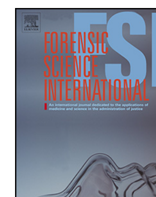
Forensic Science International 265 (2016) 182–185

Contents lists available at ScienceDirect

Forensic Science International

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



Sensitive liquid chromatography/tandem mass spectrometry method for the simultaneous determination of nine local anesthetic drugs

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ARTICLE INFO

Article history:
Available online 4 March 2016

Keywords:
LC/MS/MS, Local anesthetic, Analysis, Solid phase extraction

ABSTRACT

A high-performance liquid chromatography–tandem mass spectrometry (LC/MS/MS) with electrospray ionization (ESI) procedure for the simultaneous determination of nine local anesthetic drugs (procaine, mepivacaine, lidocaine, ropivacaine, oxybuprocaine, tetracaine, bupivacaine, T-caine and dibucaine) in human serum is described. The chromatographic separation was performed on a Mightysil-RP-18 GP II column (2.0 mm × 150 mm, particle size 5 μm). The mobile phase consisted of 10 mM acetic ammonium buffer (pH 5.4) and acetonitrile and was delivered at a flow rate of 0.20 mL/min. The triple quadrupole mass spectrometer was operated in positive ion mode, and multiple reaction monitoring was used for drug quantification. Solid-phase extraction of the nine local anesthetic drugs added to the human serum was performed with an Oasis® HLB extraction cartridges column. The method was linear for the investigated drugs over the concentration range of 10–100 ng/mL. The recoveries of these drugs were in the range of 81.4–144%. The standard deviation (SD) values for all analytes were <0.10 for both intraday and interday accuracy and precision. The selectivity, accuracy and precision of this method are satisfactory for clinical and forensic applications. The sensitive and selective method offers the opportunity for the simultaneous screening and quantification, for clinical and forensic purposes, of almost all local anesthetics available in Japan.

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1. Introduction

Intoxication by the anesthetic drugs, although significantly less frequent compared with the other drugs, occurs often due to medical accident or malpractice and has become a major social problem. Local anesthetic drugs can be classified into ester-type and amide-type drugs according to their structures. The two types of the drugs differ in the mode of metabolism and chemical stability. Local anesthetic drug by applicable law surface anesthesia, anesthesia, transfer anesthesia, spinal anesthesia and epidural also should note that can increase blood concentrations of local anesthetic drug toxicity is divided into anesthesia for many medical accidents in the present situation [1–3]. Various intoxicatuin cases have been reported by local anesthetic drugs [4–10]. In the case of local anesthetic drugs, the frequency of occurrence of drug shock is also high, lidocaine, procaine, dibucaine often becomes problem. In recent years, several new low-dose drugs have entered the market and have put further demands on the assays used to detect them. Cases of fatal intoxication caused by these medicines are commonly due to the combination of several drugs by the patient. Therefore, it is necessary to develop a simple and sensitive method for the screening of these drugs.

Several methods for the determination of local anesthetic drugs using high-performance liquid chromatography (HPLC) [11], LC/MS and LC/MS/MS have been reported [12–15]. However, the simultaneous determination of nine local anesthetic drugs (procaine, mepivacaine, lidocaine, ropivacaine, oxybuprocaine, tetracaine, bupivacaine, T-caine and dibucaine) using LC/MS/MS with ESI has not been reported.

In this paper, we report a selective and sensitive method for the simultaneous determination of nine local anesthetic drugs using LC/MS/MS with ESI, and a simple procedure for solid-phase column extraction of the drugs from human serum using an Oasis® HLB cartridge column.

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http://dx.doi.org/10.1016/j.forsciint.2016.02.044
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2. Experimental

2.1. Chemicals

Nine local anesthetic drugs were examined in this study. Drug standards for lidocaine and oxybuprocaine hydrochloride, procaine hydrochloride, dibucaine hydrochloride and tetracaine hydrochloride were purchased from Waco Pure Chemical Industries (Osaka, Japan). Mepivacaine hydrochloride and bupivacaine hydrochloride were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Ropivacaine hydrochloride was purchased from Sigma-Aldrich Japan (Tokyo, Japan). Lidocaine-d_{10} (internal standard: IS) was purchased from C/D/N ISOTOPES Inc. (Central Chemicals Co., Inc.) (Tokyo, Japan). Normal human serum was purchased from Millipore (Temecula, CA, USA). Methanol (HPLC grade), water (HPLC grade), acetonitrile (HPLC grade), acetic acid (analytical grade) and ammonium acetate (analytical grade) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Oasis® HLB extraction cartridges were purchased from Waters (Milford, MA, USA). All other chemicals were commercially available and of reagent grade.

2.2. Preparation of the standard solutions and calibration standards

Individual stock solutions of the analyte compounds at a concentration of 1 mg/mL were prepared in methanol (stable for at least three months when stored at −20 °C). Serum standards were prepared with concentrations of 10, 25, 50, 75 and 100 ng/mL of each compound by diluting appropriate aliquots of the stock solution with drug-free serum (normal human serum). The calibration curves were obtained by simple linear regression analysis of each drug's concentration and corresponding peak area ratio. The regression equations for the nine local anesthetic drugs extracted from human serum are based on the ratio of peak area of each drug to that of the IS.

2.3. Apparatus and chromatographic conditions

LC/MS/MS experiments were performed with an HPLC system, which consisted of Shimadzu LC-20AD pumps (Shimazu, Kyoto, Japan), a SIL-20AC autosampler (Columbia, MD, USA) and the 4000 QTRAP mass spectrometer (Applied Biosystems, Foster City, CA, USA).

The chromatographic separation was performed on a Mightysil-RP-18 GP II column (2.0 mm × 150 mm, particle size 5 μm; Kanto Chemical Co., Inc., Tokyo, Japan) with guard cartridge (2.0 mm). For the gradient elution, two solvents were used: (A) 10 mM acetic ammonium buffer, pH 5.4 and (B) acetonitrile. The mobile phase composition was held initially at 65% (A) for 10 min and then changed during the linear gradient elution from 65% (A) to 40% (A) over 20 min. The mobile phase was then returned to 65% (A) over 5 min for the next run. The flow rate was set to 0.20 mL/min and 10 μL of sample was injected for each analysis. The column and autosampler were maintained at 37 and 4 °C, respectively.

All experiments were conducted in the positive ion electrospray mode. The TurboIonSpray source was operated at 600 °C with the capillary voltage set at 5500 V. Nitrogen was used as the nebulizer gas, curtain gas (40 psi) and collision gas (4 psi). The collision energy was set between at 27 and 41 V. The instrument was used in MRM mode (Table 1).

2.4. Extraction procedure of the drugs from human serum using an Oasis® HLB cartridge column

The human serum samples were stored at −20 °C until analysis time. These samples were analyzed to determine possible endogenous interferences and were used as "blanks". An Oasis® HLB cartridge column with a capacity of 1 mL was placed in an Agilent Vac Elut system (Agilent Technologies, CA, USA). Each column was activated by washing with 1 mL of methanol followed by 1 mL of water.

A cholinesterase inhibitor, such as neostigmine bromide (100 μg), was added to the human serum. Five microliters of phosphoric acid and 500 μL of water were added to 1.0 mL of human serum containing 10–100 ng of each of the nine local anesthetic drugs and 100 ng lidocaine-d_{10} (IS). The mixture was applied to the Oasis® HLB cartridge column and allowed to pass through. The column was then washed with 1 mL of 2% ammonium hydroxide in a 10% methanol solution. The local anesthetic drugs were eluted with 1 mL of a methanol:2% acetic acid (70:30) solution. The eluent was dried under N_2 gas at 40 °C. The residue was dissolved in 200 μL of mobile phase, and an aliquot (10 μL) was then analyzed by the chromatographic system.

2.5. Linearity, accuracy, precision, and recovery

For the evaluation of the linearity of the standard calibration curve, the analyses of the nine local anesthetic drugs in serum samples were performed on three separate days using freshly prepared samples and solutions. The calibration curves were prepared over a linear range of 10–100 ng/mL using five concentrations (10, 25, 50, 75 and 100 ng/mL).

The intraday assay precision and accuracy were obtained by analyzing six aliquots of the quality control samples in duplicate using a calibration curve constructed on the same day. The interday assay precision and accuracy were evaluated using six replicate determinations for each concentration with solutions made on different days. The recoveries were calculated by comparing the chromatographic peak areas obtained from the extracts of the serum samples (containing the nine local anesthetic drugs and the IS) with those obtained by extracting the drugs from water instead of serum.

Table 1
Retention time and tandem mass spectrometry parameters of local anesthetic drugs.

Drugs	Retention time (min)	MW	Q1	Q3	CE (V)	DP (V)	EP (V)	CXP (V)
Procaine	2.70	236.32	237	100	21.0	56.0	10.0	8.0
Mepivacaine	3.75	246.35	247	98	25.0	61.0	10.0	8.0
Lidocaine-d_{10} (I.S.)	5.01	244.40	245	96	29.0	76.0	10.0	6.0
Lidocaine	5.75	234.34	235	86	27.0	71.0	10.0	6.0
Ropivacaine	6.46	274.40	275	126	29.0	66.0	10.0	10.0
Oxybuprocaine	8.60	308.42	309	80	71.0	56.0	10.0	14.0
Tetracaine	9.07	264.37	265	176	21.0	56.0	10.0	14.0
Bupivacaine	9.82	288.43	289	140	29.0	76.0	10.0	10.0
T-caine	12.99	292.42	293	176	23.0	61.0	10.0	16.0
Dibucaine	17.67	343.46	344	116	71.0	51.0	10.0	8.0

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