Talanta 128 (2014) 23–30

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Analysis of trimethoprim, lincomycin, sulfadoxin and tylosin in swine manure using laser diode thermal desorption-atmospheric pressure chemical ionization-tandem mass spectrometry

Morgan Solliec [a,b], Daniel Massé [b], Sébastien Sauvé [a,*]

[a] Department of Chemistry, Université de Montréal, Montreal, QC, Canada
[b] Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada

ARTICLE INFO

Article history:
Received 7 February 2014
Received in revised form
15 April 2014
Accepted 16 April 2014
Available online 24 April 2014

Keywords:
Laser diode thermal desorption
Veterinary antibiotics
Mass spectrometry
Swine manure

ABSTRACT

A new extraction method coupled to a high throughput sample analysis technique was developed for the determination of four veterinary antibiotics. The analytes belong to different groups of antibiotics such as chemotherapeutics, sulfonamides, lincosamides and macrolides. Trimethoprim (TMP), sulfadoxin (SFX), lincomycin (LCM) and tylosin (TYL) were extracted from lyophilized manure using a sonication extraction. McIlvaine buffer and methanol (MeOH) were used as extraction buffers, followed by cation-exchange solid phase extraction (SPE) for clean-up. Analysis was performed by laser diode thermal desorption-atmospheric pressure chemical-ionization (LDTD-APCI) tandem mass spectrometry (MS/MS) with selected reaction monitoring (SRM) detection. The LDTD is a high throughput sample introduction method that reduces total analysis time to less than 15 s per sample, compared to minutes when using traditional liquid chromatography (LC). Various SPE parameters were optimized after sample extraction: the stationary phase, the extraction solvent composition, the quantity of sample extracted and sample pH. LDTD parameters were also optimized: solvent deposition, carrier gas, laser power and corona discharge. The method limit of detection (MLD) ranged from 2.5 to 8.3 $\mu g\ kg^{-1}$ while the method limit of quantification (MLQ) ranged from 8.3 to 28 $\mu g\ kg^{-1}$. Calibration curves in the manure matrix showed good linearity ($R^2 \geq 0.996$) for all analytes and the interday and intraday coefficients of variation were below 14%. Recoveries of analytes from manure ranged from 53% to 69%. The method was successfully applied to real manure samples.

1. Introduction

In the past few decades, veterinary antibiotics have been widely used in swine breeding [1]. They have been administered routinely at therapeutic doses to prevent diseases, improve feed efficiency and accelerate growth, and as a result, huge quantities have been used for swine husbandry. However, all these antibiotics are not absorbed by the animals and a significant portion is excreted in the feces and urine and end up in the manure. Those antibiotics enter the environment through the land application of manure as organic fertilizer and can potentially contribute to bacterial resistance [2–4]. For several years, researchers have studied the anaerobic digestion of swine manure slurry [5]. They are now trying to understand the biodegradation of veterinary antibiotics in swine manure. Thus, robust analytical methods are necessary in order to quantitate and measure the degradation of these compounds. Various authors have dealt with the analysis of veterinary antibiotics in manure or other matrices (soils, wastewaters, animal meat, etc.) and they almost systematically use liquid chromatography (LC) before analysis by tandem mass spectrometry (MS/MS) [6–11]. This article explores an original analytical approach for the analysis of veterinary antibiotics in manure which switches from using a time consuming method based on LC-MS/MS (measured in minutes) with an ultrafast analytical method based on laser diode thermal desorption (LDTD) coupled to MS/MS (measured in seconds).

Most of the methods proposed in the literature for the analysis of antibiotics use LC and require time-consuming preparation steps such as solid phase extraction (SPE) with solid liquid extraction (SLE), followed by evaporation to dryness [6,12,13], and reconstitution in the solvent selected for analysis. Moreover, for LC techniques using ultraviolet or fluorescent detection, a derivatization step is usually required prior to analysis [12]. Therefore sample preparation and chromatography require several minutes. The global objective of this study is to develop an

* Corresponding author. Tel.: +1 514 343 6749; fax: +1 514 343 7586.
E-mail address: sebastien.sauve@umontreal.ca (S. Sauvé).

http://dx.doi.org/10.1016/j.talanta.2014.04.023
0039-9140/

original, simple, sensitive, robust and fast method to extract and quantify veterinary antibiotics from a complex dirty matrix like swine manure. This method requires the use of an LDTD interface to permit high throughput sample introduction. The LDTD is coupled to an atmospheric pressure chemical ionization (APCI) source which precedes a triple-quadrupole MS instrument capable of MS/MS determinations. This method was applied to four different antibiotics: trimethoprim (TMP), sulfadoxin (SFX), lincomycin (LCM) and tylosin (TYL) which are among the most widely used antibiotics in veterinary medicine for swine production. The sample preparation time is minimized to an ultrasonic extraction followed by SPE and only one evaporation step. Ultrasonic extraction using a solution of methanol (MeOH), McIlvaine buffer and ethylenediaminetetraacetic acid (EDTA) was used for the extraction of the target analytes [12,14,15]. Efficient clean-up was required, therefore a cation exchange cartridge was used to strongly retain the target analytes to the sorbent and allow the use of organic solvents to remove a large portion of the interfering matrix. This clean-up is crucial because no chromatographic separation occurs using the LDTD-APCI prior to MS/MS detection. The method was tested and validated with freeze-dried manure from the experimental farm of Agriculture and Agri-food Canada (Lennoxville, QC, Canada). Method performance was evaluated by the determination of extraction recovery, linearity, precision, repeatability and limits of detection and quantification. The determination of targets compounds at micrograms per kilogram in pig manure was performed to confirm the applicability of the method in real environmental samples.

LDTD-APCI is an alternative sample introduction technique without a separation step like LC or gas chromatography (GC) prior to detection. For that reason, LDTD-APCI technology permits the virtual elimination of chromatographic columns and mobile phase, thus drastically reducing analysis time, sample preparation and analysis costs while increasing sample throughput. In fact, the LDTD-APCI is coupled to MS/MS and reduces total analysis time to 15 s compared to minutes with LC coupled to MS/MS. The LDTD technology is based on the volatilization and on the physicochemical properties of the compound. An IR laser diode beam hits the back of the sample well (metal bottom) and the target sample is volatilized by the heat-gradient that is thus generated. In a second step, the compounds are transferred with a gas flow and ionized in the APCI before entering the MS/MS. To the best of our knowledge, there are no published methods using LDTD or similar APCI-based approaches to quantify veterinary antibiotics in swine manure. Some analytical methods have been published on LDTD-APCI-MS/MS and it has so far been applied in toxicology [16,17], pharmaceutical [18], environmental samples such as endocrine disruptors in wastewaters [19], municipal sludge and aquatic sediments [20], and sulfonamides in dairy milk [21]. The schematic and assembly of the LDTD-APCI source apparatus have previously been detailed [22].

2. Materials and methods

2.1. Chemicals, reagents and stock solutions

LCM (purity ≥ 89.1%), SFX (purity ≥ 99.9%), TMP (purity ≥ 99.5%), TYL (purity ≥ 83.8%) and spiramycin (SPI, purity ≥ 90.0%) were purchased from Sigma Aldrich (St. Louis, MO, USA). Isotopically-labeled trimethoprim, [$^{13}C_3$]-trimethoprim ([$^{13}C_3$]-TMP) used as internal standard (IS, purity ≥ 99%), was obtained from ACP Chemical Inc. (Montreal, QC, Canada). All solvents used were of HPLC grade purity from Fisher Scientific (Whitby, ON, Canada) and deionized/distilled water (dd-H_2O) was used for dilutions. Individual stock solutions were prepared in MeOH at a concentration of 1000 mg L^{-1} and kept at −20 °C for a maximum of 6 months. Individual intermediate solutions were prepared by dilution of the 10 mgL^{-1} stock solution in MeOH. Given the potential for degradation of the target analytes [23], working solutions were prepared daily at a concentration of 1 mg L^{-1} by dilution in MeOH from individual intermediate stock solution. Sodium phosphate dibasic (Na_2HPO_4, purity ≥ 99.0%) and citric acid (purity ≥ 99.5%) were purchased from Sigma Aldrich (St Louis, MO, USA).

2.2. Agricultural soil

Swine manure was obtained from the Dairy and Swine Research and Development Center of Agriculture and Agri-Food Canada (Lennoxville, QC, Canada). Manure samples were collected in plastic flasks, homogenized and freeze-dried in Lennoxville. All the samples were kept at 6 °C until analysis. This manure does not contain target analytes and was used for all method validation tests.

2.3. Extraction and cleanup

Approximately 100 mg of freeze-dried manure was weighted into a 15 mL conical-bottom centrifuge tubes from Kimble Chase (Rockwood, TN, USA) and 5 mL of extraction buffer MeOH/McIlvaine/EDTA (50:45:5, v/v/v) at pH 5 was added. McIlvaine buffer (20 mL) was prepared by mixing 9.70 mL of 0.1 M citric acid and 10.30 mL of 0.2 M Na_2HPO_4. The tubes were mixed for 1 min on a vortex and were subsequently placed into an ultrasonic bath for 15 min. They were then centrifuged at approximately 2750 g for 15 min. The supernatant was collected into a 15 mL brown glass tube. These extractions were repeated twice but 50 μL of acetonitrile (MeCN) was added for the second extraction, before the centrifugation step, to help precipitate proteins [11].

SPE was done using a 12-position manifold manufactured by Phenomenex (Torrance, CA, USA). Strong cation mixed mode phase Strata-X-C (surface-modified styrene divinylbenzene polymer) cartridges with a total volume of 6 mL and a 200 mg bed mass from Phenomenex were used to wash the sample extracts. Multiple SPE parameters were optimized: cartridge type, loading step, loading flow rate, washing step and sample pH. The SPE cartridges were conditioned with 2 × 5 mL of MeOH and 2 × 5 mL of distilled-deionized water (dd-H_2O) acidified at pH 4. Samples were loaded on the cartridge column at a flow rate of 2–3 mL min^{-1} by applying negative pressure using a mechanical pump. The SPE cartridges were washed with 2 × 5 mL of MeOH followed by 2 × 5 mL of ethyl acetate (EtAc). The analytes were eluted with 2 × 5 mL of MeOH/NH_4OH (95:5, v/v) at pH 9.0 into conical-bottom centrifuge tubes. Before evaporation, eluates were filtered on 0.45 μm pore size fiberglass membranes from Whatman (Piscataway, NJ, USA) to eliminate particulate materials. The eluates were then evaporated to total dryness under a gentle stream of nitrogen at 40 °C with a nine-port Reacti-vap unit from Pierce (Rockford, IL, USA) and then reconstituted to 200 μL with MeOH/H_2O (90:10, v/v) for LDTD-APCI-MS/MS analysis.

2.4. LDTD-APCI-MS/MS

Desorption and ionization of target veterinary antibiotics were performed with the T-960 LDTD-APCI ionization source controlled by the LazSoft 4 Software, developed and manufactured by Phytronix Technologies (Quebec, Canada) and data integration was performed using the Xcalibur™ 2.0 software (Thermo Fisher Scientific, Watthan, MA). For analyte detection, LDTD-APCI was mounted on a Quantum Ultra AM triple quadrupole mass spectrometer by Thermo Fisher Scientific (Waltham, MA). Ionization was

performed in positive mode (PI). Sample solutions (3 μL) were spotted into a 96-well LazWell plate (Phytronix Technologies, QC, Canada) containing inserts made of proprietary stainless steel alloy and then dried at 40 °C for 5 min until complete solvent evaporation. The loaded LazWell plate was then transferred into the LDTD housing unit. An infrared (IR) laser diode (980 nm, 20 W) was then focalized to impact the back of the inserts, thermally desorbing the dried sample which is vaporized into the gas phase. The uncharged molecules travel along the transfer tube with a carrier gas (medical grade purified air). Upon reaching the corona discharge region, they are ionized by the APCI and then transferred to the MS inlet (see details in Supporting information Fig. S-1).

The LDTD-APCI optimization for MS/MS conditions in positive ionization mode (PI) was performed by depositing the standard analytes of interest and the corresponding internal standard (IS) at a concentration of 1 mg L^{-1} in the well plate inserts. Analytes were spotted into the sample well once reconstituted in a MeOH/H_2O (90:10, v/v) solution following SPE with a deposition volume of 3 μL. Optimization of LDTD-APCI parameters was performed in pre-concentrated extraction samples in order to account for the matrix effects. The LDTD-APCI source parameters were set as follow: capillary temperature at 350 °C, a discharge current of 3.6 μA in PI mode, a carrier gas temperature of 50 °C, a carrier gas flow set at 3.0 L min^{-1}, and the ion sweep gas set at 0.3 (arbitrary unit). The sheath gas, the auxiliary gas, the skimmer offset and the vaporizer temperature were set to 0. The final laser pattern for SDX, LCM and TMP consisted of a 0.5 s initial linear ramp from 0% to 40% with no holding time at the maximum power before shut-down. For TYL, the laser pattern consisted of a 0.5 s initial linear ramp from 0% to 50% with a holding time of 1 s before shut-down. The two laser patterns of the target analytes are illustrated in Fig. 1.

The respective relative intensity ratios per compound were used in selected reaction monitoring (SRM) mode for detection and quantification. Optimization of MS/MS parameters was done with the following settings: collision gas (Ar) pressure at 1.5 mTorr, resolution for Q_1/Q_3 was set at 0.7 u and scan time was set at 0.005 s. The different SRM transitions and their optimized parameters for the quantification and confirmation of target analytes are shown in Supporting information Table S-1.

2.5. Validation of LDTD-APCI-MS/MS method

A minimum of two SRM transitions and their relative intensity ratios were used to avoid false positive and confirmed the presence of the compounds of interest. The instrument response was determined as the ratio of the analyte area to that of the IS.

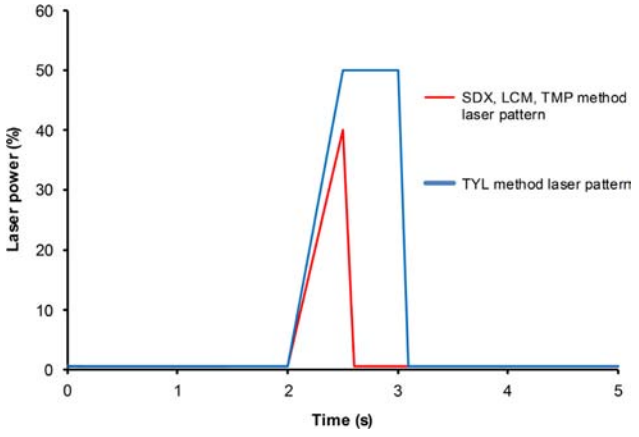

Fig. 1. Illustration of LDTD laser patterns for SDX, LCM, TMP method and TYL method.

The method limit of detection (MLD) and the method limit of quantification (MLQ) were determined as 3.3 and 10 times, respectively, the standard deviation of the *y* intercept divided by the slope of the calibration curve in matrix sample. The most intense transition was used for quantification and the second weaker transition was used for confirmation.

The recovery values for the SPE method were evaluated using concentrations of 100, 250 and 500 μg kg^{-1}. During the SPE optimization, three sets of samples were prepared to determine the extraction recovery. Extraction recoveries were determined by comparing mean peak area ratios of target analytes spiked prior to extraction in manure samples with those of the target analytes spiked in post-extraction manure samples in triplicate and were reported in percentage. Un-spiked manure samples were also extracted to account for their presence in the recovery values. The IS was added after the extraction and before the reconstitution step to calculate the extraction recovery. In the case of manure samples containing antibiotics, the IS was added before the extraction step to correct for analytes losses through handling.

The repeatability (intra-day precision, expressed as relative standard deviation in %) was evaluated by analysis of the same spiked sample at 100 μg kg^{-1} five times on a single workday. Reproducibility (inter-day precision) was also calculated by spiking an extracted sample at 100 μg kg^{-1} freshly prepared each day during 3 days. The Statistical Package for Social Science (SPSS 13.0, Chicago, IL), ANOVA test was used to compare the signal intensities for the optimization of the LDTD-APCI parameters, and a post-hoc Tukey's *b* test was performed with statistical significance defined as $p < 0.05$.

3. Results and discussion

3.1. Manure characteristics

Because swine manure is a complex matrix consisting of feces, urine, feed residues, straw and wash waters, several analyte-matrix interactions and possible impurities must be considered during method development. Manure consists largely of organic matter and nutrients but may also contain substantial amounts of proteins and lipids. The organic matter of swine manure is mainly composed of low molecular weight compounds and a high content of functional groups such as carboxylic acids and phenols which contain a lot of potential binding sites for the target molecules based on hydrogen bonding and ion-exchange [24]. The higher molecular weight organic matter constituents are more hydrophobic substances which could make hydrophobic interactions with van der Walls forces. The sorption coefficient (K_d) for TYL in manure is between 39 and 108 L kg^{-1} [25]. To the best of our knowledge, sorption coefficients of TMP, LCM and SFX in manure have not been investigated, but K_d for sulfonamide in soil-manure (50:1) mixture was 0.59–1.18 L kg^{-1} [26]. Thus, TYL is expected to show stronger retention on manure than SFX. TYL is excreted as the parent compound and metabolites from livestock with desmycosin, macrosin and relomycin (TYL B, C and D) as the most commonly observed. Moreover, TYL degrades rapidly into its various metabolites in swine manure [27,28].

3.2. Manure extraction method development

A number of different techniques deal with the extraction of organic chemicals such as veterinary antibiotics from manure. Traditional non-instrumental techniques such as soxhlet extraction and shake flask extraction are well established but can be time consuming and they use large volumes of organic solvents [29]. An alternative technique which is rapid and does not

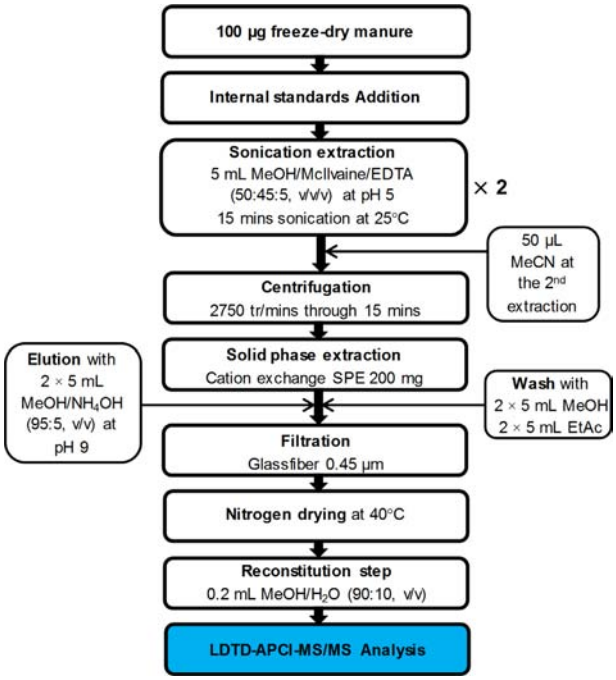

Fig. 2. Sample-preparation and clean-up procedure for analysis of SFX, LCM, TMP and TYL.

Table 1
Physicochemical properties of SDX, LCM, TMP and TYL including molecular weight, pK_a [30,31] and log *P*.

Compounds	MW	pK_a	Log *P*
SDX	310.3	6.01	0.58
LCM	406.5	7.79	0.56
TMP	290.3	6.76	1.28
TYL	916.4	7.50	2.32

consume large volumes of solvents or expensive instruments is ultrasonic extraction [10,12]. This technique has been successfully applied to veterinary antibiotics and it has been adopted in the current study.

The first step was to select a buffer suitable for extracting the target compounds. Sulfonamide is known to form chelate complexes with metal ions and therefore, the use of chelating agents such as EDTA and McIlvaine buffer were suitable. McIlvaine buffer is a mixture of citric acid and Na_2HPO_4 and has previously been used in the extraction of sulfonamides from manure [8,15]. For that reason, this extraction mixture was chosen for this study.

The extraction was optimized with the procedure described in Fig. 2. Before the clean-up step, target analytes have to be protonated to create a positive charge that allows the formation of an ionic bond with the cationic phase. The pK_a of the target analytes is in the same range (Table 1), the pK_a of SDX is the lowest at 6.01 [30] and the pK_a of LCM is the highest at 7.79 [31]. The pH must be below the pK_a of the compounds to allow protonation. But TYL is instable below pH 4 [32], so the pH cannot be lowered below 4. Initial experiments with a single extraction gave poor recoveries, so a second extraction was added to the procedure. Extractions were also carried out using a pH 5 McIlvaine buffer with a mixture of MeOH and EDTA to improve performances with our target analytes. A vortexing stage was also added to the procedure before the ultrasonic extraction step to suspend the particles and homogenize the solution. Ultrasonic extraction was done at 25 °C during 15 min for TMP, LCM, SDX and TYL. After centrifugation, the supernatant liquids produced by the two extraction processes were added one to the other and combined in a single 15 mL glass vial.

3.3. Sample clean-up and elution

A solid phase extraction procedure was used to enable the manure extracts to be cleaned-up. We use a Strata X-C cartridge, a cation exchange cartridge that permits the use of organic solvents during the washing step so as to remove large portions of the interfering matrix [12,33]. The pH of the supernatant solutions was checked and adjusted with citric acid, if necessary, before clean-up because it could change due to the multiple compounds present in the samples. The pH of the solution has to be below the pK_a of the target analytes (Table 1) [31]. Thus, citric acid was added to the solution to protonate the target compounds and maintain slightly acidic conditions. The supernatant containing TMP, LCM, TYL and SDX was maintained to a pH of 5 because SDX has a lower pK_a (Table 1). The pH of the supernatant should not be below 4 because TYL is instable under such conditions. Maintaining a pH between 4 and 5 thus allows an ionic bond between the protonated target compounds and the cationic phase. Ionic bonds are stronger than van der Walls interactions and further allow us to use a strong wash with organic solvents to remove a maximum of interfering compounds. Various solvents and solvent mixtures were tested at different pH. After a series of experimental washes, MeOH and EtAc were found to remove maximum interferences without affecting analytes recovery. Additional washing stages were also tested to try to remove interfering compounds that had not been removed from the Strata X-C.

Target analytes were eluted with a solution of MeOH/NH_4OH (95:5, v/v) at pH 9.0, above the pK_a of the compounds. The pH of the eluting solution should not be above pH 9 because TYL is instable at higher pH [32,33]. This basic solution breaks ionic bonds between the protonated compounds and the cationic phase thus releasing the target analytes.

It is important to properly control pH conditions at this step. First, it allows the retention and release of the analytes from the cartridge. Secondly, TYL is unstable at extreme pH, therefore, the pH has to be maintained between 4 and 9. Outside this range of pH, TYL will degrade rapidly into various metabolites. The stability of TYL was studied by varying the pH and measuring the amount of TYL. This shows that the pH used in this study to treat the samples did not affect TYL stability (Fig. S-2). Moreover, it was shown that TYL degrades rapidly outside this range [32] but for exposure below 24 h, degradation is not significant.

3.4. LDTD-APCI parameters optimization

Analysis of different pharmaceuticals by LDTD-APCI necessitates the optimization of different parameters to improve signal intensity. Therefore, several LDTD parameters were optimized to achieve signal enhancement while minimizing variability: the laser power, the laser pattern, the deposition volume, the carrier gas flow rate and the solvent of deposition. LDTD parameters were optimized in positive ionization mode (PI). Aliquots of extracts of manure were spiked with target analytes at 500 $\mu g\,kg^{-1}$ to account for matrix effects. Each spiked sample was analyzed 6 times ($n=6$) in SRM mode with the corresponding *m/z* precursor and product, and optimized tube lens and collision energy.

Fragmentation of precursor ions at *m/z* 311 for SFX, 291 for TMP, 407 for LCM, and 916 for TYL gave very clean MS/MS spectra, consistent with the literature of MS/MS spectra from the same ions obtained using APCI and ESI. The major product ions obtained correspond to the losses of part from $[M+H]^+$ and were observed at *m/z* 156 and 92 for SFX, *m/z* 123 and 230 for TMP, *m/z* 126 and 359 for LCM and *m/z* 174 and 772 for TYL, and those transitions

could be used for detection. Tube lens and collision energy for each quantification transition are presented in Supporting information Table S-1.

The laser power and the laser pattern were used to control the power of the laser diode applied to the back of the metal well during a short period of time, approximately 1–3 s. Therefore, increasing the percentage of laser power will increase the laser radiation power hitting the back side of the Lazwell plate and ultimately the amount of energy transferred to the sample. This fast heat transfer (as high as 3000 °C s^{-1}) generates a temperature of roughly 200 °C induced by the laser and allows the thermal desorption of the target compounds, induces sublimation and transfers them by the gas flow to the APCI ionization [34]. The amount of energy transferred is dependent on the laser power setting of the instrument (%). The laser power should not be set too high in order to maintain a low background signal generated by the thermal degradation of matrix components and cause the degradation or fragmentation of target compounds. Depending on the matrix, increasing laser power can cause a decrease of S/N response because of a larger amount of interfering compounds are being desorbed and transferred to the corona discharge along with the compounds of interest. By adjusting the laser ramp pattern and the hold time at maximum laser power, it is possible to increase the amount of desorbed analytes and control the amount of desorbed matrix components. Laser power was evaluated between 10% and 60%. Fig. 3 shows the various laser patterns tested with their corresponding signal. The optimal laser power for maximum compound desorption was set at 40% for SFX, LCM and TMP but it was set at 50% for TYL. Lower laser power did not allow for maximum compound desorption and a higher laser power caused higher variability and decreased signal intensity which we attribute to heat-promoted compound degradation. Optimized laser pattern for SFX, LCM and TMP was a 2 s at 0%, with a ramp of 0.5 s from 0% to 40%, with no hold time required at maximum power, and a direct decrease of 0.1 s from 40% to 0% with finally 2 s at 0% (Fig. 1). Previous work used the same type of laser pattern for desorption of various pharmaceutical compounds in wastewater [18]. For TYL, optimized laser pattern was 2 s at 0%, with a ramp of 0.5 s from 0% to 50%, with a hold time of 1 s before a direct decrease of 0.1 s from 50% to 0% with 2 s at 0% (Fig. 1). The hold time of 1 s was necessary to completely desorb TYL, presumably because of its higher molecular weight. The total analysis time is 15 s per sample, which compares very favorably with the minutes usually required for chromatography analysis.

LDTD does not permit compound separation prior to analysis. Therefore, during desorption, a large amount of compound including matrix components and target analytes will simultaneously reach the APCI region. A competition will then occur between the compounds for ionization. Consequently, it is beneficial to try to desorb part of the interfering matrix components before the desorption of the target analytes. TYL starts to desorb at 35%, thus several laser patterns were tested in two different steps. Initially, a short laser impulsion at 25% laser power is used to desorb a part of the matrix without desorbing the analyte. In a second step, the target analyte will be desorbed with the optimized laser pattern (Fig. 1). Laser patterns are presented in Fig. 4 (subfigure numbers 1–4) and the peak area measurements with their respective laser patterns are presented in Fig. 5. SFX, LCM and TMP start to desorb at a low laser power (15%). Subsequently, it was not possible to use this "pre-desorption" technique for these analytes. Three different laser patterns were tested on a matrix solution spiked with 250 μg L^{-1} of TYL (Fig. 4) and peak areas were measured in each case. Number 1 is the reference laser pattern. The best results seem to be the laser patterns 2 and 3 for which variability is lower and peak area is greater. Laser pattern number 4 gives the smallest peak area. Therefore, laser pattern numbers 2 and 3 were compared to the reference with an ANOVA post-hoc Tukey *b* test. The result of this test showed that there is a significant difference between number 3 and the reference ($P<0.05$). Laser pattern number 3 was therefore chosen for the TYL method.

The solvent of deposition for analytes has an important impact on the MS/MS peak shape, peak intensity and signal variation [19]. Depending on the solvents used for deposition, those parameters could change significantly. Therefore, optimization of deposition solvent is required to optimize the LDTD-APCI method. Different types of solvents and mixtures were tested and the coefficient of variation (CV, $n=5$) was compared for the 8 different solutions. Fig. 6 shows that the MeOH/H_2O (90:10, v/v) mixture gave the best peak intensity and the lowest CV. Thus, this mixture was selected for all optimization experiments.

The deposition volume has an important influence on the APCI ionization and on the MS signal intensity of the target compounds. Previous work showed the influence of deposition volume in the Lazwell plate upon the effectiveness of the APCI [19]. It was shown that, by adding a large volume of sample, a higher amount of analyte would reach ionization, increasing the signal and sensitivity, and improving the MLD. Consequently, more compounds in the ionization area can induce a proton affinity competition during the APCI discharge and would also affect signal intensity. Also, non-volatile matrix products could trap the target compounds and affect signal intensity. The deposition volume was tested from 2 to 8 μL in steps of 1 μL (see details in Supporting information Fig. S-3). The peak area progressively decreases with increasing deposition volume in spiked matrix aliquots. These results suggest

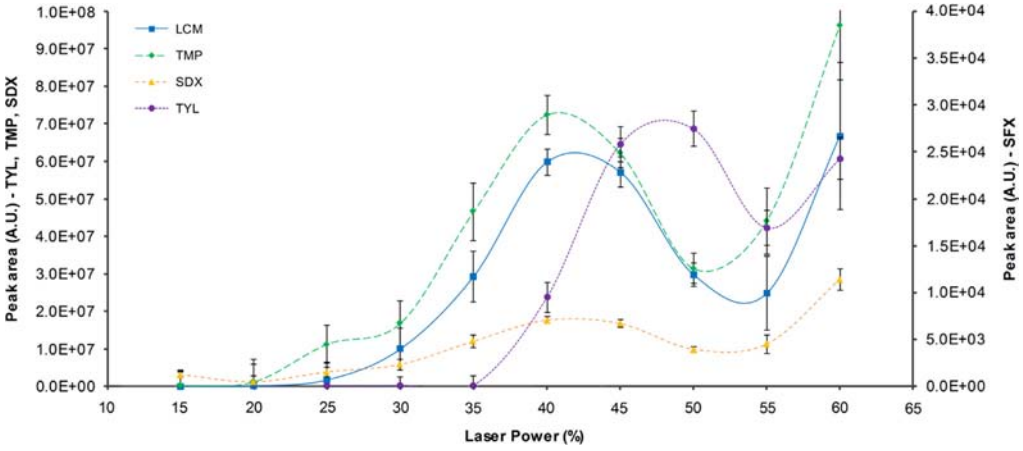

Fig. 3. Effect of laser power on peak area of target analytes spiked in matrix solution (SDX use the right *y*-axis scale, others compounds use the left axis-scale).

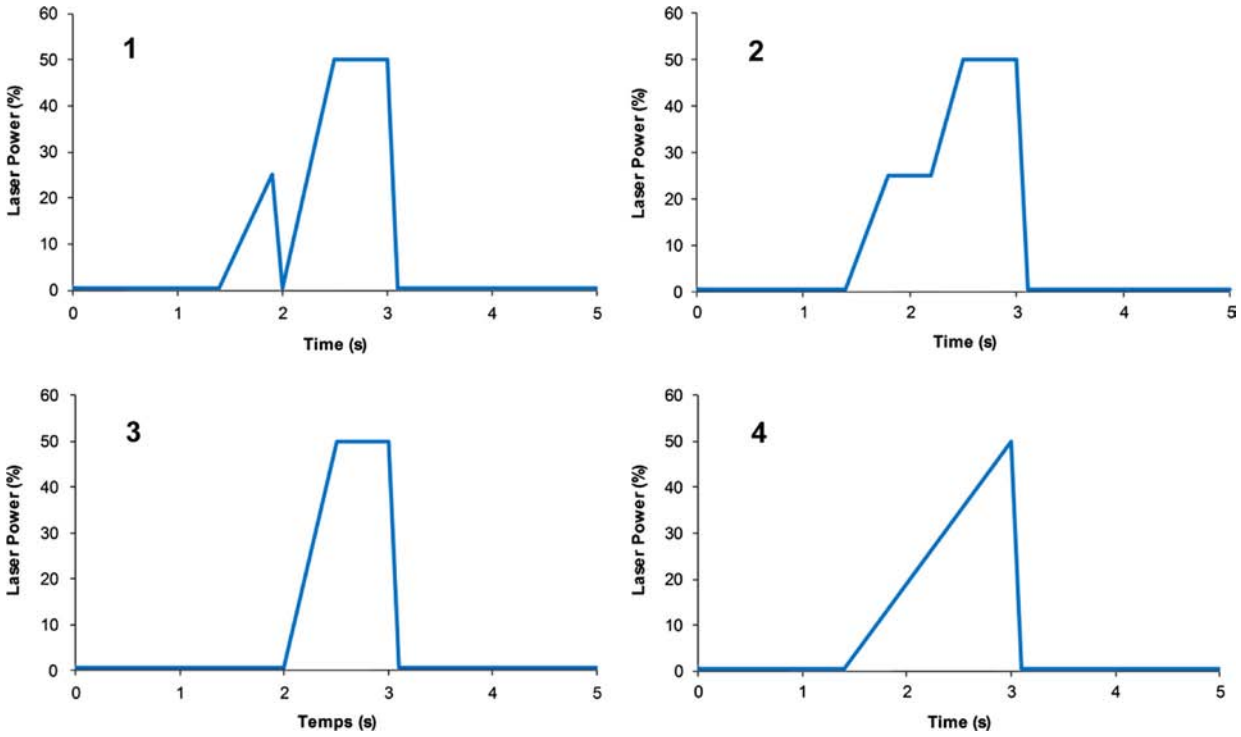

Fig. 4. Illustration of different LDTD laser patterns tested for the TYL method.

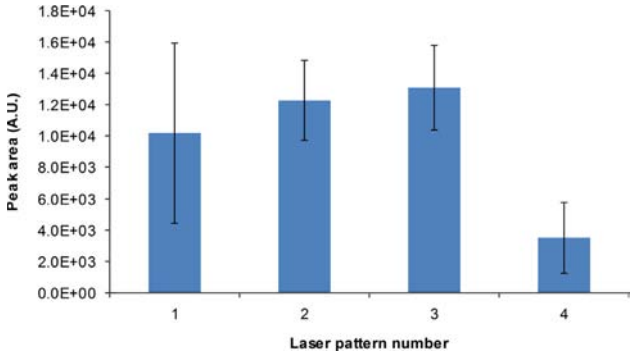

Fig. 5. Effect of laser pattern (presented in Fig. 4) on TYL peak area measured with 250 μg kg^{-1} of target analyte in matrix solution (n=5).

minimizing the deposition volume to minimize matrix effects and to maximize the peak area and the S/N. An optimal deposition volume of 3 μL was chosen for the analysis of all target compounds with significantly higher S/N response ($p < 0.05$).

For the carrier gas optimization, the same samples were used to determine the highest S/N signal. The carrier gas flow has two main functions: transferring the thermally desorbed analytes to the corona discharge region for ionization and thermalization of the vaporized analytes. Carrier gas flow has an influence on the APCI ionization. A higher carrier gas flow reduces the residence time of neutral compounds in the ionization source and a lower gas flow limits the amount of molecules reaching the source. It has to be optimized to bring enough water molecules in the corona discharge area and permits formation of charged water clusters $(H_2O)_nH^+$ through a chain of ion–molecule reactions starting with N_2^+, O_2^+, NO^+, and H_3O^+. Those clusters react with target analytes by proton-transfer and induce the ionization [35]. Therefore, it becomes important to control the time of residence in the ionization area to optimize ionization and thus improve sensitivity. Carrier gas flow rate was optimized between 1 and 8 L min^{-1} and was set to 3 L min^{-1} which gave significantly higher peak area response ($p < 0.05$) and showed the highest S/N values for the SRM of all target analytes. Additionally, a gas flow of 3 L min^{-1} provided a smaller signal variability than using 2 L min^{-1}. This optimal flow rate corresponds with what has been observed previously for hormones and pharmaceutical compounds [18,19].

A sample of resulting peak shapes is illustrated in the Supporting information Figs. S-4 and S-5. Peak shapes of LCM, SFX and TMP are symmetrical and reproducible for quantitative analysis. For TYL and SPI, peak shapes are asymmetrical with significant peak tailing which might be caused by the laser pattern of the TYL method which has a hold time of 1 s at maximum laser power. This hold time is necessary to desorb all TYL and SPI. Different hold times were tested at 0.5 s, 1 s and 1.5 s with 50% and 55% laser power (Fig. 7). The peak shapes are still asymmetrical when those parameters were modified. Despite being asymmetrical, the peaks are reproducible. A hold time of 1 s with 50% laser power gives the best intensity and seemed to be optimal for TYL (Fig. 7).

The target compounds were analyzed in two desorption events (two plate wells) in PI mode using two LDTD methods. Several LDTD methods were necessary because of the limitation of the maximum MS/MS transitions that can be handled simultaneously in the same method. Therefore, 8 SRM transitions can be handled to keep enough acquisition point per peak. Moreover, TYL requires a higher laser power than the other target compounds, thus two LDTD methods are required. In the first method TMP, SFX, LCM and [$_{13}C^3$]-TMP (as IS) were analyzed with the laser pattern described in Fig. 1. The second method allows the analysis of TYL and SPI (as IS) using the other laser pattern described in Fig. 4 (number 3).

3.5. Method validation

The LDTD-APCI-MS/MS method with optimized parameters was applied to spiked freeze-dried manure. The analysis took 15 s per well, with 2 separate LDTD methods per sample. In consequence, for a sample, two wells were desorbed. The samples were concentrated using SPE and gave acceptable extraction

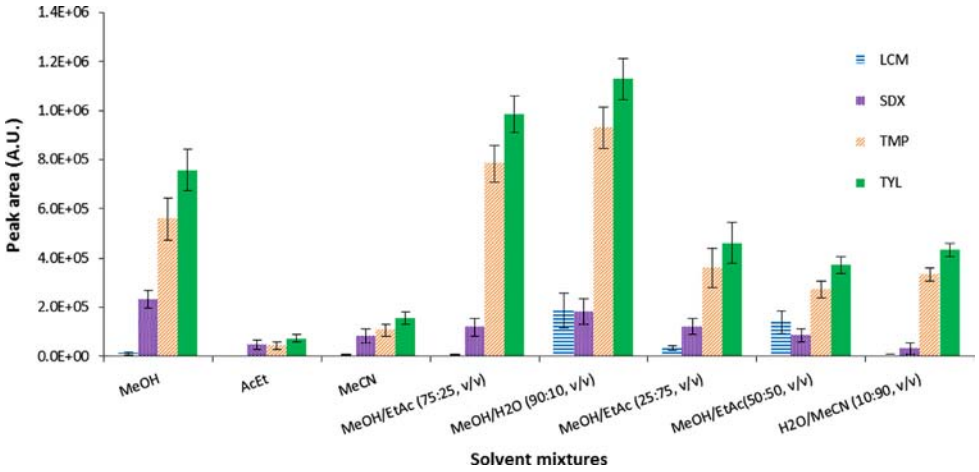

Fig. 6. Effect of solvent mixture on peak area of target analytes.

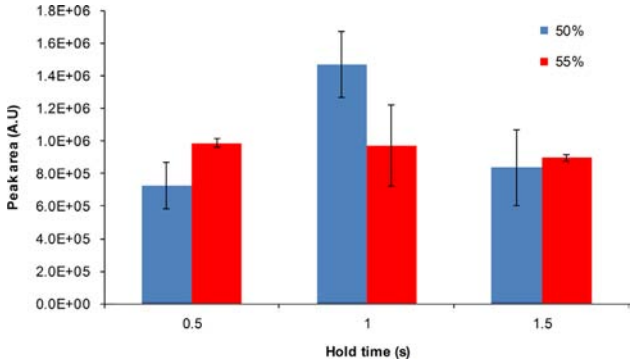

Fig. 7. Effect of hold time in laser pattern and laser power on TYL peak area measured with 250 μg kg^{-1} of target analyte (n=3).

recoveries for a concentration of 250 μg kg^{-1} ranging from 53% to 69% in spiked freeze-dried manure for all tested antibiotics.

To validate the method, a five-point standard addition calibration curve was analyzed in a freeze-dried manure sample that was spiked one night before the SPE procedure. Each point of the linear dynamic range (0–500 μg kg^{-1}) was analyzed using triplicates. Method validation parameters are presented in Table 2. The calibration curves showed good linearity, with coefficients of determination ranging from 0.984 to 0.996. MLD are calculated from calibration curves and range from 2.5 to 8.3 μg kg^{-1} and MLQ ranged from 8.3 to 28 μg kg^{-1}. The MLD is comparable to several other analytical methods applied to veterinary antibiotics, including off-line SPE coupled to LC-MS/MS [8,10].

SPE followed by LDTD-APCI-MS/MS was tested with success to the spiked freeze-dried manure. Accuracy and precision are presented in Table 3. Accuracy was determined at three concentration levels as percent bias (%) between the concentration added and that found in the spiked freeze-dried manure (n=3). Accuracy was good with bias under 11% for each concentration levels (100, 250, 500 μg kg^{-1}) for all compounds. Intraday precision calculated for 3 concentration levels (n=10) ranged from 5% to 10% and interday precision calculated for 3 concentrations levels (n=10) ranged from 8% to 14%. The entire method with optimized parameters was applied to real manure sample containing TYL. The compound was detected and quantified in the sample with concentrations ranging from 30.3 to 543 μg kg^{-1}.

Overall, the LDTD-APCI-MS/MS is a robust method that allows high throughput analysis with less than 15 s/sample and could be a powerful tool for fast screening of veterinary pharmaceuticals in manure. TYL and SPI are well desorbed by LDTD and macrolides have practically the same structure. We can presume that other macrolides could potentially be analyzed using LDTD-APCI-MS/MS. Moreover, TYL metabolites are well documented and could be analyzed with this method.

Table 2
Method validation parameters including linearity ranges, coefficient correlation (R^2), sensitivity, the limit of detection (MLD), the limit of quantification (MLQ) and recoveries.

Compounds	Linearity range (μg kg^{-1})	R^2	Sensitivity	MLD (μg kg^{-1})	MLQ (μg kg^{-1})	Recovery (%) ± STD[a]
SDX	1.4–500.0	0.984	0.0011	8.3	28	61 ± 2
LCM	0.6–500.0	0.989	0.0048	3.3	11	53 ± 3
TMP	0.5–500.0	0.988	0.0015	3.1	10	69 ± 1
TYL	0.4–500.0	0.996	0.0075	2.5	8.3	63 ± 3

[a] Recovery is measured in 100 μg kg^{-1} matrix solution with n=3.

Table 3
Mean concentration (mean analysis ± SD, n=3) of target analytes measured in freeze-dried manure samples including bias values and method interday/intraday precision (n=10).

Compounds	Accuracy			Precision	
	Amount added (μg kg^{-1})	Amount found ± SD (μg kg^{-1})	Error (%)	Intraday (%)	Interday (%)
SDX	100	90 ± 3	10	6	12
	250	228 ± 6	9	6	8
	500	459 ± 7	8	8	12
LCM	100	93 ± 2	7	6	10
	250	234 ± 7	6	5	9
	500	470 ± 7	6	7	12
TMP	100	96 ± 2	4	5	13
	250	242 ± 6	3	9	11
	500	480 ± 8	4	10	14
TYL	100	89 ± 3	11	8	14
	250	230 ± 5	8	6	8
	500	465 ± 7	7	9	14

4. Conclusion

In summary, this new analytical method for antibiotics in swine manure is simple, sensitive, fast and allows high-throughput analysis with a simple extraction and cleanup step. The LDTD-APCI-MS/MS is suitable for the rapid detection and quantification of 4 veterinary antibiotics targeting some of the mostly commonly used antibiotics for hog production. Target analytes were determined at $\mu g\ kg^{-1}$ levels using 100 mg of freeze-dried manure. This is proposed as an alternative to the traditional LC-MS/MS which gives comparable results. Only ultrasonic extractions followed by a SPE step with cleanup are necessary to remove interfering matrix components from this complex matrix. We used a solid phase extraction method using a cation exchange cartridge that allows a strong organic solvent wash and removes a large part of matrix components. SPE recovery ranged from 53% to 69%. The calibration curves were linear with R^2 values ranging from 0.984 to 0.996. MLD and MLQ ranged from 2.5 to 8.3 $\mu g\ kg^{-1}$ and from 8.3 to 28 $\mu g\ kg^{-1}$. The resulting accuracy bias was less than 11% for all compounds at 3 different concentrations levels. Interday and intraday precision was good with CV from 8% to 14% and 5% to 10%, respectively for all compounds. The ultra-fast analysis combined with a robust quantification and simple sample pretreatment makes this method a useful alternative for further environmental analysis and monitoring.

Acknowledgment

Agriculture and Agri-food Canada and the Natural Sciences and Engineering Research Council of Canada (NSERC) are acknowledged for their financial support. We thank Thermo Fisher Scientific and Phytronix Technologies for their support. We thank Audrey Roy-Lachapelle, Paul Fayad and Sung Vo Duy for their technical help and scientific support.

References

[1] A.K. Sarmah, M.T. Meyer, A.B.A. Boxall, Chemosphere 65 (2006) 725–759.
[2] N. Kemper, Ecol. Indic. 8 (2008) 1–13.
[3] B. Halling-Sørensen, S.N. Nielsen, P.F. Lanzky, F. Ingerslev, H.C.H. Lützhøft, S. E. Jørgensen, Chemosphere 36 (1998) 357–394.
[4] A.G. Mathew, R. Cissell, S. Liamthong, Foodborne Pathogens Dis. 4 (2007) 115–133.
[5] D.I. Masse, R.L. Droste, Water Res. 34 (2000) 3087–3106.
[6] M. Dousa, Z. Sikac, M. Halama, K. Lemr, J. Pharm. Biomed. 40 (2006) 981–986.
[7] M.Y. Haller, S.R. Müller, C.S. McArdell, A.C. Alder, M.J.F. Suter, J. Chromatogr. A 952 (2002) 111–120.
[8] A.M. Jacobsen, B. Halling-Sørensen, Anal. Bioanal. Chem. 384 (2006) 1164–1174.
[9] T. Christian, R.J. Schneider, H.A. Färber, D. Skutlarek, M.T. Meyer, H. E. Goldbach, Acta Hydrochim. Hydrobiol. 31 (2003) 36–44.
[10] E. Martínez-Carballo, C. González-Barreiro, S. Scharf, O. Gans, Environ. Pollut. 148 (2007) 570–579.
[11] Z.X. Cai, Y. Zhang, H.F. Pan, X.W. Tie, Y.P. Ren, J. Chromatogr. A 1200 (2008) 144–155.
[12] P.A. Blackwell, H.C.H. Lützhoft, H.P. Ma, B. Halling-Sorensen, A.B.A. Boxall, P. Kay, Talanta 64 (2004) 1058–1064.
[13] A. Boscher, C. Guignard, T. Pellet, L. Hoffmann, T. Bohn, J. Chromatogr. A 1217 (2010) 6394–6404.
[14] Y.S. Chen, H.B. Zhang, Y.M. Luo, J. Song, Chin. Sci. Bull. 57 (2012) 606–614.
[15] A. Karcı, I.A. Balcıoğlu, Sci. Total Environ. 407 (2009) 4652–4664.
[16] I. Beattie, A. Smith, D.J. Weston, P. White, S. Szwandt, L. Sealey, J. Pharm. Biomed. 59 (2012) 18–28.
[17] P. Lemoine, A. Roy-Lachapelle, M. Prévost, P. Tremblay, M. Solliec, S. Sauvé, Toxicon 61 (2013) 165–174.
[18] M. Boisvert, P.B. Fayad, S. Sauvé, Anal. Chim. Acta 754 (2012) 75–82.
[19] P.B. Fayad, M. Prévost, S. Sauvé, Anal. Chem. 82 (2010) 639–645.
[20] L. Viglino, M. Prévost, S. Sauvé, J. Environ. Monit. 13 (2011) 583–590.
[21] P.A. Segura, P. Tremblay, P. Picard, C. Gagnon, S. Sauvé, J. Agric Food Chem. 58 (2010) 1442–1446.
[22] J. Wu, C.S. Hughes, P. Picard, S. Letarte, M. Gaudreault, J.F. Levesque, D. A. Nicoll-Griffith, K.P. Bateman, Anal. Chem. 79 (2007) 4657–4665.
[23] J.J. Bergh, J.C. Breytenbach, P.L. Wessels, J. Pharm. Sci. 78 (1989) 348–350.
[24] B.J. Kerr, C.J. Ziemer, S.L. Trabue, J.D. Crouse, T.B. Parkin, J. Anim. Sci. 84 (2006) 1584–1592.
[25] A.C. Kolz, S.K. Ong, T.B. Moorman, Chemosphere 60 (2005) 284–289.
[26] S. Thiele-Bruhn, M.O. Aust, Arch. Environ. Contam. Toxicol. 47 (2004) 31–39.
[27] J.S. Teeter, R.D. Meyerhoff, Environ. Res. 93 (2003) 45–51.
[28] A.C. Kolz, T.B. Moorman, S.K. Ong, K.D. Scoggin, E.A. Douglass, Water Environ. Res. 77 (2005) 49–56.
[29] N. Saim, J.R. Dean, M.P. Abdullah, Z. Zakaria, J. Chromatogr. A 791 (1997) 361–366.
[30] L. Geiser, Y. Henchoz, A. Galland, P.A. Carrupt, J.L. Veuthey, J. Sep. Sci. 28 (2005) 2374–2380.
[31] Z.M. Qiang, C. Adams, Water Res. 38 (2004) 2874–2890.
[32] J. Paesen, W. Cypers, K. Pauwels, E. Roets, J. Hoogmartens, J. Pharm. Biomed. 13 (1995) 1153–1159.
[33] M. Cherlet, S. De Baere, S. Croubels, P. De Backer, Anal. Chim. Acta 473 (2002) 167–175.
[34] P. Picard, P. Tremblay, R. Paquin, in: 56th ASMS, 2008.
[35] K. Badjagbo, P. Picard, S. Moore, S. Sauvé, J. Am. Soc. Mass Spectrom. 20 (2009) 829–836.