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Journal of Chromatography B, 690 (1997) 359–362

JOURNAL OF CHROMATOGRAPHY B

Short communication

Stereoselective determination of mepivacaine in human serum using a brush-type chiral stationary phase and solid-phase extraction

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Received 25 April 1996; revised 14 June 1996; accepted 23 July 1996

Abstract

A stereoselective high-performance liquid chromatography assay method was developed for the quantitation of *R*-(+)- and *S*-(−)-mepivacaine in human serum. The assay uses a Pirkle brush-type (*S*)-*tert.*-leucine, (*R*)-1-(α-naphthyl)ethylamine stationary phase (Sumichiral OA-4700, 250×4 mm I.D.) at ambient temperature with a mobile phase of hexane–ethylenedichloride–absolute methanol (85:10:5, v/v) for the separation of *R*-(+)- and *S*-(−)-mepivacaine. The eluents were monitored using UV detection at 220 nm. Isolation of the analytes from serum was performed using a 1-ml C_{18} solid-phase extraction cartridge with high recovery and selectivity. The detection limits were 100 ng/ml for each enantiomer and the limits of quantitation were 150 ng/ml for both enantiomers. Linear calibration curves in the 150–2400 ng/ml range showed good correlation coefficients ($r>0.9994$, $n=3$). Precision and accuracy of the method were within 2.1–5.3 and 2.0–3.6%, respectively, for *R*-(+)-mepivacaine and 2.7–5.7% and 1.7–4.2%, respectively, for *S*-(−)-mepivacaine.

Keywords: Enantiomer separation; Mepivacaine

1. Introduction

Mepivacaine is a racemic mixture (50:50) of *R*-(+)- and *S*-(−)-mepivacaine, commonly used in Psoas compartment block and sciatic nerve block technique [1]. The *S*-(−) enantiomer is biologically more active than the *R*-(+) enantiomer and the plasma concentrations of *S*-(−)-mepivacaine are higher than those of the *R*-(+)-mepivacaine [2]. Stereospecific separation of *R*-(+)- and *S*-(−)-mepivacaine has been performed using a protein high-performance liquid chromatography (HPLC) column, but there is only one report of the quantitation of *R*-(+)- and *S*-(−)-mepivacaine in biological matrices [2,3]. The method uses a liquid–liquid extraction for sample clean-up and the run times are around 16 min. This report describes a chiral HPLC method using a brush-type OA-4700 stationary phase and solid-phase extraction to measure ng/ml concentrations of *R*-(+)- and *S*-(−)-mepivacaine with good sensitivity and selectivity. The method is linear up to 2400 ng/ml of each enantiomer. The assay possesses the required sensitivity for monitoring the individual isomers of mepivacaine in serum.

2. Experimental

2.1. Reagents

Mepivacaine was obtained from Sigma (St. Louis, MO, USA). *R*-(+)- and *S*-(−)-mepivacaine and *S*-

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PII S0378-4347(96)00347-7

bupivacaine were gifts from Astra (Westborough, MA, USA). Blank human serum was obtained from Biological Specialty Corporation (Colmar, PA, USA). The solvents used were all HPLC grade. Concentrated HCl, absolute methanol, hexane, dichloroethane and sodium dihydrogen phosphate monohydrate were obtained from Baker (Phillipsburg, NJ, USA). All chromatographic mobile phases were filtered through a 0.45-μm filter (Alltech, Deerfield, IL, USA) and degassed prior to use.

2.2. Preparation of stock and spiked standard solutions

Individual stock solutions were prepared in absolute methanol to give concentrations of 200 μg/ml for *R*-(+)- and *S*-(−)-mepivacaine and 200 μg/ml of *S*-bupivacaine (internal standard, I.S.). Appropriate volumes of the *R*-(+)- and *S*-(−)-mepivacaine stock solutions were pipetted into 2-ml volumetric flasks and evaporated. Then 1 ml of serum was added to each flask to give the desired final serum concentrations of each analyte. To each serum sample, 350 μl of 100 m*M* phosphate buffer (pH 4.5) and 40 μl of the I.S. solution were added and mixed well.

3. Instrumentation

A Spectroflow Model 400 solvent delivery system (Kratos Analytical Instruments, Ramsey, NJ, USA) and a Model 728 autosampler (Micromeritics Instruments, Norcross, GA, USA) equipped with a 100-μl loop were used for the analysis. A Model 481 Lambda-Max LC spectrophotometer (Waters, Milford, MA, USA) was used to monitor the eluent. A Spectra-Physics Model 4290 integrator (Spectra Physics, San Jose, CA, USA) was used to record each chromatogram and peak-area responses.

3.1. Chromatographic conditions

The stationary phase was a brush-type (*S*)-*tert.*-leucine, (*R*)-1-(α-naphthyl)ethylamine (Sumichiral OA-4700, 5 μm, 250×4 mm I.D, YMC, Wilmington, NC, USA). The mobile phase was hexane–ethylenedichloride–absolute methanol (85:10:5, v/v). The flow-rate was 0.8 ml/min and the column was at ambient temperature (22±1°C). The eluent was monitored at 220 nm.

3.2. Assay procedure

The solid-phase extraction (SPE) was performed using a literature method with minor changes [4]. A 350-μl volume of phosphate buffer and 40 μl of the I.S. were added to 1-ml serum samples containing *R*-(+)- and *S*-(−)-mepivacaine. The samples were vortexed and then passed through a C_{18} Bond-Elut SPE column attached to a vacuum manifold (1 ml, Vac-Elut, Varian, Harbor City, CA, USA). The column was previously conditioned with 1 ml each of 0.1 *M* HCl, methanol and water. The column was washed with 4×250 μl of water and 1×500 μl of acetonitrile and allowed to air dry for 1 min between each wash. The analytes of interest were eluted with 4×250 μl of acidified methanol (2% HCl in methanol). The tubes were evaporated under a nitrogen stream. The samples were reconstituted in 1 ml of mobile phase after which 100 μl was injected into the liquid chromatography. Linear regression analysis of drug/I.S. peak-area ratios versus concentration gave slope and intercept data for each analyte, which were used to calculate the concentration of each analyte in the serum samples. For absolute recovery experiments, spiked samples were compared to unextracted stock solutions which had been injected directly into the HPLC system. Drug peak-area ratios were used to calculate the absolute recoveries of each analyte.

4. Results and discussion

The chemical structures of *R*-(+)- and *S*-(−)-mepivacaine and *S*-bupivacaine (I.S.) are shown in Fig. 1. Baseline separation of *R*-(+)- and *S*-(−)-mepivacaine was achieved using a brush-type OA-4700 column with retention times of 9.3 and 11.4 min, respectively. *S*-Bupivacaine (I.S.) gave a retention time of 7.7 min. *S*-Bupivacaine was chosen as internal standard because it is structurally similar to mepivacaine and gave good recoveries from serum using the SPE method. Earlier work in this laboratory on the development of chiral separations on various types of chiral stationary phases and mobile

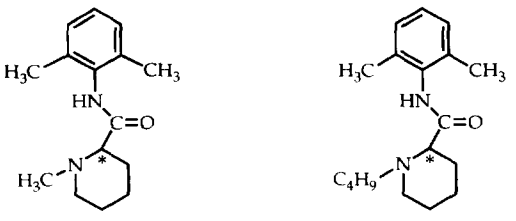

Fig. 1. Chemical structures of mepivacaine and bupivacaine.

phases showed that mepivacaine enantiomers were easily separated using a OA-4700 stationary phase and a mobile phase of hexane–ethylenedichloride–absolute methanol (85:10:5, v/v). It was found that ethylenedichloride could be replaced by methylene chloride, but the replacement of methanol with ethanol or propanol led to an increase in retention times and broad peaks were obtained. Addition of trifluoroacetic acid increased the retention times of the analytes with no significant increase in the resolution. The stock solution of mepivacaine in methanol was stable since no racemization was observed upon prolonged storage. Fig. 2A shows the chromatogram of a serum blank and Fig. 2B shows a chromatogram of the analytes and I.S. spiked in serum. The order of elution for the enantiomers was determined to be *R*-(+)-, then *S*-(−)-mepivacaine.

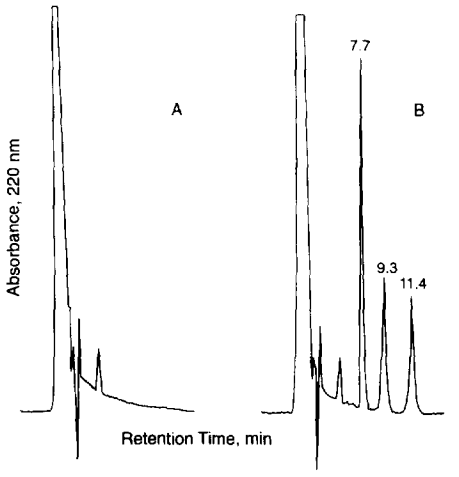

Fig. 2. (A) Representative chromatogram of serum blank. (B) Representative chromatogram of spiked serum sample containing *R*-mepivacaine (9.3 min), *S*-mepivacaine (10.1 min) and *S*-bupivacaine (internal standard, 7.7 min).

An SPE procedure was used for sample clean-up to decrease the sample preparation time involved in liquid–liquid extraction. The C_{18} sorbent allowed the elution of mepivacaine and bupivacaine using acidified methanol, which was evaporated to dryness under a nitrogen stream and reconstituted with the mobile phase. Absolute recoveries greater than 90% were obtained for all three analytes using 1.0 ml of methanol as eluent. The mean absolute recoveries using C_{18} SPE cartridge were 92.3±4.3% for *R*-(+)-mepivacaine, 91.2±3.3% for *S*-(−)-mepivacaine and 91.1±2.8% for *S*-bupivacaine (n=3). The limits of detection based on a signal-to-noise ratio of 3 were 100 ng/ml for *R*-(+)- and *S*-(−)-mepivacaine (n=3).

The calibration curves showed good linearity in the range of 150–2400 ng/ml for *R*,*S*-mepivacaine. The correlation coefficients were higher than 0.9994 (n=3). Representative linear regression equations obtained for *R*-(+)- and *S*-(−)-mepivacaine were $y=0.001026x-0.00008$ and $y=0.001073x+0.000081$, respectively, where y and x are the drug to I.S. peak-area ratios and concentration of each analyte, respectively. The intra-day precision and accuracy (n=3) as expressed by % R.S.D. and % error were 2.95–4.41% and 3.1–3.6%, respectively, for *R*-(+)-mepivacaine and 2.7–5.7% and 1.7–4.2% for *S*-(−)-mepivacaine, respectively. The inter-day precision and accuracy (n=9, over three days) expressed by % R.S.D. and % error were 2.1–5.3% and 2.0–3.25%, respectively, for *R*-(+)-mepivacaine

Table 1
Accuracy and precision of serum samples with added *R*- and *S*-mepivacaine

Enantiomer	Concentration added (ng/ml)	Concentration found (ng/ml)[a,b]	Error (%)	R.S.D. (%)
Intra-day				
R	160	154.3±6.81	3.6	4.4
	2375	2301±67.85	3.1	3.0
S	160	153.3±4.16	4.2	4.2
	2375	2336.0±133.9	1.7	1.7
Inter-day				
R	160	156.9±8.31	2.0	5.3
	2375	2297.7±49.1	3.3	2.1
S	160	164.0±9.41	2.5	5.7
	2375	2442.0±93.0	2.8	3.8

[a] Based on n=3, for intra-day assay.
[b] Based on n=9, for inter-day assay.

and 3.8–5.7% and 2.5–2.8%, respectively, for *S*-(−)-mepivacaine. The detailed data is listed in Table 1.

In conclusion, a sensitive and selective isocratic chiral HPLC method utilizing SPE clean-up has been developed for the analysis of *R*-(+)- and *S*-(−)-mepivacaine in human serum. The method is sensitive to 100 ng/ml of each isomer and the total chromatographic run time is 11.5 min.

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