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Determination of bupivacaine and metabolites in rat urine using capillary electrophoresis with mass spectrometric detection

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Abstract

A method using capillary electrophoresis-mass spectrometry (CE-MS) was developed for the structural elucidation of bupivacaine and metabolites in rat urine. Prior to CE-MS analysis, solid-phase extraction (SPE) was used for sample cleanup and preconcentration purposes. Exact mass and tandem mass spectrometric (MS/MS) experiments were performed to obtain structural information about the unknown metabolites. Two instruments with different mass analyzers were used for mass spectrometric detection. A quadrupole time-of-flight (Q-TOF) and a magnetic sector hybrid instrument were coupled to CE and used for the analysis of urine extracts. Hydroxybupivacaine as well as five other isomerically different metabolites were detected including methoxylated bupivacaine.

Keywords

Bupivacaine; Capillary electrophoresis-mass spectrometry; Metabolites; Urine

1 Introduction

Bupivacaine is a local anesthetic used extensively for chronic and acute pain treatment, and its metabolism has been studied in several animal models [1–8]. Desbutylbupivacaine, 3'-hydroxybupivacaine, and 4'-hydroxybupivacaine as well as a number of other metabolites of bupivacaine have been reported [1–8]. The development of analytical methods for the analysis of bupivacaine in biological samples is important for understanding the metabolic fate and tissue distribution following administration of the drug. Bupivacaine and its metabolites have been studied in biological samples using a variety of techniques including thin-layer chromatography (TLC), gas chromatography (GC), and high-performance liquid chromatography (HPLC) [1–19]. For the analysis of bupivacaine in rat urine, TLC has been used following liquid-liquid extraction [1]. Bupivacaine metabolites were identified by extraction of the sections of interest on a TLC plate followed by GC-MS analysis. Desbutylbupivacaine and three hydroxylated metabolites were reported as the major metabolites. A major disadvantage of TLC is that the required sample preparation is more time-consuming since the GC-MS analysis is performed off-line. This ultimately complicates the analysis considering that GC-MS can be performed without a prior TLC separation.

GC-MS analysis combined with liquid-liquid extraction has been reported for the analysis of bupivacaine, 3'-hydroxybupivacaine, and desbutylbupivacaine [3]. In conjunction with nuclear magnetic resonance spectroscopy, the method was able to identify three additional metabolites, *N*-butyl-pipecolyl-2-amide as well as two hydroxylated species. GC-MS with selected ion

monitoring has been reported for the analysis of bupivacaine and desbutylbupivacaine in fetal, maternal, and neonatal plasma following administration as an anesthetic for cesarean section [5]. More recently, GC-MS with selected ion monitoring (SIM) was used for the determination of bupivacaine and the metabolites, 3'- and 4'-hydroxybupivacaine [17]. Even though GC-MS provides for adequate analysis, the sample preparation is time-consuming since it required both liquid-liquid extraction and a derivatization procedure in order to volatilize the sample. Furthermore, structural information is lost when SIM was applied making it more difficult to identify minor metabolites.

More recent applications have relied upon the use of HPLC and liquid-liquid extraction, solid-phase extraction (SPE), or with column-switching strategies [9–16]. The analysis of bupivacaine, desbutylbupivacaine, and 4'-hydroxybupivacaine in human serum, plasma, and urine has been reported by several authors using HPLC [12,13,18,19]. Although separation of all three species was achieved, no separation or quantitation of 3'-hydroxybupivacaine was reported. Arvidsson and co-workers [19] reported an HPLC method for the separation of the 3'- and 4'-hydroxylated metabolites of bupivacaine in human urine [19]. However, the method was not compatible with mass spectrometric detection and no structural elucidation of any of the sample components could be performed. Additionally, the run time for the analysis was nearly three times longer than that of the CE method reported in this paper.

CE is becoming a more frequently utilized separation technique for bioanalytical challenges including pharmaceutical applications. There are several advantages of CE over other separation techniques such as GC and HPLC including lower sample volume requirements, higher separation efficiencies, speed of analyses, and generation of less sample waste. The combination of these attributes makes CE an attractive option for pharmaceutical applications since it can provide high resolution of analytes in a limited sample volume consisting of a complex biological matrix. Coupling CE to the power of MS detection enhances the capabilities of both techniques. There are a number of reviews in the literature discussing the application of CE-MS to pharmaceutical and bioanalytical challenges [20–22].

CE has been used for the analysis of pharmaceutical drugs and metabolites in biological samples including separation and quantitation of bupivacaine enantiomers in human plasma, and for the monitoring of bupivacaine in drainage fluid collected after pulmonary surgery [23,24]. A CE method for the analysis of bupivacaine and its major metabolites in rat urine has been reported [25]. However, no structural information was obtained for any metabolites since MS detection was not utilized. In this paper, a method for the separation of bupivacaine and its metabolites in rat urine by CE-MS is reported using two types of mass spectrometers.

CE has been coupled to a large variety of mass analyzers since CE was first coupled to MS (CE-MS) in 1988 [26]. In recent years, time-of-flight mass spectrometers (TOF-MS) have become more compatible for coupling to CE due to fast acquisition rates and excellent mass resolution without losing sensitivity. Spectra acquisition rates in TOF-MS can be on the order of a few milliseconds in order to obtain a complete mass spectrum. In addition, TOF-MS instruments duty cycles are significantly better than scanning analyzers increasing the sensitivity of ion detection. Due to these characteristics, TOF-MS is capable of adequately sampling across a narrow CE peak (on the order of a few seconds) while maintaining reasonable limits of detection. Despite limitations imposed by scanning acquisitions, magnetic sector instruments still provide superior mass accuracy, consistently better than 5 ppm.

In this paper, CE-MS was used to identify bupivacaine metabolites in rat urine using two hybrid instruments, a sector-quadrupole configuration and a Q-TOF. Exact mass data were obtained to determine the molecular formulas of unknown metabolites and was compared to data obtained with CE-MS experiments using a Q-TOF mass analyzer.

2 Materials and methods

2.1 Chemicals

Bupivacaine hydrochloride and prilocaine hydrochloride were obtained from Sigma-Aldrich (St. Louis, MO, USA). Desbutylbupivacaine, 3'-hydroxybupivacaine, and 4'-hydroxybupivacaine were gifts from Astra Pain Control AB (Södertälje, Sweden). Structures of each compound are shown in Fig. 1. HPLC-grade methanol was used in all experiments. Polyethylene glycol with an average molecular weight of 300 amu (PEG 300) was used for calibration of the mass scale and was obtained from Aldrich Chemical (Milwaukee, WI, USA). All other chemicals were reagent grade or better and used as received.

2.2 Urine collection

Female Sprague-Dawley rats weighing 300–350 g were housed in metabolism cages (Lab Products, Maywood, NJ, USA) and were provided food and water *ad libitum*. Urine samples were collected over 24 h time intervals and were frozen in dry ice unless otherwise specified. Rats were then given a 15 mg/kg intramuscular injection of bupivacaine, and urine was collected for 24 h unless specified otherwise. All urine samples were kept frozen prior to analysis.

2.3 SPE of urine samples

Aliquots of urine (5 mL) were spiked with 0.2 mL of a 4 mM solution of prilocaine and loaded onto Varian 3 mL, 200 mg Bond Elut™ C₁₈ SPE cartridges (Phenomenex, Torrance, CA, USA) previously conditioned with 1 mL of methanol and 3 mL of deionized water. The SPE cartridges were then washed with 0.5 mL of deionized water and drained completely followed by elution with 1 mL of chloroform. All washes, sample application, and elutions were aided by vacuum at a flow rate of approximately 1 mL/min. Chloroform extracts were evaporated to dryness and reconstituted in 200 µL of 0.1% v/v formic acid, 20% v/v methanol.

2.4 CE-UV experiments

CE-UV experiments were performed on an Isco Model 3850 Capillary Electropherograph™ (Isco, Lincoln, NE, USA) using Turbochrom 4.0 software (PE Nelson, Norwalk, CT, USA) as the data acquisition system. A fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 73.5 cm total length, 53 cm effective length, 360 µm OD, and 50 µm ID was used for separation. The run buffer consisted of 20% v/v methanol and 80% v/v 125 mM ammonium acetate, pH 7.0. Between CE runs, the capillary was flushed with run buffer for 5 min at 13.8 MPa, and was replaced after every 5 runs. Injection was performed electrokinetically for 90 s at 5 kV followed by separation at 20 kV. The detection wavelength was 210 nm.

2.5 CE-MS interface

Fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 105 µm OD and 40 µm ID was used in all CE-MS experiments. The CE-MS interface was of a liquid sheath flow design, and the CE capillary was inserted through a microvolume mixing 'T' (Valco Instruments, Houston, TX, USA). The inlet side of the T was sealed using 1/16" PEEK tubing (Upchurch Scientific, Oak Harbor, WA, USA) and a 1/16" ferrule. At the outlet of the interface, the CE capillary was inserted through a 2 cm length of 30 gauge stainless steel tubing (Small Parts, Miami, FL, USA) and secured with a 0.3 mm ID fused-silica adapter (Valco Instruments, Houston, TX, USA). The CE capillary was positioned such that it extended approximately 80 µm past the outer stainless steel electrospray needle. Sheath flow consisting of 95% v/v methanol and 1% v/v formic acid was supplied through the sidearm of the mixing T using a Harvard 55-1111 syringe pump (Harvard Apparatus, South Natick, MA, USA) at a flow rate of 0.5–1 µL/min. Electrospray voltage was applied to the mixing T using a Spellman

CZE1000R high-voltage power supply (Spellman High Voltage Electronics, Hauppauge, NY, USA).

2.6 CE-MS using a magnetic sector-quadrupole hybrid instrument

The fused-silica separation capillary was 73 cm long, and a voltage of 27.7–30 kV was applied to the anodic end of the capillary. Typical electrospray voltages were on the order of 9–10 kV, and a difference between the anodic and cathodic ends of the capillary was maintained at 17.7 kV unless specified otherwise. The run buffer consisted of 20% v/v methanol and 80% v/v 31 mM ammonium acetate (pH 7.0) unless otherwise specified. Between CE runs, the capillary was flushed with run buffer for 5 min at 13.8 MPa, and was replaced after every 5 runs. Samples were electrokinetically injected for 30 s at 5 kV unless specified. During injection, the electrospray voltage was turned off and the cathodic end of the capillary was grounded. Mass spectra were recorded on a VG AUTOSPEC-Q mass spectrometer (sector hybrid of EBEoQ configuration) equipped with Mark III ESI source (VG Analytical, Manchester, UK). The standard “pepper pot” counter electrode was removed and replaced with a sampling cone flange on which the CE-MS interface was attached. This arrangement is similar to that of a design reported by Woolfit *et al.* [27] in which the sampling cone serves as the counter electrode. For normal CE-MS operation, the sampling cone and skimmer lens were set at 4035 V and 4045 V, respectively. In order to acquire cone fragmentation spectra, the sampling cone and skimmer lens were set at 4083 V and 4098 V, respectively. The instrument was set at a resolving power of 1800 and scanned from 100–500 amu at a rate of 5 s/dec. Exact mass experiments were performed through the addition of PEG 300 serving as a mass calibrant in the make up flow liquid [28]. Exact mass CE-MS was carried out at 1:10 000 resolution and the sample mass was determined by using the OPUS data system interpolation routine [28]. Spectra were acquired using linear voltage scans over a 50 amu mass range encompassing two PEG ions. Off-line collision-induced dissociation (CID) mass spectra were obtained by infusion of the urine extracts through the CE capillary after identification of ions of interest from CE-MS experiments. CID-MS experiments were performed with precursor ions attenuated by 50% with xenon introduced into the collision octopole. The collision energy was 35–45 eV, and the quadrupole mass analyzer was scanned at a rate of 100 amu/s and 40 scans were integrated during infusion of urine extract at approximately 250 nL/min and 1 µL/min make up flow rate.

2.7 CE-MS with a Q-TOF instrument

The CE interface was coupled to a Micromass Q-TOF₂TM mass spectrometer equipped with a Z-Spray ionization source (Manchester, UK) by attaching the CE-MS interface to the Micromass nanospray *x, y, z* positioning stage. The CE separation capillary was approximately 65 cm in length and a voltage of 23 kV was applied to the anodic end of the capillary. An electrospray voltage of 4.3 kV was applied to the cathodic end of the capillary. The sheath make-up flow consisted of 95% v/v methanol and 0.1% v/v formic acid and was pumped at a rate of 1.0 µL/min. The CE run buffer consisted of 20% v/v methanol and 80% v/v 62 mM ammonium acetate (pH 7.0). All samples were electrokinetically injected for 30 s at 5 kV applied while keeping the cathodic end of the capillary grounded. For CE-MS experiments, the instrument was operated for maximum resolution (10 000RP FWHH) with all lenses optimized on the M2H²⁺ ion from the cyclic peptide Gramicidin S. The cone voltage was 30 eV and Ar was admitted to the collision cell at a pressure that attenuates the beam to about 20%, and the cell was operated at 5 eV (maximum transmission). Spectra were acquired at 22 727 Hz pusher frequency covering the mass range 50–975 amu and accumulating data for 1 s per cycle. A daily time to mass calibration was made with CsI cluster ions acquired under the same conditions and a mass correction for metabolite peaks was made using a background phthalate ion (MH⁺ 391.2848). MS/MS CID spectra were acquired by setting the MS1 quadrupole to transmit a precursor mass window of ±1.5 amu centered on the most abundant

isotopomer. Ar pressure was the same as above and the collision energy was varied 20 eV. CID spectra were acquired in 1 s cycles.

3 Results and discussion

3.1 CE-UV and CE-MS of bupivacaine and metabolites in urine extracts

Initial CE experiments using UV detection showed separation of desbutylbupivacaine, bupivacaine, prilocaine, and hydroxybupivacaine. Figure 2 depicts electropherograms of a blank urine extract (Fig. 2a), standards of desbutylbupivacaine, bupivacaine, hydroxybupivacaine, and prilocaine (Fig. 2b), and a urine extract from a bupivacaine dosed rat (Fig. 2c). From the urine of a dosed rat, it was determined that bupivacaine, hydroxybupivacaine, and a relatively low concentration of desbutylbupivacaine were present. In addition, several unknown nonendogenous peaks were detected labeled as peaks 5–9 in Fig. 2.

In order to further characterize the structures of the compounds detected by CE-UV analysis, CE-MS analyses were performed. Figures 3 and 4 show selected ion electropherograms using the magnetic sector mass analyzer (Fig. 3) and the Q-TOF mass analyzer (Fig. 4) of a urine extract of a dosed rat. Peaks corresponding to bupivacaine and hydroxybupivacaine were detected in addition to unknown species. Interestingly, the metabolite corresponding to a molecular ion of m/z 321 resulted in two peaks for the Q-TOF instrument as shown in Fig. 4 (peaks 9a and 9b) but in only one peak for the sector instrument shown in Fig. 3. This is most likely due to the better sensitivity that the Q-TOF instrument possesses. Desbutylbupivacaine was also detected but because of its weak ion intensity, was omitted for clarity. Relatively clean mass spectra of the MH^+ molecular ions were observed for the CE peaks shown in Figs. 3 and 4. Urine extracts collected from nondosed rats resulted in no detectable signals for each of the metabolites detected in dosed animals.

3.2 Fragmentation studies

Due to the difficulty of tuning the magnetic sector instrument for on-line MS/MS experiments, CID fragmentation spectra were obtained for the urine extracts “off-line” (no separation) with the Autospec-Q whereas the Q-TOF instrument easily acquired fragmentation spectra by CE-MS/MS for separated extracts. The advantage of obtaining mass spectra in an off-line mode is that the composite mass spectrum generated by the summation of multiple spectra is not limited by the acquisition of MS data during the elution of a CE peak. Thus, it is much easier to detect small signals from minor fragmentation products in the off-line mode. The major disadvantage of obtaining mass spectrometric data without a prior separation is that it is not possible to differentiate mass spectra from structural isomers of the same molecular weight.

For fragmentation studies the precursor ion of interest was selected by the first mass analyzer followed by fragmentation in the collision cell, and the resulting product ion spectra were acquired by the second mass analyzer. Figure 5a shows the CID spectra of components in unseparated urine extracts for the ions of interest determined by CE-MS using the quadrupole on the hybrid magnetic sector instrument, and Fig. 5b shows the CID spectra of the separated components using the Q-TOF. Structural information can be obtained from the fragmentation spectra acquired from the sector hybrid instrument shown in Fig. 5a. The mass spectra for each of the precursor ions resulted in a product ion with an m/z of 140 amu as a major product ion. In addition, precursor ions of m/z of 321 and 351 gave rise to an additional product ion of m/z 156. The appearance of m/z 156 may be the result of hydroxylation of the m/z 140 product ion that corresponds to the piperidine ring/butyl side chain. A product with an m/z of 170 was found for the unknown precursor ions of m/z 335 and 365 indicating an addition of 30 amu to the m/z 140 product ion. This can be explained by methoxylation or methylation and

hydroxylation of the piperidine ring moiety. The m/z 172 product ion present for the unknown precursor ion of m/z 321 is indicative of dihydroxylation of the piperidine ring functionality.

Since the acquired spectra were obtained from an unseparated sample, detection of both isobaric isomers for the m/z 321 metabolite was not possible for the sector instrument. The CE-MS/MS experiments resulted in the using the Q-TOF analyzer detection of two distinct isomers of the m/z 321 precursor ion that were separated by CE (Fig. 4). One of the spectra lacked the m/z 172 product suggesting that one of the isomers (peak 9b in Fig. 4) contained dihydroxylation on the piperidine ring.

Figure 6 shows cone fragmentation spectra of the first scan of the hydroxybupivacaine peak (Fig. 6b) of a urine extract from a dosed rat. As seen in Fig. 6, a product ion of m/z 156 was present in the last scan (the fourth scan across the peak) that was absent in the first scan. CID-MS studies of 3'- and 4'-hydroxybupivacaine did not result in a product ion of m/z 156 whereas CID-MS of crude urine extract resulted in the detection of this ion. The m/z 156 ion corresponds to hydroxylation of the piperidine ring. That the m/z 156 ion appeared only in the trailing region of the peak indicates that the leading edge of the peak consisted of a hydroxybupivacaine with hydroxylation occurring on the aromatic ring of the molecule. The trailing edge of the peak is composed of a metabolite of bupivacaine with hydroxylation occurring on the piperidine ring as well as the aromatic region. Although aromatic hydroxylation of bupivacaine is well known [1,2,12–14], only a few papers have reported hydroxylation on the piperidine ring [3,4].

3.3 Exact mass CE-MS experiments

Exact mass determinations were performed in order to further characterize the structures of bupivacaine and its metabolites. For CE-MS experiments using the magnetic sector instrument, the mass of unknown peaks were determined by calibrating the mass scale with bracketing $\text{PEG}(\text{NH}_4)^+$ ions generated by introducing PEG into the sheath flow. Exact mass data were obtained for the hydroxybupivacaine peaks as well as unknown peaks corresponding to $(\text{MH})^+$ ions with nominal masses 321, 335, and 351. Table 1 shows data obtained from the exact mass determinations with the magnetic sector mass analyzer. For CE-MS experiments using the Q-TOF mass analyzer, exact mass data were obtained for bupivacaine, hydroxybupivacaine and unknown peaks corresponding to (MH^+) ions of nominal masses 321, 335, 351, and 365 by correcting the mass scale with a known background ion. Table 2 shows data obtained from the exact mass experiments with the Q-TOF analyzer.

Data from the peak of nominal mass 305 suggest a molecular formula of $\text{C}_{18}\text{H}_{29}\text{N}_2\text{O}_2$ and is consistent with the hydroxylation of bupivacaine. The postulated structure hydroxybupivacaine is depicted in Fig. 7a. Exact mass and MS/MS data suggest the presence of aromatic hydroxylation and cone fragmentation mass spectra suggest hydroxylation of the piperidine ring. The exact mass of the m/z 321 isomers suggested two molecular formulas. The formula with the lower deviation ($\text{C}_{16}\text{H}_{27}\text{N}_5\text{O}_2$, 1.2 ppm deviation) was rejected due to its whole number of double bond equivalents. Although the molecular, $\text{C}_{18}\text{H}_{29}\text{N}_2\text{O}_3$, has a deviation of 5.3 ppm, it was selected since it has fractional number of double bond equivalents. This molecular formula corresponds to dihydroxylation of bupivacaine. The MS/MS data obtained from the Q-TOF instrument suggest that one isomer contained dihydroxylation of the piperidine ring. Fig. 7b shows proposed structures of the m/z 321 isomers. The exact mass data for the m/z 335 ion corresponds to a molecular formula of $\text{C}_{19}\text{H}_{31}\text{N}_2\text{O}_4$ that suggests the addition of CH_2O_2 to bupivacaine. This can be explained by methylation and dihydroxylation of bupivacaine or by methoxylation and hydroxylation as indicated in Fig. 7c. The molecular formula of the m/z 351 ion was determined to be $\text{C}_{19}\text{H}_{31}\text{N}_2\text{O}_4$ and suggests addition of CH_2O_3 to bupivacaine as shown in Fig. 7d. This may be indicative of methylation and trihydroxylation or methoxylation and dihydroxylation of bupivacaine. The exact mass of the m/z 365 ion was not determined with the magnetic sector analyzer due to an interfering PEG

ion of the same nominal mass. The Q-TOF exact mass data suggested three different molecular formulas of fractional double bond equivalents that are $C_{25}H_{33}O_2$ (−2.9 ppm deviation), $C_{21}H_{29}N_6$ (4.5 ppm deviation), and $C_{20}H_{33}N_2O_4$ (8.1 ppm deviation). It would be difficult to positively identify the exact molecular formula of the m/z 365 metabolite without any further data. However, the molecular formula for this molecule is likely to be $C_{20}H_{33}N_2O_4$ based upon the assumption that is a metabolite of bupivacaine. The MS/MS data obtained from the sector instrument suggest addition of $C_1H_3O_2$ to the aromatic ring as depicted in Fig. 7e. This suggests methylation and dihydroxylation or methoxylation and hydroxylation of the aromatic ring. Additionally, the data suggest modification of the piperidine with either methylation and hydroxylation or methoxylation as indicated in Fig. 7e.

4 Concluding remarks

Although no quantitative determination of bupivacaine metabolites was demonstrated in this paper, a CE-MS method for the identification of bupivacaine and its metabolites in rat urine was developed for magnetic sector hybrid and Q-TOF instruments. Quantitative measurements are to be performed for future experiments once an internal standard is found. Since most of the metabolites detected in rat urine were unknown, using a stable isotope analog for each analyte is not possible. However, a reasonable choice of internal standard may be a stable isotope analog of bupivacaine that would allow for quantitative determinations of the unknown metabolites.

The exact mass and fragmentation data obtained suggested the presence of five metabolites of different molecular weights. In addition, there were multiple isomers detected for several of the metabolites that mean there were up to nine metabolites detected in rat urine using the CE-MS method. In addition to determining the metabolites of bupivacaine, a comparison between the two different mass analyzers can be made. The Q-TOF is superior to the sector instrument as far as acquisition rates, sensitivity, and ease of use are concerned. However, the exact mass capability of the sector analyzer was better than that of the current generations of Q-TOFs, making it easier to determine the molecular formulas of unknowns.

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Abbreviation

Q-TOF
quadrupole time-of-flight

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Figure 1. Structures of (a) bupivacaine, (b) desbutylbupivacaine, (c) 3'-hydroxybupivacaine, (d) 4'-hydroxybupivacaine, and (e) prilocaine.

Figure 2. (A) CE-UV of a blank urine extract; (B) 11 μ M standard of (3) bupivacaine, (1) desbutylbupivacaine, (4) hydroxybupivacaine and (2) 4 μ M prilocaine; (c) a urine extract from a dose rat. Peaks (5)–(9) are metabolite peaks of unknown identity.

Figure 3.

CE-MS superimposed selected ion electropherograms of (3) bupivacaine ($MH^+ = 289$), (4) hydroxybupivacaine ($MH^+ = 305$), and unknown metabolites of MH^+ molecular ions of (6) 335, (7) 365, (8a) and (8b) 351, and (9) 321 in urine extract using the magnetic sector mass analyzer.

Figure 4.

CE-MS selected ion electropherograms of bupivacaine and metabolites in urine extract using the Q-TOF mass analyzer. Peak identifications are the same as in Fig. 3.

Figure 5.

(a) CID spectra of bupivacaine and metabolites of interest in urine extract using the quadrupole on the sector instrument from an unseparated extract and (b) on-line CE-MS/MS with the Q-TOF instrument.

Figure 6. Cone fragmentation spectra m/z 305 ion showing the spectra (A) at the leading edge of the peak and (B) the trailing edge of the peak.

Figure 7. Postulated structures of bupivacaine and its metabolites according to exact mass and fragmentation experiments.

Table 1
Exact mass data for hydroxybupivacaine and unknown metabolites of nominal mass of 321, 335, and 351 by CE-MS with a magnetic sector mass analyzer

Nominal mass	Determined mass	Calculated mass	Deviation (PPM) ^a	Molecular formula (MH ⁺)	Double bond equivalents
289	—	289.2280	—	C ₁₈ H ₂₉ N ₂ O	5.5
305	305.2279	305.2290	3.6	C ₁₈ H ₂₉ N ₂ O ₂	5.5
321	321.2161	321.2178	5.3	C ₁₈ H ₂₉ N ₂ O ₃	5.5
335	335.2324	335.2335	3.2	C ₁₉ H ₃₁ N ₂ O ₃	5.5
351	351.2293	351.2284	-2.6	C ₁₉ H ₃₁ N ₂ O ₄	5.5

^a Deviation was calculated by: deviation = [(determined mass - calculated mass)/(calculated mass)]×10⁶

Table 2
Exact mass data for bupivacaine, hydroxybupivacaine, and unknown metabolites of nominal mass of 321, 335, 351, and 365 by CE-MS with a TOF mass analyzer

Nominal mass	Determined mass	Calculated mass	Deviation (PPM) ^a	Molecular formula (MH ⁺)	Double bond equivalents
289	289.2300	289.2280	6.9	C ₁₈ H ₂₉ N ₂ O	5.5
305	305.2271	305.2290	-6.2	C ₁₈ H ₂₉ N ₂ O ₂	5.5
321	321.2189	321.2178	3.4	C ₁₈ H ₂₉ N ₂ O ₃	5.5
335	335.2348	335.2335	3.9	C ₁₉ H ₃₁ N ₂ O ₃	5.5
351	351.2308	351.2284	6.8	C ₁₉ H ₃₁ N ₂ O ₄	5.5
365	365.2470	365.2440	8.2	C ₂₀ H ₃₃ N ₂ O ₄	5.5

^a Deviation was calculated by: deviation = [(determined mass – calculated mass)/calculated mass]×10⁶