Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography A, 1116 (2006) 193–203
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Determination of acidic drugs and caffeine in municipal wastewaters and receiving waters by gas chromatography–ion trap tandem mass spectrometry

Sergei S. Verenitch *, Christopher J. Lowe, Asit Mazumder

University of Victoria, Water and Watershed Research Program, P.O. Box 3020 STN CSC, Victoria, BC, Canada V8W 3N5

Received 8 December 2005; received in revised form 27 February 2006; accepted 1 March 2006
Available online 20 March 2006

Abstract

Some aspects of both sample preparation and instrumental techniques for analysis of such acidic drugs as acetylsalicylic acid, ibuprofen, gemfibrozil, fenoprofen, naproxen, ketoprofen, and diclofenac, as well as caffeine in surface water and municipal wastewater have been studied and further developed. Water samples were filtered and target analytes were extracted by solid-phase extraction (SPE). Supelco LC-18 and Oasis HLB SPE cartridges were used to pre-concentrate samples for acidic drugs and caffeine, respectively. A methylation process was applied to acidic drugs prior to analysis while caffeine was analyzed directly. A method of gas chromatography–ion trap tandem mass spectrometry (IT–MS/MS) for analysis of the target acidic pharmaceuticals and caffeine is presented here in detail. Such parameters as collision-induced dissociation (CID) voltage, isolation time, excitation time, excitation storage level, and electron energy were adjusted in order to optimize the instrument analytical performance. After optimization, an instrument detection limit of 0.5–20 pg/μL with signal-to-noise (S/N) not less than 5 was achieved for all target analytes. It was shown that this method has good linearity within the range of 10–2000 pg/μL. The application of the optimized IT–MS/MS parameters conjointly with the sample preparation procedure resulted in method detection limits (MDLs) of 0.1–1.0 and 20 ng/L for the determination of acidic drugs and caffeine, respectively in such samples as surface water, effluent from municipal wastewater plants, as well as receiving waters.

Keywords: Pharmaceuticals; Caffeine; GC–MS/MS; Municipal wastewater; Surface water

1. Introduction

During the last three decades, the impact of chemical pollution has focused almost exclusively on the conventional "priority" pollutants, especially acutely toxic/carcinogenic compounds such as pesticides and industrial intermediates that display persistence in the environment. This spectrum of chemicals, as it has been recently found, is only one piece of the larger puzzle in risk assessment. Another diverse group of bioactive chemicals receiving comparatively little attention as potential environmental pollutants comprises pharmaceuticals and the active ingredients in personal care products (PPCPs), both human and veterinary, including not just prescription drugs and biologics (drugs derived from living sources), but also diagnostic agents, "nutraceuticals", fragrances, and numerous others. Approximately 3000 compounds are approved as constituents in medicinal products. Prescription and nonprescription pharmaceutical drugs have been estimated [1,2] to be produced in volumes that exceed hundreds of metric tons annually. These compounds and their bioactive metabolites have been continually introduced to the aquatic environment as complex mixtures via a number of routes, but primarily by both untreated and treated municipal wastewater.

Manufactured and used in large quantities, PPCPs comprise a diverse array of pollutants. Usage rates of many are on par with agrochemicals. Escalating introduction to the marketplace of new pharmaceuticals, as well as expanding usage of existing drugs, is exponentially increasing and already a large array and amount of PPCP chemical classes have been found in the environment. Several acidic and neutral drugs were detected at μg/L concentrations in the effluents of Canadian sewage treatment plants (STPs) [3,4]. In the UK [5,6], several PPCPs at

* Corresponding author. Tel.: +1 250 472 4833; fax: +1 250 721 7120.
E-mail address: ssv@uvic.ca (S.S. Verenitch).

0021-9673/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2006.03.005

the level of 1 μg/L were found in the aquatic environment. A recently conducted study by US Geological Survey (USGC) [7] provides data for the occurrence of PPCPs, hormones, and other organic wastewater contaminants (OWCs) in water resources of the USA. The most frequently (from 60 to 90%) detected compounds in the study by USGC were some specific steroids, insect repellants, stimulants, antimicrobial disinfectants, fire retardants and nonionic detergent metabolites.

Numerous analytical methods for the determination of pharmaceuticals and their metabolites in aqueous solutions have been described in the literature. Liquid chromatography–mass spectrometry (LC–MS) and gas chromatography-mass spectrometry (GC–MS) are the most widely used techniques. Buerge et al. [8] used GC–MS/MS methodology to show that caffeine can be used as a chemical marker for surface water pollution by domestic wastewater and Loffler and Ternes [9] applied a LC–MS/MS technique to determine acidic pharmaceuticals, antibiotics, and ivermectin in river sediments. Koutsouba et al. [10] have developed a methodology based on the use of GC–MS in selective ion monitoring (SIM) mode to determine polar pharmaceuticals in sewage water of Greece. Brooks et al. [11] were the first to report the data regarding pharmaceutical accumulation in fish of effluent-dominated ecosystems. Select antidepressants such as the select serotonin reuptake inhibitors fluoxetine and setraline and their metabolites norfluoxetine and desmethylsetraline were detected means of GC–MS in SIM mode at levels greater than 0.1 ng/g in all tissues examined from fish residing in a municipal effluent-dominated stream. Detection limits achieved by LC–MS and GC–MS methods for a group of acidic drugs and caffeine vary depending on sample origin and the objectives of a specific application. They cover a wide range from ng/L to μg/L [3,4,10,12–15]. The complexity of a sample matrix may complicate the sample preparation procedure and cause high levels of interference in the final extract, which can make the proper identification and quantification of target parameters difficult. A traditional way to reduce the effects of both background and potential interferences on the accuracy of the quantification process of target analytes is to use tandem or some times even triple mass spectrometry techniques. The advantages of these methods, in comparison to single MS, are increased sensitivity and greater selectivity. This approach also allows method detection limits (MDLs) to be achieved in the range from 0.1 to 1 μg/L even for such samples as municipal wastewater or solid matrices.

The aim of this study was to establish a routine method based on ion trap GC–MS/MS to deliver MDLs in the range of lower ng/L for analysis of acidic pharmaceuticals and caffeine in surface water near municipal wastewater treatment plants and in municipal wastewater itself. The behavior of acidic drugs and caffeine, in terms of their fragmentation by electron ionization (EI) in an ion trap instrument, was studied and the precursor and corresponding product ions for all analytes have been determined. The MS/MS parameters and collision induced dissociation amplitudes have been determined and optimized for the greatest yield of daughter ions. Calibration curves for each analyte were created for a wide range of concentration levels. The developed GC–MS/MS method was then used to analyze a number of water samples collected near municipal wastewater outfalls in Western Canada and directly from municipal wastewater.

2. Experimental

2.1. Materials

All acidic drugs studied in this work are listed in Table 1. Acetylsalicylic acid (ASA), ibuprofen (IBU), gemfibrozil (GEM), fenoprofen (FEN), naproxen (NAP), ketoprofen (KET), diclofenac (DCF), caffeine, and the internal (IS) and surrogate standards (SS) meclofenamic acid (MCF) and 2,3-dichlorophenoxyacetic acid (2,3-D), respectively, were purchased from Sigma–Aldrich Canada (Oakville, Canada). The derivatization reagent, BF_3/MeOH, and all solvents (methanol (MeOH), light petroleum (PE), methyl *tert*-butyl ether (MTBE), acetone, hexane and dichloromethane (DCM)) were of HPLC grade and were purchased from Fisher Scientific (Ottawa, Canada).

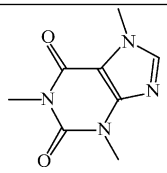
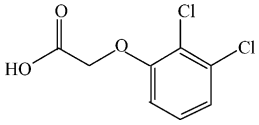
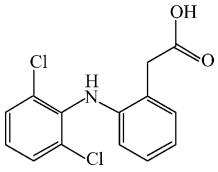
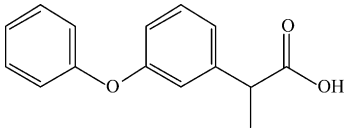
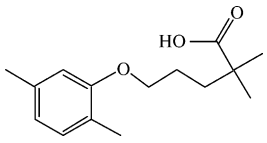
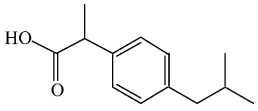
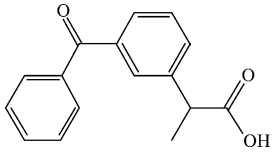
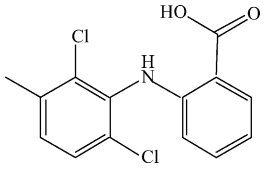
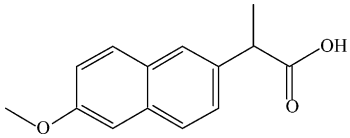
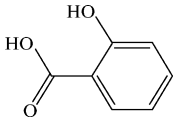
Standard (STD) stock solutions of methylated individual acidic analytes and caffeine were prepared in methanol (Table 1). Calibration solutions of a mixture of all the methylated analytes and caffeine at different concentration levels in methanol were used for determination and optimization of GC–MS/MS conditions (Table 1). Anhydrous sodium sulfate (Na_2SO_4) was baked at 400 °C for 4 h prior to use. All glassware including sampling amber bottles were rinsed with DCM, acetone, MeOH, and as a last step, double distilled deionized water and baked at 300 °C overnight. Solid-phase extraction (SPE) cartridges were Oasis HLB 6 mL, 0.2 g from Waters (Milford, MA, USA) for caffeine, and Supelclean LC-18, 6 mL 0.5 g from Supelco (Bellefonte, PA, USA) for the acidic drugs.

Two liters of grab samples were collected by hand at the water surface at different locations on the West coast of Vancouver Island, British Columbia, Canada. The sampling sites included effluent from two STP, as well as upstream and downstream surface water samples at different distances from the STP effluent outfall, and lake water samples. All samples were collected in March 2005, placed in plastic bottles and immediately transported to the laboratory where they were frozen and kept at −20 °C until the extraction procedure took place. Samples were processed usually within 3 months period after their collection.

2.2. Standard solutions

All standards of methylated acidic drugs were synthesized in a macro scale and used as absolute standards for identification and calibration purposes. For this study, standard solutions of caffeine and methyl esters of individual acidic pharmaceuticals, as well as a mixture of them, were used (Table 1). Individual standard solutions were used for identification purposes. The retention time (t_R) of each analyte, as well as its mass spectrum, was determined at this stage. A mixture of methyl esters of acidic drugs and caffeine at the concentration level 2 (Table 1)

Table 1
Commercial names, chemical structures, use and concentrations of standard (STD) stock and calibration solutions of the drugs investigated, as well as surrogate and internal standards

Analyte (abbreviation); MW; formula; CAS number	Chemical structure	Use and origin	Stock STD concentration calibration solution concentrations (levels 1 through 5) (pg/μL)
Caffeine (CAF); 194; $C_8H_{10}N_4O_2$; 58-08-2		Constituent of coffee psychostimulant	5240; 1–786, 2–393, 3–196.5, 4–98.3, 5–49.1
2,3-Dichlorophenoxyacetic acid (2,3-D); 220;$C_8H_6Cl_2O_3$; 2976-74-1		Surrogate standard	2560; 1–770, 2–385, 3–192.5, 4–96.3, 5–48.1
Diclofenac (DCF); 295; $C_{14}H_{11}Cl_2NO_2$; 15307-79-6		Antophlogistic	6000, 1–1800, 2–900, 3–450, 4–225, 5–112.5
Fenoprofen (FEN); 242, $C_{15}H_{14}O_3$; 53746-45-5		Analgesic and antiphlogistic	7600; 1–2280, 2–1140, 3–570, 4–285, 5–142.5
Gemfibrozil (GEM); 250; $C_{15}H_{22}O_3$; 25812-30-0		Lipid regulator	4800; 1–1440, 2–720, 3–360, 4–180, 5–90
Ibuprofen (IBU); 206; $C_{13}H_{18}O_2$; 15687-27-1		Analgesic and anti-inflammatory	8000; 1–2400, 2–1200, 3–600, 4–300, 5–150
Ketoprofen (KET); 254; $C_{16}H_{14}O_3$; 22071-15-4		Analgesic and anti-inflammatory	8000; 1–2400, 2–1200, 3–600, 4–300, 5–150
Meclofenamic acid (MCF); 309; $C_{15}H_{13}Cl_2NO_2$; 6385-02-0		Internal standard	2640; 1–1580, 2–790, 3–395, 4–197.5, 5–98.8
Naproxen (NAP); 230; $C_{14}H_{14}O_3$; 22204-53-1		Analgesic and anti-inflammatory	6800; 1–2040, 2–1020, 3–510, 4–255, 5–127.5
Salicylic acid (SA); 8, $C_7H_6O_3$; 69-72-7		Metabolite of acetylsalicylic acid (ASA) (aspirin) analgesic	3240; 1–970, 2–485, 3–242.5, 4–121.3, 5–60.6

was used for determination and optimization of MS/MS parameters.

2.3. Filtration and solid-phase extraction of acidic drugs

A 2 L sample was split into two, so to have 1 L each for analysis of acidic drugs and caffeine separately. All samples for analysis of acidic drugs, including laboratory spikes and field replicates were filtered using 0.45 μm glass-fiber (GF/C) filters. Prior to filtration, the filters had been soaked overnight in a mixture of hexane/DCM (1:1), dried in the oven and then ashed at 500 °C for 1 h. Laboratory spike samples were 1 L of tap and surface water spiked with each of the seven acidic drugs and caffeine STD stock solutions (for the concentrations of the STD stock solutions see Table 1) so that the final concentration of the analytes was in the range between 50 and 200 ng/L. After filtration, 10 μL of 2.56 μg/mL stock solution of 2,3-D (SS) was added to each 1 L of filtrate. The pH of the filtrate was adjusted to 2.0 with 1 mL of concentrated H_2SO_4 and the solution was passed through a pre-conditioned SPE cartridge (SupelcoLC-18 6 mL). For conditioning the SPE cartridges, 6 mL of hexane, 3 mL of acetone, 6 mL of DCM, followed by 2 mL of deionized water adjusted to pH 2.0 with concentrated H_2SO_4, were used. All samples were passed through the SPE cartridges at a flow rate of approximately 10 mL/min. Each sample bottle was rinsed three times with 10 mL of pH 2.0 distilled water, and the rinses were also passed through the SPE cartridge. After extraction, the SPE cartridges were dried under vacuum for approximately 2 min. The target analytes were eluted from the SPE cartridges with three successive 3 mL aliquots of methanol at a flow rate of approximately 0.5 mL/min. Prior to methylation, the extract was concentrated to 0.2 mL using a gentle stream of N_2 at room temperature.

2.4. Solid-phase extraction of caffeine

The second 1 L portion of each sample was also filtered through 0.45 μm GF/C filters which had been pre-washed with hexane and DCM, oven dried and ashed for 1 h at 500 °C. A known amount of caffeine standard solution was added into 1 L of a fortified sample so that the final concentration of the analyte in the extract was 494 ng/L. If necessary, the pH of the filtrate was adjusted with 1 M NaOH to pH 7.5. The SPE cartridge (Oasis HLB) was conditioned with 3 mL of MTBE, then 3 mL of MeOH followed by 3 mL of deionized water, and then the sample was passed through the cartridge at approximately 10 mL/min. All sample bottles were rinsed with10 mL of pH 7.5 deionized water three times and the rinses were combined and passed through the SPE cartridge. After the extraction was completed, the cartridges were washed with 2 mL of 25% MeOH/water to remove the polar co-extractives and further dried under full vacuum for 5 min. The elution of caffeine was performed using 1 mL of MeOH followed by 6 mL of MeOH/MTBE (1:9, v/v). The extract was dried to dryness and then dissolved in 0.1 mL of PE. For highly contaminated samples, such as the municipal wastewater effluent, the volume of PE added was 1 mL instead. The sample was then analyzed on GC–MS/MS.

2.5. Derivatization of acidic drugs

The methylation procedure of acidic drugs used in this study was based on the method described by Metcalfe et al. [4]. Individual acidic drugs, internal and surrogate standards, their mixture and SPE sample extracts were methylated with methanol in the presence of BF_3 reagent. 10 μL of a 2.64 μg/mL solution of MCF (IS) in methanol was added to each sample extract prior to the derivatization procedure. Each solution was dried under nitrogen at room temperature to a volume of 0.2 mL to which 2 mL of 14% BF_3/MeOH reagent was added for methylation. The methylation process was carried out at 85 °C for 2 h in a heating block. After methylation, the derivatized sample was cooled and evaporated under nitrogen to a volume of 500–750 μL. Following evaporation, 3 mL of 1% potassium carbonate in water and 2 mL of PE were added to the sample. After vortexing for 1 min, the organic and aqueous layers were allowed to separate, and the organic layer was removed with a pipette. The extraction process with PE was repeated two more times using 2 mL of PE each time. The PE extract was transferred into a glass test tube containing anhydrous Na_2SO_4. After drying over Na_2SO_4, the extract was transferred into a clean glass test tube and the Na_2SO_4 was rinsed with 1 mL of PE three times. The rinses were combined with the PE extract which was evaporated to a final sample volume of 0.1 or 1 mL depending on the origin of a sample. This solution was then analyzed by GC–MS/MS.

2.6. Instrumentation

The instrument used in this work was a Varian CP-3800 gas chromatograph equipped with a Saturn 2200 ion trap mass spectrometer and a Varian CP-8200 autosampler. The flow of He through a GC column was constant and set at 1 ml/min. The programmable temperature of the vaporization injector was maintained at 250 °C, the transfer line at 290 °C, and the ion trap at 200 °C. The injector was operated at splitless conditions for 0.5 min, then turned to the split mode at the ratio of 100:1. All the compounds, except caffeine and the methyl ester of gemfibrozil, were completely separated on a fused-silica capillary column (CP-SIL 8CB-MS from Supelco) that had a 30 m × 0.25 mm inner diameter and a film thickness of 0.25 μm. The column temperature program was as follows: initial temperature 50 °C, maintained for 0.75 min, then ramped at 20 °C/min to 120 °C and at 2 °C/min to 200 °C, then at 9 °C/min to 290 °C and held at this temperature for 10 min. Total run time was 64.25 min.

2.7. Ion preparation and analysis

The capability of ion trap technology to manipulate the ion population following ion creation, but prior to ion analysis, is very advantageous especially when there is a need to quantify very low level of pollutants with high levels of background interference. It is accomplished by means of an ion preparation method (IPM). The ion preparation parameters control how the basic electron ionization scan functions are modified to prepare the ions for scanning. These parameters allow for control of the ions that are ejected, those that are retained, and those

that will undergo collision-induced dissociation (CID). The ion preparation technique determines how the scan function is constructed and what custom waveforms are created to complete the analysis.

Scan functions optimized for caffeine and each of the derivatized acidic drugs, the IS and SS, were used on the pre-selected retention time windows called 'segments'defined by the retention times of the ten target analytes (Table 2). Due to co-elution of caffeine and gemfibrozil at the chosen GC conditions, multiple reaction monitoring (MRM) mode had to be used to quantify these analytes. MRM was also applied to analyze ketoprofen and diclofenac, for which the t_R values were also very close. MRM mode uses different precursor ion masses and different dissociation parameters throughout all four stages of the MS/MS analysis. It is capable of doing MS/MS of up to ten different precursor ions and is especially useful, for example, when target compounds co-elute but have mass differences larger than the maximum allowed isolated window.

Besides the common MS parameters used for all the analytes, each segment was associated with an ion preparation mode (IPM) which defined the MS/MS parameters and a *m/z* scan range. The MS/MS parameters used in this study and also common for all the analytes were the following: Fil/Mul delay: 5 min; peak threshold: 0; mass defect: 0 mmu/100 u; background mass: 45 *m/z*; RF dump value: 650 *m/z*; filament current: 80 μA; AGC target: 2000; prescan ionization time: 1500 μs; scan time: 0.50 s/scan; multiplier offset: ±300 V.

The scan range of each segment was adjusted to include the product ions of interest. IPM parameters adjusted to maximize the performance of the instrument and common to all the analyte segments were as follows: isolation windows: 3 *m/z*; low/high offset: 6/2 DAC steps; ionization storage level: 48 *m/z*; ejection amplitude: 20 V; isolation time: 5 ms; excitation time: 20 ms; modulation rate: 3000 μs/step; modulation range: 2 steps; CID frequency offset: 0 Hz, electron energy: 70 eV.

3. Results and discussion

3.1. Efficiency of methylation process

The efficiency of the methylation of individual acidic drugs versus a mixture of them was studied using GC–MS method with no ion preparation technique involved, single MS mode. All acidic analytes studied in this work were methylated individually at the concentration range between 2560 and 8000 pg/uL. The methyl esters of acidic pharmaceuticals were extracted using PE followed by evaporation under N_2 stream to the level of 1 mL. The final products of this process were used for identification and creation of the calibration curves for each target compound. The concentration range of these calibration standards after dilution was from about 50 to 2000 pg/uL. The calibration curves created using individually produced analytes were applied to the quantification of the same analytes methylated as a mixture. The results obtained indicate that no significant differences in the efficiencies of the methylation process were observed when comparing the derivatization process of individual standards versus a mixture of them.

Table 2
Optimized parameters for ion preparation mode (IPM) for the identification of drug analytes

Compound	t_R (min)	Segment start-end (min)	Precursor ion (PI) (*m/z*)	Excitation storage level (*m/z*)	CID (V)	Product ion (*m/z*)	Quantification mode
Salicylic acid (ASA metabolite)	7.12	6.5–7.5	152, M^+	85.7	0.30	120, $[M\text{-}O\text{-}CH_3]^+$	MS/MS
Ibuprofen	16.91	16.2–17.2	220, M^+	115	0.50	161, $[M\text{-}COO\text{-}CH_3]^+$	MS/MS
2,3-D (SS)	23.01	22.3–23.5	199, $[M\text{–}Cl]^+$	87.6	1.00	156, $[PI\text{-}H3C\text{-}CO]^+$	MS/MS
Caffeine	30.67	29.5–32.0	194, M^+	85.4	1.10	165, $[M\text{-}NCH_3]^+$; 150, $[M\text{-}CH_3\text{-}NCH_3]$+; 138 $[M\text{-}H_3C\text{-}N\text{-}CO]^+$	MRM
Gemfibrozil	30.66	29.5–32.0	143, $[CH_2CH_2CH_2C(CH_3)_2COOCH_3]^+$	53.6	0.55	83, $[PI\text{-}COOCH_3]^+$	MRM
Fenoprofen	33.77	33.0–34.5	256, M^+	140.7	0.45	197, $[M\text{-}COOCH_3]^+$	MS/MS
Naproxen	38.57	38.0–39.0	244, M^+	132.1	0.65	185, $[M\text{-}COOCH_3]^+$	MS/MS
Ketoprofen	43.98	43.0–44.2	209, $[M\text{-}COOCH_3]^+$	92	0.55	194, $[PI\text{-}CH_3]^+$; 105, $[C_6H_5CO]^+$	MRM
Diclofenac	44.48	44.2–45.0	277, $[M\text{-}OCH_3]^+$	172.9	0.92	242, $[PI\text{-}Cl]^+$	MRM
Meclofenamic acid (IS)	48.16	47.5–48.7	311, M^+	172.9	0.91	242, $[M\text{-}Cl_2]^+$	MS/MS

We also compared the efficiencies of extraction procedures of the methylated analytes from aqueous solution by two different organic solvents: MTBE and PE. The method of GC and single MS was used in this part of the study. This work was carried out on individual analytes. The calibration curves created on the basis of individually methylated acidic standards extracted by PE solvent were applied to quantify the recoveries of methyl esters of individual acidic drugs extracted by MTBE. The results obtained in this study indicate that the chosen solvents (MTBE and PE) differ in their extraction efficiencies. Although, PE demonstrated higher extraction efficiencies for most of the parameters studied in this work, methylated ibuprofen showed a greater affinity to MTBE, which was reflected on its higher extraction recovery, three times higher than by PE as an extraction solvent.

We found that caffeine, if not processed separately from acidic drugs gets completely destroyed during the methylation process. For this reason, caffeine was analyzed directly in the SPE extracts.

3.2. Ion dissociation

Due to the differences in chemical structures of our target analytes, the choice of precursor ion and corresponding daughter ion(s) for quantification in their MS/MS analysis was based on the relative abundance of the main fragments, as well as the intensity of the product ions in the mass spectrum of each compound quantified.

The typical behaviors of caffeine and the methyl esters of the acidic pharmaceuticals in the ion trap instrument and electron ionization (EI) are presented in the Table 3. Due to differences in their chemical structures, the main fragments of the target analytes are quite different. None of the acidic drugs had a molecular ion M^+ as the most abundant ion in its mass spectrum obtained by electron ionization.

Acetylsalicylic acid (ASA), which is relatively unstable in aqueous matrices, degrades during the methylation process at high temperature to salicylic acid, which produces methyl salicylate. For this reason, the precursor ion chosen for MS/MS analysis of ASA was the molecular ion of methyl salicylate with $m/z = 152$ and the corresponding daughter ion with $m/z = 120$ (Tables 2 and 3).

For the methylated esters of ibuprofen, fenoprofen, naproxen, meclofenamic acid (IS), and the neutral drug caffeine, their molecular ions were used as precursors. The daughter ions for these compounds used for MS/MS quantification were chosen on the basis of principle of their highest relative abundance (Tables 2 and 3). For the first three derivatized acidic drugs (ibuprofen, fenoprofen and naproxen), the formation of their most intense daughter ion was a result of the loss of the ester group [–$C(OCH_3)O$], $m/z = 59$. The daughter ion, with $m/z = 242$, of the methyl ester of the meclofenamic acid was formed via a loss of two chlorine atoms. For caffeine, a few daughter ions were used for MS/MS quantification to increase the sensitivity of the MS/MS method for this parameter.

Molecular ions of the methyl esters of gemfibrozil and diclofenac readily decayed in the source. For this reason, their molecular ions with $m/z = 264$ and 309, respectively were not visible in the full mass spectra of these compounds. To increase the sensitivity of the method for these analytes, the precursors with $m/z = 143$ [–CH_2–CH_2–CH_2–$C(CH_3)$–$COOCH_3$] for the gemfibrozil derivative and $m/z = 277$ for the diclofenac ester were selected for further fragmentation at the MS/MS conditions. The precursor ion chosen for MS/MS analysis of gemfibrozil upon electron ionization, via a loss of an ester group, produced a high-intensity daughter ion with $m/z = 83$ which was used for

Table 3
Relative abundances of the main fragments in EI full mass spectra of caffeine, the methyl esters of acidic pharmaceuticals and their method standards

Analyte	Molecular ion (*m/z*)	Product ions (*m/z*)				
Caffeine	194[a]	[165]	[138]	[109]	82	
Abundance	100	6	5	59	27	
2,3-D (SS)	234	199[a]	175	147	111	[156]
Abundance	34	100	32	30	26	10
Diclofenac	309	277[a]	[242]	214	179	151
Abundance	0	100	84	94	24	13
Fenoprofen	256[a]	[197]	181	103	91	
Abundance	59	100	6	11	20	
Gemfibrozil	264	233	194	143[a]	122	[83]
Abundance	0	1	12	100	22	60
Ibuprofen	220[a]	177	[161]	117	91	
Abundance	29	34	100	24	18	
Ketoprofen	268	209[a]	[194]	[105]	77	
Abundance	45	100	27	58	46	
Meclofenamic acid (IS)	311[a]	277	[242]	214	179	151
Abundance	23	13	100	16	18	12
Naproxen	244[a]	[185]	170	153	141	
Abundance	34	100	15	10	15	
Salicylic acid	152[a]	[120]	92	65	39	
Abundance	41	100	58	26	35	

[]: product ("daughter") ion(s) used for characterization.
[a] Precursor ion chosen for MS/MS analysis.

final quantification. The precursor ion of the diclofenac methyl ester under EI upon the loss of one chlorine atom produced a very intensive daughter ion with $m/z = 242$, which was used in the final MS/MS quantification of this compound.

Although the molecular ions of the methyl esters of the SS (2,3-D) and ketoprofen had relatively high abundances (Tables 2 and 3), the ions with $m/z = 199$ and 209, respectively were selected as the precursor ions because of their greater intensities. To further increase method sensitivity for the methyl ester of ketoprofen, two daughter ions were also used for the final MS/MS quantification of this product (Table 3).

The identification of the target analytes in the samples was based on two parameters: peak window and spectrum match. A peak window of an analyte was centered on the retention time of a corresponding standard and was set at ±0.400 min of its t_R for every compound of interest. Match threshold of a spectrum fit between the reference and sample analyte was set at 700 out of 1000. Three most intensive ions of MS/MS spectrum of a target

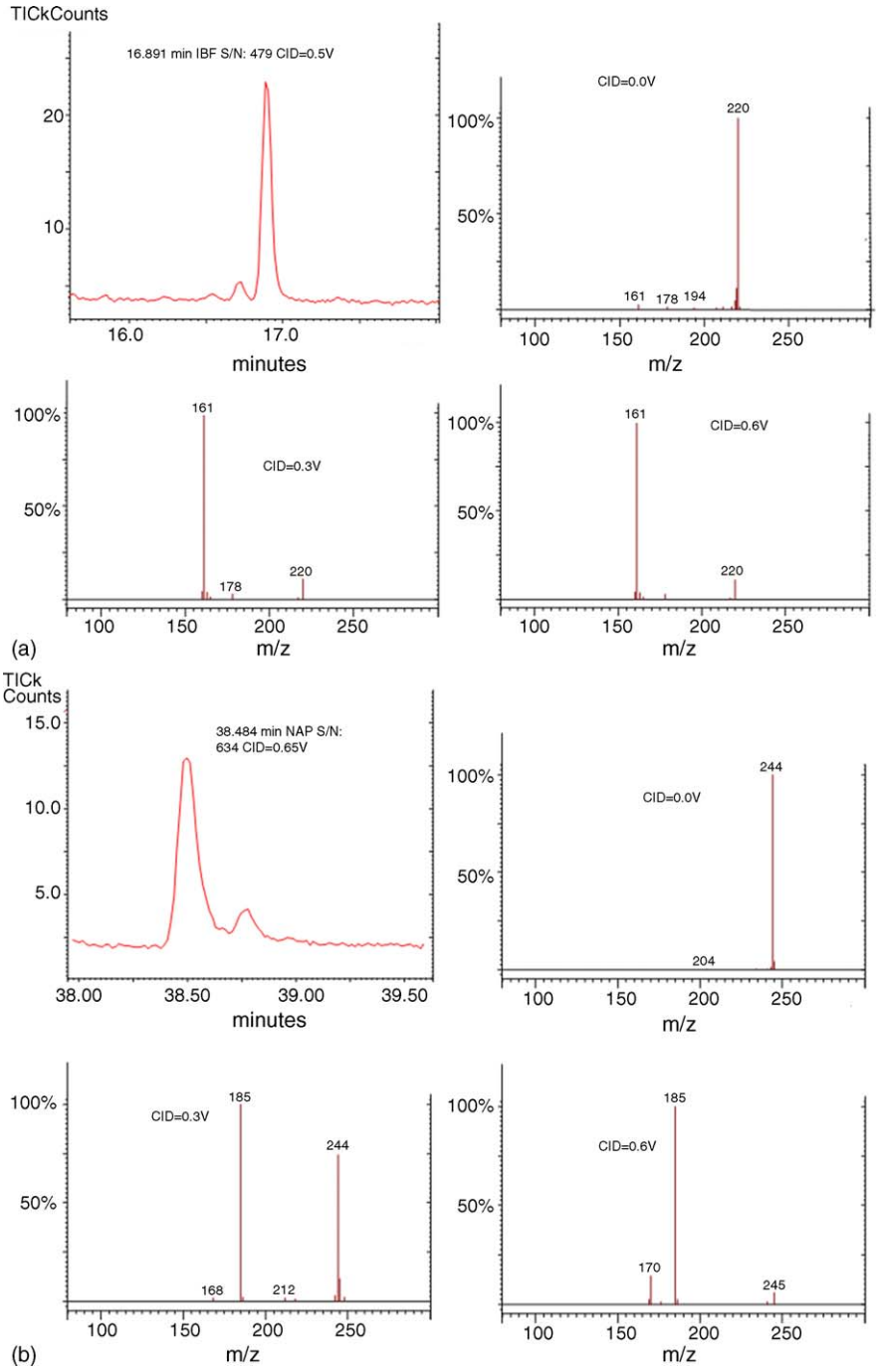


Fig. 1. Daughter ion full mass spectra of methyl ester of ibuprofen (a) obtained in AMD mode at different CID amplitudes and naproxen (b). The intensities of both chromatograms are presented as total ion counts (TIC), *m/z*.

analyte in a reference and a sample run were used to verify a correct identification.

3.3. CID amplitudes

MS/MS conditions for the dissociation of the selected precursor ions were optimized using the automated method development (AMD) option built into the Varian Saturn GC–MS/MS software. AMD uses up to ten different CID voltages for the same precursor ion. The optimization was performed at the resonant conditions in two steps. For the first step, the CID voltage was incrementally raised by using the AMD option of 0–0.9 V and full mass spectra of daughter ions were acquired at each CID amplitude. Fig. 1a and b present full mass spectra obtained by MS/MS for two structurally different analytes, the methyl derivatives of ibuprofen and naproxen, respectively. These mass spectra were produced at different dissociation voltages (CID) using the AMD function. The best CID was the one that gave the highest yield of the daughter ions ($m/z = 161$ and 185 for ibuprofen and naproxen, respectively) and very little of the precursor ions ($m/z = 220$ and 244 for ibuprofen and naproxen, respectively). Once a rough estimate of the most suitable CID amplitude was determined, the voltage was optimized using the AMD function at lower increments. Fig. 2a and b present two ion abundance curves for the methylated derivatives of ibuprofen and naproxen, respectively, obtained by plotting initial ion and fragment ion abundances as a function of the CID amplitudes. The optimum CID voltages for these parameters that were determined on the basis of these plots were 0.50 V for ibuprofen and 0.65 V for naproxen (Table 2).

The optimized energies and CIDs required for the dissociation of the other selected precursor ions of the target analytes were found to be in the range of 0.3 and 1.1 V (Table 2). Higher energies were required for the analytes that had two chlorine atoms in their structure, such as the methylated derivatives of 2,3-D, diclofenac and meclofenamic acid. Caffeine also required higher CID amplitude (1.1 V) to produce daughter ions of lower m/z.

When compared to standard (i.e. single) MS mode methods, the principal differences of this MS/MS method, such as selective precursor ion trapping and quantitative structural information through a formation of the product ion spectrum, allowed significant increase of selectivity of MS/MS technique toward target analytes. By eliminating interfering matrix ions in the product ion spectrum, MS/MS also greatly increased the signal-to-noise (S/N) ratio. In Fig. 3, two ion chromatograms of the STD stock mixture of the methyl derivatives of the acidic drugs are presented. The concentrations of the analytes in the mixture were at 'level 4' concentrations (Table 1). The intensities of the peaks on both chromatograms were presented as total ion counts of m/z of the characteristic precursors and fragmentation ions of the analytes (see Table 2).

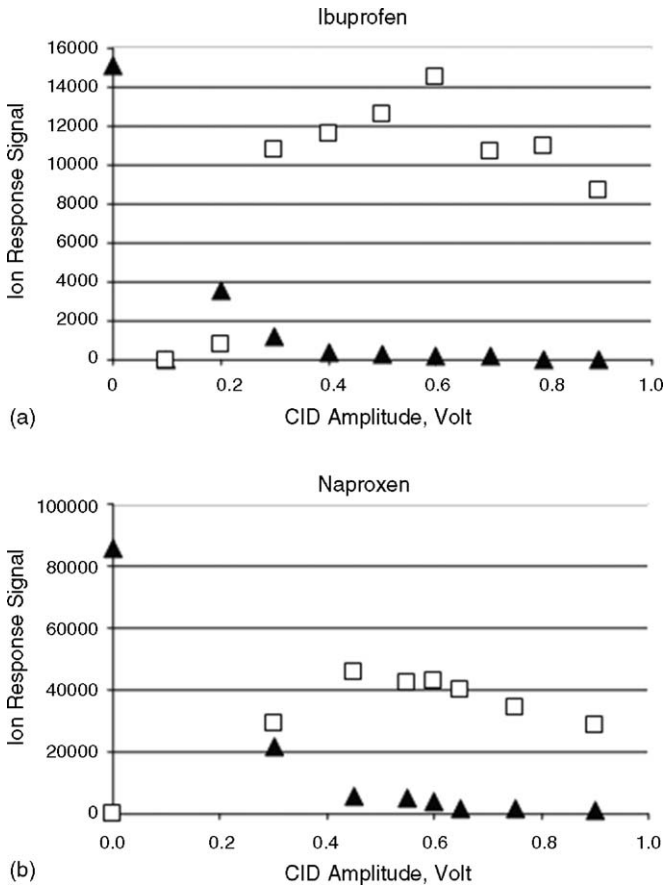


Fig. 2. Ion abundance curves obtained by plotting precursor ion and daughter ion abundances (expressed as the response signal) as a function of the CID amplitudes: (a) precursor ion (▲) 220 m/z and daughter ion (□) 161 m/z for ibuprofen methyl derivative; and (b) precursor (▲) 244 m/z and daughter (□) 185 m/z ions for naproxen methyl derivative.

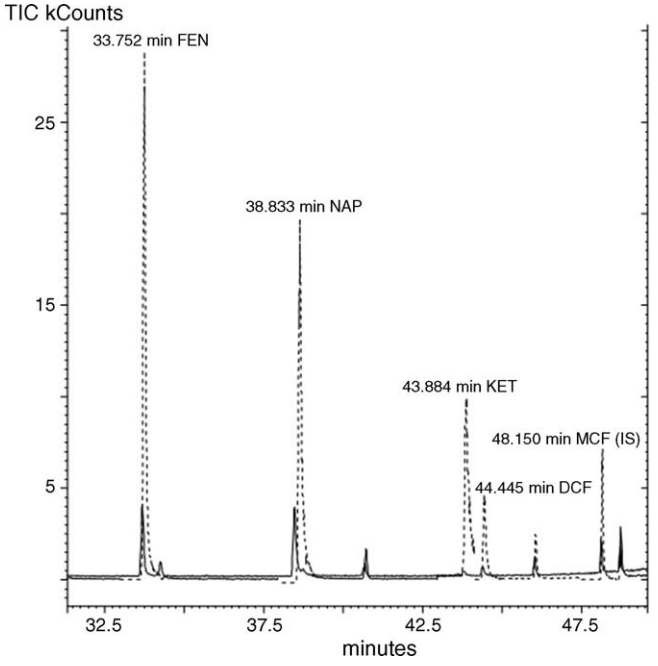


Fig. 3. A comparison of the chromatograms in MS (solid line) and MS/MS (dashed line) modes for methylated acidic pharmaceutical STDs at the concentration 'level 4' (see Table 1). The intensities of both chromatograms are presented as total ion counts of m/z of the characteristic precursors and fragmentation ions (see Table 2).

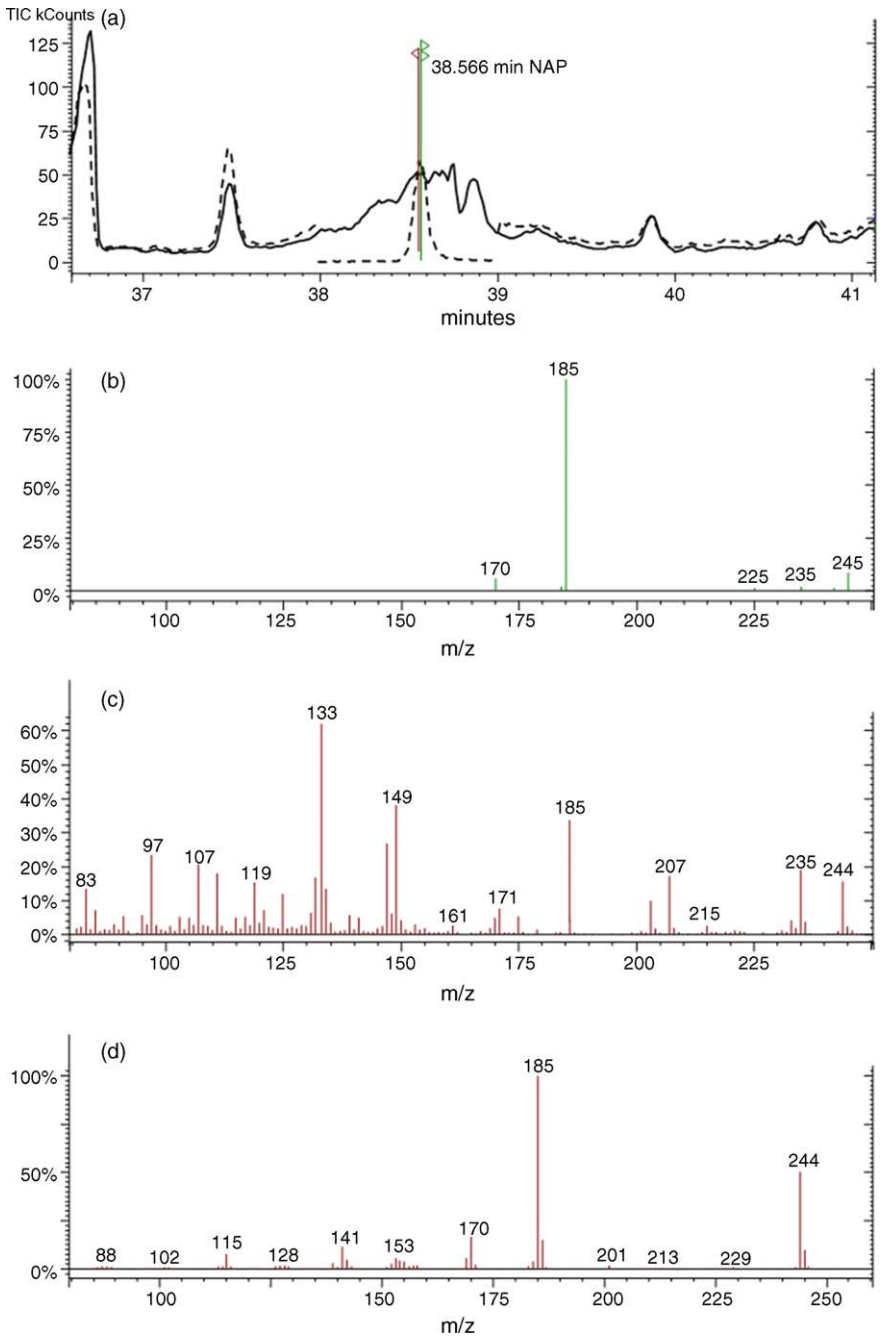


Fig. 4. A segment of the chromatograms of the analysis of STP influent using MS (solid line) and MS/MS (dashed line) methods: (a) mass spectra at t_R of naproxen for MS/MS (b) and MS (c) analysis are compared with the library mass spectrum and (d) of naproxen methyl derivative.

Table 4
Calibration parameters for methyl derivatives of acidic pharmaceuticals, caffeine and method standards (SS and IS) obtained in MSMS mode

Analyte	t_R (min)	Calibration range (pg/μL)	IDL (pg)	Calibration equation (r^2)	RSD (%)
Salicylic acid (ASA metabolite)	7.12	10–2000	0.5	$y = +28.8878x$; (0.995)	6.5
Ibuprofen	16.91	10–2000	8	$y = +10.4599x$; (0.993)	5.4
2,3-D	23.01	10–2000	10	$y = +17.2328x$; (0.991)	8.2
Caffeine	30.67	10–2000	20	$y = 0.05693x^2 + 9.8932x$; (0.989)	8.1
Gemfibrozil	30.66	10–2000	3	$y = + 58.7096x$; (0.997)	5.1
Fenoprofen	33.77	10–2000	1.5	$y = +76.4972x$; (0.994)	6.2
Naproxen	38.57	10–2000	5	$y = +76.3303x$; (0.995)	6.8
Ketoprofen	43.98	10–2000	10	$y = +83.3742x$; (0.990)	6.9
Diclofenac	44.48	10–2000	10	$y = +23.6889x$; (0.995)	6.5
Meclofenamic acid (IS)	48.16	10–2000	10	$y = +17.1048x$; (0.995)	8.9

As shown in Fig. 3, due to higher selectivity of the MS/MS technique target analytes the peak intensities of the compounds analyzed in MS/MS mode were significantly higher than in single MS mode. In the case of complex matrices such as the municipal wastewater, for instance, where the levels of interference were very high, the MS/MS approach was undoubtedly more advantageous. Fig. 4 presents: (a) a segment of the chromatograms obtained on the same sample using MS (solid line) and MS/MS (dashed line) methods. Mass spectra at the retention time of the methyl ester of naproxen received in MS/MS (b) and MS (c) modes are compared with the library mass spectrum (d) of the naproxen methyl derivative. The fact that the MS/MS method can select and isolate a precursor ion from the rest of the matrix and then let it undergo a fragmentation under collision induced dissociation conditions not only significantly reduced the background interference and increased the sensitivity, but also improved the confidence of identification. The spectrum fit between the sample (b) and its reference MS/MS spectra was more than 700 out of 1000. The effect of the matrix background has resulted in much lower spectrum fit, 400 between the sample (c) and its reference library (d) spectra obtained in the single MS mode. On the basis of our results and depending on the abundance of precursor and daughter ions of a selected analyte, instrument detection limits (IDLs) in the range of 0.5–20 pg injected were reached with the MS/MS method for acidic pharmaceuticals and caffeine in water samples (Table 4). The differences in IDLs obtained for the analytes at GC–MS/MS conditions can be attributed to their structural differences. In this study, IDL was defined as a signal-to-noise ratio >5.

3.4. Calibration and sample results

Having optimized CID parameters for all the compounds of interest and the surrogate and internal standards, the MS/MS method was used to create five point calibration curves for all analytes. The concentration levels of each analyte in the calibration mixtures covered a wide range from a few pg/μL to a few thousand pg/μL, Table 1. A good linearity was achieved for each analyte. All the correlation coefficients (r^2) were >0.99, except for caffeine $r^2 = 0.989$ (Table 4). Six samples of tap water and four samples of surface water collected near STP outfalls have been fortified with known amounts of the acidic drugs and caffeine. The concentration level of each analyte spiked into a fortified sample was in the range between 50 and 200 ng/L. The final results for fortified samples have been corrected against the corresponding blanks. The recoveries of the analytes from the spiked samples varied depending on the type of sample water and analyte and ranged from 55 to 128%. The results obtained on the fortified tap water samples have been averaged and presented in the Table 5. The highest average recovery obtained on the fortified samples was for naproxen, at the level of 112.1% while the lowest recovery was for ibuprofen, 63.9% (Table 5).

The calculated MDLs of the acidic drugs determined from the spiked water samples ranged from 0.1 to 1.0 ng/L, Table 5 compared with from 6 to 45 ng/L reported previously [16]. Caffeine showed relatively poor MDL in this study, 20 ng/L. Low MDLs achieved in the developed method can be explained by high

Table 5
Concentrations (ng/L) of acidic drugs and caffeine, as well as the recoveries (%) of IS and SS determined in sewage treatment plant (STP) effluent and receiving waters upstream (u/s) and downstream (d/s) of different STPs by GC–MS/MS

Analyte (ng/L)	MDL ng/L	Recoveries in fortified samples,% (average of 6 tap water samples) (%)	STP 1 Effluent	STP 1dup Effluent	STP 2 Effluent	STP 2dup Effluent	STP receiving waters							
							Near STP C.	T.River u/s of Creek	T.River 400m d/s of Creek	T.River 400m d/s of Creek, dup.	Creek u/s of T.River	Lake Water	C.River u/s of STP	C.River 200m d/s of STP	Near STP D.
Salicylic acid	0.1	75.6 ± 6.2	2178.2	2107.1	554.3	819.0	243.4	130.4	282.0	234.4	363.9	286.7	371.5	262.8	227.9
Ibuprofen	0.8	63.9 ± 5.6	6718.3	6523.2	3588.1	2235.2	9.5	ND	ND	ND	ND	ND	ND	ND	2.9
Caffeine	20	110.6 ± 7.1	2263	1742	8059	8132	1590	53	29	ND	ND	ND	ND	ND	ND
Gemfibrozil	0.3	73.8 ± 6.1	403.1	478.2	104.1	80.1	18.4	9.0	3.9	5.9	ND	ND	ND	ND	35.3
Fenoprofen	0.2	90.3 ± 7.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Naproxen	0.5	112.1 ± 6.8	7098.2	7962.3	1043.8	633.1	271.4	ND	ND	ND	ND	ND	ND	ND	17.8
Ketoprofen	1.0	108.4 ± 8.9	268	351	10	8	ND	ND	ND	ND	ND	ND	ND	ND	ND
Diclofenac	1.0	99.1 ± 7.5	448	457	42	32	ND	ND	ND	ND	ND	ND	ND	ND	ND
Meclofenamic acid (IS), %	1.0	76.8 ± 8.9	79	83	86	90	112	109	80	97	113	115	96	107	80
2,3-D (SS) (%)	1.0	79.1 ± 8.4	82	96	103	80	96	91	110	95	115	113	139	87	80

MDLs and relative standard deviations (%) determined from the spiked samples are also provided.

efficiency of IT–MS/MS methodology in suppressing matrix background.

A number of surface water and municipal wastewater samples from different locations were analyzed using the MS/MS method developed in this study. The list of samples analyzed included two duplicates of effluent samples from two different STPs (STP1 and STP2), six spiked tap water samples, as well as nine surface water samples collected within 500 m radius up and downstream of small size STP outfalls. The results obtained including % recovery of both IS and SS, are presented in Table 5. High reproducibility was possible to achieve on the replicates of STP effluent samples, ranging between 75.5 and 113.4% for NAP and KET, respectively. The analgesic/anti-inflammatory drugs such as ibuprofen, naproxen, as well as the metabolite of acetylsalicylic acid (salicylic acid) were found in the samples of STP wastewater and also in those surface water samples collected near STP outfalls. It should be noted that the ASA metabolite (salicylic acid) was present in each sample at an elevated level. This may be attributable to natural occurrence of salicylic acid in surface waters. It was also not a surprise that caffeine was found at various levels in almost every sample.

4. Conclusions

This work has demonstrated the capability of tandem mass spectrometry using an ion trap with electron ionization source for the analysis of low levels of acidic pharmaceuticals and caffeine. The method can also be successfully used as an instrumental tool to analyze these compounds in surface water and STP wastewater samples. The application of the optimized IT–MS/MS parameters conjointly with the sample preparation procedure resulted in MDLs of 0.1–1.0 ng/L and 20 ng/L for the determination of the acidic pharmaceuticals and caffeine, respectively in effluent samples of municipal wastewater plants, as well as receiving waters. The use of successive fragmentation of the selected precursor ions, with the formation of corresponding daughter ions, gives supplementary information for the identification of the target compounds analyzed in these complex matrices.

Acknowledgements

This research was supported by the Natural Sciences and Engineering Research Council (NSERC), Industry Research Chair Grant, Capital Region District (CRD) Environmental Services Department, CRD Water Services Department and the Ministry of Environment. This manuscript benefited from comments by Dr. Chris Metcalfe and two anonymous referees.

References

[1] B. Halling-Sorenson, S.N. Nielsen, P.F. Lanzky, F. Ingerrslev, H.C. Holten Lutzhoft, S.E. Jorgensen, Chemosphere 36 (1998) 357.
[2] T.A. Ternes, Water Res. 12 (1998) 3245.
[3] C.D. Metcalfe, X.S. Miao, B.G. Koenig, J. Struger, Environ. Toxicol. Chem. 22 (2003) 2881.
[4] C.D. Metcalfe, B.G. Koenig, D.T. Bennie, M. Servos, T.A. Ternes, R. Hirsch, Environ. Toxicol. Chem. 22 (2003) 2872.
[5] M.L. Richardson, J.M. Bowron, J. Pharm. Pharmacol. 37 (1985) 1.
[6] A. Waggott, in: W.J. Cooper (Ed.), Chemistry in Water Reuse, vol. 2, Ann Arbor Science, MI, 1981, p. 55.
[7] D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, H.T. Buxton, Environ. Sci. Technol. 36 (2002) 1202.
[8] I.J. Buerge, T. Poiger, M.D. Muller, H.R. Buser, Environ. Sci. Technol. 37 (2003) 691.
[9] D. Loffler, T.A. Ternes, J. Chromatogr. A 1021 (2003) 133.
[10] V. Koutsouba, Th. Heberer, B. Fuhrmann, K. Schnidt-Baumler, D. Tsipi, A. Hiskia, Chemosphere 51 (2003) 69.
[11] B.W. Brooks, C.K. Chambliss, J.K. Stanley, A. Ramirez, K.E. Banks, R.D. Johnson, R.J. Lewis, Environ. Toxicol. Chem. 24 (2005) 464.
[12] X.S. Miao, B.G. Koenig, C.D. Metcalfe, J. Chromatogr. A 952 (2002) 139.
[13] T. Ternes, Trends Anal. Chem. 9 (2001) 419.
[14] G.R. Boyd, H. Reemtsma, D.A. Grimm, S. Mitra, Sci. Total Environ. 311 (2003) 135.
[15] H.B. Lee, K. Sarafin, T.E. Peart, M.L. Svoboda, Water Qual. Res. J. Can. 38 (2003) 667.
[16] P.M. Thomas, G.D. Foster, J. Environ. Sci. Health A 39 (2004) 1969.