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Resolution of the enantiomers of ibuprofen; comparison study of diastereomeric method and chiral stationary phase method

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Abstract

In this study, an indirect diastereomeric method and a direct method utilizing a chiral stationary phase (CSP) were investigated for the resolution of ibuprofen enantiomers. In the indirect method, ethylchloroformate (ECF) and 2-ethoxy-1-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) were utilized as first-step derivatizing reagents in acetonitrile or toluene. In the direct CSP method, ibuprofen enantiomers were derivatized to *p*-nitrobenzyl ureides and then resolved on an (*R*)-(−)-(1-naphthyl)ethylurea CSP column. The derivatization procedure took place in 10 min with an overall inversion efficiency of 90.3%. Racemization was not observed under the derivatization conditions used. The HPLC-CSP method was utilized to study the pharmacokinetics of ibuprofen enantiomers in dog plasma after a single oral administration of 200 mg of ibuprofen racemate.

1. Introduction

There has been considerable interest in the stereospecific pharmacokinetics, metabolism and clinical pharmacology of chiral drug molecules. This interest has been stimulated in part by the development of highly efficient chromatographic methods for the analytical determination of enantiomers in biological fluids. The pharmaceutical industry has also placed new emphasis on the synthesis, isolation and analysis of enantiomerically pure drugs. Accordingly, it is not only important but now also feasible to determine low levels of each of the enantiomers of a chiral drug.

A variety of HPLC methods for the resolution of the enantiomers of ibuprofen have been reported. The majority have employed indirect chiral chromatographic methods based on the formation of diastereomeric derivatives [1–4]. However, it has been reported that diastereomeric approaches may introduce inaccuracies into the determination of enantiomeric ratios due to chiral impurities in the derivatizing agent or to racemization during the derivatization procedure [5]. An alternative approach which

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avoids these problems is direct enantiomeric analysis using enantioselective chiral stationary phases (CSPs). Over the past decade, many different CSPs have become available and have been applied to problems of stereochemical analysis [6–12].

In the present study, a direct approach for the resolution of the ibuprofen enantiomers, as their *p*-nitrobenzylurea derivatives, by chromatography on a chiral stationary phase was investigated as well as an indirect chiral chromatographic method. The chiral stationary phase consisted of *R*-1-(1-naphthyl)ethylurea, covalently bound to silica through a propyl linkage. The derivatizing agent, *p*-nitrobenzylamine is achiral. Therefore, there is no possible analytical error from enantiomeric contamination of the reagent. The procedure is based on a CSP, originally described by Oi *et al.* [11], which we prepare by a simple *in situ* technique as described by Doyle *et al.* [12]. The derivatization is based on a convenient method for urea formation introduced by Pirkle *et al.* [9]. The suitability of this new procedure for determination of ibuprofen in plasma was demonstrated by analysis of spiked plasma samples, and by analysis of samples from a dog. The extent of racemization during the derivatizing procedure of one of the previously described indirect methods [4] was also investigated. We found that the degree of racemization for such methods was directly affected by the nature of the derivatizing agents and by the reaction solvents. This was confirmed by the analysis employing the direct CSP method.

2. Experimental

2.1. Indirect method

Chemicals

Racemic ibuprofen (secondary reference standard) was obtained from Boots Pharmaceuticals (Shreveport, VA, USA). (*S*)-(+)-Ibuprofen (Lot No. 152-20, optical purity >99.9%) and (*R*)-(−)-ibuprofen (Lot No. 111-78c, optical purity 99.0%) were kindly donated from Sepracor (Marlborough, MA, USA). Ethylchloroformate (ECF) was purchased from Aldrich (Milwaukee, WI, USA) and (*S*)-(−)-l-(l-naphthyl)ethylamine (S-NEA) was obtained from Sigma (St. Louis, MO, USA). All other materials were reagent or HPLC grade.

Chromatographic apparatus and conditions

The HPLC system (Spectra Physics, San Jose, CA, USA) consisted of an SP 8800 ternary HPLC pump, Spectra FOCUS forward optical scanning detector, SP 8880 autosampler fitted with a 100-μl loop and Spectra FOCUS software for data analysis. An Ultrasphere ODS (octadecylsilane), particle size 5 μm, 100 × 4.6 mm I.D. analytical column (Beckman, San Ramon, CA, USA) was used. The mobile phase consisted of acetonitrile–water–acetic acid–triethylamine (55:45:0.1:0.02, v/v) [4], and was delivered at a flow-rate of 1.2 ml/min.

Standard solutions

Solutions of triethylamine (TEA, 50 m*M*), ethylchloroformate (ECF, 6 m*M*), (*S*)-naphthylethylamine (S-NEA, 1 ml/l) and 2-ethoxy-l-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ, 1 mg/ml) were prepared in acetonitrile and toluene, separately. Ibuprofen isomers (100 μg/ml) were dissolved in methanol.

Sample preparations

When ethylchloroformate was used as a first-step derivatizing agent, the sample was prepared as described by Mehvar *et al.* [4] with a few modifications. Ibuprofen enantiomers were extracted from the plasma (0.5 ml) with 3 ml of isooctane–isopropanol (95:5, v/v) and derivatized by adding 0.1 ml of TEA solution, 50 μl of ECF solution and 25 μl of S-NEA solution. Two solvents, acetonitrile and toluene, were separately evaluated for this purpose. After 3 min, the reaction was stopped by adding 0.5 ml of 0.25 mmol/l of HCl and the derivatives were extracted with 3 ml of chloroform. The extracts were evaporated to dryness and the residue was reconstituted in the mobile phase for HPLC injection.

To study the effect of the derivatizing agent, the following procedure was employed: Ibupro-

fen isomers in 0.5 ml of distilled water or dog plasma were extracted with 3 ml of isooctane–isopropanol (95:5, v/v). After the organic solvent was evaporated, 0.1 ml of TEA, 200 μl of EEDQ, and 100 μl of S-NEA were added and the sample was heated for 1 h at 80°C. The reaction was stopped and derivatives were extracted with 3 ml of chloroform. The extracts were evaporated to dryness and the residue was reconstituted in 200 μl of the mobile phase, 100 μl was injected.

2.2. Direct method

Chemicals

The derivatizing reagents, 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) and *p*-nitrobenzylamine hydrochloride (PNBA), and an internal standard, tridecanoic acid, were purchased from Aldrich. Racemic ibuprofen and ibuprofen isomers were obtained as above. All other materials were reagent or HPLC grade.

Preparation of the chiral stationary phase

A 100 × 4.6 mm I.D., 3 μm, aminopropylsilanized silica column (Regis, Morton Grove, IL, USA) was installed in the analytical HPLC system, except that the detector was by-passed. A solution of 2 g (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate (98% purity, Aldrich) in 100 ml of methylene chloride was pumped through the column at 2 ml/min, at ambient temperature. After the first 25 ml, the remaining eluate was recycled; pumping was continued for two hours. The column was washed with 200 ml of methylene chloride at 2 ml/min. The detector was then reconnected, and washing was continued with an additional 100 ml of methylene chloride and then with isopropanol–hexane (20:80, v/v) until a steady baseline level was achieved [11]. For improved resolution two separately prepared columns were connected in series (total 200 mm length).

Chromatographic apparatus and conditions

The HPLC system was as described above; the mobile phase consisted of hexane–isopropanol (35:5, v/v). The flow-rate was 1.5 ml/min and the detection wavelength was set at 235 nm.

Standard solutions

EEDQ (2.4 mg/ml) solution was prepared in ethylene chloride. PNBA was prepared by the following procedure: 5 mg of PNBA was dissolved in 5 ml of 0.2 *M* NaOH, and then extracted with 5 ml of ethylene chloride. The ethylene chloride layer was dried with sodium sulfate. Tridecanoic acid (0.2 mg/ml) solution was prepared in ethylene chloride and used as internal standard.

Sample preparation

To 0.5 ml of dog plasma containing ibuprofen, 0.1 ml of internal standard solution (0.2 mg/ml) and 0.2 ml of sulfuric acid (0.6 *M*) were added. Ibuprofen and internal standard were extracted with 3 ml of isooctane–isopropanol (95/5, v/v). The organic layer was transferred to a clean tube and evaporated to dryness. To the residue 1 ml of EEDQ solution and 5 ml of PNBA solution were added. The solution was refluxed for 10 min, diluted to 10 ml with ethylene chloride, and then washed with equal volumes of 0.2 *M* NAOH, 1 *M* HCl and water. The organic layer was dried with sodium sulfate and evaporated to dryness, and the residue was reconstituted with mobile phase for HPLC injection.

Derivatization yield

To estimate the unreacted fraction of ibuprofen, 100 μg of racemic ibuprofen was derivatized as described above and washed with 10 ml of 0.2 *M* NaOH solution. Underivatized ibuprofen (NaOH aqueous layer) was separated from the derivatized portion (organic layer). The aqueous layer was acidified with concentrated HCl solution and underivatized ibuprofen was extracted twice with an equal volume of isooctane–isopropanol. After the solvent was evaporated, ibuprofen was derivatized again and washed with 0.2 *M* NaOH, 1 *M* HCl and water in sequence.

3. Results and discussion

3.1. Racemization in indirect method

Although diastereomeric derivatization has been widely used in the HPLC resolution of ibuprofen enantiomers, the potential for racemization always exists. As shown in Table 1, the racemization was markedly dependent on solvent, when ECF was used as first-step derivatizing agent. Acetonitrile induced more racemization of ibuprofen enantiomers than toluene. By contrast, solvent effects were not found when EEDQ was used as a first-step derivatizing agent. Acetonitrile induced the same degree of racemization as toluene. Although ECF has been commonly used as a first-step derivatizing reagent in the formation of diastereomeric derivatives [4], EEDQ in acetonitrile appears to be a better choice than ECF for minimizing the racemization. However, derivatization with EEDQ requires a longer time and involves heating. Table 1 shows that the degree of racemization of ibuprofen enantiomers with ECF in toluene was similar to that with EEDQ in toluene. The degree of racemization was calculated based on the simple area-percent method. Although the data is not available, it was observed that the degree of racemization was sensitive to the reaction conditions, including the temperature and duration of the evaporation. Despite these disadvantages, there are some advantages as well; these include improved peak symmetry and resolution, since the separation occurs on achiral columns, compared to the direct method [13].

3.2. Derivatization in direct method

Although some chiral compounds can be separated on CSPs without derivatization, frequently it is essential to modify the compounds. Three objectives are attained by derivatization:
(a) Attenuation of the polarity of the functional groups of the solute, so that individual polar interactions are not of a magnitude to overwhelm the influence of other interactions.
(b) Introduction of an aromatic functionality with π-basicity or acidity, complementary to the aromatic function in the CSP.
(c) Enhancement of the ultraviolet/visible or fluorescence detectability of the solutes.
The first two objectives are often essential for enantiomeric resolution; the third is non-essential but often desirable [14]. Since the CSP in the present study contains a π-basic naphthyl group, chiral resolution is most effective when the solutes contain a π-acidic group. The introduced nitrobenzyl group, in addition to being π-acidic, is also achiral. Therefore, the resulting amides are enantiomeric rather than diastereomeric.

During exploratory experiments to determine the most appropriate ibuprofen derivative, it was found that aromatic amines such as 3,5-dinitroaniline and *p*-nitroaniline produced amides of ibuprofen which afforded high enantioselectivity on the CSP. However, the derivatization was unacceptably slow, and yields were poor under non-racemizing conditions. By contrast, benzylamine reagents, including PNBA, produced high conversions in reasonable time, most likely due to the increased basic character of the aliphatic amine functionality. The derivatization

Table 1
Effects of derivatizing reagents (ECF *vs.* EEDQ) and solvents (acetonitrile *vs.* toluene) on the degree of racemization of ibuprofen enantiomers

	ECF		EEDQ	
	Acetonitrile	Toluene	Acetonitrile	Toluene
S to *R*	4.32 ± 0.19	0.21 ± 0.37	1.27 ± 0.08	1.27 ± 0.03
R to *S*	5.06 ± 0.24	3.15 ± 0.22	2.89 ± 0.85	3.34 ± 0.08

Reported as the mean ± S.D. percentages of three experiments.

procedure takes place in 10 min with an overall efficiency of 90.3% yield ($n = 5$, 6.98% C.V.). Prolongation of the derivatization time did not significantly affect the overall derivatization yield.

3.3. Chromatographic results

Fig. 1 shows typical chromatograms of the blank dog plasma and the resolution of (*R*)-(−)- and (*S*)-(+)- ibuprofen as the *p*-nitrobenzyl ureides, resulting from resolution on the CSP. Chromatographic parameters were calculated and the results of a comparison of a 1-column (100 mm, 3 μm) *versus* a 2-column system (two 100 mm identical columns in series) system for the resolution of the enantiomeric ibuprofen derivatives are in excellent agreement with theory under the same experimental conditions

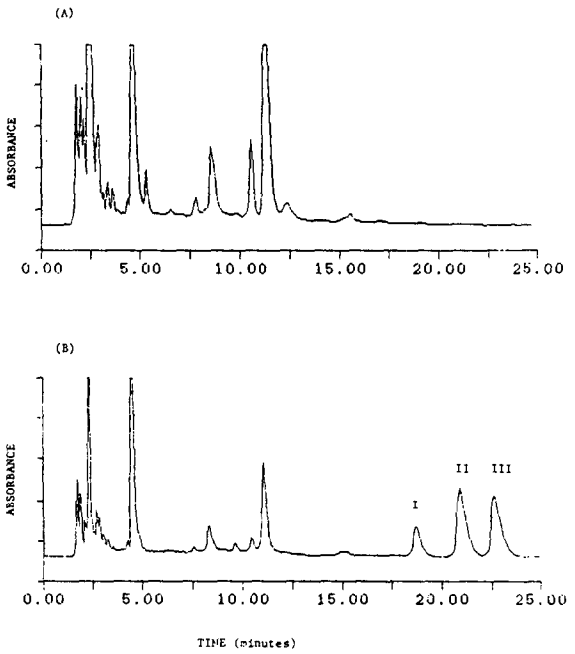

Fig. 1. Resolution of *R*(−)- and *S*(+)-ibuprofen on the (*R*)-NEU CSP. Chromatograms of a blank plasma (A) and a blank plasma spiked with ibuprofen racemate (B). Key: I, internal standard; II and III, (S)- and (R)-ibuprofen, respectively.

(mobile phase, flow-rate, etc.). The capacity factors ($k'_1 \approx 13$ and $k'_2 \approx 15$) and the separation factors ($\alpha = 1.09$) are found to be independent of column length.

In theory the number of theoretical plates (N) is directly proportional to column length. The 2-column system ($N = 9820$) showed nearly twice the plate count of the one-column system ($N = 5060$). Thus, there was no observable peak broadening resulting from the extra column factor of the additional coupling. The net effect is reflected in the observed resolution factors, (R_s), which in theory are proportional to the square root of the plate count, other parameters remaining constant. The theoretical values, calculated from the equation:

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k'}{k' + 1}$$

are in reasonable agreement with the observed values. More significantly, the increase in resolution upon doubling the effective column length of the system is expected to be 41%; the observed increase (R_s 1.47 to 2.00) was 36%.

The practical consequences are that the 100-mm system is adequate for most applications, affording an R_s value of close to the accepted benchmark of 1.5, which is required for baseline resolution. However, the 2-column, 200-mm system provides the necessary ruggedness and margin for error required for detection of small amounts of one enantiomer in the presence of the other. Thus, this system is ideal for studies of racemization of ibuprofen. This result was actually achieved with a shorter retention time than that required for analysis using a more traditional (and less satisfactory) 250-mm, 5-μm column.

No peak corresponding to the antipode was observed in this experiment; this suggests that no racemization occurs under the derivatization conditions. Excellent linearity was observed between the peak-area ratios (*R*- and *S*-IB/I.S.) and the corresponding plasma concentrations over the examined concentration range ($r^2 > 0.990$). A typical standard curve fits the equations $y = -0.040 + 0.023x$ and $y = -0.043 + 0.023x$ for the (*R*)- and (*S*)-enantiomers, respectively, where y is the peak-area ratio (*R* or

S-IB/I.S.) and x is the IB enantiomer concentration. Quantitation limit was 2.5 μg/ml. The coefficient of variation (C.V.%) and relative error for the assay are reported in Table 2.

3.4. Application to pharmacokinetic studies

Although the present study was primarily concerned with the chemical and chromatographic aspects of a new chiral resolution technique, some results concerning the feasibility of the procedure for pharmacokinetic studies of ibuprofen enantiomers were obtained by analysis of plasma samples from a dog after oral administration of a single 200 mg dose of ibuprofen

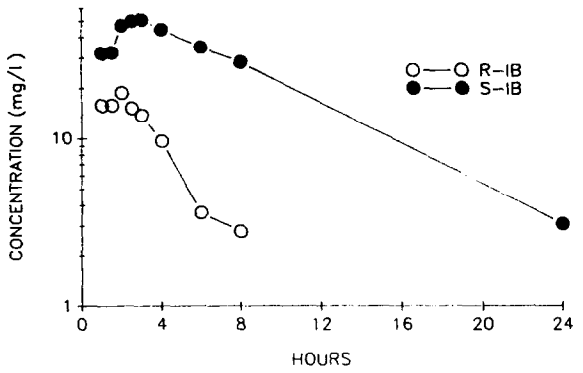

Fig. 2. Plasma concentration–time profiles of *S*- and *R*-ibuprofen in a dog after a single 200 mg oral dose of racemic ibuprofen.

Table 2
Validation of direct method HPLC (chiral stationary phase) on two separated days (1 and 2) for assay of *R*- and *S*-ibuprofens in dog plasma

Spiked concentration (μg/ml)		Observed concentration (mean ± S.D., $n = 3$) (μg/ml)	C.V. (%)	Mean relative error (%)
S-Ibuprofen				
5.0	1	5.63 ± 0.06	1.1	+ 13
	2	4.37 ± 0.06	1.4	− 13
10.0	1	8.36 ± 0.22	2.6	− 16
	2	10.4 ± 1.08	10	+ 4.0
25.0	1	23.3 ± 0.25	1.1	− 6.8
	2	24.2 ± 0.62	2.6	− 3.2
50.0	1	46.2 ± 3.32	7.2	− 7.6
	2	50.4 ± 1.56	3.1	+ 0.8
100	1	95.6 ± 8.18	8.6	− 4.4
	2	99.9 ± 3.27	3.3	− 0.1
R-Ibuprofen				
5.0	1	5.40 ± 0.05	0.9	+ 8.0
	2	4.27 ± 0.10	2.3	− 14
10.0	1	8.59 ± 0.73	8.5	− 14
	2	10.6 ± 2.30	22	+ 6.0
25.0	1	23.0 ± 0.32	1.4	− 8.0
	2	24.6 ± 1.13	4.6	− 1.6
50.0	1	46.0 ± 3.30	7.2	− 8.0
	2	50.2 ± 2.26	4.5	− 0.4
100	1	95.0 ± 8.04	8.5	− 5.0
	2	101 ± 3.97	4.0	+ 0.1

racemate. The time courses of the isomers are depicted in Fig. 2.

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