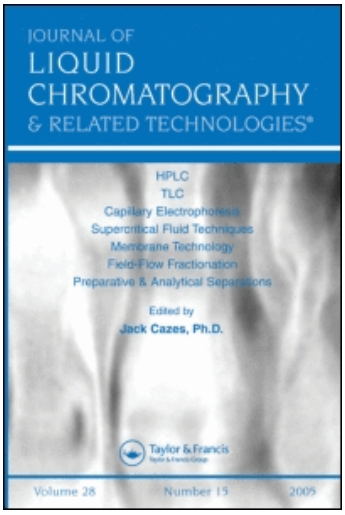

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Determination of Plasma Levels of Bupivacaine by High-Performance Liquid Chromatography

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DETERMINATION OF PLASMA LEVELS OF BUPIVACAINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

We evaluate a method for determination of bupivacaine using HPLC, and the possibility of pharmacological interferences produced by seven commonly used drugs administered before, during and after surgery: diacepam, midazolam, epinephrine, naloxone, flumacenil, atropine and ephedrine. The method has an average recovery of 102.8 +/- 5.4 %. The detection limit is 0.125 µg/mL. The within-day coefficient of variation is 5.88 % and the between-day coefficient of variation is 15 %. We haven't found drug interferences at generally encountered serum concentrations.

INTRODUCTION

Bupivacaine is an anilide-type local anesthetic frecuently used with postoperative nervous blockage, after abdominal and thoracic surgery.(1-4)

The determination of bupivacaine in plasma is important because of the undeserable effects of this drug, specially those of the central nervous system and cardiac toxicity (5-7). These effects are directly related to its concentration in the systemic circulation.(8-10)

We propose a method for determination of bupivacaine and lidocaine in serum or plasma by high-performance liquid chromatography with ultraviolet detection. Our method has advantages over previously published methods (11-13) with respect to simplicity of use.

MATERIALS AND METHODS

Apparatus

The HPLC instrumentation was from Perkin-Elmer (Norwalk Conn.), composed of a Model Series 2 pump, a model 7105 Rheodyne liquid chromatograph injector and

a model LC-75 spectrophotometric detector (size cell 8 µL), attached to the Sigma 10B chromatography data station. We used a 25 cm x 4.6 mm inverse phase column of 10 um Nucleosil-ODS (Perkin-Elmer).

Reagents

Bupivacaine and lidocaine hydrocloride were obtained from Sigma Chem. Co. (St. Louis, Mo, USA). Methanol, ethanol, acetonitrile and n-hexane for HPLC were from Scarlan (F.E.R.O.S.A, Spain) and analytical grade potassium biphosphate (KH2PO4) from Merck-Igoda (Spain).

Standards

A stock solution of bupivacaine HCl (16.6 µg/mL) was prepared in bidistilled water and a stock solution of internal standard, lidocaine HCl (16.6 µg/mL) was also prepared in bidistilled water. The two reference solutions were stored at -20° C.

Working standard solutions were prepared from stock solutions by dilution 1:10 with distilled water.

Procedures

50 µL of internal standard (1.66 µg/mL of lidocaine HCl), 0.8 mL of methanol, and 0.8 mL of

ethanol were added to 1 mL of serum or plasma. After throrough vortexing for 5 seconds, the mixture was extracted once with 3 mL of n-hexane by vortexing for 60 seconds. Pyrex tubes, 100 x 10 mm, were used for the extraction procedure. The tubes were centrifuged (1000 g, 5 minutes) to separate the phases and we transfered the supernate to a separate tube with a Pasteur pipette, and evaporated it to dryness under a stream of nitrogen. The dry residues were redissolved in 200 µL of acetonitrile. A 50 µL aliquot of this solution was injected into the chromatograph.

The standards were processed in the same way, using 1 mL of physiological serum instead of serum, 50 µL of internal working standard, 0.8 mL of ethanol and 0.8 mL of methanol.

Chromatography was carried out using acetonitrile - K2HPO4 0.04 M/L (40:60) as eluent at a flow rate of 1 mL/min. The effluent was monitored at 210 nm and a detection sensitivity of 0.02 a.u.f.s. All chromatography was performed at 55 C.

To quantify bupivacaine in the samples, the working standard was always analyzed along with the samples and peak-area ratios were used for calculations following the internal standard method.

TABLE 1. Plasma Levels of Bupivacaine.

Time after begining perfusion	[bupivacaine](ug/mL)
5 (minutes)	0.75 +/- 0.32 (x +/- 2 SD)
15 (minutes)	1.08 +/- 0.40 "
30 (minutes)	1.05 +/- 0.48 "
60 (minutes)	0.94 +/- 0.36 "
6 (hours)	1.09 +/- 0.58 "
18 (hours)	1.89 +/- 0.58 "

APPLICATION OF THE METHOD

We determined plasma concentrations of bupivacaine of 16 patients who had undergone abdominal or thoracic surgery. We evaluated plasma levels of bupivacaine (0.375 %) after intrapleural infusion, at 5, 15, 30, and 60 minutes and 6, 18 hours after begining administration. (Table 1)

RESULTS AND DISCUSSION

The resolution of the chromatographic system was checked daily and the response factor was 1.09 +/-

Figure 1. Liquid Chromatogram showing Lidocaine (1) and Bupivacaine (2).

0.06 . Retention times were 10 minutes for bupivacaine and 6 minutes for lidocaine (Figure 1). This retention time is similar to that wich was obteined by Tucker with gas cromatography (11), but Tucker needs more than an hour for every sample because a peak of

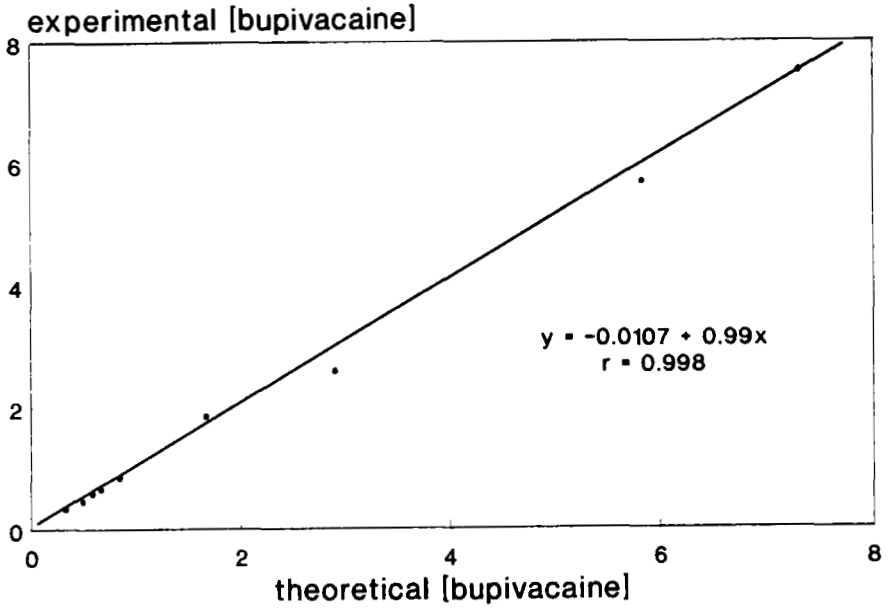

Figure 2. Regression Line for Bupivacaine.

cholesterol appears at 101 minutes, and it must be eluted.

Calibration curves

Calibration curves obteined from standard solution of increasing concentrations of bupivacaine (0.3 - 8 µg/mL) gave the following regression line equation and coefficient of correlation:

$$y = -0.0107 + 0.99x \quad r = 0.998$$ (Figure 2)

TABLE 2. Recovery of Bupivacaine HCl, μg/mL.

Theoretical	Experimental	Recovery (%)
0.330	0.340	103
0.490	0.470	95.9
0.580	0.590	101.7
0.660	0.660	100
0.830	0.850	102.4
0.1660	0.1850	111.4
0.2600	0.2900	111.5
0.5700	0.5840	102.4
0.7500	0.7300	97.3

Recovery

The recovery experiment was carried out by adding increasing amounts of bupivacaine to a serum sample, which was treated identically to the samples. The recoveries at different concentrations of bupivacaine are shown in Table 2. Average recovery was 102.8 +/- 5.4 % (n= 9). We observed a recovery better than other authors, Wegand (12) reported an average recovery of 94 +/- 4.4 %

Detection limit

The detection limit of the method was 0.125 μg/mL.

Precision

The within-day coefficient of variation was 5.88 % and the between-day coefficient of variation was 15 % .

We have proposed here a simpler test than other reported methods (11, 12, 13) having similar precision and accuracy.

Interferences

Endogenous plasma components did not interfere with either bupivacaine or lidocaine using the extraction method described.

We evaluated the possibility of farmacological interferences produced by seven commonly used drugs administered before, during and after surgery: diacepam, midazolam, epinephrine, naloxone, flumacenil, atropine and ephedrine.

We based our study on the "in vitro" drug interferences evaluation protocol, stablished by the "Spanish Drug Effects Comission of Clinical Chemistry" (14).

A pooled human serum was prepared without drugs and an aliquot for each drug, and a control aliquot was analyzed. Drugs were diluted in the aliquot to

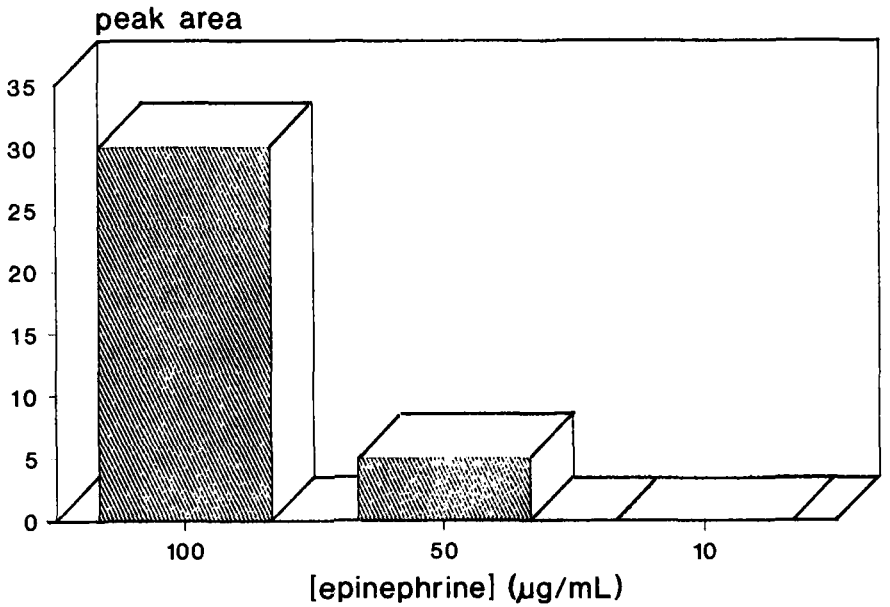

Figure 3. Interferences with Epinephrine.

obtain serum concentrations at least ten times higher than therapeutic serum concentration, and then, decreasing dilutions were prepared.

Samples were treated in the same manner as for the determination of bupivacaine. If peaks didn't appear at the retention time of bupivacaine or its internal standard (lidocaine), interferences were considered to be absent. We found no interferences with: diacepam, midazolam, ephedrine, flumacenil and atropine. A 30 mm peak height was found at 100 µg/mL

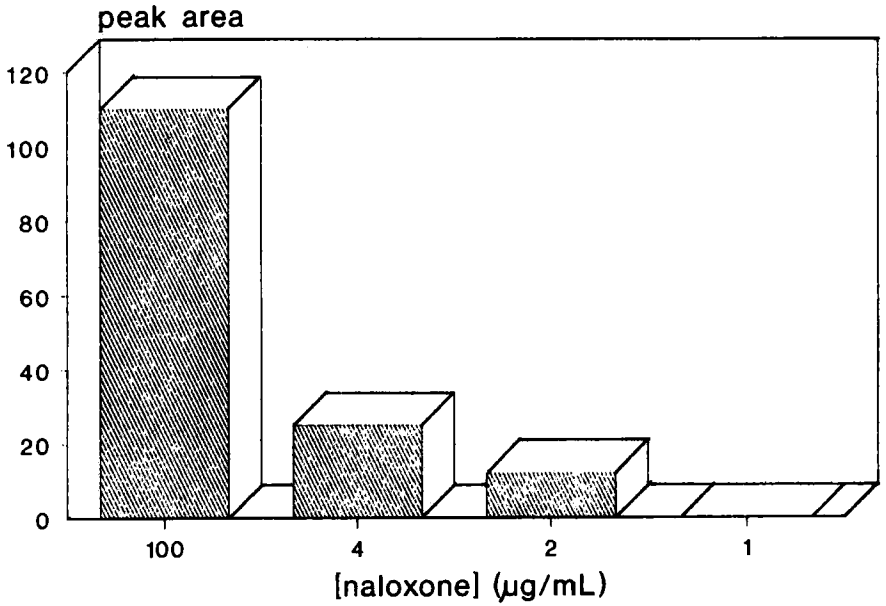

Figure 4. Interferences with Naloxone.

epinephrine concentration, at bupivacaine retention time, but it disappeared at 10 µg/mL (Figure 3) (plasma epinephrine concentration 10 minutes after a epidural injection of anesthetic with epinephrine 1/200.000 , is 0.3 ng/mL) (15). With 100 µg/mL naloxone concentration there was a peak (110 mm height) at the retention time of lidocaine, but it disappeared at 1 µg/mL (Figure 4) (plasma naloxone concentration 40 minutes after a 35-70 µg/mL intravenous injection is 7-20 ng/mL respectivily)

(16). So we conclude that there is not any interference at therapeutic levels with other drugs used in surgery.

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