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Simultaneous analysis of select pharmaceuticals and personal care products in fish tissue using pressurized liquid extraction combined with silica gel cleanup

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ABSTRACT

Analytical improvements were developed and validated for measuring select personal care products (PCPs) and two pharmaceuticals in fish tissue. The method was validated using fortified fillet tissue for twelve PCPs including fragrance materials, alkylphenols, photo initiators, and triclosan as well as two pharmaceuticals including carbamazepine (anti-seizure) and diazepam (anti-convulsant). The analytical method utilized pressurized liquid extraction (PLE) combined with silica gel cleanup, gel permeation chromatography, and gas chromatography ion-trap tandem mass spectrometry. Silica gel cleanup was combined with the PLE to produce one automated extraction/cleanup technique. This analytical improvement served to reduce the incurred cost, time, and loss of potential target analytes associated with independent cleanup steps. The combined extraction/cleanup technique resulted in an average increase of 10% in analyte recoveries. Average triplicate recoveries and relative standard deviations for the entire method, using 2.5 g of fish fillet tissue, were 92 ± 9% (recoveries ranged from 64 to 131%). The sensitivity of the analytical methods was improved by optimizing the resonant collision induced dissociation energy to the hundredths place (0.01 V). Improvements in ion production range from 24 to 122% for six of the 12 PCPs. Statistically derived method detection limits (MDLs) were also lowered on average by a factor of 8 and ranged from 1.2 to 38 ng/g wet weight. MDLs for carbamazepine and diazepam were 18 and 3.7 ng/g wet weight, respectively. Galaxolide and tonalide were measured in an environmental sample at concentrations of 81 and 5.5 ng/g wet weight, respectively.

1. Introduction

Pharmaceuticals and personal care products (PPCPs) are an emerging class of environmental contaminants that include pharmaceuticals (human and veterinary), active ingredients in personal care products (PCPs) such as neutraceuticals, cosmetics, surfactants, contrasting agents, and many others [1]. Wastewater treatment plants (WWTP) are capable of removing select PPCPs at appreciable percentages [2]; however, WWTP effluent is the major source of these compounds in aquatic environments [3,4]. PPCPs are continuously discharged to the aquatic environment without any restrictions [1,5]. A wide range of PPCPs have been detected at levels ranging from ppt to ppm in wastewater effluents [3,6] as well as river water [7], suspended particulate matter [8], and lake water [9,10], which are impacted by wastewater effluent. PPCPs have also been detected in fish tissues: fillets in Denmark [11], Switzerland [12,13], Germany [14–17], Japan [18,19], USA [20–22]; liver in Japan [18] and USA [20]; blood plasma in Sweden [23], and brain in the USA [24]. As wastewater-derived compounds, PCPs are continually replenished to the aquatic environment, which causes them to be 'pseudopersistant' despite their relatively short environmental half-lives [25]. Select PPCPs such as galaxolide, tonalide, triclosan and octocrylene are relatively lipophilic ($\log K_{ow}$ = 5.17–7.53 [20]) and have demonstrated the ability to bioaccumulate through the food chain [14,20].

A wide range of analytical methods have been developed to analyze PCPs in fish tissue. Analytical methods have utilized a wide assortment of extraction techniques (Soxhlet, microwave assisted extraction, focused ultrasound-solid liquid extraction, and pressurized liquid extraction (PLE)) and cleanup techniques (silica gel, florisil, and/or gel permeation chromatography (GPC) cleanup) prior to the analysis with GC–MS or GC–MS^n [13,15,16,26–28]. Pharmaceuticals in fish tissues are typically analyzed utilizing liquid chromatography triple quadrupole tandem mass spectrometry with an electrospray ionization interface [20,22]. However, a single method capable of analyzing PCPs and pharmaceuticals has not

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been reported. Typically, silica gel and GPC are required for the analysis of PCPs in a complex biological matrix such as fish tissues [21]. Combining PLE with different cleanup techniques such as silica and florisil has been reported for more nonpolar analytes such as dioxins and polycyclic aromatic hydrocarbons [29–31]. PLE combined with the silica gel cleanup technique reduced the sample preparation time and extraction solvents by 15% and 52%, respectively, as compared to extraction followed by an independent silica gel cleanup step [29]. However, the improvements in sample preparation and sensitivity associated with combining PLE and silica gel cleanup have not been reported in the literature for relatively more polar analytes, such as PPCPs.

Additional sensitivity and lower method detection limits (MDLs) can be achieved with increased fragmentation efficiencies, through the optimization of the collision induced dissociation (CID) voltage. Kemmochi et al. demonstrated that the optimization of damping gas flow rate and CID voltage on the analysis of tetrachlorodibenzo-dioxin decreased the limit of detection by a factor of 3.2 [32]. Mottaleb et al. reported the optimizations of CID to the tenths place (100 mV) for the analysis of PCPs using GC–MS/MS ion-trap [21]. However, additional improvements in instrumental sensitivity and MDLs for the analysis of PPCPs maybe achieved with the optimizations of CID voltages to the hundredths place (10 mV).

The objective of this research was to develop and validate a single analytical method capable of quantifying a wide range of PPCPs in fish tissue at low ng/g concentrations. Twelve PCPs and six pharmaceuticals were selected as environmentally relevant molecular markers of urban wastewater effluent. Sample preparations were improved for simultaneously extracting and purifying PPCPs in fish tissue by combining PLE and silica cleanup techniques. PLE also allows increased tissue mass to be utilized in extractions resulting in lower detection limits by increasing the overall contaminant mass extracted. Optimizations of CID to the hundredths place increase the ion abundance of select PPCPs. Overall, sensitivity was increased and lower MDLs were achieved through the development of new extraction/cleanup techniques and excitation amplitude optimization.

2. Experimental

2.1. Chemicals and materials

All chemicals were purchased as reagent grade or better from commercial vendors and have been previously described by Mottaleb et al. [21] and Ramirez et al. [20,22]. Personal care products analytes include *m*-toluamide, benzophenone, 4-methylbenzylidine camphor (4-MBC), octocrylene, celestolide®, galaxolide®, tonalide®, musk xylene, musk ketone, *p*-octylphenol, *p*-nonylphenol, and triclosan. Surrogates and internal standard include benzophenone-d_{10}, pentachloronitrobenzene (PCNB), *p*-nonylphenol-$^{13}C_6$, carbamazepine-d_{10} and phenanthrene-d_{10} (internal standard). Pharmaceuticals analytes include carbamazepine, diazepam, fluoxetine, sertraline, diphenhydramine, and diltiazem. These target analytes were selected based on use, environmental relevance, and the analytical capabilities of the GC–MS/MS [20,21].

2.2. Tissue samples

Tilapia fillets, small mouth bass fillets, and bream fillet were selected for method development, optimization and/or validation. Tilapia fillets were purchased locally, while small mouth bass were collected from a remote site of the East Fork Gila River in New Mexico. German Environmental Specimen Bank (GESB) bream fish composites were provided by German Federal Environment Agency from two sites (Belauer See and Rhine River, Bimmen). As part of method validation, triplicate recovery and MDL studies were performed on tilapia and the GESB fish composites, respectively. Tilapia, bass, and bream (Belauer See) tissue used in this study were assumed to have low concentrations of PCPs due to their perceived lack of exposure to wastewater effluent. Fish tissue fillets homogenization has been previously described [21] and followed standard USEPA protocols. Samples were stored at −85 °C prior to extraction.

Table 1
Percentage recovery[a] with different solvents and their combinations (v/v).

Solvent(s)	% Recovery						
	PCP[b]	CBZ	DZP	DPH	FLX	DTZ	STL
HEX	54 ± 22	ND	74	ND	ND	ND	ND
CHX	47 ± 29	<10	ND	33	ND	ND	ND
DCM	73 ± 21	<10	ND	<10	ND	ND	ND
EA	76 ± 12	81	82	<10	ND	ND	ND
DCM:EA (1:1)	75 ± 12	92	80	<10	ND	ND	ND
DCM:MeOH (8:2)	66 ± 14	86	79	72	<10	<10	ND
EA:MeOH (8:2)	65 ± 11	88	76	27	ND	ND	ND
DCM:MeOH (9:1)	71 ± 12	90	89	<10	<10	ND	ND
EA:MeOH (9:1)	77 ± 10	103	93	18	10	<10	ND
ACE	73 ± 13	96	94	ND	<10	ND	ND

[a] With silica gel in ASE-cell.
[b] Average percentage recovery of all personal care product analytes. CBZ (carbamazepine), DZP (diazepam), DPH (diphenhydramine), FLX (fluoxetine), DTZ (diltiazem), STL (sertraline), and ND (not detected).

2.3. Pressurized liquid extraction solvent optimization

Extraction solvent efficiency was examined in terms of PPCPs percentage recoveries. Extraction efficiencies of hexane (HEX), cyclohexane (CHX), DCM, EA, acetone (ACE), methanol (MeOH), and solvent combinations (DCM:EA, DCM:MeOH, and EA:MeOH) were examined using PLE combined with silica gel cleanup (Section 2.4). Small mouth bass tissue composites (~3 g) were fortified prior to PLE with target analytes and surrogates at continuous calibration verification (CCV) concentrations (Section 2.7). Extracts were concentrated and solvent exchanged to DCM (if necessary) for GPC cleanup. GPC eluate underwent concentration, derivatization, and analysis as described in Sections 2.5 and 2.6.

2.4. Pressurized liquid extraction combined with silica gel cleanup

The analysis of semi-volatile organic compounds by GC–MS in matrices such as fish tissue usually requires the removal of the more polar interferences [21]. As part of the sample preparation, silica-gel chromatography has been widely used to remove such polar interferences [12,15,16,21]. The analysis of PCPs in fish tissue also requires a silica gel cleanup technique. Excluding this cleanup step resulted in additional instrument maintenance and reduced analyte response. Typically, sample extracts are allowed to pass through a packed silica gel (SiO_2) column after extraction. During the silica cleanup step, columns are conditioned with the necessary solvent(s) and extracts are concentrated both prior to and after silica gel cleanup (for more information about solvents selection: Table 1). During the combined extraction/silica gel cleanup technique, the fish tissue homogenate was placed at the top of precleaned silica gel. Within the Acceleration Solvent Extractor (ASE) cell, the extracting solvent passes through the tissue homogenates where target analytes and interference are extracted and then the extract passes through pre-cleaned silica gel where polar interferences are removed.

The combined silica gel cleanup technique was validated for PCPs in fish tissue using ~2.5 g of tilapia fish tissue composite. Six fish tissue samples homogenized with sodium sulfate were spiked with known concentrations of target analytes and

surrogates within the ASE cell. Spiked homogenates were allowed ~30 min to equilibrate and evaporate solvent prior to extraction, and then underwent PLE using an ASE 350 (Dionex, Sunnyvale, CA), silica cleanup, and GPC cleanup (see below). Three of the six samples were extracted using PLE combined with silica gel cleanup described above while the remaining three fish tissue samples were extracted without silica gel present in the ASE cells under the same PLE conditions. These three extracts were subsequently passed through a packed silica column. Silica gel column cleanup for the analysis of PCPs in fish tissues was previously explained by Mottaleb et al. [21]. After silica gel cleanup, all extracts were followed by GPC cleanup and spiked with an internal standard prior to GC–MS/MS analysis.

Under final conditions, samples were allowed to thaw in the dark for 1 h and ~2.5 g composites were homogenized with anhydrous Na_2SO_4 (1:24 ratio) to remove excess water using a mortar and pestle. Sodium sulfate was baked at 500 °C for 5 h and allowed to cool prior to use. The fish tissue homogenate was packed into a 66 mL ASE cell (Dionex, Sunnyvale, CA) that contained 14 g of pre-cleaned silica gel (pore size 40 Å, 70–230 mesh). Samples were extracted with a ASE 350 using 1:1 (v/v) dichloromethane (DCM):ethyl acetate (EA) (80 °C, 1500 psi, 2 cycles of 5 min static time, 75% flush volume). Silica gel was pre-cleaned using the ASE under identical sample extraction conditions. DCM rinses were used between sample extractions. Prior to extractions, samples were spiked in the ASE cell with target analytes and surrogates. The fish tissue extract was concentrated to 0.5 mL in the Turbo-Vap II (Zymark, Hopkinton, MA) under a gentle stream of nitrogen at 30 °C and solvent exchanged to DCM. The concentrated extract was transferred to a GPC vial with a final volume of approximately 700 μL.

2.5. GPC cleanup and derivatization

The higher molecular weight interferences were removed using a Waters GPC Cleanup System (Milford, MA) as previously described [21]. Briefly, the Waters GPC system consisted of a 600 Controller, 717 plus Autosampler, 486 Autotunable Absorbance detectors, HP 3398 Series integrator, Envirogel GPC guard column (4.6 mm × 30 mm) and two analytical Envirogel columns (19 mm × 150 mm and 19 mm × 300 mm, Waters, MA, USA) connected in series. DCM was used as the mobile phase at a flow rate of 5 mL/min. Sample volume of 700 μL was analyzed with 24 min total run-time. The retention time of each of the target analytes was determined individually. PPCPs were collected from 11 to 19 min and the target analyte cutoff times were verified before each batch of samples using individual PPCPs standard.

The target analyte fraction collected from the GPC (~40 mL) was concentrated to ~200 μL with nitrogen and solvent exchanged to hexane using the Turbo Vap II. Select target analytes were derivatized with 100 μL of N-methyl-N-(trimethylsilyl)-trifluoroacetamide. Mixtures were incubated at 70 °C for 1 h and then allowed to cool. Derivatization was followed by concentration under a gentle stream of nitrogen at 21 °C within a 500 μL glass insert and spiking with 20 μL of 2 ng/μL phenanthrene-d_{10} to a final volume of approximately 200 μL.

2.6. Extract analysis

Target PCPs were quantified using gas chromatography–tandem mass spectrometry (GC–MS/MS). Fish tissue extracts were analyzed on a Varian (Palo Alto, CA, USA) CP-3900 GC system coupled with a Saturn 2100T ion trap mass spectrometer. One microliter from ~200 μL extracts was injected with a CP-8410 autoinjector (pulsed splitless at 270 °C). Target PCPs and surrogates along with carbamazepine, diazepam, diphenhydramine, diphenhydramine-d_3,

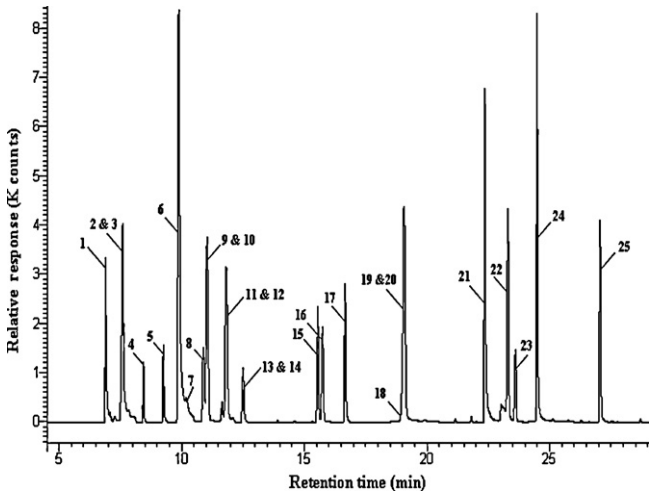

Fig. 1. A representative GC-chromatogram for PPCPs standard solution containing all analytes, surrogates and internal standards: (1) *m*-toluamide, (2) benzophenone, (3) benzophenone-d_{10} surrogate, (4) celestolide, (5) PCNB, (6) phenathrene-d_{10} internal standard, (7) *p*-octylphenol, (8) galaxolide, (9) tonalide, (10) musk xylene, (11) diphenhydramine, (12) diphenhydramine-d_3 surrogate, (13) *p*-nonylphenol, (14) *p*-nonylphenol-$^{13}C_6$ surrogate, (15) 4-MBC, (16) musk ketone, (17) triclosan, (18) fluoxetine, (19) carbamazepine, (20) carbamazepine-d_{10} surrogate, (21) diazepam, (22) sertraline, (23) mirex, (24) octrocrylene, and (25) diltiazem.

diltiazem, fluoxetine, and sertraline were chromatographically separated using 30 m × 0.25 mm, 0.25 μm of HP-5 MS capillary column (Agilent technologies, West Chester, PA, USA) with helium carrier gas (99.999%) at a flow rate of 1 mL/min. The GC oven temperature program was: 100 °C held for 1 min, ramped at 15 °C/min to 180 °C, held for 5 min, ramped at 6 °C/min to 280 °C, ramped at 15 °C/min to 310 °C, and held for 15 min, total run-time of 44.8 min. A representative total ion chromatogram for the solution containing all target analytes, surrogates, and internal standard under the above stated conditions is shown in Fig. 1.

PPCPs precursor ions were identified using GC–MS with electron impact ionization and the mass analyzer operated in full-scan mode. Precursor ions were selected based on ion abundance, uniqueness, and secondary fragmentation patterns. Selected precursor ions were fragmented via CID at different voltages (excitation amplitudes) by an automated method of dissociation. Excitation amplitudes in resonant mode ranged from 0.0 to 1.0 V and are typically optimized to the tenths place [21]. The precursor ions, fragmentation ions (quantitative and qualitative ion), excitation storage, excitation amplitudes, and optimized excitation amplitudes for the target analytes and surrogates are provided in Table 2. In this study, a secondary optimization step allowed for the excitation amplitudes to be optimized to the hundredths place. This additional optimization step resulted in increased analyte response for approximately 75% of the PPCPs analytes (Table 2).

2.7. Analyte identification and quantitation

Analyte peak identification was dependent upon on retention time (±0.05 min) and the ratio of qualitative to quantitative ion response (±20%). An internal standard method of quantification was employed. Calibration curves with at least seven points were prepared by plotting the concentration-dependent response factor of each target analyte (peak area of analyte divided by peak area of internal standard) versus the response dependent concentration factor (concentration of analytes divided by concentration of internal standard). Coefficient of determination (r^2) was greater than

Table 2
Optimization of excitation amplitude of PPCPs.

Compounds	MS/MS transitions (*m*/*z*)	Excitation storage (*m*/*z*)	Excitation amplitude (V)	Optimized excitation amplitude (V)	Quantitative ion response increase (%)
m-Toluamide	190 > **145**[a], 175[b]	83.6	0.7[b]	0.69	3
Benzophenone	182 > **153**[b]	80.1	0.8[b]	0.83	8
Celestolide	229 > **173**, 131[b]	101	0.7[b]	0.65	122
p-Octylphenol	278 > **179**[b]	123	0.6[b]	0.63	1
Galaxolide	243 > **213**, 171[b]	107	0.8[b]	0.78	18
Tonalide	243 > **187**, 159[b]	107	0.8[b]	0.78	27
Musk xylene	282 > **265**, 248[b]	124	0.8[b]	0.77	42
p-Nonylphenol	292 > **179**[b]	128	0.6[b]	0.57	5
4-MBC	211 > **169**, 155[b]	134	0.8[b]	0.81	13
Musk ketone	304 > **214**, 287	92.9	0.7[b]	0.72	44
Triclosan	347 > **200**, 310[b]	153	0.8[b]	0.82	24
Octocrylene	250 > **248**, 221[b]	110	0.8[b]	0.76	31
Benzophenone-d_{10}[c]	192 > **190**, 163[b]	84.5	1.0[b]	0.95	51
PCNB[c]	295 > **237**, 263	130	0.8[b]	0.78	19
p-Nonylphenol-$^{13}C_6$[c]	298 > **185**[b]	131	0.9[b]	0.87	17
Carbamazepine	193 > **191**, 165	85.0	0.9	0.90	–
Diazepam	285 > **248**, 268	126	1.8	1.78	29
Sertraline	274 > **239**, 259	121	0.5	0.50	–
Diltiazem	222 > **207**, 192	97.8	0.5	0.53	13
Fluoxetine	264 > **160**, 115	116	0.6	0.56	58
Diphenhydramine	165 > **163**, 115	72.6	0.9	0.90	–
Carbamazepine-d_{10}[c]	203 > **201**, 175	89.4	0.7	0.70	–
Diphenhydramine-d_3[c]	152 > **150**, 126	66.8	0.8	0.80	–

[a] Bold denotes quantitation ion.
[b] Reported by Mottaleb et al. [21].
[c] Surrogate.

0.99, and average response factor relative standard deviation (RSD) was 7.4%, however, response factor RSDs for triclosan and tonalide were 17%.

A CCV standard was run at regular intervals (every 4th injection) to ensure quality assurance and quality control in every batch of samples analyzed. The CCV standard was prepared with analyte concentrations near the middle point of the corresponding calibration curve and used to verify the accuracy of the target analyte calibration curves. Observation of analyte recoveries resulting from CCV analyses beyond the accepted ±25% range required maintenance such as replacing the injector liner, removal of a few centimeters of the head of the capillary column, and/or constructing a new matrix-matched calibration curve. Solvent blanks that were analyzed both before and after each sample batch showed no carryover of target analytes.

2.8. Triplicate recovery and method detection limit (MDLs) studies

The entire analytical method was validated using fortified control matrices (~2.5 g of tilapia fish composites) with analytes spiked at the second lowest calibration level and surrogates spiked near the middle of the calibrated linear range. Composites were extracted, cleaned, and analyzed with GC–MS/MS as explained in Sections 2.4–2.6 for recovery and statistically derived MDLs studies. Background analyte concentrations were determined using a single 2.5-g composite that was spiked with surrogates prior to extraction.

Statistically derived MDLs were determined using seven replicates of ~2.5 g of bream fish composite (Belauer See) that were fortified with target analytes (spiking level ≤10 × MDL) (Table 3). MDLs were determined by multiplying the standard deviation times the one-sided Student's *t*-statistic (99% confidence) [33]. Composite samples were spiked with analytes and surrogates prior to extraction at the concentrations specified above. Background analyte concentrations were determined using a single 2.5 g composite that was spiked with surrogates prior to extraction. Spiked composites were extracted, cleaned, and analyzed with GC–MS/MS as explained above. Background corrected triplicate recovery data and MDLs are reported in Table 3.

2.9. Analysis of environmental samples

Applicability of the analytical method was demonstrated by examining PPCPs concentrations in GESB fish tissue (~2.5 g). Samples were collected from a GESB site (Rhine River, Bimmen), homogenized, and spiked with surrogates prior to PLE. Extractions, cleanup, and analyses were performed using the analytical method described above. Duplicate matrix spikes of GESB tissue were fortified with target analytes at CCV concentrations prior to PLE. Matrix spikes concentrations were background corrected.

3. Results and discussions

3.1. Pressurized liquid extraction solvent optimization

Solvent effectiveness was evaluated based on PPCPs percentage recoveries and silica gel cleanup efficiency, which varied among solvents and solvent combinations (Table 1). PCPs percentage recoveries were fairly consistent over a wide range of extraction solvents; however, HEX and CHX provide average PCPs recoveries of 54% and 47%, respectively (Table 1). Percentage recoveries for pharmaceuticals varied widely from solvent to solvent. Carbamazepine and diazepam yield higher percentage recovery (within 76–103%) with more polar solvent and solvent combinations, such as ACE or MeOH:EA. These two pharmaceuticals were not detected in eluate using DCM or CHX. The highest diphenhydramine percent recovery was 72% with 20% MeOH in DCM. Nonpolar solvents (polarity ≤ DCM), did not provide adequate pharmaceuticals recoveries; however HEX recovered 74% of diltiazem. A wide range of extraction solvents, ranging from relatively polar (MeOH) to nonpolar (HEX), provided recoveries of <10% for sertraline, diltiazem, and fluoxetine. This apparent loss of select pharmaceuticals may potential be a result of reduced extraction efficiency of nonpolar solvents, such as HEX and/or the increase matrix interference associated with more polar solvents. Additional interferences, extracted

Table 3
Spiking recoveries, statistically derived method detection limits, and environmental sample analysis (ww data).

Analytes	Triplicate recovery[a]		Environmental sample[b]			Reported MDL[c] (ng/g)	Reported fish tissue concentration (ng/g)
	Spiking level (ng/g)	Recovery (%) ± RSD	Tissue concentration (ng/g)	Matrix spiked recovery (%) (n = 2)	MDL (ng/g) (n = 7)		
m-Toluamide	20	89 ± 6	ND	84.76	4.0	5.1	NR
Benzophenone	38	131 ± 19	ND	75.77	35	16	0.66–90 [13,21,36]
4-MBC	80	82 ± 7	ND	67.79	12	120	0.44–27 [12,13]
Octocrylene	20[d]	91 ± 6	ND	57.69	1.5	36	0.10–69 [12,13]
Celestolide	11	101 ± 13	ND	56.73	4.0	18	0.03–34 [15]
Galaxolide	8	109 ± 5	81.2	72.78	1.6	12	0.52–2500 [15,20,21]
Tonalide	14	103 ± 4	5.5	61.02	3.0	13	0.44–730 [15,20,21]
Musk xylene	220	90 ± 28	ND	76.23	38	397	0.05–41 [14,15]
Musk ketone	360	84 ± 7	ND	57.15	35	321	0.07–66 [14,15]
p-Nonylphenol	8	77 ± 3	ND	70.24	1.2	9.7	3.3–566 [16,34,37]
p-Octylphenol	12	77 ± 5	ND	72.39	3.1	8.2	0.2–6 [16,34,37]
Triclosan	10	64 ± 9	ND	66.07	3.4	38	0.3–31 [17,21,38]
Carbamazepine	16	88 ± 6	ND	64.81	18	0.54[e]	2.3–3.1 [20]
Diazepam	32	97 ± 9	ND	58.35	3.7	8.2[e,f]	23–110[g] [35]

ND denotes non detect; NR denotes not reported.
[a] Tilapia fillet composite (~2.5 g).
[b] Bream fillet composite (~2.5 g).
[c] Reported by Mottaleb et al. employing GC–MS/MS [21].
[d] 13 × MDL (n = 2).
[e] Employing LC–MS/MS technique [20].
[f] LOQ in fish liver tissue [36].
[g] Fish liver concentration.

with ACE and MeOH, also resulted in decreased PCPs percent recoveries and increased instrumentation maintenance.

Overall, the use of more polar extraction solvents appreciably decreased the extraction efficiency of select PCPs as well as decreased silica gel effectiveness as a cleanup technique. DCM:EA (1:1) was the lowest polarity extraction solvent capable of extracting carbamazepine and diazepam with recoveries >80%. DCM:EA (1:1) was selected as the preferred extraction solvent based on the overall PPCPs percent recovery and silica gel cleanup efficiency. This decision resulted in the loss of diphenhydramine, fluoxetine, diltiazem, and sertraline as viable target analytes.

3.2. Pressurized liquid extraction combined with silica gel cleanup

Triplicate recovery experiments using fortified fish tissue were used to compare the combined silica gel cleanup technique to that of a typically packed silica gel column (Fig. 2). Individual target analyte recoveries between the two cleanup techniques were not statistically different ($\rho_{\alpha=0.005} > 0.05$). However, recoveries were significantly increased ($\rho_{\alpha=0.005} < 0.05$) for benzophenone, triclosan, octocrylene, carbamazepine, and diazepam using the combined silica gel cleanup technique as compared to that of a packed silica gel column. PLE combined with silica gel cleanup (DCM:EA) resulted in less than 10% recoveries for diphenhydramine, fluoxetine, diltiazem, and sertraline (Table 1). Due to insufficient recoveries using both silica gel techniques, these analytes were not included in further method development and validation. The average percent recoveries and RSDs for combined silica gel cleanup and packed silica column techniques were 83 ± 21% and 73 ± 15%, respectively. Samples cleanliness was assessed using CCV as well as qualitatively assessed using GPC chromatograms. CCV standards demonstrated less than 20% deviation in target analyte concentrations and were within the accepted ±25% recovery range. GPC was required after both techniques to remove high molecular weight interferences such as cholesterols and long chain fatty acids. GPC chromatograms for both techniques were virtually identical and provided no comparative differences (chromatograms not shown). Combining the necessary silica gel cleanup step with the extraction step improved the cost and time associated with sample preparation as well as resulted in a single automated step.

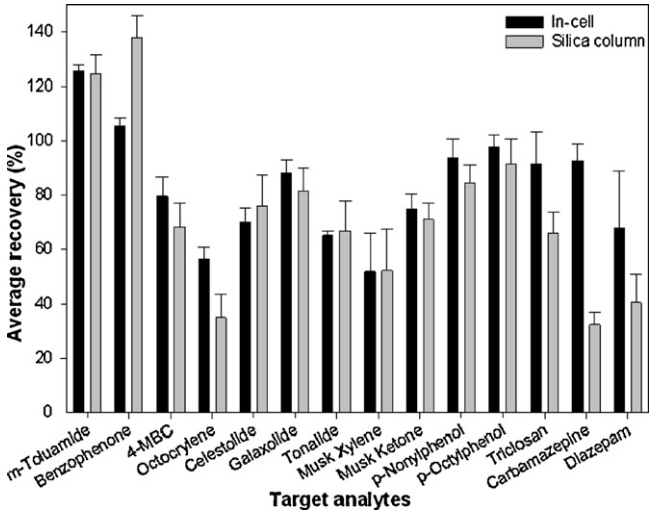

Fig. 2. Triplicate recovery in tilapia fish fillet composites with relative standard deviations for combined silica gel cleanup and typically pack silica column cleanup.

3.3. Excitation amplitude optimization

Optimization of the excitation amplitude to the hundredths place resulted in an increase in ion production (>20%) for six of the 12 PCPs and two of the six pharmaceuticals. The largest increases in the quantitative ion production were for celestolide (122%), fluoxetine (58%), musk ketone (44%), musk xylene (42%), octrocrylene (31%), diazepam (29%), tonalide (27%), and triclosan (24%) (Table 2). The average percentage increase in quantitative ion response for PCPs' surrogates was 29%. A representative chromatogram for the

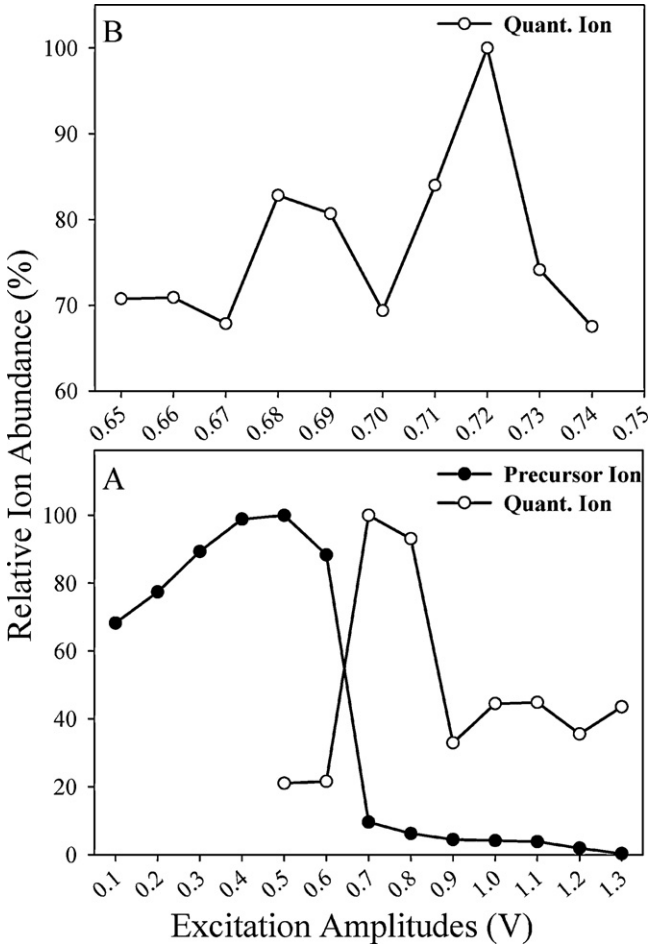

Fig. 3. Differential ion response (normalized to maximum peak area counts) for musk ketone: (A) first step excitation amplitude optimization by 100 mV and (B) second step excitation amplitude optimization by 10 mV.

optimization of excitation amplitudes for musk ketone in resonance mode is given below (Fig. 3). A small change in CID voltage did not consistently provide an increase in ion abundance for all PCPs. For example, CID voltage change for musk ketone by 0.02 V from 0.70 V to 0.72 V resulted in an increase in ion abundance by 44%; however, CID voltage change for nonylphenol by 0.03 V (from 0.60 to 0.57) resulted in only an ~5% increase. Therefore, the percentage increment in quantitative ion response on changing the particular magnitude of voltage was analyte dependent. In general, optimization of the excitation amplitude to the hundredths place increased analyte sensitivity and lowered the corresponding method detection limit.

3.4. Method validation

3.4.1. Triplicate recoveries

The entire analytical method was validated with the triplicate recovery study using 2.5 g of fortified tilapia fish composites (Section 2.8). In general, the PPCPs recoveries were fairly similar; the average percentage recovery was 92% and the RSDs ranged from 3% to 28% (Table 3). However, the average recoveries and RSDs for benzophenone and triclosan were 131% ± 19 and 64% ± 9, respectively. Average PPCPs surrogates' recovery and RSDs were 63 ± 7%.

3.4.2. MDL study

Method sensitivity for select PCP analytes was evaluated by measuring the statistically derived MDLs. Lower MDLs for PCPs were measured as compared to the similar study on sonora sucker reported by Mottaleb et al. [21] (Table 3). MDLs were improved approximately on average by a factor of 8. Lowering MDLs by the factor of ~2.5 is expected due to increased tissue mass from 1.0 g to 2.5 g employing PLE. MDLs improvement of individual target analyte could be a matrix dependent. However, MDLs for nitromusk, musk xylene and musk ketone, decreased by approximately one order of magnitude which are in accordance with increased sensitivity through optimization of excitation amplitude to the hundredths place (42 and 44%, respectively) (Table 2). However, MDL for celestolide was only increased by the factor of ~5 although the sensitivity was increased by 122%. Therefore, the consequences of lowering MDLs and increasing sensitivity by excitation amplitude optimization cannot be generalized for all analytes. MDLs for musk xylene, musk ketone, 4-MBC, and triclosan in this study are an order of magnitude lower than reported by Mottaleb et al. [21] (Table 3). Typically, the reported fish tissue concentrations at or below MDLs reported by this study were measured considering the limit of quantification (LOQ) [14] or limit of detection (LOD) [34]. It has already been demonstrated that the MDLs are typically higher than the LOQ and LOD for pharmaceuticals [22]. It is also important to note, that PCPs fish tissue studies typically employed a single analytical method optimized for particular class of compounds. However, this study allows for the simultaneous analyses of a diverse class of PPCPs.

Carbamazepine and diazepam were the only two pharmaceuticals able to be incorporated with the PCPs for simultaneous analysis of select PPCPs in fish tissue with an acceptable percentage recovery (88% ± 6 and 97% ± 9, respectively) (Table 3). The MDLs for carbamazepine and diazepam were 18 and 3.7 ng/g ww, respectively (Table 3). The carbamazepine MDLs reported in this study, are greater than previously reported fish fillet tissue concentrations [20]. MDLs are less than previously reported fish liver tissue concentrations [35]. Typically, pharmaceuticals fish liver tissue concentrations are greater than fish fillet tissue concentrations [20]. Unfortunately, few analytical methods are capable of measuring diazepam and carbamazepine in fish tissue, and as a result environmentally relevant concentrations reported in the literature in fish tissue are limited. Based on current literature, this method may be more prudent for measuring carbamazepine and diazepam in highly contaminated sampling sites.

3.4.3. Analysis of environmental samples

This analytical method was used to examine environmentally relevant PPCPs concentrations in GESB fish tissue (Rhine River, Bimmen). Two of the fourteen PPCPs were detected above the MDLs. PPCPs surrogate recoveries in GESB fish tissue samples averaged 63%. Galaxolide and tonalide, two polycyclic musk fragrance materials, were measured at 81 and 5.5 ng/g ww, respectively (Table 3). The average PPCPs recoveries for the matrix spikes (GESB bream fish tissues, $n = 2$) ranged from 57 to 85% (Table 3). Therefore, the analytical method describing the simultaneous analysis of select PPCPs in fish tissue is capable of measuring environmentally relevant fish tissue concentrations.

4. Conclusions

An analytical method was developed and validated for the simultaneous analysis of select PCPs, carbamazepine, and diazepam in fish fillet composite using GC–MS/MS. The combined PLE with silica gel cleanup technique reduced the intrinsic cost associated with conventional PCP sample preparation protocol. Analyte sensitivity for select analytes was increased upon optimization of excitation amplitude. The reported methodology is capable of measuring a

wide range of PPCPs at environmentally relevant fish tissue concentrations.

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