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Note

# Simultaneous determination of bupivacaine and 2,6-pipecoloxylidide in serum by gas—liquid chromatography

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Bupivacaine (Marcaine®) is a local anesthetic of the anilide type used for epidural analgesia during labor and delivery. Bupivacaine is a highly lipid-soluble drug which can cross the placenta quite easily. The major metabolite of bupivacaine is 2,6-pipecoloxylidide (PPX) which is formed in the liver by oxidative dealkylation of the parent drug. It is important to be able to measure bupivacaine and PPX in the maternal and fetal circulation in order to evaluate the toxicity and disposition kinetics of bupivacaine.

Several gas chromatographic (GC) methods have been reported for the measurement of serum bupivacaine. Some of these methods are time-consuming, laborious, or not specific or sensitive enough to measure PPX [1—4]. A modified GC procedure for bupivacaine has been reported to detect PPX in blood but the authors provided no information describing the linearity, reproducibility, sensitivity and recovery of the method. Furthermore, this method required 2 ml of whole blood and a double-extraction procedure [5].

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A recent method for the determination of bupivacaine by GC appears to be rapid and simple, but it, too, does not measure PPX [6]. In all of these GC methods of assay a flame-ionization detector was used and no attempts to use the more sensitive nitrogen—phosphorus detector have been reported.

This report describes a GC method designed for rapid, simultaneous determination of bupivacaine and PPX in serum using a nitrogen—phosphorus detector. The method is suitable for monitoring bupivacaine and PPX serum concentrations in maternal and fetal circulation during labor and delivery.

## EXPERIMENTAL

### *Reagents and chemicals*

The following reagents and chemicals were used: bupivacaine hydrochloride (Sterling-Winthrop Research Institute; lot No. XB0-4-68), 2,6-pipecoloxylidide (Sterling-Winthrop Research Institute, New York, N.Y., U.S.A.) and lidocaine hydrochloride (Astra Pharmaceutical Products, Framingham, Mass., U.S.A.; lot No. XH 99 A). Diethyl ether (Fisher Scientific, Pittsburgh, Pa., U.S.A.), methanol (Burdick and Jackson Labs., Muskegon, Mich., U.S.A.) and all other solvents and reagents were of analytical grade quality and were used as received.

*Internal standard.* A solution of lidocaine hydrochloride (500 mg/l) was prepared by dissolving 50.0 mg of lidocaine hydrochloride (equivalent to 43.3 mg of lidocaine base) in 100 ml of methanol. A second solution containing lidocaine hydrochloride (5 mg/l) was prepared by dilution with methanol.

*Drug standards.* Stock solutions of bupivacaine hydrochloride (500 mg/l) and PPX (500 mg/l) were prepared by dissolving 50 mg of bupivacaine hydrochloride (equivalent to 45 mg of bupivacaine base) and 50 mg of PPX, respectively, in 100 ml of methanol. Working drug solutions were prepared by diluting the original stock solutions 100-fold with methanol. Drug serum standards were prepared by spiking blank control serum with appropriate microliter volumes of working drug solution to obtain five serum standards with the following concentrations of bupivacaine and PPX: 0.1, 0.2, 0.4, 0.8 and 1.6 μg/ml.

### *Instrument and chromatographic conditions*

A Perkin-Elmer Sigma 3 gas chromatograph fitted with a nitrogen—phosporus detector in the nitrogen mode was used for chromatography. A glass column, 1.8 m × 2 mm I.D., packed with 3% OV-17 on Chromosorb W HP (100—120 mesh) was used for separation (Perkin—Elmer, Norwalk, Conn. U.S.A.) The column was conditioned for 16 h at 280° with a nitrogen flow-rate of 20 ml/min. The detector end of the column was disconnected during the conditioning process. The recorder was a Perkin—Elmer Model 023 with a range of 0—1 mV; the chart speed was 0.5 cm/min.

The zone temperature (injector and detector block) was 260° and the rubidium bead temperature in the detector was set at 450°. The oven temperature was programmed from 180° to 240° at a ramp rate of 20°/min.

The initial hold time was 8 min at 240°. The gas flow-rates were as follows: nitrogen (carrier gas) 16 ml/min, hydrogen 2.25 ml/min and air 120 ml/min. Peak heights were measured to the nearest half millimeter with a metric ruler.

## PROCEDURE

A 0.1-ml sample of patient serum and 0.1 ml of lidocaine hydrochloride stock solution (5 mg/l) were added to a small, disposable, acid-washed glass test-tube. The serum was made alkaline (pH adjusted to 11) by adding 0.5 ml of sodium carbonate solution (0.2 mole/l) and extracted with 1.0 ml of diethyl ether by vortex mixing for 15 sec. The organic phase (upper) was separated by centrifugation at 1600 *g* for 10 min and transferred to a clean, disposable, glass test-tube. The ether was evaporated at ambient temperature under a gentle stream of nitrogen. The residue was reconstituted with 20 µl of methanol by vortex mixing, and 10 µl were injected into the gas chromatograph. The concentrations of bupivacaine and PPX in the serum sample were determined from a calibration curve of peak height ratio (drug/internal standard) versus drug concentration in serum standards carried through this procedure.

## RESULTS

A chromatogram obtained for a spiked serum sample containing lidocaine, PPX and bupivacaine extracted and assayed as previously described is shown in

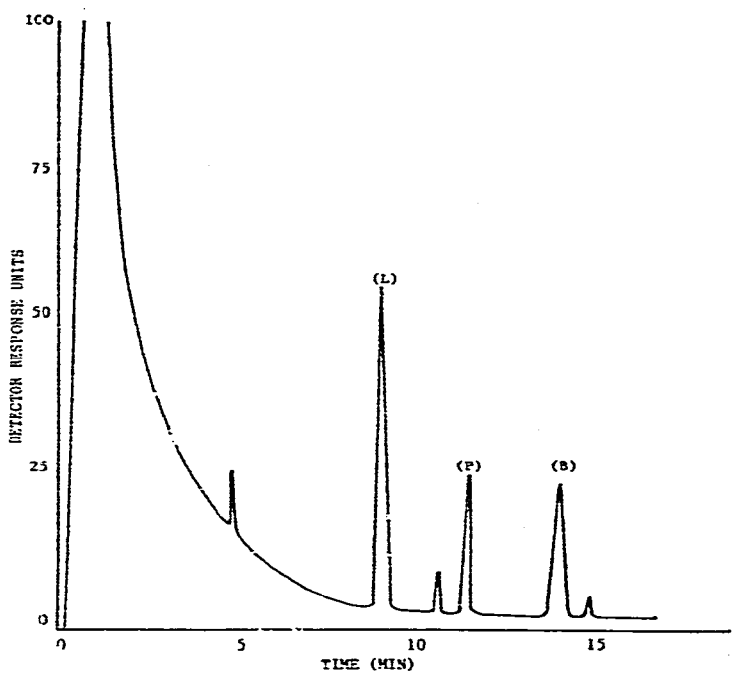


Fig. 1. Gas chromatogram of a mixture of lidocaine (L), PPX (P) and bupivacaine (B) added to drug-free serum; the concentration of each is 0.8 µg/ml.

Fig. 1. Three symmetrical drug peaks appear in the chromatogram with lidocaine emerging first from the column followed in sequence by PPX and bupivacaine. The retention times for lidocaine, PPX and bupivacaine were 9, 11.5 and 14 min, respectively. The drug peaks were well-removed from the solvent front and completely resolved from any extraneous serum peaks.

Calibration curves of peak height ratio versus concentration were obtained by analyzing serum standards containing bupivacaine and PPX in concentrations ranging from 0.1 to 1.6 μg/ml. The calibration curves were linear over the concentration range studied. The least-squares linear regression line which represents the best fit of the bupivacaine data has a slope of 0.89 and a $y$-intercept (where $y$ = peak height ratio of drug to internal standard) of 0.07 ($r$ = 1.0). The corresponding line for the PPX data had a slope of 0.77 and a $y$-intercept of 0.02 ($r$ = 1.0).

The precision of the method was determined by repeated analysis of spiked serum samples containing low (0.1 μg/ml) and high (0.8 μg/ml) concentrations of bupivacaine and PPX. The mean concentration, standard deviation and coefficient of variation for intra- and inter-day analysis are shown in Table I. The within-day coefficient of variation for low concentrations of bupivacaine and PPX was 9.6 and 7.9%, respectively, while at high concentrations the coefficient of variation was 6.1 and 3.6%, respectively. The day-to-day precision for low and high concentrations of bupivacaine and PPX was between 1.7 and 11.8%.

TABLE I

PRECISION DATA FOR THE DETERMINATION OF BUPIVACAINE AND PPX IN SERUM

| Drug | Intra-day | | | Inter-day | | |
|---|---|---|---|---|---|---|
| | $n$ | Mean (μg/ml ± S.D.) | C.V. (%) | $n$ | Mean (μg/ml ± S.D.) | C.V. (%) |
| Bupivacaine | | | | | | |
| Low conc. | 5 | 0.093 ± 0.0088 | 9.46 | 10 | 0.110 ± 0.0100 | 9.09 |
| High conc. | 8 | 0.800 ± 0.0489 | 6.11 | 10 | 0.790 ± 0.0212 | 2.68 |
| PPX | | | | | | |
| Low conc. | 5 | 0.089 ± 0.0070 | 7.86 | 10 | 0.110 ± 0.0126 | 11.4 |
| High conc. | 8 | 0.840 ± 0.0300 | 3.57 | 10 | 0.840 ± 0.0092 | 1.10 |

The analytical recovery of bupivacaine, PPX and lidocaine from serum was determined by comparing the peak heights for each drug obtained by analyzing extracted, spiked serum specimens, to the peak heights obtained by analyzing unextracted methanolic solutions of each drug containing an amount of drug equivalent to the amount in the spiked serum specimens. The recovery data for each drug are shown in Table II.

The sensitivity (least amount of bupivacaine and PPX measurable) of this method was 0.005 μg. Using 0.1 ml of patient serum and the analytical procedure described in this paper, the lowest concentration that can be measured accurately is 0.1 μg/ml. Greater sensitivity may be possible by increasing instrument sensitivity, increasing serum volume, using whole blood,

TABLE II

ANALYTICAL RECOVERY OF BUPIVACAINE, PPX, AND LIDOCAINE ADDED TO SERUM

| Drug | n | Present (μg/ml) | Found (μg/ml) | Mean recovery (%) | C.V. (%) |
|---|---|---|---|---|---|
| Bupivacaine | 5 | 0.80 | 0.76 | 95.1 | 3.8 |
| PPX | 5 | 0.80 | 0.73 | 91.2 | 10.7 |
| Lidocaine | 5 | 0.80 | 0.64 | 79.9 | 3.7 |

or increasing injection volume. Concentrations of bupivacaine and PPX of 0.05 μg/ml in serum were readily measurable by including such modifications in the method of analysis.

## DISCUSSION

The ability to simultaneously measure the serum concentration of bupivacaine and PPX may be of particular value to the clinician who may attempt to correlate serum levels of these substances with signs of toxicity in the neonate after delivery or the ability of the neonate to metabolize bupivacaine in the post-partum period. The method described is relatively simple, rapid and well-suited for the clinical or pharmacokinetics laboratory. Only 0.1 ml of serum is required for analysis.

Previously published assay procedures for bupivacaine did not measure the concentration of PPX conveniently, if at all. The analysis of PPX by this procedure is based on the use of a nitrogen—phosphorus detector which has greater selectivity and sensitivity than the flame-ionization detector utilized in previously reported assays. The resolution of the bupivacaine, PPX and lidocaine peaks from each other and from extraneous serum or solvent peaks after only a single extraction step is made possible by temperature programming.

The use of an internal standard maximizes the precision and accuracy of the assay by making quantitation independent of transfer volumes or injection volumes. Lidocaine was selected as an internal standard because of its structural similarity to bupivacaine and PPX, because it is extracted and chromatographs well, and because of the good detector response to lidocaine. It is unlikely that a maternal or neonatal serum sample will contain lidocaine and bupivacaine simultaneously but, if that were to occur, virtually any other anilide-type local anesthetic may be used as an internal standard.

OV-17 was selected for the GC column because it had been used for the separation of local anesthetics in the past. In conjunction with the nitrogen–phosphorus detector drug-free serum extracts were analyzed and were found not to produce any extraneous peaks at retention times that would interfere with the quantitation of bupivacaine and PPX. Using the experimental conditions described herein, the relative retention times for lidocaine, PPX and bupivacaine were 0.64, 0.82 and 1.0. Extraneous serum peaks in the chromatogram may be avoided in one of two ways: first, the serum aliquot may be mixed with an equal volume of methanol to precipitate protein, and after centrifuging, extract the supernatant with diethyl ether; second, back-extract

the ether phase with hydrochloric acid (0.1 mole/l) and re-extract with a fresh aliquot of ether. The recovery of the drugs was not reduced when the first approach was used but when back-extracting in the second approach the recovery of PPX was noticeably decreased. Organic solvents other than ether (for example, chloroform and hexane) were not suitable in the single extraction procedure.

It is worthwhile to use small, disposable, glass test-tubes in this assay to facilitate evaporation of the ether phase and complete reconstitution of the residue with only a small volume of methanol. Disposability also minimizes the problem of contamination arising from the use of phosphorus-containing soaps used to clean reusable glassware. Phosphorous contaminants may be adequately removed by acid washing and methanol rinsing. If such a contaminant is present it will emerge most often under the lidocaine peak.

Whenever bupivacaine is present care must be taken to evaporate the ether phase at ambient temperature under nitrogen rather than at elevated temperatures in order to minimize any loss of bupivacaine through volatilization encountered in this procedure.

This method has been used to measure the concentration of bupivacaine and PPX in serum samples obtained from the maternal vein, umbilical artery and umbilical vein at the time of delivery. Interference from endogenous substances has not been observed. We have not determined the potential interference of other co-administered drugs in the measurement of bupivacaine and PPX. Approximately 20—25 serum samples may be conveniently assayed in a day by one analyst. This method has also been found to be useful for therapeutic monitoring of serum lidocaine concentrations.

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