# Matrix effects on mass spectrometric determinations of four pharmaceuticals and personal care products in water, sediments, and biota

**Ève B. Dussault, Vimal K. Balakrishnan, Keith R. Solomon, and Paul K. Sibley**

**Abstract:** Simple analytical methods were developed for the extraction and determination of four pharmaceuticals and personal care products (PPCPs) from water, sediments, and biota. PPCPs were determined using tandem LC–MS in electrospray ionization mode, and interactions with matrix co-eluents were investigated. Extractions of water samples were performed using solid-phase extraction (SPE), sediments were extracted by pressurized liquid extraction (PLE), and biota was extracted by liquid extraction. The selected analytical methods yielded recoveries ≥ 61% in all matrixes. Matrix interactions were investigated throughout the linear range of quantification of each compound, revealing that dissolved salts had relatively minor effects on ionization (between 14% suppression to 12% enhancement), but that sediment and biota extracts caused significant matrix effects (ranging from 56% suppression to 25% enhancement). The direction and magnitude of matrix interactions reflected the physico-chemical properties of each analyte, particularly their p$K_a$. Among the compounds analyzed in electrospray positive mode, carbamazepine was insensitive to matrix interactions, because it is a strong proton acceptor (p$K_a$ = 14.0). In contrast, atorvastatin (p$K_a$ = 4.5), a weaker proton acceptor, was particularly sensitive to matrix effects. For those compounds analyzed in negative-ion mode, sample alkalinity was found to be important. With a p$K_a$ of 10.4, 17α-ethinylestradiol generally exhibited matrix enhancement with increased sample alkalinity. However, the presence of acidic co-eluents contributed to matrix suppression. Lastly, TCS was particularly sensitive to matrix suppression, as its circumneutral p$K_a$ (7.9) caused even slight changes in sample pH to considerably impact ionization. We conclude that while different matrixes have clear impacts on ionization of these PPCPs, matrix effects can be quantified and overcome.

*Key words:* pharmaceuticals and personal care products, matrix effects, LC–MS/MS, sediments, biota.

**Résumé :** On a développé des méthodes analytiques simples pour l'extraction et la détermination de quatre produits pharmaceutiques et de soins personnels (PPSP) à partir de solutions aqueuses, de sédiments et du biote. On a déterminé les PPSP en faisant appel à la chromatographie liquide opérant en tandem avec spectrométrie de masse en mode d'ionisation par électronébulisation (CL–SM) et on a étudié les interactions avec les coéluants de la matrice. Les extractions à partir de solutions aqueuses ont été effectuées en utilisant l'extraction en phase solide (EPS) alors que les sédiments ont été soumis à une extraction avec un liquide pressurisé (ELP) et que le biote a été soumis à une extraction liquide. Dans l'ensemble des matrices, les méthodes analytiques choisies ont permis d'obtenir des taux de récupération d'au moins 61 %. On a étudié les interactions avec la matrice pour la plage entière de quantification de chaque composé et ces études ont révélé que les sels dissous n'ont que des effets mineurs sur l'ionisation (allant de 14 % de suppression à 12 % de renforcement); toutefois, dans les cas des extractions des sédiments et du biote, on observe des effets de matrice importants allant de 56 % de suppression à 25 % de renforcement. La direction et l'amplitude des interactions de matrice sont des reflets des propriétés physico-chimiques de chaque analyte, en particulier de leur p$K_a$. Parmi les composés analysés en mode d'électronébulisation positive, la carbamazépine est insensible aux interactions de matrice dû au fait qu'il s'agit d'un produit fortement accepteur de proton (p$K_a$ = 14,0). À l'opposé, l'atorvastatine (p$K_a$ = 4,5), un accepteur de proton beaucoup plus faible, est particulièrement sensible aux effets de matrice. Pour les composés analysés en mode d'ion négatif, on a trouvé que l'alcalinité de l'échantillon est importante. Avec un p$K_a$ de 10,4, le 17α-éthinylestradiol présente généralement un renforcement de matrice avec une augmentation de l'alcalinité de l'échantillon. Toutefois, la présence de coéluants acides contribue à la suppression de l'effet de matrice. Enfin, le « TCS » est particulièrement sensible à la suppression de la matrice dû au fait que son p$K_a$ pratiquement neutre (7,9) provoque des changements considérables dans l'ionisation même pour de faibles changements dans le pH des échantillons. On en conclut que même si les diverses matrices ont une influence certaine sur

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**È.B. Dussault.**[1] Department of Environmental Biology, University of Guelph, Guelph, ON N1G 2W1, Canada; Water Science and Technology Directorate, Aquatic Ecosystem Protection Research Division, Environment Canada, Burlington, ON, L7R 4A6, Canada.
**V.K. Balakrishnan.** Water Science and Technology Directorate, Aquatic Ecosystem Protection Research Division, Environment Canada, Burlington, ON, L7R 4A6, Canada.
**K.R. Solomon and P.K. Sibley.** Department of Environmental Biology, University of Guelph, Guelph, ON N1G 2W1, Canada.

[1]Corresponding author (e-mail: Eve.Dussault@ec.gc.ca).

l'ionisation de ces PPSP, les effets de matrice peuvent être quantifiés et surmontés.

*Mots-clés :* produits pharmaceutiques et de soins personnels, effets de matrice, CL–SM/SM, sédiments, biote.

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## Introduction

Environmental contamination of aquatic systems by pharmaceuticals and personal care products (PPCPs) has been of increasing concern in the last decade. However, limited availability of adequate analytical tools, mass spectral libraries, and analytical standards had, until the 1990s, significantly hindered investigations.[1,2] Since then, hundreds of products have been detected in wastewater effluents and surface water, including lipid regulators, analgesics, antimicrobials, and endocrine disruptors.[2–4] In addition, as the fate, effects, and environmental risks posed by the presence of PPCPs in aquatic systems are evaluated, there is an increasing demand for reliable analytical methods permitting their quantification in various matrixes.

Monitoring studies have generally detected PPCPs at low concentrations. However, some compounds are relatively persistent, due to their continuous usage and release into aquatic environments.[1–3] Consequently, aquatic organisms may be exposed to a multitude of these bioactive compounds at sublethal concentrations over the duration of their life cycle.

Despite the important role they play in aquatic food webs, potential effects of PPCPs on aquatic invertebrates, such as the midge *Chironomus tentans* and the freshwater amphipod *Hyalella azteca*, have been less frequently investigated than vertebrate species (e.g., fish). To execute studies into the effects of PPCPs on these aquatic organisms, it is critical to first establish and validate analytical methods for their extraction and determination in environmental matrixes.

PPCPs differ from many other pollutants in that they are specifically designed to elicit responses from target species with the potential for unintended consequences on non-target species. Accordingly, these compounds are generally moderately water-soluble, non-volatile, and thermally labile, hindering analysis by GC–MS without prior derivatization.[1] In contrast, analysis by LC–MS provides a more facile alternative to GC–MS analysis. However, a complication associated with LC–MS analysis is the potential for interactions with other matrix co-eluents, believed to arise from competition between the analyte ions and co-eluents for gas-phase emission in the electrospray ionization source.[5,6] As the number of co-eluents increases, their interactions with the target analytes interferes with adequate quantification, thereby hindering investigations.[5,7,8]

Several approaches have been suggested for the resolution of matrix effects. Quantification via matrix-matched calibration has been suggested, but the availability of non-contaminated material is problematic when studying contaminated sites. Another method consists of the addition of an internal standard (typically isotopically labelled) to each sample prior to analysis to correct for variations in ionization efficiency brought about by interactions with matrix co-eluents. Although widely accepted, this technique is most effective when the co-eluents have an identical effect on both the internal standard and the target analyte(s).[7] However, in their study of matrix effects on sulfonamide antibiotics, Balakrishnan et al.[9] recently showed that such an assumption is not necessarily valid, even in comparing $^{13}C_6$-sulfamethazine with unlabelled sulfamethazine. In cases where the internal standard interacts with matrix components in a different manner than do the target analytes, the use of this approach would incorporate a non-systematic bias into calculations, resulting in quantification errors.

The objectives of this study were two-fold: to develop separate extraction and analytical methods for the determination of four PPCPs in water, sediment, and biota, and to investigate the impact of these matrixes (water, sediment, and biota) on compound ionization. First, various extraction techniques were explored for each PPCP of interest, via extraction of samples spiked with known concentrations of analytical standards, and for which the recovery of each compound was calculated. Once satisfactory extraction methods were established, the matrix interactions of these extracts were individually assessed and quantified for each target analyte.

## Experimental

### Materials

#### *Compound selection*

The four compounds of interest were selected based on various criteria, including usage in Canada, persistence, and previous work in our laboratory, as described in Dussault et al.,[10] and included the lipid-regulator atorvastatin, the antiepileptic drug carbamazepine, the synthetic hormone 17α-ethinylestradiol, and the antimicrobial triclosan. The structures are shown in Table 1.

#### *Chemicals*

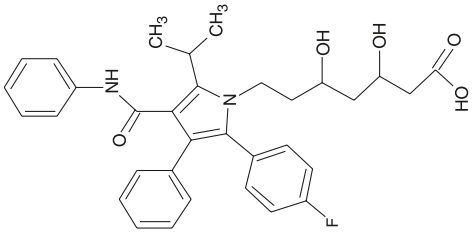
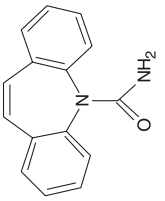
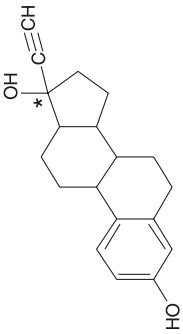
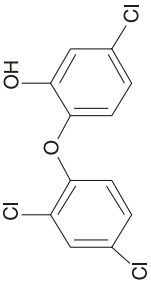
Atorvastatin (ATO, ≥99%) was obtained from Rugao Foreign Trade Corp. (Shanghai, China), carbamazepine (CBZ, ≥99%) was purchased from China Jiangsu Textiles (Nanjiing, China), and 17α-ethinylestradiol (EE2, ≥98%) and triclosan (TCS, ≥97%) were acquired from Sigma-Aldrich (Oakville, ON, Canada).

Calcium chloride ($CaCl_2$), calcium sulfate dihydrate ($CaSO_4 \cdot 2H_2O$), magnesium sulfate ($MgSO_4$), potassium chloride (KCl), sodium bicarbonate ($NaHCO_3$), and sodium bromide (NaBr), were purchased from Fisher Scientific (Whitby, ON, Canada). Ammonium carbonate was obtained from Caledon Laboratories (Georgetown, ON, Canada).

Acetonitrile (MeCN, HPLC grade), methanol (MeOH, HPLC grade), and acetone ($(CH_3)_2CO$, distilled in glass) were acquired from Caledon Laboratories (Georgetown, ON, Canada). Deionized water was obtained by Milli-Q (Millipore) filtration.

Isotopically labelled $^{13}C_5$-atorvastatin ($^{13}C_5$-ATO, ≥98%) was purchased from Toronto Research Chemicals (North

**Table 1.** Chemical structures and $pK_a$ of the four pharmaceuticals and personal care products used in this study.

| | Atorvastatin (ATO) | Carbamazepine (CBZ) | 17α-Ethinylestradiol (EE2) | Triclosan (TCS) |
|---|---|---|---|---|
| Molecular structure |  |  |  |  |
| CAS reference number | 134523–00–5 | 298–46–4 | 57–63–6 | 3380–34–5 |
| $pK_a$ | 4.46[11] | 14.0[12] | 10.4[13] | 7.9[11] |

*denotes C-17.

York, ON, Canada), $^2H_{10}$-carbamazepine ($^2H_{10}$-CBZ, ≥98%) was purchased from Cambridge Isotope Laboratories (Andover, MA, USA), and $^2H_2$-17β-estradiol ($^2H_2$-E2, atomic purity 98%) was obtained from Sigma-Aldrich (Oakville, ON, Canada). $^{13}C_6$-Triclosan ($^{13}C_6$-TCS, ≥99%) was purchased from Wellington Laboratories (Guelph, ON, Canada).

### *Standard solutions*

A 10 mg/L stock solution of each analyte was prepared in 1:1 MeOH/$H_2O$. Calibration standards corresponding to the linear range of detection (10–5000 μg/L) were prepared by dilution in MeOH/$H_2O$. To correct for instrumental variability, an internal standard was added to each vial prior to analysis, by addition of 50 μL of either 10 mg/L $^{13}C_5$-ATO or $^2D_2$-E2, 5 mg/L $^2D_{10}$-CBZ or 1 mg/L $^{13}C_6$-TCS, as appropriate, to 250 μL of sample. Standards and QC samples were stored at –20 °C until analysis. QC samples were analyzed with the study samples; the results of which formed the basis of accepting or rejecting a run.

### *Reconstituted water*

Two types of reconstituted water for standard toxicity testing were selected and prepared, representing the range of variability commonly found in southern Ontario waters. Recon A represented Lake Ontario water (Ontario, Canada; pH 7.7, conductivity 395 mS/cm, alkalinity 0.11 mequiv., hardness 0.13 mmol), and contained 147 mg $CaCl_2$, 84 mg $NaHCO_3$, 1 mg NaBr, 3.7 mg KCl, and 30.1 mg $MgSO_4$ per litre of distilled water.[14] Recon B was selected to represent the water hardness of the Grand River watershed (Ontario, Canada; pH 8.4, conductivity 606 mS/cm, alkalinity 0.35 mequiv., hardness 0.25 mmol), and contained 192 mg of $NaHCO_3$, 120 mg of $CaSO_4{\cdot}2H_2O$, 120 mg of $MgSO_4$, and 8 mg of KCl in 1 L of distilled water.[15]

## Analytical methods

### *Separation*

Individual isocratic liquid chromatographic methods were developed for each PPCP, using an Acquity UPLC (Waters, Milford, MA, USA). Ten microlitre aliquots were injected into an Xterra MS-C8 column (particle size 3.5 μm, 2.1 id × 100 mm) fitted with a column guard, kept at 35 °C. For all compounds, HPLC separation involved the use of two solvents: $H_2O$ (A) and MeCN (B), at a flow rate of 300 μL/min, with the exception of TCS, for which a flow rate of 200 μL/min was used. For ATO, an isocratic mixture of 20% A: 80% B was used, where A and B both contained 0.1 formic acid (*v*/*v*). The CBZ and EE2 solvent mixture was 40% A: 60% B, and the TCS solvent mixture was 10% A: 90% B. EE2 required the post-column infusion of 0.5% ammonium carbonate, at a rate of 10 μL/min.

### *Detection*

Analyses were performed using a Quatro Ultima tandem LC triple quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with a Z-Spray electrospray ionization (ESI) source. A positive-ionization mode was used for the determination of ATO and CBZ, while a negative-ionization mode was used for analysis of EE2 and TCS. Nitrogen was used as both drying and nebulizing gas, at flow

rates of 500 and 70 L/h, respectively. Argon was used as the collision gas, at a pressure of $2.5 \times 10^{-3}$ mbar (1 bar = 100 kPa), with the exception of TCS, where the pressure was lowered to $2.5 \times 10^{-4}$ mbar. Source and desolvation temperatures were 120 and 350 °C, respectively.

For the detection of TCS, ion fragmentation did not provide satisfactory quantifiable transitions, even at low ion energy, a phenomenon that has been observed in other studies.[6,16] Quantification was therefore performed using precursor-to-precursor transitions (i.e., both quadrupoles were set to select the $[M - H]^-$ mass) for the TCS ions (*m/z* 288.8 and 286.8, found in proportion to the isotopic ratio of chlorine) and internal standard (*m/z* 299.1 and 301.1). Tandem LC–MS conditions are summarized in Table 2.

## Extraction procedures: development and validation

### *Water extractions*

For each solid-phase extraction (SPE) cartridge tested, triplicate 500 mL aliquots of ultrapure water were spiked with a known quantity of each individual PPCP, using 100 μL of either a 5 or 10 mg/L solution of ATO, CBZ, EE2, or TCS in MeOH. Samples were acidified to pH 2 using 50% sulfuric acid, after which they were extracted through a SPE cartridge. For comparison purposes, SPE cartridges used were Supelco ENVI-18 (Sigma-Aldrich, Oakville, ON, Canada), OASIS MAX and OASIS HLB (Waters, Mississauga, ON, Canada). Cartridges were first conditioned with 6 mL of $(CH_3)_2CO$, followed by 6 mL of MeOH, and 12 mL of deionized water, adjusted to pH 2 with 50% $H_2SO_4$. Cartridges were loaded at a rate of approximately 10 mL/min, and eluted with $3 \times 2$ mL MeOH. Samples were then evaporated to 1 mL, and reconstituted to 2 mL with ultrapure water. Samples were frozen at –20 °C until analysis, usually performed within 2 weeks (with the exception of EE2 samples, which were analyzed after 9 weeks).

### *Sediment extractions*

For each compound investigated, triplicate samples of 10–15 g of wet sediment from Environment Canada Reference site No. 112, pH 8.17, alkalinity 89 mg/L, 36% clay, 55% silt, 9% sand, 1.9% total organic carbon,[17] was added to pressurized liquid extraction (PLE) cells previously filled with 10–15 g of Ottawa sand reference material (Fisher Scientific, Whitby, ON, Canada). The sediments were then spiked with 1 μg of ATO, CBZ, EE2, or TCS, by adding 100 μL of 10 mg/L in MeOH of the analyte to the sediments. The remaining volume of the cells was filled with Ottawa sand, and samples were extracted in MeOH by PLE (ASE 300 Accelerated Solvent Extraction System; Dionex, Oakville, ON, Canada) at a temperature of 100 °C and pressure of 1500 psig. The extraction procedures included four cycles of 15 min (static), using a solvent delivery of 60% of cell volume. The extracts were evaporated to 1–5 mL, after which an equal volume (1–5 mL) of $CaCl_2$ (0.5 mol/L) was added to facilitate the precipitation of suspended solids.[18] Samples were vortexed and centrifuged at 1000*g* for 30 min at 4 °C. The supernatant was collected, filtered through 0.45 μm syringe filters (Chromatographic Specialties, Brockville, ON, Canada), and stored at –20 °C pending analysis, which typically took place within 2 months of extraction (however, due to unforeseen complications, the EE2 samples were stored for 12 months prior to analysis).

To compare the results of the PLE of ATO and in the absence of data from the literature, a triplicate Soxhlet extraction was performed using MeOH. Approximately 2 g of sediments were spiked with 0.1 μg of ATO by addition of 1 mL of a 0.1 mg/L ATO solution in MeOH, after which the sediments were extracted for 24 h using a Soxhlet extraction apparatus. The extracts were then evaporated following the same procedures as with the PLE extractions.

### *Biota extractions*

Extractions were performed on two benthic invertebrates, the midge *Chironomus tentans* and the freshwater amphipod *Hyalella azteca*. For each organism and compound investigated, the extraction consisted of the addition of 9–12 animals to 2 mL of $(CH_3)_2CO$ ($n = 6$), into which 10 μL of a 10 mg/L solution of ATO, CBZ, EE2, or TCS in MeOH was added. Samples were thoroughly mixed and sonicated for 30 min (Branson Ultrasonic, Danbury, CT, USA). After additional mixing, samples were centrifuged at 1000*g* for 30 min at 4 °C, and the supernatant was collected and evaporated to dryness. Samples were reconstituted in 1 mL of 50:50 MeOH/$H_2O$, filtered through 0.45 μm syringe filters, and stored at –20 °C pending analysis that was performed within 5 days.

### *Precision and accuracy*

Method precision was determined using replicate extractions ($n = 3$) of spiked samples in all matrixes investigated in the present study. For biota (*C. tentans* and *H. azteca*), six replicate sample extractions were performed ($n = 6$) because of the expected greater matrix complexity. The linearity of all standard curves was verified in each matrix by plotting the peak area of the analyte versus its concentration, and the Response ($R$) of the analyte against concentration was calculated using the equation

$$[1] \qquad R = \frac{\text{Peak area}_{\text{Std}} \times \text{Concn}_{\text{IS}}}{\text{Peak area}_{\text{IS}}}$$

The accuracy of the method was estimated using the standard error of the mean and the relative standard deviation. The standard error of the mean was calculated using the equation

$$[2] \qquad \text{SEM} = \frac{\text{SD}}{\sqrt{n}}$$

where SD is the standard deviation, and $n$ is the number of replicates. The relative standard deviation (RSD) was calculated using the equation

$$[3] \qquad \text{RSD}(\%) = \frac{\text{SD}_\text{A}}{\text{Concn}_\text{A}} \times 100\%$$

where $SD_A$ is the standard deviation of the analyte, and $Concn_A$ is the average concentration of the analyte.

### *Method selectivity*

For each PPCP, the selectivity (susceptibility to cross-contamination) of the LC–MS/MS methods employed in the

**Table 2.** Optimized LC–MS/MS conditions for the analysis of four pharmaceuticals and personal care products and their internal standards in water and sediments. Argon served as the collision gas, at a pressure of $2.5 \times 10^{-3}$ mbar.

| Analyte | ES mode (+/–) | Retention time (min) | Parent ion (*m/z*) | Daughter ions | | Cone voltage (kV) | Collision energy (eV) |
|---|---|---|---|---|---|---|---|
| | | | | Quantitation (*m/z*) | Confirmation (*m/z*) | | |
| Atorvastatin | ES+ | 1.10 | 559.2 | 440.3 | 292.3 | 44 | 19 |
| $^{13}C_5$-Atorvastatin[a] | | 1.08 | 564.2 | 445.2 | 297.2 | 65 | 18 |
| Carbamazepine | ES+ | 1.22 | 237.1 | 194.2 | 179.1 | 39 | 27 |
| $^{2}D_{10}$-Carbamazepine[a] | | 1.20 | 247.2 | 204.2 | 186.0 | | 32 |
| 17α-Ethinylestradiol | ES– | 1.47 | 295.1 | 145.0 | 159.0 | 20 | 37 |
| $^{2}D_2$-17β-Estradiol[a] | | 1.44 | 273.1 | 147.0 | 185.0 | 20 | 36 |
| Triclosan[b] | ES– | 1.60 | 288.8 | 288.8 | — | 20 | 0 |
| | | | 286.8 | 286.8 | — | 20 | 0 |
| $^{13}C_{10}$-Triclosan[a] | | 1.59 | 299.1 | 299.1 | — | 20 | 0 |
| | | | 301.1 | 301.1 | — | 20 | 0 |

**Note:** Collision-gas pressure for triclosan was $2.5 \times 10^{-4}$ mbar to avoid excessive fragmentation.

[a]Internal standard.

[b]Quantification of triclosan was performed using only the precursor-to-precursor transition.

present study was investigated by analyzing solutions that contained the individual PPCP, or its internal standard, across all MRM channels. For all PPCPs, a solution concentration of 5000 μg/L was used, while the internal standards were used at concentrations of 1667 (ATO, EE2), 833 (CBZ), or 167 (TCS) μg/L, corresponding to the final concentrations of each internal standard to be employed during sample analysis. The extent of cross-contamination was calculated using the equation

$$[4] \qquad \text{CC}\ (\%) = \left[\frac{\text{Area}_\text{A}}{\text{Area}_\text{C}}\right] \times 100\%$$

where $\text{Area}_\text{A}$ is the peak area for the analyte quantitation ion, and $\text{Area}_\text{C}$ is the peak area for the quantitation ion of the suspected cross-contaminant.

## Matrix effects

Given the well-established susceptibility of LC–MS/MS techniques to matrix effects,[5,7,8] the impact of reconstituted water, sediments, and biota on the MS/MS ionization responses of the four PPCPs was carefully assessed in each extracted matrix as follows. Spiked samples were frozen at –20 °C and analyzed within 1 week.

### *Water*

For each analyte, matrix interactions were investigated by spiking the target analyte in 50:50 MeOH/Recon (A or B) at concentrations corresponding to those of the standard curve prepared in MeOH/Milli-Q water (10–5000 μg/L). Each series of spikes was prepared in duplicate. The appropriate internal standard (see standard solutions Section) was added to each vial prior to analysis.

### *Sediments*

A 13 g sample of wet sediment was extracted by PLE, as outlined above. The sediment extracts were used to spike various concentrations of the target analyte (10–5000 μg/L) for comparison with the standard curve prepared in Milli-Q water. For each PPCP, each series of spikes was prepared in duplicate. The suitable internal standard was added to each sample prior to analysis.

### *Biota*

A pooled sample of 10–15 animals each of *Chironomus tentans* or *Hyalella azteca* was extracted following the procedures outlined above. Twelve biota extracts were pooled in two groups of six, to provide duplicates of 6 mL, for biota tissue concentrations of 10.5 mg/mL (*C. tentans*) and 1.9 mg/mL (*H. azteca*). A series of spiked samples (10–5000 μg/L) were prepared from both duplicates, for each PPCP investigated, and the appropriate internal standard was added to each vial prior to analysis.

### *Calculations*

For each solution sample (in 50:50 MeOH/Milli-Q) and corresponding spiked matrix sample, the matrix effect was calculated at each concentration as

$$[5] \qquad M_x(\%) = \left[\frac{\left(\frac{\text{Area}_\text{A}}{\text{Area}_\text{IS}}\right)_\text{matrix}}{\left(\frac{\text{Area}_\text{A}}{\text{Area}_\text{IS}}\right)_\text{standard}} - 1\right] \times 100\%$$

where $M_x$ is the matrix effect at concentration $x$ (%); $M_x > 0$ corresponds to matrix enhancement and $M_x < 0$ corresponds to matrix suppression. According to this calculation, the standard solvent, by definition, produces no matrix effects. In addition, if the internal standard fully corrects for the variation in ionization efficiency, the ratio $\frac{\text{Area}_\text{A}}{\text{Area}_\text{IS}}$ will be the same, regardless of the matrix, and the matrix effect will be zero ($M_x = 0$). This approach resembles the method proposed by Matuszewski et al.,[19] which is calculated using the equation

$$[6] \qquad M_x(\%) = \left[\frac{\text{Area}_\text{matrix}}{\text{Area}_\text{MilliQ}} - 1\right] \times 100\%$$

with the exception that we have chosen to include an internal standard (IS) in our calculations, thus permitting differentiation between those effects caused by actual matrix

components from effects arising from simple variations in the ionization efficiency of the mass spectrometer.

### Statistical analyses

For each analyte, differences between concentrations and matrixes were determined using a multiple factor Analysis of Variance and Tukey's adjustment for multiple comparisons.[20] The interactions of the following factors were analyzed: concentration, matrix, and concentration × matrix, the results of which are presented in the Matrix effects Section (below). All statistical computations were performed using SAS® (Version 9.1, Cary, NC, USA).

## Results and discussion

### Extraction procedures: development and validation

The calibration curves for each matrix extract are presented in the Supplementary data, Fig. S1 (peak area vs. concentration) and Fig. S2 (response vs. concentration). All calibration curves exhibited satisfactory linearity throughout the range of concentrations examined (limit of quantification: 5000 μg/L), with $R^2$ values of 0.9881–0.9999 (Table 3). 3). The limit of detection (LOD) and limit of quantification (LOQ), calculated for each matrix investigated, are also presented in Table 3.

#### *Water extractions*

The use of solid-phase extraction for the determination of PPCPs in aqueous environmental samples has been investigated in several studies.[21–23] In the present study, the recovery of PPCPs after SPE varied with cartridge selection (Table 4). The Supelco ENVI-18 yielded the most satisfactory accuracy for the extraction of CBZ, EE2, and TCS with average recoveries of 91%, 87%, and 84%, respectively. Given the better accuracy and precision provided by the Oasis HLB cartridge, the latter was selected for the recovery of ATO (71%; Table 4), a finding in agreement with those of Miao and Metcalfe.[24] Our recoveries are consistent with those obtained in similar studies, where recoveries for ATO ranged from 77% to 86%,[24,25] CBZ varied between 57% and 100%,[4,21,26–29] EE2 ranged from 76% to 96%,[30] and TCS varied between 36% and 95%.[16,22,31,32] Satisfactory linearity was obtained, with $R^2$ values of 0.9964 to 0.9999 (Table 3).The limit of detection for each analyte in Milli-Q water was 1.12, 1.12, 3.45, and 4.33 μg/L for ATO, CBZ, EE2, and TCS, respectively, while the limit of quantification was 3.76, 3.76, 11.50, and 14.42 μg/L, respectively (Table 3). For Recon A, the limit of detection was 0.65, 0.82, 6.29, and 5.37 μg/L, and the limit of quantitation was 2.18, 2.74, 20.97, and 17.91 μg/L for ATO, CBZ, EE2, and TCS, respectively (Table 3). For Recon B, the limit of detection was 0.61, 1.00, 5.41, and 3.03 for ATO, CBZ, EE2, and TCS, while the limit of quantitation was 2.03, 3.35, 18.06, and 10.10 μg/L, respectively (Table 3).

#### *Sediment extractions*

PLE proved to be a straightforward and reliable technique for the extraction and determination of PPCPs in sediments, with recovery rates varying between 70% (ATO) and 116% (EE2) (Table 5). The recovery of ATO following Soxhlet extraction was 42 ± 9.3% (mean ± SEM, $n$ = 3), considerably lower than that obtained via PLE. Although PLE is a widely used technique for the analysis of several organic contaminants (e.g., PAHs, PCBs, surfactants, and pesticides),[33,34] there are notably fewer studies using this method for the extraction of PPCPs. Miao et. al.[35] successfully used PLE for the extraction of CBZ from biosolids, and had similar recoveries, while another study reported EE2 recovery of 87% from sediments.[36] Lastly, three studies successfully extracted TCS by PLE, and obtained 100%–120% recovery in sediments,[16,32,37] which is consistent with our values. To our knowledge, this study is the first to report extraction of ATO by PLE. Linearity varied between 0.9918 and 0.9997 (Table 3). The limit of detection of each analyte in sediment extracts was 4.72, 7.99, 7.62, and 17.76 μg/L for ATO, CBZ, EE2, and TCS, respectively. The limit of quantification was 15.72, 26.63, 25.39, and 59.19 μg/L, respectively (Table 3).

#### *Biota extractions*

Although the use of PLE presents clear advantages for the analysis of sediments and biota, the large sample sizes required (e.g., several grams) preclude its use with very small animals, such as benthic invertebrates, due to limitations in the availability of animal tissue. The liquid extraction we present here is a simple and flexible technique, which could be adapted to various sample sizes. All compounds exhibited satisfactory recoveries, ranging from 61% to 129% (Table 6). 6). Few studies to date have investigated the extraction of PPCPs from non-target organisms. Liquid–liquid extraction methods have been proposed for the analysis of estrogens in algae,[38] and for a wide variety of PPCPs and triclosan in fish,[39,40] with recoveries varying between 40% and 119%. Recently, a study proposed the extraction of selective serotonin re-uptake inhibitors from fish by PLE.[39] It is noteworthy that the studies reporting methods for estrogens and triclosan proposed GC–MS analyses, which involved preliminary derivatization of the target analytes. To the best of our knowledge, this study is also the first to propose extraction procedures for both ATO and CBZ in biota. In addition, the quantification we propose by tandem LC–MS represents an alternative to the derivatization processes involved in the analysis of PPCPs by GC–MS. Linearity ($R^2$ values) varied between 0.9881 and 0.9993 (*C. tentans*) and 0.9888 and 0.9998 (*H. azteca*; Table 3). For the *C. tentans* extracts, the limit of detection for ATO, CBZ, EE2, and TCS was 0.34, 1.12, 3.74, and 7.67 μg/L, respectively; the limit of quantification of each analyte was 1.12, 2.87, 12.47, and 25.58 μg/L (Table 3). For the *H. azteca* extracts, the limit of detection was 1.29, 1.10, 6.50, and 7.99 μg/L for ATO, CBZ, EE2, and TCS, respectively, while the limit of quantification was 4.31, 3.66, 21.68, and 26.65 μg/L, respectively (Table 3).

#### *Method selectivity*

Cross-talk between the MRM channels of each analyte and internal standard was investigated using individual solutions of each PPCP and its internal standard, the results of which are presented in Supplementary data Figs. S3–S10. We observed negligible cross-talk between each analyte and their respective internal standard. For ATO, a cross-talk of 0.05% was calculated (S3), representing the percentage of

**Table 3.** Limit of detection (LOD; μg/L), limit of quantification (LOQ; μg/L), and linearity ($R^2$; 5–5000 μg/L) for four pharmaceuticals and personal care products in various matrixes.

| | Atorvastatin | | | Carbamazepine | | | 17α-Ethinylestradiol | | | Triclosan | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LOD[a] | LOQ | $R^2$ | LOD | LOQ[b] | $R^2$ | LOD | LOQ | $R^2$ | LOD | LOQ | $R^2$ |
| Milli-Q | 1.13 | 3.76 | 0.9996 | 1.13 | 3.76 | 0.9999 | 3.45 | 11.50 | 0.9999 | 4.33 | 14.41 | 0.9989 |
| Recon A | 0.65 | 2.18 | 0.9996 | 0.82 | 2.74 | 0.9965 | 6.29 | 20.97 | 0.9999 | 5.37 | 17.91 | 0.9985 |
| Recon B | 0.60 | 2.03 | 0.9999 | 1.01 | 3.35 | 0.9975 | 5.42 | 18.06 | 0.9999 | 3.03 | 10.10 | 0.9964 |
| Sediment | 4.72 | 15.72 | 0.9997 | 7.99 | 26.63 | 0.9918 | 7.62 | 25.39 | 0.9994 | 17.76 | 59.19 | 0.9964 |
| Biota A (*Chironomus tentans*) | 0.34 | 1.12 | 0.9881 | 0.86 | 2.87 | 0.9988 | 3.74 | 12.47 | 0.9993 | 7.67 | 25.58 | 0.9926 |
| Biota B (*Hyalella azteca*) | 1.29 | 4.31 | 0.9990 | 1.10 | 3.66 | 0.9993 | 6.50 | 21.68 | 0.9998 | 7.99 | 26.65 | 0.9888 |

[a]The LOD was calculated as 3σ, where σ is the relative standard deviation of 6–7 analyses of a low-concentration standard in the matrix of interest.
[b]The LOQ was calculated as 10σ, where σ is the relative standard deviation of 6–7 analyses of a low-concentration standard in the matrix of interest.

the peak area detected in the quantitation MRM channel of its internal standard. For $^{13}C_5$-ATO, a cross-talk of 0.03% was calculated; in other words, the peak area detected in the quantitation MRM channel of ATO was 0.03% of that detected for the quantitation ion of $^{13}C_5$-ATO (Fig. S4). This level of cross-talk is neglible in comparison with the purity of the internal standard, which was ≥98%. CBZ exhibited a cross-talk of 0.01% (Fig. S5), while that of $^2H_{10}$-CBZ was 0.05% (Fig. S6), both are also negligible compared with the purity of the chemical (≥98%). For EE2 and $^2D_2$-E2, no cross-talk was detected (Figs. S7–S8), because the analyte and its internal standard were different compounds. For TCS, the cross-talk was calculated for both precursor transitions; the cross-talk of TCS was 0.4% for both transitions (Fig. S9), but that of $^{13}C_6$-TCS was 6.1% and 3.1% for the heavier and lighter precursors, respectively (Fig. S10). While these values are greater than those expected based on the purity of the internal standard (≥99%), they remain well under the RSD (precision) associated with the various extractions (9.2%–21.8%), and thus further correction against the impact of cross-talk was deemed unnecessary.

## Matrix effects

To further minimize the potential impact of matrix interactions, we selected an internal standard that closely resembled the properties of each target analyte. For ATO, CBZ, and TCS, an isotopically labelled version of the compound of interest was used, while for EE2, a deuterated estradiol standard was selected, from which the target analyte differs only by the presence of an ethinyl group on C-17 (Table 1). The physico-chemical properties of both compounds are relatively similar, and they exhibited nearly identical elution times (Table 2). The impact of various matrixes on each analyte was investigated at concentrations ranging from 10 to 5000 μg/L. The greatest matrix effects were generally detected at lower concentrations of analyte, since the co-eluents (i.e., matrix) are present in a greater proportion relative to the analyte. The calibration curves for each analyte and matrix prior to correction for variations in ionization efficiency are presented in Fig. S1, while the calibration curves corrected for each analyte using their respective internal standard are presented in Fig. S2.

To illustrate the benefits of integrating an internal standard into the calculation of matrix interactions, the absolute matrix effects, calculated using the approach of Matuszewski et al.,[19] are represented in Fig. S11 for comparison with the approach used in the present study (Fig. 1). Although the differences are minimal for the two aqueous matrixes, the absolute matrix effects calculated using the approach of Matuszewski et al.[19] were greater than those calculated using our method, especially with complex matrixes such as sediment and biota, (note the change in *y* axis, increased to 250%–400% in Figs. S11C–S11E). In the absence of internal standards, the variation in ionization efficiency brought about by the presence of matrix co-eluents is incorporated into the absolute matrix effect, thus increasing its intensity. These data are in agreement with the calibration curves presented in Fig. S1, where, for example, a significant matrix enhancement was associated with the presence of sediment or biota with TCS (see Fig. S1D).

**Table 4.** Recovery (accuracy ± SEM (precision)) of four pharmaceuticals and personal care products in aqueous samples, and cartridge selected for each PPCP investigated. In each case, $n = 3$ and optimum recoveries are denoted in bold font.

| | Recovery[a] (%) | | | |
|---|---|---|---|---|
| | ENVI-18 | MAX | HLB | Cartridge selected |
| Atorvastatin | 62±1.3 (3.6) | 71±4.9 (11.9) | **71±1.9 (4.7)** | HLB |
| Carbamazepine | **95±3.4 (6.1)** | 82±1.2 (1.5) | 75±4.5 (6.0) | ENVI-18 |
| 17α-Ethinylestradiol | **92±7.9 (14.7)** | 79±5.2 (11.4) | 86±8.1 (16.3) | ENVI-18 |
| Triclosan | **103±5.5 (9.2)** | 96±13.2 (33.5) | 43±3.8 (8.9) | ENVI-18 |

**Note:** Precision was expressed as RSD × 100.

[a]Cartridges selected for recovery experiment were Supelco ENVI-18, Waters Oasis MAX, and Waters Oasis HLB.

**Table 5.** Recovery (% ± SEM (precision)) of four pharmaceuticals and personal care products in sediments by pressurized liquid extraction (PLE). In each case, $n = 3$.

| Compound | Recovery (%) |
|---|---|
| Atorvastatin | 70±2.5 (6.2) |
| Carbamazepine | 89±2.7 (5.2) |
| 17α-Ethinylestradiol | 116±4.0 (6.0) |
| Triclosan | 109±6.2 (9.9) |

**Note:** Precision was expressed as RSD × 100.

**Table 6.** Recovery (% ± SEM (precision)) of four pharmaceuticals and personal care products in biota, after sonication. In each case, $n = 6$.

| | Compound | Recovery (%) |
|---|---|---|
| *Chironomus tentans* | Atorvastatin | 61±2.8 (11.0) |
| | Carbamazepine | 118±2.2 (4.5) |
| | 17α-Ethinylestradiol | 98±5.7 (14.3) |
| | Triclosan | 69±7.0 (21.8) |
| *Hyalella azteca* | Atorvastatin | 108±2.8 (6.3) |
| | Carbamazepine | 129±1.4 (2.6) |
| | 17α-Ethinylestradiol | 95±2.9 (7.4) |
| | Triclosan | 81±3.3 (17.0) |

**Note:** Precision was expressed as RSD × 100.

### *Water*

When the compounds of interest were dissolved in reconstituted water, the presence of salts generally had minor effects on compound ionization, which were usually adequately corrected by the selected internal standards (Fig. 1). CBZ was generally insensitive to either Recon A or B, as reflected by the low amplitude of matrix effects (5% enhancement) and the absence of differences in matrix interactions between the two water types. With a p$K_a$ of 14, CBZ is a very strong proton acceptor compared with other bases in solution, such as $HCO_3^-$ (p$K_a$ 6.1). Consequently, CBZ will be preferentially protonated, regardless of the differences in the composition of Recon A or B. In contrast, the presence of salts in the softer water (Recon A) caused, on average, 4% and 8% suppression with TCS and ATO, respectively (Fig. 1). This interaction is not surprising, given the known ability of sulfates and phosphates to cause matrix suppression.[8] However, effects were more noticeable with EE2, where the addition of salts caused, on average, 12% matrix enhancement, which was most pronounced at lower concentrations (Fig. 1). The addition of further salts, as found in Recon B, did not appear to significantly affect CBZ and EE2, leaving matrix effects relatively unchanged. Meanwhile, Recon B yielded greater matrix suppression (14% on average) of ATO than did Recon A (Fig. 1). Matrix suppression was not dependent on ATO concentration ($p = 0.99$), suggesting that the increased suppression is attributable to differences between the two matrixes (Recon A and B). The main difference in these two systems lies in their relative alkalinity and buffering capacity, with Recon A having an alkalinity of 0.11 mequiv. (compared with 0.35 mequiv. for Recon B), and a buffering capacity of 84 mg $NaHCO_3$ (vs. 192 mg $NaHCO_3$ for Recon B). In effect, the higher alkalinity and buffering capacity of Recon B would serve as a "proton sponge", steadily reducing the protonation of ATO, regardless of concentration. The reduced protonation of ATO in solution would thus lead to a greater suppression in Recon B than in Recon A, due to the presence of a larger quantity of a stronger base ($HCO_3^-$).

Addition of 0.5 mol/L ammonium carbonate base post-injection facilitated the ionization of EE2 in $ES^-$ mode, resulting from the increased alkalinity of the mobile phase. The presence of carbonate (via $HCO_3^-$) in Recon A and B likely further increased the alkalinity of the mobile phase, thus facilitating the ionization of the target analyte even further, and resulting in matrix enhancement. In the case of TCS (p$K_a$ 7.9), it is suspected that the alkalinity in Recon B (pH 8.4) may have facilitated ionization, thus causing a slight matrix enhancement (3%), whereas Recon A (pH 7.7) may not have been sufficiently alkaline to fully ionize a compound of comparable p$K_a$ (as expected under the Henderson–Hasselback equation), as seen by the overall 6% matrix suppression.

### *Sediments*

In contrast with the generally minor matrix effects in water, the presence of sediment extracts had a noticeable impact on compound ionization, especially with ATO, where the signal amplitude was reduced 10 fold in comparison with Milli-Q water (see Fig. S1). This is also reflected in the higher limits of detection and quantitation associated with analyses in sediment extracts, which increased by a factor of 2–8 compared with the standards prepared in Milli-Q water (Table 3). When the appropriate internal standards were used to correct for these variations in ionization efficiency, the matrix effects varied, on average, be-

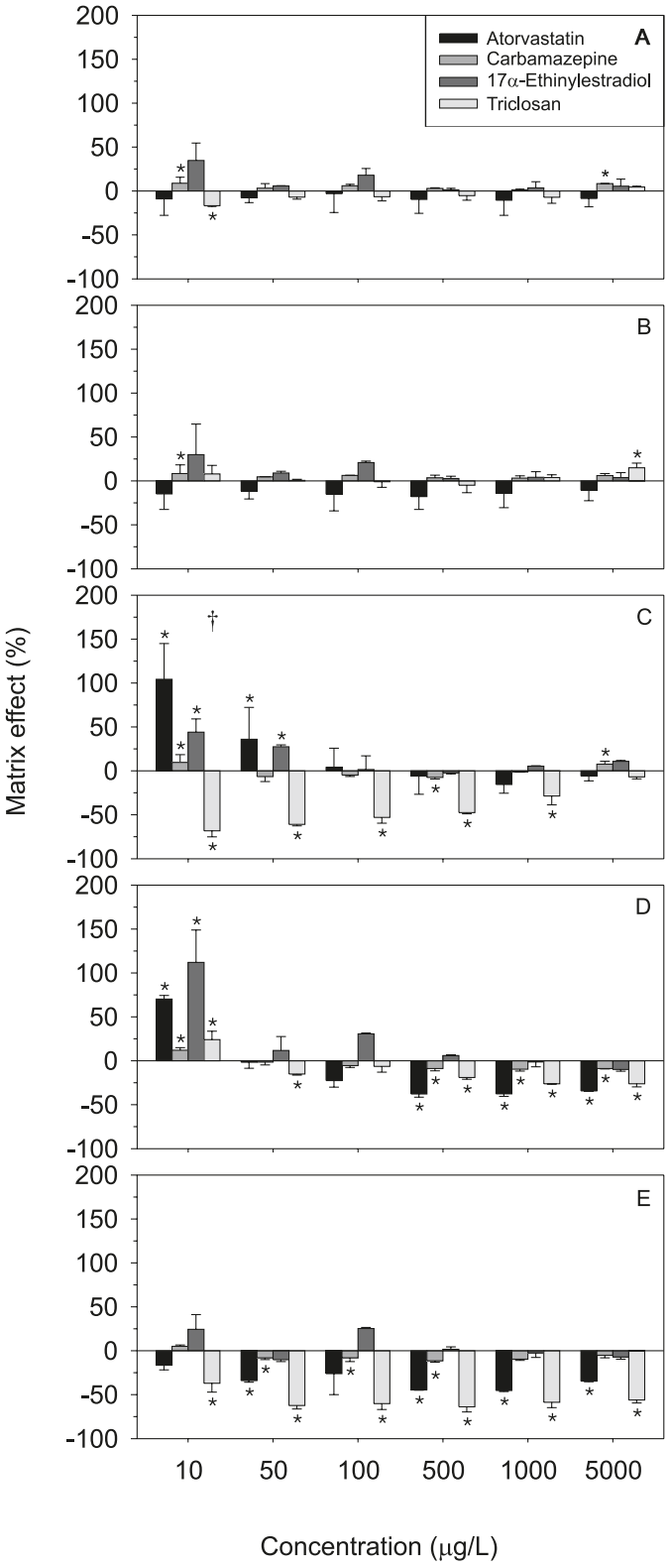



**Fig. 1.** Matrix effects (mean ± SEM; $n = 2$) in two types of reconstituted water (A, B), sediment extracts (C), and biota extracts (D, *Chironomus tentans*; E, *Hyalella azteca*) with increasing standard concentration on four pharmaceuticals and personal care products. The matrix effect signifies the compound is *X*% more or less recoverable than in the absence of the matrix of interest. * denotes values significantly different from zero ($p < 0.05$). †denotes concentrations below the limit of detection (LOD) and should be interpreted with caution.

tween 39% suppression (TCS) and 19% enhancement (ATO). There were negligible matrix effects associated with CBZ (no overall matrix effect), while EE2 showed, on average, a 15% enhancement (Fig. 1). All effects were concentration-dependent, with most significant interactions at the lowest concentration investigated (10 μg/L, $p \leq 0.012$), where the relative amount of matrix constituents to target analytes were highest. The presence of humic and fulvic acids have been reported to cause matrix suppression in the LC–MS analysis of the pesticide dichlorvos, as well as naphthenic acids,[41,42] and may have been responsible for the matrix interactions observed in the present study. Given the comparatively low p$K_a$ of ATO, the addition of such acidic components to the ionization matrix would increase the protonation of ATO, leading to the observed matrix enhancement. At low concentrations of ATO, the higher [acid]:[ATO] ratio would lead to proportionately greater ATO protonation than would be seen at higher ATO concentrations. In contrast, because of its high p$K_a$, the presence of additional acids had no impact on CBZ ion formation. Lastly, suppression was observed at all but the highest TCS concentration (Fig. 1). The p$K_a$ (7.9) of TCS was closest to the pH of the sediments (8.17), and was the compound most affected by the presence of dissolved acids.

### *Biota*

Matrix interactions in the presence of biota extracts were remarkably similar to observations made with other matrixes. The presence of *C. tentans* extracts had neglible effects on the analysis of CBZ, with an overall matrix suppression of 4% (Fig. 1). A 25% matrix enhancement was observed with EE2, while an average matrix suppression of 11% and 14% was observed with ATO and TCS, respectively (Fig. 1). However, these values were mainly due to a significant matrix enhancement observed at the lowest concentration with all analytes in *C. tentans* extracts: CBZ (15%), TCS (24%), ATO (70%), and EE2 (112%; $p \leq 0.0024$). The effects then became of lower magnitude with increasing concentrations (Fig. 1), as the relative ratio of matrix:analyte decreased. Note that while the lowest concentrations used for EE2 and TCS with *C. tentans* were below the LOQ, the concentrations exceeded the LOD. Since the compounds could be detected, the impact of the matrix on the ionization response could thus be determined.

Given the smaller animal size, and hence, lower biomass extracted, we expected the magnitude of effects in *H. azteca* extracts to be lower than in *C. tentans*. Indeed, matrix effects of low magnitude were observed with CBZ (6% suppression) and EE2 (5% enhancement; Fig. 1). However, greater matrix suppression was observed with ATO and TCS, throughout the range of concentrations investigated, with average suppressions of 33% and 56%, respectively, indicating that the composition of the matrix plays a greater role than its quantity. Given the wide variety of possible co-eluents associated with each species (particularly ionizable groups, such as amines from amino acids or phenolic moieties found in lipids, which are present in greater relative abundance in *H. azteca*, 6%,[43] than in *C. tentans*, 1%[44]), we

recommend that further investigations be conducted to identify the origin of this matrix suppression.

Recent studies dedicated to the extraction of PPCPs from fish tissues have reported the presence of significant matrix effects.[39,45] While investigating the extraction of the selective serotonine re-uptake inhibitors from fish tissue, Chu and Metcalfe[45] reported matrix suppression of 19%, 35%, and 39% for paroxetine, fluoxetine, and norfluoxetine, respectively. Another study also describing analytical methods for the extraction of PPCPs from fish tissue reported matrix effects varying between –95% for miconazole and +867% for erythromycin.[39]

In some respects, the observation of matrix effects in the presence of isotopically labelled analogues of the target analytes themselves may appear somewhat surprising. After all, it has been posited that the structural similarity of isotopically labelled substrates to their unlabelled analogues should lead to both compounds exhibiting similar behaviour, and hence, similar matrix effects. However, it has been well-established that acid dissociation constants (much like kinetic rate constants) are subject to often significant isotope effects. For example, laccase has been shown to have a p$K_a$ of 8.98 in water, whereas in deuterated water, the p$K_a$ becomes 9.24.[46] Meanwhile, the p$K_a$ of chloracetic acid differs from that of $^2$H-chloracetic acid labelled in the alpha position.[47] If the labelled and unlabelled compounds have different susceptibilities to ionization (as suggested by their differing p$K_a$ values), then matrixes that can affect ionization in the mass spectrometer will have different impacts on the labelled and unlabelled compounds; hence, differing matrix effects will be observed.[9]

## Conclusions

This study aimed to develop simple and straightforward methods for the extraction of four PPCPs from water, sediments, and biota, with the objective of subsequently applying these techniques to toxicological studies. The four compounds of interest were successfully extracted from water by solid-phase extraction with recoveries of at least 71%. Meanwhile, the pressurized liquid extraction method, developed to extract PPCPs from sediments, yielded recoveries > 70%. Lastly, the liquid extraction method, developed specifically to accommodate small tissue samples, yielded recoveries for the four compounds ranging from 61% to 129%.

Despite the use of carefully chosen internal standards to appropriately correct for variation in the ionization efficiency of each analyte, matrix effects of variable direction and amplitude were detected in the four PPCPs investigated. Although matrix suppression of greater amplitude has been observed in more polar compounds,[7] we found that physicochemical properties of each analyte, especially pH–p$K_a$ interactions, could explain the direction and even magnitude of the matrix effect. For example, because of its high p$K_a$ of 14.0, CBZ was not particularly sensitive to matrix effects at circumneutral pH. Meanwhile, matrix suppression was generally observed in ATO, which had the lowest p$K_a$ (4.46), and for which the addition of alkaline salts likely caused significant matrix suppression. On the other hand, sediments having low concentrations of ATO were subject to matrix enhancement, presumably due to the additive effect of acidic co-eluents, such as humic and fulvic acids. Conversely, EE2 was generally susceptible to matrix enhancement in the presence of alkaline salts, which improved the ionization efficiency in negative electrospray mode. However, the presence of sediment co-eluents (presumably of acidic nature) had suppressive effects at low analyte concentrations. Lastly, TCS was the analyte most susceptible to matrix effects, exhibiting considerable matrix suppression in sediment and biota matrixes. We believe that its circumneutral p$K_a$ (7.9) makes this compound more sensitive to pH-related changes. Overall, we have shown that matrix effects exist and may not be fully corrected using isotopically labelled analogues of target analytes. Our results demonstrate that potential matrix interactions should be taken in consideration during the development and application of analytical methods for pharmaceuticals and personal care products in environmental and biological matrixes, since failure to do so could drastically bias quantification and undermine study validity.

## Supplementary data

Supplementary data for this article are available on the journal Web site (canjchem.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0R6, Canada. DUD 3925. For more information on obtaining material, refer to cisti-icist.nrc-cnrc.gc.ca/cms/unpub_e.shtml.

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