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Determination of ibuprofen in erythrocytes and plasma by high performance liquid chromatography

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Abstract

A high-performance liquid chromatographic (HPLC) method is described for the determination of ibuprofen in isolated erythrocytes and plasma. Before HPLC analysis ibuprofen was isolated by liquid–liquid extraction from these biological matrices; methylene chloride proved to be the best of the organic solvents tested. For the sample of erythrocytes it was necessary to carry out haemolysis prior to their extraction. HPLC was performed on a C-18 column with a mobile phase of methanol–water (220:100, v/v) acidified with perchloric acid to pH 3. Ultraviolet detection was at 222 nm. This method has been applied to the quantification of ibuprofen in rabbit erythrocytes and plasma for a pharmacokinetics study.

Keywords: Erythrocytes; Ibuprofen; Pharmacokinetics; Plasma; Rabbit; Reversed-phase HPLC

1. Introduction

Ibuprofen, 2-(4-isobutylphenyl) propionic acid, a non-steroidal anti-inflammatory drug, is used in the treatment of several forms of arthritis and other disorders. The chromatographic methods presently available to measure ibuprofen include gas chromatography [1,2] and HPLC [3–28]. A HPLC method has been used to determine ibuprofen in urine [3–8], saliva [8], tissues of laboratory animals [9], and mainly in plasma [4,5,9–22] and serum [8,10,23,24]. In a few published HPLC methods [25–27], ibuprofen has been analyzed in whole blood.

Ibuprofen is isolated from plasma or serum by deproteination [4,5,11,12,14,15,18,22], liquid–liquid extraction [9,12,13] or solid–liquid extraction [16,17,21]. Timmoney et al. [24] have compared both extraction procedures for the analysis of ibuprofen in serum. In the HPLC analysis of ibuprofen in serum a column-switching method [23] has been used.

Pharmacokinetic studies of ibuprofen have been performed by HPLC primarily in plasma [4,5,11,12,14–16,18,22]. Ibuprofen was determined simultaneously with other drugs in a sample of plasma [8]. Ibuprofen, as a metabolite of pimoprofen, was assayed simultaneously with pimoprofen in plasma after solid–liquid extraction [28].

This paper presents an assay for ibuprofen in rabbit erythrocytes and plasma. The aim was to develop an extraction procedure and to optimize chromatographic conditions, suitable for both biological matrices. The method has been applied to the determination of ibuprofen lev-

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els in erythrocytes and plasma in a pharmacokinetic study.

2. Experimental

2.1. Chemicals and reagents

Ibuprofen and the internal standard (indomethacin) were supplied by Léčiva (Praha, Czech Republic). Methanol, methylene chloride and hydrochloric acid (35% w/w) were purchased from Lachema (Brno, Czech Republic). Perchloric acid was obtained from Merck (Darmstadt, Germany).

All these reagents were analytical-reagent grade, methanol was HPLC-grade and methylene chloride was distilled. Double-distilled water was used.

2.2. Instrumentation

The HPLC system comprised of Model 8500 Varian pump and a Varichrom UV–Vis detector (Varian, Palo Alto, CA, USA) with an SP 4100 integrator (Spectra Physics, Santa Clara, CA, USA). Analytical samples were introduced on to the column using a Model LCI 30 injection valve (Laboratory Instruments, Prague, Czech Republic) with a 10-μl loop. The analytical glass column (150 × 3.3 mm i.d.) was packed with 5-μm (Tessek, Prague, Czech Republic). The UV absorbance of the column eluent was monitored at 222 nm.

The mobile phase was methanol–water (220:100, v/v), with a final apparent pH of 3.0 adjusted with 5% w/w perchloric acid; the mixture was filtered and helium degassed prior to use. The flow-rate was set at 1.3 ml min^{-1}.

2.3. Biological samples

Erythrocytes and plasma were obtained from Department of Pathological Physiology, Faculty of Medicine, Charles University. Here also three rabbits were treated with a solution of ibuprofen (25 mg kg^{-1}). Blood samples were withdrawn 3, 6, 15, 30, 60 and 120 min after drug administration in a pharmacokinetics study.

Erythrocytes were isolated from plasma by centrifugation at 1500*g* for 10 min in each sample of withdrawn blood (heparinized). The samples of erythrocytes and plasma were immediately frozen.

2.4. Standard solutions

Stock solutions of ibuprofen and internal standard (indomethacin) were prepared by dissolving 10 mg of each drug in 10 ml of methanol.

Erythrocyte and plasma standards were prepared by adding appropriate volumes of the stock solutions to 0.5 ml of control rabbit erythrocytes and to plasma (drug-free) to produce a concentration series of 2–15 μg ml^{-1} for erythrocytes and 20–200 μg ml^{-1} for plasma.

2.5. Extraction procedure

Erythrocytes

0.5 ml of erthrocytes was transferred by pipette into a 10 ml glass-stoppered centrifuge test tube and 5 μl of the internal standard solution was added. The sample was haemolysed by adding 0.9 ml of water, shaking for 5 min, placing in an ultrasonic bath for 5 min, and leaving at room temperature for 5 min. The mixture was then acidified with 0.4 ml of 3 M hydrochloric acid and, after 5 min shaking, 6 ml of methylene chloride was added. After shaking and centrifugation (5 min at 1930*g*), methylene chloride (5 ml) was separated and evaporated to dryness under a gentle stream of nitrogen. The dry residue was dissolved in 100 μl of the mobile phase and 10 μl was injected into the HPLC column.

Plasma

0.5 ml of plasma was transferred by pipette into a 10 ml glass-stoppered centrifuge test tube and 5 μl of the internal standard solution was added. The sample was acidified gradually with 0.9 ml of 0.1 M hydrochloric acid and, after shaking, a further 0.2 ml of 3 M hydrochloric acid was added. After 5 min shaking, 6 ml of methylene chloride was added. The procedure was then continued in the same manner as described for the erythrocyte sample.

3. Results and discussion

The isolation technique and HPLC method were developed for the determination of ibuprofen in isolated erythrocytes and plasma.

Under the chromatographic conditions described in the experimental section, ibuprofen and the internal standard were completely separated from the endogenous constituents

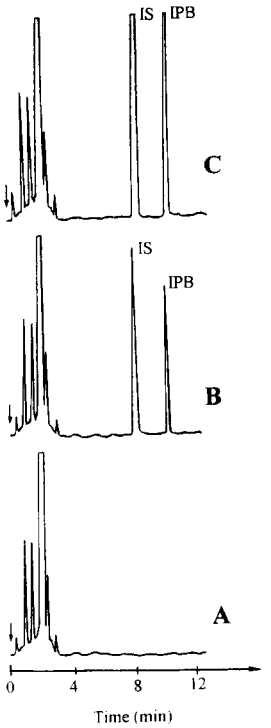

Fig. 1. Typical chromatograms for ibuprofen (IBP) and internal standard (IS) in rabbit erythrocytes. (A) Blank sample; (B) control sample spiked with a standard solution of ibuprofen (15 μg ml^{-1}) and internal standard (10 μg ml^{-1}); (C) 30 min sample from a rabbit given a single dose of 25 mg kg^{-1} of ibuprofen.

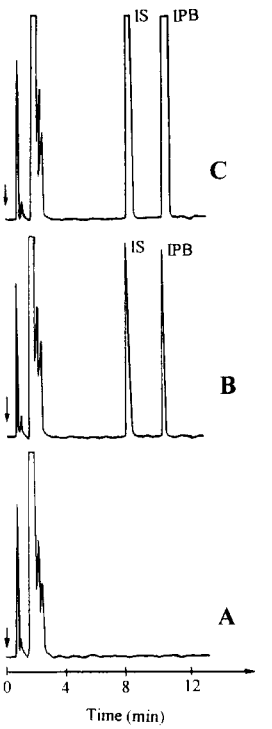

Fig. 2. Typical chromatograms for ibuprofen (IBP) and internal standard (IS) in rabbit plasma. (A) blank sample; (B) control sample spiked with a standard solution of ibuprofen (10 μg ml^{-1}) and internal standard (10 μg ml^{-1}); (C) 30 min sample from a rabbit given a single dose of 25 mg kg^{-1} of ibuprofen.

(Figs. 1 and 2). The chromatographic conditions gave sharp, symmetrical and well-resolved peaks for ibuprofen and the internal standard with retention times of 9.9 min and 7.8 min, respectively. No potential metabolite peaks were observed in the extracted post-dose rabbit erythrocytes or plasma samples. That is why indomethacin, with a shorter retention time than that of ibuprofen, was used as the internal standard. Satisfactory results have also been achieved in testing other potential internal standards (diazepam, phenylanthranilic acid).

An isolation procedure was developed for erythrocytes, which represent a more complex biological material than plasma. A comparison of Figs. 1 and 2 indicates that the erythrocyte background was not richer in endogenous constituents than the plasma background when the proposed extraction procedure was used. In order to increase the efficacy of the extraction procedure to the maximum, it was necessary to carry out the haemolysis of erythrocytes before their extraction to an organic solvent. Chloroform, methylene chloride and diethylether were tested. Methylene chloride proved to be the best of the organic solvents tested because relatively little of the residues from erythrocytes was extracted by this solvent. The recovery of ibuprofen was 78% for erythrocytes and 95% for plasma (see Table 1).

Table 1
Recovery of ibuprofen for erythrocytes and plasma

Biological Material	Added concentration (μg ml^{-1})	Recovery ($n = 5$) (mean ± SD) (%)
Erythocytes	2.0	78.3 ± 2.2
	8.5	77.5 ± 2.5
	15.0	79.1 ± 2.0
Plasma	20.0	96.2 ± 2.1
	100.0	95.5 ± 1.9
	200.0	94.3 ± 2.2

Quantification was based on the least-squares linear regression analysis of peak–area ratio (y) versus concentration (x). The calibration curves displayed good linearity over the range examined. The linear regression equations were $y = 0.0699x + 0.0492$ ($r = 0.9987$)

Table 2
Precision and accuracy of determination of ibuprofen in spiked erythrocytes and plasma

Biological Material	Concentration added ($\mu g\ ml^{-1}$)	Intra-day ($n = 5$)		Inter-day ($n = 16$)	
		Concentration found (mean ± SD) ($\mu g\ ml^{-1}$)	RSD (%)	Concentration found (mean ± SD) ($\mu g\ ml^{-1}$)	RSD (%)
Erythrocytes	2.0	2.0 ± 0.1	3.0	1.9 ± 0.1	3.7
	8.5	8.6 ± 0.2	2.3	8.4 ± 0.2	2.8
	15.0	14.6 ± 0.3	2.1	14.6 ± 0.4	2.9
Plasma	20.0	20.3 ± 0.5	2.6	21.0 ± 0.5	2.3
	100.0	100.9 ± 1.4	1.4	102.2 ± 1.6	1.6
	200.0	201.7 ± 2.0	1.0	200.8 ± 2.2	1.1

and $y = 0.1014x + 0.1008$ ($r = 0.9989$) in erythrocytes and plasma, respectively.

Both intra- and inter-day precision and accuracy of the calibration curves were examined. Intra-day precision was calculated from the analysis of five samples in both biological materials for four concentrations of ibuprofen. Inter-day precision was investigated over a four-month period. Measured concentrations and relative standard deviations (RSDs) are presented in Table 2. The RSD values were all less than 5%.

The detection limits for ibuprofen were 30 ng ml^{-1} in isolated erythrocytes and 20 ng ml^{-1} in plasma. To determine the detection limits, the stock solution of ibuprofen was diluted to a concentration of 0.1 mg ml^{-1} or 0.01 mg ml^{-1}. These limits were determined by the usual process (from the standard deviation of the mean) on the basis of experimental measuring of the above mentioned solutions of ibuprofen. In the present work, however, the determined levels of ibuprofen were higher owing to the administration of 25 mg ibu-profen kg^{-1} body weight of the rabbit. This dose was required by collaborators from the Faculty of Medicine for another pathophysiological study.

The level of ibuprofen was investigated in three laboratory rabbits in a 2-h pharmacokinetic study. The samples of isolated erythrocytes and plasma were analyzed and the amounts of ibuprofen determined. The observed erythrocyte and plasma levels of ibuprofen are shown in Figs. 3 and 4. The results show that 15–20% of ibuprofen was found in erythrocytes compared to that in plasma (related to 1 ml of withdrawn blood with regard to the haematocrit value). During a 2-h pharmacokinetic study the erythrocyte level of

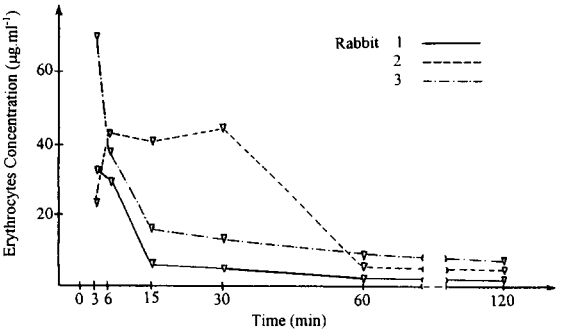

Fig. 3. Concentration of ibuprofen in rabbit erythrocytes following intravenous administration.

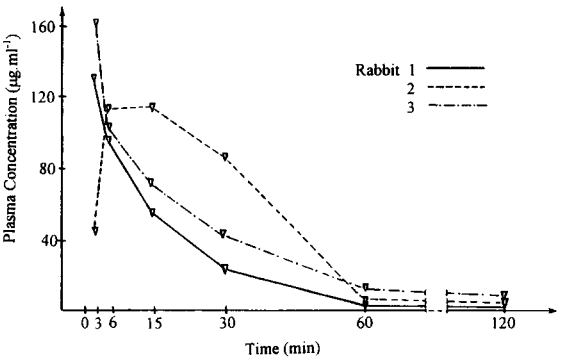

Fig. 4. Concentration of ibuprofen in rabbit plasma following intravenous administration.

ibuprofen decreased only slightly in comparison with a rapid initial fall in the plasma level of the drug.

4. Conclusions

Application of these methods enables a more detailed picture to be obtained of the distribution of ibuprofen in blood between erythrocytes and plasma. In the analysis of a plasma

sample, the level of ibuprofen in plasma is determined whereas in the analysis of whole blood sample, the sum of ibuprofen in plasma and erythrocytes is determined. The method developed in the present paper makes it possible to determine the levels of ibuprofen both in erythrocytes and in plasma, which contributes to a more objective view of the behaviour of ibuprofen in the body.

The HPLC method developed will be further used to determine the immediate erythrocyte and plasma levels of ibuprofen in laboratory rabbits, in which other physiological and pathophysiological parameters influenced by ibuprofen will be investigated simultaneously.

References

[1] E.G. De Jong, J. Kiffers and R.A.A. Means, J. Pharm. Biomed. Anal., 7 (1989) 1617–1622.
[2] P.J. Nayak and G.K. Pillai, Ind. J. Pharm. Sci., 51 (1989) 141–144.
[3] S.R. Binder, M. Regalia, M. Biaggi-Mc Eachern and M. Mazhar, J. Chromatogr., 473 (1988) 325–341.
[4] A. Shah and D. Young, J. Chromatogr., 378 (1986) 272–276.
[5] A. Avgerinos and A.J. Hutt, J. Chromatogr., 380 (1986) 468–471.
[6] Chai Baoling, E.P. Minkler and Ch. L. Hoppel, J. Chromatogr., 430 (1988) 93–101.
[7] R.T. Patel, J.R. Benson, D. Hometchko and G. Marshall, Am. Lab. (Fairfield, Conn.), 22 (1990) 92, 94–99.
[8] R.W. Slingsby and M. Rey, J. Liq. Chromatogr., 13 (1990) 107–134.
[9] G. Berner, R. Staab and M.M. Wagener, Fresenius' J. Anal. Chem., 336 (1990) 238.
[10] P.J. Streete, J. Chromatogr., 495 (1989) 179–183.
[11] P.E. Minkler and Ch.L. Hoppel, J. Chromatogr., 428 (1988) 388–394.
[12] J.H. Satter White and F.D. Boupinot, J. Chromatogr., 497 (1989) 330–335.
[13] R. Ginman, H.T. Karnes and J. Perrin, J. Pharm. Biomed. Anal., 3 (1985) 439–445.
[14] M. Lalande, D.L. Wilson and I.J. McGilveray, J. Chromatogr., 377 (1986) 410–414.
[15] J. Askholt and F. Nielsen-Kudsk, Acta Pharmacol. Toxicol., 59 (1986) 382–386.
[16] J.H.G. Jonkmann, R. Schoenmaker, A.H. Holtkamp and J. Hempenius, J. Pharm. Biomed. Anal., 3 (1985) 433–438.
[17] H.T. Karnes, K. Rajasekhariah, R.E. Small and D. Farthing, J. Liq. Chromatogr., 11 (1988) 489–499.
[18] A.M. Rustum, J. Chromatogr. Sci., 29 (1991) 16–20.
[19] G.S. Owen, S.M. Roberts and W. Friesen, J. Chromatogr., 416 (1987) 293–302.
[20] F. Lapicque, P. Netter, B. Bannwarth, P. Trechot, P. Gillet, H. Cambert and R.J. Royer, J. Chromatogr., 496 (1989) 301–320.
[21] M. Shulz and A. Schmoldt, Pharm. Ztg. Viss., 2 (1989) 41–44.
[22] C.M. Nahata, J. Liq. Chromatogr., 14 (1991) 187–192.
[23] Kenji Yamashita, Michio Motohashi and Takatsuka Yashiki, J. Chromatogr., 570 (1991) 329–338.
[24] P. Timmoney, S. Newton and P. Beals, Adv. Lab. Autom. Rob., 5 (1989) 249–265.
[25] A.M. Rustum, J. Chromatogr., 526 (1990) 246–253.
[26] C.M. Moore and I.R. Tebbet, Forensic Sci. Int., 34 (1987) 155–158.
[27] E.M. Chan and S.C. Chan, J. Anal. Toxicol., 8 (1984) 173–176.
[28] B.D. Kaluzny and C.A. Bannow, J. Chromatogr., 414 (1987) 228–234.