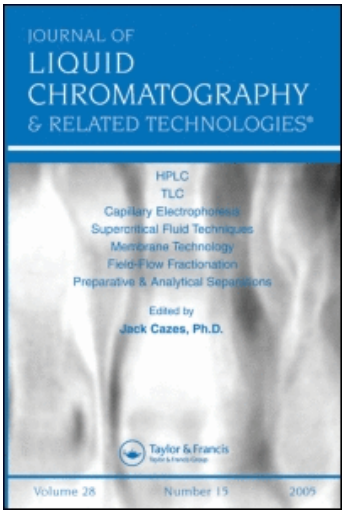

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Determination of Bupivacaine in Human Plasma by HPLC

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DETERMINATION OF BUPIVACAINE IN HUMAN PLASMA BY HPLC

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ABSTRACT

A rapid, sensitive, selective and reproducible reversed- phase high-performance liquid chromatographic method has been developed and validatd for the quantification of bupivacaine in human. Bupivacaine and the internal standard, lidocaine, are extracted from alkalinized plasma using n-hexane before chromatographing on a reversed-phase system. The mobile phase comprises 62 parts of 0.05 M phosphate buffer and 38 parts of methanol at pH 5.9 and it is pumped at 1.0 ml/min. The retention times of bupivacaine and lidocaine were observed to be 3.8 and 5.9 min respectively. Ultraviolet detection at 254 nm enabled a limit of detection of 25 ng/ml to be achieved. The reproducibility of the method was good at 1000 ng/ml (C.V.=4.3%, n=6). The method is linear from 50 to 3200 ng/ml. The high sensitivity and the speed at which this assay can be performed makes it especially useful for estimating bupivacaine in human plasma.

INTRODUCTION

A long-acting anilide anesthetic agent, bupivacaine (1-n-buthyl-DL-piperidine-2-carboxylic acid-2,6-dimethylanilide hydrochloride), was synthesized in 1957 by Ekenstam et al. (1). Bupivacaine is one of the most frequently used local anesthetics in many regional anesthetic techniques, but the undesirable effects of this drug, especially those on the central nervous system and cardiac toxicity, can still cause severe problems (2, 3).

Since these effects are directly related to the concentrations of local anesthetics in the systemic circulation, their determination in plasma is of paramount importance (4).

This paper describes a rapid, selective and sensitive high-performance liquid chromatographic assay for the analysis of bupivacaine in human plasma, with advantages over previously published methods (5-9). This method has been applied to the pharmacokinetic study of pediatric and ophthalmology patients who received peridural, caudal or retrobulbar administration.

MATERIALS AND METHODS

Chemicals

HPLC-grade methanol and n-hexane from Probus (Barcelona, Spain) were used. Acetonitrile Far UV was from Romil Chemicals (England). Sodium hydroxide, sodium dihydrogenphosphate and potassium dihydrogenphosphate were obtained from Merck (Darmstadt, Germany). Bupivacaine hydrochloride was supplied by INIBSA (Barcelona, Spain). The internal standard (IS), lidocaine hydrochloride, was obtained from Laboratories Palex (Jaen, Spain).

Standard solutions

Stock solutions (5 mg/ml) of bupivacaine hydrochloride without vasoconstrictor and the IS (50 mg/ml) were prepared in distilled water. Standard solutions contain 5 ng/µl to 320 ng/µl. The IS solution was diluted to a final concentration 100 ng/µl. These solutions could be stored up to one month without signs of decomposition.

HPLC instrumentation

The liquid chromatography system consisted of a model 510 pump, a U6K injector equipped with a 100 µl loop injection and a Lambda Max Model 481 LC-UV detector connected to a Data Module 740 integrator (Waters Assoc., Milford, MA., USA). The analysis was performed using a Spherisorb ODS-2, 25 x 0.4 cm I.D., 5 µm particle size (Tecknokroma, Barcelona, Spain). Chart speed of 0.2 cm/min was used.

Selection of mobile phase

An aqueous solution of bupivacaine hydrochloride was initially chromatographed to select a suitable mobile phase. Binary mixtures comprising varying proportions of acetonitrile and phosphate buffer, methanol and phosphate buffer of different pH, were initially investigated as potential mobile phases. All aqueous components of the mixtures were passed through a 0.45 µm HW filter (Millipore, Bedford, MA., USA) prior to mixing with acetonitrile or methanol. The binary mixtures which gave a desirable retention time and peak shape for bupivacaine were then adjusted to provide satisfactory resolution between bupivacaine and the internal standard, lidocaine.

The mobile phase used for quantification of bupivacaine in plasma was 0.05 M KH_2PO_4 (ph=5.9)-methanol (62:38), prepared daily and delivered at flow-rate of 1.0 ml/min resulting in an inlet pressure of approximately 3000 psi. All chromatographic separations were carried out at ambient temperature. The effluent was monitored at 254 nm and with an attenuation of 4 mV.

Extraction procedure

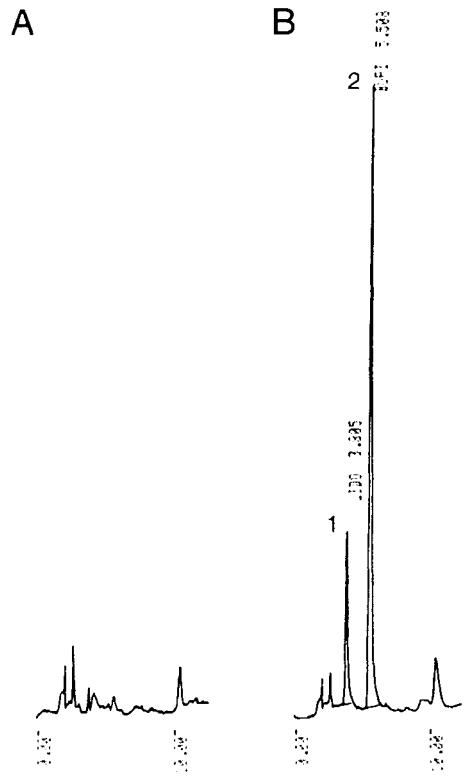

Figure 1. Chromatograms from human plasma extracts. (A) a blank plasma. (B) a blank plasma spiked with 1000 ng/ml IS (1) and 3200 ng/ml bupivacaine (2).

Plasma (1 ml) samples were mixed with 10 µl of the IS and 100 µl of 2 N sodium hydroxide in a glass tube. N-hexane (3 ml) was added to all the samples, and the tubes were shaken for 1 min, separation of the two phases was achieved by centrifugation at 3500 rpm for 10 min. The organic phase was transferred to a second tube and evaporated to dryness with nitrogen under vacuum. The sample were then reconstituted with 200 µl of mobile phase and mixed on a vortex agitator and 100 µl were injected into the chromatograph.

The internal standard method was used to quantify bupivacaine in the samples.

RESULTS AND DISCUSSION

The resolution of the chromatography system was checked daily and the response factor was 1.74 ± 0.02. The retention times for bupivacaine and lidocaine were 5.9 and 3.8 min respectively (Fig.1). These times are lower than those obtained by other authors (5-9).

The assay was validated by analyzing six bupivacaine standard solutions three-fold for each concentration. The results were peak-height ratio = $8.9111 * 10^{4} x - 0.0229$ ($r=0.9977$), where x is the bupivacaine concentration.

The accuracy and precision of the calibration curves were determined from the variation of the standard solutions. The within-day coefficient of variation was 4.6% (n=5) and the between-day coefficient of variation was 4.0% (n=4). Based on these results, the method is linear from 50 to 3200 ng/ml. The detection limit for plasma was 25 ng/ml. The extraction efficiency of IS was 99.9% for the concentration used for plasma sample.

No endogenous compounds or metabolites were observed near the retention time corresponding to bupivacaine or the IS. There were no interferences with other drugs administered before, during or after surgery.

We have proposed here a test simpler than other reported methods (5-9) with a lower retention time and more sensitivity. This method has been used extensively for measuring bupivacaine in patients's plasma.

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