TITLE: AN IMPROVED HPLC METHOD FOR DETERMINATION OF BUPIVACAINE IN HUMAN PLASMA

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INTRODUCTION: Bupivacaine is an amide type local anesthetic used in a variety of clinical settings. The toxic effects of local anesthetics are directly related to their plasma concentration. Several HPLC methods have been reported to detect bupivacaine in plasma. However, these assays require approximately 1 ml of plasma which may not always be feasible, particularly if the pharmacokinetics of bupivacaine is to be studied in pediatric patients. We report a simple and accurate HPLC method using 100 ul of plasma which detect minute quantities of bupivacaine.

METHODS: A stock solution containing 1 mg/ml of bupivacaine (astra pharmaceutical) was prepared and was further diluted to final concentrations of 5, 10, 40, 100, 200 and 300 mcg/ml. 10 ul of each solution was then added to 990 ul of drug free human plasma to be used as standard. A 20 mcg/ml solution of etidocaine (astra pharmaceutical) was also prepared to be used as internal standard. 100 ul aliquots of standard plasma samples were used in the assay to get final concentrations of of 5, 10, 40 100, 200 and 300 ng of bupivacaine. 5 ul of etidocaine (100 ng) solution was added to each 100 ul aliquot of plasma samples as an internal standard. The plasma samples were then alkalinized by addition of 30 ul of ammonium acetate (1 M, pH=9) and vortexed for 30 seconds. 3ml of diethylether (Fisher Scientific) was added to each plasma sample and samples were shaken for 20 minutes. Following centrifugation the ether phase was transfered into conical test tube containing 50 ul of 0.1 N HCL. The ether and acid phases were then separated by centrifugation for 10 minutes at 2500 RPM. The ether phase was discarded and the acid phase was allowed to dry under nitrogen gas. The dried samples were reconstituted with 100 ul of mobile phase [Methanol: 0.1 M ammonium phosphate, (43/57 ratio), pH=3]. 70 ul of each reconstituted sample was injected onto a C-8 supelocosil (25 cm X 4.6 mm) column at a flow of 1 ml/min. The effluent was monitored by a UV detector set at 216 nm. Peak heights of bupivacaine and internal standard were measured and the calibration curve was constructed by plotting the ratio of the peak height of bupivacaine and etidocaine against the respective concentrations of bupivacaine. All reagents used were HPLC grade.

RESULTS: The experimentally determined concentrations agreed well with the theorectical concentrations of bupivacaine with coefficient of variation of 5.4% and 0.5% at 5 ng and 300 ng, respectively. Figure 1 is a typical chromatograph obtained from a pediatric patient receiving bupivacaine. The retention times of etidocaine and bupivacaine are 11 and 15 minutes respectively. Bupivacaine peak is well separated from etidocaine and there is no interference by any plasma components. There was also no interference with the following drugs at their respective therapeutic concentrations: Atracurium, Vecuronium, Succinylcholine, Tubocurarine, Pancuronium, Neostigmine, Atropin, Naloxone, Glycopyrrolate, Ephedrine, Droperidol, Epinephrine, Neosynephrine, Midazolam, Sufentanil, Diazepam, Thiopental, Morphine, Fentanyl and Meperidine.

DISCUSSION: In summary, we present a rapid and simple method for analysis of bupivacaine plasma concentration using a reversed-phase high performance liquid chromatography assay. Total retention time between successive samples is 17 minutes and only 100 ul of plasma is needed. This method is particularly useful in pediatric patients.

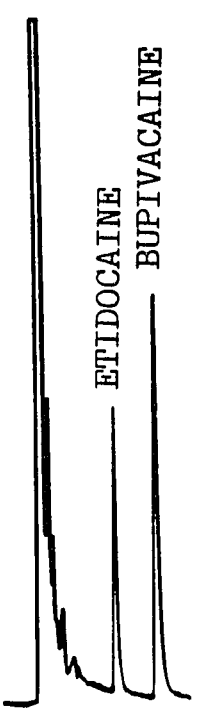

FIGURE 1