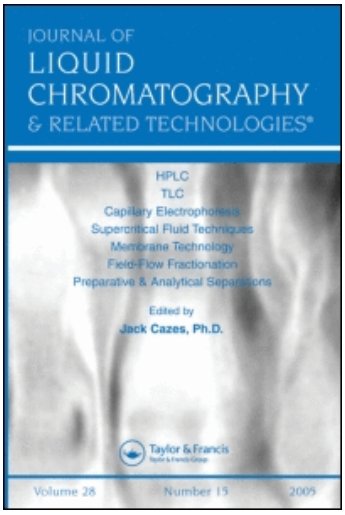

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SIMULTANEOUS DETERMINATION OF LIDOCAINE (LIGNOCAINE) AND THIOPENTAL IN PLASMA USING HIGH PRESSURE-LIQUID CHROMATOGRAPHY

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ABSTRACT

A sensitive and precise high-pressure liquid chromatographic method for the simultaneous quantitation of lidocaine (lignocaine) and thiopental in 0.5 ml samples of plasma is described. Extraction was conducted at pH 7.0 using ethyl acetate, and bupivicaine was employed as an internal standard. The drugs were eluted from a reversed-phase column using a mobile phase consisting of acetonitrile/0.2 M phosphate buffer pH 4.0 (1:9) at a flow rate of 1.0 ml/min. The drugs were detected and quantified by their UV absorption at 205 nm. The sensitivity limits of detection for lidocaine and thiopental were 1 µg/ml and 5 µg/ml, respectively. Atropine, caffeine and meperidine were found to have interfering retention times.

INTRODUCTION

Lidocaine is the most widely used local anesthetic. It is the standard against which other local anesthetics are evaluated. Lidocaine is used intravenously as a general anesthetic and for therapy in cardiac arrhythmias. In view of the introduction of the oral, topical form of lidocaine, and the fact that plasma concentrations relate more accurately to the clinical response than does the dose (1-3), we became interested in monitoring plasma levels of lidocaine.

Barbiturates are commonly administered with lidocaine for sedation. Thiopental is a short-acting barbiturate with a rapid hypnotic action, and is widely used as an intravenous anesthetic (4).

Lidocaine and thiopental are frequently administered simultaneously for general anesthesia in cardiac patients and a number of other procedures and disease states (5). The quantification of these two agents in the same plasma sample has become of interest to us in our studies on the influence of lidocaine on malignant hyperthermia, utilizing pigs susceptible to this disease.

While procedures are available for the independent analysis of lidocaine and thiopental or the analysis of either anesthetic in combination with other drugs (6-8), no assay procedures have been reported in which these two drugs are assayed simultaneously.

This paper describes a simple, sensitive and specific high pressure liquid chromatographic procedure for the simultaneous determination of lidocaine and thiopental.

MATERIALS AND METHODS

Instrumentation

A model 601 Perkin-Elmer high pressure liquid chromatograph equipped with a model LC-55 UV variable wavelength detector and a 1 mv recorder was employed. The detector was set at 205 nm. We used a 25 cm x 2.6 mm (i.d.) column packed with Sil-X-1 ODS manufactured by Perkin-Elmer Corporation. The mobile phase was acetonitrile/0.2 M phosphate buffer pH 4.0 (1:9), with a flow rate of 1.0 ml/min. Oven temperature was maintained at 40°C.

Reagents

Ultraviolet grade acetonitrile and methanol were purchased from Burdick and Jackson Laboratories, Inc. Ethyl acetate was of reagent grade and obtained from Mallinckrodt. Aqueous phosphate buffer, 0.20 M, pH 4.0, was prepared from reagent grade phosphoric acid and monobasic potassium phosphate. Lidocaine standard, 1.0 mg/ml, was prepared by diluting 2% lidocaine hydrochloride

(Xylocaine® obtained from Astra Pharmaceuticals) with methanol. Thiopental standard, 1.0 mg/ml, was prepared by diluting thiopental (from U.S.P.C., Rockville, Md.) with methanol. The internal standard bupivicaine (obtained from Sterling-Winthrop) was prepared at a concentration of 0.05 mg/ml in methanol.

Extraction Procedure

Plasma (0.50 ml), 0.50 ml of the internal standard and 0.20 ml of 3.0 M Tris buffer pH 7.0 were transferred to 12 ml teflon-lined screw-capped tubes. After the addition of 7.0 ml ethyl acetate, the tubes were agitated for 10 minutes on a mechanical shaker at 240 oscillations/min. The tubes were centrifuged for 1 min at 2200 x G, the organic phase was removed and evaporated to dryness *in vacuo* at 37°C. The residue was redissolved in 1.0 ml hexane and 1.0 ml acetonitrile, transferred to a 12 ml tube, vortexed for 1 min and the phases were again separated by centrifugation. The hexane (upper phase) was discarded, the acetonitrile phase was transferred to a Silli-vial and evaporated to dryness under a stream of nitrogen at 40°C. The residue was dissolved in 20 µl of methanol and 5 µl was injected onto the liquid chromatographic column.

Chromatography

Plasma samples were spiked with increasing amounts of lidocaine and thiopental, mixed with the internal standard, and extracted as described above. Lidocaine was added to plasma samples to give the following concentrations: 0, 2, 4, 6, 8, 10, 12, 14 and 16 µg/ml. Thiopental was added to plasma samples to yield the following concentrations: 0, 10, 20, 40, 60 and 80 µg/ml. Samples at each concentration were assayed in triplicate, and the internal standard at each level was computed. Standard curves were then constructed for each drug showing the relationship between the amount of the drug added to the plasma and the peak height ratios.

RESULTS

Separation

Figure 1 illustrates a typical chromatogram for a plasma sample spiked with the two drugs at 10 μg/ml and the internal standard. It is clear that the three peaks of interest were well resolved. Control plasma showed no peaks at the retention times of the drugs or the internal standard. However, a small peak under II was observed for extracted plasma. Furthermore, pentobarbital which is a decomposition product of thiopental, also had the retention time of peak II. Therefore, peak II is composed of a component extracted from the plasma and pentobarbital. Identification of pentobarbital was made based on both HPLC and TLC. Quantitation of pentobarbital was not desired in these investigations, but could have been made since the contribution to peak II due to the plasma component was constant.

Linearity

Figures 2 and 3 depict the linear relationship between the drug plasma levels and the peak height ratios for lidocaine and thiopental, respectively. The correlation coefficient (r) for lidocaine was 0.999 and for thiopental was 0.994.

For all concentrations of lidocaine which we examined, the extraction efficiency was approximately 40 $\pm$ 3%. Similarly, the extraction efficiency for thiopental was approximately 20 $\pm$ 2%. By appropriate pH adjustment, the extraction efficiency of either drug could be enhanced, to the detriment of the extraction efficiency of the other drug.

Interference

The drugs listed in Table 1 were subjected to the same extraction procedures and were chromatographed under the indentical chromatographic conditions as described above. Atropine, caffeine and meperidine were the only drugs that we examined which had interfering retention times, and would interfere if a patient were concomi-

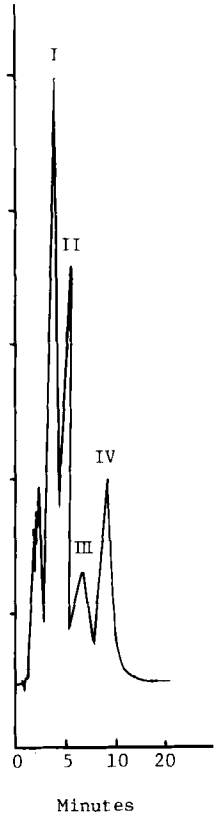

FIGURE 1

Chromatogram of a plasma extract of lidocaine (I, 10 µg/ml), thiopental (III, 10 µg/ml) and bupivicaine (IV, internal standard). Peak II is composed of a plasma component and pentobarbital, a decomposition product of thiopental. The following chromatographic conditions were employed: column - 25 x 0.26 cm ID, ODS-Sil-X-1 (Perkin-Elmer Corp.), maintained at 40°C; mobile phase - 10% acetonitrile in 0.20 M phosphate buffer, pH 4.0, at a flow rate of 1.0 ml/min.; detection - UV absorbance at 205 nm, with 0.10 absorbance units full scale.

tantly taking one of these drugs. Although a diazepam standard solution could be chromatographed in this system, diazepam was not extracted from plasma and therefore, no retention time is reported.

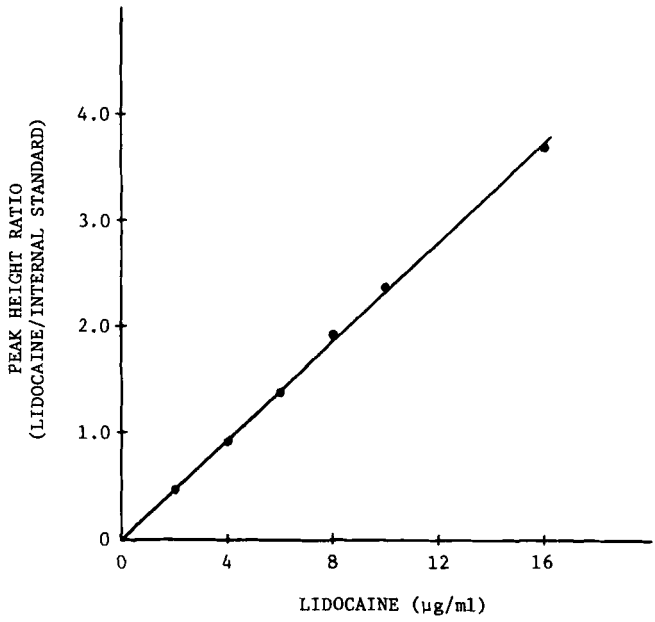

FIGURE 2

Standard curve depicting the ratio of the peak heights of lidocaine and the internal standard (bupivicaine) against the concentration of lidocaine (µg/ml). Triplicate plasma samples were seeded with the appropriate concentrations of lidocaine and extracted as described in the Materials and Methods. The correlation coefficient $r = 0.999$ ($p < 0.005$).

The retention time of pentazocine interfered with bupivicaine, the internal standard.

Biological Applications

In studies on the influence of lidocaine on the induction of hyperthermia in crossbred Poland/China Large White malignant hyperthermia susceptible pigs weighing approximately 20 kg, we have employed thiopental as the general anesthetic. Plasma levels of thiopental in the range of 40-60 µg/ml were determined to produce the desired state of anesthesia, and could be produced by the intravenous administration of 26 mg of thiopental per kg. Infusion of

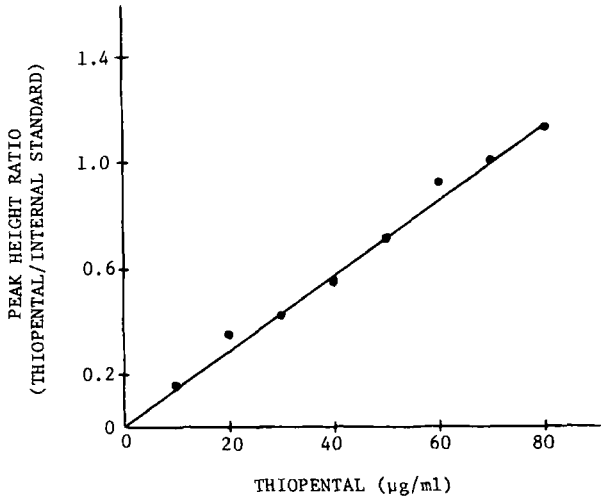

FIGURE 3

Standard curve depicting the ratio of the peak heights of thiopental and bupivicaine, the internal standard, against the concentration of thiopental (µg/ml). The appropriate concentrations of thiopental were added to plasma samples in triplicate and the samples were extracted as described in the Materials and Methods. The correlation coefficient $r = 0.994$ ($p < 0.005$).

lidocaine at a rate of 0.20 mg/kg/min to provide a total dose of 24 mg/kg over a two hour period resulted in the maintenance of plasma levels of lidocaine at 7-9 µg/ml.

DISCUSSION

We have developed a procedure for the simultaneous extraction, separation and quantitation of an acidic (thiopental) and a basic (lidocaine) compound. The drugs were extracted at pH 7.0, which rendered the procedure effective for both compounds of interest. Reverse phase, high pressure liquid chromatography provided the versatility necessary for the separation of both acidic and basic compounds in the same sample mixture. Bupivicaine was chosen as

TABLE I

Retention Times of Selected Drugs

Drug	Relative Retention Time
Aspirin	0.21
Atropine	0.59
Bupivicaine	1.00 (7.5 min)
Caffeine	0.43
Codeine	0.16
Diazepam	unextracted
Dropoperidol	2.15
Fentanyl	2.08
Lidocaine	0.43
Meperidine	0.72
Morphine	0.25
Pentazocine	1.15
Propoxyphene	2.07
Scopolamine	0.24
Thiopental	0.79

The retention times are relative to bupivicaine, the internal standard. All drugs listed in the Table were added to plasma samples, extracted, and chromatographed as described in the Materials and Methods section. Atropine, caffeine and meperidine had interfering retention times. The retention time of pentazocine interfered with bupivicaine, the internal standard.

an internal standard since its use in the presence of lidocaine is not very likely, and it was adequately separated from the two drugs of interest.

The use of UV absorption at 205 nm wavelength for the detection of the drugs provides adequate sensitivity. It must be pointed out that many drugs absorb at that wavelength. However, the chromatographic separation adequately offsets this lack of specificity.

The technique for simultaneously determining plasma levels of thiopental and lidocaine has been successfully applied to studies

in pigs which were susceptible to malignant hyperthermia. In patients, lidocaine plasma levels of approximately 1-5 µg/ml are required to suppress ventricular arrhythmias, and these levels are within our limits of detectability. Serum levels above 35 µg/ml for short-acting barbiturates, including thiopental, are usually fatal (9). As such, the procedure described above is useful in thiopental overdoses and forensic cases.

In conclusion, we report a simple, specific and sensitive procedure for the simultaneous quantitation of lidocaine and thiopental in the same plasma sample.

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