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Carbamazepine in municipal wastewater and wastewater sludge: Ultrafast quantification by laser diode thermal desorption-atmospheric pressure chemical ionization coupled with tandem mass spectrometry

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ABSTRACT

In this study, the distribution of the anti-epileptic drug carbamazepine (CBZ) in wastewater (WW) and aqueous and solid phases of wastewater sludge (WWS) was carried out. A rapid and reliable method enabling high-throughput sample analysis for quicker data generation, detection, and monitoring of CBZ in WW and WWS was developed and validated. The ultrafast method (15 s per sample) is based on the laser diode thermal desorption-atmospheric pressure chemical ionization (LDTD-APCI) coupled to tandem mass spectrometry (MS/MS). The optimization of instrumental parameters and method application for environmental analysis are presented. The performance of the novel method was evaluated by estimation of extraction recovery, linearity, precision and detection limit. The method detection limits was 12 ng L^{-1} in WW and 3.4 ng g^{-1} in WWS. The intra- and inter-day precisions were 8% and 11% in WW and 6% and 9% in WWS, respectively. Furthermore, three extraction methods, ultrasonic extraction (USE), microwave-assisted extraction (MAE) and accelerated solvent extraction (ASE) with three different solvent condition such as methanol, acetone and acetonitrile:ethyle acetate (5:1, v/v) were compared on the basis of procedural blank and method recovery. Overall, ASE showed the best extraction efficiency with methanol as compared to USE and MAE. Furthermore, the quantification of CBZ in WW and WWS samples showed the presence of contaminant in all stages of the treatment plant.

1. Introduction

Large volumes of pharmaceuticals are used for the prevention, diagnosis and treatment of diseases in human and animals. The worldwide average per capita consumption of active pharmaceutical ingredients per year is estimated to be about 15 g and in industrialized countries, the value is expected to be in between 50 and 150 g [1]. Due to their extensive use, presence in the aquatic environment and potential for impacts on wildlife and humans, pharmaceutical compounds are becoming an environmental issue [2]. Therefore, study of the fate of these compounds is becoming very important component of assessing the environmental risks associated with them.

Carbamazepine (CBZ) is one such important drug used: (i) as an antieliptic and anticonvulsant; (ii) for the treatment of epilepsy, as well as for various psychotherapeutic applications and; (iii) in combination with other drugs for the treatment of alcohol withdrawal [3]. The physico-chemical and pharmacological properties of CBZ are summarized in Table 1. CBZ has been proposed as an anthropogenic marker in water bodies [6]. Annually, about 1014 t of CBZ is consumed worldwide (estimated value is in accordance with Intercontinental Marketing Services (IMS) Health data: 942 t of CBZ were sold in 2007 in 76 major countries which are believed to account for 96% of the global pharmaceutical market) and this yields to more than 30 t of CBZ which have to be removed from effluents [7]. In Canada, approximately 28 t of CBZ was sold as prescriptions in 2001 [8].

Following human administration (excreted unchanged and/or as metabolies with feces and urine), CBZ has been detected in wastewater (WW) and wastewater sludge (WWS). Studies in Europe and North America have shown that CBZ is one of the most frequently detected pharmaceuticals in wastewater treatment plants (WWTPs) effluents and in river water [9–11]. As WWTPs provide the first and perhaps the most important opportunity for removing CBZ that are destined for discharge into the environment, it is important to characterize the fate

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Table 1
Physico-chemical and pharmacological properties of CBZ.

Molecular formula, CAS No. and molecular weight	$C_{15}H_{12}N_2O$, 298-46-4 and 236.27 g mol^{-1}
Water solubility[a]	17.7 mg L^{-1} (20 °C)
Log K_{ow} (octanol-water partitioning)[a]	2.45
Henrys law constant[a]	1.09×10^{-5} Pa m^3 mol^{-1} (25 °C)
p*K*a	Neutral
Melting point	189–193 °C
Usage	Analgesic, anticonvulsant, antimanic agent
Elimination half-life[b]	25–65 h
Appearance	White, light yellowish powder
Toxicity	Mild ingestion cause vomiting, drowsiness, ataxia, slurred speech, nystagmus, dystonic reactions, and hallucinations. Severe intoxications may produce coma, seizures, respiratory depression and hypotension
Affected organisms	Human and aquatic organisms

[a] [4].
[b] [5].

of CBZ during the treatment of municipal wastewater. The most important process to study the fate of CBZ in WW and WWS includes to know whether CBZ will primarily enter the aquatic or terrestrial environment or get partitioned from aqueous sewage into sludge and disposed with further application of sludge. Several groups have investigated the elimination of CBZ during sewage treatment and also fate of the compound in different contaminated media including WW and WWS [12,13]. However, to the best of our knowledge there have been no studies conducted on partitioning of CBZ in different compartment of WWTPs which is very important to know the fate of compound and to select different treatment processes for effective degradation of compound.

Furthermore, the measurement problems associated with quantification of these pharmaceutical compounds including CBZ in WW and WWS is to detect the analyte in trace levels (ng L^{-1} or below) and to avoid the impact on the analyte signals caused by matrix components. Identification and quantification of different pharmaceutical compounds including CBZ are usually performed by LC or GC-MS/MS [13,14]. To reduce sample preparation, analysis time and concentration of organic solvents (during quantification), the development of an ultrafast method for the analysis of CBZ in WW and WWS has been carried out using a laser diode thermal desorption (LDTD) coupled to an atmospheric pressure APCI source for tandem mass spectrometry (MS/MS). LDTD-APCI-MS/MS analysis has been recently developed to enhance the high throughput capacity in MS by reducing LC-MS/MS runs of 5 to 30 min to 10 to 30 s in LDTD-APCI-MS/MS run [15].

In this study, we developed a suitable ultrafast method based on LDTD-APCI-MS/MS method for quantification of CBZ in WW and WWS which has never been studied earlier. Furthermore, three extraction methods namely, ultrasonic extraction (USE), microwave-assisted extraction (MAE) and accelerated solvent extraction (ASE) were compared for extraction of CBZ from WWS by spiked recovery experiments. Furthermore, the concentration of CBZ was monitored in WW and WWS at various stages of treatment in the WWTP for the Quebec City, Qc, Canada.

2. Materials and methods

2.1. Chemicals

CBZ was obtained from Sigma Aldrich (St Louis, MO, USA). The internal standard (IS), carbamazepine-d_{10} was purchased from C/D/N Isotopes Inc. (Montreal, Quebec, Canada). HPLC-grade methanol (MeOH), acetonitrile, ethyl acetate, and acetone, were purchased from Fisher Scientific (Ontario, Canada). Sep-Pak Plus C18 environmental cartridges used for solid phase extraction (SPE) clean-up was purchased from Waters (Milford, MA, USA). HPLC grade water was prepared in the laboratory using a Milli-*Q*/Milli-RO Millipore system (Milford, MA, USA).

2.2. Wastewater treatment plants and sampling

Samples were collected from Quebec Urban Community (CUQ) wastewater treatment plant (Beauport, Quebec city, Quebec, Canada) which receives wastewater originating from domestic zones, industries, commercial enterprises and institutions present in the city. The eastern station of WWTP serves a population of 528,016 (2006 estimate) and has a treatment capacity of 400,000 m^3 day^{-1}. The CUQ treatment plant accomplishes primary and physical-chemical treatment of sewage before discharging the treated water into the Saint-Lawrence River.

Samples of WW and WWS were collected as grab samples during the sampling periods (August 20th, 21st, 22nd, 23rd, 24th, 2011). Fig. 1 represents a schematic of the treatment process and the different sampling locations. Samples were collected in pre-cleaned glass amber bottles with aluminum foil-lined caps. After sample collection, in order to study the partitioning of CBZ in WWS, the liquid fraction of WWS was separated from the solid fraction by centrifugation at 7650 *x* g for 15 min. Later, WWS (primary sludge, secondary sludge, mixed sludge, dewatered sludge) and the solid fraction of WWS (primary sludge solids, secondary sludge solids and mixed sludge solids) were stored at 4 ± 1 °C in a cold room until preparation for analysis, which generally occurred within 24 h of collection. Furthermore, WW (influent, grit influent, effluent) and liquid fraction of WWS (primary sludge liquid, secondary sludge liquid and mixed sludge liquid) samples were filtered through a 0.45 μm glass-fiber (Fisherbrand G6 filter circles, Fisher Scientific, Ontario, Canada) and immediately stored at 4 ± 1 °C until analysis.

2.3. Extraction

Different WWS and solid fraction of WWS were frozen using liquid nitrogen prior to lyophilization by the freeze-dry system (Dura Freeze Dryer, Kinetics). Three types of extraction methods, ultrasonic extraction (USE), microwave-assisted extraction (MAE) and accelerated solvent extraction (ASE) were carried out in order to optimize the extraction method for higher recovery of CBZ from WWS. The experiments were carried out by spiking the known concentration of CBZ (100 ng g^{-1}) to WWS samples.

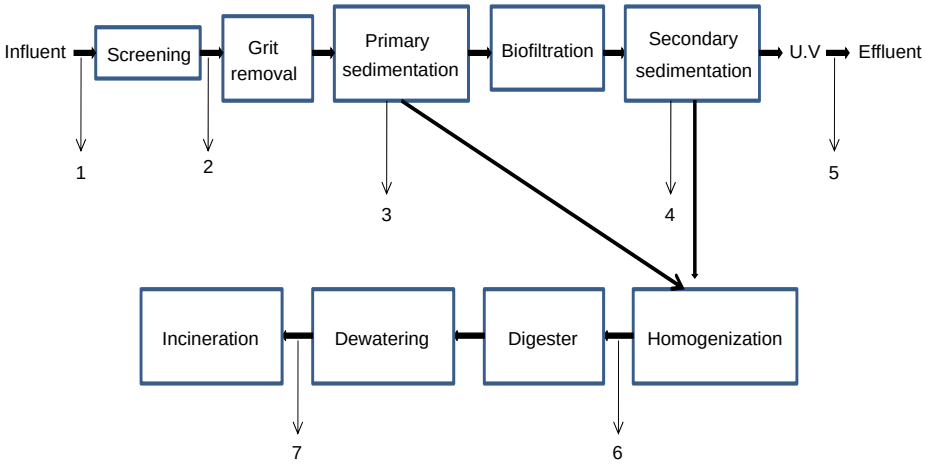

Fig. 1. Schematic of the treatment steps of WWTP located in Quebec (Quebec), Canada with different sampling points (1: Influent; 2: Grit influent; 3: Primary sludge; 4: Secondary sludge; 5: Effluent; 6: Mixed sludge; 7: Dewatered sludge).

2.3.1. Ultrasonic extraction (USE)

Ultrasonic extraction process was carried out by using a Fisher 100 Model Ultrasonicator. An amount of 0.5 g of lyophilized and homogenized sludge was transferred to a glass tube followed by addition of 20 mL of solvent. The tubes were shaken for 20 min in a mechanical shaker and sonicated for 15 min. The extract was separated by centrifugation at $7650 \times g$ for 15 min and the procedure was repeated three times. The extracts were combined, concentrated to an approximate volume of 1 mL with gentle stream of nitrogen and re-dissolved in 100 mL of HPLC grade water. After the extraction process, the sample was transferred for clean-up procedure.

2.3.2. Microwave-assisted extraction (MAE)

A CEM MARS Microwave Accelerated Reaction System (CEM Corporation, North Carolina, USA) was used. This system allowed up to 14 extraction vessels to be simultaneously irradiated. An amount of 0.5 g of lyophilized and homogenized sludge was transferred to a vessel equipped with Teflon-sealed lip-tight caps and polyetheretherketone (PEEK)-liners followed by addition of 20 mL of solvent. Microwave power was 1200 W (100%) and the extraction was performed in a temperature-controlled mode. The extraction temperature was 110 ± 1 °C and programmed as follows: ramp to 110 ± 1 °C for 10 min, holding at 110 ± 1 °C for 10 min. The extract was separated by centrifugation at $7650 \times g$ for 15 min. Furthermore, the extracts were concentrated to an approximate volume of 1 mL with gentle stream of nitrogen and re-dissolved in 100 mL of HPLC grade water. The sample was then subjected to the clean-up procedure.

2.3.3. Accelerated solvent extraction (ASE)

Accelerated solvent extraction was carried out by using an ASE 350 extractor (Dionex, Sunnyvale, CA, USA). Lyophilized and homogenized sludge sample (0.5 g) was extracted in a 34 mL stainless steel vessel. Extraction was performed with 20 mL of solvent at 140 °C and 1500 psi for a 6 min heat-up followed by a 5 min static extraction. The vessel was then rinsed with 10 mL of solvent and the extract was separated by centrifugation at $7650 \times g$ for 15 min. Furthermore, the extract was concentrated to an approximate volume of 1 mL with gentle stream of nitrogen and dissolved in 100 mL of HPLC grade water. The sample was then subjected to the clean-up procedure.

2.4. Clean-up

Solid phase extraction (SPE) method was used for clean-up and pre-concentration of extract obtained from USE, MAE and ASE methods. Sep-Pak Plus C18 environmental cartridges were fitted into the vacuum manifold (Welch, USA) which was connected to a vacuum pump (Welch Rietschle Thomas, USA) to dispense samples through the cartridges. Cartridges were pre-conditioned by passing 7 mL of methanol and 3 mL of HPLC water at a flow rate of 1 mL min^{-1}. WWS and solid fraction of WWS extracts and filtered WW and liquid fraction of WWS (100 mL each) were passed at a flow rate of 5 mL min^{-1}. After pre-concentration, the sorbents were dried by using a vacuum system set at (−15) psi.

The elution was performed by adding 2×4 mL of methanol to the cartridge at a flow rate of 1 mL min^{-1} and giving it a wait time of 10 min in order to give enough duration of contact between the solvent and the adsorbed compounds. The extracts were later evaporated to dryness with a gentle stream of nitrogen and reconstituted with methanol to a final volume of 200 μL prior to Laser Diode Thermal Desorption-Atmospheric Pressure Chemical Ionization-Mass Spectrometry/Mass Spectrometry (LDTD-APCI-MS/MS) analysis. The overall scheme of the analytical procedure used for the quantification of CBZ in WW and WWS, solid and liquid fraction of WWS is presented in Fig. 2.

2.5. LDTD-APCI-MS/MS analysis

Quantification of CBZ in WW and WWS was achieved with the LDTD-APCI ionization source (Phytronix Technologies, Quebec, Canada) mounted on a TSQ Quantum Ultra AM Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Samples were first spotted (2 μL) into the LazWell 96-well polypropylene plate cavities containing inserts made of proprietary stainless steel alloy and left to dry at room temperature. The designed well shape allows the sample to concentrate in the heating zone while drying. The loaded plate is then transferred to an *X–Y* movable stage of the LDTD housing unit. An infrared (IR) laser diode (980 nm, 20 W, continuous) is then focalized to impact the back of the inserts, thermally desorbing the dried sample, which is vaporized into gas phase. The desorbed gas phase neutral molecules are carried over by a carrier gas (medical grade purified air) into a corona discharge region to undergo APCI and then introduced directly into the mass spectrometer.

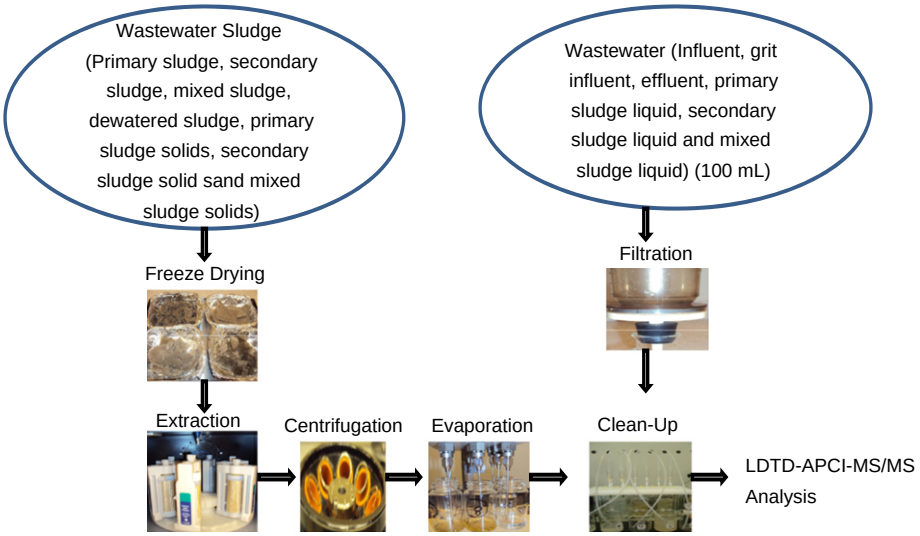

Fig. 2. Sample processing and chemical analysis scheme used for analysis of CBZ in WW and WWS samples.

Optimization of LDTD-APCI-MS/MS method for analysis of CBZ in WW and WWS was carried out in following steps: (a) LDTD-APCI sample optimization for MS and MS/MS conditions in negative ionization mode (NI) and positive ionization mode (PI) with following conditions: corona discharge voltage (5000 V in PI mode and 5500 V in NI mode); vaporizer temperature (450 °C); sheath gas pressure (0 arbitrary units); auxiliary gas pressure (0 arbitrary units); capillary temperature (270 °C); collision pressure (1.5 mTorr) and scan time (0.1 s); (b) optimization of the LDTD/APCI physical parameters using the defined SRM scans.

2.6. Data analysis and method validation

The LDTD-APCI source was controlled by the LazSoft 4.0 software (Phytronix Inc., Quebec, Canada). Quantitative analysis was performed using selected reaction- monitoring mode (SRM). Quantification of CBZ was performed by the internal standard method based on peak areas and relative retention time, using carbamazepine d_{10} as internal standard. A three-point identification approach was adopted with peak areas from the first transition (SRM1) for the quantitative analysis and a second transition (SRM2) along with the ratio of areas obtained from the first to second transitions were used for confirmation with sample tolerance established by the relative standard deviation of the ratio obtained from the standards [16]. Resulting MS/MS peaks were integrated by using the ICIS algorithm of the Xcalibur 1.2 software from Thermo Fisher Scientific (Waltham, MA, USA). Experiments with spiked samples and blank (solvent with IS) (minimum two experiments) were carried out in parallel for each set of extractions, clean-up and quantification studies.

The matrix effects were studied by the evaluation of signal suppression or enhancement. The signal suppression was calculated as the percentage of the decrease or increase in signal intensity in a sample matrix versus in methanol-water (1:9, v/v). Eq. (1) was used to calculate the signal suppression:

$$signal\ suppression(\%) = \left(1 - \frac{S_m}{S_s}\right) \times 100 \quad (1)$$

where, S_m is the slope of the calibration curve of CBZ in the sample extract (WW or WWS) spiked after extraction and S_s is the slope in the solution standard (methanol-water (1:9, v/v)) at the same concentration than the spiked sample. To overcome the effect of matrix, standard addition was used and performed as described in Ellison and Thompson [17].

The recoveries of CBZ in different samples of WW and WWS through the method were evaluated by analyzing five replicates and then applying Eq. (2):

$$recovery(\%) = \frac{C_m - C_o}{C_s} \times 100 \quad (2)$$

where, C_m=measured concentration of the CBZ observed in the spiked matrix, C_o=initial concentration of CBZ observed in matrix and C_s=concentration of CBZ spiked in the matrix. In order to measure the recovery, the spiked samples were stirred to homogenize the analytes within the matrix and were left to equilibrate for 24 h before extraction. A five point standard addition was carried out for calibration curve (triplicate injections) ranging from 50 ng L^{-1} and 700 ng L^{-1} for WW and 10 ng g^{-1} and 500 ng g^{-1} for WWS. The area ratio (analyte:internal standard) was plotted against the analyte additions to obtain the calibration curve.

The limits of detection (LOD) were determined using the standard error of the intercept and the slope of the calibration curve. LODs were defined as the concentration of a compound giving a signal-to-noise ratio of 3. The intra-day method repeatability was expressed as the relative standard deviation (in %) was calculated by analysis of the same spiked sample for each type of matrix at 100 ng L^{-1} (three times) on a given working day. Reproducibility (inter-day precision) was calculated by extracting a sample spiked at 100 ng L^{-1} freshly prepared each day for three days.

3. Results and discussion

3.1. LDTD-APCI-MS/MS optimization

MS/MS optimization showed a good sensitivity for quantification of CBZ in WW and WWS using APCI mode. The signal found with APCI showed a good intensity in positive ionization mode. Hence, the positive mode was selected for analysis of CBZ in WW and WWS. Once SRMs were fixed in the APCI mode, MS and MS/MS scan by the LDTD source were performed. The optimization of LDTD-APCI physical parameters was then calculated using the defined SRM scans.

The LDTD-APCI physical parameters were optimized for the quantification of CBZ in WW (influent was considered due to presence of higher concentration of CBZ) and WWS (secondary sludge was considered due to low matrix effect) samples.

The deposition solvent, laser power, laser pattern and carrier gas flow rate are parameters affecting the thermal desorption and the ionization of the analytes in LDTD [18]. Thus, all parameters mentioned above were optimized for analysis of CBZ in WW and WWS.

The solvent used for analyte deposition should have a surface tension superior to 27 mN/m to prevent the sample droplet to flow outside the designed cavity of the sample well [19]. The peak intensity of CBZ analyzed in WW and WWS for six different solvents used for analyte deposition is presented in Fig. 3. Maximum peak intensity of CBZ was observed with methanol:water (2:1, v/v) with relative standard deviation below 10% in WW and WWS. Therefore, methanol:water (2:1, v/v) was the preferred solvent used for analyte deposition into the well cavity for all type of WW and WWS samples.

The laser power, laser duration and pattern as well as the carrier gas flow rate were optimized with methanol:water (2:1, v/v) as the selected solvent. Laser power was optimized in both WW and WWS by using CBZ and internal standard (carbamazepine d_{10}). Each sample was analyzed in triplicate from 5 to 50% laser power. The maximum peak intensity was observed at 25% and 35% laser power (supplementary material, Fig. 1S) in WW and WWS, respectively.

Carrier gas flow rate was optimized in WW and WWS by using CBZ and internal standard (carbamazepine d_{10}). The peak intensity of CBZ analyzed in WW and WWS for a carrier gas flow rate varied from 1 to 10 L min^{-1} as presented in Fig. 4. Maximum peak intensity of CBZ was observed for a carrier gas flow rate of 3 L min^{-1} in WW and WWS. The results were in agreement with previously published LDTD optimization studies for hormones in WW [20].

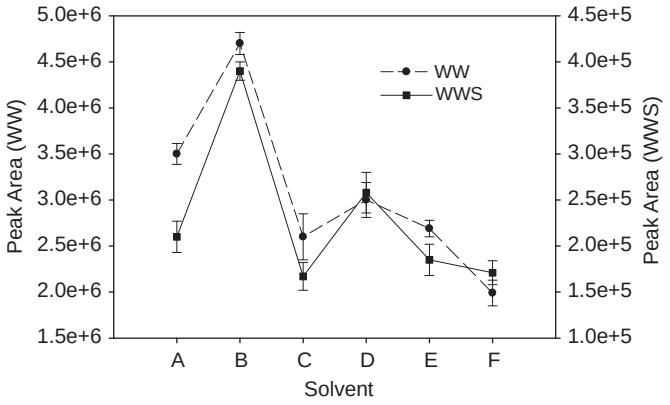

Fig. 3. LDTD-APCI optimization of different solvents used for analyte deposition in plate well. The results are the mean of triplicate spikes and the error bar lengths represent the relative standard deviations. A, methanol; B, methanol:water (2:1, v/v); C, acetonitrile; D, acetonitrile:water (2:1, v/v); E, methanol:acetonitrile (2:1, v/v); F, ethyl acetate.

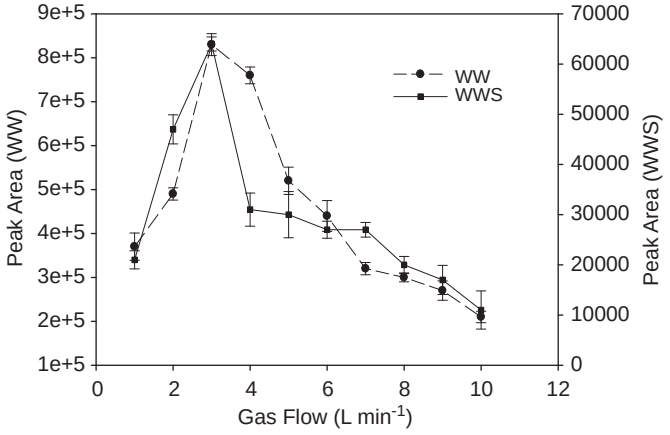

Fig. 4. Gas flow (L min^{-1}) optimization for CBZ analysis in WW and WWS samples. The results are the mean of triplicate spikes and the error bar lengths represent the relative standard deviations.

Furthermore, other factor that affects the optimization of LDTD method is the laser pattern. Different laser patterns were tried with the optimum laser power obtained in WW and WWS. The laser pattern programming that gave the maximum peak area intensity in WW was 1 s ramp from 0% to 25%, held for 3 s in 25% before shutting off the laser. In WWS, the laser pattern was 2 s ramp from 0% to 35%, held for 2 s at 35% before shutting off the laser. Increasing holding time past 3 s (at 25%) and 2 s (at 35%) in WW and WWS samples, respectively, with the same laser pattern did not improve peak area intensities (data not shown) and would have lengthened the analysis time.

3.2. Method validation

The LDTD/APCI-MS/MS method with the optimized physical parameters was applied to spiked WW and WWS samples. For each matrix, three spiked samples (50 ng L^{-1} for WW samples and 100 ng g^{-1} for WWS samples) and three non-spiked samples were extracted and analyzed. Good recoveries of CBZ was observed and ranged from 98% to 113% and 96% to 107% for WW and WWS samples, respectively. The calibration curve observed for quantification of CBZ in WW and WWS showed good linearity with correlation coefficients (R^2) of 0.9997 and 0.994 in WW and WWS, respectively (Table 2).

The repeatability (intra-day precision) and reproducibility (inter-day precision) were observed to be 8% and 11%, respectively in WW (Table 2). In case of WWS, the repeatability and reproducibility was observed as 6% and 9%, respectively. In both WW and WWS, the results of repeatability and reproducibility showed robustness and stability. Method detection limits (MDL) calculated from the calibration curves were 12 ng L^{-1} and 3.4 ng g^{-1} for WW and WWS, respectively (Table 2). The resulting MDL were comparable to several other analytical methods applied to analysis of CBZ in WW and WWS, including GC-MS or LC-MS/MS methods [21,22].

3.3. Comparison of USE, MAE and ASE methods

The experiment was carried out using the comparison of procedure blanks of the three extraction methods, namely USE, MAE and ASE. A series of procedure blanks of the three extraction methods were performed in order to check the contamination due to extraction procedure. The experiments were carried out in triplicate. The procedural blank values (data not reported) obtained were up to two orders of magnitude smaller than the concentration of CBZ in actual samples and thus did not exhibit notable influence to the experimental results. In order to investigate whether the blank values obtained from the three different extraction methods were statistically significant; a STATISTICA

Table 2
Linearity (R^2), repeatability, reproducibility and method detection limit (MDL) of the LDTD-APCI-MS/MS method for CBZ in positive ionization (PI) mode.

	R^2	Intra-day precision (% RSD)	Inter-day precision (% RSD)	MDL
WW	0.9997	8	11	12 ng L^{-1}
WWS	0.994	6	9	3.4 ng g^{-1}

7 of STATSOFT Inc. (Thulsa, USA), ANOVA test was performed at the significance level of 0.05. There was no significant difference in the blank values were observed among the three extraction method. Overall, no contamination to target compound was observed during the whole experimental procedure of extracting the CBZ from different WWS samples.

Furthermore, the three extraction methods, USE, MAE and ASE were compared on the basis of recovery efficiency of CBZ from WWS samples. The recovery experiments were performed using spiked (100 ng g^{-1}) WWS samples. Table 3 represents recoveries of CBZ from spiked WWS samples for different type of solvents with the methods of USE, MAE and ASE. The recoveries obtained from USE with methnol, acetone and acetonitrile:ethyl acetate (5:1, v/v) were 85.4–90.1%, 80.1–86.3% and 77.6–84.2%, respectively. The recoveries obtained from MAE with methanol, acetone and acetonitrile:ethyl acetate (5:1, v/v) were 91.1–95.3%, 82.1–87.9% and 70.0–88.2%, respectively. The recoveries obtained from ASE with methanol, acetone and acetonitrile:ethyl acetate (5:1, v/v) were 96.9–107.0%, 84.2–93.0% and 82.5–90.8%, respectively. Higher extraction recoveries of CBZ from different WWS samples were observed with ASE ($P < 0.01$) under methanol as solvent. To investigate the statistical significance of the difference in recovery efficiency of the three extraction methods, a statistical software, STATISTICA 7 of STATSOFT Inc. (Thulsa, USA), ANOVA test was used. Statistical significance was defined as a P value < 0.05. The statistical significance was determined based on comparison of F-value (F) with critical F-value (Fc) at 95%, 99% and 99.9% confidence. The results showed that the differences in extraction recoveries were significant (Supplementary material, Table 1S) among ASE and MAE and not significant in some results of USE extraction methods.

Furthermore, the recovery test was also carried out with the internal standard (carbamazepine d_{10}) under three different extraction methods. Higher recovery was also observed (data not reported) with ASE as compared to USE and MAE under methanol as solvent.

Among three type of extraction methods, the order of recovery of CBZ from different WWS samples by using three type of solvents, methanol, acetone and acetonitrile:ethyl acetate was ASE > MAE > USE. The method recoveries observed are in accordance with the values from most of other studies [23,24,25]. Higher recoveries observed in ASE and MAE as compared to USE may be due to higher extraction temperature (140 °C and 110 °C, respectively) used. During the extraction process, the rate-limited step was desorption of the analytes from the active spots of the matrix. The higher temperature condition helps to improve the dissolving capability and to minimize the force between the analytes and the active spot of the matrix. Furthermore, the higher temperature lowered the surface tension and viscosity of the solvent, thus making a full contact between the solvent and analytes. Furthermore, the higher recovery of CBZ observed in ASE as compared to MAE was due to the combination of high temperature and pressure conditions. Higher pressure leads to extraction of analytes from the pore water.

An additional comparison of the three extraction methods was performed based on desorption peak. LDTD-APCI-MS/MS desorption peaks obtained from extracting CBZ from secondary sludge with methanol as solvent using USE, MAE and ASE is presented in Fig. 5. It was observed that the desorption peaks of CBZ and internal standard obtained in all the three type of extraction methods were clear without any other interference. The slightly different desorption peaks observed for the same secondary sludge samples may be due to different extraction intensities of the three methods.

3.4. Application of optimization method

The applicability of the method was tested by the determination of CBZ in WW and WWS of CUQ wastewater treatment plants. Results are shown in Table 4. CBZ was detected in all the process streams of the wastewater treatment plant. It was detected at the highest concentration in the influent where a concentration of 420 ng L^{-1} was observed. The higher concentration of CBZ in influent suggests that the sources of CBZ in urban wastewater are very significant. Under the sampling conditions of this study, since no precipitation was recorded in the region served by the municipal sewer system in the days preceding the sampling, the flows mainly consisted of domestic, commercial and industrial discharges. The concentration of CBZ in the aqueous phase of WW declined from 420 ng L^{-1} in influent to 261 ng L^{-1} (38% decline) in effluent, indicating moderate removal of CBZ from the aqueous phase during different steps of treatment. Removal rate of CBZ over the total treatment process observed in this study (38%) was higher than the average removal rate of 1–10% reported for WWTP [7]. Further investigation should be conducted to determine the specific mechanisms for removal of CBZ within the WWTP.

To investigate if the concentration of CBZ observed in different samples of WWTP were statistically significant, a STATISTICA 7 of STATSOFT Inc. (Thulsa, USA), ANOVA test was performed at the significance level of 0.05. The results (Suplementary material, Table 2S) showed that the CBZ concentrations were significantly different ($P < 0.05$) among the different samples and highly significantly different in effluent ($P < 0.01$).

CBZ was detected in all types of sludge and sludge solids (Table 4). Among the four types of sludge (primary sludge, secondary sludge, mixed sludge and dewatered sludge), the

Table 3
Mean recoveries (%) and R.S.D. (%) of CBZ by USE, MAE and ASE method with different solvents.

	Methanol						Acetone						Acetonitrile:ethyle acetate (5:1, v/v)					
	USE		MAE		ASE		USE		MAE		ASE		USE		MAE		ASE	
	Reco	RSD	Reco	RSD	Reco	RSD	Reco	RSD	Reco	RSD	Reco	RSD	Reco	RSD	Reco	RSD	Reco	RSD
P.S	89.17	2.9	93.77	5.3	97.19	2.5	82.8	2.5	85.7	1.7	92.64	5.9	80.1	3.5	76.19	5.1	82.51	2.9
S.S	90.03	4.8	95.3	2.9	107	1.8	86.3	1.1	87.93	4.2	92.99	1.0	83.6	1.3	72.51	2.6	86.03	4.0
M.S	89.95	5.6	93.97	4.0	100.1	0.86	83.2	3.7	85.91	7.1	90.71	7.2	80.95	4.7	75.9	2.2	90.71	4.6
D.S	88.26	2.1	91.08	4.6	99.83	1.93	80.1	4.2	82.1	2.3	84.15	2.5	77.58	8.1	70.01	4.7	83.90	2.9
P.S.S	84.98	5.3	92.51	2.7	96.92	1.25	82.53	3.7	82.8	5.2	86.21	1.9	79.1	2.4	88.19	6.1	88.15	2.2
S.S.S	86.0	1.4	94.03	4.9	103	2.0	83.8	1.9	84	1.9	88.05	4.1	84.15	1.3	79.05	4.0	90.08	1.5
M.S.S	85.41	3.7	93.10	3.5	98.25	1.21	83.97	1.0	86.1	4.5	86.93	3.3	82.75	1.0	83.91	1.9	90	7.3

Recoveries are the average of the three determinations at spiked concentrations of 100 ng g^{-1}. P.S:primary sludge;S.S:secondary sludge;M.S:mixed sludge; D.S: dewatered sludge; P.S.S:primary sludge solids;S.S.S:secondary sludge solids;M.S.S:mixed sludge solids.

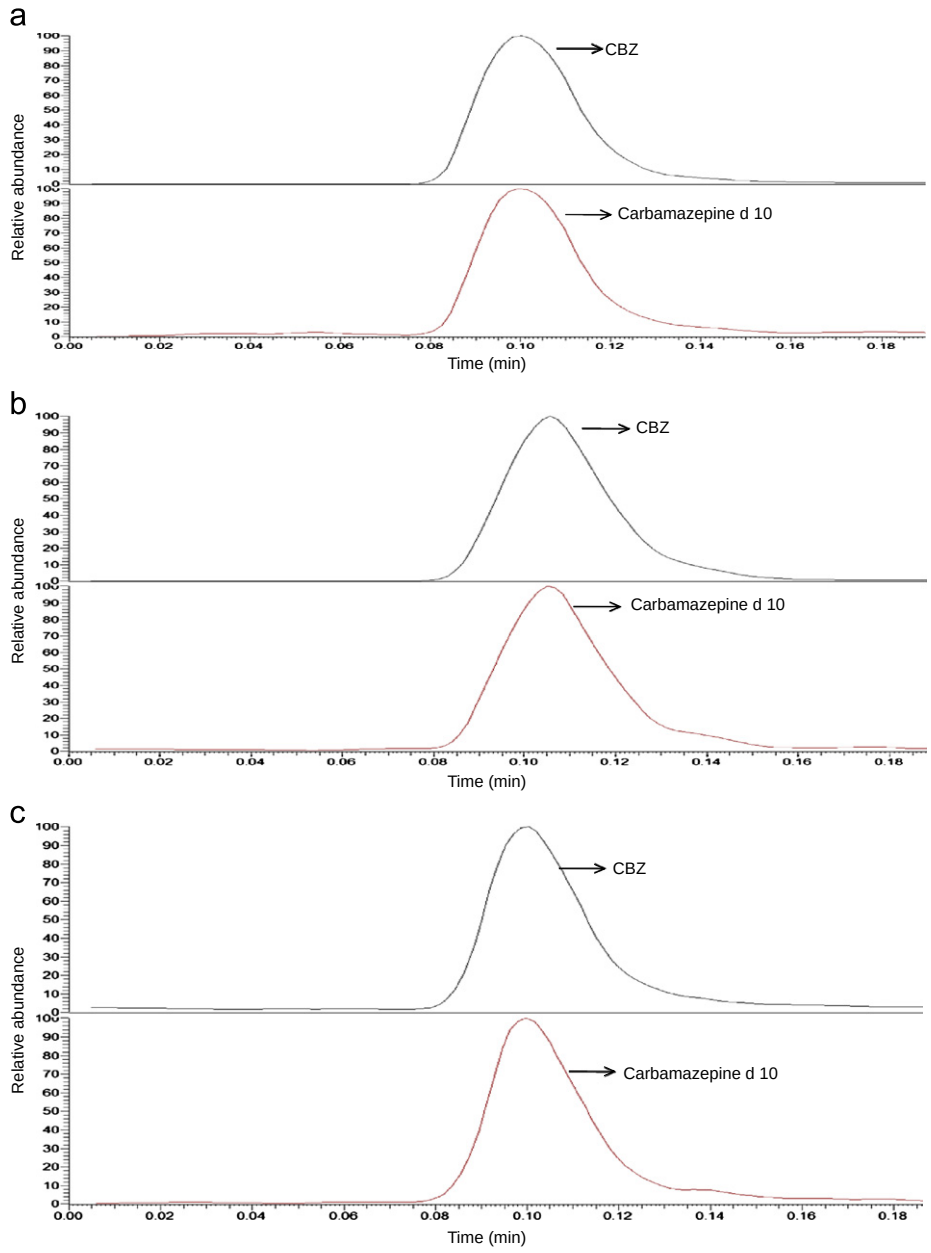

Fig. 5. Desorption peak of CBZ in secondary sludge extracted by USE, MAE and ASE under methanol as solvent: (a) USE; (b) MAE; and (c) ASE.

highest concentration of CBZ was found in primary sludge and lowest in secondary sludge. However, the concentration detected in the solid fraction of sludge was found to very low as most of CBZ was removed with the liquid fraction. The higher concentration of CBZ detected in liquid fraction of sludge as compared to their solid counterpart was mainly due to low *log* K_{ow} value (2.45) (Table 1) of CBZ. The results point towards the liquid portion or wastewater requiring more treatment for degradation of CBZ and also in selection of different treatment methods.

The estimation of daily mass flows of CBZ is presented in Table 5. The results presented can be considered as preliminary estimates of mass balance which is a reasonable estimation of quantity of CBZ distributed in various streams of WWTP. Approximately 146 g d^{-1} of CBZ was received by WWTP. Following treatment, approximately 62% of CBZ was discharged in the effluent into receiving surface water (Saint-Lawrence River), in this case CBZ is present in the effluent in environmentally important concentrations that may cause physiological effects in aquatic life. Finally, there is a possibility of ingestion by aquatic organisms, such as invertebrates resulting in potential aquatic toxicity. Ferrari et al. [26] studied the toxic effects of CBZ on bacteria, algae, microcrustaceans and fish and observed that CBZ had a limited acute ecotoxicity on the tested organism. According to the results of the present European legislation on the classification and labeling of chemicals (92/32/EEC), Jos et al. [27] classified CBZ as "R52/53 Harmful to aquatic organisms and may cause long term adverse effects in the aquatic environment.

With respect to the analyzed solid residues, grit residues, secondary and mixed sludge solids did not represent an important output for CBZ. In contrast, 1.2% of CBZ was found in dewatered sludge, which was subsequently incinerated. The dewatered sludge from the CUQ WWTP is incinerated at approximately 900 °C, which mineralizes the organic components. Hence, it is presumed that CBZ would be absent from ash

Table 4
Mean concentrations of CBZ (mean ± SD, $n=3$) in WW and WWS of Quebec Urban Community (CUQ) wastewater treatment plant.

Sample	Concentration ($ng\,L^{-1}$)	Sample	Concentration ($ng\,g^{-1}$)
Influent	420 ± 17	Primary sludge	94 ± 12
Grit liquid	327 ± 9	Secondary sludge	56 ± 9
Primary sludge liquid	73 ± 3	Mixed sludge	81 ± 4
Secondary sludge liquid	29 ± 12	Dewatered sludge	15 ± 7
Mixed sludge liquid	46 ± 9	Grit residue	69 ± 11
Effluent	261 ± 7	Primary sludge solid	21 ± 3
		Secondary sludge solid	13 ± 5
		Mixed sludge solid	18 ± 9

Table 5
Estimation of daily mass flows of CBZ in wastewater and sludge of the CUQ treatment plant and quantities of CBZ in these streams expressed relative to those observed in the influent (in %).

Compartments	Stream flow	Mass flows ($g\,d^{-1}$) (residual quantities relative to influent, in %)
Influent	346951 $m^3\,d^{-1}$	146
Effluent	346951 $m^3\,d^{-1}$	91 (62%)
Grit residues	2.8 tons d^{-1}	0.2 (~0%)
Primary sludge	63.8 tons d^{-1}	6.0 (4%)
Secondary sludge	24.71 tons d^{-1}	1.4 (1%)
Mixed sludge	47.93 tons d^{-1}	3.9 (3%)
Dewatered sludge	76 tons d^{-1}	1.2 (1%)

The mass balance of CBZ along the different units of WWTP was carried out by using the equation: $m=Q\times S$ where m is the mass flow ($g\,d^{-1}$), Q is stream flow ($m^3\,d^{-1}$ or tons d^{-1}) and S is the concentration of CBZ ($ng\,L^{-1}$ or $ng\,g^{-1}$). Value of Q was calculated by averaging the stream flow in each unit during the sampling periods (August 20th, 21st, 22nd, 23rd, 24th, 2011).

residues. The reduction in sludge volume by incineration produces secondary environmental pollution and is cost intensive. Hence, if incineration was discontinued for beneficial end use of sludge, such as land application (agriculture) and land spreading, the presence of CBZ might raise question in sludge reuse with further possibility of contamination of groundwater aquifers.

4. Conclusions

The study on optimization of LDTD-APCI-MS/MS method, extraction methods for CBZ and fate of CBZ in WW and WWS lead to following conclusions:

1. Different optimization and operation parameters for LDTD-APCI-MS/MS is suitable for the rapid detection and quantification of CBZ in WW and WWS and help to overcome the traditional use of liquid chromatography which composes use of expensive organic solvents, cost of maintenance of chromatography pumps and replacing chromatography columns.
2. The different optimum conditions for analysis of CBZ in WW and WWS included: (a) optimal solvent used for analyte deposition to the sample well cavities as methanol:water (2:1, v/v); (b) desorption laser power as 25% for wastewater and 35% for wastewater sludge and; (c) carrier gas flow rate as $3\,L\,min^{-1}$.
3. Due to the simplicity of this system, generic methods can be developed and applied for the high-throughput analysis of wide variety of organic compounds in WW and WWS without using the solvents (no mobile phase) during quantification respecting the green chemistry principles.
4. For extraction of CBZ from WWS samples, among the three extraction methods, accelerated solvent extraction with methanol as solvent offered the best extraction efficiency (96.9–107.0%) as compared to ultrasonic and microwave-assisted extraction.
5. Quantity of CBZ present in effluent ($261\,ng\,L^{-1}$) and dewatered sludge ($15\,ng\,g^{-1}$), questioning the release of effluent to river and reuse and recycle of sludge, thus requiring the efficient removal of CBZ from WW and WWS streams.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.05.047.

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