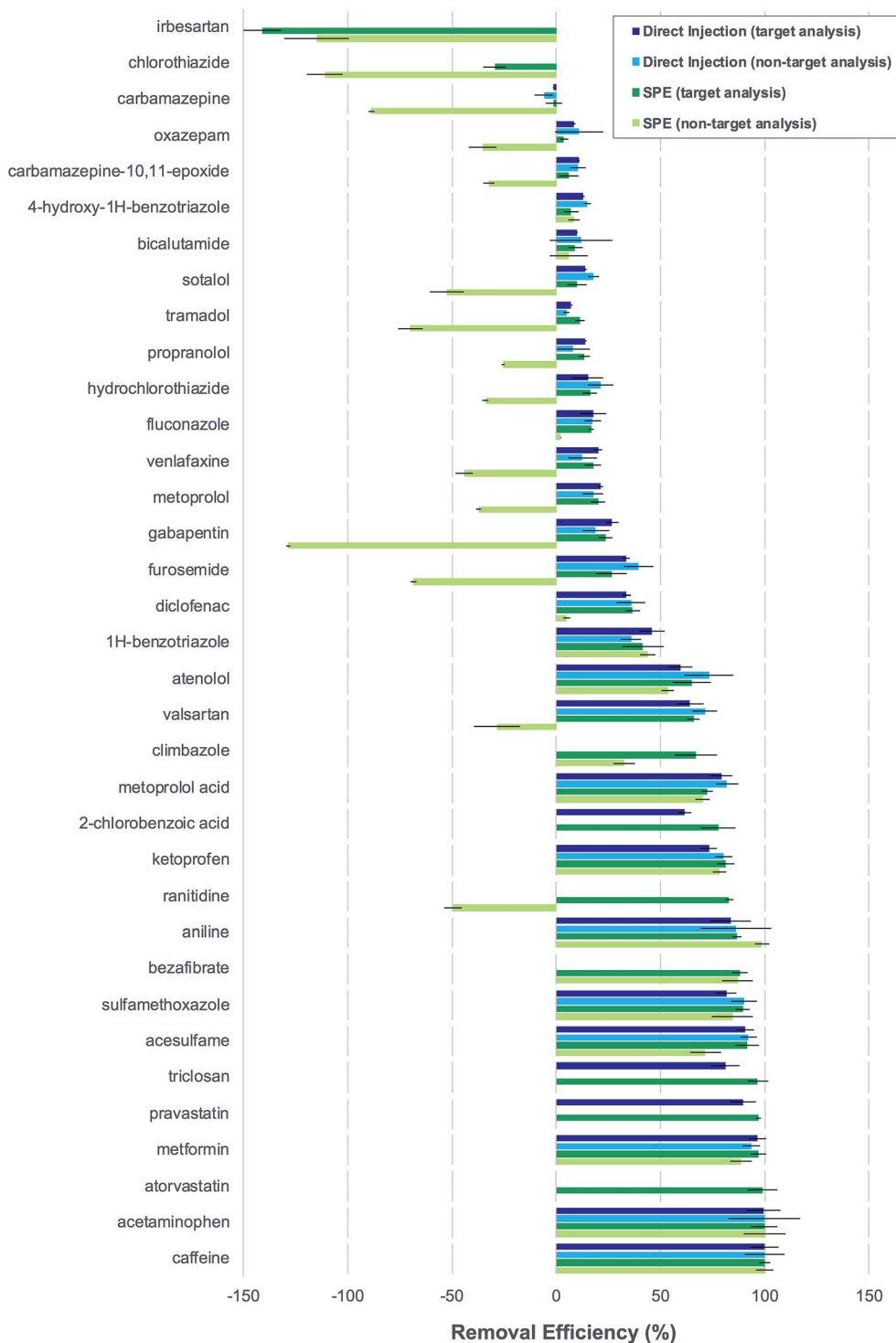*Environ. Sci.: Processes Impacts*, 2018, **20**, 561–571 | **567**

Fig. 4 Comparison of removal efficiency (RE, %) calculated using target analysis and eqn (1) to the RE for the same compounds estimated using non-target analysis and eqn (2). The comparison includes the results from the two sample processing methods (i.e., direct injection and SPE enrichment). Error bars represent standard deviation (n = 9).

obtained from the SPE method. We explored whether the SPE/non-target data could be corrected with the SPE/target data by conducting correlation analysis between the retention time of each compound and the quotient of the SPE/target and SPE/non-target data. However, no correlation was found.

3.4. Method evaluation

With this study, we expanded the domain of process-oriented applications of non-target analysis by developing a high-throughput approach for evaluating wastewater treatment efficiency. Similar concepts were applied recently by Parry and

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Young, who compared target and non-target HRMS approaches for assessing the performance of a pilot-scale advanced oxidation reactor to treat wastewater effluent.[18] Good agreement was also observed in that study between the RE values of the target analytes obtained using target and non-target approaches. Additionally, Blum et al. used non-target screening to prioritize potentially persistent, bioaccumulating, and toxic domestic wastewater contaminants.[42] While in that study target analytical methods were developed for 26 prioritized compounds and used to quantify the removal of these compounds in WWTPs, in the present study we expanded the approach to use the non-target screening data to determine RE and to apply it to the entire domain of the detected compounds. Nürenberg et al. also presented a generic non-target screening approach that can be employed to study the effects of wastewater treatment.[31] Compared to our study, the data processing workflow in Nürenberg et al. (2015) used the picked peaks instead of compounds to assess relationships and differences within a sample set. An advantage of their workflow is the amount of time saved by not conducting compound annotation and confirmation. By comparison, the workflow developed in this study has integrated automatic compound searching from an extensive online database. This can generate a list of compounds with a relatively high identification confidence level (level 2, albeit without desirable additional evidence for retention behavior, which was judged established for GC-MS but not for LC-MS),[43] because of the comprehensive Orbitrap-MS/MS information pre-stored in mzCloud that takes both collision energies and the instrument type into consideration. Therefore, among the 319 compounds that were identified using the non-target analysis, while level 1 was achieved in our study for 31 compounds by confirming their identity with reference standards, level 2 was achieved for the remaining compounds.

This study also demonstrates that direct injection is a valid and efficient approach to both target and non-target analyses of contaminants in WWTPs. False negatives occurred in our study with the non-target screening approach combined with the direct injection method due to the relatively low concentration levels of these compounds (2-chlorobenzoic acid, pravastatin, and triclosan). It may be possible to address this by using large volume injection to increase the injected chemical amount to achieve higher responses, but this might also increase the matrix background.

While direct injection clearly resulted in matrix effects for some chemicals (Fig. 2), it was found that for the majority of our tested compounds the influent and the effluent water from the WWTP were so similar in terms of matrix that it was possible to calculate RE with peak area ratios. PCA was performed to assess the uncertainty in extrapolating our findings of the matrix effect tests from the standards to the 319 identified compounds for which structures could be assigned. In total, 195 molecular descriptors were calculated from the molecular structure to visualize the representativeness of the 40 standards for the chemical domain of the identified compounds. The scores plots (Fig. S42†) of PC1 vs. PC2 and PC2 vs. PC3 provide a summary of the structural variation among the 359 compounds (i.e. 319 contaminants in addition to the 40 standards). Compounds

with a similar molecular structure are located close to each other in the PCA space. According to the congenericity principle,[44] structurally similar compounds are expected to give similar responses and therefore to behave similarly during wastewater treatment. The PCA results (Fig. S43†) show that for 275 of the identified compounds (about 85%) the chemical domain was well covered by the 34 isotope-labeled standards for which matrix effects did not differ between influent and effluent. This supports the applicability of the method to chemicals in the portion of the domain covered by the 34 standards. In total, 40 compounds, including all PEGs and PPGs and chemicals with long chains, were out of the chemical domain cover by the 34 standards. The RE calculated for these chemicals by eqn (2) may be less reliable than that for the majority of the dataset. Furthermore, the random distribution within this chemical domain of the six labeled standards (five of them showed ion suppression, see Fig. 2) with different matrix effects shows that the existence of a matrix effect cannot be predicted from PC1, PC2, or PC3. In summary, the extrapolation of our finding of similar matrix effects in influent and effluent is reasonable for a large portion of the chemical domain of the identified compounds, but within that domain we still expect that ~15% of the chemicals will have different matrix effects. Additional studies are needed to explore the reproducibility of our findings for other WWTPs.

While all spiked standards were recovered by the workflow, there are chemicals outside the analytical method and screening approach domain. A major reason for chemicals not being identified is the instrument sensitivity, since peaks with too low intensity are discarded during the isotopic peak screening step. This can potentially be overcome by including an enrichment step prior to instrumental analysis, but the non-target results can be more subject to matrix effects as shown in this study, making complementary quantitative analysis using internal standards indispensable to ensure the accuracy. Furthermore, most enrichment procedures inevitably result in the discrimination of certain compounds, e.g., those that are not retained on a sorbent. Other compounds that fall outside the method domain are those that cannot be retained on the LC column and/or cannot be ionized in ESI. This limitation can potentially be addressed by implementing other separation methods and ionization techniques.

4. Conclusions

The RE data of organic contaminants in WWTPs are available for only a few chemicals due to the time and cost required for conventional target analysis. In the present study, we show that non-target analysis, combining a direct injection method and UHPLC-Orbitrap-MS/MS, can reliably estimate the RE of large numbers of contaminants in WWTPs based on the ratio of peak areas in the influent and the effluent water. The accuracy of the overall approach was demonstrated by the confirmation of the identification of all the 31 target compounds, for which we had reference standards, in the wastewater samples, as well as by the observed good agreement with RE determined from the target analysis of the target compounds.

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Despite the effectiveness and reliability of the presented screening approach, extrapolating our finding of equal response factors in influent and effluent for our labeled standards to all chemicals is a source of uncertainty. As a result of this uncertainty, it is advisable to use target analysis to assess the RE of specific chemicals. Nonetheless, the power of the presented method lies in its ability to estimate RE for a very large number of compounds with comparatively low effort and material requirements (e.g., no standards are needed for the chemicals studied), as compared to many traditional approaches using non-target screening analysis followed by developing target analytical methods. Our method is expected to be particularly useful in applications where RE values for a large number of chemicals are needed. Such applications could include:

- Evaluating the performance of existing WWTPs. In addition to a quality control function, the method could be applied to assess the influence of operating conditions, flow, temperature and other variables on WWTP performance.

- Studying how treatment technology influences the WWTP performance. For instance, the method could be used to compare the performance of WWTPs that employ different treatment technologies.

- Developing new wastewater treatment technologies. The method could play a key role in evaluating the effectiveness of the new technology.

- Generating quantitative structure–property relationships (QSPRs) to predict RE from chemical structures, thereby generating predictive capacity for the release of chemicals from the technosphere to the environment.

- Identification of chemicals for potential upstream management. Chemicals with low RE will be released to surface waters, where they are also more likely to be persistent. Although upgrading conventional WWTPs by using advanced treatment techniques (e.g., activated carbon and ozone) can potentially increase the RE of these chemicals, this may not be sufficient or cost effective. Upstream management of emissions to wastewater may be the best option for such chemicals.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

References

1 T. A. Ternes, Water Res., 1998, 32, 3245–3260.
2 U. Hass, U. Duennbier and G. Massmann, Water Res., 2012, 46, 6013–6022.
3 K. Nödler, O. Hillebrand, K. Idzik, M. Strathmann, F. Schiperski, J. Zirlewagen and T. Licha, Water Res., 2013, 47, 6650–6659.
4 C. Tixier, H. P. Singer, S. Oellers and S. R. Müller, Environ. Sci. Technol., 2003, 37, 1061–1068.
5 R. P. Schwarzenbach, B. I. Escher, K. Fenner, T. B. Hofstetter, C. A. Johnson, U. von Gunten and B. Wehrli, Science, 2006, 313, 1072–1077.
6 T. Reemtsma, U. Berger, H. P. H. Arp, H. Gallard, T. P. Knepper, M. Neumann, J. B. Quintana and P. de Voogt, Environ. Sci. Technol., 2016, 50, 10308–10315.
7 G. Knopp, C. Prasse, T. A. Ternes and P. Cornel, Water Res., 2016, 100, 580–592.
8 P. Falås, A. Wick, S. Castronovo, J. Habermacher, T. A. Ternes and A. Joss, Water Res., 2016, 95, 240–249.
9 J. Hollender, S. G. Zimmermann, S. Koepke, M. Krauss, C. S. McArdell, C. Ort, H. Singer, U. von Gunten and H. Siegrist, Environ. Sci. Technol., 2009, 43, 7862–7869.
10 A. J. Watkinson, E. J. Murby and S. D. Costanzo, Water Res., 2007, 41, 4164–4176.
11 J. Margot, L. Rossi, D. A. Barry and C. Holliger, WIREs Water, 2015, 2, 457–487.
12 S. H. López, M. M. Ulaszewska, M. D. Hernando, M. J. Martínez Bueno, M. J. Gómez and A. R. Férnandez-Alba, Environ. Sci. Pollut. Res., 2014, 21, 12583–12604.
13 Z. Li, M. P. Maier and M. Radke, Anal. Chim. Acta, 2014, 810, 61–70.
14 D. E. Helbling, J. Hollender, H.-P. E. Kohler, H. Singer and K. Fenner, Environ. Sci. Technol., 2010, 44, 6621–6627.
15 A. C. Chiaia-Hernandez, M. Krauss and J. Hollender, Environ. Sci. Technol., 2013, 47, 976–986.
16 P. Gago-Ferrero, E. L. Schymanski, A. A. Bletsou, R. Aalizadeh, J. Hollender and N. S. Thomaidis, Environ. Sci. Technol., 2015, 49, 12333–12341.
17 C. Hug, N. Ulrich, T. Schulze, W. Brack and M. Krauss, Environ. Pollut., 2014, 184, 25–32.
18 E. Parry and T. M. Young, Water Res., 2016, 104, 72–81.
19 M. Krauss, H. Singer and J. Hollender, Anal. Bioanal. Chem., 2010, 397, 943–951.
20 E. L. Schymanski, H. P. Singer, J. Slobodnik, I. M. Ipolyi, P. Oswald, M. Krauss, T. Schulze, P. Haglund, T. Letzel, S. Grosse, N. S. Thomaidis, A. Bletsou, C. Zwiener, M. Ibáñez, T. Portolés, R. de Boer, M. J. Reid, M. Onghena, U. Kunkel, W. Schulz, A. Guillon, N. Noyon, G. Leroy, P. Bados, S. Bogialli, D. Stipaničev, P. Rostkowski and J. Hollender, Anal. Bioanal. Chem., 2015, 407, 6237–6255.
21 E. L. Schymanski, H. P. Singer, P. Longrée, M. Loos, M. Ruff, M. A. Stravs, C. Ripollés Vidal and J. Hollender, Environ. Sci. Technol., 2014, 48, 1811–1818.
22 J. E. Schollée, E. L. Schymanski, S. E. Avak, M. Loos and J. Hollender, Anal. Chem., 2015, 87, 12121–12129.
23 Z. Li, S. L. Kaserzon, M. M. Plassmann, A. Sobek, M. J. Gómez Ramos and M. Radke, Environ. Sci.: Processes Impacts, 2017, 19, 488–498.
24 S. Kern, R. Baumgartner, D. E. Helbling, J. Hollender, H. Singer, M. J. Loos, R. P. Schwarzenbach and K. Fenner, J. Environ. Monit., 2010, 12, 2100–2111.

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This journal is © The Royal Society of Chemistry 2018

25 M. Wang and D. E. Helbling, *Water Res.*, 2016, **102**, 241–251.

26 S. Kern, K. Fenner, H. P. Singer, R. P. Schwarzenbach and J. Hollender, *Environ. Sci. Technol.*, 2009, **43**, 7039–7046.

27 M. Ruff, M. S. Mueller, M. Loos and H. P. Singer, *Water Res.*, 2015, **87**, 145–154.

28 H. P. Singer, A. E. Wössner, C. S. McArdell and K. Fenner, *Environ. Sci. Technol.*, 2016, **50**, 6698–6707.

29 C. Moschet, A. Piazzoli, H. Singer and J. Hollender, *Anal. Chem.*, 2013, **85**, 10312–10320.

30 M. Schwientek, G. Guillet, H. Rügner, B. Kuch and P. Grathwohl, *Sci. Total Environ.*, 2016, **540**, 444–454.

31 G. Nürenberg, M. Schulz, U. Kunkel and T. A. Ternes, *J. Chromatogr. A*, 2015, **1426**, 77–90.

32 C. Wahlberg, B. Björlenius and N. Paxéus, *Läkemedelsrester i Stockholms vattenmiljö*, Stockholm Vatten, 2010.

33 K. Langford and K. V. Thomas, *J. Environ. Monit.*, 2011, **13**, 416–421.

34 S. Huntscha, D. M. Rodriguez Velosa, M. H. Schroth and J. Hollender, *Environ. Sci. Technol.*, 2013, **47**, 11512–11521.

35 C. Moschet, E. L. M. Vermeirssen, H. Singer, C. Stamm and J. Hollender, *Water Res.*, 2015, **71**, 306–317.

36 A. Bahlmann, W. Brack, R. J. Schneider and M. Krauss, *Water Res.*, 2014, **57**, 104–114.

37 P. Falås, H. R. Andersen, A. Ledin and J. la Cour Jansen, *Water Sci. Technol.*, 2012, **66**, 783–791.

38 R. H. Lindberg, M. Östman, U. Olofsson, R. Grabic and J. Fick, *Water Res.*, 2014, **58**, 221–229.

39 M. Östman, J. Fick, E. Nässtrӧm and R. H. Lindberg, *Sci. Total Environ.*, 2014, **472**, 862–871.

40 M. Brigante, M. DellaGreca, L. Previtera, M. Rubino and F. Temussi, *Environ. Chem. Lett.*, 2005, **2**, 195–198.

41 Z. Li, A. Sobek and M. Radke, *Environ. Sci. Technol.*, 2015, **49**, 6009–6017.

42 K. M. Blum, P. L. Andersson, G. Renman, L. Ahrens, M. Gros, K. Wiberg and P. Haglund, *Sci. Total Environ.*, 2017, **575**, 265–275.

43 E. L. Schymanski, J. Jeon, R. Gulde, K. Fenner, M. Ruff, H. P. Singer and J. Hollender, *Environ. Sci. Technol.*, 2014, **48**, 2097–2098.

44 R. Todeschini and V. Consonni, *Handbook of Molecular Descriptors*, Wiley-VCH Verlag GmbH, Weinheim, Germany, 2000.

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