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Letter to the Editor

Simple, rapid high-performance liquid chromatographic determination of bupivacaine in human plasma

Sir,

The analysis of bupivacaine has been described by many gas chromatographic methods [1–9] and more recently by high-performance liquid chromatographic (HPLC) methods [10,11]. The method described here has a similar limit of detection as the two previously published HPLC methods and has the advantage of a quick, simple extraction process with an accuracy of 93.1%. The plasma sample processing is similar to that used by Perez-Gonzales and Weiner [12] for *p*-aminohippurate.

EXPERIMENTAL

Reagents and chemicals

The chemicals were of analytical grade: sodium phosphate (Mallinckrodt, St. Louis, MO, U.S.A.) and 1-pentanesulfonic acid, sodium salt (Aldrich, Milwaukee, WI, U.S.A.). The methanol was of HPLC grade (Omnisolve, EM Science, Cherry Hill, NJ, U.S.A.). The bupivacaine hydrochloride was supplied by Astra Pharmaceuticals (Worcester, MA, U.S.A.).

Sample preparation

Human plasma samples (0.2 ml), obtained through venipuncture into heparinized tubes, were placed into conical centrifuge tubes with 0.3 ml of methanol. The tubes were covered and vortexed. After a 10-min equilibration period, the samples were vortexed again and then centrifuged for 10 min at 1640 *g* and 20 μl of the resulting supernatant were injected directly into the chromatograph.

High-performance liquid chromatography

The analysis was performed using an M45 solvent delivery system with a U6K injector (Waters Assoc., Milford, MA, U.S.A.), a μBondapak CN column (15 cm $\times$ 3.9 mm, Waters Assoc.), a Spectroflow 773 variable-wavelength absorbance detector (Kratos, Westwood, NJ, U.S.A.) and an Omniscribe recorder (Houston Instruments, Austin, TX, U.S.A.). The mobile phase consisted of a mixture of

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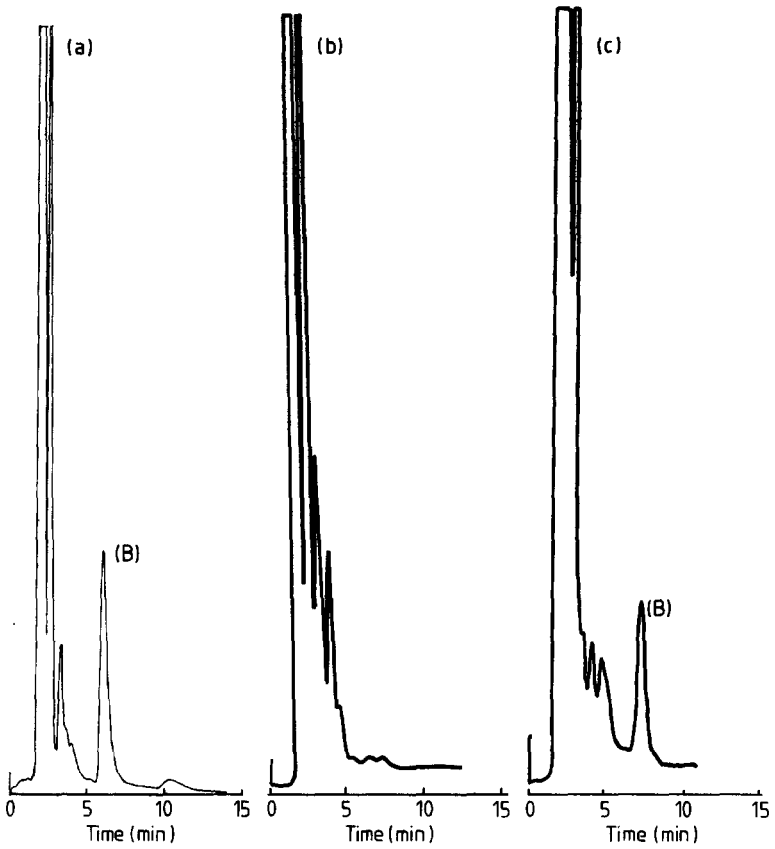

Fig. 1. Chromatograms of (a) human plasma spiked with 2 μg/ml bupivacaine (B), (b) blank plasma and (c) a patient sample 1.5 h after an epidural block.

methanol–0.05 *M* phosphate (5:95) containing 0.5 m*M* 1-pentanesulfonic acid, adjusted to pH 3.3 with phosphoric acid. The flow-rate was 1 ml/min and the detector wavelength was set at 210 nm. The analysis was executed at ambient temperature.

RESULTS AND DISCUSSION

The chromatograms of human plasma spiked with bupivacaine hydrochloride, blank plasma and a patient sample after an epidural block are shown in Fig. 1. The bupivacaine peak is well separated and has no interference from endogenous plasma components. The retention time is 6.25 min. The limit of sensitivity, defined at a signal-to-noise ratio of 4, is approximately 50 ng/ml.

A calibration graph was determined for aqueous samples over a concentration range of 0.5–5 μg/ml and was shown to be linear. A least-squares linear regression analysis was performed and resulted in the equation $y = 31.1\,x - 3.9$ (y being the peak height and x being the concentration of bupivacaine). The correlation coefficient r was > 0.999.

The recovery of bupivacaine was studied using spiked human plasma samples, yielding concentrations of 10–40 μg/ml. The extracts of the plasma samples were compared chromatographically with extracted aqueous samples. The recovery of bupivacaine was determined to be 93.1% with a standard deviation of 3.7% ($n=12$). The coefficient of variation for the recovery was 4.0% ($n=12$).

For future reference in clinical use, we tested the following drugs in our chromatographic system to determine any possible interference. We assayed naproxen, sulindac, indomethacin, tolmetin, fenoprofen, ibuprofen, piroxicam, amiloride, cortisone, furosemide, hydrochlorothiazide and salicylate with no interference detected.

In conclusion, this HPLC method for the analysis of bupivacaine has been found to be simple, accurate and time-effective.

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Department of Anesthesiology,
State University of New York Health Science Center
at Syracuse, Syracuse, NY 13210 (U.S.A.)

N.W. SULLIVAN*

Department of Pharmacology,
State University of New York Health Science Center
at Syracuse, Syracuse, NY 13210 (U.S.A.)

L. WEINER

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