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Research Article

Comparison of preconcentration methods for nontargeted analysis of natural waters using HPLC-HRMS: Large volume injection versus solid-phase extraction

Nontargeted analysis of water samples using liquid chromatography combined with high-resolution mass spectrometers is an emerging approach for surface water monitoring and evaluation of water treatment processes. In this study, sample preconcentration via direct, large volume injection with 500 μL and 1000 μL injection volumes was compared to SPE regarding analytical performance parameters in targeted and nontargeted workflows. In targeted analysis, the methods were evaluated in terms of LOD and intrabatch precision of the selected compounds, whereas in nontargeted analysis, the number of detected unknown compounds, the method's intra-batch precision, and the retention time versus molecular mass pattern of the detected unknowns were evaluated. In addition, a novel intensity drift correction method was developed that is not based on quality control samples and makes use of the signals obtained for continuously infused reference compounds, which are conventionally utilized for online mass drift correction. It could be demonstrated that the new correction method significantly reduced the bias introduced by instrumental drift and is important for the reliable intercomparison of different nontargeted methods. Intercomparison of results showed that the 1000 μL large volume injection method revealed the best performance in terms of precision under repeatability conditions of measurement as well as lower LODs for targeted compound analysis. In nontargeted analysis, the SPE method detected a higher number of unknown compounds but exhibited also a higher uncertainty of measurement caused by matrix effects.

Keywords:
High-resolution MS / Large volume injection / Natural waters / Nontargeted analysis / Signal drift correction
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1 Introduction

Contaminants of emerging concern (CECs) are anthropogenic organic chemicals, for example, personal care products, hormones, food additives, pharmaceuticals, plasticizers, pesticides, disinfectants, flame retardants, and surfactants, which are released into the environment without established regulations [1–4]. The main sources of CECs are discharges of municipal, industrial, and agricultural wastewaters into surface water [3]. Prioritization of CECs is difficult due to the chemical diversity and the limited knowledge on ecotoxicity and human toxicity [2]. However, the high polarity and persistent fraction of CECs can pass wastewater treatment and potentially also drinking water treatment processes, which increases the probability of ending up in drinking water [5]. This risk elevates the importance of developing sensitive and comprehensive analytical methods to analyze polar contaminants in water.

Analysis of polar contaminants in water matrices is usually conducted via LC combined with MS, or GC-MS with fit-for-purpose derivatization procedures [6]. In targeted analysis, LC-MS/MS or GC-MS/MS methods enable the

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Article Related Abbreviations: AM, arithmetic mean; **CECs**, contaminants of emerging concern; **FA**, formic acid; **HLB**, hydrophilic lipophilic balanced; **LVI**, large volume injection; **QC**, quality control

Color online: See article online to view Figs. 1–5 in color.

accurate, absolute quantification of selected compounds, which demands for well-characterized authentic standards and is therefore limited regarding the number of measurable compounds [7]. As a general drawback, unknowns as well as transformation products are not covered by this approach. Nontargeted analysis via high-resolution MS (HRMS) in MS1 (or "full-scan") mode is a conditional approach offering the detection of all molecules, which are retainable and ionizable with the selected chromatographic and mass spectrometric set-up [3,7]. As an advantage, this data acquisition mode is that it allows retrospective analysis of archived data [8]. After acquisition of data in high-resolution mode, data processing can be performed (i) in terms of suspect screening, where the exact monoisotopic masses of thousands of contaminants are extracted from the data, or (ii) in terms of "true" nontargeted analysis, where the data are processed with peak picking and alignment algorithms without any preselection of compounds. In this approach, single ions are detected and isotopologues, adducts, and multiply charged ions are aligned into unknown compounds (molecular features). These compounds are then aligned between samples according to their accurate mass spectra and retention time. Subsequently, statistical tools are applied to evaluate the results. In the field of water analysis, nontargeted analysis is highly valuable for process evaluation and monitoring purposes. Nevertheless, at this information state, the level of confidence of identity confirmation is poor, and only relative quantification can be performed [6,8,9].

The detection of all compounds of interest present in a water sample is not feasible by performing only one single LC-HRMS method [5]. The coverage of a method depends on the selected sample preparation, and chromatographic and ionization methods employed. Sample preparation methods involving steps such as filtration, pH adjustment, extraction, clean up, or pre-concentration, are used for reducing/removing interfering matrix and/or for concentrating compounds in order to achieve lower limits of detection [10]. SPE is a well-established analytical technique for analyte preconcentration and matrix removal [11]. The selection of appropriate sorbent material plays a key role in clean-up and enrichment of the target molecules. The sorbent material Oasis® HLB (hydrophilic lipophilic balanced) is based on macroporous poly(*N*-vinylpyrrolidone-divinylbenzene) copolymer and often used in targeted [7,12–14] and nontargeted [14–16] water analysis due to its high extraction efficiency for highly polar compounds [17].

In large volume injection (LVI) (also known as direct injection), samples with a volume $\geq$ 10% of the void volume of the analytical column are injected directly onto the chromatographic separation column. In comparison to analytical techniques involving SPE, LVI is cheaper and more environmentally friendly due to reduced consumption of extraction materials (i.e., SPE cartridges), extraction solvents, and labor time [11]. LVI is frequently employed in targeted analysis of pesticides, biocides, neurotoxins, pharmaceuticals corrosion inhibitors, and artificial sweeteners in various matrices such as drinking water, groundwater, surface water, treated water, and wastewater [18–24]. Additionally, it has also been employed in nontargeted analysis of waste and surface water [25–27].

It has been demonstrated that LVI is a valuable alternative to SPE in targeted analysis of selected contaminants [11,28] and nontarget screening [29,30], which is also reflected by its application in the standard DIN 38407–47s [31]. However, to the best of our knowledge, the preconcentration methods SPE and LVI were not compared in the context with nontargeted analysis using LC-HRMS so far. The present study therefore addressed the comparison of the two approaches for natural waters. To enable this comparison, a novel method for signal intensity drift correction has been developed and successfully applied.

2 Materials and methods

2.1 Chemicals

The LC-MS-grade ACN and methanol (Chromasolv™ HPLC solvents series) were purchased from Honeywell Riedel-de-Haën™ (Bucharest, Romania). The formic acid (FA) (98%) was purchased from Honeywell Fluka™ (Bucharest, Romania). Ultra-pure water used for the HPLC-TOFMS was prepared in house using Milli-Q® IQ 7000 Ultrapure Lab Water System (Darmstadt, Germany) combined with a Milli-Q LC-Pak® Polisher.

Atrazine-d5 (99.54%), carbamazepine (99.52%), chloridazon (PAC) (99%), clothianidin (99.5%), DEET (98.1%), pethoxamid (99%), propiconazole (99%), thiacloprid (99.2%), and thiamethoxam (99%) were purchased from Dr. Ehrenstorfer GmbH (Wesel, Germany). Acetamiprid-d3 (99.7%) and thiamethoxam-d3 (99.1%) were purchased from Honeywell Fluka™. Caffeine (pure) was purchased from Merck (Darmstadt, Germany). Alachlor (99%), isoproturon (99%), linuron (99%), metazachlor (99%), metobromuron (99%), monolinuron (Phenylurea) (99%), propazin (99%), and terbuthylazine (99%) were purchased from Honeywell Riedel-de-Haën™. Acetaminophen (99%), atenolol (98.5%), atrazine (99.5%), dexamethasone (98%), diclophenac (98.5%), IPC/propham (99.7%), thiamphenicol (99%), and triethyl phosphate (99.8%) were purchased by Sigma Aldrich (Vienna, Austria). LC/MS Pesticides Calibration Mix 4 consisted of the chemicals 3-hydroxycarbofuran (99.9%), aclonifen (99.8%), azoxystrobin (99.4%), carboxin (99.9%), clodinafop-propargyl (99.5%), cycluron (99.1%), diuron (99.9%), oxamyl (99.5%), picoxystrobin (99.9%), pymetrozin (99.9%), pyracarbolid (99.8%), and pyraclostrobin (99.9%) and was purchased from Sigma Aldrich. Pesticide residue mix SPEXPR-2 consisted of the chemicals azinphos-methyl (99.5%), coumaphos (99.4%), dicrotophos (99.5%), dimethoate (99.5%), dyfonate (Fonofos) (98.5%), imidan (Phosmet) (99.3%), malathion (99.5%), methidathion (99.5%), phosalone (99.5%), prophos (98.8%), quinalphos (99.5%), and triazophos (98.3%) and was purchased from SPEX (Stanmore, United Kingdom).

2.2 Precleaning of materials

All glassware used for sampling and sample preparation were washed in a laboratory dishwasher (Miele, Wals, Austria) with demineralized water and laboratory washing agents ProCare Lab 10 MA and ProCare Lab 30 C (Miele). Later, the glassware was rinsed twice with ultra-pure water and heated in a muffle furnace (Nabertherm GMBH, Lilienthal, Germany) for 4 h at 480°C.

2.3 Sampling and sample storage

The surface water samples were collected in 2-L glass bottles using the grab sampling method on September 23, 2019 (sample 1) and October 1, 2019 (sample 2) from the Danube Canal, within the city of Vienna, Austria. The groundwater samples were pumped in a 2-L glass bottle on September 23, 2019 from a groundwater well, which is within 40 m distance of the Danube Canal. For procedural blanks, a 2-L glass bottle was filled with ultra-pure water. After sample collection, 1 L of each sample was filtered within 2 h. While sample 1 (surface water) and groundwater sample were used in method comparison experiments, sample 2 (surface water) was used for the determination of SPE recovery.

For filtration, a Sartorius 16249 stainless steel filter holder (Göttingen, Germany) was connected to a pressurized synthetic air bottle. Before filtration, 0.45 μm cellulose filters (PALL-Life Sciences, Vienna, Austria) were conditioned with 400 mL of 90°C ultra-pure water and 200 mL of sample. Filtered samples were kept at 4°C overnight.

2.4 Sample preparation

2.4.1 Large volume injection

For large volume injection, 950 μL of sample was spiked with 50 μL of spiking solutions, which were selected according to the sample type. Surface water, groundwater, procedural blank, and instrument blank (ultra-pure water) samples were spiked with a spiking solution, containing 2% (v/v) FA, 20% (v/v) ACN, and the internal standards (acetamiprid-d3 [10 μg/L], atrazine-d5 [2 μg/L], thiamethoxam-d3 [10 μg/L]). Internal standards were not considered in calculations and served for controlling the overall process in general. For calibration samples (spiked surface water), spiked ultra-pure water samples and quality control (QC) samples (spiked surface water) spiking solutions were also containing reference substances. The reference substance concentration in the QC samples and spiked ultra-pure water samples was 0.100 μg/L, while the concentrations in the five calibration standards were 0.010 μg/L, 0.050 μg/L, 0.100 μg/L, 0.150 μg/L, and 0.200 μg/L. The concentration of the reference substances in the spiking solutions was 0.200 μg/L, 1.00 μg/L, 2.00 μg/L, 3.00 μg/L, and 4.00 μg/L to obtain 0.010 μg/L, 0.050 μg/L, 0.100 μg/L, 0.150 μg/L, and 0.200 μg/L, respectively.

2.4.2 Solid-phase extraction

Prior to SPE, approximately 30 mL of sample was spiked in 62-mL glass vials with 100 μL of an internal standard mixture containing atrazine-d5 and thiamethoxam-d3 at a concentration of 120 μg/L and 900 μg/L, respectively. QC, spiked ultra-pure water, and calibration samples were additionally spiked with the reference standard mixture. The concentration of reference substances in QC and spiked ultra-pure water samples was 0.100 μg/L, while the concentration in the five calibration samples was 0.010 μg/L, 0.050 μg/L, 0.100 μg/L, 0.150 μg/L, and 0.200 μg/L. For the preparation of the sample with 0.010 μg/L, a 10.0 μg/L reference standard mixture was used, while for the preparation of 0.050 μg/L, 0.100 μg/L, 0.150 μg/L, and 0.200 μg/L samples, a 100 μg/L reference standard mixture was used. After addition of the internal standards and reference substances, the glass vials were filled to 62 g with the corresponding sample. SPE extractions were performed using a Gilson GX-271 ASPEC® (Middleton, USA) liquid handling instrument and 60 mg 3 cc Oasis HLB cartridges (Waters, Vienna, Austria). The cartridges were preconditioned with 3 mL methanol followed by 3 mL ultra-pure water. Note that 60.0 mL of sample was loaded at a flow rate of 3 mL/min. The cartridges were then washed with 250 μL water and dried under 1 bar nitrogen gas for 60 s. The analytes were eluted with 3 mL methanol. After elution, the eluates and empty vials for instrument blanks were spiked with 100 μL internal standard mixture containing acetamiprid-d3 (150 μg/L) in ACN. Spiked eluates were dried with a centrifugal vacuum concentrator (GeneVac®, Warminster, USA) and stored at –80°C. Prior to measurement, samples were reconstituted in 150 μL of 1% (v/v) ACN and 0.1% (v/v) FA and then shaken with IKA® VXR B orbital shaker (Staufen, Germany) first 3 min at 1500 rpm and then 30 min at 750 rpm.

2.4.3 SPE recovery experiment

For the determination of SPE recovery, three procedural replicates of surface water (sample 2) containing internal standards and 0.100 μg/L reference substances and three procedural replicates of surface water (sample 2) containing only internal standards were prepared and processed according to section 2.4.2. Additionally, three technical replicates of reference substance mixtures with a concentration corresponding to 100% SPE recovery (40.0 μg/L) were prepared and measured for the assessment of compound recovery.

2.5 HPLC-TOFMS

LC was performed using a 1290 Infinity II LC system (Agilent Technologies, Santa Clara, USA) combined with an XSelect ® HSS T3 (2.1×150 mm, 3.5 μm particle size) column (Waters) and a XSelect® HSS T3 (2.1×5 mm, 3.5 μm particle size) precolumn (Waters).

For the comparison of SPE, 500 μL LVI, and 1000 μL LVI methods, a full loop injection method was applied using 5 μL, 500 μL, and 1000 μL stainless steel injection loops with loading volumes of 15 μL, 550 μL, and 1000 μL, respectively. After sample injection, gradient elution with 0.1% (v/v) FA (mobile phase A) and ACN with 0.1% (v/v) FA (mobile phase B) was performed. The flow rate was 0.250 mL/min and the column oven temperature 40°C. The gradient started at 1% B and was increased to 100% in 15.5 min. After 5.5 min, the mobile phase composition was decreased to 1% B within 0.1 min and the column was equilibrated 9.5, 11.5, and 13.5 min according to the applied injection volumes of 5 μL, 500 μL, and 1000 μL, respectively. The corresponding total run times were 30.6, 32.6, and 34.6 min.

Mass spectrometric analysis was performed on an 6230B LC-TOFMS system (Agilent Technologies) equipped with an Agilent Jet Stream electrospray ionization interface. Ionization parameters were set as follows: 180°C drying gas temperature, 10 L/min drying gas flow, 35 psig nebulizer pressure, 350°C sheath gas temperature, 12 L/min sheath gas flow, 3500 V capillary voltage, and 120 V fragmentor voltage. The TOF detector was operated in the low mass range ($\leq$1700 m/z) in 2 GHz extended dynamic range mode with an acquisition rate of 2.5 spectra per second (accumulation of 5361 TOF transients per spectrum). Spectral data were recorded over a mass range of 90–1700 m/z. A solution with reference masses (m/z = 121.050873 and 922.009798) (Agilent PN: G1969-85001) for mass calibration was continuously introduced via a second reference sprayer using a reference pump (Agilent 1200 Binary Pump SL (G1312B)). The flow rate was set to 0.050 and 0.100 mL/min for the methods with 5 μL injection and for LVI, respectively. In order to achieve a stable flow rate, a backpressure regulator (approximately 70 bar) was inserted between the reference pump and reference sprayer.

In order to reduce long-term drift, the column effluent was directed to MS only between 2–15.3 min, 5–17.3 min, and 7.4–19.4 min, according to the injection volumes of 5 μL, 500 μL, and 1000 μL, respectively. Outside of these time intervals, the column effluent was directed to waste and the ESI interface was purged with 0.1% (v/v) FA at a flow rate of 0.250 mL/min.

Each sequence commenced with three instrument blanks 1% (v/v) ACN, 0.1% (v/v) FA, and two QC samples for the conditioning of the system. After the conditioning, all samples were randomized and measured between six equidistant technical replicates of a QC sample.

2.6 Data processing workflow

2.6.1 Targeted analysis

Targeted analysis of spiked compounds was performed by MassHunter Workstation Quantitative Analysis for TOF version B10.1 (Agilent Technologies). A data processing method utilizing the compound retention times and m/z values ($[M+H]^+$) was applied. All extracted chromatograms were optically evaluated for peak integration and manually reintegrated if necessary. Outlier parameters were set as: mass accuracy > 10 ppm and signal to noise ratio (S/N) < 3. Peaks that did not fulfill these criteria were manually set to 0 abundance.

2.6.2 Nontargeted analysis

The nontargeted data workflow started with feature finding and alignment via batch-recursive MFE method in MassHunter Profinder version B10.00 SP1 (Agilent Technologies). The batch-recursive molecular feature extraction method was configured to search for peaks with spectral intensities $\geq$300 counts between the retention times in which the column effluent was directed to MS. For positive ionization mode, protonated and sodium adducts were set as possible ion features. Isotopes were grouped according to the isotope model for common organic molecules. The compound ion count threshold was set to "two or more ions." For the intersample compound alignment, retention time tolerance and mass tolerance were set to ±0.15 min and ±(10 ppm + 2.00 mDa), respectively. After molecular feature extraction, molecular features, which had a molecular feature extraction score above 80 in any sample, were processed in the recursive feature extraction step. Molecular features revealing a target score above 80 in any sample were accepted and automatically integrated. The integration of every extracted molecular feature was controlled manually and if needed peaks were manually integrated. MassHunter Profinder results were then exported to Mass Profiler Professional Version 15.0 (Agilent Technologies).

2.6.3 Signal intensity drift correction and data filtering

Signal intensity drift correction compensating the continuous loss of sensitivity was applied to all targeted and nontargeted data, which has been processed by MassHunter Workstation Quantitative Analysis for TOF and MassHunter Profinder. For signal intensity drift correction, the relative sensitivity of the TOFMS was assessed using the intensities of the mass calibration reference ions (m/z: 121.050873 and 922.009798), which were continuously infused by a secondary sprayer (Fig. S1A). For each reference ion, the average intensity was calculated from all mass spectra obtained from the start of the acquisition until the switch of the column effluent to the main sprayer (Fig. S1B and C). The initial sensitivity (i.e., the two reference values) was determined at the beginning of the measurement sequence after adequate equilibration/conditioning of the system (the first three instrument blanks for the conditioning were excluded in this calculation). In each measurement sequence, that is, the consecutive measurement of a larger number of samples, standards, and QC samples with the identical LC-TOFMS method, the relative

intensity of the system for each of the two reference ions was calculated for each sample by dividing their average intensities in the samples by the initial reference values (Fig. S1D and E). Subsequently, both of these relative intensities were averaged (Fig. S1F). Finally, the signal intensity drift was corrected in each sample by dividing the exported targeted and nontargeted compound intensities by the corresponding average relative intensity of the system (Fig. S1G).

Data from targeted and nontargeted analysis results were subjected to several filters to reduce false positive results. In NTA, false positive results can occur in the feature finding step, for example, if noise is identified as a positive feature. Additionally, compounds (contaminants) that are unintentionally added to the samples during sampling, sample preparation (procedural blank), or at the LC-TOFMS system (instrument blank) do not belong to the sample and are considered as false-positive results [32]. In targeted analysis, the signal intensity drift corrected data was first subjected to a 1000 counts intensity cut-off, in which every peak, which did not exceed an area of 1000 counts, was set to 0. Following this step, additional filtering was performed by calculation of LOQ [33]. For each compound, two separate LOQ values were calculated by applying instrument and procedural blank values (Eq. 1). Equation 1 calculates LOQ using SD and arithmetic mean (AM) of the intensities. If the standard deviation was not calculable due to the detection of blank only in one replicate, the intensity of the single blank was set as LOQ. Compound entities from each sample, which were below the highest LOQ value of the corresponding compound were removed.

In nontargeted analysis, two additional steps were included. First, before the signal intensity drift correction, entries of possible compounds comprising more than two ions were eliminated for that sample using Mass Profiler Professional. Second, an alignment artifact identification and elimination step was performed between the 1000 counts abundance cut-off and LOQ elimination steps. In this step, first, compound chunks were built for compounds, which laid within the mass tolerance of 10 ppm and retention time tolerance of 0.15 min. Consequently, from each chunk, the compound that was detected in most of the samples was selected. If this number was equal, then the compound with the highest average intensity was selected. All compounds that passed the described data filtration steps were considered as *valid compounds*.

$$\mathrm{LOQ} = 10 \times \mathrm{SD\,(Blanks)} + \mathrm{AM\,(Blanks)} \quad (1)$$

2.6.4 Calculation of signal intensity drift and SPE recovery

To assess the significance of the intensity fold changes determined for different samples, relative signal intensity drifts have been calculated for all compounds, which were detected in all of the six technical replicates of the QC samples. The QCs were equally distributed over a sequence and the signal intensity drifts were calculated according to Eq. (2). In this equation, min (intensity) and max (intensity) refer to the smallest and the largest peak areas of the respective compound detected in the six replicates.

$$\text{Signal intensity drift}\,[\%] = \left(1 - \frac{\min\left(\text{intensity}\right)}{\max\left(\text{intenstiy}\right)}\right) \times 100 \quad (2)$$

The SPE recovery [%] for each compound was calculated using signal intensity drift corrected values. For the SPE recovery calculation, the AM intensity of the compounds detected in surface water (SW) was subtracted from the AM intensity of spiked SW and then divided by the AM intensity of the reference solution (Eq. 3).

$$\text{Recovery}\ \ [\%] = \frac{(\text{AM (spiked SW)} - \text{AM (SW)})}{\text{AM (reference } (40\ \mu\text{g/L})} \times 100 \quad (3)$$

3 Results and discussions

The present study compares three different analytical techniques for targeted and nontargeted analysis of natural waters using HPLC-TOFMS. In summary, 500 μL LVI, 1000 μL LVI, and a SPE-method, where 60 mL of sample is concentrated 400 times (equivalent to 2000 μL LVI), were compared using the same HPLC-TOFMS method with adapted chromatographic equilibration times according to the increase in system volume arising from the different injection loop volumes. Additionally, a new signal intensity drift correction method that is not based on QC samples was introduced and applied to targeted and nontargeted analysis. With the help of the successful signal intensity drift correction, the comparison of the intrabatch precision between analytical methods was less biased by alterations of the mass spectrometers sensitivity during the measurement sequence.

3.1 Targeted analysis

In most cases, in targeted analysis of natural waters LC-MS/MS is applied for selective and sensitive compound separation and quantification. At the front end, during the last years, SPE is continuously substituted by LVI approaches in this field, as the instrumentation has been significantly improved regarding sensitivity and, consequently, the LODs and LOQs. This is reflected by several ISO standards recommending the application of LVI strategies (often named "direct injection methods") in water analysis [31].

In this section, we are comparing the two well-established approaches, that is, SPE and LVI using the LC-TOFMS set-up we are applying for evaluation of LVI in the context with NTA. The motivation for this step is to proof that LC-TOFMS, although usually not applied for targeted quantification, meets the recommendations of the EU regulations

for drinking water quality (EC 1998, see below). For this purpose, the analytical performance of the methods was analyzed and compared using 49 reference substances, that is, a mixture of 41 pesticides, seven pharmaceuticals, and an industrial chemical. The substances were chosen as they are representing anthropogenic contaminants character and cover a large range of chemical properties with diverse octanol/water partition coefficients. The substances were spiked to obtain a concentration of 0.100 μg/L, in accordance with the chemical parametric value for pesticides in the European Union regulations for drinking water quality (EC 1998) [34]. Compounds detected in all replicates were chosen as eligible for determination of measurement repeatability. The LOD was calculated, according to the standard from the German Institute for Standardization (DIN 32645:2008-11, calibration curve method) [35]. In an additional experiment (Section 2.4.3), the SPE recovery of each compound was determined by measuring three procedural replicates of surface water samples, which were spiked to a final concentration of 0.100 μg/L of each compound. For the calculation, Eq. (3) is used and the result for each compound is shown in Table 1. In total, 22% (11 compounds) were recovered with 60% and 140%, which is the accepted range according to SANTE Guidelines [36]. A further 49% (24 compounds) were recovered between 30% and 60%, while 27% (13 compounds) exhibited recoveries of below 30%. The 37 compounds belonging to the last two categories were not within the accepted recovery range, but the intrabatch precision of 23 compounds was satisfactory with RSD values below 20%.

After the application of postacquisition filters (Section 2.6.3), the 500 μL LVI, 1000 μL LVI, and SPE methods resulted in 41, 43, and 44 detected target compounds across all replicates of the QC samples, respectively. Both the instrumental and the procedural precision under repeatability conditions of measurement were determined. The influence of the two LVI methods and of the SPE procedure on the instrumental precision of the HPLC-TOFMS was assessed by measuring six equally distributed technical replicates of a single QC sample (spiked surface water). The effect of sample preparation was determined via measurement of three matrix-free procedural replicates, that is, spiked ultra-pure water. Figure 1A and B show the evaluation of these uncertainty sources using the cumulative frequency of the RSD of the concentrations of target compounds. The analysis of the technical replicates from the QC samples resulted in very similar RSD values for the three methods (Fig. 1A) with all of the RSD values being below 15%, which clearly indicates that, after drift correction (see Section 3.2.1), neither the surface water matrix nor the SPE enrichment procedure have a negative impact on instrumental precision. The investigation of the influence of sample preparation yielded a different result. For the two LVI methods, the RSDs were below 10% for more than 90% of the compounds, whereas only 29% of the compounds for the SPE method revealed an RSD lower than 10%. In total 50% of compounds had RSDs over 20% (Fig. 1B). These results clearly indicate that, in comparison to LVI, the performed SPE method involved a step, which significantly increased the uncertainty for several compounds. A comparison of the RSDs with water solubility data (Fig. S2) indicates a positive correlation of decreasing solubility and increasing RSDs. Considering the satisfactory repeatability of SPE for many compounds in nontargeted analysis (see Fig. 4 later), this correlation indicates that the variation is probably caused by the reconstitution step in the SPE-method.

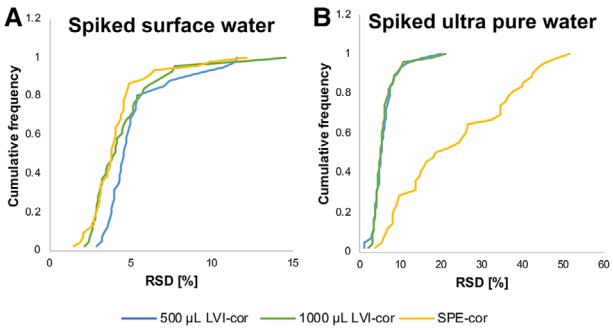

Figure 1. Cumulative frequencies of target compound RSDs of the two LVI methods and the SPE method. The RSDs were calculated from signal intensity drift corrected values by the method described in Section 2.6.3. (A) RSDs from six equidistant technical replicates of spiked (0.100 μg/L) surface water (QC sample). (B) RSDs from three procedural replicates of spiked (0.1 μg/L) ultrapure water.

Besides analytical precision, LOD is an important criterion for evaluation of analytical method performance. The EU regulations for drinking water quality (EC 1998) request that analytical methods for pesticides achieve an LOD value of at least 25% of the parametric value of 0.1 μg/L. In this study, LOD was calculated by applying DIN 32645:2008-11 (calibration method) from intensities of calibration samples obtained from single measurements of surface water samples with five different concentration levels (0.010, 0.050, 0.100, 0.150, and 0.200 μg/L). Figure 2 shows the number of target compounds detected by each method in five different LOD intervals. The 500 LVI, 1000 LVI, and SPE methods revealed a LOD below the value required for pesticides by EC 1998 for 33, 35, and 32 target compounds, respectively. The most significant difference in the LOD results can be seen at the LOD level ≤ 0.01 μg/L. At this level, 1000 μL LVI revealed 23 compounds, whereas 500 μL LVI and SPE method revealed only eight and five compounds, respectively. Although the SPE-method has the highest enrichment (equivalent to 2000 μL of LVI), it resulted in the lowest number of target compounds with a LOD level ≤ 0.01 μg/L. This is caused by the increased uncertainty of the SPE method (Fig. 1B), as in the DIN 32645 calibration method, increased uncertainty of measurement directly increases the methods LOD due to the lower coefficient of determination.

3.2 Nontargeted analysis

In nontargeted analysis, the evaluation and intercomparison of analytical methods regarding their analytical figures of

Table 1. SPE recovery at 0.1 μg/L, coefficient of determination (*R*-squared) of the calibration curve from the SPE method, and LOD values of target compounds obtained by the different analytical methods

Compound	Pollutant family	*m/z*	LOD (μg/L)			Recovery (%)	RSD (%)	*R*-squared
			500 μL LVI	1000 μL LVI	SPE-method	SPE-method	SPE-method	SPE-method
3-Hydroxycarbofuran	Pesticide	238.1074	0.020	0.033	0.024	44	2	0.996
Acetaminophen	Drug	152.0706	0.104	NA	0.022	25	4	0.983
Aclonifen	Herbicide	265.0374	NA	NA	NA	NA	NA	NA
Alachlor	Herbicide	270.1255	0.017	0.011	0.014	72	16	0.984
Atenolol	Drug	267.1703	0.024	0.031	0.021	53	8	0.954
Atrazine	Herbicide	216.1010	0.010	0.020	0.013	59	7	0.992
Azinphos-methyl	Pesticide	318.0130	0.015	0.004	0.027	51	16	0.951
Azoxystrobin	Fungicide	404.1241	0.014	0.010	0.026	62	22	0.990
Caffeine	Drug	195.0877	0.034	0.016	0.021	38	3	0.994
Carbamazepine	Drug	237.1022	0.005	0.010	0.025	69	43	0.978
Carboxin	Fungicide	236.0740	0.027	0.007	0.023	39	5	0.947
Chloridazon (PAC)	Herbicide	222.0429	0.008	0.007	0.010	24	3	0.997
Clodinafop-propargyl	Herbicide	350.0590	0.006	0.006	0.028	19	52	0.983
Clothianidin	Pesticide	250.0160	0.012	0.039	0.005	22	5	0.994
Coumaphos	Pesticide	363.0217	0.115	0.042	0.024	11	57	0.999
Cycluron	Herbicide	199.1805	0.014	0.003	0.013	74	6	0.991
DEET	Pesticide	192.1383	0.013	0.024	0.037	66	7	0.992
Dexamethasone	Drug	393.2072	0.013	0.014	0.078	26	5	0.989
Diclophenac	Drug	296.0240	0.053	0.043	0.043	26	28	0.974
Dicrotophos	Pesticide	238.0839	0.010	0.008	0.012	61	6	0.991
Dimethoate	Pesticide	230.0069	0.014	0.003	0.009	47	40	0.989
Diuron	Herbicide	233.0243	0.030	0.022	0.026	48	7	0.968
Dyfonate (Fonofos)	Pesticide	247.0375	0.022	0.005	0.119	25	24	0.882
Imidan (Phosmet)	Pesticide	318.0018	0.463	0.003	NA	2	173	NA
IPC/propham	Herbicide	180.1019	NA	NA	NA	35	10	NA
Isoproturon	Herbicide	207.1492	0.016	0.004	0.030	65	3	0.962
Linuron	Herbicide	249.0192	0.023	0.028	0.008	53	51	0.974
Malathion	Pesticide	331.0433	0.032	0.012	0.013	59	19	0.996
Metazachlor	Herbicide	278.1055	0.014	0.011	0.021	90	7	0.989
Methidathion	Pesticide	302.9691	0.031	0.003	0.027	61	12	0.953
Metobromuron	Herbicide	259.0077	0.008	0.005	0.017	44	9	0.984
Monolinuron	Herbicide	215.0582	0.023	0.016	0.023	46	4	0.982
Oxamyl	Pesticide	220.075	NA	0.243	0.030	58	4	0.993
Pethoxamid	Herbicide	296.1412	0.011	0.009	0.022	69	16	0.993
Phosalone	Pesticide	367.9941	NA	0.041	NA	18	45	NA
Picoxystrobin	Fungicide	368.1104	0.013	0.006	0.018	29	29	0.992
Propazin	Herbicide	230.1167	0.006	0.004	0.007	59	12	0.989
Prophos	Pesticide	243.0637	0.012	0.008	0.014	52	45	0.997
Propiconazole	Fungicide	342.0771	0.011	0.001	0.017	31	25	0.991
Pymetrozin	Pesticide	218.1036	0.031	0.100	0.017	99	40	0.991
Pyracarbolid	Fungicide	218.1176	NA	NA	0.017	54	4	0.992
Pyraclostrobin	Fungicide	388.1059	0.015	0.011	0.017	11	55	0.966
Quinalphos	Pesticide	299.0614	0.011	0.008	0.017	36	12	0.988
Terbuthylazine	Herbicide	230.1167	0.008	0.019	0.013	53	15	0.992
Thiacloprid	Pesticide	253.0309	0.009	0.007	0.014	35	6	0.988
Thiamethoxam	Pesticide	292.0266	0.013	0.009	0.006	26	7	0.994
Thiamphenicol	Drug	356.0121	NA	NA	0.052	58	36	0.980
Triazophos	Pesticide	314.0723	0.010	0.004	0.020	39	30	0.995
Triethyl phosphate	Industrial catalyst	183.0781	0.011	0.005	0.017	38	4	0.931

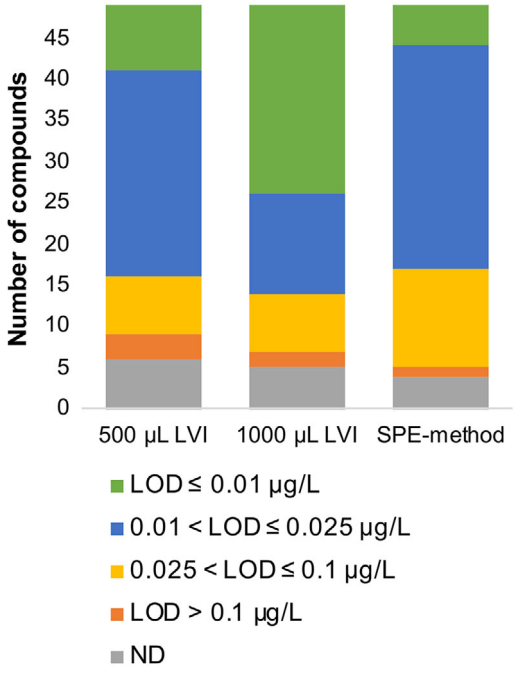

Figure 2. Stacked column diagram showing the number of target compounds at five different concentration intervals of LOD (ND: not detected, greater than 0.1 µg/L, between 0.025 and 0.1 µg/L, between 0.01 and 0.025 µg/L, and less than 0.01 µg/L).

merit is a difficult task. Each change in the settings of the analytical method can have a strong influence on the covered polarity range [5]. Dependent on the chemical composition of the investigated water sample, this change may significantly reduce or increase the number and nature of detected compounds. At the same time, changes regarding the enrichment factors may alter the matrix effect, which could in turn affect the methods sensitivity and LOD.

In this section, the analytical performance of 500 µL LVI, 1000 µL LVI, and SPE method was compared in the context of nontargeted analysis. As mentioned in the introduction section, the intercomparison was performed after the signal intensity drift was corrected with the novel method. As elaborated in the subsequent section, this step is shown to be a necessity as it removes unwanted instrumental errors and fluctuations, which could falsify the interpretation of the intercomparison results. After the correction step, the three methods were compared according to compound coverage, technical and procedural precision under repeatability conditions of measurement, as well as the polarity-range of detectable compounds.

3.2.1 Signal intensity drift correction and instrumental precision

Signal intensity drift is a systematic error of instrumental origin, which can cause a significant bias of relative quantitative results in nontargeted analysis [37]. An estimation

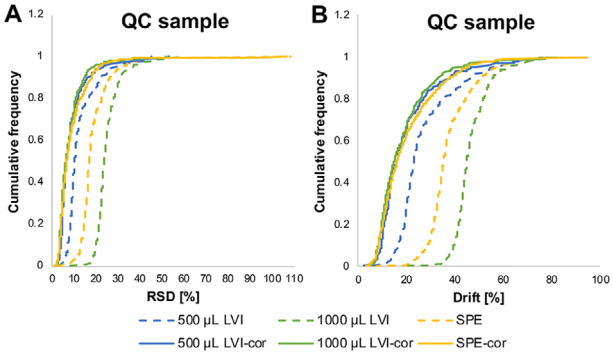

Figure 3. Cumulative frequencies of nontarget compound RSDs and signal intensity drifts from 500 µL LVI, 1000 µL LVI, and SPE-method. (A) RSDs of nontarget compounds calculated from six equally distributed technical replicates of QC sample. (B) Maximum signal intensity drifts of nontarget compounds between six equally distributed technical replicates of QC sample. The RSDs and drifts were calculated from signal intensity drift-corrected ("-cor") and uncorrected values.

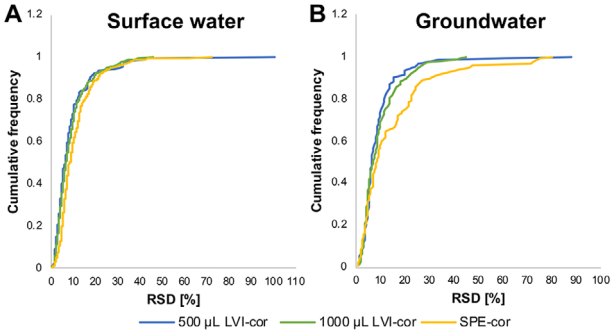

Figure 4. Cumulative frequencies of nontarget compound RSDs from 500 µL LVI, 1000 µL LVI, and SPE-method. (A) RSDs of nontarget compounds calculated from three procedural replicates of surface water. (B) RSDs of nontarget compounds calculated from three procedural replicates of groundwater. The RSDs were calculated from signal intensity drift-corrected values only.

of signal intensity drift for each single measurement in a sequence can be used for correction of this systematic error and increase the precision. In this investigation, the average intensities of the reference masses (m/z = 121.050873 and 922.009798) at the beginning of each measurement were used for the estimation of signal intensity drift in each measurement (Section 2.6.3). According to the results obtained for the reference masses, the average signal loss over time for the 500 µL LVI, 1000 µL LVI, and the SPE method was 1.9%, 2.4%, and 2.8% per hour corresponding to 27%, 36%, and 37% over the total individual sequence times of 14.2, 15.2, and 13.2 h, respectively. The initial sensitivity of the instrument could not be restored during the measurement of a sequence but between the sequences by proper cleaning and conditioning of the interface.

Figure 3A and B show the impact of the new signal intensity drift correction method by plotting the cumulative frequencies of RSD and signal intensity drift of compounds detected in all equidistant replicates of QC samples. The

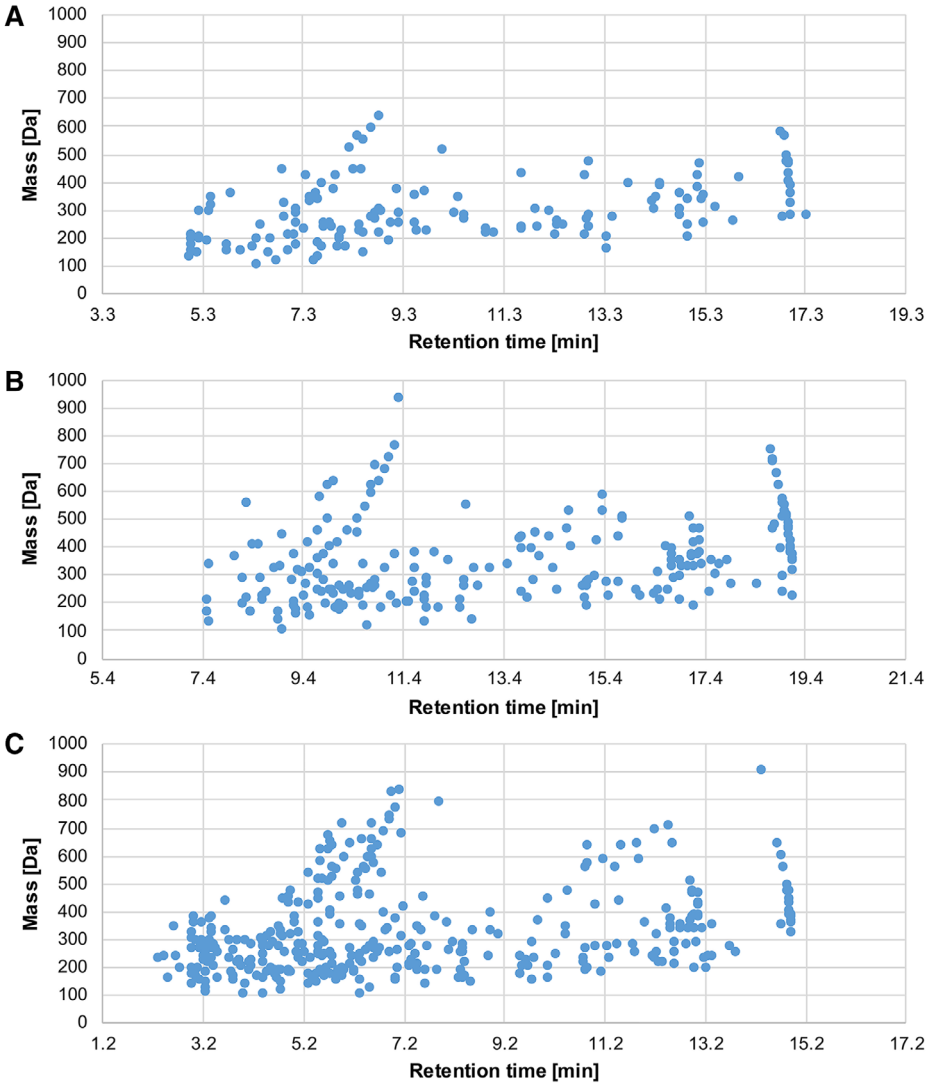

Figure 5. Mass [Da] and retention time [min] plot of compounds detected in surface water with (A) 500 μL LVI, (B) 1000 μL LVI, and (C) SPE-method. The *x*-axis (retention time) was shifted to compensate the dwell time caused by the different injection volumes of each method.

application of 500 μL LVI, 1000 μL LVI, and SPE methods resulted in the detection of 209, 298, and 439 valid compounds in the QC sample, respectively. The significant difference of the RSD and signal intensity drift values between uncorrected intensities of each method indicate a method-dependent effect, which is mainly due to the different amount of sample matrix introduced [38]. The analysis of uncorrected data resulted in the highest signal intensity drift for 1000 μL LVI and this was followed by SPE-method and 500 μL LVI, respectively. After the application of the signal intensity drift correction, the RSD and signal intensity drift could be significantly reduced and the performance differences between the methods caused by the mass spectrometer were eliminated. After the drift correction, more than 70% of the compounds resulted in an RSD less than 10% for each method and the median drift for each method was between 15% and 16%. These results indicate that the application of this new signal intensity drift correction method reduces a large part of the systematic bias caused by the instrumental side.

3.2.2 Analytical precision and compound coverage

The drift-corrected intensities of compounds detected in groundwater and surface water samples were investigated to study the variations caused by sample preparation methods. In summary, 148, 216, and 356 valid compounds in surface water and 60, 77, and 97 valid compounds in groundwater were detected by 500 μL LVI, 1000 μL LVI, and SPE methods, respectively. The SPE method detected substantially more compounds in both water samples without a significant reduction of intrabatch precision (Figure 4A and B). In groundwater, the relatively higher RSDs for the SPE-method may be caused by the poorly water-soluble compounds (e.g., humic substances), which are found in groundwater in higher proportion compared to surface water.

In Figure 5, compounds detected in all procedural replicates of surface water by each analytical method are plotted according to retention time versus mass. The similarity of the plots indicates that the SPE sorbent material (HLB) has a

similar range of coverage as the chromatographic phase in terms of polarity, but accesses more compounds due to the higher enrichment factor (absolute sample volume of 2000 μL in SPE versus 1000 μL and 500 μL in LVI) and efficiency. In addition, it can be seen that the SPE method detects more compounds in the first minutes of the chromatogram. Apparently, this increase is not only caused by the higher enrichment factor, but is also due to the lower injection volume of 5 μL in SPE, which is directly linked to better chromatographic performance in terms of efficiency and compound retention.

4 Concluding remarks

The results of this study indicate that LVI is a valuable alternative to SPE in nontargeted analysis. As LVI does not contain any evaporation and reconstitution steps, it is advantageous regarding measurement uncertainty. However, if highly polar compounds are in the focus of interest, SPE utilizing fit-for-purpose materials is still the method of choice. The integration of the described signal intensity drift correction method into the nontargeted analytical workflow significantly improves the precision and significance of results. This method can also be implemented into systems without secondary nebulizers via addition of a reference compound solution to the column effluent with an external pump and a T-piece.

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The authors have declared no conflict of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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