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Short Communication

High-performance liquid chromatographic assay for ibuprofen in whole blood using solid-phase extraction

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Abstract

A precise, accurate, reproducible one-step method for the high-performance chromatographic determination of ibuprofen in whole blood is described. Samples were, after haemolysis, prepared by solid-phase extraction. Analyses were performed using reversed-phase chromatography on a Separon SGX C_{18} column with a mobile phase of methanol–water (pH 3) and ultraviolet detection at 222 nm. The method was used for pharmacokinetic studies in rabbits.

1. Introduction

Ibuprofen, 2-(4-isobutylphenyl)propionic acid, is widely used in anti-inflammatory therapy. Analysis of ibuprofen in biological fluids is frequently performed with the use of high-performance liquid chromatography (HPLC).

Most HPLC determinations of ibuprofen are carried out in plasma [1–18] or serum [18–21]. Only a few papers [23–26] describe a HPLC assay of ibuprofen in whole blood.

Ibuprofen was isolated from plasma or serum by deproteination [1,2,9,10,18,22], liquid–liquid extraction [3–6,11,15,17] or solid-phase extraction [7,8,12,13,16]. A column-switching technique for HPLC is described [20] after prior liquid–liquid extraction. Timmoney *et al.* [21] have compared both extraction procedures for ibuprofen analysis in serum.

Post-mortem blood was prepared by liquid–liquid extraction for screening of acidic and neutral drugs, including ibuprofen, by HPLC [23]. An analogous procedure was described by Levine and Caplan [24]. Ibuprofen was analysed in whole blood after deproteination of the sample by ion-pairing HPLC [25]. Solid–liquid extraction was used to clean-up the blood sample for HPLC analysis of ibuprofen [26].

In the present paper, a HPLC analysis of ibuprofen in whole blood is described. Blood

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samples were cleaned-up by solid-phase extraction before HPLC analysis. The method was applied to the determination of ibuprofen in blood samples arising from a pharmacokinetic study.

2. Experimental

2.1. Chemicals and reagents

Ibuprofen standard and internal standard (I.S.) (indomethacin) were supplied by Léčiva (Prague, Czech Republic). Methanol, methylene chloride and hydrochloric acid (35%) were purchased from Lachema (Brno, Czech Republic). Perchloric acid was obtained from Merck (Darmstadt, Germany). All reagents were analytical-reagent grade, methanol was HPLC grade and methylene chloride was distilled. Water was doubly distilled. The phosphate buffer (pH 2) used for sample preparation was prepared from 66 mmol KH_2PO_4 (pH 5), adjusted to pH 2 with phosphoric acid.

The extraction columns Silica cart C_{18} and Separon SGX C_{18}, 60 μm, (1 ml cartridge) were supplied by Tessek (Prague, Czech Republic).

2.2. Chromatography

The HPLC system consisted of a Model 8500 Varian pump, Varichrom UV-Vis detector (both: Varian, Palo Alto, CA, USA) and SP 4100 integrator (Spectra Physics, Santa Clara, CA, USA). Analytical samples were introduced onto the column using a Model LCI 30 injection valve (Laboratory Instruments, Prague, Czech Republic) with a 10-μl loop. The analytical glass column contained Separon SGX C_{18} (150 × 3.3 mm I.D., 5 μm, Tessek). The mobile phase was methanol–water (220:100, v/v) with a final apparent pH of 3.0 adjusted with 5% perchloric acid solution and was filtered and helium degassed prior to use. The flow-rate was set at 1.3 ml/min. The UV absorbance of the column effluent was monitored at 222 nm.

The concentration of ibuprofen was calculated using a calibration curve. The calibration curve was made by plotting the peak-area ratio (y) *versus* the concentration of the ibuprofen (x).

2.3. Preparation of standard

Stock solutions of ibuprofen and indomethacin (internal standard, I.S.) were prepared in methanol by dilution of 1 mg/ml and O.5 mg/ml, respectively.

Drug-spiked blood standards were prepared by aliquoting appropriate volumes of stock solutions to 0.25 ml of control rabbit blood (concentration range 20–100 μg/ml).

2.4. Biological samples

Samples of whole blood (heparinized) were obtained from the Department of Pathological Physiology, Faculty of Medicine, Charles University (Hradec Králové, Czech Republic). Rabbits were treated with a solution of ibuprofen (25 mg/kg weight) and blood samples were withdrawn in a pharmacokinetic study. Samples were withdrawn 3, 6, 15, 30, 60 and 120 min after drug administration. All blood samples were immediately frozen.

2.5. Sample preparation

Blood samples were prepared for analysis by solid-phase extraction using vacuum. A 0.25-ml volume of blood sample was pipetted into a 5-ml glass stoppered centrifuge test tube, 5 μl of the internal standard solution were added and sample was haemolysed by adding 0.5 ml of water. The sample was shaken for 5 min, placed in an ultrasonic bath for 5 min and a left at room temperature for 5 min. A 1-ml volume of phosphate buffer (pH 2) was added to the haemolysed sample and, after 5 min shaking, the sample was centrifuged for 10 min at 1930 g. The supernatant was transferred onto an extraction cartridge, which had been prewashed sequentially with 5 ml of methanol, then with 5 ml of water, and 5 ml of phosphate buffer (pH 2). The loaded cartridge was washed with 5 ml phosphate buffer followed by 10 ml of water. The

extraction cartridge was dried with vacuum for 5 min. Ibuprofen and internal standard were eluted with 4 ml methylene chloride. The eluate was evaporated to dryness under a gentle stream of nitrogen. Prior to liquid chromatography, the residue was dissolved in 100 μl of the mobile phase.

2.6. Recovery

The recovery of ibuprofen from whole blood was examined. Spiked samples were prepared by adding known amounts of ibuprofen to blank whole blood. The concentrations of the samples were 20, 50 and 100 μg/ml. The samples were analysed as described above. The peak areas obtained were compared with those obtained by direct injection of ibuprofen without extraction.

3. Results and discussion

An isolation technique was developed and HPLC conditions were optimized for determination of ibuprofen in whole blood samples. Over the last years the importance of solid-phase extraction has grown considerably. Solid-phase extraction columns have been used to isolate ibuprofen from samples of plasma [7,8,12,13,16], serum [21], urine [27,28] or blood [26]. In the present paper solid-phase extraction was used to clean-up blood samples. Ibuprofen was isolated on C_{18} extraction columns. Of the eluents tested, methylene chloride was preferred to methanol, because the latter gave a low extraction efficiency. In order to increase the efficacy of the solid-phase extraction, it was necessary to carry out haemolysis of the blood sample. Adding phosphate buffer and subsequent centrifugation of the sample improved the extraction procedure. The mean recovery of ibuprofen over the range 20–100 μg/ml was 91.7% (Table 1)

Under the chromatographic conditions described, ibuprofen and internal standard (indomethacin) gave sharp, symmetrical and well-resolved peaks with retention times 9.9 min and 7.8. min, respectively. No endogenous blood components interfered with either peak. The chromatograms obtained from a blank blood sample, a control spiked sample and a sample from the rabbit are shown Fig. 1. No potential metabolite peaks were observed in the solid-phase extracted post-dose rabbit blood sample. Satisfactory results have been achieved also when testing other potential internal standards (diazepam, phenylanthranilic acid).

Table 1
Recovery of ibuprofen in whole blood

Added concentration (μg/ml)	Recovery (means ± S.D., $n = 5$) (%)
20.0	89.3 ± 2.0
50.0	92.5 ± 2.3
100.0	93.3 ± 2.7

Quantitation of ibuprofen was based on the least-squares linear regression analysis. The calibration curves displayed good linearity over the range examined. The linear regression equations were $y = 0.0460 \times -0.063$ ($r = 0.999$). The limit of detection for ibuprofen in blood was 100 ng/ml, and the limit of quantitation was 300 ng/ml.

Both within-day and day-to-day precision and accuracy of the calibration curves were examined. Within-day precision was calculated from the analysis of five blood samples of four concentrations of ibuprofen. Day-to-day preci-

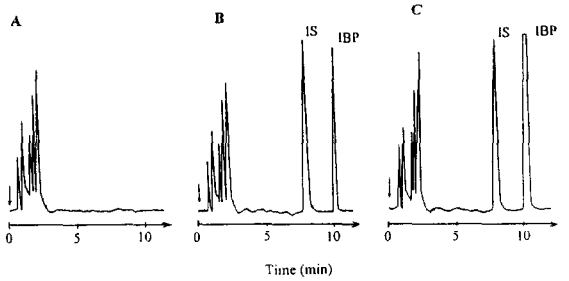

Fig. 1. Typical chromatogram for ibuprofen (IBP) and internal standard (I.S.) in rabbit whole blood. (A) Blank sample, (B) control sample spiked with standard solution of ibuprofen (10 μg/ml) and I.S. (10 μg/ml), (C) 30-min sample from a rabbit given a single dose of 25 mg/kg of ibuprofen.

Table 2
Assay precision and accuracy of determination of ibuprofen in whole blood

Concentration (μg/ml)		n	C.V. (%)
Added	Found		
Within-day			
10.0	10.2 ± 0.2	5	2.0
30.0	29.7 ± 0.5	5	1.7
90.0	91.2 ± 1.2	5	1.3
120.0	121.1 ± 1.5	5	1.2
Day-to-day			
10.0	9.8 ± 0.4	16	4.1
30.0	30.8 ± 0.7	16	2.3
90.0	89.0 ± 1.7	16	1.9
120.0	122.4 ± 2.4	16	2.0

sion was investigated during a four-months period. Measured concentrations and coefficients of variation (C.V.) are presented in Table 2. The C.V. values were all less than 5%. The good precision and accuracy of the method allow it to be used successfully in pharmacokinetic studies.

The level of ibuprofen was investigated with laboratory rabbits in a two-hour pharmacokinetic study. The blood samples were analyzed and the amounts of ibuprofen determined. A typical plot of the concentration of ibuprofen in blood *versus* time obtained after i.v. administration of ibuprofen in a single dose of 25 mg/kg to rabbits is illustrated in Fig. 2.

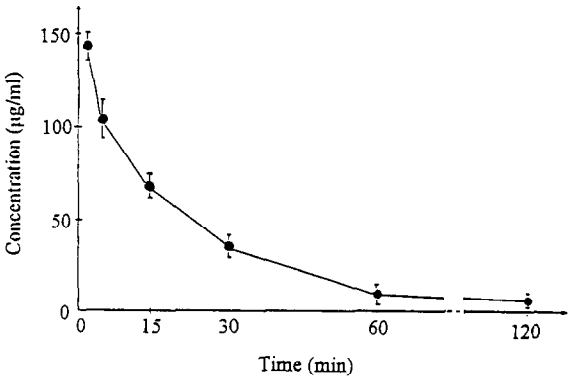

Fig. 2. Concentration of ibuprofen in rabbit blood following intravenous administration.

4. Conclusions

The HPLC method described in this paper allows the determination of ibuprofen in whole blood using solid-phase extraction. The advantage of this method is that whole-blood samples can be analyzed. After withdrawing the blood sample it is not necessary to separate the plasma; the sample can be directly analyzed with the method described in the present paper. The method is precise, accurate and reproducible, even when a complex biological matrix like plasma or serum has to be analyzed. The method is suitable for the monitoring of ibuprofen levels in blood.

5. References

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