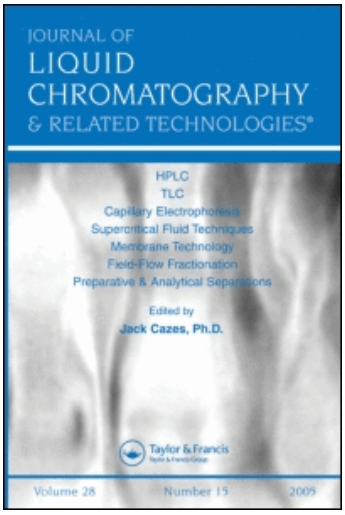

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Determination of Ibuprofen in Human Plasma by High-Performance Liquid Chromatography

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DETERMINATION OF IBUPROFEN IN HUMAN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Ibuprofen is being used with increasing frequency in children to control fever. A high performance liquid chromatographic (HPLC) method was developed to measure ibuprofen in small volumes of plasma to conduct pharmacokinetics studies in infants and children. The assay involved precipitation of plasma proteins with 0.5 N perchloric acid in methanol, using isobutyl phenyl acetate as an internal standard. Chromatographic separation was accomplished under isocratic conditions using a reverse-phase ultrasphere C18 column and mobile phase consisting of 53% acetonitrile at a detection wave length of 214 nm. The retention time of ibuprofen and the internal standard was 6.6 and 9.8 minutes, respectively. The method was suitable for quantitation of ibuprofen at concentrations ranging from 0.25 to 70 mcg/ml. The interday and intraday coefficient of variation at these concentrations was <5%. The method was used successfully to measure ibuprofen in 100 microliters of plasma samples obtained from infants and children.

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INTRODUCTION

Ibuprofen was approved recently by the U.S. Food and Drug Administration for the treatment of fever in children. In a double-blind placebo controlled study of children between 2 and 11 years of age, the antipyretic efficacy for temperature >102.5^{0}F was ibuprofen 10 mg/kg>ibuprofen 5 mg/kg>acetaminophen 10 mg/kg>placebo. All treatments were also found to be safe in these children.[1]

Despite the availability of ibuprofen liquid dosage form for children, limited data are available about its pharmacokinetics in this population. It would be most desirable to have a simple, accurate and reproducible HPLC method utilizing small volume of plasma to conduct the pharmacokinetics studies. Numerous HPLC methods have been published and reviewed for ibuprofen,[2-5] but these may require large volumes of plasma, extraction steps or have not used plasma specimens from children to show their direct application. One method was developed for pediatric application, but it required extraction and maintenance of nonambient column temperature.[6]

This article describes a simple, accurate, sensitive, reproducible reverse-phase HPLC method for the determination of ibuprofen in small volume of plasma. The method was used successfully to perform a pharmacokinetics study in children with fever.

Materials and Methods

Equipment

The chromatographic system consisted of a model 110 A Beckman pump, an ultrasphere C18 column (5 mcm, 4.6 mm x 25 cm) (Beckman

Instruments, San Ramon, CA), a detector (V^4 variable wavelength Absorbance Detector, ISCO, Lincoln, NE), an integrator (Hitachi Model D-2000 Chromato-Integrator, Tokyo, Japan), and an automated sample injector (WISP 712, Waters Associates, Milford, MA). All equipments were maintained at room temperature.

Mobile phase

The mobile phase consisted of 53% acetonitrile (HPLC grade, Fisher Scientific), which was prepared with 530 ml of acetonitrile, 470 ml of distilled water and 1 ml of 85% phosphoric acid.

Standard preparation

Ibuprofen, 1 mg/ml in methanol (Sigma) was used to prepare a stock solution of 100 mcg/ml. Measured aliquots of this solution was added to human plasma to yield the ibuprofen concentrations of 0.25, 0.5, 1.0, 2.5, 5.0, 10, 25, 50 and 70 mcg/ml. These were stored in Nunc vials and stored at -70^0C for future use. The standards were stable for at least six months under these conditions. Isobutyl phenyl acetate (Pfaltz-Bauer) was used as an internal standard. A stock solution of 4% concentration was made in methanol. One milliliter of this solution was added to 10 ml of methanol to prepare a working solution.

Sample preparation

One hundred microliters of plasma (standard from Blood Bank or patient's), 50 mcl of internal standard working solution, and 100 mcl of cold 0.5 N perchloric acid in methanol were added to disposable 1.5 ml polypropylene micro-centrifuge tubes (Eppendorf). The samples were vortexed for 10 seconds and centrifuged in a microfuge (Beckman) for 5 minutes. The 300 mcl supernatant samples

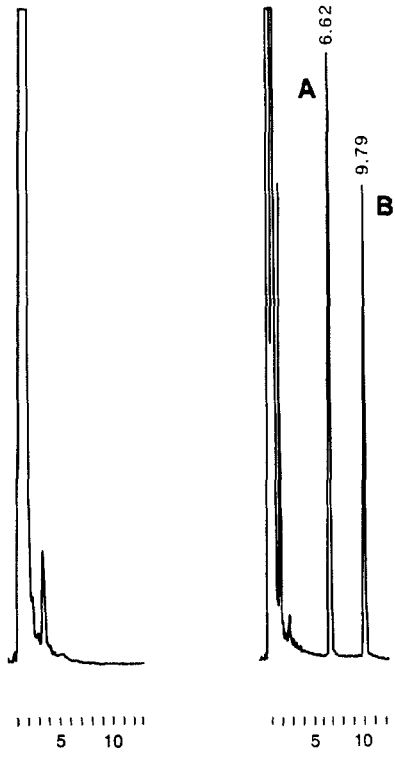

Figure 1. Left: chromatogram of blank human plasma

Right: Chromatograms of ibuprofen (A) and internal standard, isobutyl phenyl acetate (B) in human plasma

were placed in the autosampler vials and 100 mcl were injected onto the column for each measurement.

Chromatographic conditions

The flow rate of mobile phase was 1.7 ml/min. The detector was set at 214 nm, and the chart speed was 2.5 mm/min.

Data Analysis

Data acquisition and calculations were performed by the integrator. A linear least squares analysis of the ratios of the areas under the drug's and internal standard's curves in spiked standards constituted daily standardization. Interpolation from the linear regression line were used to determine ibuprofen concentrations in patient's and quality control specimens.

Results

Each chromatographic run required about 12 minutes. Ibuprofen eluted at 6.62 minutes and the internal standard at 9.79 minutes. A typical chromatogram of blank plasma, the plasma containing ibuprofen, and the internal standard are shown in Figure 1.

The standard curves were linear for ibuprofen concentration ranging from 0.25 to 70 mcg/ml (r=0.999). The limit of detection was 0.25 mcg/ml. The interday and intraday coefficient of variation was <5%.

Application

Two children (age 2 and 10 years) with fever due to Streptococcal pharyngitis received ibuprofen liquid 5 mg/kg and 10 mg/kg. Blood samples (0.5 ml) were collected just before (0 hr), and at 0.5, 1.0, 2.0, 3.0, 4.0, 6.0 and 8.0 hr after the dose. The maximum plasma concentration of ibuprofen was 39 mcg/ml at 10 mg/kg dose, and 28 mcg/ml at 5 mg/kg dose. These occurred at 1 hour after the dose. The elimination half-life was 1.5 and 1.7 hours.

The HPLC method described here has proved to be simple, accurate, sensitive, and reproducible for the measurement of ibuprofen in human plasma. Further, it has been used successfully in conducting the pharmacokinetics studies in children with fever.

Acknowledgement

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