Liquid-Chromatographic Assay of Ibuprofen Enantiomers in Plasma
Reza Mehrvar, Fakhraddin Jamali, and Franco M. Pasutto

This stereospecific "high-performance" liquid-chromatographic (HPLC) assay is suitable for pharmacokinetic studies of ibuprofen (IB). Very efficient extraction of the drug and internal standard, (±)-3-ethylcarbodiimide, from plasma with isooctane/isopropanol (95/5, by vol) is followed by sequential reaction of the enantiomers with ethyl chloroformate and (S)-(−)1-(1-naphthyl)ethylamine. The reactions take place at ambient temperature in <4 min. The naphthylethylamide derivatives of IB enantiomers and the internal standard are then extracted into chloroform. After the organic layer is evaporated, the reconstituted residue is chromatographed at ambient temperature on a C18 reversed-phase column with a mobile phase of acetonitrile/water/acetic acid/triethylamine (55/45/0.1/0.02 by vol) at a flow rate of 1 mL/min. The IB diastereoisomers, detected at 232 nm, are free of interferring peaks and have a resolution factor of 2.2. Within the examined enantiomer concentration range of 0.1 to 20 mg/L in plasma, the peak-area ratios varied linearly with the corresponding IB concentrations. We used the assay to study the pharmacokinetics of IB enantiomers in plasma of a subject who took a single 600-mg dose of racemic drug.

Additional Keyphrases: chromatography, reversed-phase · stereospecific assay · pharmacokinetics

Ibuprofen (IB) is a 2-arylpropionic acid nonsteroidal anti-inflammatory drug, marketed as the racemate. The enantiomers, however, differ in their pharmacokinetic properties (J–8), mainly because of a marked unidirectional bioinversion of the pharmacologically inactive R enantiomer to the active S antipode. Several GC (6, 7) and HPLC (2, 8, 9) methods for analyzing individual isomers of IB have been reported. However, most involve lengthy procedures for pre-column derivatization and sample preparation, or lack suitable sensitivity. Very recently, a normal-phase HPLC assay (10) was used for analysis of naphthylethylamide derivatives of IB enantiomers; the derivatization reaction reportedly took 2 h in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide. Here we report the application of a very rapid derivatization technique utilizing ethyl chloroformate and (S)-(−)1-(1-naphthyl)ethylamine in analysis of IB enantiomers in plasma.

Materials and Methods

Apparatus and Chromatographic Conditions

The HPLC (Waters, Mississauga, Ont., Canada) consisted of a Model 590 pump, a Model 481 variable-wavelength ultraviolet detector (set at 232 nm), a 710B Wisp auto-


1 Address correspondence to this author.

This work was presented in part at the 2nd Annual Meeting of the Am. Assoc. of Pharmaceutical Scientists, Boston, MA, June 1987.

Received October 14, 1987; accepted December 2, 1987.
Analytical Recovery and Extraction Yields

We determined how much IB was recovered from plasma by comparing the peak areas for the underivatized drug after extraction from plasma with the areas observed after extraction from aqueous solutions.

To determine the extraction yield for underivatized IB, we compared peak areas before and after extraction. The chromatographic conditions described for the assay of diastereoisomers were also suitable for analysis of underivatized IB; under these conditions, IB was eluted in 4.7 min.

To determine extraction efficiency for the diastereoisomers, we derivatized the IB dissolved in the 50 mmol/L solution of TEA in acetonitrile and compared the areas of derivatized drug injected directly into the HPLC with those obtained after injection of extracted samples. The above studies \( n = 5 \) were carried out at concentrations of 0.5 and 5 mg/L, in the absence of the internal standard. Where necessary, we evaporated exact volumes of the organic layers.

Derivatization Yield

To estimate the unreacted fraction of IB, we derivatized 0.02 mg of racemic IB as described above. The solution was then alkalized with 0.5 mL of 1 mol/L NaOH solution, and 3 mL of isooctane/isopropanol (95/5 by vol) mixture was added. The mixture was vortexed (15 s), centrifuged (2 min), and the organic layer discarded. To 0.5 mL of the aqueous portion was added 0.2 mL of 5 mol/L HCl and the underivatized drug extracted with another 3 mL of the isooctane/isopropanol mixture. Two-milliliter aliquots of the organic layer were transferred to clean glass tubes, the solvent was evaporated, and the residue was taken up in 0.2 mL of the mobile phase. Concentrations of IB were determined against a standard curve obtained after analysis of samples prepared as described above but without derivatization.

Analysis of the underivatized IB was carried out under the HPLC conditions stated above but with a mobile phase consisting of water/acetonitrile/acid/TEA (55/45/0.1/0.02 by vol).

Accuracy and Precision

Six blank plasma samples were supplemented with IB, and concentrations of the individual isomers were determined against a standard curve. The difference between the mean of estimated and the mean of added concentrations was considered as the accuracy of the method. The precision was estimated by calculating the inter-day CVs.

Results and Discussion

Chromatographic resolution of enantiomers is generally accomplished on chiral stationary phases (9), by addition of a chiral reagent to the mobile phase (11), or by pre-column derivatization of the enantiomers with a chiral reagent (2, 7, 8) followed by separation of the resulting diastereoisomers on an achiral phase. Several chiral GC and HPLC stationary phases are now available. However, these columns have limitations, including relatively low thermal stability of many of the GC phases and significant racemization at the critical temperature (12). In the case of HPLC, some columns can only be used with non-aqueous solvents (12), or pre-column achiral derivatization may be necessary to shorten very long retention times (9). Ibuprofen enantiomers in equine urine have been resolved on a chiral HPLC phase after achiral derivatization with benzylamine (9). For the chiral arylpropionic nonsteroidal anti-inflammatory drugs, the presence of a reactive carboxylic acid moiety allows for facile pre-column derivatization with chiral reagents, and this technique has enjoyed extensive applications (2, 6, 13–21). Several GC (6, 7) and HPLC (2, 8) methods have been reported for the separation and quantification of individual IB enantiomers. All of these involve lengthy sample preparation and derivatization procedures. Furthermore, some of these methods are unsatisfactory with respect to resolution and sensitivity (2, 6). Lee et al. (2) derivatized IB by reacting the drug with (S)-2-octanol at 150 °C for 1 h. The diastereoisomers were then separated by normal-phase HPLC on two columns of 5-μm silica, connected in series. However, using 1 mL of plasma, these investigators were not confident in measuring concentrations of individual isomers below 0.5 mg/L because of "significant interference from the contaminating peaks." Furthermore, after each run, it was necessary to purge the system for 20 min at a flow rate twice as fast as that used during the analytical run.

Goto et al. (13) synthesized a highly fluorescent chiral reagent, 1-(4-dimethylamino-1-naphthyl)ethylamine and utilized it for the separation of IB, indoprofen, and naproxen enantiomers. This reagent, however, is not yet commercially available, and a derivatization time of 3 h is required. In subsequent studies the method was applied to normal-phase HPLC determination of naproxen enantiomers in serum, with use of a 45-min derivatization time (14).

Commercially available reagents including (S)-(−)-1-phenylethylamine (6, 8), as well as (R)- and (S)-amphetamine (7) have been used for chiral derivatization and chromatographic resolution of IB enantiomers. In all of these methods, 1,1′-carbonyldimidazole was used as the coupling reagent, and in some cases (7) the reaction required heating for 2 h at relatively high temperatures (85 °C). These conditions are time consuming, and may also cause chemical decomposition of unstable drugs. On the other hand, for the procedure described in this report the maximum yield is reached within 4 min at ambient temperature. Ethyl chloroformate-mediated derivatization of the carboxylic acid group with S-NEA gave distereoisomers that were easily separated by the reversed-phase system. Figure 1 depicts chromatograms of blank and supplemented (0.1 mg of each isomer per liter) plasma, as well as a sample taken 12 h after oral administration of 600 mg of racemic IB (Motrin) to a healthy volunteer. Under the stated conditions, S- and R-IB were eluted with retention times of 18.5 and 21.0 min, respectively, and were separated with a resolution factor of 2.2. Optically pure isomers were unavailable for comparison, so the elution order was assigned on the basis of the relative peak magnitudes of the enantiomers in the volunteer’s plasma. The concentration of the S isomer in plasma reportedly (4, 5) is greater than that of the R isomer after oral administration of racemic IB. As expected, derivatization with R-NEA reversed the elution order of IB diastereoisomers. We preferred to use S-NEA, because derivatization with the antipode resulted in the appearance of interfering peaks that co-eluted with derivatized IB. Other peaks in the blank plasma, presumably byproducts of the reaction, did not interfere with the assay when S-NEA was used. Applicability of this method to analysis of other nonsteroidal anti-inflammatory drugs and (or) the possibility of interference with IB peaks was tested by subjecting ketoprofen, flurbiprofen, etodolac, and tiaprofenic acid to the assay. Ketoprofen and flurbiprofen enantiomers may be conve-
ciently assayed by this method because their derivatized enantiomers are eluted with retention times of 9.6 and 11.1 min for ketoprofen and 14.5 and 17.2 min for furbiprofen. For etodolac, only one peak was observed, with a retention time of 15 min, which was different from that observed after injection of underivatized drug. This implies that although diastereoisomers of etodolac could be produced, the procedure is not capable of resolving the two. For tiaprofenic acid, on the other hand, the drug did not seem to undergo derivatization, because the retention time (7.3 min) was the same before and after derivatization of the drug. Nevertheless, none of the peaks representing enantiomers of these related drugs interfered with those of IB.

In order for us to determine the efficiency of the derivatization method, it was necessary to measure the unreacted IB remaining after the procedures were completed. When the stereospecific method was used for analysis, unreacted IB eluted in 4.7 min, but it could not be measured in the derivatized samples, owing to the presence of interfering peaks representing the reagents and their byproducts. Consequently, to estimate the derivatization yield we used a mobile phase incorporating a decreased proportion of the organic modifier. Under these conditions racemic IB was eluted with a retention time of 9.7 min, free of the interfering peaks.

The derivatization procedure takes place in <4 min with an overall efficiency of 68.7 ± 2.0%. Prolongation of the derivatization time (for the ethyl chloroformate IB reaction to 5 min from 30 s, and for the NEA reaction to 24 h from 3 min) did not significantly affect the overall derivatization yield. However, increasing the concentration of the NEA solution from 1 to 2 mL/L increased the yield to 92.4 ± 1.9% (n = 5). Unfortunately, this increase was associated with a corresponding increase in the intensities of byproduct peaks. Consequently, the 1 mL/L solution was used for subsequent analyses.

During preparation of this manuscript, a new stereospecific HPLC assay for IB was reported (10). In this method, normal-phase chromatography was used for analysis for (S)-naphthylethylamide derivatives of IB enantiomers. Although the final derivatives are the same as those prepared in the present study, the derivatization procedure is entirely different. Their reaction required a 2-h exposure to 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, while ours took place in <4 min on using ethyl chloroformate. Furthermore, their extraction method, which utilizes benzene, was found to be 80% efficient (10). In our hands, however, extraction of IB-containing plasma samples with isooctane/isopropanol (95/5 by vol) was essentially quantitative; 99.6 ± 7.6% and 108 ± 2.2% of the drug was extracted at concentrations of 0.5 and 5 mg/L, respectively. A more efficient extraction procedure reported here counterbalances the less-efficient derivatization yield as compared with those reported in the above study (10).

No significant difference was observed between the extraction of the drug from plasma and water, indicating complete analytical recovery of the drug from plasma (95 ± 7.2% and 102 ± 2.0% for 0.5 and 5 mg/mL concentrations, respectively). The extraction efficiency of IB diastereoisomers was 100 ± 5.3% and 92.5 ± 8.2% at concentrations of 0.5 and 5 mg/L, respectively.

Liquid chromatographic resolution of amide derivatives of N-acetyl-L-amino acids reportedly was possible only on a

![Chromatograms](image)

Fig. 1. Chromatograms of a blank plasma (A), a blank plasma with added isomers, 0.1 mg/L of each (S), and a plasma sample from a subject 12 h after oral administration of a single 600-mg dose of racemic ibuprofen (C)

Peaks 1 and 2: internal standard diastereoisomers; 3 and 4: diastereoisomers of (S)- and (R)-ibuprofen, respectively

<table>
<thead>
<tr>
<th>Table 1. Accuracy and Precision of the Method*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conc., mg/L</strong></td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>0.10</td>
</tr>
<tr>
<td>0.50</td>
</tr>
<tr>
<td>1.00</td>
</tr>
<tr>
<td>2.00</td>
</tr>
<tr>
<td>5.00</td>
</tr>
<tr>
<td>10.0</td>
</tr>
<tr>
<td>20.0</td>
</tr>
</tbody>
</table>

*n = 6; one set in each day.
Normal-phase system (13); the reversed-phase HPLC was then suggested (13) to be less efficient in separation of amide diastereoisomers in general. Recent reports from our laboratory and other laboratories, however, indicate that the reversed-phase HPLC can be efficiently applied for separation of amide diastereoisomers of ketoprofen (18, 19) and fenoprofen (21). The present study is also in agreement with our previous experience.

Excellent linearity was observed between the peak area ratios (R- and S-IB/IS) and the corresponding plasma concentrations over the examined concentration range (r > 0.999). A typical standard curve could be described by the equations: y = 0.0073 + 0.1439x and y = 0.0065 + 0.1403x, for the S and R enantiomers, respectively, where y is the peak-area ratio (R or S IB/IS) and x is the IB enantiomer concentration.

The observed limited inter-day variations and differences between added and measured concentrations (Table 1) indicate acceptable reproducibility and accuracy for the assay, respectively. The lowest examined concentration in plasma, 0.1 mg/L, was associated with an error of 8.0% and 7.0% and an inter-day variation of 6.7% and 8.5% for the S and R isomers, respectively. However, if the criterion of signal-to-noise ratio were used, a sensitivity of greater than 0.1 mg/L could be claimed (Figure 1).

The applicability of the method to pharmacokinetic studies of IB enantiomers after administration of the usual doses was examined by analysis of plasma sampled from a healthy subject after oral administration of a single 600-mg dose of the racemic drug. The time courses of the isomers are depicted in Figure 2. As expected, the concentrations of the more-active S isomer in plasma exceeded those of the less-active antipode, and the enantiomers had similar elimination half-lives (5).

This study was supported by Grant No. MA9669 of the Medical Research Council of Canada. R. M. was the recipient of an Alberta Heritage Foundation for Medical Research Studentship.

References