Talanta 113 (2013) 82–92

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Simultaneous determination of PPCPs, EDCs, and artificial sweeteners in environmental water samples using a single-step SPE coupled with HPLC–MS/MS and isotope dilution

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ARTICLE INFO

Article history:
Received 6 March 2013
Received in revised form
27 March 2013
Accepted 28 March 2013
Available online 6 April 2013

Keywords:
Artificial sweeteners
Endocrine disrupting chemicals
Pharmaceuticals and personal care products
Simultaneous determination
High performance liquid chromatography–tandem mass spectrometry
Solid-phase extraction (SPE)

ABSTRACT

A high-throughput method for the simultaneous determination of 24 pharmaceuticals and personal care products (PPCPs), endocrine disrupting chemicals (EDCs) and artificial sweeteners (ASs) was developed. The method was based on a single-step solid phase extraction (SPE) coupled with high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) and isotope dilution. In this study, a single-step SPE procedure was optimized for simultaneous extraction of all target analytes. Good recoveries (≥70%) were observed for all target analytes when extraction was performed using Chromabond® HR-X (500 mg, 6 mL) cartridges under acidic condition (pH 2). HPLC–MS/MS parameters were optimized for the simultaneous analysis of 24 PPCPs, EDCs and ASs in a single injection. Quantification was performed by using 13 isotopically labeled internal standards (ILIS), which allows correcting efficiently the loss of the analytes during SPE procedure, matrix effects during HPLC–MS/MS and fluctuation in MS/MS signal intensity due to instrument. Method quantification limit (MQL) for most of the target analytes was below 10 ng/L in all water samples. The method was successfully applied for the simultaneous determination of PPCPs, EDCs and ASs in raw wastewater, surface water and groundwater samples collected in a local catchment area in Singapore. In conclusion, the developed method provided a valuable tool for investigating the occurrence, behavior, transport, and the fate of PPCPs, EDCs and ASs in the aquatic environment.

1. Introduction

Recently, pharmaceuticals and personal care products (PPCPs), endocrine disrupting chemicals (EDCs) and artificial sweeteners (ASs) have increasingly gained attention due to their ubiquitous occurrence in the water environment and their potential to cause undesirable ecological effects [1–4]. The main source of PPCPs, EDCs and ASs into the environment is raw wastewater or insufficiently treated wastewater effluents [5,6]. Other important sources include landfill leachate and leaking storage tanks. The widespread occurrence of PPCPs, EDCs and ASs in the aquatic environment such as rivers, lakes and groundwater has been reported over the past decade [4,7–9]. The presence of PPCPs and EDCs in the water environment has been reported to potentially affect aquatic organisms and produce changes that threaten the sustainability of aquatic ecosystem [10–13]. Recently, risk assessment of artificial sweeteners (e.g. sucralose) in the water environment to the aquatic organisms has also gained concerns [14,15]. Although the toxicity of PPCPs, EDCs and ASs to human health is relatively unknown at trace levels, continuous discharge and chronic exposure to these compounds may pose a risk to human health associated with exposure to very low concentrations of pharmaceuticals in drinking-water over a lifetime [16,17]. Due to the frequent detection and persistence of PPCPs, EDCs and ASs in the aquatic environment, many of these compounds have been proposed as chemical makers of wastewater contamination in surface water and groundwater [3,8,9,18–20]. However, no reports on the occurrence of artificial sweeteners in Asian countries are available in the open literature. Hence, in order to better evaluate the occurrence, fate, and environmental risk of these compounds in the environment to the aquatic ecosystems, development of a robust sensitive analytical method for simultaneous determination and extraction of these chemicals at trace levels in various environmental matrices is critically required.

Currently, several advanced analytical methods, such as gas chromatography–mass spectrometry (GC–MS) [21,22], GC–MS/MS [23] liquid chromatography–mass spectrometry (LC–MS) [24] or LC–MS/MS [25–30] have been reported. However, most of them have some drawbacks, such as a lack of selectivity and sensitivity. Most of the developed methods just focused on determining a

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http://dx.doi.org/10.1016/j.talanta.2013.03.072

specific class of compounds [4,23,29,30] with limited method validation data reported. In environmental analysis of trace pollutants, it is challenging to extract the target analytes present in sample at very low concentrations from complex environmental matrices. At present, solid phase extraction (SPE) has been widely used for enriching and purifying a wide range of organic emerging contaminants from the environmental samples [31]. However, it is obvious that besides enriching target analytes, SPE may either enrich some interference or eliminate matrix constitutes, which may influence LC separation and tandem MS detection. Thus, SPE extraction and HPLC–MS/MS operating conditions affect directly the sensitivity and accuracy of analytical method.

To improve recoveries of analytes in environmental samples, one of the options is to use different SPE sorbents and perform extraction under various conditions for different classes of target analytes [4,32]. It is obvious that choosing a proper SPE cartridge plays a key role in enhancing recovery of analytes in the environmental water samples. Normally, the selection is frequently based on the physicochemical properties of target analytes and SPE sorbent characteristics. For example, Kasprzyk-Hordern et al. [32] found that Oasis MCX—a strong cation-exchange mixed-mode polymeric sorbent was the best out of the 8 tested SPE sorbent types for extracting basic/neutral pharmaceuticals and illicit drugs in surface water samples. In another study, Scheurer et al. [4] reported that styrene—divinylbenzene (Bakerbond SDB 1) provided notably high recoveries ($>75\%$) for the artificial sweeteners compared to other SPE cartridges such as Isolute ENV+, C18, Varian Bond Elut PPL, Strata X, Strata X-AW, Oasis HLB, WAX, MAX, and HCX. Although using different SPE cartridges used for enrichment of different classes of analytes may provide a relatively high extraction recovery for analytes compared to a single-step SPE procedure [31], this approach is time-consuming once analyzing with a huge number of target analytes with different physicochemical properties (such as $\log K_{ow}$ and pK_a) and is quite expensive due to SPE cartridge consumption. Most of the published methods did not use isotopically labeled internal standards (ILIS) to correct the matrix effect in analysis of real environmental water samples [4,23,24,33]. This is also one of the drawbacks in applying those methods to quantify the target analytes in different environmental matrices. Because of these reasons, the development of a high-throughput method for the determination of multiple target analytes with different physicochemical properties under different matrices is of importance.

The first objective of this study was to develop a fast and reliable method for the detection and quantification of 24 PPCPs, EDCs and ASs in water samples by using a single-step SPE coupled with HPLC–MS/MS and isotope dilution. The second objective was to investigate stability of the target analytes during sample storage and determine an appropriate preservation procedure. Finally, this study aimed to test the applicability of the developed method for the monitoring of PPCPs, EDCs and ASs in wastewater, surface water and groundwater samples.

2. Experimental

2.1. Chemicals and materials

The target analytes studied were 24 PPCPs, EDCs and ASs with different chemical structures and physicochemical properties (i.e. acidic, neutral, hydrophilic, and hydrophobic properties). These compounds were acetaminophen [ACT], caffeine [CF], carbamazepine [CBZ], ibuprofen [IBP], ketoprofen [KEP], fenoprofen [FEP], naproxen [NPX], propyphenazone [PPZ], clofibric acid [CA], gemfibrozil [GFZ], diclofenac [DCF], indomethacin [IDM], salicylic acid [SA], crotamiton [CTMT], trimethroprim [TMP], and diatrizoic acid [DTZ], diethyltoluamide [DEET], bisphenol A [BPA], acesulfame [ACE], aspartame [ASP], cyclamate [CYC], saccharin [SAC], sucralose [SUC], and neohesperidin dihydrochalcone [NHDC]. All these PCCPs, EDCs and ASs were purchased from Sigma Aldrich (Sigma Aldrich, Singapore). Their physicochemical properties are summarized in Table A1 (Supplementary Information). Thirteen ^{2}H-isotope labeled compounds were used as internal/surrogate standards to correct the loss during SPE extraction procedures and ESI–MS/MS. These isotopically labeled internal standards (ILIS) included acesulfame-d_4 [ACE-d_4], acetaminophen-d_4 [ACT-d_4], aspartame-d_5 [ASP-d_5], bisphenol A-d_{16} [BPA-d_{16}], carbamazepine-d_8 [CBZ-d_8], caffeine-d_9 [CF-d_9], cyclamic acid-d_{11} [CYC-d_{11}], diclofenac-d_4 [DCF-d_4], diethyltoluamide-d_{10} [DEET-d_{10}], ketoprofen-d_3 [KEP-d_3], salicyclic acid-d_4 [SA-d_4], saccharin-d_4 [SAC-d_4] and sucralose-d_6 [SUC-d_6], which were purchased from Toronto Research Chemicals Ins. (Toronto, Canada). The solvents, HPLC grade methanol and acetonitrile, were provided by Merk (Darmstadt, Germany). The individual stock solutions of each target compounds as well as the ILIS solution were prepared in methanol–water (50/50, v/v) at 2.0 g/L and 0.05 g/L, respectively. The solutions were stored in the dark condition at −18 °C. A mixture of all PPCPs, EDCs and ASs was prepared by diluting individual stock solutions with methanol and it was renewed before each analytical run. A separate mixture of ILIS used for internal standard quantification was prepared in methanol–water (75/25, v/v) and further diluted in MeOH/H_2O (75/25, v/v) solution.

2.2. Sample collection and pretreatment

Grab water samples (including raw wastewater, surface water and groundwater) were collected from a local water catchment area in Singapore and were used for development and validation of the method. All water samples were filled in 1 L amber glass bottles immediately carried to the laboratory in ice-packed containers. Once in the laboratory, the samples were filtered using 1.2 μm glass fiber filters (GF/C, Whatman, UK), followed by 0.45 μm membrane filters (PALL corporation, US), subsequently to separate dissolved and particulate phases and stored at 4 °C until SPE extraction, which was performed within 24 h in order to avoid any degradation.

2.3. Solid-phase extraction

Due to the wide range of the target analytes with different chemical characteristics, it is challenging to extract all the target analytes in a single-step SPE procedure with good method performance. Therefore, optimization of a single-step SPE procedure is critically required. In this study, the following five cartridges, Chromabond® HR-X (500 mg, 6 mL), Chromabond® HR-XAW (500 mg, 6 mL), an Bond Elut Plexa (200 mg, 6 mL), and Oasis HLB Plus (225 mg), and SampliQ C18 (500 mg, 6 mL), were used in screening for enrichment purpose. The characteristics of these SPE cartridges are presented in Table A2 (Supplementary Information). The experiment was performed to evaluate extraction efficiency by spiking 200 ng/L of analytes into 500 mL Milli-Q water. The effect of sample pH on recovery of target analytes in Milli-Q water samples was investigated at various pH values ranging from 2 to 10.

For real water samples, prior to SPE extraction, water samples were spiked with the 13 ILIS (including ACE-d_4, ACT-d_4, ASP-d_5, BPA-d_{16}, CBZ-d_8, CF-d_9, CYC-d_{11}, DCF-d_4, DEET-d_{10}, KEP-d_3, SA-d_4, SAC-d_4 and SUC-d_6). The SPE cartridges were preconditioned with 6 mL methanol, followed by 6 mL of Milli-Q water at a flow rate of 3 mL/min. After the conditioning step, 250 mL of wastewater samples or 500 mL of groundwater/surface water samples were

passed through the wet cartridge at a flow rate of 10 mL/min, after which the cartridges were rinsed with 6 mL of ultrapure Milli-Q water. On the completion of extraction, the SPE cartridges were dried for 30 min under vacuum. Next, the SPE cartridges were eluted by 2×5 mL of methanol at a flow rate of 1 mL/min. The resulting extracts were dried under a gentle stream of nitrogen at 40 °C. The dried extracts finally were dissolved again in a MeOH/H_2O (75/25, v/v) solution to a final volume of 1.0 mL.

2.4. HPLC–ESI–MS/MS analysis

The measurement of the target analytes was performed using a high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (Shimadzu, Japan). Agilent ZORBAX SB-C18 (150×2.1 mm i.d; 3.5 μm particle size) column was used for separation and quantification of the target analytes. The tandem MS analyses were performed on a triple quadrupole mass spectrometer equipped with a Z-spray electrospray interface (LCMS-8030, Shimadzu, Japan). Ions were acquired in multiple reaction monitoring (MRM) modes with a dwell time of 7.0 ms. Collision induced dissociation (CID) was performed using argon at approximately 230 kPa. The electrospray source block and desolvation temperature were set at 300 and 250 °C, respectively. Drying and nebulizing gas flow rates were set at 15 and 3 L/min, respectively. Interface voltage and interface current were set at 3.5 kV and 0.2 μA, respectively. After choosing the precursor ions, product ions were obtained and optimized with three key parameters: entrance potential (EP), collision energy (CE), and collision exit potential (CXP).

A combined method was used for the identification and quantification of the 24 target analytes in environmental samples by a single injection. All the 24 target analytes and the ILIS were separated using gradient method and the following mobile phase composition optimized: 2 mM NH_4Ac in ultrapure Milli-Q water (mobile phase A) and 2 mM NH_4Ac in HPLC methanol (mobile phase B). The gradient program started with 30% mobile phase B and kept isocratic for 1.0 min, and then rose to 95% mobile phase B at 6.0 min and held until 12 min. At the end of chromatographic run, the column was re-equilibrated to the initial conditions in 1 min and stabilized for 5 min. The total mobile phase flow rate was 0.25 mL/min. This flow rate was selected for optimum separation during method development and kept constant thereafter. The column temperature was kept at 40 °C and autosampler at 15 °C. An injection volume of 5.0 μL was used for all analyses. The MS analyses were performed simultaneously in the positive electronspray ionization ESI (+) and negative electronspray ionization ESI (−) modes. Optimized ESI–MS/MS parameters for the detection of the target analytes and the ILIS by MRM are presented in Tables A3 and A4 (Supplementary Information).

2.5. Quantification and method validation

Two MRM transitions between the precursor ion and two most abundant fragment ions were monitored for each analyte. The highest characteristic precursor ion/product ion MRM transition was used for quantification purpose, while the second one was selected to confirm the presence of target analytes in the samples. However, the following analytes ACT, ACT-d_4, ASP-d_5, CYC, CYC-d_{11}, GFZ, KEP, KEP-d_3, IBP, SA, and SA-d_4, exhibited only one MRM transition due to their poor fragmentation. Besides the monitoring of the MRM transitions, comparing the LC retention time of analytes with the corresponding reference/internal standards was used as a criterion to identify the presence of analyte in environmental samples. Calibration curves were established by injecting pooled solutions prepared from the standard mixtures. The linearity of the method was estimated by fitting a linear mode, least-squares regression analysis ($y = ax + b$) in the concentration range studied.

Recovery tests were carried out by spiking the analytes at appropriate concentrations in various water samples before extraction and in 1 mL of the extract, after extraction. For instance, the spiking level for control water, groundwater and surface water samples was 1.0 μg/L, while the spiking level for raw wastewater was 5.0 μg/L. The spiked samples were added with the ILIS as the surrogate standard and mixed before being extracted and analyzed in the same way with the correspondingly non-spiked samples. The recovery was calculated by comparing the analyte concentration of samples spiked prior to and after extraction using the following equation as proposed by Laven et al. [34]:

$$\%\ \text{Recovery} = \frac{C_{\text{Pre-Extr}} - C_{\text{Non-Spiked}}}{C_{\text{Post-Extr}} - C_{\text{Non-Spiked}}} \times 100 \quad (1)$$

where $C_{\text{Pre-Extr}}$ and $C_{\text{Non-Spiked}}$ are measured concentration in the spiked and corresponding non-spiked samples, respectively. $C_{\text{Post-Extr}}$ is the measured concentration of sample spiked after extraction in the reconstitution step. The recovery calculated represents the loss arising from SPE extraction, excluding any losses by matrix effects in HPLC–ESI–MS/MS or other instrumental variations. To compensate for the losses of target analytes during SPE extraction and matrix effects during HPLC–ESI–MS/MS, the ILIS were added to the water samples prior to the whole analytical procedure in this study.

To investigate the matrix effect (ME) on signal intensity of the analytes during ionization in the ESI source as well as the efficiency of the ILIS in correction of the ME, a known amount of the analytes (100 ng/mL) and the ILIS (100 ng/mL) was added to sample extracts. The ME was calculated using the following equation as the percentage of analyte signal suppression or enhancement.

$$\%\ \text{ME} = \left[1 - \frac{(A_S - A_N)}{A_{\text{Sp-Sol}}}\right] \times 100 \quad (2)$$

where A_S is the peak area of the analyte in the sample extracts spiked with analyte standard and ILIS mixtures (100 ng/mL). A_N is the peak area of the analyte in the corresponding sample extracts without spiking with standard mixtures, $A_{\text{Sp-Sol}}$ is the peak area of the analyte in the spiking solution (100 ng/mL) dissolved in MeOH/H_2O (75/25, v/v). The signal of the analyte is enhanced if $ME < 0$, whereas the signal of analyte is suppressed if $ME > 0$.

Instrumental detection limits (IDL) and instrumental quantification limits (IQL) for each analyte were determined as the minimum detectable amount of analyte giving a signal to noise ratio (S/N) of 3 and 10, respectively. These were determined by directed injection of decreasing amounts of the standard mixture.

The method detection limit (MDL) and method quantification limit (MQL) were defined and determined as the lowest observable concentration of analyte from water samples spiked extract in multiple reaction monitoring (MRM) mode giving a signal-to-noise (S/N) of 3 and 10, respectively. For the analytes existed initially, MQL and MDL were evaluated by determining S/N of the lowest measured concentrations and extrapolating to S/N values of 10 and 3.

2.6. Blank analysis

To check whether there was any instrumental and sample contamination that could interfere with the method detection and quantification, blank control samples were examined. For instance, to determine whether there was existing contamination of unlabeled target analytes in the isotopically labeled internal standards, Milli-Q water blanks were spiked only with ILIS and used to check for any possible background concentration of target

analytes. It would be noted that none of the target analytes were detected in these spiked blanks, suggesting that the ILIS used were appropriate for this study. To check any possible cross contamination during sample preparation, several Milli-Q water blanks were extracted and analyzed. Similarly, these blanks resulted in concentrations below respective MDLs. In addition, a solvent blank (MeOH) was injected at regular interval of every ten injection. As expected, no carryover of the target analytes as well as the respective ILIS was observed.

2.7. Stability tests

Stability tests were performed with both filtered (0.45 μm) and unfiltered environmental water samples (wastewater, surface water and groundwater) in 250 mL amber glass bottles containing 200 mL of samples and spiked with the target analytes at concentration of 50 μg/L. At the same time, Milli-Q water sample spiked at the same concentration of each analyte was used as a control test. The closed bottles were stored in the dark at 4 °C up to 3 weeks. Samples (1.5 mL) were taken at variable intervals, filtered through 0.45 μm membrane filters, and stored frozen until analyzed by HPLC–MS/MS without SPE extraction.

2.8. Statistical analysis

A one-way analysis of variance (ANOVA Tukey's post hoc) test was used to examine the statistically significant difference between the mean values of control and experiment groups. While unpaired *T*-test was used to evaluate whether the mean concentrations of the PPCPs, EDCs and ASs between wastewater, surface water and groundwater are statistically different. A significance level of 0.05 is used for all statistical tests in this study.

3. Results and discussion

3.1. Optimization of HPLC–ESI–MS/MS analysis

It has been found that organic mobile phases and the mobile phase additives can play a crucial role in chromatographic separation and detection sensitivity of the HPLC–ESI–MS/MS method [32,33]. Batt et al. [35] found that the presence of an additive might either enhance or suppress the signal intensity of the analyte in ionization process, which seemed to depend on its concentration. To address this, in preliminary experiments we tested the effects of the different organic mobile phases and the addition of additives on the separation efficiency and detection sensitivity. Firstly, to evaluate the role of organic mobile phases in chromatographic separation and detection sensitivity of the analytes, three types of organic phases were examined, including methanol (MeOH), acetonitrile (ACN), and a mixture of MeOH–ACN (50/50, v/v). As it can be seen in Fig. 1A (Supplementary Information), MeOH showed notably high signal intensity for all the analytes compared to ACN or ACN–MeOH (50/50, v/v) solution. Consequently, MeOH was chosen as an organic phase for the further experiments. Next, to investigate the effects of mobile phase additives in the detection sensitivity and chromatographic separation of analytes, ammonium acetate (NH_4Ac) and formic acid (FA) were added into both water phase (Milli-Q water) and organic phase (MeOH) with different concentrations. As expected, the addition of NH_4Ac or FA into mobile phases resulted in good peak shapes of the analytes and ILIS. The presence of FA in mobile phases suppressed signal intensity of most analytes, whereas the addition of NH_4Ac in mobile phases to its final concentration of 2 mM enhanced signal intensity of many analytes and particularly improved chromatographic separation efficiency and peak shapes of all the analytes. Therefore, the optimal chromatographic separation of the 24 target PPCPs and ASs and the 13 corresponding ILIS detected simultaneously in positive ionization (PI) mode and negative ionization mode (NI) was achieved using 2 mM NH_4Ac in Milli-Q water (mobile phase A) and 2 mM NH_4Ac in methanol (mobile phase B). Additionally, to improve the chromatographic separation, three HPLC columns were tested, including Waters: XBridge™ C18 (100 × 2.1 mm i.d; 3.5 μm particle size), Agilent ZORBAX SB-C18 (150 × 2.1 mm i.d; 3.5 μm particle size), and ZORBAX RRHD Eclipse Plus C18 (100 × 2.1 mm i.d; 1.8 μm particle size). Out of the three columns tested, Agilent ZORBAX SB-C18 column provided the best peak shape of the analytes and the ILIS in both NI and PI modes. Representative chromatograms of a standard mixture of the analytes and their ILIS analyzed in NI and PI modes are presented in Fig. 2A (Supplementary Information). Overall, it is not required to get a complete separation of the analytes for the selective MS/MS detection. However, an efficient chromatographic separation may improve detection sensitivity and reduce matrix effects. The overall HPLC–MS/MS method was found to be high throughput and high sensitivity for the simultaneous quantification of 24 target analytes in a single injection with total run time of 18 min.

3.2. Instrumental performance

The IDL and IQL of the target analytes are presented in Table 1. The IDL and IQL values of the analytes ranged from 0.005 to 2.5 ng/mL and from 0.01 to 5.0 ng/mL, respectively. To minimize the

Table 1
Instrumental performance and validation data.

Analytes	IDL (ng/mL)	IQL (ng/mL)	Linearity	
			R^2	Range (ng/mL)
ACE[a]	0.005	0.01	0.9998	0.1–100
ACT[b]	0.25	0.2	0.9981	2.5–250
ASP[c]	0.10	0.25	0.9992	10–1000
BPA[d]	0.4	2.0	0.9975	5–5000
CA[l]	0.03	0.2	0.9998	1–1000
CBZ[e]	0.005	0.01	0.9998	0.1–500
CF[f]	0.4	2.0	0.9992	10–5000
CTMT[e]	0.02	0.03	0.9984	0.5–1000
CYC[g]	0.05	0.1	0.9998	5–1000
DCF[h]	0.06	0.2	0.9997	2.5–500
DEET[i]	0.01	0.02	0.9998	0.2–500
DTZ[e]	0.01	0.1	0.9966	0.5–500
FEP[k]	0.4	0.3	0.9993	5–1000
GFZ[h]	0.08	0.2	0.9993	1–500
IBP[h]	0.3	1.0	0.9983	10–1000
IDM[h]	0.06	0.25	0.9985	1–500
KEP[k]	0.6	1.0	0.9998	5–1000
NHDC[n]	0.13	0.25	0.9995	3–1000
NPX[k]	0.2	0.6	0.9992	5–1000
PPZ[e]	0.02	0.02	0.9995	0.5–500
SA[l]	0.2	0.5	0.9984	2.5–1000
SAC[m]	0.5	0.025	0.9954	0.5–100
SUC[n]	2.5	5.0	0.9954	25–1000
TMP[e]	0.02	0.01	0.9960	0.1–500

The corresponding ILIS:

[a] ACE-d_4.
[b] ACT-d_4.
[c] ASP-d_5.
[d] BPA-d_{16}.
[e] CBZ-d_8.
[f] CF-d_9.
[g] CYC-d_{11}.
[h] DCF-d_4.
[i] DEET-d_{10}.
[k] KEP-d_3.
[l] SA-d_4.
[m] SAC-d_4.
[n] SUC-d_6.

impact of analyte losses and instrumental fluctuation on the quantification of an analyte, an isotopically labeled internal standard (ILIS) was added in equal amount to both known concentration (calibration standards and quality control) and unknown sample prior to sample treatment. It was noted that the ideal internal standard for an analyte should be its ^{2}H-isotope or ^{13}C-isotope labeled compound [36,37]. In this study, for the analytes without their isotopically labeled analogs, the selection of internal standard for an analyte was based on the following criteria: (i) the internal standard should be an isotope compound, which shares the same or very similar physicochemical properties/chemical structure as the analyte; (ii) it should have a chromatographic retention time close to that of the analyte and should mimic the analyte in sample treatment steps; and (iii) the internal standard should have a similar extraction recovery, ionization response in ESI–MS/MS to the analyte. The corresponding ILIS for the analytes are shown in Table 1. After choosing the appropriate ILIS for each analyte, calibration curves were constructed by plotting the concentrations of each analyte versus the ratios between analyte peak area and the corresponding ILIS peak area using linear regression analysis. A ten-point calibration curve in the range of 0.1–1000 ng/mL or 1.0–5000 ng/mL was generated with satisfactory correlation coefficient ($r^2 > 0.995$, Table 1).

3.3. Optimization of SPE procedure

Due to the wide spectrum of analytes with different physicochemical characteristics (such as their pK_a values varying 0.9–14 and the polarity ranging from log $K_{ow} < 0$ to log K_{ow} of 4.9) the extraction of these analytes by using a single-step SPE procedure can be really challenging. The choice of the best SPE sorbent that gives an acceptable recovery for all the analytes plays a crucial role in method development. Five different SPE cartridges, including HR-X, HLB Plus, HR-WAX, SampliQ C18, and Elut Plexa, were investigated in this work. The experiment was conducted using 500 mL Milli-Q water spiked at 1 μg/L level of each analyte. Experimental results showed that reserved-phase polymeric sorbent-based cartridges such as HLB Plus and HR-X exhibited the same behavior for most analytes (Fig. 1). However, HR-X cartridge was found to be the most effective for most of the studied analytes (> 75%), whereas it was found that recoveries for most of the analytes were very low in the case of SampliQ C18 cartridge (< 50%). The mixed-mode sorbent cartridges such as Plexa and HR-XAW also showed low recoveries for several compounds such as ACE, ACT, ASP, CYC, DTZ and TMP. The present study results are consistent with those reported in the previous literature [38], in which it was demonstrated that Plexa, Plexa PAX, Oasis WAX and Oasis MAX cartridges had low recoveries for the artificial sweeteners such as ACE, ASP, and CYC.

To investigate the effect of sample pH on the recovery of analytes, a series of Milli-Q water samples spiked with 200 ng/L of the target analytes were extracted under different sample pH values ranging from 2 to 10. From Fig. 1, it was seen that different pH conditions could affect either the overestimation or underestimation of recoveries for different target analytes. For instance, the recoveries of DEET and CBZ were over 110% at pH 2, while NPX and IBP were over 110% at pH 10. It was clear that several analytes had very low recoveries at both basic and neutral pH values (ACE, ACT, CYC, ASP, SUC and SAC). The low recoveries of some analytes at neutral or basic pH values were not clear and might be attributed to poor cartridge retention. A possible explanation could be due to the pH-induced molecular conformation changes, which would increase or decrease the formation of ions in the ESI source. Hence, further experiments for real environmental water samples should be extracted by using HR-X catridges under sample pH of 2.

For real environmental water samples, absolute recoveries were higher than 70% for most of the analytes when SPE procedure was performed by using HR-X cartridges under pH of 2. The average absolute and relative (relative to the recovery of surrogate/internal standard) recoveries and relative standard deviations for the target analytes in various water samples are shown in Table 2. Recovery varied among analytes and environmental matrices. For example, absolute recoveries of target analytes ranged from 76.3 to 113% in Milli-Q water samples, from 66.4

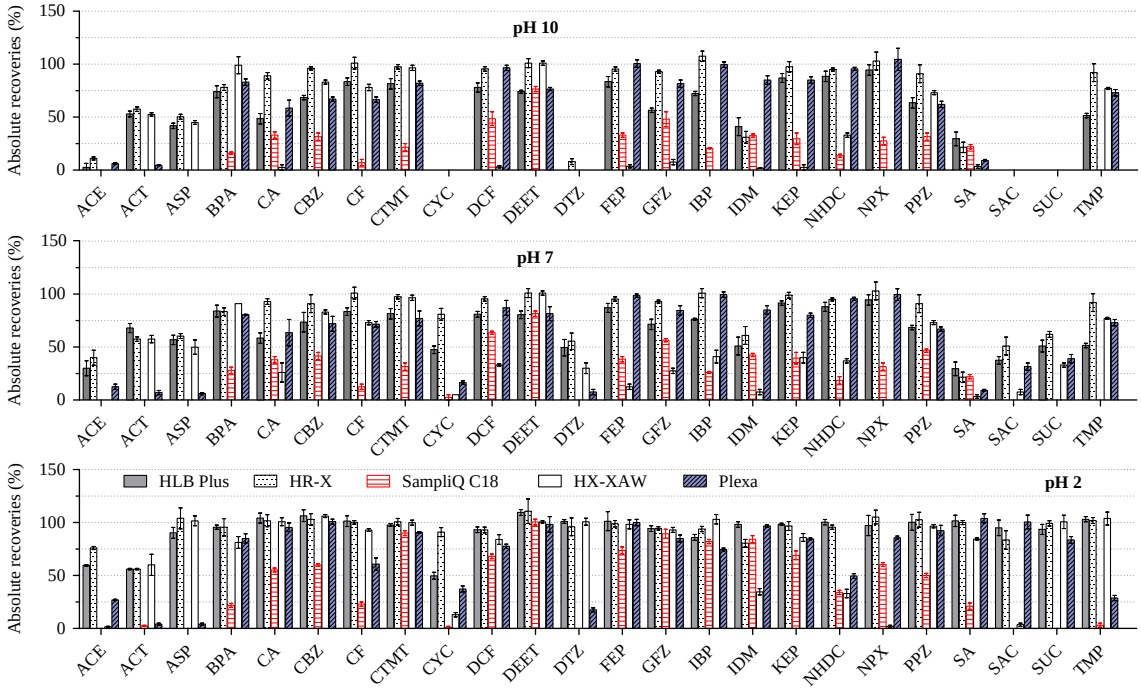

Fig. 1. Effects of pH and SPE sorbents on percentage recovery of analytes in Milli-Q water sample spiked with target analytes at 1 μg/L.

Table 2
Absolute and relative recoveries of the target analytes in environmental water samples using HR-X cartridge under extraction pH of 2.

Analytes	Absolute recoveries (RSD) (%)				Relative recoveries (RSD) (%)*			
	Milli-Q water	Ground water	Surface water	Raw wastewater	Milli-Q water	Ground water	Surface water	Raw wastewater
ACE[a]	76.3 (7.2)	68.7 (4.5)	66.4 (7.4)	62.5 (4.3)	101 (5.0)	103 (7.8)	99.4 (3.2)	99.3 (2.6)
ACT[b]	85.7 (4.9)	82.1 (3.8)	83.1 (5.3)	66.5 (6.1)	99.6 (4.5)	98.5 (4.7)	99.6 (2.8)	97.4 (3.6)
ASP[c]	104 (5.8)	96.4 (4.8)	98.1 (3.5)	101.5 (8.9)	102 (6.5)	100 (2.8)	100 (3.3)	102 (4.0)
BPA[d]	96 (6.3)	89.1 (6.1)	92.4 (8.2)	92.7 (13.1)	101 (8.7)	97.4 (1.9)	105 (3.1)	101.9 (3.3)
CA[l]	104 (6.3)	95.7 (1.2)	102 (3.5)	97.6 (1.2)	105 (8.7)	99.7 (2.9)	99.6 (5.1)	105.8 (1.9)
CBZ[e]	103 (7.0)	97.7 (1.4)	97.8 (2.4)	96.4 (4.5)	101 (4.5)	100 (2.3)	101 (1.3)	101.4 (4.4)
CF[f]	102 (7.0)	88.1 (7.4)	92.1 (5.2)	76.8 (9.0)	101 (6.3)	96.3 (2.5)	101 (4.4)	104.7 (6.1)
CTMT[e]	98.7 (6.1)	94.3 (2.9)	94.7 (2.3)	87.3 (7.0)	101 (8.1)	96.8 (1.5)	97.1 (1.7)	109 (6.7)
CYC[g]	91 (6.6)	81 (2.4)	83.6 (5.3)	76.3 (5.7)	103 (4.6)	98.9 (3.7)	101 (1.9)	100.4 (5.7)
DCF[h]	88.7 (3.6)	75.7 (3.5)	73.3 (6.0)	67 (4.0)	99 (2.2)	99.8 (5.3)	98 (2.6)	99.8 (2.2)
DEET[i]	113 (6.4)	102 (3.9)	101 (4.2)	93.8 (6.6)	102 (6.1)	102 (3.7)	99.4 (2.3)	104 (5.0)
DTZ[e]	101 (3.6)	98.5 (9.5)	98.2 (4.4)	99.2 (8.9)	99.4 (2.0)	101 (10)	101 (6.0)	104.5 (7.2)
FEP[k]	90.3 (3.4)	77.3 (1.0)	78.3 (4.2)	66.6 (8.1)	99.8 (3.4)	102 (3.3)	103 (2.9)	97.1 (8.5)
GFZ[h]	95 (2.8)	77.9 (5.2)	79.6 (5.4)	63.3 (2.7)	106 (8.3)	103 (1.1)	105 (9.1)	94.5 (7.9)
IBP[h]	94 (3.2)	78.7 (8.2)	80 (6.0)	73 (5.5)	105 (5.9)	104 (4.3)	105 (2.2)	106.7 (9.9)
IDM[h]	91.3 (4.9)	78 (3.4)	78.9 (5.1)	71.3 (7.7)	102 (6.2)	103 (6.0)	104 (6.3)	106.4 (7.7)
KEP[k]	94.3 (4.8)	76.2 (1.7)	76.5 (3.7)	68.2 (8.2)	104 (1.9)	101 (3.4)	101 (3.0)	99.2 (5.1)
NHDC[n]	98 (6.7)	94.4 (4.2)	93.4 (5.5)	92 (6.9)	112 (12)	108 (3.9)	103 (8.6)	105 (11.7)
NPX[k]	105 (5.8)	73.6 (3.4)	74.3 (3.5)	66.6 (3.8)	116 (12)	96.6 (11)	98 (5.1)	97.1 (5.2)
PPZ[e]	103 (5.1)	98.9 (3.1)	96.6 (2.1)	92.6 (4.4)	102 (7.9)	101 (1.0)	99 (1.4)	97.5 (2.1)
SA[l]	100 (4.0)	95.7 (3.6)	95.4 (2.6)	90.7 (3.8)	98.7 (4.8)	98.7 (3.5)	98.4 (3.1)	98.4 (4.5)
SAC[m]	86 (4.5)	81.3 (8.5)	81 (5.8)	78.3 (12.2)	102 (3.5)	101 (5.5)	100 (5.1)	99.8 (6.5)
SUC[n]	100 (3.0)	91.5 (3.3)	89.8 (4.1)	87.5 (5.8)	104 (4.9)	101 (1.7)	98.5 (1.0)	98.7 (1.7)
TMP[e]	101 (8.1)	99.7 (4.6)	101 (6.4)	102 (6.8)	99.7 (12)	102 (7.9)	105 (7.8)	107.5 (5.3)

Data in bracket indicate the relative standard deviation (RSD) of triplicate samples.

* Relative to the recovery of the corresponding ILIS.

[a] ACE-d_4.
[b] ACT-d_4.
[c] ASP-d_5.
[d] BPA-d_{16}.
[e] CBZ-d_8.
[f] CF-d_9.
[g] CYC-d_{11}.
[h] DCF-d_4.
[i] DEET-d_{10}.
[k] KEP-d_3.
[l] SA-d_4.
[m] SAC-d_4.
[n] SUC-d_6.

to 102% in surface water and from 62.5 to 102% in raw wastewater. In general, lower absolute recoveries of most of the analytes were observed in the environmental water samples compared to those in Milli-Q water samples. The decrease of recoveries might result from both reduction of sorption efficiency of SPE cartridges and also signal suppression in ESI due to the presence of matrix components [33,39]. In this study, 13 ILIS were added to the water samples prior to the whole analytical procedure in order to compensate for losses of analytes during SPE procedure and correct the matrix effects on signal in ESI source. As a result, after correction by the ILIS, relative recoveries of most target analytes were enhanced considerably. For instance, relative recoveries ranged from 94.5% to 109% for target analytes in raw wastewater samples. Additionally, the precision of SPE procedure was assessed by calculating the relative standard deviation (RSD, %) of triplicates. RSD was below 10% for most analytes in various matrices. Exceptions to this behavior were BPA and SAC in raw wastewater samples. Taken together, the results indicated a good precision of SPE procedure.

3.4. Matrix effect on method performance

Due to the complexity of environmental sample matrix, HPLC–MS/MS analysis may be subject to signal suppression or enhancement due to the presence of co-eluting matrix components in the sample that affects analyte ionization. Therefore, the evaluation of matrix effects is absolutely important to provide accurate and reproducible quantitative data. In this study, the matrix effect was evaluated by comparing the signal intensity of the analytes spiked in the sample extract and the response of the standard in solvent at the same concentration (50 ng/mL). Table 3 shows the matrix effect on signal suppression or enhancement of the analytes in various environmental matrices. As expected, matrix effects in raw wastewater were higher than those in surface water and groundwater samples for most of the analytes. While, some of the analytes, such as ACE, ACT, ASP, CF and FEP, showed relatively high signal suppression in both groundwater and surface water compared to those in raw wastewater. This may be related to the characteristics of matrix components, which can enhance or reduce the signal intensity of some analytes in ESI–MS/MS. Slight signal suppression was observed for DCF, NPX and KEP in any of the samples tested.

It has been known that matrix effects are one of the main factors that cause the decrease of method sensitivity and accuracy and if not characterized well may lead to incorrect quantification of analytes. As a result, matrix effect correction is critically needed for the wide majority of PPCPs and ASs to obtain acceptable results. In this study, the use of 13 ILIS for the correction for matrix effects was evaluated. Thirteen analytes (including ACE, ACT, ASP, BPA, CBZ, CF, CYC, DEET, DCF, KEP, SA, SAC, and SUC) were corrected highly satisfactory in all environmental matrices since their ILIS were available. For instance, ACE suffered 57.3% absolute

Table 3
Effect of matrix components on the analytical method performance.

Analytes	Absolute ME (%)				Relative ME (%)*			
	Milli-Q water	Ground water	Surface water	Raw wastewater	Milli-Q water	Ground water	Surface water	Raw wastewater
ACE[a]	1.9	65.1	57.3	27.1	0.9	−0.8	1.7	3.1
ACT[b]	8.5	84.6	82.6	61.2	−0.6	−4.3	4.0	−3.1
ASP[c]	−1.5	84.1	9.7	4.0	−2.9	0.7	−2.4	0.9
BPA[d]	8.4	22.4	9.3	13.6	1.7	0.0	−0.9	−3.6
CA[l]	−2.4	−23.4	−1.5	6.3	2.2	−35	1.1	−3.0
CBZ[e]	4.8	13.6	57.1	58.1	2.4	0.4	−1.1	−2.6
CF[f]	7.0	72.2	83.5	60.2	9.0	−0.8	5.1	2.7
CTMT[e]	7.1	−7.5	22.7	54.8	−3.5	8.7	9.5	−8.5
CYC[g]	3.3	46.1	36.1	61.9	1.3	−3.5	2.5	−0.7
DCF[h]	2.0	−10.5	0.9	5.1	−2.0	−12.1	−1.1	−1.9
DEET[i]	5.1	12.2	41.0	40.4	0.9	−0.2	1.8	1.1
DTZ[e]	−46	24.1	22.0	37.1	−20.0	15.1	11.2	23.0
FEP[k]	0.7	18.6	37.8	9.7	−10.0	10.5	10.1	0.67
GFZ[h]	9.5	−1.8	0.9	19.4	8.5	−12.1	−9.0	11.4
IBP[h]	4.8	−6.8	4.6	22.3	2.9	−17.5	−4.9	14.5
IDM[h]	−2.6	10.9	24.6	49.0	−0.4	2.0	17.1	23.9
KEP[k]	2.9	0.5	9.9	4.9	−1.5	−0.6	0.9	0.8
NHDC[n]	6.9	14.2	29.2	47.5	−27	−28.9	−6.3	−10.2
NPX[k]	3.5	0.7	9.7	5.4	7.0	−9.2	0.6	−4.0
PPZ[e]	2.0	11.7	53.1	77.1	−6.7	4.5	6.7	10.7
SA[l]	2.2	58.3	26.0	77.9	1.0	−0.3	−1.7	1.2
SAC[m]	3.6	41.3	−1.8	57.1	−0.9	−1.7	−1.9	1.6
SUC[n]	−10	77.1	68.9	85.0	−1.1	−1.9	−3.2	5.3
TMP[e]	2.7	54.1	56.3	51.5	2.8	32.1	17.9	25.0

* The corresponding ILIS.
[a] ACE-d_4.
[b] ACT-d_4.
[c] ASP-d_5.
[d] BPA-d_{16}.
[e] CBZ-d_8.
[f] CF-d_9.
[g] CYC-d_{11}.
[h] DCF-d_4.
[i] DEET-d_{10}.
[k] KEP-d_3.
[l] SA-d_4.
[m] SAC-d_4.
[n] SUC-d_6.

signal suppression in surface water; however when using ACE-d_4 to correct matrix effects, the signal suppression reduced to 1.7% relative suppression. For the remaining compounds, the selection of ILIS for correction was based on the similarity in their physico-chemical properties (such as pK_a and log K_{ow}) and chemical structures. Such as, both CBZ-d_8 and DEET-d_{10} could be used to correct matrix effects for the following compounds: CTMT, PPZ, and TMP; however CBZ-d_8 showed more efficient in correction for matrix effects than DEET-d_{10} (data not shown). Thus, CBZ-d_8 was chosen to correct for these compounds in further studies. SA-d_4 was used to correct matrix effects for both SA and CA. Similarly, DCF-d_4 was used to correct matrix effects for the following compounds: IDM, GFZ, and IBP, while NPX and FEP was corrected by using KEP-d_3. Taken together, the usage of 13 ILIS for analysis of 24 PPCPs and ASs proved to be efficient for the correction for matrix effects without need for further treatment or the use of time-consuming standard addition method.

3.5. MDL, MQL, repeatability and reproducibility

MDL and MQL values for the analytes in various matrices are presented in Table 4. The MQLs of most analytes except for the 4 analytes (ASP, DTZ, NHDC, and SUC) ranged 0.5–10 ng/L for raw wastewater, 0.3–10 ng/L for both surface water and groundwater, and 0.1–10 ng/L for Milli-Q water. It can be seen that the MDL and MQL values for real environmental water samples were higher than those for Milli-Q water sample. This is probably due to the impacts of matrix effects. To ensure a correct quantification of analytes, method precision and accuracy expressed as relative standard deviation (RSD, %), were obtained from the repeated injection (five times) of a spiked extract (100 ng/mL) during the same day (repeatability) and in different days (reproducibility). As it can be seen in Table 5, repeatability and reproducibility of analytical method ranged from 1.3% to 8.8% and from 2.1% to 14.5%, respectively.

3.6. Stability of the analytes during sample storage

In environmental monitoring and analysis of organic micro-pollutants (e.g. PPCPs, EDCs, and ASs) with large numbers of water samples collected, it is often not feasible to analyze such samples within a day. Then the question arises: how long the samples can be stored under laboratory conditions prior to analysis without causing adverse effects on the accuracy of monitoring results. To address this, the stability of the 24 target PPCPs, EDCs and ASs was investigated in different environmental matrices under the same storage conditions at 4 °C in dark. Fig. 2 shows the stability of the analytes in groundwater, surface water and wastewater samples without filtration using filter membrane (0.45 μm). It could be seen that no significant losses were observed after 21 days of storage for all the analytes except for the four compounds (i.e. ASP, BPA, NHDC, and SA). The higher loss rate of ASP, NHDC and SA was observed in raw wastewater and surface water samples compared to that in groundwater samples. Control tests showed minor

Table 4
Method detection limits (MDL) and method quantification limits (MQL).

Analytes	MDL (ng/L)				MQL (ng/L)			
	Milli-Q water	Ground water	Surface water	Raw wastewater	Milli-Q water	Ground water	Surface water	Raw wastewater
ACE	0.3	1.5	1.5	2.5	1.0	3.0	3.5	5.0
ACT	1.5	2.5	2.5	3.0	2.0	5.0	5.0	5.0
ASP	3.0	7.0	4.0	10	5.0	10	10	15
BPA	1.0	1.5	1.5	5.0	4.0	5.0	5.0	10
CA	0.5	0.5	0.8	1.5	1.0	2.0	2.5	3.0
CBZ	0.1	0.1	0.15	0.15	0.2	0.3	0.3	0.5
CF	1.0	5.0	3.0	5.0	5.0	6.0	5.0	10
CTMT	0.1	0.1	0.1	0.2	0.2	0.3	0.3	0.5
CYC	0.6	1.0	1.0	1.5	2.0	3.0	4.0	5.0
DCF	0.3	0.5	0.3	1.0	0.6	1.5	1.5	3.0
DEET	0.1	0.15	0.2	0.2	0.1	0.2	0.2	0.5
DTZ	0.5	10	10	10	2.0	10	15	30
FEP	1.0	1.5	2.0	2.0	2.0	3.0	2.0	5.0
GFZ	0.15	0.1	0.25	0.35	0.3	0.3	0.4	1.0
IBP	2.5	3.0	5.0	5.0	4.0	5.0	5.0	10
IDM	0.1	0.25	0.3	0.4	0.5	1.0	1.5	2.0
KEP	1.0	1.5	1.5	2.0	2.0	3.0	3.0	5.0
NHDC	5.0	5.0	7.0	10	10	12	25	30
NPX	0.8	1.0	1.0	1.5	1.5	2.0	2.0	5.0
PPZ	0.1	0.15	0.2	0.3	0.2	0.4	0.7	1.0
SA	0.8	2.5	2.0	3.0	1.5	3.0	4.0	5.0
SAC	1.0	2.5	1.0	3.0	2.0	3.0	3.0	5.0
SUC	10	10	16	15	25	30	50	50
TMP	0.3	0.8	0.9	1.0	1.0	2.0	2.5	2.5

Table 5
Repeatability and reproducibility of analytical method.

Analytes	Repeatability RSD (%) ($n=5$)				Reproducibility RSD (%) ($n=5$)			
	Milli-Q water	Ground water	Surface water	Raw wastewater	Milli-Q water	Ground water	Surface water	Raw wastewater
ACE	2.5	3.5	3.5	3.8	3.7	4.1	5.0	5.5
ACT	3.6	3.9	3.8	4.5	5.8	5.9	6.3	7.5
ASP	4.5	5.1	5.7	6.5	9.5	10	9.5	10.5
BPA	6.6	7.9	7.5	8.8	10.5	11.2	9.7	12.5
CA	4.0	5.5	4.8	5.6	6.7	6.9	7.1	7.4
CBZ	2.1	2.7	2.2	2.9	3.2	3.5	4.1	5.3
CF	4.5	5.8	5.5	6.7	7.8	8.1	8.6	9.5
CTMT	2.1	2.3	2.0	2.7	3.1	3.0	4.7	5.9
CYC	1.8	2.0	2.3	2.7	3.5	3.7	5.2	5.0
DCF	3.5	3.1	3.8	4.1	4.9	4.8	5.2	5.5
DEET	3.3	2.1	5.0	4.5	4.7	5.8	5.5	5.7
DTZ	5.2	5.9	6.3	7.4	9.5	11.0	12.7	14.5
FEP	2.3	2.6	2.1	3.7	6.4	6.5	8.2	8.5
GFZ	5.6	4.1	5.5	6.1	8.4	8.0	8.5	9.3
IBP	1.3	2.5	3.5	4.7	11	12.1	11.7	13.8
IDM	4.1	5.0	4.8	5.1	5.2	5.6	7.8	8.2
KEP	1.9	2.1	2.3	2.9	5.5	5.6	6.3	7.4
NHDC	1.8	3.1	2.0	2.1	2.1	2.4	3.2	3.5
NPX	3.3	3.5	4.7	4.8	6.1	5.8	6.3	7.5
PPZ	1.9	2.4	2.6	3.1	3.2	3.5	4.7	5.5
SA	3.5	5.0	3.3	4.9	3.3	4.0	5.1	5.4
SAC	2.6	5.5	4.9	5.8	6.1	6.0	6.4	6.5
SUC	3.9	4.5	4.7	4.7	5.3	4.7	5.3	5.0
TMP	3.1	3.2	3.0	4.5	5.1	6.4	9.1	11.5

RSD: Relative standard deviation (%).

changes in concentration of all the analytes (except for ASP) after 21 days of storage as shown in Fig. 3A (Supplementary Information). The losses of the analytes in real environmental water samples during storage may be attributed to hydrolysis, sorption onto suspended particulate matters, chemical degradation by oxidative-free radicals present in natural waters, or microbial degradation [31,40]. The results are in agreement with those reported by Ordonez et al. [38], in which it was observed that NHDC and ASP concentration were significantly decreased with the storage time.

For the filtered water samples, it was observed that most of the target analytes seemed to be stable during sample storage time up to 21 days. However, ASP showed a high instability and severe losses during sample storage. The loss of ASP might be related to its physicochemical properties rather than biodegradation/sorption because most of the suspended solids/bacteria were eliminated by using filter membranes (0.45 μm). This finding is consistent with the one reported by Berset and Ochsenbein [41], who found that ASP was hydrolyzed at sample pH below 3.4 or above 5.0. To obtain a reliable quantification of ASP, Berset and

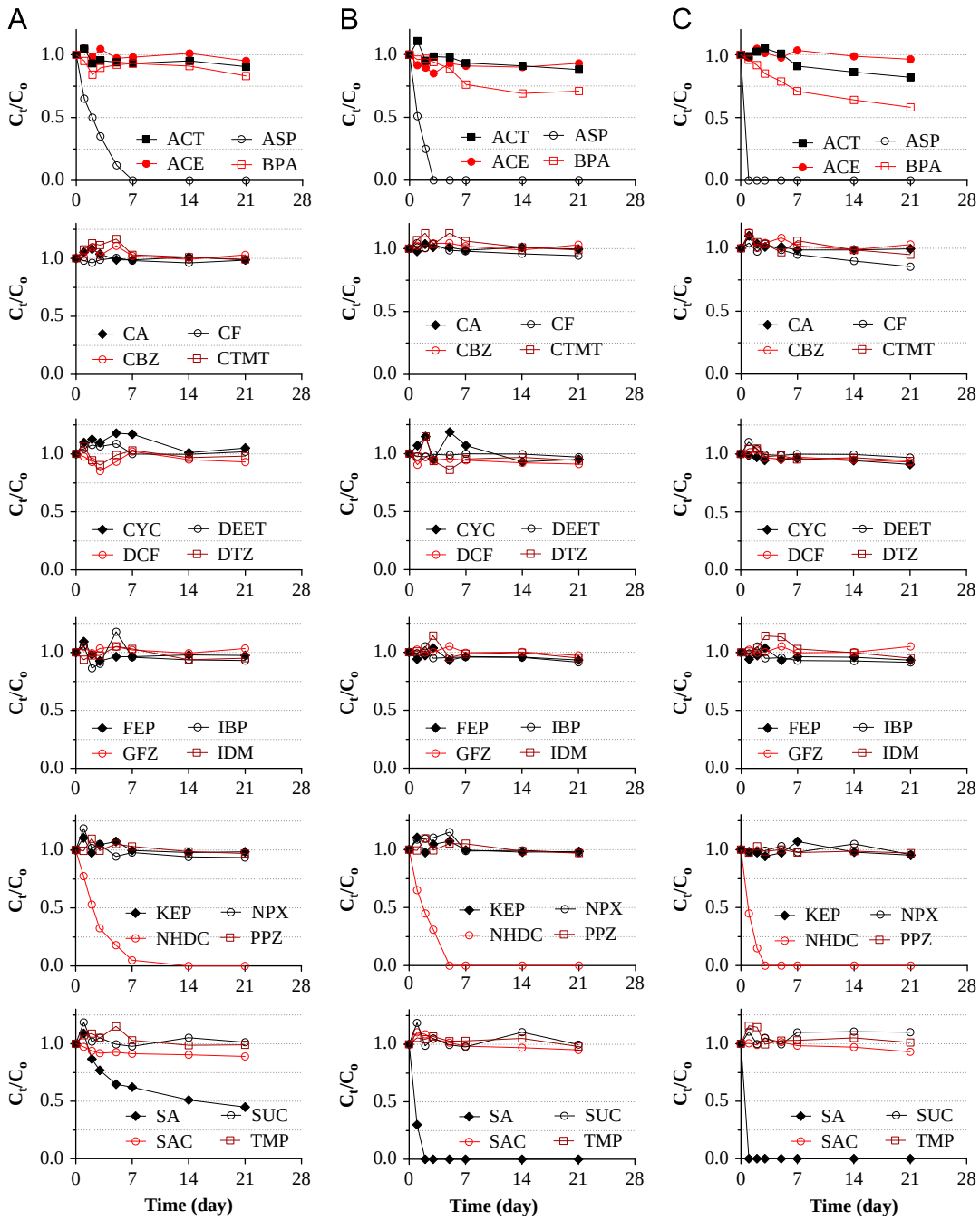

Fig. 2. Stability of the analytes in various environmental water samples during sample storage: (A) unfiltered groundwater; (B) unfiltered surface water; and (C) unfiltered wastewater.

Ochsenbein [41] suggested pH should be adjusted to 4.3 in order to avoid formation of the metabolite [41]. In short, most of the analytes in both filtered and unfiltered samples showed a good stability during sample storage for at least 7 days in the dark room and temperature at 4 °C. Exceptions to this behavior were ASP, BPA, NHDC and SA. These compounds were not stable even in filtered samples. Therefore, it is best to analyze the samples as soon as possible after collection to obtain the most accurate data.

3.7. Environmental application

The method developed in this study was applied to identify and quantify the occurrence of 24 PPCPs, EDCs and ASs in various environmental water samples including raw wastewater, surface water and groundwater. The monitoring results are presented in Table 6. It is clear that a wide spectrum of PPCPs, EDCs and ASs was detected in raw wastewater and surface water samples. In wastewater samples, 21 out of the 24 PPCPs, EDCs and ASs were detected at least one time. The levels of those compounds in wastewater were generally in the range of a few ng/L to several hundred μg/L depending on compound and sampling point. The most abundant compounds detected in raw wastewater samples were consumer products and nonprescription drugs or personal care products, such as CF (4119–359,400 ng/L), ACT (3026–337,035 ng/L), CYC (300–250,348 ng/L), SAC (500–135,759 ng/L), ACE (187–75,093 ng/L), and SA (300–42,972 ng/L), whereas CA, DTZ, and NHDC were below MQL in all raw wastewater samples. For the determination of ACT, ACE, CF, CYC, DEET, SAC and SA in most of the raw wastewater samples in this study, it might be quickly performed by direct injection of the raw wastewater

Table 6
Concentration of PPCPs and ASs detected in different environmental water samples.

Analytes	Concentration range (ng/L)		
	Ground water ($n=30$)	Surface water ($n=42$)	Raw wastewater ($n=20$)
ACE	< MQL–95	5.0–350	187–75,093
ACT	< MQL–485	25–1163	1530–337,035
ASP	< MQL	< MQL	< MQL–2262
BPA	< MQL–239	< MQL–324	< MQL–839
CA	< MQL	< MQL	< MQL
CBZ	< MQL–9.3	0.5–53.5	6.1–939
CF	97–5066	265–14,418	4219–359,400
CTMT	< MQL–11.3	< MQL–52	< MQL–6369
CYC	< MQL–87	28–1406	300–250,348
DCF	< MQL	< MQL	< MQL–950
DEET	20–2570	55–3050	88–2238
DTZ	< MQL	< MQL	< MQL
FEP	< MQL	< MQL–95	< MQL–3091
GFZ	< MQL–68	< MQL–81	< MQL–6861
IBP	< MQL	< MQL–111	< MQL–2445
IDM	< MQL	< MQL	< MQL–183
KEP	< MQL	< MQL–105	< MQL–7221
NHDC	< MQL	< MQL	< MQL
NPX	< MQL	< MQL	< MQL–2691
PPZ	< MQL–2.0	< MQL	< MQL–180
SA	< MQL–147	11–278	300–42,972
SAC	< MQL–210	40–810	500–135,759
SUC	< MQL	< MQL	100–4719
TMP	< MQL	< MQL	< MQL–880

MQL: Method quantification limits.

samples, which were filtered through 0.45 μm membrane filters and were mixed with ILIS into HPLC–MS/MS system without SPE. However, when quantifying these compounds in surface water and/or groundwater samples, it would be necessary to perform SPE prior to HPLC–MS/MS analysis. Similarly, for analysis of other target analytes in raw wastewater, surface water and groundwater samples, it is required to enrich these analytes by using SPE coupled with HPLC–MS/MS and isotope dilution.

Regarding surface water samples, 15 out of the 24 PPCPs, EDCs and ASs were detected in surface water, in which the 8 compounds including ACE, ACT, CBZ, CF, DEET, SA, and SAC were found in all surface water samples. On the contrary, the following 9 compounds: ASP, CA, DTZ, IDM, NHDC, NPX, PPZ, SUC and TMP, were not found in any surface water sample. The absence of these analytes in surface water can be interpreted as being due to more dilution in canals/reservoirs and natural attenuation. The difference in concentrations of analytes between wastewater and surface water were statistically significant (T-test: $p<0.05$), except for the case of DEET. In relation to groundwater, the concentrations of PPCPs and ASs in groundwater samples were lower than those in surface water. Similar situation in surface water, there was no evidence on the presence of nine compounds (i.e. ASP, CA, DTZ, IDM, NHDC, NPX, PPZ, SUC and TMP) in groundwater samples. Only two compounds (CF and DEET) were ubiquitously present in groundwater. The presence of the analytes in surface water and groundwater indicated the impacts of wastewater contamination on receiving water bodies. The monitoring of the 24 target analytes aimed to identify and evaluate the effects of sewer leaks on surface water and groundwater quality. These analytes are highly specific to sewage and have been suggested to serve as molecular markers indicating the sewage contamination in receiving water bodies.

4. Conclusion

A robust sensitive method based on a single-step SPE coupled with HPLC–ESI–MS/MS was developed to determine simultaneously the 24 PPCPs, EDCs and ASs with different chemical structures and physicochemical properties (i.e. acidic, neutral, hydrophilic, and hydrophobic properties). A single-step SPE procedure and HPLC–MS/MS parameters were investigated and optimized. The method sensitivity was validated with the real environmental water samples (i.e raw wastewater, surface water and groundwater). The MQL value of most target analytes was below 10 ng/L, which is low enough for analysis of these target analyte in surface water and groundwater. Consequently, the method was successfully applied to determine simultaneously the 24 PPCPs, EDCs and ASs in real environmental water samples collected in urban water catchment area in Singapore. Taken together, the results confirmed applicability of the developed method in environmental monitoring of these compounds in wastewater, surface water and groundwater.

Acknowledgment

This research is supported by PUB Singapore under the Project (R-302-000-005-490). The authors sincerely thank Ms. Shivasorupy Barthiban from NUS and staffs of Operation and Maintenance Division, Water Reclamation (Network) Department, PUB Singapore for the assistance in sample collection.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.03.072.

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