Figures of Merit in the Quantification of Ibuprofen Enantiomers by Chiral HPLC

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Abstract

The determination of ibuprofen (IBU) enantiomers by chiral high-performance liquid chromatographic is described. The methodology is based on chiral recognition of ibuprofen by a chiral column based on cellulose tris(4-methylbenzoate) coated on silica gel (Chiralcel OJ-H). The mobile phase is *n*-hexane–2-propanol–trifluoroacetic acid (98:2:0.1, v/v/v). The flow rate was 1.0 mL/min, and UV detection was 254 nm. The samples of ibuprofen were prepared in *n*-hexane in the concentration range 50–100% of (*S*)-IBU 1×10^{-3} mol/L. Calibration and validation method were performed with six and nine samples, respectively. Goodness-of-fit measures represented by correlation coefficient, y-intercept, and slope of the regression line were 0.9836, 21373, 2162, respectively. Average of the relative error of the proposed method was 3.0%, 0.9% (*S*)-IBU selectivity, and 2162% (*S*)-IBU^{-1} sensitivity. The minimum concentration difference between two samples that could be determined in the linear dynamic range was 0.4% (*S*)-IBU. Limits of detection and quantification were 8.1 and 27.0% (*S*)-IBU, respectively. These results indicate that the proposed method can be employed for determination of the enantiomeric composition of IBU.

Introduction

Ibuprofen (IBU), [(±)-2-(4-isobutylphenyl)-propionic acid] is a non-steroidal anti-inflammatory drug, which presents two enantiomeric forms due to the presence of an asymmetric carbon atom (Figure 1). It is commercialized as a racemic mixture, but it has been demonstrated that the anti-inflammatory activity is due to the (*S*)-enantiomer (1–3). There has been considerable interest in the stereospecific pharmacokinetics, pharmacodynamics, metabolism, and clinical pharmacology of chiral drug molecules. The pharmaceutical industry has placed new emphasis on the synthesis, isolation, and analysis of enantiomers and to determine low levels of every enantiomer of a chiral drug is extremely important, and it is feasible to do (4).

The high-performance liquid chromatographic (HPLC) methods for the resolution of ibuprofen enantiomers reported in literature are based on the formation of diastereomeric derivatives (4–21). However, this approach may introduce inaccuracy into the determination of enantiomeric ratios due to chiral impurities in the derivatizing agent or to racemization during the derivatization procedure (4). Moreover, other possible disadvantages of this approach are that the rates of reaction for the formation of the two diastereomers may be different, thus resulting in an incorrect ratio of the two diastereomers; and impurity of the chiral reagent may produce four diastereomers instead of only two (13).

Cellulose-based chiral stationary phases (Chiralcel OJ) have been used for chiral separation of ibuprofen enantiomers after derivatization into their amide (22) and resolution of ibuprofen esters (7). Chiralcel OJ stationary phase was utilized for the thermodynamic study of enantioseparation of arylpropionic acids (23). The significance of the mobile phase composition in enantioseparation based on cellulose-based chiral stationary phase was evaluated (24).

The purpose of this study was to quantify the ibuprofen enantiomers by HPLC with cellulose-based chiral stationary phase. We will present the results of several validation parameters which were tested.

Materials and Methods

Chemical and materials

(±)-Ibuprofen and (–)-ibuprofen were purchased from Sigma Aldrich (St. Louis, MO). *N*-Hexane 85% and 2-propanol was purchased from Tedia (Fairfield, OH). Trifluoroacetic acid was purchased from Fluka (St. Louis, MO).

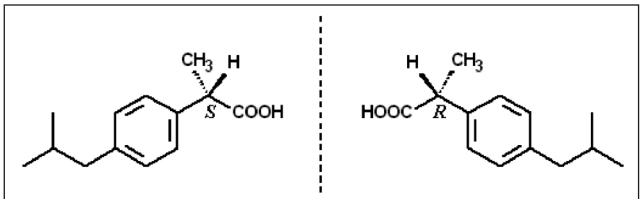

Figure 1. Chemical structures of IBU enantiomers.

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Apparatus and chromatographic conditions

The HPLC was carried out using a Shimadzu LC-20AT HPLC system, which consisted of a binary gradient pump model LC-20AT, a SPD-M20A diode-array detector, a CBM-20A communications bus module, and a DGU-20A5 degasser. The apparatus was interfaced with a compatible computer using LC solution software (Kyoto, Japan).

The separation was performed on a chiral column based on cellulose tris(4-methylbenzoate) coated on 5 mm silica-gel Chiralcel OJ-H (Daicel Chemical Industries, Osaka, Japan) (150 mm × 4.6 mm, 5 mm). The mobile phase consisted of *n*-hexane–2-propanol–trifluoroacetic acid (98:2:0.1, v/v/v) and was filtered through a 0.45-mm polyvinylidene fluoride membrane filter prior to use. The mobile phase was delivered at a flow rate of 1.0 mL/min. Detection was performed at a wavelength of 254 nm. The sample injection volume was 20 mL.

Sample preparation procedures

A 1.0×10^{-2} mol/L stock solution of (±)-ibuprofen was prepared by dissolving 0.02063 g in 10 mL of *n*-hexane. This procedure was repeated for the (–)-ibuprofen enantiomer. For the analysis, a data set of six solutions for calibration, and nine solutions for validation in the concentration of 1.0×10^{-3} mol/L of the ibuprofen enantiomers were prepared in 10-mL flasks by dilution of the stock solution in *n*-hexane. The range from 50 to 100% of the (–)-ibuprofen was considered in the sample preparation as shown in Table I.

Figures of merit

The estimation of figures of merit for univariate calibration was calculated in this work in accordance with the following definitions:

Accuracy reports the closeness of agreement between the reference value and the value found in the determined assay (25).

Precision represents the degree of scatter between a series of measurements for the same sample under prescribed conditions. It is expressed as the standard deviation of a series of measurements (25).

$$s = \sqrt{\frac{\Sigma(x_i - \bar{x})^2}{n-1}} \qquad \text{Eq. 1}$$

where $\bar{x}$ is the arithmetic mean of a small number of measurements, x_i is the value of the individual measurement and n is the number of measurements. In agreement with ICH (25), it should be determined as the mean of the standard deviation of a minimum of three measurements on a minimum of three samples.

Linearity is the capacity of the method in supplying results directly proportional to the concentration of the substance of interest inside the application range (25–27). The correlation between the peak-area ratio and the concentration is called analytical calibration curve. The least squares linear regression analysis is used to determine the equation that supplies the slope, intercept, and correlation coefficient, and represents the linearity of the univariate model.

Sensitivity this parameter informs what fraction of the analytical signal is due to the increase of the concentration of a particular analyte at unitary concentration (28). In a univariate calibration model, it is defined as the slope of the analytical calibration curve.

Analytical sensitivity is defined as the ratio between the sensitivity and the instrumental noise (29–31). In univariate calibration, the instrumental noise is defined as the standard deviation of a blank sample ($s_{y/x}$) as (32):

$$s_{y/x} = \left\{ \frac{\sum_i (y_i - \hat{y}_i)^2}{n-2} \right\}^{1/2} \qquad \text{Eq. 2}$$

where y_i is the value of the peak-area of an individual measurement of the calibration sample, $\hat{y}_i$ is the arithmetic mean of the measurements of the calibration samples, and n is the number of the measurements in the calibration set. The inverse of the analytical sensitivity reports the minimum concentration difference between two samples, which can be determined by the model (29–31).

Selectivity has been defined by IUPAC as the ratio of the slopes of the calibrations lines of the analyte of interest and a particular interference (31):

$$\hat{i}_{i,a} = \frac{s_a}{s_i} \qquad \text{Eq. 3}$$

where s_a and s_i denote the sensitivity of the analyte and interference, respectively. Ideally, the selectivity indices should be evaluated for each important interferences likely to be present in varying amounts because this will lead to biased predictions.

Limit of Detection (LOD), following the IUPAC recommendations, can be defined as the minimum detectable value of concentration for which the probability of false negatives (b) and false positives (a) is 0.05 (28). More simply, it is the analyte concentration giving a signal equal to the blank signal, y_B, plus three standard deviations of the blank, $s_{y/x}$ (32):

$$y - y_B = 3s_{y/x} \qquad \text{Eq. 4}$$

Table I. Composition of the Enantiomers Used for Calibration and Validation

Sample	Mole fraction (+)-IBU	Volume of the (±)-IBU (µL)	Mole fraction (–)-IBU	Volume of the (–)-IBU (µL)
Calibration				
1	0.50	1000	0.50	0
2	0.40	800	0.60	200
3	0.30	600	0.70	400
4	0.20	400	0.80	600
5	0.10	200	0.90	800
6	0	0	1	1000
Validation				
1	0.45	900	0.55	100
2	0.43	860	0.57	140
3	0.38	760	0.62	240
4	0.35	700	0.65	300
5	0.33	660	0.67	340
6	0.25	500	0.75	500
7	0.18	360	0.82	640
8	0.15	300	0.85	700
9	0.05	100	0.95	900

where y_B is the intercept of the analytical calibration curve.

From y results, the LOD is obtained from the equation of the analytical calibration curve.

Limit of quantification (LOQ) is expressed in terms of the analyte concentration value that will produce estimates having a specified relative standard deviation (28). The LOQ can be calculated by the analyte concentration giving a signal equal to the blank signal, y_B, plus ten standard deviations of the blank, $s_{y/x}$:

$$y - y_B = 10 s_{y/x} \quad \text{Eq. 5}$$

Analogously, as for LOD from y results, the LOQ is obtained from the equation of the analytical calibration curve.

Results and Discussion

Figure 2 shows the chromatograms of racemic IBU and pure (S)-IBU. The retention times of (*R*)-IBU and (*S*)-IBU were approximately 8.6 and 9.6 min, respectively, in racemic samples, and approximately 9.3 min in (*S*)-IBU pure form samples. The peaks were sharp and symmetrical with good baseline resolution.

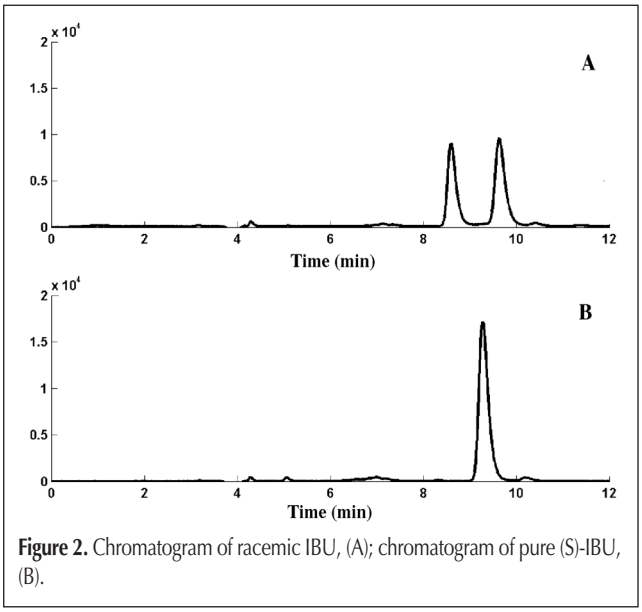

Figure 2. Chromatogram of racemic IBU, (A); chromatogram of pure (S)-IBU, (B).

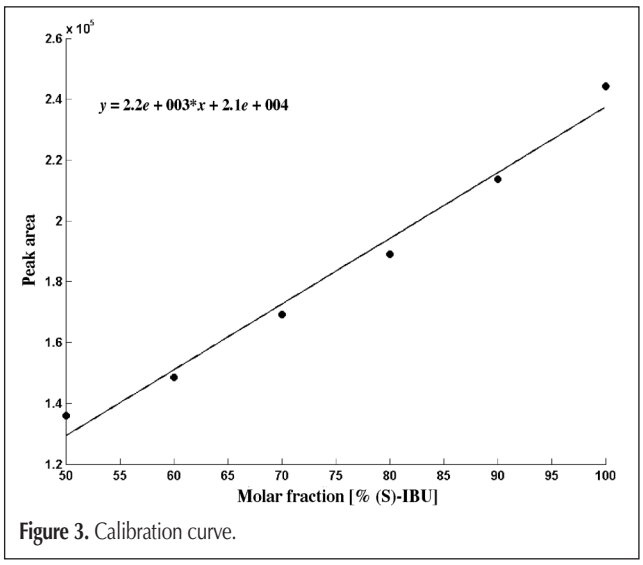

Figure 3. Calibration curve.

The calibration curve in the concentration range from 50–100% of the (*S*)-IBU was constructed by plotting the peak-area ratio of (*S*)-IBU versus (*S*)-IBU nominal concentration (Figure 3). The model was based on (S)-IBU concentration because it is 160-fold more active than its antipode, and nowadays, there is an increasing interest in obtaining and analyzing this pure enantiomeric form.

The least squares linear regression analysis was used to determine the linearity of the univariate model and is represented by the slope, intercept, and correlation coefficient. Their values are shown in Table II. Good linearity was achieved with correlation coefficient higher than 0.99.

Figure 4 shows the satisfactory fit of the univariate model, presented by plotting the reference values against the estimates for pure enantiomers.

Apart from the linearity, several validation parameters such as accuracy, precision (repeatability), LOD, LOQ, sensitivity, analytical sensitivity, and selectivity were also examined. The results are shown in Table II.

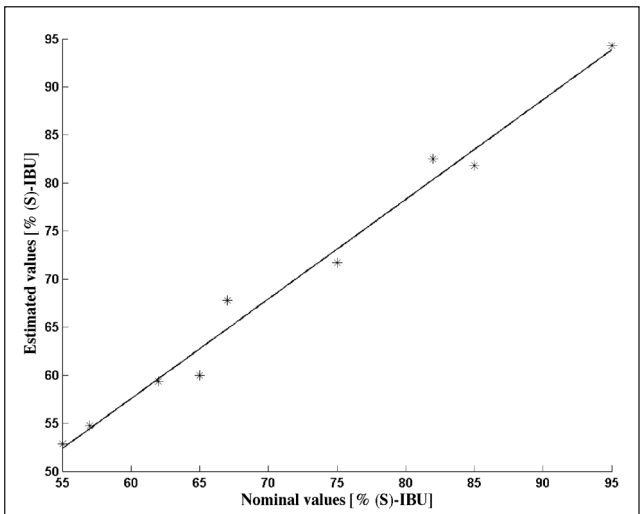

Figure 4. Nominal values against the values estimated by univariate model.

Table II. Analytical Figures of Merit for Univariate Model

Figures of merit		Pure form (S)-IBU
Accuracy*		3.0
Precision*		7.0
Sensitivity†		0.022×10^5
Analytical Sensitivity^{-1} *		0.4
Selectivity		0.9
	Slope	0.022×10^5
Fit	Intercept	0.21×10^5
	Correlation. Coef.	0.9918
LOD*		8.1
LOQ*		27.0

* % (S)-IBU;
† % (S)-IBU^{-1}.

The accuracy of the model presented a good agreement with nominal values. The prediction error was 3%. Precision, at the level of repeatability, was assessed by analysis of three samples with three replicates each. Measurements were made on the same day and showed results around 7%.

For sensitivity and analytical sensitivity, good results were observed taking into account the linear dynamic range. Analytical sensitivity is simpler and more informative for comparison and judgment of the sensitivity of an analytical method. It is possible to establish a minimum concentration difference, which is discernible by the analytical method, to the range it was applied. Based on this result, it is possible to distinguish between samples with value differences around 0.37% (*S*)-IBU.

The respective LOD and LOQ of 8.11% and 27.02% (*S*)-IBU, which were obtained, showed coherent compatibility with the measured quantities.

Conclusion

The proposed chiral HPLC method is simple and shows good results for quantification of the ibuprofen enantiomers. The methodology was validated by determination of the figures of merit. Errors in the order of 3.00% were obtained, and the model showed significant sensitivity, differentiating samples with less than 0.4% difference in concentration.

Acknowledgements

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