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Optical Purity Determination of (*S*)-Ibuprofen in Tablets by Achiral Gas Chromatography

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An optical purity test was indirectly performed on (*S*)-ibuprofen as its diastereomeric (*R*)-(+)-1-phenylethylamide derivative using achiral gas chromatography (GC). The method for the determination of trace (*R*)-ibuprofen (optical impurity), within the range 1.0 to 50 ng, from a racemic ibuprofen standard was linear (r = 0.9997) with acceptable precision (% RSD ≤ 5.3) and accuracy (% RE = 0.7 ~ -3.9). Similar results were obtained with the method validation for the quantification of (*S*)-ibuprofen within the range 0.1 to 2.0 μg using a (*S*)-ibuprofen standard. When applied to seven different commercial (*S*)-ibuprofen products, their optical purities (98.7 ~ 99.1%) were determined with good precision (% RSD ≤ 4.0).

Key words: Enantioseparation, Ibuprofen, Optical purity, (*R*)-(+)-1-Phenylethylamime, Achiral gas chromatography, Chiral separation

INTRODUCTION

The anti-inflammatory activity of chiral ibuprofen, (±)-2-(4-isobutylphenyl) propionic acid, is ascribed to the (*S*)-(+)-enantiomer (Lombardino, 1985; Tracy and Hall, 1992). Its antipode, (*R*)-(-)-ibuprofen, exhibits different toxicological and pharmacological properties (Tracy and Hall, 1992; Jamali and Wainer, 1993). Hence, for the production of active (*S*)-ibuprofen in an enantiomerically pure form, the study of its optical purity control and stereoselective pharmacokinetics have become important tasks (Davis, 1997; Bhunshan and Martens, 1998).

For the direct enantioseparation of ibuprofen without derivatization, chiral capillary electrophoresis (CE) permits faster method development, with easier reversal of the enantiomer migration order (Bjornsdottir *et al.*, 1998; Blanco *et al.*, 1998; Abushoffa *et al.*, 2002; Jabor *et al.*, 2002; La *et al.*, 2003). However, this has a precision problem with regard to the migration times of runs compared to high-performance liquid chromatography (HPLC) or gas chromatography (GC). The majority of selective, sensitive and versatile HPLC methods have been based on the indirect enantioseparation, as diastereomeric derivatives, on conventional reversed-phase columns (Rudy *et al.*, 1990; Wright and Jamali, 1993; Pehourcq *et al.*, 1995; Thomason *et al.*, 1997; Santa *et al.*, 1998; Yasaka *et al.*, 1998). However, the responses of diastereomers with UV or fluorescence detection are not necessarily identical, but this is not a problem when using GC with flame ionization detection, as explained elsewhere (Carlson and Gyllenhaal, 1990; Davis, 1997). If their indirect separation as diastereomeric derivatives were to be used, GC combined with mass spectrometry (MS), employing achiral capillary columns, with incomparably higher resolving power and long-term durability, would offer more rapid and robust analyses with positive peak identification suitable for pharmacokinetic studies (Baillie *et al.*, 1989; Blessington *et al.*, 1989; Carlson and Gyllenhaal, 1990; Paik *et al.*, 2004). In a recent report (Paik *et al.*, 2004), chiral profiling and screening analysis of multiple profens was studied employing achiral dual-capillary columns with different polarities. Eight profens were studied and rapidly (within 3 min) converted to their diastereomeric (*R*)-(+)-1-phenylethylamide derivatives with no evidence of racemization. Moreover, the achiral GC system provided simultaneous enantioseparation of eight profens, including ibuprofen, with high enantioresolution within a single 30 min run. However, achiral GC methods have rarely been employed in routine optical purity tests for active (*S*)-ibuprofen in tablets.

The present study was undertaken to determine the optical purity of active (*S*)-ibuprofen in seven different products employing our previous achiral GC method (Paik

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et al., 2004). In this study, a single DB-17 MS column of intermediate polarity was employed to achieve higher enantioresolution of ibuprofen. The reaction conditions for (*R*)-(+)-1-phenylethylamidation were further optimized to reduce the regent bumping peak for the accurate measurement of (*R*)-ibuprofen within the optical impurity range 0.1 to 5.0%. The optical purity (99.9%) of (*R*)-(+)-1-phenylethylamine was also taken into account in all quantifications.

MATERIALS AND METHODS

Chemicals

Racemic ibuprofen, (*S*)-ibuprofen, 3-phenylpropionic acid, (*R*)-(+)-1-phenylethylamine (-1-PEA), triethylamine (TEA), ethyl chloroformate (ECF) and L-alanine were obtained from Sigma-Aldrich (St. Louis, MO, USA). Diethyl ether, acetonitrile, ethyl acetate, toluene, dichloromethane of spectroanalyzed grade were purchased from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals were of analytical-reagent grade and used as received.

Preparation of standards, reagent and aqueous calibration solutions

Each stock solution of (*S*)-ibuprofen, racemic ibuprofen and 3-phenylpropionic acid used as internal standards (IS) were made up at 10 mg/mL in acetonitrile in their free acid forms. The working solutions were then prepared by diluting each stock solution to 50 mg/mL with acetonitrile. The (*R*)-(+)-1-PEA solution was prepared at 0.5 M in methanol. The TEA and ECF solutions were prepared in acetonitrile at 50.0 and 60.0 mM, respectively. Five calibration samples for (*R*)-ibuprofen measurement were prepared containing (*R*)-ibuprofen in the range 1.0 to 50 ng from the racemic ibuprofen working solution. For measurement of (*S*)-ibuprofen, four calibration samples were prepared containing (*S*)-ibuprofen in the range 0.1 to 2.0 μg. All prepared standard solutions were stored at 4°C.

Gas chromatography and gas chromatography-mass spectrometry

The GC analyses were performed on an Agilent 6890 gas chromatograph equipped with a split/splitless inlet system, flame ionization detector (FID) and GC Chemstation (Agilent Technologies, Atlanta, GA, USA). The injector was installed with a DB-17 MS (OV-17 bonded) fused-silica capillary column (15 m × 0.25 mm I.D., 0.25 mm film thickness; J & W Scientific, Folsom, CA, USA). The injector and detector temperatures were 260 and 290°C, respectively. Samples (*ca* 1.0 μL) were injected in the splitless mode with purge delay time of 0.7 min. The flow rate of the helium carrier gas was initially set at 0.8 mL/min. The initial oven temperature was 150°C (1 min) and programmed at 30°C/min to 260°C and finally to 300°C (3 min) at 10°C/min.

GC-MS analyses were performed on an HP 5890A Series II gas chromatograph interfaced to an HP 5970B mass-selective detector (70 eV, electron impact mode) and installed with an HP-50+ (OV-17 bonded) fused-silica capillary column (25 m × 0.20 mm I.D., 0.16 mm film thickness, Hewlett-Packard, Avondale, PA, USA). The temperatures of the injector, interface and ion source were 260, 280 and 230°C, respectively. The helium inlet pressure was set to 85 kPa. Samples were introduced in the split-injection mode (10:1), the oven temperature maintained at 100°C (2 min) and programmed to 260°C at 3°C/min and finally to 300°C (10 min) at a rate of 20°C/min. The mass range scanned was 50-650 *u* at a rate of 0.99 scan/s.

Diastereomeric (*R*)-(+)-1-phenylethylamide formation

Racemic ibuprofen and (*S*)-ibuprofen were subjected to reaction with (*R*)-(+)-1-phenylethylamidation, as described elsewhere (Paik *et al.*, 2004). Briefly, each sample containing racemic ibuprofen or (*S*)-ibuprofen was added with IS at a constant amount (100 ng) and evaporated to dryness (under gentle nitrogen stream). The residue was dissolved in dichloromethane (200 μL) and then sonicated (1 min) after addition of 0.06 mmol TEA (50 mM, 1.2 μL) and 0.24 mmol ECF (60 mM, 4 μL). Subsequently, 0.5 M (*R*)-(+)-1-PEA (2 μL) was added and the mixture sonicated (2 min). After acidification (pH ≤ 2) with 0.1 M hydrochloric acid (200 μL), the amide derivative was extracted with diethyl ether (600 μL) and ethyl acetate (600 μL) in sequence. The combined extracts were evaporated to dryness (under a gentle nitrogen stream) and the residue reconstituted in a mixture (20 μL) of toluene and ethyl acetate (1:1) for direct GC and GC-MS analyses.

Calculations of corrected peak area ratios for quantitation

The quantitative calculations of (*R*)-ibuprofen and (*S*)-ibuprofen were based on the corrected peak area ratios relative to that of IS. The optical purity of (*R*)-(+)-1-PEA was measured as 99.9%, with high precision (better than ± 0.1% RSD), employing *N*-ethoxycarbonyl-L-alanine as the chiral resolving agent, as in a previous study (Paik *et al.*, 2004). Hence, the peak corresponding to (*R*)-ibuprofen contained (*S*)-ibuprofen at 0.1%, while that of the (*S*)-ibuprofen contained (*R*)-ibuprofen at 0.1%. Thus, the peak area ratios of (*R*)- and (*S*)-ibuprofens were corrected according to the following equations in all calculations:

Corrected peak area ratio for (*R*)-ibuprofen = (0.999 X + 0.001 Y) / Z

Corrected peak area ratio for (*S*)-ibuprofen = (0.001 X + 0.999 Y) / Z

Here X and Y were the measured peak areas of (*R*)-ibuprofen and (*S*)-ibuprofen, respectively, and Z the peak area of IS.

Method validation for optical purity determination

The method validation for the measurement of trace (*R*)-ibuprofen was conducted with five calibration racemic ibuprofen samples (1.0, 5.0, 10.0, 25.0, and 50.0 ng) with a constant amount (100 ng) of IS. These amounts correspond to the optical impurity within the range 0.1 to 5.0% for (*S*)-ibuprofen. The linearity was tested using least-squares regression analysis on the corrected peak area ratios against the increasing ratios of (*R*)-ibuprofen. The precision, expressed as the percentage of the relative standard deviation (% RSD), and the accuracy, as the percentage of the relative error (% RE) of the method, were determined in triplicate with three different amounts (5.0, 10.0 and 50.0 ng) of (*R*)-ibuprofen.

The linearity test for (*S*)-ibuprofen quantification was performed with five calibration samples (0.1, 0.5, 1.0, 1.5 and 2.0 μg) containing a constant amount (0.1 μg) of IS. The precision and accuracy tests were conducted in triplicate with three different amounts (0.5, 1.0 and 2.0 μg). Each amount of (*S*)-ibuprofen examined was corrected for trace (*R*)-ibuprofen prior to quantification.

Sample preparation for optical purity determination of (*S*)-ibuprofen in tablets

An aliquot (100 mg) of finely ground tables from each of seven different commercial (*S*)-ibuprofen products was dissolved in alkaline water (2 mL, pH ≥ 12). The aqueous solution, after washing with diethyl ether (3 mL × 3), was adjusted to a pH ≤ 2, saturated with sodium chloride and the (*S*)-ibuprofen subsequently extracted in the free acid form with diethyl ether (3 mL × 3). After evaporation to dryness under a gentle nitrogen stream, a free acid form sample stock solution was made up at 10 mg/mL in acetonitrile. The working sample solution was then prepared by diluting the stock solution to 100 μg/mL with acetonitrile.

A constant aliquot (1.0 μg) from each free (*S*)-ibuprofen sample solution was added to IS (100 ng) and evaporated under a gentle stream of nitrogen. The residue was then subjected to diastereomeric (*R*)-(+)-1-phenylethylamide formation, with subsequent GC and GC-MS analysis for the determination of the optical purity, as described in the preceding sections.

RESULTS AND DISCUSSION

(*R*)-(+)-1-phenylethylamidation and enantioresolution by achiral GC analysis

From a number of preliminary experiments, one tenth of each the optimal amounts of TEA, ECF and (*R*)-(+)-1-PEA, used as multiple profens, as reported elsewhere (Paik *et al.*, 2004), was found to be optimal for the (*R*)-(+)-1-phenylethylamidation of a single ibuprofen. On chiral analysis using the GC conditions described earlier, complete enantioresolution of ibuprofen at the sub-microgram level was achieved within 9 min, as demonstrated using a 20 ng racemic ibuprofen standard (Fig. 1-A). When 1 μg of (*S*)-ibuprofen standard was analyzed, the trace level of (*R*)-ibuprofen as an enantiomeric impurity was well resolved from the major (*S*)-ibuprofen peak (Fig. 1-B). An enantioresolution similar to the pure (*S*)-ibuprofen standard was obtained from each (*S*)-ibuprofen product, as exemplified by one product (Fig.1-C). The impurity peaks originating from the solvent and reagents did not interfere

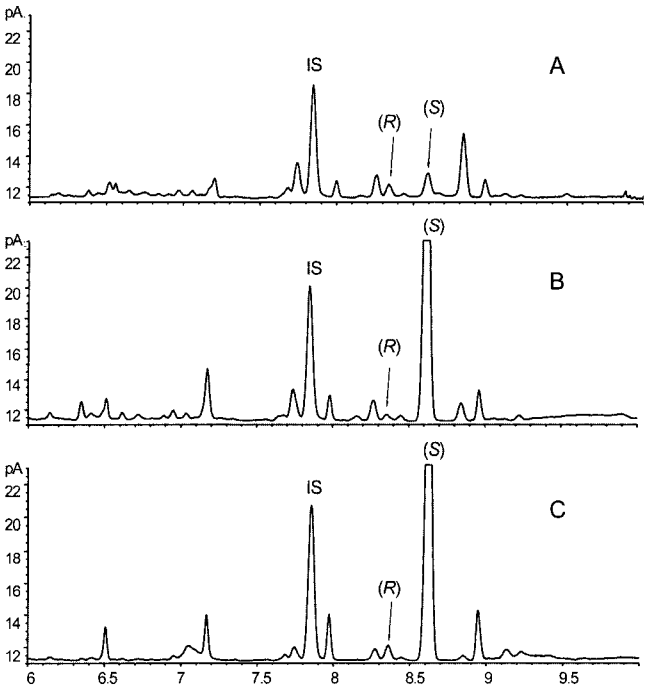

Fig. 1. GC chromatograms of the ibuprofen enantiomers, as their (*R*)-(+)-1-phenylethylamide derivatives, as: (A) the racemic ibuprofen standard (B) the pure (*S*)-ibuprofen standard and (C) a commercial (*S*)-ibuprofen product. Phenylpropionic acid was used as the IS. GC conditions: DB-17MS fused-silica capillary column (15 m × 250 mm I.D., 0.25 mm film thickness), initial temp 150°C (1 min), raised to 260°C at 30°C/min, to 280°C at 4°C/min and finally to 300°C (3 min) at 30°C/min; 1.0 μL sample injected in the pulsed splitless injection mode (purge delay time 42 sec); helium as carrier gas at 0.8 mL/min in constant flow mode; injector and detector temperatures at 260 and 300°C, respectively.

with the measurement of each enantiomer, indicating that the present method is specific for the optical purity determination of ibuprofen.

Method validation for optical purity measurement of ibuprofen

When the detector response (expressed as peak area ratios) of the resolved (*R*)-ibuprofen at the sub microgram level for racemic ibuprofen calibration samples was plotted against increasing amount (1.0~50.0 ng) of (*R*)-ibuprofen (expressed as weight ratios), a good linear relationship (r = 0.9997) was obtained (Table I). The precision (% RSD) and accuracy (% RE) of the method measured with three different amounts (5.0, 10.0, and 50.0 ng) varied from 0.6 to 5.3 and from 0.7 to -3.9, respectively, indicating that the level of (*R*)-ibuprofen as an optical impurity in (*S*)-ibuprofen could be measured with good precision and accuracy (Table I).

The average optical purity of the (*S*)-ibuprofen standard when four different amounts (0.5, 1.0, 1.5, and 2.0 μg) were tested was 99.54%, thus each amount was corrected prior to the linearity test. The present method for (*S*)-ibuprofen quantification was found to be linear (r = 0.9993) within the range 99.5 to 1990.8 ng (corrected amounts) (Table I). The precision and accuracy with the three amounts ranged from 1.1 to 7.2% and from -1.8 to 2.5%, respectively (Table I), supporting that the level of pure (*S*)-ibuprofen could be measured with excellent precision and accuracy.

Optical purity determination of (*S*)-ibuprofen in tablets

Table II. Optical purity test on seven different commercial (*S*)-ibuprofen products, as their (*R*)-(+)-1-phenylethylamide derivatives

Product No.	Enantiomer	Amount ± SD (ng)[a]	Optical purity ± SD(%)[a]
1	(*R*)-Ibuprofen	9.2 ± 0.3	
	(*S*)-Ibuprofen	850.9 ± 3.4	99.0 ± 0.6
2	(*R*)-Ibuprofen	11.4 ± 0.4	
	(*S*)-Ibuprofen	902.9 ± 12.7	98.8 ± 2.0
3	(*R*)-Ibuprofen	8.1 ± 0.1	
	(*S*)-Ibuprofen	799.0 ± 8.2	99.0 ± 1.4
4	(*R*)-Ibuprofen	8.0 ± 0.4	
	(*S*)-Ibuprofen	886.0 ± 14.0	99.1 ± 2.2
5	(*R*)-Ibuprofen	10.1 ± 0.2	
	(*S*)-Ibuprofen	1049.5 ± 16.9	99.0 ± 2.3
6	(*R*)-Ibuprofen	11.6 ± 0.5	
	(*S*)-Ibuprofen	863.7 ± 24.8	98.7 ± 3.9
7	(*R*)-Ibuprofen	11.5 ± 0.1	
	(*S*)-Ibuprofen	1091.3 ± 11.7	99.0 ± 1.5

[a] Standard deviation for n = 3

When the present method was applied to seven different commercial (*S*)-ibuprofen products, the optical purities were measured with the range 98.7 to 99.1%, with good precision (% RSD ≤ 4.0) (Table II).

CONCLUSIONS

The present achiral GC method for the analyses of trace (*R*)-ibuprofen and active (*S*)-ibuprofen in seven different commercial (*S*)-ibuprofen products, as their (*R*)-

Table I. Linearity, precision and accuracy of the measurements of (*R*)-ibuprofen and (*S*)-ibuprofen as their (*R*)-(+)-1-phenylethylamide derivatives

Enantiomer	Calibration range (ng)	Regression line		r^e	Amount tested (ng)	Precision (% RSD)[f]	Accuracy (% RE)[g]
		m^c	b^d				
(*R*)-Ibuprofen	1-50[a]	1.04 ± 0.01	-0.014 ± 0.003	0.9997	5	5.3	-3.9
					10	3.5	-1.2
					50	0.6	0.7
(*S*)-Ibuprofen	99.5-1990.8[b]	1.19 ± 0.03	0.7 ± 0.3	0.9993	497.7	7.2	-1.8
					995.4	2.6	2.5
					1990.8	1.1	-1.7

[a] Calibration range corresponding to (*R*)-ibuprofen in racemic ibuprofen
[b] Calibration range corrected for the trace (*R*)-ibuprofen measured in (*S*)-ibuprofen standard
[c] Slope; (mean ± standard deviation)
[d] Intercept; (mean ± standard deviation)
[e] Correlation coefficient
[f] Relative standard deviation for n = 3
[g] Relative error; {(measured mean value - nominal value) / nominal value} × 100
All quantitative calculations were based on peak area ratios relative to that of the IS (3-phenylpropionic acid, 100 ng), measured in triplicate on a DB-17 MS column.

(+)-1-phenylethylamide derivatives, employing an intermediately polar DB-17 column, provided for rapid determination, within 20 min. The optical purities ranged from 98.7 to 99.1%, and were measured with good precision (% RSD ≤ 4.0), indicating the present method to be suitable for the optical purity testing of other chiral profens and similar acidic drugs.

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