

RP-HPLC ASSAY OF IBUPROFEN IN PLASMA USING DIFFERENT EXTRACTION PROCEDURES

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ABSTRACT

An isocratic reverse phase high performance liquid chromatographic (RP-HPLC) method with ultra violet detection at 220 nm was used for a simple, selective and precise quantitation of a non-steroidal anti-inflammatory drug i.e. ibuprofen (IBF) from rat plasma. The procedure employed mefenamic acid as an internal standard (IS). A good chromatographic separation between IBF, the internal standard and interfering endogenous peaks was achieved using a Octadecyl Silane column and acetonitrile (ACN)-water-methanol-ortho phosphoric acid (in ratio of 58: 37: 5: 0.05 v/v/v/v) as the mobile phase at a flow rate of 1.0 ml/min. Two extraction procedures for RP-HPLC quantitation of IBF were analysed. In the first method acetonitrile was used for extraction of the drug from plasma whereas the second method involved extraction of drug from plasma using diethyl ether. The second method proved to be more suitable for quantitation of the drug from plasma. The applicability of procedure includes the requirement of less sample volume, lesser organic solvent consumption and good plasma clean up for pharmacokinetic studies, in case of small animals, neonates and children as well.

KEY WORDS: RP-HPLC, Ibuprofen, Plasma, Extraction procedure

1. INTRODUCTION

Ibuprofen (2-(4-isobutylphenyl) propionic acid) (IBF) (Fig. 1 a) is a widely prescribed non-steroidal anti-inflammatory drug that shows anti-inflammatory, analgesic and anti-pyretic properties¹. It is commonly used as an analgesic, especially in case of inflammation for relieving symptoms of arthritis, gout, fever and headache etc. Ibuprofen is a known non-selective cyclooxygenase (COX) inhibitor. This drug inhibits the COX enzyme and thus exerts its anti-inflammatory activity by inhibition of prostaglandin synthesis. COX converts arachidonic acid to

prostaglandin H₂, which in turn, is converted to several other prostaglandins (which are mediators of pain, inflammation and fever) by other enzymes and to thromboxane A₂ (which stimulates platelet aggregation, leading to the formation of blood clots). Furthermore, IBF inhibits both isoforms of cyclooxygenase, COX-1 and COX-2. The analgesic, anti-pyretic and anti-inflammatory activity of non-steroidal anti-inflammatory drugs appears to be achieved mainly through inhibition of COX-2, whereas inhibition of COX-1 would be responsible for unwanted effects on platelet aggregation and the gastrointestinal tract². IBF is absorbed

well from the stomach and intestinal mucosa. It is highly protein-bound in plasma (99%), usually to albumin, so that its volume of distribution typically approximates to plasma volume. Like most of the non-steroidal anti-inflammatory drugs, it is metabolized in the liver by oxidation and conjugation to inactive metabolites which are typically excreted in the urine.

Various analytical procedures have been employed earlier for determination of IBF in body fluids, plasma and serum³⁻¹⁸. However many of these assays comprise of complex extraction procedure and require more sample volume leading to difficulties in pharmacokinetic studies in small animals, neonates and children. In the present study, a modified method for extraction of drug from plasma with diethyl ether and isocratic RP-HPLC method have been utilized to quantify concentration of IBF has advantage of small sample volume, simple optimized extraction procedure using inexpensive chemicals and short run time. The extraction procedure was selected because it had good plasma clean up property, was easy to perform and did not require specialized equipments.

2. EXPERIMENTAL

2.1. MATERIALS AND METHODS

IBF and Mefenamic acid (2-(2, 3-dimethylphenyl) aminobenzoic acid) (used as an internal standard) (Fig. 1 b) were procured from Sigma-Aldrich (MO, USA). HPLC grade Acetonitrile, water and methanol were purchased from Merck Chemicals (India), Ortho-phosphoric acid was obtained from Thermo-Fisher (India) and diethyl ether was obtained from Qualigen Ltd. (India).

2.2. Instruments and chromatographic conditions

The HPLC system consisted of Waters (Milford, MA, USA) 600-solvent delivery pump, Waters 7(1)7plus auto sampler, Waters 2487 dual λ Absorbance Detector and 150 x 4.6 mm ID x 5 μ m Altex-Ultasphere- ODS (Octadecyl Silane) (Mount Holly, NJ, USA) analytical column. The chromatographic separation was performed through injection of 20 μ l samples at ambient room temperature, detected at 220 nm. The mobile phase composed of 58% acetonitrile, 37% water, 5% methanol and 0.05% Ortho-phosphoric acid filtered through nylon membrane filter and delivered in isocratic mode at a flow rate of 1.0 ml/min.

2.3. Preparation of drug stock solutions

The stock solutions of IBF and IS were prepared in acetonitrile at concentration of 1 mg/ml and 0.5 mg/ml respectively. The working solutions of 100 and 40 μ g/ml were prepared by appropriately diluting the stock solutions of IBF and IS. Standard curve of high, medium and low concentrations viz. 10 μ g/ml, 5 μ g/ml and 2 μ g/ml respectively of IBF were constructed for reference. The stock solutions were refrigerated (2-8 °C) when not in use.

2.4. Preparation of calibration standards

Ibuprofen working solution was used to prepare the spiking stock solutions for construction of calibration curve at concentrations of 1.2, 0.8, 0.6, 0.4 and 0.2 μ g/ml by serial dilution of the 40 μ g/ml calibration standard using drug-free rat plasma.

2.4.1. Sample preparation method

Blood was collected in heparinised vacutainers from orbital sinus of Sprague Dawley rats and plasma was separated after centrifugation at 2500 rpm (419 x g). The plasma was stored at -80 °C for further analysis. In the first extraction procedure employed for sample preparation, 100 µl of 40 µg/ml internal standard was added to 50 µl of plasma. After vigorous mixing for 1 minute, it was kept at 4 °C for 30 minutes and then centrifuged at 2500 rpm (419 x g) for 20 minutes. The clear supernatant was used for HPLC analysis. In the second method of sample preparation, 100 µl of 40 µg/ml internal standard and 1 ml cold diethyl ether (4 °C) were added to 50 µl of plasma in extraction glass tubes (graduated borosilicate centrifuge tubes with conical bottom and a stopper,) and vigorously mixed for 2 minutes. After keeping the extraction glass tubes at -80 °C for about 10 minutes the organic layer was separated and kept overnight at 37 °C to dry up in the incubator. The dried layer was dissolved in 100 µl ACN and shaken for 2 minutes and used for analysis and this extraction method was designated as single extraction procedure. Further modifications were done in the single extraction procedure to avoid extra peaks. In the modified method designated as double extraction procedure, 1 ml cold diethyl ether was added to the volume of 50 µl of plasma and 100 µl of 40 µg/ml internal standard and vigorously mixed for 2 minutes and the tubes were kept at -80 °C for about 10 minutes and the organic layer (1) was separated. The extraction was repeated in the left over residue in order to achieve good recovery. This organic layer was added to the earlier collected organic layer (1) and evaporated to dryness at 37 °C overnight. Then dried

layer was reconstituted with 100 µl acetonitrile, transferred to vial and placed in autosampler for analysis.

2.5. Assay method

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. This procedure was repeated for the plasma samples. To calculate the linear regression, the peak area was plotted against drug concentration in micrograms per milliliter.

3. RESULTS AND DISCUSSION

In the present study, under the described chromatographic conditions, retention time for ibuprofen was 4.3 ± 0.3 min and for mefenamic acid 6.2 ± 0.3 min at the detection wavelength of 220 nm. With the chromatographic conditions ibuprofen and internal standard were completely separated (Fig. 2 a). An assay performed on drug-free plasma spiked with internal standard (mefenamic acid) did not show any interfering peak at the retention time of interest with the modified extraction procedure for sample preparation. The calibration curve in plasma ranging from 0.2-1.2 µg/ml was analyzed. The modified extraction method developed represents a less complex procedure for analysis of ibuprofen in blood plasma. Comparison of Fig. 2 b with Fig. 2 c showed less number of interfering peaks after single extraction method. However a comparison of Fig. 2 b and Fig. 2 d indicates that with the modified extraction procedure (double extraction method) there was further more reduction in the background caused due to endogenous constituents. Sharp, symmetrical, well resolved peaks were

obtained in modified extraction procedure.

4. CONCLUSION

The observations suggest that our procedural conditions are suitable for accurate quantitation of ibuprofen in plasma. This modified technique thus developed not only for resolving the occurrence of extra peaks but also to obtain good resolution during analysis of the drug in plasma. The advantages of the modified method render it to be more useful, convenient and accurate for application in pharmacokinetic studies where small sample volume is available especially conducting studies on small rodents or animals simulated or exposed to various environmental vagaries like heat, cold, hypoxia, microgravity and deal with these stresses with pharmacological interventions.

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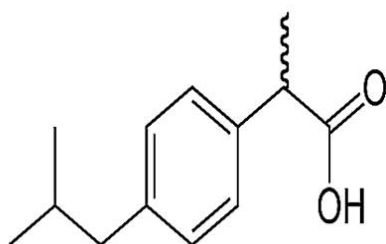


Fig. 1 (a)

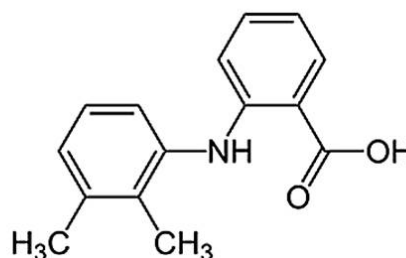


Fig. 1 (b)

Fig. 1 Structure of: (a) Ibuprofen and (b) Mefenamic acid

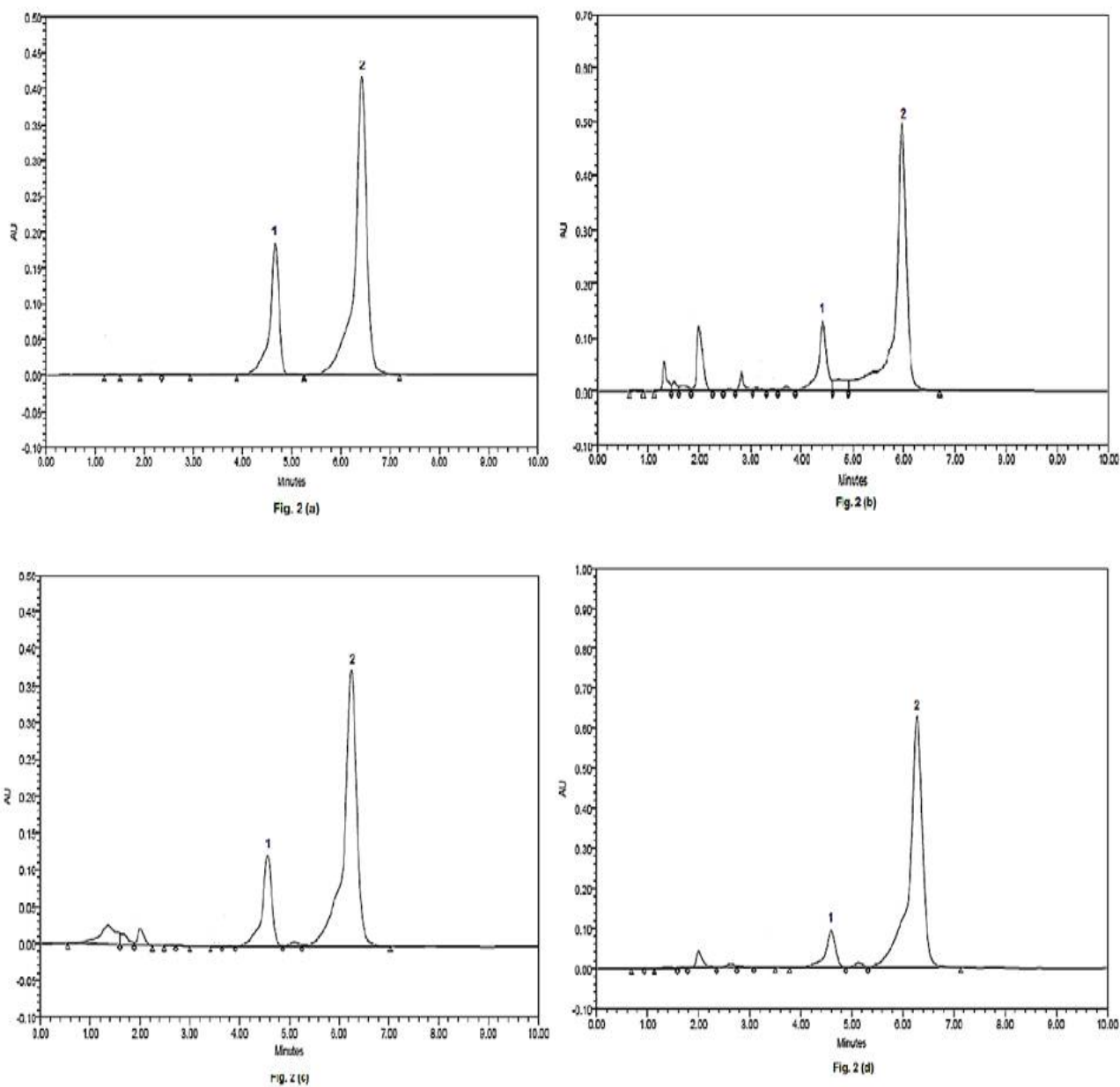


Fig. 2 HPLC chromatogram of Ibuprofen: (a) IBF + I.S. (100µg/ml) (b) Plasma + STD mix (100µg/ml) [Extra peaks appeared after extraction with ACN] (c) Plasma + STD mix (100µg/ml) (Single extraction) [Extra peaks appeared after single extraction] (d) Plasma + STD mix (40µg/ml) (Double extraction) [Extra peaks resolved after double extraction]. Peaks identified were: (1) Ibuprofen standard (IBF) (2) Internal standard (I.S.) - Mefenamic acid.