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Multiresidue analysis of 88 polar organic micropollutants in ground, surface and wastewater using online mixed-bed multilayer solid-phase extraction coupled to high performance liquid chromatography–tandem mass spectrometry

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ABSTRACT

An automated multiresidue method consisting of an online solid-phase extraction step coupled to a high performance liquid chromatography–tandem mass spectrometer (online-SPE–HPLC–MS/MS method) was developed for the determination of 88 polar organic micropollutants with a broad range of physicochemical properties (log D_{OW} (pH 7): −4.2 to 4.2). Based on theoretical considerations, a single mixed-bed multilayer cartridge containing four different extraction materials was composed for the automated enrichment of water samples. This allowed the simultaneous analysis of pesticides, biocides, pharmaceuticals, corrosion inhibitors, many of their transformation products, and the artificial sweetener sucralose in three matrices groundwater, surface water, and wastewater. Limits of quantification (LOQs) were in the environmentally relevant concentration range of 0.1–87 ng/L for groundwater and surface water, and 1.5–206 ng/L for wastewater. The majority of the compounds could be quantified below 10 ng/L in groundwater (82%) and surface water (80%) and below 100 ng/L in wastewater (80%). Relative recoveries were largely between 80 and 120%. Intraday and inter-day precision, expressed as relative standard deviation, were generally better than 10% and 20%, respectively. 50 isotope labeled internal standards were used for quantification and accordingly, relative recoveries as well as intraday and inter-day precision were better for compounds with corresponding internal standard. The applicability of this method was shown during a sampling campaign at a riverbank filtration site for drinking water production with travel times of up to 5 days. 36 substances of all compound classes investigated could be found in concentrations between 0.1 and 600 ng/L. The results revealed the persistence of carbamazepine and sucralose in the groundwater aquifer as well as degradation of the metamizole metabolite 4-acetamidoantipyrine.

1. Introduction

In recent years, the number of studies about the occurrence and fate of emerging contaminants such as pesticides, pharmaceuticals, personal care products, industrial chemicals, hormones, flame retardants, and disinfection by-products in the aquatic environment have increased steadily. Analytical methods containing one or more compound classes of emerging contaminants were developed in many of these studies, often referred to as multiresidue methods [1]. Most recently, the interest in transformation products (TPs) of these compounds has risen and with it the need for analytical methods that include both active substances and their TPs [2]. The trace analysis of such polar and medium polar emerging contaminants is usually carried out by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS). However, the inclusion of diverse groups of chemicals as well as their TPs into one multiresidue method widens the range of physicochemical properties that have to be covered. Different polarity, molecular mass and speciation of the target compounds put high requirements on the analytical method and often the chosen experimental conditions are a compromise in terms of sensitivity and selectivity [3]. However, multiresidue methods have the potential to provide broader knowledge about the occurrence and fate of emerging contaminants in the environment [4].

Due to low environmental concentrations, a crucial step is the enrichment of the sample, which is traditionally performed using solid-phase extraction (SPE). Nowadays, the time and resource-consuming offline SPE is increasingly replaced with online enrichment (online-SPE) or large-volume injection (LVI) prior to liquid chromatography. Both methods are typically coupled to tandem (MS/MS) or multistage (MS^n) mass spectrometers because of their enormously high selectivity and sensitivity. Online-SPE is very advantageous compared to manual offline SPE because of the short analysis time, minimal interference and highly robust performance [5], largely due to easier handling and higher automation,

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with similar or better detection limits [6,7]. Additionally, it has economic and ecological benefits due to smaller sample volumes, smaller volumes of organic solvents for elution, shorter total analysis time, and less material and personnel costs [8]. In the last decade, several multiresidue online-SPE methods for the determination of emerging contaminants in the aquatic environment have been reported. However, most methods include only one class of compounds; such as pesticides [9–16] or pharmaceuticals [8,16–26], and only some of the mentioned methods incorporate the respective TPs. Few methods exist in literature that include both pesticides and pharmaceuticals along with their TPs [27,28].

In contrast to SPE methods, LVI is carried out without prior enrichment. Instead, low detection limits in the ng/L-range are achieved by injecting several microliters up to a few milliliters of a filtrated or centrifuged sample directly into a chromatographic column. This procedure requires less instrumentation and reduces material and solvent consumption, with the corresponding cost advantages. Additionally, it involves fewer sample treatment steps, which is beneficial for the accuracy [29]. However, the use of only one analytical column for both sample enrichment and chromatographic separation can be a drawback of LVI for multiresidue methods including compounds with a large variety in physicochemical properties especially in samples with high matrix load. Very polar compounds are especially prone to breakthrough on the commonly used reversed-phase columns as reported for the small, polar compound creatinine (octanol–water partition coefficient $\log P_{OW}$: −1.8, molecular mass: 113.1 g/mol) on a C18 column [30]. In online-SPE methods, this problem can be tackled by the use of enrichment materials with different sorbing properties compared with the chromatographic column. This allows the application of different eluent conditions for the sample enrichment and the chromatography by varying, for example, pH, buffer type and concentration. Moreover, it is possible to combine two or more SPE phases to enhance the range of compounds that can be enriched, as demonstrated in the multiresidue methods for 95 pesticides [10] and 68 pharmaceuticals [21]. This results in more flexible methods that can easily be extended to new emerging polar contaminants, eliminating the need to develop multiple analytical methods for the same compound classes. The thorough investigation of diffuse and point sources, entry pathways, and fate of emerging contaminants in the aquatic environment, however, requires the inclusion of compounds from different compound classes, as well as their TPs. Therefore, there is a need for reliable analytical methods with high throughput, low limits of quantification and high precision that have a broad compound coverage and, thus, cover a wide range of physicochemical properties.

The aim of this study was to establish a single online-SPE–HPLC–MS/MS method for the simultaneous, automated analysis of various pharmaceuticals, pesticides, biocides, corrosion inhibitors, an artificial sweetener and several of their TPs. The method should be able to deal with various liquid matrices such as groundwater, surface water, and wastewater. The biggest challenge for the online enrichment is the wide range of physicochemical properties and the high polarity of many of the target compounds, especially the TPs. Therefore, one focus of this article is on the choice of the SPE material. The analytical method is then demonstrated for several compounds in groundwater samples from a riverbank filtration site with short travel times.

2. Materials and methods

2.1. Chemicals

HPLC grade methanol, acetonitrile and water were purchased from Acros organics (Geel, Belgium). Ethanol (absolute), formic acid (98–100%), hydrochloric acid (32%) and ammonium acetate (ACS grade) were purchased from Merck (Darmstadt, Germany). Nanopure water for SPE was obtained using a Barnstead ultrapure water system (Thermo Scientific, Basel, Switzerland). Disodium hydrogen citrate sesquihydrate (p.a.) and trisodium citrate dihydrate (p.a.) were purchased from Fluka Chemicals (Buchs, Switzerland).

Analyte standards (Table 1) and isotope labeled internal standards were supplied by Sigma-Aldrich (Buchs, Switzerland), Toronto Research Chemicals (North York, Canada), Riedel-de-Häen (Seelze, Germany), CDN Isotopes Inc. (Pointe-Claire, Canada), Dr. Ehrenstorfer (Augsburg, Germany), ReseaChem (Burgdorf, Switzerland), CIL (Andover, USA), Solvias AG (Kaiseraugst, Switzerland), Novartis (Basel, Switzerland), and Monsanto (Antwerp, Belgium) (see Table S2 for detailed information). Stock solutions (1 mg/mL) of all analytes and internal standards were prepared in appropriate organic solvents (ethanol, methanol, acetonitrile, methanol/water, ethanol + 0.1 M HCl, methanol + 0.1 M HCl). Mixed spike solutions (1, 0.1 and 0.01 μg/mL) of most analytes were prepared in ethanol. Standard solutions containing acid (atrazine-2-hydroxy, atrazine-desethyl-2-hydroxy, simazine-2-hydroxy, terbutylazine-2-hydroxy) were prepared in a separate mixture to avoid the loss of the other compounds susceptible to acid hydrolysis. An internal standard mixture solution containing 0.5 ng/μL of all 50 isotope labeled compounds was prepared in ethanol.

Physicochemical properties such as the acid dissociation constant (pKa), speciation at pH 7, $\log P_{OW}$ and the pH-dependent octanol–water distribution coefficient $\log D_{OW}$ at pH 7 were predicted using JChem for Excel 5.6, 2011, ChemAxon Ltd. (Budapest, Hungary) (http://www.chemaxon.com) and are listed in Table S1. JChem implements the quantitative structure–activity relationship (QSAR) model given in Viswanadhan et al. [31]. The overall uncertainty of predicted octanol–water partition coefficients using different QSAR models range in the order of ±1 log units [32].

2.2. Field site and sampling

For the method validation, grab samples of surface water were taken from the river Thur (September 23, 2010) and groundwater was pumped from several piezometers at a riverbank filtration site at the river Thur close to Niederneunforn/Altikon, Switzerland (November 4, 2010) [33]. A 24-h composite effluent sample from the wastewater treatment plant of Frauenfeld, Switzerland was used for the evaluation of wastewater.

Field observations were made at a groundwater test field with 18 piezometers close to Felben-Wellhausen, Switzerland, which is part of a riverbank filtration site at the pre-alpine river Thur. Fig. 4a shows the setup of the piezometers and the groundwater contour lines based on water level measurements on the sampling day (November 2, 2010). The river water infiltrates approximately at an angle of 45° to the river flow direction into a sandy gravel aquifer of about 10 m thickness (7 m thick gravel and sand layer, overlain by 3 m of alluvial loam; unconfined except during high discharge events). A detailed description of the field site can be found in Coscia et al. [34]. Mean groundwater travel times were determined between May and September 2009 [35] based on cross-correlation of electrical conductivity time series of the river and the respective piezometer as described by Vogt et al. [36] and reported for the transect P03–C2–C3–P12 to be between 67 and 113 h for an intermediate depth (approximately 6.5–6.7 m below ground) of the aquifer. The oxygen saturation of the river Thur and the short subsurface residence times result in oxic groundwater conditions [37].

Sampling of 12 piezometers was performed on November 2, 2010 as indicated in Fig. 4a. For each piezometer, the water level was measured and a submersible pump was installed 6 m below

Table 1
Compound classes and names of the 88 compounds. CAS-numbers and further details can be found in the supplementary data (Appendix A; Table S1).

Pesticides (18)
2,4-D*
Atrazine*
Azoxystrobin
Bentazon*
Chloridazon*
Dichlorprop*
Dimethachlor
Dimethenamid*
Isoproturon*
MCPA*
Mecoprop*
Mesotrione*
Metazachlor
Metolachlor*
Simazine*
Sulcotrione*
Terbutylazine*
Tritosulfuron

Pesticide TPs (25)
2,6-Dichlorobenzamide
Alachlor ESA
Alachlor OXA
Atrazine-2-hydroxy*
Atrazine-desethyl*
Atrazine-desethyl-2-hydroxy
Atrazine-desisopropyl*
Azoxystrobin acid
Chloridazon-methyl-desphenyl
Dimethachlor ESA
Dimethachlor OXA
Dimethenamid ESA
Dimethenamid OXA
Isoproturon-didesmethyl
Isoproturon-monodesmethyl
Metazachlor ESA
Metazachlor OXA
Metolachlor ESA
Metolachlor OXA
Metolachlor-morpholinon
Propachlor ESA
Simazine-2-hydroxy
Terbutylazine-2-hydroxy
Terbutylazine-desethyl
Terbutylazine-desethyl-2-hydroxy

Pharmaceuticals (20)
Antipyrine (Phenazone)*
Atenolol*
Bezafibrate*
Carbamazepine*
Clarithromycin*
Diclofenac*
Eprosartan*
Irbesartan*
Lidocaine*
Mefenamic acid*
Metoprolol*
Naproxen*
Primidone*
Propranolol*
Sotalol*
Sulfamethazine*
Sulfamethoxazole*
Tramadol*
Valsartan*
Venlafaxine*

Pharmaceutical TPs (13)
4-Acetamidoantipyrine
4-Aminoantipyrine
4-Formylaminoantipyrine
Atenolol acid (metoprolol acid)*
Atenolol-desisopropyl
Carbamazepine epoxide*
Carbamazepine-10,11-dihydro-10,11-dihydroxy
N,*N*-Didesmethylvenlafaxine
N,*O*-Didesmethylvenlafaxine*
N-Desmethylvenlafaxine*
O-Desmethylvenlafaxine*
Ritalinic acid*
Valsartan acid*

Biocides (3)
Carbendazim*
Diethyltoluamide (DEET)*
Diuron*

Biocide TPs (4)
2-Aminobenzimidazole
Diuron-monodesmethyl (DCPMU)
Diuron-didesmethyl (DCPU)
Diuron-deschloro (MCPDMU)

Corrosion inhibitors (3)
1H-Benzotriazole*
4-Methyl-1H-benzotriazole
5-Methyl-1H-benzotriazole*

Corrosion inhibitor TP (1)
1-Methyl-1H-benzotriazole

Artificial sweetener (1)
Sucralose*

* Compounds with a corresponding isotope labeled internal standard are marked with an asterisk. All other compounds were quantified with closest-matching internal standard according to retention time and structure.

ground, which is the middle of the gravel layer. A minimum of 3 piezometer volumes was pumped until the temperature and electrical conductivity were stable. The samples were subsequently filled into single-use 250 mL glass bottles after rinsing with three sample volumes. The bottles were kept horizontally, half-filled at −20 °C until analysis. Groundwater temperature was between 9.9 and 11.9 °C, while electrical conductivity and the dissolved organic carbon (DOC) were in the range of 483–496 μS/cm and 1.4–1.6 mg C/L, respectively.

Sampling of the river Thur was performed on October 30, 2010, three days before the groundwater sampling. Time-proportional 4-h composite samples (eight aliquots every 30 min) were taken using an automatic water sampler equipped with 24 PE bottles (ISCO 6712, Teledyne Inc., LA, USA). Exposure tests over six days with a field blank, a river sample, and a spiked river sample revealed that no significant dissipation of the compounds due to sorption or other processes took place in the sampler bottles under natural conditions. No target compounds were found with quantifiable concentrations in the field blanks.

2.3. Sample preparation

The samples were thawed at room temperature. An aliquot of 50 mL was filtered with a bottle-top vacuum filtration unit through a glass microfiber filter (GF/F, 0.7 μm average pore size, 47 mm diameter) into 100 mL glass bottles. After filtration, 20 μL of the internal standard mixture was added, resulting in a concentration of 200 ng/L of each isotope labeled compound in the sample. 20 mL aliquots of nanopure water, groundwater and surface water were used for the automated enrichment of samples and standards. To minimize matrix effects for wastewater, 5 mL aliquots of wastewater were diluted with 15 mL of nanopure water before addition of the internal standard mixture. To achieve stable pH conditions for the sample enrichment, samples were adjusted to pH 7 prior to enrichment by the addition of 80 μL of a 0.5 M citrate buffer (pH 7) via the autosampler. The 0.5 M citrate buffer was prepared by mixing a 0.5 M disodium hydrogen citrate solution (13.16 g disodium hydrogen citrate sesquihydrate in 100 mL nanopure H_2O) with a 0.5 M trisodium citrate solution (14.71 g trisodium citrate dihydrate in 100 mL nanopure H_2O) in a ratio of 1:30 (v/v). The resulting pH in

the samples after buffer addition was checked regularly. All glassware used for the sample preparation was cleaned with laboratory glassware washers and post-rinsed with HPLC grade methanol and nanopure water.

2.4. Online-SPE and HPLC

The instrumental set-up was similar to the one reported earlier [16] and consisted of a tri-directional autosampler (HTC PAL, CTC Analytics, Zwingen, Switzerland), a dispenser syringe, a sample loop of 20 mL, three LC pumps, two six-port valves, and an on-line extraction cartridge. The HPLC pump system was composed of a binary pump (load pump, Surveyor LC, Finnigan), a quaternary low-pressure mixing gradient pump (elution pump, Rheos 2200, Flux instruments, Switzerland) for the SPE elution with the methanol gradient, and an isocratic pump (Rheos, 2000; Flux instruments, Switzerland) for the water gradient as well as a column oven (Portmann Instruments AG, Biel-Benken, Switzerland).

The online-SPE procedure involved three steps: loading, enrichment, and elution. The 20 mL loop was loaded with two times 10 mL of sample via a dispenser syringe. A self-made mixed-bed multilayer extraction cartridge and two six-port valves were used for sample enrichment. The SPE cartridge was prepared in-house by filling an empty cartridge (stainless steel, 20 mm × 2.1 mm, BGB Analytik AG, Germany) with 10 mg Oasis HLB (15 μm, Waters) as first material in enrichment flow direction. As second material, 10 mg of a mixture of Strata X-AW (33 μm), Strata X-CW (25 μm, both from Phenomenex, Brechbühler AG, Schlieren, Switzerland) and Isolute ENV+ (70 μm, Biotage, Uppsala, Sweden) in a ratio of 1/1/1.5 (X-AW/X-CW/ENV+) was used. One SPE cartridge was used for up to 200 injections, i.e. one SPE cartridge per measurement sequence. The sample was loaded with a flow rate of 2 mL/min and subsequently eluted in back-flush mode with methanol containing 0.1% formic acid at a flow rate of 30 μL/min for 7 min. The acidic methanol SPE eluate was then diluted with water (HPLC grade) containing 5 mM ammonium acetate by an additional pump with an active mixer (Portmann Instruments AG, Biel, Switzerland) with a low-volume (15 μL) mixing chamber. This procedure enables the refocusing of the eluted analytes on the analytical column. The HPLC gradient was formed by changing the mixing ratio of 5 mM ammonium acetate in water (solvent A) and acidic methanol containing 0.1% formic acid (solvent B). The gradient was initiated with 10% B for 8 min, followed by a 18 min linear gradient to 95% B. Afterwards the column was washed with 95% B for 3 min. Initial conditions were re-established in 2 min, and the column was equilibrated for 5 min prior to the next analysis. The total run time for one sample including on-line SPE and LC–MS/MS was 36 min. Chromatographic separation was performed using an Atlantis T3 column (3.0 (I.D.) × 150 mm, 3 μm particle size; Waters, Baden-Dättwil, Switzerland), which is reported by the manufacturer to achieve stable results in a pH range of 2–8. The HPLC column was equipped with an in-line filter (0.5 μm pore size, BGB Analytik AG, Germany) to prevent particulate matter entering the column. Optimal separation was achieved at 30 °C with a total flow rate of 300 μL/min. To prevent carry-over between two runs, the sample loop and the extraction cartridge were flushed with acetonitrile after each extraction and conditioned with water and 2 mM ammonium acetate prior to enrichment of the next sample.

2.5. Mass spectrometry

The HPLC was connected to an electrospray ionization probe (ESI) of a TSQ Quantum Ultra triple quadrupole MS (Thermo Scientific, San Jose, CA, USA) that was operated under unit resolution in the selected reaction monitoring (SRM) mode. Analyses were performed in the polarity-switching mode, which allows the use of positive and negative ionization during the same run. For all analytes, protonated ($[M+H]^+$) or deprotonated ($[M-H]^-$) molecular ions were selected as precursor ions except for sucralose, for which the formic acid adduct ($[M-H+FA]^-$) was the most intense ion. The most specific or most intense product ion of each target analyte was used for quantification, and a secondary product ion was used as a qualifier ion for confirmation.

Nitrogen was used as the sheath gas (50 arbitrary units) and auxiliary gas (10 arbitrary units) with argon as the collision gas (1.5 mTorr). Analyses were performed at a spray voltage of +3800 V (positive mode)/−3000 V (negative mode), a capillary temperature of 350 °C, a cycle time of 1 s, and a scan width of 0.002 m/z. For the acquisition of 274 SRM transitions, the scheduled SRM mode was used, which opens a freely adjustable acquisition window that is adjusted to the expected retention time of each compound. Details of the substance specific parameters for the ionization and detection of the analytes are given in Table S2.

2.6. Quantification and method validation

For quantification, the internal standard method was used. Isotope-labeled internal standards were available for 50 analytes. For the remaining 38 analytes, quantification was performed using the closest-matching internal standard according to retention time and structure. The concentration for each product ion in a sample was calculated by comparing the peak area ratio of the analyte and its assigned internal standard to the corresponding ratio in the calibration standard curve. Calibration curves were obtained by a weighted ($1/x$) linear least square regression of 11 standards spiked in nanopure water between 0.1 and 1000 ng/L. If the deviation between the concentration of the quantifier and qualifier ion did not exceed 20%, the concentration of the quantifier ion was reported as the final result. The calibration standards were measured twice, at the beginning and end of a sequence. Both calibration measurements were used for the calibration curve. Laboratory blank samples of nanopure water were used to prevent potential carryover after highly concentrated standards or samples. Nanopure water spiked with internal standard was used to quantify potential carryover. Maximum carryover rates were less than 1% after the injection of a 1000 ng/L standard and two laboratory blanks.

The limit of quantification (LOQ) for each compound was determined on the basis of the lowest calibration standard with a signal to noise ratio of the qualifier ion higher than 10:1 and a correction factor for each matrix, which represents matrix effects resulting from enrichment, ion suppression or ion enhancement in the ESI source. The difference of the peak areas of a spiked sample and the corresponding unspiked sample divided by the peak area of a calibration standard with the corresponding spike level was used as matrix correction factor. Because of the dilution factor of 1:4 for wastewater, the LOQs of wastewater were multiplied by 4.

Relative recoveries in the different matrices were determined with samples spiked at different concentrations. Spiking of the analytes was performed simultaneously to the internal standard solution after the sample filtration, allowing the validation of all sample treatment steps after the filtration. The filtration with glass fiber filters was validated in Singer et al. for a set of 24 pesticides, biocides, and pharmaceuticals and caused only minor losses for single compounds [28]. For groundwater, four samples were spiked with four different concentrations (10, 50, 100, 250 ng/L), while for surface water three samples were spiked with 25, 100 and 500 ng/L, and two wastewater effluent samples with 500 ng/L. The concentration of the respective unspiked sample was subtracted from the concentration in the spiked sample and then divided by the spike level. The relative recovery for each matrix was calculated using the average of all spiked samples. For compounds without

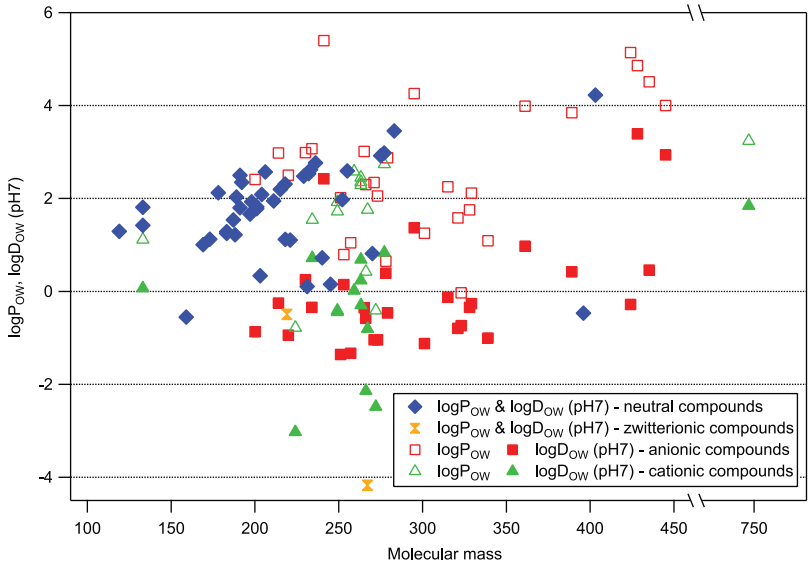

Fig. 1. The log P_{OW}, log D_{OW} (pH 7), molecular mass and speciation at pH 7 are shown for all 88 compounds.

corresponding isotope labeled internal standard, the results of environmental samples were corrected by the relative recovery.

The precision of the method was tested in two ways. For the intraday precision, a 250 ng/L calibration standard was measured sevenfold to determine the variation between several runs. For the inter-day precision, a spiked groundwater and a spiked surface water sample were frozen in small portions and an aliquot was measured in every sequence. These samples reveal additional variations caused by different operators, cartridges, and calibrations, storage stability, and other procedural losses. We chose to report the respective relative standard deviations.

3. Results and discussion

3.1. Choice of SPE material

The LC hardware setup used here allows the refocusing of analytes on the chromatographic column after elution from the enrichment column by adding water prior to the analytical column. As a result, the full variety of conventional SPE and LC materials can be applied for online sample enrichment without compromising the separation performance. The use of SPE material comes with the advantage that numerous literature data and many sorbent materials with different mode of interactions are available. Furthermore, the cost benefit is significant if large volumes of samples with high matrix loads are analyzed frequently, due to the relatively low cost of SPE materials. As this method is designed for the high-throughput analysis of wastewater samples, SPE copolymers (15–70 μm particles) instead of chromatographic C18 silica material (3–5 μm particles) were chosen as the adsorbent material in the enrichment column. The SPE material needs to fulfill many requirements to enable the development of a comprehensive online-SPE multiresidue method for compounds with highly diverse physicochemical properties. Traditionally, several materials are tested empirically for a set of compounds when optimizing the SPE material, and the material with the best performance is chosen. It is thus often not possible to include anionic and cationic compounds into an online-SPE procedure with only one enrichment material as described elsewhere [3].

In the method here, the physicochemical properties of the 88 selected compounds were evaluated to find a combination of SPE materials that is capable of enriching all compounds sufficiently. Important physicochemical properties for this evaluation were the molecular mass, the log P_{OW}, the pH-dependent octanol–water distribution coefficient log D_{OW} and the pKa value, which governs the speciation of a compound. The evaluated compounds are in the range of 119–748 Da (average: 250 Da) for molecular mass, −4.2 to 5.4 (average: 1.9) for log P_{OW} and −4.2 to 4.2 (average: 0.7) for log D_{OW} (pH 7) (see Fig. 1, Table S1). At pH 7, 43 compounds are present as neutral species, 29 as anionic species, 14 as cationic species and 2 as zwitterionic species. For non-ionizable compounds, values for log P_{OW} and log D_{OW} (pH 7) are identical, whereas for ionized compounds the log D_{OW} is up to 5.4 units smaller than the log P_{OW} (1.2 units in average) at the enrichment pH of 7. This shows the importance of incorporating the speciation into the evaluation of the hydrophobicity of ionizable compounds.

Hydrophobicity is the driving force for the enrichment of a compound on reversed-phase materials such as alkyl-modified silica or poly(styrene-divinylbenzene) polymers, which are traditionally used for SPE of compounds with a log P_{OW} greater than one. However, proper adjustment of sample pH is necessary to avoid losses of the target analytes on those SPE materials by deprotonation of acidic compounds or protonation of basic compounds [38]. The introduction of new polymeric sorbents with novel functional groups in the polymeric structure extended the applicability of solid-phase extraction to more hydrophilic compounds (log P_{OW} < 1). Such materials are e.g. the widely used Oasis HLB (hydrophilic–lipophilic balance) material (Waters), which provides lipophilic (divinylbenzene-rings) and hydrophilic (*N*-vinyl-pyrrolidone) groups for retention of non-polar and polar compounds, or the Strata-X material (Phenomenex), which provides similar sorption properties via polydivinylbenzene resin containing piperidone groups. However, Weigel et al. [39] showed, for example, poor recovery (30–40%) for the anionic carboxy-ibuprofen (log D_{OW} (pH 7) = −2.8) with an offline Oasis HLB approach at pH 7. Freitas et al. [40] showed poor recoveries (16–28%) for the anionic compounds dimethachlor oxa (log D_{OW} (pH 7) = −1.4) and dimethachlor esa (log D_{OW} (pH 7) = −1.1) with extraction using Oasis HLB at pH 4. Additionally, several studies report partly low extraction recoveries (40–90%) for the cationic beta-blockers atenolol, sotalol, and metoprolol (log D_{OW} (pH 7) = −2.1, −2.5, and −0.8, respectively) [41–43]. Since half of the compounds in this study are completely or partly present as ionic species and as many have a low log D_{OW} at pH 7 (27 compounds: log D_{OW} < 0), other interactions had to be taken into account.

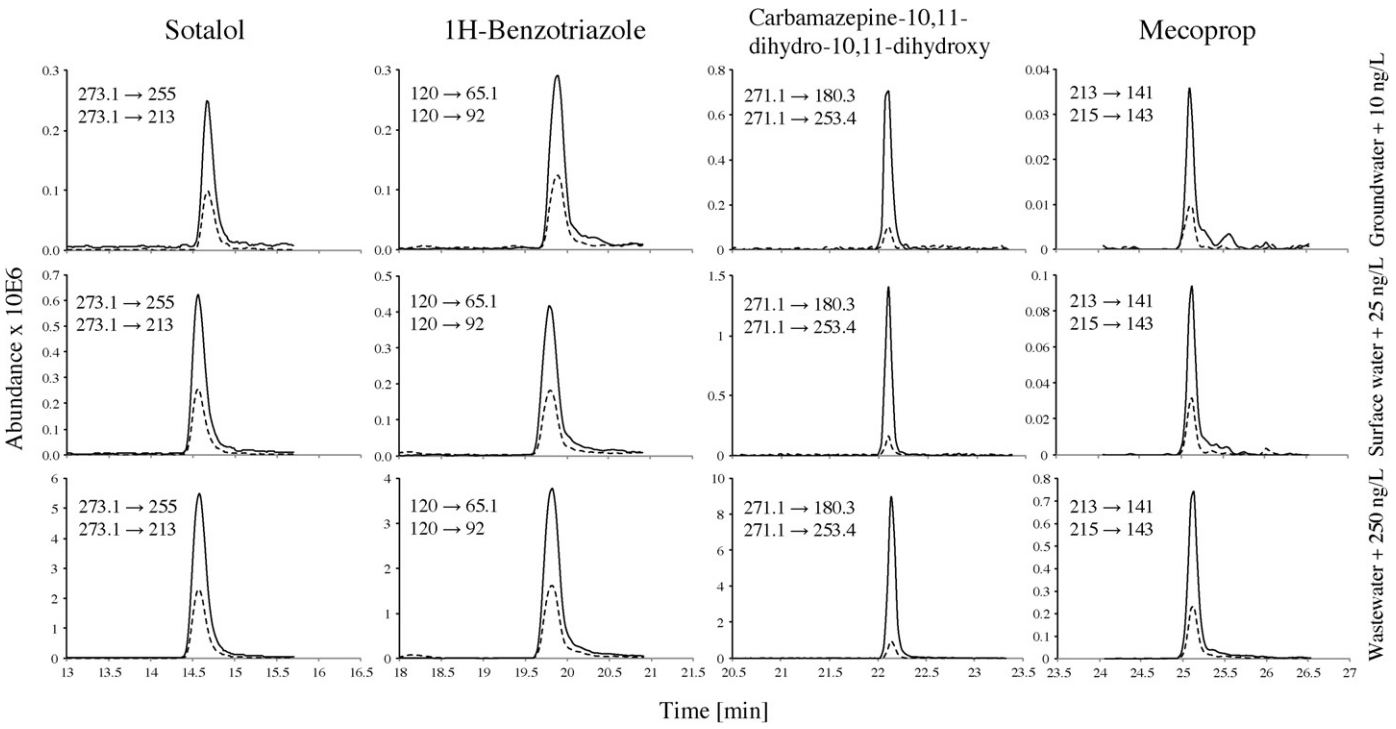

Fig. 2. Chromatograms for sotalol, 1H-benzotriazole, carbamazepine-10,11-dihydro-10,11-dihydroxy and mecoprop (quantifier ion: solid line; qualifier ion: dashed line) in spiked groundwater (top, 10 ng/L), spiked surface water (middle, 25 ng/L) and spiked wastewater (bottom, 250 ng/L).

To cover the enrichment of ionic hydrophilic compounds, mixed mode sorbents containing ion-exchange groups were added to the SPE material. The weak anion exchange material Strata X-AW was selected to target anionic compounds at pH 7. This material provides anion exchange at di-amino ligands as well as hydrophobic interactions at phenyl rings and aliphatic chains together with pi−pi interactions at phenyl rings. The weak cation exchange material Strata X-CW was chosen for cationic compounds at pH 7, which provides cation exchange at carboxylic acid ligands. Those two ion exchange materials are mixed with the material Isolute ENV+, which is a hyper cross-linked hydroxylated polystyrene-divinylbenzene copolymer with a high surface area (1000 m^2/g). This material was shown to be complementary to Oasis HLB for numerous hydrophilic compounds [44]. Zwitterionic compounds have the capability to sorb at any of the aforementioned SPE materials. Oasis HLB material was chosen as the first SPE material in flow direction to cover the unspecific molecular interactions of neutral and medium polar compounds with the sorbent. The mixture of the two ion exchange materials and the Isolute ENV+ is used as the second SPE material to extract all compounds that are not covered by the Oasis HLB material.

For the elution of this mixed-bed multilayer cartridge, the organic solvent methanol has to be either acidic or basic, to exploit the use of the two ion exchange materials. Acidic elution leads to protonation of anionic analytes, destroying the interactions with the weak anion exchange sorbent. Acidic elution also deactivates the cation exchange material through protonation of the carboxylic acid ligands, which disrupts the ionic interaction between the sorbent and cationic analytes. Correspondingly, basic elution leads to deprotonation of the anion exchange material and the cationic analytes that are retained by the cation exchange material. Due to the stability of the chromatographic column in the lower pH range, acidic elution conditions were chosen for this method.

3.2. LC–MS/MS performance

The chromatographic setup resulted in sharp peaks with baseline widths generally lower than 30 s (Fig. 2). Retention times (RT) for the 88 compounds were between 9.9 and 28.8 min. RT shifts within a sequence were generally lower than 30 s. Nonetheless, it is necessary to measure the calibration curve at the beginning and the end of a sequence as well as to include single calibration standards after every 8–10 samples for correct peak assignment.

Polarity switching and scheduled SRM allowed the simultaneous acquisition of 274 ion transitions in the same chromatographic run (Table S2). 79 compounds were measured in positive ionization mode, 8 compounds in negative ionization mode. For the five compounds containing chlorine 2,4-D, diuron, MCPA, mecoprop and sucralose, no suitable confirming fragment from the monoisotopic molecular ion was available. Therefore, the protonated ^{37}Cl isotope was used as precursor for the respective qualifier ion of these compounds. The two isomers 4- + 5-methyl-1H-benzotriazole could not be separated sufficiently on the column (RT difference: 0.1 min) and have the same precursor and product ions. Therefore, they are reported as the sum of both compounds (4- and 5-methyl-1H-benzotriazole, often referred to as tolyltriazole). The third isomer 1-methyl-1H-benzotriazole had a lower RT (1.8 min less) and can thus be quantified separately.

3.3. Method validation

The method developed was validated on three matrices groundwater, surface water, and wastewater. Relative recoveries and LOQs are reported for 81 compounds for all matrices and for six additional compounds, which were added later to the method and only evaluated for groundwater and surface water (Table S3). For the majority of compounds, LOQs were below 10 ng/L in groundwater (n = 71) and surface water (n = 70) and below 100 ng/L in wastewater (n = 70) (Fig. 3a). All other compounds showed LOQs below

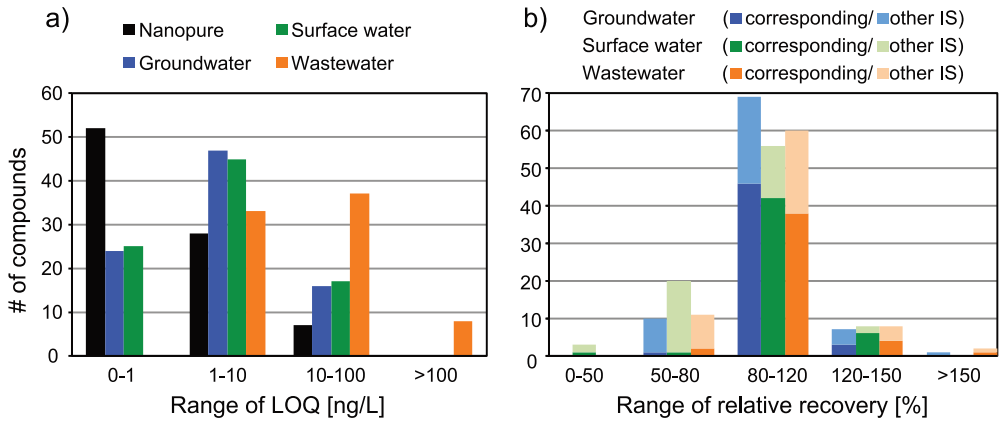

Fig. 3. (a) Ranges of the limits of quantification (LOQ) in nanopure water (n = 87), groundwater (n = 87), surface water (n = 87) and wastewater (n = 78); (b) ranges of the relative recoveries with and without corresponding isotope labeled internal standard (IS) in groundwater (n = 87), surface water (n = 87), and wastewater (n = 81). (Please note that 4-methylbenzotriazole and 5-methylbenzotriazole were evaluated together.)

100 ng/L in ground- and surface water, whereas the highest LOQ in wastewater was 206 ng/L (metazachlor oxa). Due to matrix effects during enrichment and ionization, nanopure standards yielded the best LOQs, followed by groundwater, surface water, and then wastewater. The differences between ground- and surface water were very small. Median values for the signal suppression through matrix effects were 38%, 34%, and 14% for groundwater, surface water, and diluted wastewater, respectively. This shows that the fourfold lower sample volume for wastewater indeed led to smaller matrix effects, but resulted in higher LOQs by a factor of 4. However, the LOQs determined with the chosen approach are only valid for the specific matrix of the validated samples and they also depend on the chosen standard concentration levels. Therefore, it is recommended to determine LOQs separately for every cluster of samples in every sample sequence, especially for quantification of environmental trace concentrations.

The influence of the physicochemical properties of the compounds on the LOQs can be seen in two ways. First, a slight trend of higher LOQs with lower log D_{OW} values at the enrichment pH of 7 can be observed (see Fig. S1). This may be caused by a lower enrichment efficiency on the SPE cartridge, incomplete elution from the SPE cartridge or a lower ionization efficiency in the ESI source. In contrast to this trend, sotalol, atenolol, and atenolol-desisopropyl, three of four compounds with a log D_{OW} less than −2, show LOQs lower than 5 ng/L for all matrices. These belong to the group of beta-blockers and are cationic at the enrichment pH of 7. The zwitterionic TP atenolol acid is the compound with the lowest log D_{OW} (pH 7) (−4.2), but still has adequate LOQs of 21 ng/L for groundwater and surface water and 112 ng/L for wastewater. Secondly, the speciation of the compounds influences the LOQs. All compounds with a LOQ greater than 25 ng/L in groundwater and surface water are present as anions at the enrichment pH of 7. For wastewater, 5 of 8 compounds with a LOQ higher than 100 ng/L belong to the anionic compounds. It is possible that the protonation of anionic analytes with methanol +0.1% formic acid is insufficient for strongly acidic compounds, leading to incomplete elution from the anion exchange material. All anionic compounds with a log D_{OW} (pH 7) greater than 0.25 had LOQs below 7 ng/L for ground and surface water and below 25 ng/L for wastewater, indicating that for less hydrophilic anions, the unspecific interactions with the Oasis HLB and ENV+ materials are sufficient to enable their enrichment and elution.

The relative recoveries of the compounds were very satisfying (Fig. 3b), with the majority of the relative recoveries between 80 and 120% in all matrices. In groundwater, 79% of the compounds fall into this category, in surface water and wastewater 64% and 74%, respectively. As expected, the compounds with a corresponding isotope labeled internal standard and therefore identical physicochemical properties generally showed better relative recoveries than the compounds without a corresponding internal standard. 92%, 84%, and 84% of the compounds with corresponding internal standard are in the range of 80–120% relative recovery, whereas only 62%, 38%, and 61% of the compounds without a corresponding internal standard are in this range for groundwater, surface water, and wastewater, respectively. We did not observe an influence of the speciation or the log D_{OW} (pH 7) on the relative recoveries, as values below 80% and above 120% are present in all categories of compounds. As the results of compounds without corresponding internal standard should be corrected by the relative recovery, samples with one to four different spike levels for each matrix in every sequence were measured and the average recovery or the recovery of the closest spike level was used for this correction. The standard addition method can also be used, which is advantageous especially if many samples with different matrices are to be measured.

The intraday precision of the method was tested for 78 compounds with a sevenfold measurement of a nanopure standard. The relative standard deviation (RSD, n = 7) was generally lower than 10% (65 compounds, 83%), including all compounds with a corresponding internal standard. Only six compounds (dimethenamid oxa, atrazine-desethyl-2-hydroxy, carbamazepine-10,11-dihydro-10,11-dihydroxy, metazachlor oxa, propachlor esa and dimethachlor oxa) showed RSDs between 21 and 36% (Table S3). For the inter-day precision, two spiked samples, one for groundwater and another for surface water, were measured five times over a period of eight months. The RSD (n = 3–5) was generally lower than 20% for both matrices (51 compounds in groundwater and 53 compounds in surface water), including all compounds with a corresponding internal standard except for chloridazon. Only 12 compounds showed higher RSDs than 20% in groundwater and 9 compounds in surface water (Table S3).

3.4. Application to groundwater samples of a field site

The method was applied for a sampling campaign at the Widen riverbank filtration site. Concentrations of all 88 compounds were determined for 12 groundwater samples from a piezometer test field (see Table S4). 36 compounds were found in quantifiable concentrations and the median of all quantified concentrations was 4.7 ng/L, showing the necessity for a sensitive and reliable analytical method. The highest quantified concentration was 600 ng/L for 1H-benzotriazole. Fig. 4b–d shows the concentration patterns in the groundwater for the anti-epileptic drug carbamazepine, the artificial sweetener sucralose and 4-acetamidoantipyrine (AAA),

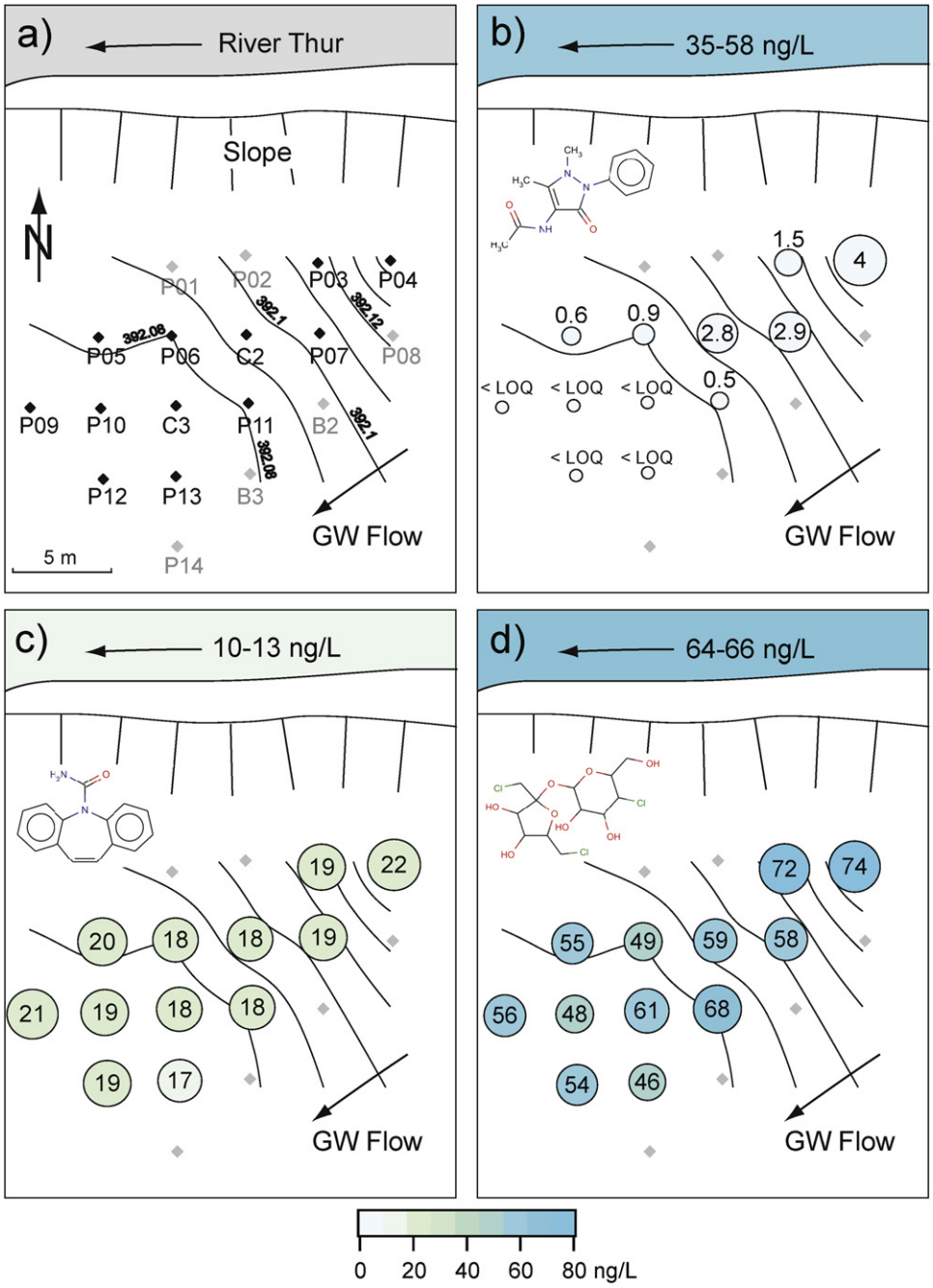

Fig. 4. Schematic plan of the Widen test field with groundwater contour lines (a). Concentrations (in ng/L) of 4-acetamidoantipyrine (b), carbamazepine (c) and sucralose (d) in the Widen test field and the river Thur. Groundwater sampling was performed at the piezometers marked in black, groundwater contour lines (in meters above sea level) origin from groundwater levels of all piezometers (a).

a human metabolite of the analgesic metamizole. For these compounds, concentrations were also determined for six 4-h composite samples from the river Thur, which were taken 58–82 h before the groundwater sampling. This corresponds to the average travel time from the river Thur to the piezometers P03 and C2. The concentration range in these six samples is given in Fig. 4.

The concentrations of carbamazepine and sucralose were relatively constant over the whole groundwater test field, with average concentrations of 19 ng/L for carbamazepine and 58 ng/L for sucralose (Fig. 4c and d). These compounds are both used as anthropogenic markers in the environment, are poorly degradable during wastewater treatment, and persistent in the environment [45,46]. For periods with stable flow conditions in the receiving stream, we can assume a constant input of compounds from wastewater treatment plant (WWTP) effluents to the river. Two rainfall events with discharges of up to 150 m^3/s took place 8 and 13 days before the groundwater sampling. The discharge of the river Thur decreased slowly from 50 m^3/s five days before sampling to 30 m^3/s on the sampling day. Thus, relatively stable concentrations in the river water can be expected and this was confirmed by the observed river concentrations for carbamazepine (10–13 ng/L) and for sucralose (64–66 ng/L). Since the groundwater is mainly fed by freshly infiltrated river water and travel times in the sampled transect are between 67 and 113 h, the concentration patterns indicate

persistence of these two compounds in the groundwater within this timeframe. The slightly lower river concentrations of carbamazepine compared to those in the groundwater may be explained by retardation of this compound in the aquifer.

In contrast, the metamizole metabolite AAA showed concentrations between 35 and 58 ng/L in the river Thur, decreasing to a concentration of 4 ng/L in the closest piezometer to the river Thur and further to concentrations below the LOQ in the groundwater test field with increasing travel time (Fig. 4b). AAA also enters the river water through WWTP effluents, shown by Kahle et al., who observed a good correlation between the wastewater burden of 8 lakes and the concentration of AAA [47]. They reported stable concentration ratios of AAA and carbamazepine in several wastewater effluents, whereas the ratios in the lakes were lower indicating slow dissipation of AAA in surface waters. The decreasing concentrations of AAA compared to carbamazepine and sucralose in the groundwater test field indicate degradation of this compound in the oxic aquifer, as other processes such as dilution and input effects would also affect carbamazepine and sucralose. Sorption of AAA to aquifer material is unlikely due to the low $\log D_{OW}$ (pH 7) of 0.15 and its occurrence as neutral species below pH 10. It was also shown that sorption of AAA plays a minor role in desorption experiments with filter material, sludge and soil samples [48]. Our findings are in agreement with investigations at a bank filtration site at lake Wannsee close to Berlin, Germany [49], where degradation of AAA was already shown to be favored under oxic conditions.

4. Conclusions

For research projects that deal with groundwater, surface water, and wastewater it is essential to have an analytical method that gives precise data over a wide concentration range with very low limits of quantification. The use of a mixed-bed multilayer cartridge for online enrichment allowed us to develop a single LC–MS/MS method for the simultaneous, sensitive, and precise measurement of 88 compounds in all three matrices. The simultaneous detection of pesticides, biocides, pharmaceuticals, corrosion inhibitors, and an artificial sweetener enables the comparative evaluation of different input sources. The inclusion of many of their transformation products facilitates the elucidation of fate processes in environmental systems. The use of 50 isotope labeled internal standards was indispensable to get high quality data with good relative recoveries and satisfactory inter-day and intraday precision. More than 80% of the compounds could be quantified with high precision below 10 ng/L in groundwater and surface water, such that the fate of these contaminants at a bank filtration site could be studied accurately in the low ng/L-range. While carbamazepine and sucralose appear to be persistent in the aquifer, the data for AAA indicate that this compound is well degraded within only 5 days resistance time.

A volume of only 20 mL is needed for the analysis of one sample, and the effort for manual sample handling is limited to filtration. The automated enrichment and the LC–MS/MS run take 36 min per sample for all compounds, which allow for high sample throughput. Based on theoretical considerations, the method is easily expandable to other polar compounds with comparable properties. The combination of the different SPE materials makes the enrichment of neutral, anionic, cationic, and zwitterionic compounds with a wide range of physicochemical properties possible ($\log D_{OW}$ (pH 7): −4.2 to 4.2). The implementation of new analytes into the method should be straightforward if the compound properties meet the general scope of LC–MS/MS methods in terms of mass range (30–1000m/z) or ionization potential (H-donor/H-acceptor moiety). As long as no analyte losses during sampling, sample handling or analysis are expected, e.g. due to hydrolysis or volatilization, the efficiency and broad applicability of this method renders the development of completely new analytical methods unnecessary in many cases. Coupled to a high-resolution mass spectrometer, this online-SPE method with its mixed-bed multilayer cartridge has enormous potential for use in non-target screening of a wide range of analytes in water matrices.

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Appendix A. Supplementary data

Additional data for the physicochemical properties of the analytes, for the MS/MS settings and for the validation data are listed in Tables S1–S3 in the supplementary data section. Table S4 shows the concentrations of all compounds for the Widen field site. In Fig. S1, the limit of quantification is plotted against the $\log D_{OW}$ for the investigated compounds.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2012.10.032.

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