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Short communication

Simultaneous determination of three local anesthetic drugs from the pipecoloxylidide group in human serum by high-performance liquid chromatography

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Abstract

A high-performance liquid chromatographic (HPLC) method has been developed for the simultaneous analysis of the local anesthetic amide drugs, bupivacaine, mepivacaine and ropivacaine, belonging to the pipecoloxylidide group using a C_{18} reversed-phase column (150 × 4.6 mm I.D.) filled with 5-μm particles and attached to a UV detector. The mobile phase was composed of acetonitrile–methanol–30 mM NaH_2PO_4 (pH 5.6) (100:100:300, v/v/v) and the flow rate was 1 ml/min. The absorbance of the eluate was monitored at 210 nm. The retention times of the three compounds were: 4.6 min (mepivacaine), 9.7 min (ropivacaine) and 16.4 min (bupivacaine). With this sample preparation method, good and consistent recoveries of the three compounds were obtained: 88–91% for mepivacaine, 87–89% for ropivacaine and 88–91% for bupivacaine. The limit of quantification for three compounds in human serum was 2 ng/ml for mepivacaine, 5 ng/ml for bupivacaine and ropivacaine. This method may be useful in clinical and forensic applications for the determination or identification of the local anesthetic drugs: bupivacaine, mepivacaine or ropivacaine.

Keywords: Local anaesthetic drug; Bupivacaine; Mepivacaine; Ropivacaine

1. Introduction

The local anesthetic amide drugs, bupivacaine, mepivacaine and ropivacaine, are the members of the pipecoloxylidide group (Fig. 1). These drugs are extensively metabolized by the liver and excreted in the urine in animals and human [1]. Several methods have been previously described for the determination of the parent drug and/or the major metabolites using GC [2], high-performance liquid chromatography (HPLC) [3–8] and LC–MS [9]. More recently, Baniceru et al. [2] reported that a method of analysis based on solid-phase extraction coupled with a capillary GC system for the determination of mepivacaine, bupivacaine and ropivacaine in human serum was developed. However, this method is time-consuming (about 27 min) and not sensitive (limit of quantification: 50 ng/ml). The simultaneous determination of these three drugs using HPLC/UV has not been reported previously.

The purpose of the present study was to develop a speedily and sensitive method for the simultaneous determination of bupivacaine, mepivacaine and ropivacaine in human serum, using HPLC/UV.

2. Experimental

2.1. Reagents and materials

Bupivacaine, mepivacaine and ropivacaine were a gift from AstraZeneca (Osaka, Japan). Tetracaine (internal standard, I.S.) was purchased from Sigma (St. Louis, MO, USA). All other chemicals and reagents used were of the highest commercially available quality.

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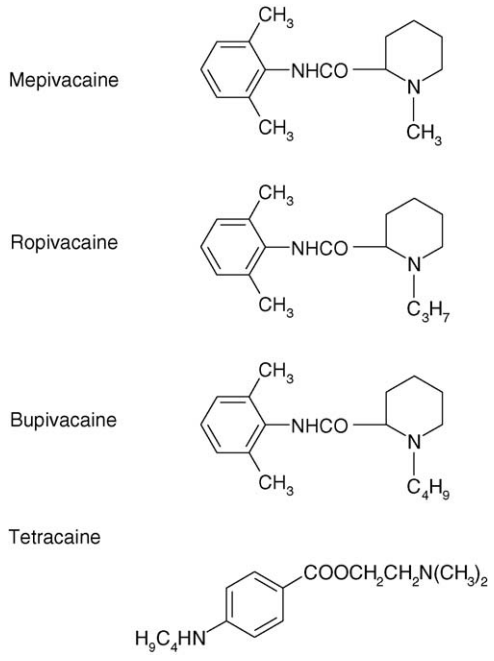

Fig. 1. Structural formula of bupivacaine, mepivacaine, ropivacaine and tetracaine.

2.2. Extraction procedure

To 0.5 ml of serum were added 100 μl acetonitrile, 3 ml *t*-butyl ethyl ether and 30 μl tetracaine (0.5 μg/ml, I.S.). After vortex mixing for 3 min, the tubes were centrifuged at 1200 × *g* for 5 min. The organic phase was transferred to a clean conical tube and evaporated to dryness in a water-bath at about 40 °C under a gentle stream of nitrogen. The residue was dissolved in 200 μl mobile phase and 50 μl injected into the HPLC.

2.3. Standard solutions and calibration

A standard stock solution, containing the three drugs, was prepared at a concentration of 1 mg/ml of each compound in methanol, and it remained stable for at least 3 months at −20 °C. Serum standards were prepared at concentrations of 2, 10, 50, 100, 200, 500 and 1000 ng/ml of each compound by diluting appropriate aliquots of the stock solution with drug-free serum. The calibration curve was obtained by linear regression of the peak-height ratio.

2.4. Chromatography

The HPLC equipment consisted of a pump (Model CCPS, Tosho, Tokyo, Japan) and a variable-wavelength UV detector (Model UV-8020, Tosho, Tokyo, Japan). Separation was achieved using a C_{18} reversed-phase column (150 mm × 4.6 mm I.D., particle size 5 μm, Inersil ODS-EP; GL Science, Tokyo, Japan). The mobile phase was acetonitrile–methanol–30 mM NaH_2PO_4 (pH 5.6) (100:100:300, v/v/v) and the flow rate was 1 ml/min. The absorbance of the eluate was monitored at 210 nm. All instruments were operated at ambient laboratory temperature (ca. 23 °C).

2.5. Accuracy and recovery

The accuracy and the recovery were calculated by comparing the peak heights of the three drugs (accuracy: 10, 50, 100, 200 and 1000 ng/ml; recovery: 10, 50, 100, 200 and 500 ng/ml) in spiked samples after extraction from serum with the peak heights of a series of unextracted reference standards. The recovery of I.S. (50 ng/ml) was also calculated by comparing the peak height of unextracted I.S.

2.6. Limit of detection and limit of quantitation

Serum standard curves were prepared at concentrations of 2, 10, 50, 100, 200, 500 and 1000 ng/ml of each compound by diluting appropriate aliquots of the stock solution with drug-free serum. The resulting peak heights were plotted against the concentrations.

2.7. Stability test

The stability of the three drugs, bupivacaine, mepivacaine and ropivacaine, and I.S. in serum was investigated. Spiked samples were prepared with drug-free serum at two concentration levels (50 and 200 ng/ml). Spiked serum samples were divided in two portions. One portion was stored at −20 °C, thawed and analyzed on weeks 0, 1, 4, 8 and 12. The other portion was treated as described in the sample preparation, divided in two portions and stored at 4 °C and room temperature, respectively. At 4, 8 and 12 h after extraction, each sample in both portions was directly analyzed by HPLC.

2.8. Clinical study

Serum samples were collected from four patients: a sample from patient A was collected at 2 h after epidural (e.d.) injection of 80 mg ropivacaine, a sample from patient B was collected at 0.25 h after e.d. injection of 10 mg ropivacaine, a sample from patient C was collected at 1 h after e.d. injection of 30 mg ropivacaine, and a sample from patient D collected at 1 h after e.d. injection of 25 mg ropivacaine.

3. Results and discussion

3.1. Retention time

Fig. 2 shows chromatograms of the three drugs, bupivacaine, mepivacaine and ropivacaine. These drugs and the I.S. were well separated. The retention times (R.T.)s of mepivacaine, ropivacaine, I.S. and bupivacaine were 4.6, 9.7, 14.3 and 16.4 min, respectively. Under the described optimized chromatographic conditions, they all eluted within 20 min.

No interfering peaks appeared when the following drugs were added to the serum: theobromine (R.T.; 1.9 min), theophylline (2.2), procaine (2.3), cimetidine (2.3), caffeine (2.5), clonidine (2.8), thiamylal (9.2), lidocaine (9.3), bromazepam (10.5), carbamazepine (13.8), nitrazepam (17.7), alprazolam (>20), diazepam (>20), estazolam (>20), etizolam (>20), haroxazolam

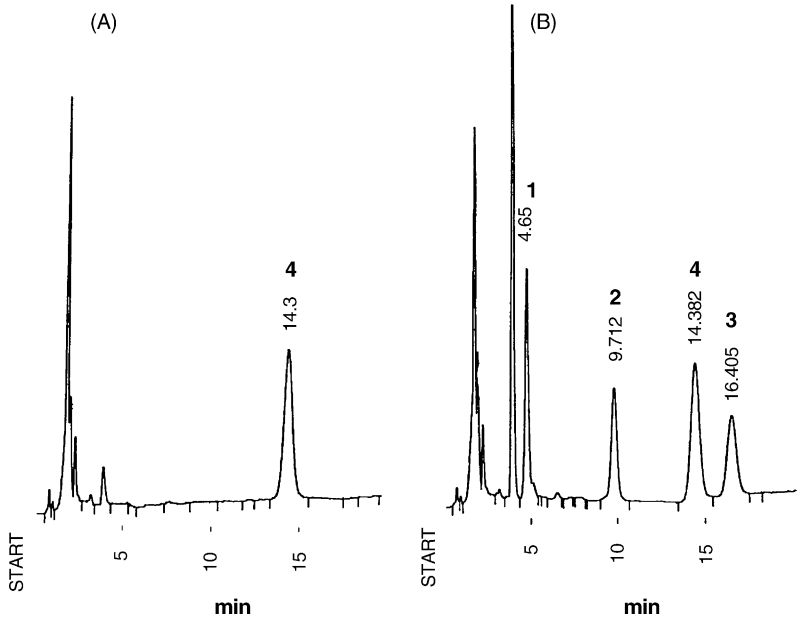

Fig. 2. Chromatograms of bupivacaine, mepivacaine and ropivacaine in human serum. Conditions: column, 150 × 4.6 mm I.D., particle size 5-μm, Inersil ODS-EP; mobile phase, acetonitrile–methanol–30mM NaH_2PO_4 (pH 5.6) (100:100:300,v/v/v); flow-rate, 1 ml/min; detection wavelength, 210 nm. (A) Pooled blank serum; (B) drugs added to the drug-free serum. The concentrations of the three compounds are 50 ng/ml. (1) mepivacaine; (2) ropivacaine; (3) bupivacaine and (4) tetracaine (internal standard).

(>20), lorazepam (>20), midazolam (>20), nitrazepam (>20), oxazepam (>20), oxazolam (>20), triazolam (>20), propofol (>20), pentobarbital (>20), trimethadione (>20), ethosuximide (>20), primidone (>20), phenobarbital (>20) and phenytoin (>20).

3.2. Limit of quantification

The limit of quantification is the lowest concentration on the standard curve that can be measured with acceptable accuracy (coefficient of variation (C.V.) <5%). The lower practical limit of quantification was 5 ng/ml for bupivacaine and ropivacaine, and 2 ng/ml for mepivacaine. This method offered an approximately two- to ten-fold higher sensitivity compared with the previous methods that have been reported [5–7,9]. All of these quantification limits are adequate for clinical and forensic analyses [1,7].

3.3. Recovery

Four liquid–liquid extraction solvents were investigated, including *t*-butyl ethyl ether, diethyl ether, hexane-ethyl acetate and ethyl acetate. *t*-Butyl ethyl ether was chosen as the extraction solvent because it provided >85% absolute recoveries for the three drugs. Actually, the recovery rate of these drugs was >88% and the coefficients of variation ranged from 2.2 to 4.4% for all drugs (Table 1). The mean recovery rate of I.S. was 92%.

3.4. Precision and accuracy

The precision and accuracy obtained using this method are shown in Table 2. The within-day reproducibility was assessed using 10 samples at 5 different concentrations (10, 50, 100, 200 and 1000 ng/ml) in 15 samples that were analyzed on the same day. The C.V.s ranged from 2.0 to 4.8%. The between-day reproducibility was determined using five different quality control samples over a 2-week period. The C.V.s ranged from 3.2 to 5.3%.

3.5. Linearity

The calibration curves (the ratio between the peak height of the drugs analyzed and that of the I.S. versus the amount of each drug) was linear over the concentration range of 2–1000 ng/ml serum. The coefficients of determination (r^2) from regression

Table 1
Recovery of bupivacaine, mepivacaine and ropivacaine added to human drug-free serum

Drug	Added (ng/ml)	Mean recovery (%)	C.V. (%)
Mepivacaine	10	88	4.1
	50	89	2.2
	100	89	3.3
	200	91	3.4
	500	90	3.8
Ropivacaine	10	87	3.6
	50	89	2.9
	100	88	2.5
	200	89	3.8
	500	88	3.5
Bupivacaine	10	90	4.4
	50	88	2.7
	100	91	3.9
	200	91	3.2
	500	90	4.1

Data of each drug were determined by measuring five concentrations in 15 samples. C.V. is the coefficient of variation.

Table 2
Precision and accuracy of the determination of bupivacaine, mepivacaine and ropivacaine added to human drug-free serum

Drug	Added (ng/ml)	Mean ± S.D. (%)	Between-day C.V. (%)	Within-day C.V. (%)
Mepivacaine	10	10.2 ± 0.31	2.9	4.8
	50	50.1 ± 2.41	4.8	5.1
	100	100.2 ± 2.52	2.5	4.5
	200	199.6 ± 5.53	2.7	3.2
	1000	1001.2 ± 37.01	3.7	4.9
Ropivacaine	10	9.8 ± 0.22	2.0	5.3
	50	50.3 ± 1.83	3.6	4.4
	100	99.9 ± 2.49	2.5	3.3
	200	202.7 ± 5.69	2.8	3.9
	1000	1002.5 ± 35.08	3.5	5.1
Bupivacaine	10	10.2 ± 0.35	3.4	4.9
	50	49.7 ± 1.36	2.7	3.3
	100	99.5 ± 3.93	3.9	4.2
	200	201.6 ± 6.48	3.2	3.7
	1000	998.5 ± 37.11	3.7	4.7

Data of each drug were determined by measuring five concentrations in 15 samples. Means ± S.D. C.V. is the coefficient of variation.

analyses were 0.999 for bupivacaine, 0.975 for mepivacaine and 0.995 for ropivacaine.

3.6. Stability test

A stability study was conducted to determine the best storage temperature for serum samples. The results demonstrated that the three drugs, bupivacaine, mepivacaine and ropivacaine, and I.S. were stable for longer than 12 h at 4 °C and for longer than 8 h at room temperature. Furthermore, four drugs were stable up to 12 weeks when stored at −20 °C. Therefore, all extracted samples were stored refrigerated at 4 °C for the same-day analysis, whereas serum samples were frozen at −20 °C until analysis by HPLC.

3.7. Clinical study

The serum level of each patient was as follows: 634 ng/ml for patient A, 138 ng/ml for patient B was, 686 ng/ml for patient C and 840 ng/ml for patient D.

4. Conclusion

The suitability of the HPLC method for the determination of three local anesthetics has been studied. The method reported by Baniceru et al. [2] is time-consuming and not sensitive. However, this HPLC method has adequate speed, sensitivity, precision and accuracy. This method may be useful for the determination of the blood levels after local anesthesia produced using bupivacaine, mepivacaine or ropivacaine in clinical and forensic applications investigations.

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