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Determination of ibuprofen in human plasma by solid phase extraction and reversed-phase high-performance liquid chromatography

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Abstract: A new, sensitive and simple method for the rapid quantitative determination of ibuprofen in human plasma has been developed. This method involves the use of a solid phase extraction on "Baker" C-18 disposable extraction columns for sample clean-up and uses mefenamic acid as an internal standard.

Separation and quantitation are performed by reversed-phase liquid chromatography using a Nucleosil C_{18} column and methanol–0.04 M phosphoric acid (80:20, v/v), as the mobile phase.

Detection was achieved by UV-absorbance measurements at 229 nm.

Keywords: *Ibuprofen; therapeutic drug monitoring; pre-column purification; HPLC.*

Introduction

Ibuprofen is a non-steroidal anti-inflammatory, analgesic and antipyretic drug that is used extensively in the treatment of several forms of arthritis and additionally in the treatment of mild to moderate pain and for antipyresis.

A number of methods for the determination of ibuprofen in plasma have been published; among them are paper chromatography [1], gas–liquid chromatography (GLC) with prior derivatization [2, 3] or electron-capture detection [4, 5], combined gas chromatography–mass spectrometry [6, 7] and high-performance liquid chromatography (HPLC) [8–14]. Paper chromatography is very time consuming. On the other hand, the more rapid gas chromatographic methods require 1.0–2.0 ml of plasma for each determination, thus making pharmacokinetic or bioavailability studies in children difficult; moreover, some GLC methods require tedious and time-consuming derivatization steps. Most of the published HPLC methods require 0.5–1.0 ml of plasma and a clean-up extraction before chromatography (including acidification of the plasma, extraction with an organic solvent, evaporation of the organic layer and dissolution of the residue in the eluent or internal standard solution), which is time consuming and may

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introduce errors. Additionally, they require prior removal of proteins from the plasma (for instance, by adding methanol and subsequent filtering or centrifugation). This method of deproteinization, however, may not always be adequate, as revealed by rapid deterioration of LC columns and the necessity to use guard columns.

Snider *et al.* [14] used an automated sample processor to perform extractions which involved a microprocessor-controlled centrifugal force to move serum samples through an extraction resin bed. However, a simple manual extraction procedure might be more appropriate for general use.

Therefore a rapid, simple, accurate and sensitive LC method has been developed, which requires only 250 μl of plasma or serum and which is suitable for the determination of large numbers of blood samples in pharmacokinetic studies.

Experimental

Apparatus

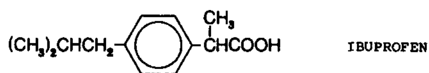
The equipment for high-performance liquid chromatography (HPLC) consisted of a Waters M45 solvent delivery system and a Model 441 fixed wavelength ultraviolet absorbance detector equipped with a 229 nm filter (Waters Assoc., Milford, MA, USA). The columns (150 × 4.6 mm i.d.) were of stainless steel and packed with 5-μm Nucleosil C-18 (Machery-Nagel; Düren, Federal Republic of Germany).

A solvent flow of 1.0 ml min^{-1} was used at room temperature.

Injections were made by means of a Waters Intelligent Sample Processor, Model 710B (Waters Assoc., Milford, MA, USA). Data analysis was performed by a Spectra-Physics SP 4100 computing integrator (Spectra Physics, Santa Clara, CA, USA), measuring peak-heights.

Chemicals and reagents

The ibuprofen used was a U.S.P. standard. The internal standard mefenamic acid was of analytical reagent grade and supplied by Sigma Chemicals (Fig. 1).



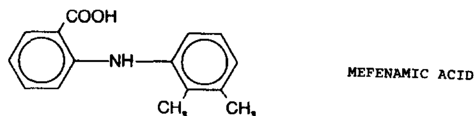


Figure 1
Structural formulae of ibuprofen and of the internal standard mefenamic acid.

Methanol, HPLC grade was obtained from Fisons (Loughborough, UK). Phosphoric acid (25% m/v) of Dutch Pharmacopoeial quality was supplied by O.P.G. (Utrecht, The Netherlands).

The system was calibrated using standard solutions prepared by dissolving ibuprofen in 5.0 ml of borax (pH = 11.0) and diluting with water. The internal standard solution was made in the same manner by dissolving the mefenamic acid.

These solutions were stored at 4°C for four weeks without deterioration.

Calibration curves (5 concentrations) were recorded daily.

The mobile phase methanol–0.04 M phosphoric acid (80:20, v/v) was filtered through a Millipore filter type HA 0.45 μm and degassed by ultrasonification for 15 min prior to use.

Extraction procedure

The extraction columns were conditioned with two 1.0-ml volumes of methanol followed by two 1.0-ml volumes of wash solvent (1% m/v phosphoric acid).

A 250-μl aliquot of plasma, 100 μl of the internal standard solution, 100 μl of water (or standard solution of ibuprofen for calibration) and 500 μl of the wash solvent were mixed on a Vortex mixer.

After centrifugation for 2 min at 15 000 rpm to remove solid particles, this mixture was transferred to the extraction column and eluted at about 0.2 ml min^{-1}. The column was washed with 1 ml of the wash solvent by applying a water-pump vacuum. The sample was eluted from the column with two volumes of 500 μl methanol under vacuum, waiting 30 sec before applying the vacuum.

An aliquot of 250 μl of 0.04 M phosphoric acid was added to adjust the composition to that of the mobile phase. This solution was mixed on a Vortex mixer and a 15-μl volume injected into the liquid chromatograph.

Results and Discussion

A chromatogram obtained from a blank human plasma is shown in Fig. 2. A chromatogram generated from a plasma sample that was drawn from the same subject after ingestion of a dosage form containing 400 mg of ibuprofen is illustrated in Fig. 3. Ibuprofen and the internal standard were well resolved and eluted with retention times of 231 and 322 s, respectively. No interfering peaks were detected in blank plasma, under these experimental conditions.

Ibuprofen is metabolized to 2-[4′-(2-hydroxy-2-methylpropyl)phenyl]propionic acid and to 2-[4′-(2-carboxypropyl)phenyl]propionic acid and conjugates. Interference from the metabolites was not expected because of their high polarity.

Absorbance measurements at 229 nm proved to be satisfactory for both ibuprofen and mefenamic acid.

The results of recovery studies are given in Table 1. The absolute analytical recovery (>93%) was satisfactory at all ibuprofen concentrations, with RSD of about 1.5%). For the internal standard mefenamic acid the recovery was in the same range (93.6 ± 1.4%).

The linearity of peak height ratios with the concentration of ibuprofen was investigated. Calibration curves from plasma spiked with 0.5–40.0 mg l^{-1} ibuprofen showed excellent linearity with correlation coefficients between 0.9999 and 1.0000 (Table 2). The regression equation was found to be: $y = 0.034x + 0.002$, where y and x are peak height ratio and ibuprofen concentration (mg l^{-1}) respectively.

The reproducibility of the entire assay was demonstrated by determination of the intra-day and the inter-day reproducibility of the calibration curve. The results are given in Table 2 and Table 3 respectively.

The intra-day reproducibility is excellent, as assessed by generating seven calibration curves on the same day, with plasma concentrations of 0.5, 1.0, 1.5, 2.0, 5.0, 10.0, 20.0 and 40.0 mg l^{-1} (see Table 2). At 0.5 mg l^{-1} the RSD was 7.9% and at all other concentrations the RSD was below 3.1%.

The inter-day reproducibility is good, as established by assaying seven calibration

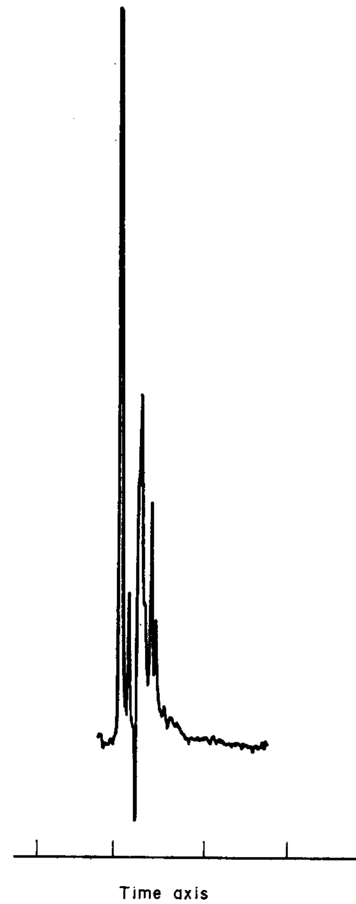


Figure 2
Chromatogram obtained from a blank human plasma.

Table 1
Analytical recovery of ibuprofen at five different plasma concentrations

Amount added ($mg\ l^{-1}$)	Absolute recovery (%) (mean ± S.D.; n= 7)
2.0	100.0 ± 1.3
5.0	93.7 ± 2.0
10.0	94.7 ± 1.3
20.0	93.3 ± 2.4
40.0	95.6 ± 1.1

Table 2
The inter-day reproducibility as indicated by calibration data

Plasma concentration: ($mg\ l^{-1}$)	2.0	5.0	10.0	20.0	40.0
Peak height ratio (mean)	0.072	0.173	0.341	0.683	1.368
S.D.	0.003	0.006	0.010	0.010	0.030
R.S.D. (%)	4.2	3.7	2.9	1.5	2.2
n	7	7	7	7	7

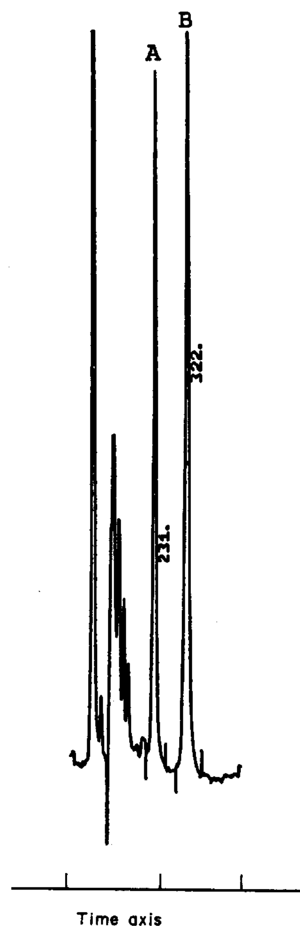


Figure 3
Chromatogram obtained from a plasma sample that was drawn from the same subject as in Fig. 2 after administration of an ibuprofen dosage form. Numbers refer to retention time in seconds. A = ibuprofen (26.6 mg l^{-1}), B = internal standard (mefenamic acid).

Table 3
The intra-day reproducibility as indicated by calibration data

Plasma concentration (mg l^{-1})	0.5	1.0	1.5	2.0	5.0	10.0	20.0	40.0
Peak height ratio (mean)	0.019	0.035	0.052	0.070	0.173	0.350	0.716	1.414
S.D.	0.001	0.001	0.001	0.001	0.002	0.004	0.006	0.016
RSD (%)	7.9	3.1	2.4	1.8	0.9	1.2	0.8	1.1
n	7	7	7	7	7	7	7	7

Table 4
The inter-day reproducibility as indicated by quality control samples over a period of forty days

Theory (mg l^{-1})	4.00	8.00	16.00	32.00	48.00
Range (mg l^{-1})	3.93–4.06	7.82–8.18	15.61–16.35	31.17–32.79	47.00–48.76
Mean (mg l^{-1})	4.02	8.09	16.04	31.96	47.96
S.D.	0.05	0.09	0.23	0.47	0.69
RSD (%)	1.3	1.1	1.5	1.5	1.4
n	8	26	42	10	12

curves on seven subsequent days (plasma concentrations between 2.0 and 40.0 mg l^{-1}). The RSD values are all less than 5%, even at the lowest plasma concentration (mean 2.9%).

The inter-day reproducibility of the assay was also demonstrated by determination of quality control samples during a period of 40 consecutive days. The results are given in Table 4. The low RSD values indicate the very good reproducibility of the assay over long periods.

The stability of the samples that have been prepared by solid phase extraction, and that are ready for HPLC-analysis in the autosampler appears to be good at about 5°C.

Samples prepared from plasma, containing 2.0, 5.0 and 10.0 mg l^{-1} ibuprofen, and stored in a refrigerator for six days, did not show any sign of compound instability as established by LC analysis. On this evidence the assay is considered to be suitable for automation.

The sensitivity of the assay is very high. Using only 250 μl of plasma, ibuprofen concentrations down to 0.5 mg l^{-1} can be accurately measured. This is adequate for the purpose for which the method is intended.

The use of the present extraction method with Baker-10 SPE™ disposable cartridges apparently resulted in clean extracts, since after injection of 3000 samples the LC-columns appeared to show no signs of deterioration.

When analysed in duplicate, about 30 plasma samples can be processed per day by one analyst (including calibration procedures). Again this indicates the suitability of the determination for pharmacokinetic studies.

Conclusion

A simple, specific and sensitive HPLC method for the rapid determination of ibuprofen in human plasma has been developed. Adequate sensitivity and excellent reproducibility of calibration data and quality control samples is demonstrated. The assay is designed for pharmacokinetic studies in adults and children that are currently being carried out in our laboratories. The results of these clinical studies will be discussed elsewhere.

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