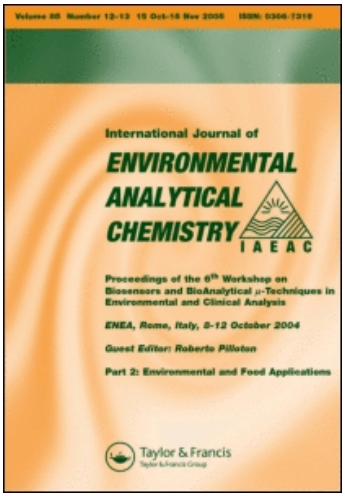

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Determination of acidic pharmaceutical products and carbamazepine in roughly primary-treated wastewater by solid-phase extraction and gas chromatography-tandem mass spectrometry

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To cite this Article Lajeunesse, André and Gagnon, Christian(2007) 'Determination of acidic pharmaceutical products and carbamazepine in roughly primary-treated wastewater by solid-phase extraction and gas chromatography-tandem mass spectrometry', International Journal of Environmental Analytical Chemistry, 87: 8, 565 — 578
To link to this Article: DOI: 10.1080/03067310701189083
URL: http://dx.doi.org/10.1080/03067310701189083

Intern. J. Environ. Anal. Chem.
Vol. 87, No. 8, 15 July 2007, 565–578

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Determination of acidic pharmaceutical products and carbamazepine in roughly primary-treated wastewater by solid-phase extraction and gas chromatography–tandem mass spectrometry

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(*Received 25 October 2006; in final form 20 December 2006*)

A gas chromatography–tandem mass spectrometry (GC–MS/MS) method has been developed for the determination of selected pharmaceutical residues (carbamazepine, salicylic acid, clofibric acid, ibuprofen, 2-hydroxy-ibuprofen, fenoprofen, naproxen, ketoprofen, diclofenac, and triclosan) in sewage influent and roughly primary-treated effluent. The method involved solid-phase extraction (SPE) with polymeric sorbents, and two SPE cartridges were compared for the extraction and elution of the targeted compounds in complex matrices. A successful chemical derivatization of carbamazepine and acidic compounds using *N*,*O*-bis(trimethylsilyl) trifluoroacetamide +10% trimethylchlorosilane is also described. The quantification limits of the analytical procedure ranged from 30 to 60 ng L^{-1} for 500 mL of wastewater. The best recovery rates (72–102%) in spiked effluent samples were obtained with Phenomenex Strata-X^{TM} cartridges. Detection limits ($S/N = 3$) were estimated at between 1 and 18 ng L^{-1}. The reported GC–MS/MS method significantly reduces the strong matrix effects encountered with more expensive LC-MS/MS techniques. Application of the developed method showed that most selected analytes were detected at concentrations ranging from low μg L^{-1} to trace level ng L^{-1} in Montreal's wastewater treatment plant effluent and influent, as well as in the receiving waters at more than 8 km downstream of the effluent outfall. The rugged alternative analytical method is suitable for the simultaneous analysis of carbamazepine and pharmaceutical acidic residues in wastewater samples from influents and effluents that have undergone rough primary treatment.

Keywords: Pharmaceutical products; GC–MS/MS; Acidic drugs; Carbamazepine; Solid-phase extraction; Wastewater

1. Introduction

In the last decade, there as been growing concern about the presence of pharmaceutical and personal-care products (PPCPs) in the aquatic environment. These chemicals range from non-prescription and prescription drugs to antibacterial agents and surfactants commonly found in household products [1, 2]. PPCPs and their metabolites are introduced into the environment via a number of routes, the primary route being the discharge of treated and untreated wastewater to rivers [3]. After ingestion by humans,

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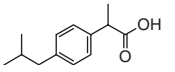
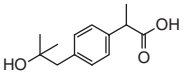
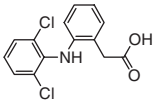
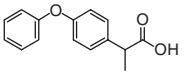
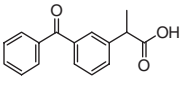
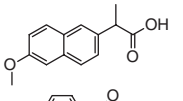
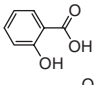
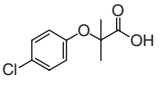
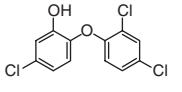
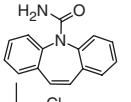
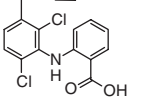
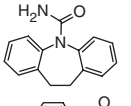
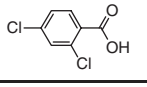
International Journal of Environmental and Analytical Chemistry
ISSN 0306-7319 print/ISSN 1029-0397 online
DOI: 10.1080/03067310701189083

Table 1. Molecular structure of investigated PPCPs with their internal and surrogate standards.

Compounds	Molecular structures	Use
Ibuprofen		Anti-inflammatory/analgesic
2-Hydroxy-ibuprofen		Anti-inflammatory/analgesic (metabolite of ibuprofen) Anti-inflammatory/analgesic
Diclofenac		Anti-inflammatory/analgesic
Fenoprofen		Anti-inflammatory/analgesic
Ketoprofen		Anti-inflammatory/analgesic
Naproxen		Anti-inflammatory/analgesic
Salicylic acid		Analgesic (metabolite of acetylsalicylic acid)
Clofibric acid		Lipid regulator (metabolite of clofibrate)
Triclosan		Antibacterial
Carbamazepine		Antiepileptic
Meclofenamic acid		Surrogate standard
10,11-Dihydro-carbamazepine		Surrogate standard
2,4-Dichlorobenzoic acid		Internal standard

pharmaceutical drugs are excreted as initial molecules, water-soluble conjugates, or metabolites, and thus freely enter the influent of municipal wastewater treatment plant (WTP) [1]. Due to their polar structure, most PPCPs are not totally removed by treatment plants [4–7]. The PPCPs selected for our study are listed in table 1 alongside their respective chemical structures.

Despite the continuous discharge of PPCPs to the environment, few data are available on their occurrence, toxicity, and environmental fate, either around the world

[8–11] or in Canada [12–15]. As such, sensitive and reliable analytical methods are needed to monitor PPCPs in wastewater samples.

Several analytical methods for PPCPs have already been published in the literature. Many of them are based on liquid chromatography–tandem mass spectrometry (LC–MS/MS) [13, 16–18]. However, interference or important signal suppression caused by sample extracts continues to be a major issue, especially with untreated or poorly treated wastewater samples. Matrix effects, for example, are frequently observed when the LC–MS/MS electrospray ionization mode is used [17–19]. Furthermore, the cost of operating an LC–MS/MS system is an important consideration for many research or monitoring laboratories.

Gas chromatography–mass spectrometry (GC–MS) instrumentation is another sound method for analysing PPCPs. Unfortunately, analysis of PPCPs by GC–MS often requires the derivatization of carboxylic acid and hydroxyl moieties to some less polar group. Various GC–MS methods have been described for the analysis of polar drugs in wastewater samples after chemical derivatization with diazomethane [8, 11]. Handling diazomethane, however, carries some risk and demands particular safety procedures as well as secure disposal. Chemical methylation with trimethylsulfonium-hydroxyde directly into the hot injector of a GC has been reported elsewhere [20, 21]. In this case, the new products of this derivatization must be injected within a few hours to ensure sample stability. As an alternative to the above-described chemicals, several silylation reagents have been widely used by researchers. Since 2000, many analytical methods have been successfully developed with *N*,*O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) or *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) in appropriate solvent mixtures [14, 22–24].

Previously reported methods for trimethylsilyl-derivatized compounds focused on only a few pharmaceutical products, including acidic drugs and triclosan. Others reported the analysis of carbamazepine by GC–MS without chemical derivatization. However, the thermal instability of the molecule in injector is such that chemical derivatization must be considered. In fact, underivatized carbamazepine decomposed inside the hot liner, giving a poor peak shape in chromatography and non-reproducible formation of iminostylbene at different carbamazepine concentrations [11, 25, 26].

The aim of this article was to develop a new reliable GC–MS/MS method analysis based on quantitative chemical derivatization with BSTFA of carbamazepine and pharmaceutical acidic residues found in roughly primary-treated wastewater. The sensitive and specific analytical method includes the optimization of a solid-phase extraction (SPE) procedure. Two commercial hydrophilic sorbents (OASIS® HLB and Strata-X™) were compared on spiked purified water and, obviously, poorly treated effluent samples. The developed method was applied to the analysis of 24-h composite sewage samples taken from the inlet and the outlet streams of Montreal's WTP, but also to its effluent outfall in the receiving waters.

2. Experimental

2.1 *Chemicals and reagents*

All pharmaceutical residues (salicylic acid, clofibric acid, ibuprofen, 2-hydroxy-ibuprofen, fenoprofen, naproxen, ketoprofen, carbamazepine, diclofenac, and

triclosan) and standards (2,4-dichlorobenzoic acid, 10,11-dihydrocarbamazepine, and meclofenamic acid) were purchased from Sigma-Aldrich Co. (St. Louis, MO) and Cerilliant Corp. (Round Rock, TX). All pharmaceutical products were of >98% purity. The HPLC-grade solvents used (methanol, acetonitrile, and ethyl acetate) were provided by Caledon Laboratories Ltd (Georgetown, Ontario, Canada) and American Chemicals Ltd (Montreal, Quebec, Canada). Concentrated hydrochloric acid, Celite® 545, and anhydrous sodium sulfate were purchased from American Chemicals Ltd (Montreal, Quebec, Canada), while *N*,*O*-bis-(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane (BSTFA + 1% and 10% TMCS) and *N*-methyl-*N*-(*tert*-butyldimethylsilyl) trifluoroacetamide with *tert*-butyldimethylchlosilane (MTBSTFA + 1% TBDMCS) were manufactured by Pierce and purchased from BioLynx Inc. (Brockville, Ontario, Canada). Solid-phase extraction (SPE) cartridges of 6 mL and 200 mg (OASIS® HLB, Strata-X^{TM}) were purchased from Waters Corp. (Milford, MA) and Phenomenex (Torrance, CA). Stock solutions of 400 mg L^{-1} of each substance were prepared in methanol and stored at 4°C in amber bottles.

2.2 *Sample collection and storage*

All 24-h composite influent and effluent samples were collected from Montreal WTP. The city of Montreal has a population of approximately 1.8 million people, about one-third of the entire population of the Province of Quebec. The treatment plant typically processes 1.3 million m^3 of raw sewage daily, with a representative mean effluent discharge of 19.8 $m^3 s^{-1}$. Montreal's treatment plant uses a physico-chemical process, which involves adding ferric chloride or alum as coagulant, and anionic polymer as a coagulant aid. Treated effluent is then discharged directly into the St. Lawrence River [27].

Composite sewage influent and effluent samples were drawn directly from the WTP, while surface water samples were collected (1-m depth) with a Teflon pump from the St. Lawrence River at −1, 0.5, 2.5, 4.5 and 8 km away of the effluent outfall. Samples were then transported to the laboratory in a Spartanburg^{TM} stainless steel container and stored in the dark at 4°C for less than 24 h until the extraction step.

2.3 *Solid-phase extraction (SPE)*

Prior to extraction, each wastewater sample was filtered under nitrogen flow from Spartanburg^{TM} container through a 142-mm glass-fibre filter (0.7 μm) retained in a Millipore stainless steel holder. Following a previously published method [14], a second filtration was completed on a 90-mm GF/F glass-microfibre filter (0.7 μm) with a fritted, all-glass filtration device and Celite 545 under tab vacuum. To each 500 mL of the filtered sewage sample was added 150 μL of a methanolic surrogate standard solution (10,11-dihydrocarbamazepine: 3.33 mg L^{-1}, meclofenamic acid: 6.67 mg L^{-1}) and 5 mL of methanol before lowering the pH value ~2 with 0.5 mL of concentrated hydrochloric acid. For outfall effluent, filtered samples (1 L) were used with a double volume of surrogate standard solution and other additives mentioned earlier. Meanwhile, polymeric cartridges (OASIS HLB and Strata-X) were conditioned with 4 mL of methanol followed by 8 mL of acidified (pH 2), purified water (1 mL of hydrochloric acid in 1 L of purified water). SPE was performed with a VAC ELUT

SPS24^{TM} manifold (Varian) at a flow rate of 10–15 mL min^{-1} [14]. After extraction, all the cartridges were washed with 2 mL of a solvent mixture of methanol and water (20% v/v) after drying for 5 min. The analytes were then eluted with 2×2 mL of ethyl acetate. The combined fractions of each cartridge were passed through 6.5 g of anhydrous sodium sulfate. The dried eluate was then mixed with 150 μL of a solution of 2,4-dichlorobenzoic acid in methanol (6.67 mg L^{-1}), as the internal standard and evaporated under a gentle stream of nitrogen gas. For outfall effluent samples, two combined fractions taken at the same sampling site were pooled prior to the addition of internal standard and evaporating to dryness.

2.4 *Derivatization*

The dried extract was reconstituted with 50 μL of acetonitrile and 100 μL of BSTFA + 10% TMCS. This solution was transferred into a reaction vial under nitrogen atmosphere, and the sealed vial was then heated at 70°C for 20 min. The remaining solution was transfered to an amber autosampler vial fitted with a 100 μL conical insert.

2.5 *GC–MS/MS analysis*

Analyses were performed on a Trace GC Ultra-PolarisQ GC–MS/MS system (Thermo Electron Corporation) fitted with a 30 m × 0.25 mm i.d. × 0.25 μm Phenomenex ZB-5ms capillary column. The carrier gas was helium (BOC, purity: 99.999%), and the flow rate was 1.0 mL min^{-1}. A TRIPLUS programmed temperature vaporizing injector (PTV) was used in splitless mode (0.40 min) with an injection volume of 0.5–1.0 μL. The oven temperature was held at 70°C for 1 min following injection, and then a temperature gradient was started at a rate of 14°C min^{-1} from 70 to 150°C. After the first temperature transition, the temperature was increased by 6°C min^{-1} (from 150 to 285°C), and then by 7.5°C min^{-1} (from 285 to 300°C). Finally, the temperature was kept at 300°C for 0.54 min (with a total run time of 31.75 min). The injector and transfer line temperatures were maintained at 210 and 285°C, respectively. The mass spectrometer was operated in positive-ion mode using electron impact (EI) ionization at 70 eV. Segments of mass acquisition were set in MS/MS for each compound. Selected reaction monitoring (SRM) was employed for the quantitative analysis. The ion-trap temperature was 230°C. Detailed ion-trap mass spectrometer parameters are listed in table 2.

3. Results and discussion

3.1 *Solid-phase extraction (SPE) procedure*

The chosen group of PPCPs displayed a wide range of chemical properties, from very hydrophilic to moderately hydrophobic. Due to these marked differences, selecting an extraction cartridge that can provide acceptable recoveries for all compounds is typically a difficult task. A search of the available literature indicates that a few reliable

Table 2 Optimal GC–MS/MS conditions for the analysis of target PPCPs

Compounds	Retention time (min)	Ions full scan[a] (m/z)	Ions for quantification MS/MS (m/z) Precursor	SRM transition	CID[b] (V)	Max. ion time (ms)	Microscans
Salicylic acid	9.69	267	267	249	3	3	3
2,4-Dichlobenzoic acid	10.31	173, 203, 247	247	105, 131, 167	2.35	4	3
Clofibric acid	10.67	128, 143, 169	143	73, 99, 115	1.3	10	3
Ibuprofen	11.32	160, 234, 263	160	117, 145	1	10	3
2-Hydroxy-ibuprofen	15.26	131, 308, 351	351	119, 161, 233	1	2	2
Fenoprofen	16.92	270, 299, 314	270	196, 255	0.6	1	5
Naproxen	18.39	185, 243, 302	185	170	2.7	2	3
Triclosan	19.04	347	347	185, 200, 312	1.2	5	3
Ketoprofen	20.18	282, 295, 311	295	267	3.2	10	3
10,11-Dihydrocarbamazepine	20.62	195	195	180	3.2	5	3
Carbamazepine	21.08	165, 193, 293	193	167, 191	3.25	25	3
Diclofenac	21.4	214, 242, 367	242	179	3.6	25	3
Meclofenamic acid	22.63	242, 277, 367	242	214	3.2	10	2

[a]Full scan range $m/z = 100$–450. [b]Collision-induced dissociation energy.

SPE methods have been developed for pharmaceuticals (with a wide range of polarity) in wastewater [11, 14, 22, 24, 28, 29] or surface water [30] using polymeric cartridges such as OASIS HLB or Strata-X. Given the difficulty of treating the matrix in question, satisfactory extraction could not be obtained by replicating any of these existing SPE methods.

All methods using single filtration prior extraction, as well as elution solution made of neat methanol or acetone-ethyl acetate (1 : 1, v/v) were discarded immediately. The resulting dark brown residue could not be wholly derivatized and injected directly into the GC system without significantly contaminating the injection port. Back-extraction with a solvent mixture such as dichloromethane/petroleum ether reported by Lee *et al.* [14] after chemical derivatization was also abandoned. The trimethylsilyl and *tert*-butyldimethylsilyl derivative, generated by the reaction of BSTFA and MTBSTFA to the amide group of carbamazepine, is likely to be unstable under aqueous conditions. Thus, back-extraction approach is not suitable for maintaining the converted structure of carbamazepine as silyl derivative with other pharmaceutical residues.

During SPE development, extraction solvents were selected, based on their elution strength. All substances were satisfactorily eluted by ethyl acetate, but some yellowish co-extractive impurities were removed by a mixture of 20% v/v methanol in purified water as documented by Öller *et al.* [11].

Recoveries for the selected compounds were better for Strata-X cartridges than OASIS HLB cartridges (table 3). Absolute recoveries for 500-mL fortified samples at a nominal concentration of 1000 ng L^{-1} were evaluated using spiked Milli-Q water and effluent samples against the non-extracted standard solution. For the recovery tests, the internal standard (2,4-dichlorobenzoic acid) was spiked just after elution, before the derivatization step.

Absolute recoveries obtained from spiked effluent samples using Strata-X cartridges were quite acceptable (72–102%). Among the compounds extracted, salicylic acid had

Table 3. Absolute recoveries of selected pharmaceutical drugs determined in spiking experiments with 500 mL of distilled water and sewage effluent (nominal conc. 1000 ng L^{-1}).

	Milli-Q water recoveries (%)		Sewage effluent[a] recoveries (%)	
Compound	Oasis HLB ($n=3$)	Strata-X ($n=3$)	Oasis HLB ($n=3$)	Strata-X ($n=3$)
Salicylic acid	73 ± 3	78 ± 3	66 ± 8	72 ± 7
Clofibric acid	80 ± 2	85 ± 3	72 ± 5	82 ± 6
Ibuprofen	91 ± 2	93 ± 2	89 ± 3	96 ± 6
2-Hydroxy-ibuprofen	89 ± 3	90 ± 2	68 ± 6	80 ± 8
Fenoprofen	99 ± 6	102 ± 2	85 ± 4	96 ± 5
Naproxen	83 ± 2	84 ± 3	70 ± 3	82 ± 7
Triclosan	88 ± 6	101 ± 4	74 ± 6	102 ± 3
Ketoprofen	93 ± 8	103 ± 2	89 ± 4	101 ± 5
10,11-Dihydro-carbamazepine	85 ± 6	91 ± 5	88 ± 2	98 ± 4
Carbamazepine	90 ± 5	95 ± 5	91 ± 3	99 ± 3
Diclofenac	84 ± 8	96 ± 4	84 ± 9	93 ± 5
Meclofenamic acid	90 ± 4	94 ± 1	74 ± 2	97 ± 3

[a]Results corrected for blanks.

the lowest recovery, possibly due to its high polarity, but also to the moderate polarity of ethyl acetate compared to methanol currently used as an elution solvent in SPE methods. However, as mentioned earlier, the OASIS HLB cartridges provided lower recoveries, and care had to be taken to prevent clogging of the SPE material. Another comparative study of OASIS HLB and Strata-X cartridges, using a different elution solution and detector, has been reported elsewhere, with similar conclusions, on estuarine water samples [31].

3.2 *Derivatization*

In order to improve the chromatography, chemical derivatization is often performed for GC–MS analysis of less volatile substances. Previous tests on pentafluorobenzyl bromide (PFBBr) yielded interesting results for acidic drugs (unpublished results). Unfortunately, PFBBr does not react with carbamazepine; consequently, unreproducible signals were monitored for this compound. As mentioned in the introduction, carbamazepine is partly degraded to iminostilbene in a hot injector [11, 25, 26]. The derivatization of carbamazepine therefore becomes necessary prior to GC–MS analysis. To achieve the chemical derivatization of carbamazepine, the silylation agents MTBSTFA + 1% TBDMCS and BSTFA + 10% TMCS were tested under absolute anhydrous conditions. Although MTBSTFA + 1% TBDMCS gave good results for all compounds, as evidenced by the formation of several well-known characteristic $[M-57]^+$ ions fragments, this option was quickly abandoned. After final MS/MS optimization of the ion-trap mass detector, a loss of sensitivity (approximately tenfold lower than other PPCPs) was observed for carbamazepine and its standard surrogate 10,11-dihydrocarbamazepine. However, BSTFA + 10% TMCS provided a better sensitivity, shorter retention times, and good chromatographic resolution, for neutral and acidic compounds alike. The amide group of the carbamazepine is not derivatized completely when 1% of TMCS is used with BSTFA, especially when a very small

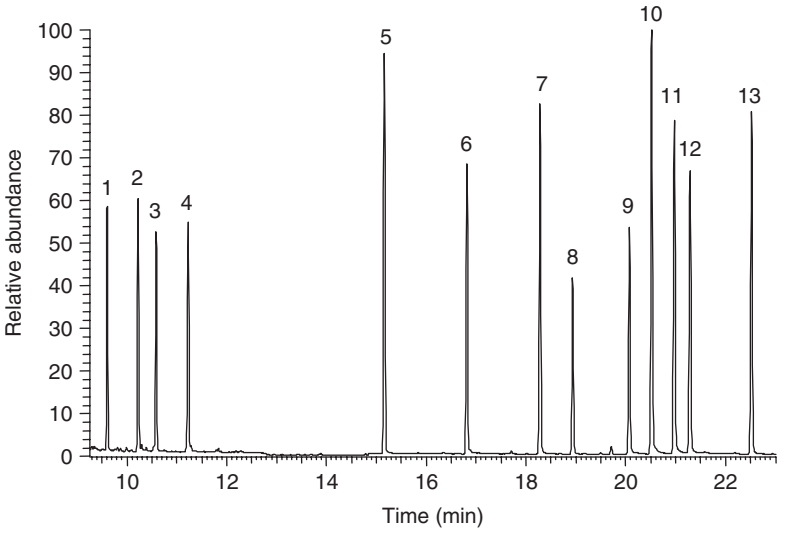


Figure 1. Total ion chromatogram for TMS derivatives of the ten studied compounds with standards. Peak identification. 1: salicylic acid; 2: 2,4-dichlorobenzoic acid (internal standard); 3: clofibric acid; 4: ibuprofen; 5: 2-hydroxy-ibuprofen; 6: fenoprofen; 7: naproxen; 8: triclosan; 9: ketoprofen; 10: 10,11-dihydrocarbamazepine (surrogate standard); 11: carbamazepine; 12: diclofenac; 13: meclofenamic acid (surrogate standard).

amount of moisture is present in sample (yields of conversion ~75–90%). However, when the percentage of TMCS is increased to 10%, quantitative conversion of carbamazepine is obtained under mild heating conditions (70°C) in only 20 min. The addition of the relatively weak silyl donor, TMCS (which acts as a catalyst), to BSTFA enhances the donor strength of the stronger donor, BSTFA [32]. Unfortunately, the mechanism for the catalytic effect of TMCS is not well understood.

A total ion chromatogram (TIC) depicting the target compounds is shown in figure 1. After derivatization, each compound formed a single trimethylsilyl (TMS) derivative confirmed by the presence in mass spectrums of characteristic $[M-15]^+$ ions relevant to the loss of a TMS methyl group from the parent molecules. In the case of salicylic acid and 2-hydroxy-ibuprofen, injection of standard solutions in full scan mode has proven that their common carboxylic and hydroxyl groups were both derivatized under selected experimental conditions. Figure 2 shows the electronic impacts (EI) ionization mass spectrum of TMS carbamazepine derivative with its characteristic ion $[M-15]^+$ represented at m/z 293.

3.3 *Validation of the analytical method*

The accuracy and precision of the method were established by triplicate analysis of the samples. Accuracy was determined through the absolute recovery of spiked analytes. Based on previous SPE results (see table 3), Strata-X cartridges were chosen over the OASIS HLB cartridges for the purpose of this study. Absolute recoveries for Milli-Q water fortified to 1000 ng L^{-1} were better than 78% when the test was performed with Strata-X cartridges. The precision of the method, which is defined by

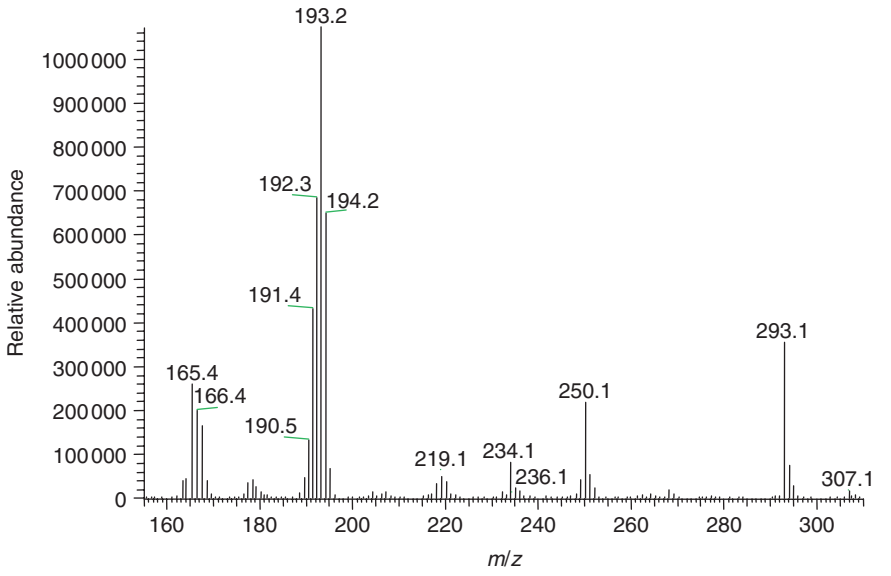


Figure 2. Full-scan EI ionization mass spectrum of carbamazepine as TMS derivative.

the standard deviation, was found to be acceptable within 1 and 5%. In fortified effluent samples, similar recoveries were obtained for all substances.

To ensure the analytical performance required for determination of the target substances in wastewater samples, 2,4-dichlorobenzoic acid was used as the internal standard (IS). Quantitation of the analytes was performed by comparing the ratios of peak areas of the compound to the IS before fitting to a calibration curve. Four-point calibration curves were developed for analytes across a range of concentrations typically retrieved in environmental samples. The response of the ion-trap mass detector for all TMS derivatives, in SRM using 1–3 transitions, was found to be linear over the concentration range from 30 to 4000 ng L^{-1} (based on a concentration factor of 3333). The substances 2-hydroxy-ibuprofen and fenoprofen tended to be less sensitive, with respective linearity ranges of 60–3000 ng L^{-1} and 60–4000 ng L^{-1} (table 4). An additional linearity test was performed on extracted effluent samples by adding set amounts of analytes from 0 to 2000 ng L^{-1} prior to derivatization. Yet again, experimental data followed a perfect linear trend, with a mean correlation coefficient $r^2 > 0.995$ for all substances. Therefore, rough primary-treated effluent matrices affected neither the MS signal nor linearity.

The limit of detection (LOD) of the method ranged from 1 to 18 ng L^{-1} (table 4). Values for LOD were defined as the minimum detectable amount of analyte in effluent extract with a signal-to-noise ratio of 3 : 1 in SRM mode. The higher LOD values were assigned to 2-hydroxy-ibuprofen and fenoprofen. Note that a higher baseline noise was seen for fenoprofen.

Two surrogate standards were also included in the extraction method. Among the surrogates selected, meclofenamic acid, which is commonly used as a nonsteroidal anti-inflammatory drug (NSAID), is found to be entirely representative of all acidic compounds. When ingested, this NSAID is completely metabolized by the body

Table 4. Mean linearity (STD solutions, spiked effluent extracts) and estimated limits of detection (LOD) for the analytical method.

	Linearity				
	STD solutions ($n=4$ curves)		Fortified effluent extracts ($n=3$ curves)		LOD ($S/N=3$)
Compound	Concentration range (ng L^{-1})	Mean r^2	Spiked level (ng L^{-1})	Mean r^2	Concentration range (ng L^{-1})
Salicylic acid	30–3000	0.999	0–2000	0.999	1
2,4-Dichlorobenzoic acid	–	–	–	–	4
Clofibric acid	30–4000	0.996	0–2000	0.996	8
Ibuprofen	30–4000	0.996	0–2000	0.995	1
2-Hydroxy-ibuprofen	60–3000	0.997	0–1000	0.995	16
Fenoprofen	60–4000	0.998	0–2000	0.996	18
Naproxen	30–3000	0.993	0–2000	0.998	2
Triclosan	30–4000	0.995	0–2000	0.999	2
Ketoprofen	30–4000	0.995	0–2000	0.997	3
10,11-Dihydrocarbamazepine	–	–	–	–	3
Carbamazepine	30–4000	0.994	0–2000	0.997	8
Diclofenac	30–4000	0.996	0–2000	0.997	7
Meclofenamic acid	–	–	–	–	3

and thus unlikely to be present in wastewater [33]. The other surrogate, 10,11-dihydrocarbamazepine, is not a pharmaceutical compound but was chosen for its molecular similarity to carbamazepine. The mean rates of recovery for surrogates retrieved following routine influent and effluent extractions are quite acceptable, ranging from 70 to 120% when Strata-X cartridges are used. The standard deviations of the mean effluent recoveries did not exceed 10%, while values for influent sometimes reached as high as 15%. The main purpose of the standard surrogates was to check the recovery process of target compounds. In the case where surrogate recovery values yielded unexpected results, the entire extraction procedure was repeated.

3.4 *Application to wastewater analysis*

A brief survey of the target compounds was carried out at the Montreal WTP using the method developed. The extraction procedure was successfully applied to the analysis of 24-h composite samples of influent and effluent. Two series of samples were taken in spring (April 2005 and 2006) and one in winter (December 2005). The results are shown in table 5; a diagram of the influent and effluent concentrations assigned to one sampling operation is represented in figure 3.

Among the PPCPs measured, salicylic acid, clofibric acid, ibuprofen, 2-hydroxy-ibuprofen, naproxen, triclosan, carbamazepine, and diclofenac were detected in all the samples. However, no fenoprofen and ketoprofen was detected in any sample. A chromatogram of an effluent sample is shown in figure 4. Concentrations of PPCPs in WTP effluent are found to be consistent with concentrations previously reported in other Canadian studies. Average concentrations of ibuprofen (0.84 μg L^{-1}), naproxen (1.1 μg L^{-1}), triclosan (0.28 μg L^{-1}), and diclofenac (0.082 μg L^{-1}) were found to be comparable with those reported in Ontario by Lee *et al.* [15] in a survey

Table 5. Concentrations ($ng\,L^{-1}$) of selected compounds in physico-chemical treatment plant of Montreal.

	Date					
	27 April 2005		7 December 2005		26 April 2006	
Compound	Influent ($n=3$)	Effluent ($n=3$)	Influent ($n=2$)	Effluent ($n=2$)	Influent ($n=3$)	Effluent ($n=3$)
Salicylic acid	3125 ± 172	3522 ± 97	1601 ± 105	1675 ± 70	1825 ± 47	1955 ± 111
Clofibric acid	220 ± 8	134 ± 8	84 ± 9	65 ± 5	41 ± 1	33 ± 4
Ibuprofen	1171 ± 64	858 ± 40	1132 ± 43	1060 ± 51	827 ± 69	609 ± 32
2-Hydroxy-ibuprofen	1069 ± 55	883 ± 39	1957 ± 117	1563 ± 122	1081 ± 74	684 ± 50
Fenoprofen	nd[a]	nd	nd	nd	nd	nd
Naproxen	3934 ± 357	2579 ± 188	449 ± 40	382 ± 44	348 ± 25	217 ± 15
Triclosan	811 ± 54	662 ± 59	126 ± 7	113 ± 11	102 ± 2	55 ± 5
Ketoprofen	nd	nd	nd	nd	nd	nd
Carbamazepine	701 ± 44	656 ± 28	98 ± 11	100 ± 5	98 ± 2	91 ± 4
Diclofenac	216 ± 5	214 ± 5	26 ± 3[b]	20 ± 3[b]	20 ± 1[b]	13 ± 1[b]

[a]Not detected. [b]Concentrations < limit of quantification (LOQ).

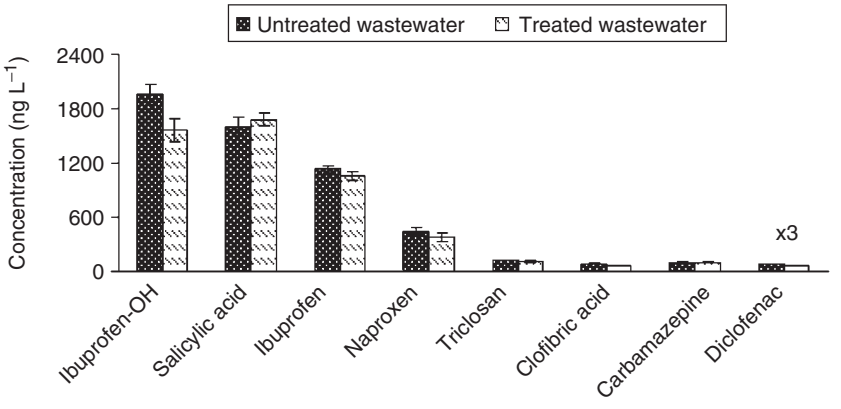


Figure 3. Mean concentrations and relative standard deviations ($n=2$) of PPCPs in treated and untreated wastewater from STP of Montreal (7 December 2005).

of eight WTP effluents (respective mean concentrations of 0.31, 0.82, 0.14, and $0.11\,\mu g\,L^{-1}$). In addition, the neutral drug carbamazepine was detected at a median concentration of $0.28\,\mu g\,L^{-1}$, compared with $0.077\,\mu g\,L^{-1}$ [12] and $0.43\,\mu g\,L^{-1}$ [17] in other Ontario effluents.

The reported results from the WTP of the city of Montreal have shown that current physical and chemical treatments processes removed little of the studied PPCPs. Other analysis data indicated that most of these substances are detected more than 8 km away from the effluent outfall in the receiving water of the St. Lawrence River (figure 5). Thus, 2-hydroxy-ibuprofen, salicylic acid, ibuprofen, triclosan, naproxen and carbamazepine were detected at all sampling sites. The higher concentrations of each PPCPs were observed at a site 0.5 km away from the effluent outfall, and concentraions then decreased along the dispersion plume.

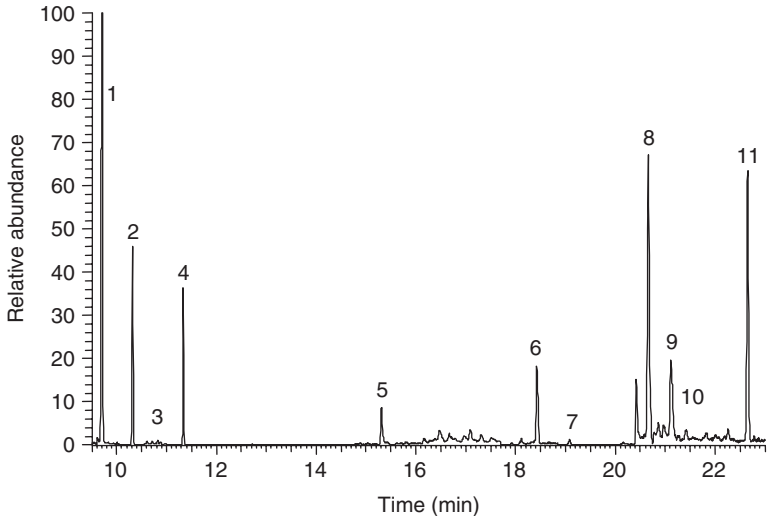


Figure 4. MS/MS chromatogram of a sewage-effluent extract depicting the detected compounds. Peak identification. 1: salicylic acid; 2: 2,4-dichlorobenzoic acid (internal standard); 3: clofibric acid; 4: ibuprofen; 5: 2-hydroxy-ibuprofen; 6: naproxen; 7: triclosan; 8: 10,11-dihydrocarbamazepine (surrogate standard); 9: carbamazepine; 10: diclofenac; 11: meclofenamic acid (surrogate standard).

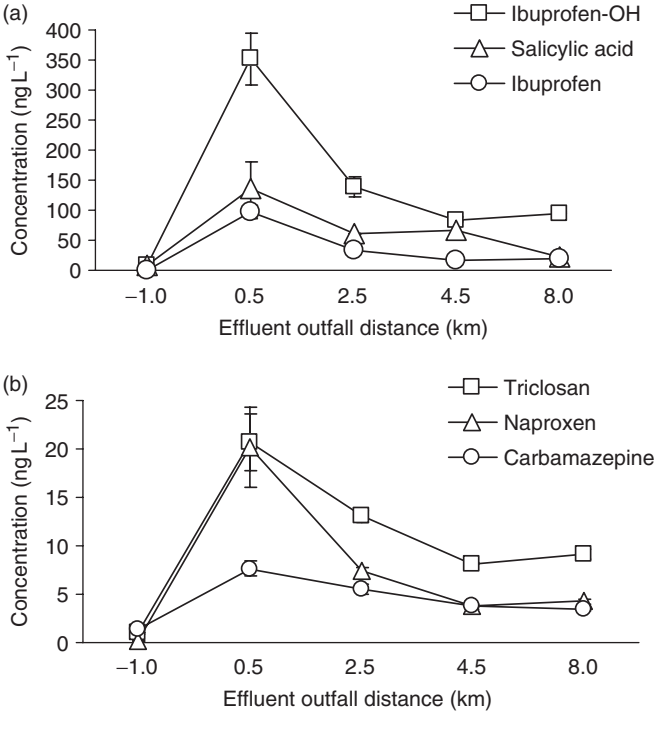


Figure 5. Mean concentrations and relative standard deviation ($n = 2$) of PPCPs retrieved along the effluent outfall in the receiving waters of the St. Lawrence River (21 November 2005).

4. Conclusion

A rugged, sensitive, and cost-effective analytical method has been developed for the simultaneous analysis of carbamazepine and pharmaceutical acidic residues in roughly primary-treated wastewater samples by GC–MS/MS. The solid-phase extraction method described, which uses Strata-X cartridges, enabled the selective extraction of all PPCPs in highly contaminated water samples of influent and effluent, even at low ng L^{-1} concentrations. The use of BSTFA + 10% TMCS as a derivatization agent proved to be a wise choice, providing remarkable chromatographic resolution and stable TMS derivatives. Despite the difficult matrices encountered, tandem mass spectrometry proved its usefulness by enhancing the specificity and sensitivity of the analysis. This alternative analytical method is suitable for laboratories providing routine analytical services and having no access to costly LC–MS instrumentation.

The samples collected at the Montreal WTP were drawn from roughly primary-treated and hence highly complex matrices. Application of the method to analysis of the studied substances revealed the presence of eight of the target compounds in all influents and effluents, from low (μg L^{-1}) to trace levels (ng L^{-1}). The occurrence of these PPCPs in the final effluent and in the receiving waters of the St. Lawrence River suggests that these compounds are not being removed by physico-chemical treatment. The method developed in the present study will allow for further investigation of the behaviour and ecotoxicological effects of these PPCPs as a necessary precursor to assessing the environmental risk they pose to aquatic organisms.

Acknowledgements

The authors are indebted to P.-J. Čejka and C. Yelle from the Montreal WTP for their technical assistance. Dr H.-B. Lee of the National Water Research Institute (Environment Canada) is also acknowledged for fruitful discussions. Finally, the authors thank P. Potvin for the edition of the manuscript. This work was funded by the St. Lawrence Action Plan.

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