Simultaneous determination of bupivacaine, mepivacain, prilocaine and ropivacain in human serum by liquid chromatography–tandem mass spectrometry

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Abstract

A liquid chromatography–tandem mass spectrometric (LC–MS-MS) method with a rapid and simple sample preparation was developed and validated for the simultaneous determination of the local anesthetics bupivacaine, mepivacaine, prilocaine and ropivacaine in human serum. An external calibration was used. The mass spectrometer was operated in the multiple reaction monitoring mode. A good quadratic response over the range of 1.0–200.0 ng/ml was demonstrated. The accuracy for bupivacaine ranged from 93.2 to 105.7%, for mepivacaine from 96.2 to 104.3%, for prilocaine from 94.6 to 105.7% and for ropivacaine from 94.3 to 104.0%, respectively. The limit of quantification was 1.0 ng/ml for all substances. This method is suitable for pharmacokinetic studies.
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1. Introduction

Drugs with rapid onset are widely used in combination with long-acting local anesthetics [1].

Some of the most commonly used local anesthetics are the substances bupivacaine, mepivacaine, prilocaine and ropivacaine. Therapeutic monitoring of patients verify the cardiac toxicity of this drugs [2]. Total and free fraction serum levels were measured for each drug alone and a correlation between the drug concentration and the cardiovascular system toxicity have been reported. These reports led to a renewed interest in the relationship between the toxicity and the combination of drugs. Recommendations for the quantity of single drugs in a mixture are still missing.

For a survey on the mixtures, a sensitive analytical procedure is necessary.

Several different methods have already been reported for the determination of these drugs in human fluids. Some of them are complicated.

High-performance liquid chromatography with ultraviolet detection (HPLCUV) is the most used method [2–13]. But also gas chromatography–mass spectrometry (GC–MS) methods are described [14,15].

For the sample preparation there is often a precolumn separating the analytes from proteins and polar endogenous compounds and therefore a direct injection of 500 μl plasma is possible [12,13,15,16,18]. Another way for separating the analytes from the matrix is the ultrafiltration or microfiltration, which are coupled with GC–MS or liquid chromatography–tandem mass spectrometry (LC–MS-MS) [10,11,15,17].

Solid-phase microextraction, solid-phase extraction, microextraction in packed syringes (MEPS) and extractions with dichlormethane or *tert*-butylmethyl ether are also often used to determine local anaesthetics in human fluids [5,8,14,17,19–22]. All these methods have an insufficient sample preparation for routine measurements of local anesthetics in human serum.

This paper describes a liquid chromatographic–tandem mass spectrometry method with a rapid and simple sample preparation by protein precipitation to determine simultane-

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ously the local anesthetics bupivacaine, mepivacaine, prilocaine and ropivacaine in biological samples, requiring small sample volumes and enabling a low limit of quantification.

2. Experimental

2.1. Chemicals

Reference substances of the local anesthetics bupivacaine (Carbostesin® 0.5%), mepivacaine (Scandiain® 1%), ropivacaine (Naropin® 10 mg/ml) and prilocaine (Xylonest® 1%) were purchased from AstraZeneca (Wedel, Germany).

Acetonitrile LiChrosolv (for chromatography), methanol LiChrosolv (for chromatography) and ammonium acetate (analytical reagent grade) were purchased from Merck (Darmstadt, Germany). Pure water (18 MΩ) was obtained using an ion-exchange system RS 40E, SG Ionenaustauscher (Barsbüttel, Germany).

2.2. LC–MS-MS analysis—apparatus and chromatographic conditions

2.2.1. Mass spectrometer

The LC–MS-MS system used was a Quattro micro (Micromass, Manchester, UK) equipped with an electrospray interface (ESI). Full scan mass spectrum were acquired by continual infusion of standard solution (concentration 200.0 ng/ml with 10 µl/min). The product ion mass spectra were obtained by choosing the molecular ions as the precursor ions and scanning product ions from m/z 80–300. For positive ionisation a capillary voltage of 3200 V and ion source temperature of 100 °C were applied. The desolvation gas flow (nitrogen) was 600 l/h at 300 °C.

The multiple reaction monitoring (MRM) was performed by monitoring the transitions between m/z 288.9 (parent ion) and m/z 140.2 for bupivacaine, between m/z 247.0 (parent ion) and m/z 98.1 for mepivacaine, between m/z 221.0 (parent ion) and m/z 86.0 for prilocaine and between m/z 275.3 (parent ion) and m/z 126.2 for ropivacaine. The collision gas was argon. The dwell time, collision energy and pressure are shown in the following table:

Compound	Dwell time (ms)	Collision energy (eV)	Pressure (mbar)
Bupivacaine	200	18	3.20e−3
Mepivacaine	200	16	3.20e−3
Prilocaine	200	12	3.20e−3
Ropivacaine	200	19	3.03e−3

The transition was used for the determination of analyte concentration.

The MassLynx Data System was applied for MS control and QuanLynx for peak area evaluation, regression analysis of standard curves and calculation of concentrations.

2.2.2. LC system

The LC equipment consisted of a Dionex P580 HP-Gradient pump and an autosampler Dionex ASI 100 T (Id-stein, Germany) with a Chromeleon Chromatography Data System (Dionex Softron, Idstein, Germany). The chromatographic separation was performed on a Synergy 4 µ Polar-RP 80A, 150 mm × 2 mm (Phenomenex, Aschaffenburg, Germany) column with a Security Guard C18, 4 mm × 2 mm i.d. (Phenomenex).

The following gradient was applied, with solvent A (5/95/0.2, v/v/v) and solvent B (95/5/0.2, v/v/v) each being a mixture of acetonitrile, 2 mM ammonium acetate and formic acid:

Time (min)	0.0	0.1	2.5	3.0	3.2	7.0
A (%)	100	100	20	20	100	100
B (%)	0	0	80	80	0	0

The flow-rate was 0.5 ml/min, and temperature was 25 °C.

2.3. Sample preparation

The frozen serum samples (−20 °C) were thawed at room temperature.

The sample volume of 0.4 ml serum was diluted with 0.8 ml of acetonitrile, mixed (Vortex, Genie2) and centrifuged for 10 min. 0.8 ml of the liquid layer was diluted with 1.2 ml solvent A (mobile phase), removed to a 1 ml autosampler vial and 10 µl were injected for LC–MS-MS.

2.4. Recovery and ion suppression

The recovery from serum was evaluated by comparing the peak areas of amounts mixed with assayed blanks and injected than directly with those of assayed samples. The ion suppression of the drugs in the mass spectrometer (effect of the matrix) were evaluated by comparing the peak areas of amounts injected directly with peak areas of amounts mixed with assayed blanks and injected than directly.

The slope of the quadratic regression line $y = ax^2 + bx + c$ of the response x of amounts mixed with assayed blanks (and than injected directly) and the response of the assayed samples y delivers the mean recovery of the concentration range investigated. The slope of the quadratic regression line of the response x of directly injected amounts and the response of the amounts mixed with assayed blanks (and than injected directly) y delivers the mean ion suppression of the concentration range investigated. The peak areas of local anesthetics at seven concentrations in the range of 1.0 up to 200.0 ng/ml were used. The graphs were evaluated using the "Trend line function" of Excel.

3. Results

3.1. Mass spectrometry

The MS-MS spectra of the local anesthetics bupivacaine, mepivacaine, prilocaine and ropivacaine are shown in Fig. 1.
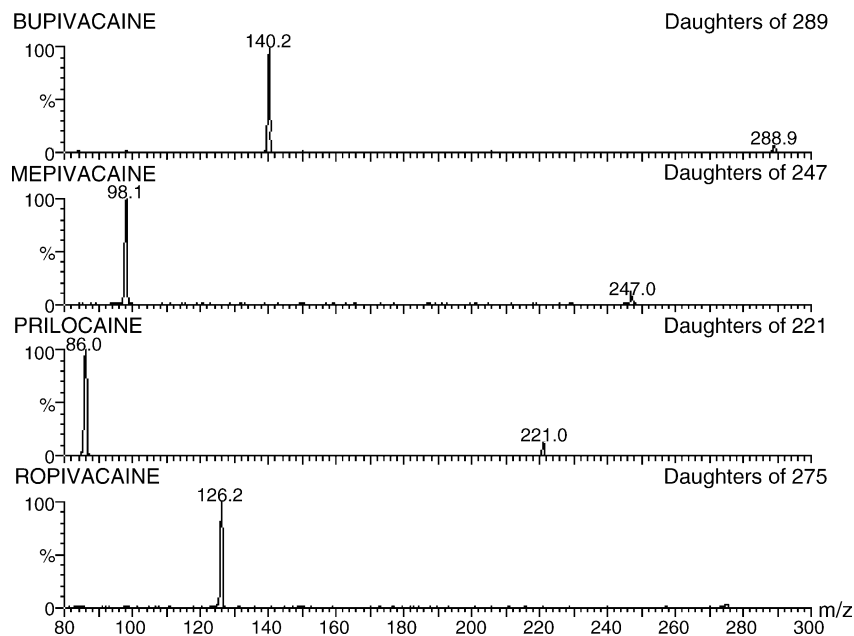
Fig. 1. MS-MS spectra of bupivacaine, mepivacaine, prilocaine and ropivacaine: the spectrum reveales a base peak at *m/z* 288.9 and daugther peak *m/z* 140.2 for bupivacaine, a base peak at *m/z* 247.0 and daugther peak *m/z* 98.1 for mepivacaine, a base peak at *m/z* 221.0 and daugther peak *m/z* 86.0 for prilocaine and a base peak at *m/z* 275.3 and daugther peak *m/z* 126.2 for ropivacaine.

The fragment ion of bupivacain observed at *m/z* 140.2 corresponds to the butane-1-piperidinyl group, the fragment ion of mepivacain observed at *m/z* 98.1 corresponds to the methane-1-piperidinyl group, the fragment ion of prilocain observed at *m/z* 86.0 corresponds to ethylpropylamine and the fragment ion of ropivacain observed at *m/z* 126.2 corresponds to the propane-1-piperidinyl group. Intensities of other fragments are negligible.

3.2. Chromatography

Because of the high specificity of the MS-MS method a complete chromatographic separation of analytes and matrix is not necessary. To exclude interferences from the biological matrix, chromatograms of all local anesthetics were controlled separately. No interferences and a low background noise were found (Fig. 2).

MS-MS does not respond to ions originating from impurities of the biological matrices that differ in *m/z* from the selected ion. The retention time of bupivacaine was about 4.6 min, of mepivacaine about 4.2 min, of prilocaine about 4.2 min and of ropivacaine about 4.4 min (Fig. 2).

3.3. Recovery and ion suppression

The recovery in the ranges from LOQ to 200.0 ng/ml was found to be 96% for bupivacaine, 93% for mepivacaine 94% for prilocaine and 97% for ropivacaine. A recovery of more than 93% means that the sample preparation, i.e. the protein precipitation, is very efficient and the ion suppression of the drugs in the mass spectrometer (effect of the matrix) is neg-

ligible. Because of the low effect of the matrix and the good reproducibility of the sample preparation an internal standard was not necessary but it is possible to use one of the drug as an internal standard for the determination of the other drugs.

3.4. Calibration graph

The calibration graphs for the local anesthetics were generated from MRM of increasing amounts of the drug standards. A quadratic calibration graph was constructed using least-squares regression of quantities versus peak area. A good response over the range of 1.0–200.0 ng/ml was demonstrated. Samples with a higher concentration of the analytes were diluted. The correlation coefficient of regression lines was 0.9974 or higher.

3.5. Reproducibility

The precision and accuracy of the method were assessed by determination of seven concentrations in six independent series of spiked serum samples (see Table 1). The accuracy was calculated to be from 93.2 to 105.7% for bupivacaine,

Table 1
Precision and accuracy of bupivacaine, mepivacaine, prilocaine and ropivacaine from six independent sets of spikes serum samples

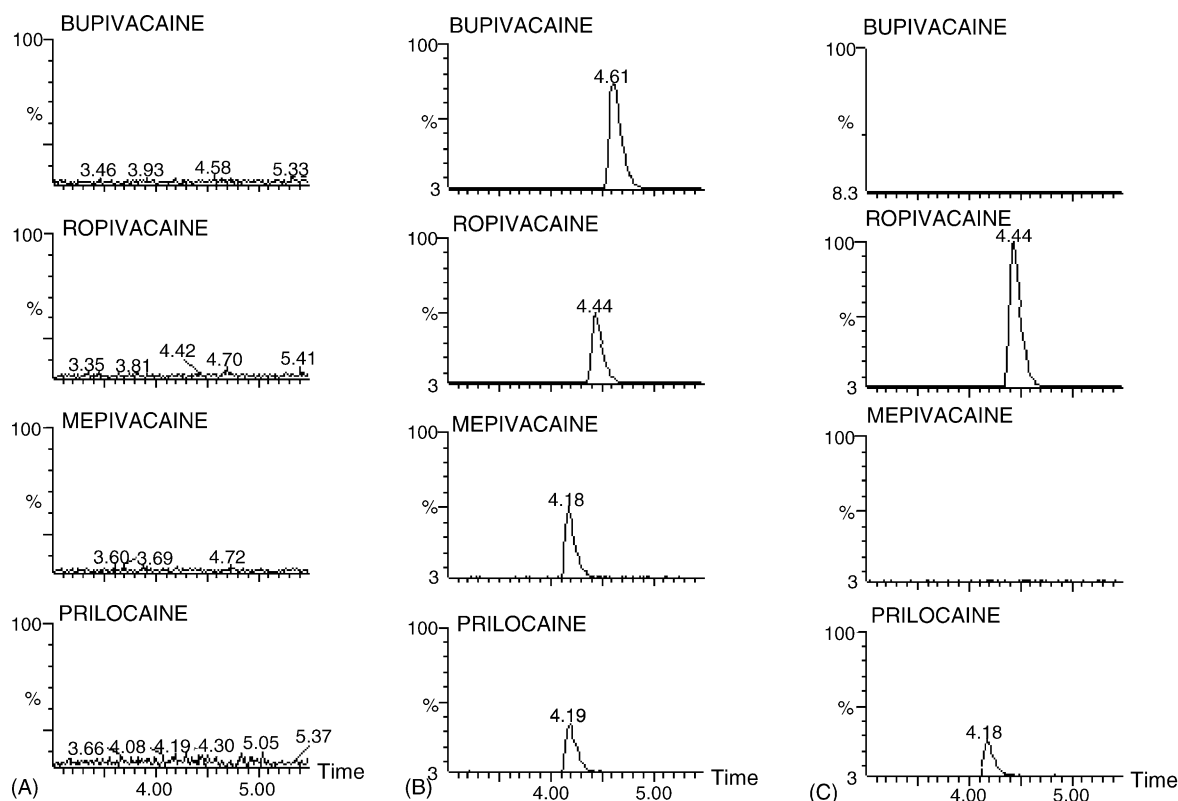|	Precision (%, *n* = 6)	Accuracy (%, *n* = 6)
Bupivacaine	0.7–6.6	93.2–105.7
Mepivacaine	0.8–9.6	96.2–104.3
Prilocaine	0.9–6.5	94.6–105.7
Ropivacaine	0.6–7.1	94.3–104.0

Fig. 2. Chromatograms of bupivacaine, mepivacaine, prilocaine and ropivacaine: (A) blank samples, (B) standards with 5 ng/ml and (C) patient sample with 56 ng/ml prilocaine and 183 ng/ml ropivacain.

from 96.2 to 104.3% for mepivacaine, from 94.6 to 105.7% for prilocaine and from 94.3 to 104.0% for ropivacaine, respectively. The coefficient of variation (CV) ranged from 0.7 to 6.6% for bupivacaine, from 0.8 to 9.6% for mepivacaine, from 0.9 to 6.5% for prilocaine and from 0.6 to 7.1% for ropivacaine, respectively. The limit of quantification (LOQ) was obtained by comparing the true and measured values for the lowest standard data points and blanks of the six standard curves. The acceptance criterion was a deviation of less than ±20%. LOQ of 1.0 ng of local anesthetics/ml could be accepted. The day-to-day recovery was calculated with three different quality control samples to be from 85.1 to 111.1% for bupivacaine, from 89.1 to 113.4% for mepivacaine, from 86.2 to 113.7% for prilocaine and from 86.4 to 113.6% for ropivacaine, respectively.

4. Discussion

Liquid chromatography tandem mass spectrometry is a powerful technique for highly specific and quantitative measurements of low levels of analytes in biological matrices. There have also been several other reports on the use of the highly sensitive and specific liquid chromatography–tandem spectrometry technique to measure local anesthetics in human plasma.

In this study an analytical method of local anesthetic determination using LC–MS-MS after simple sample preparation by protein precipitation has been developed. Only the small sample volume of 0.4 ml serum was necessary to achieve a limit of quantification of 1.0 ng/ml. A number of about 100 samples a day can be analysed in this way without any decreasing of the limit of quantification or accumulation of sample matrix in the ion-source. The method is suitable for routine measurements of local anesthetics in human serum samples.

5. Conclusion

LC–MS-MS is the most sensitive method for a quantitation of local anesthetics in serum. Furthermore, the assay requires only a rapid and simple sample preparation. To increase the sensitivity it is possible to increase the sample volume. This method is suitable for pharmacokinetic and clinical studies.

Acknowledgement

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