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Determination of enantiomeric composition of ibuprofen in bulk drug by proton nuclear magnetic resonance spectroscopy with a chiral lanthanide chelate

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Abstract

The enantiomeric composition of ibuprofen was determined in a simple and reliable manner by proton nuclear magnetic resonance spectroscopy with a chiral lanthanide chelate. Optimum complexation with the europium (III) chelate took place in CCl_4 after conversion of the enantiomeric sample into a mixture of methyl esters. The optimization of the experimental conditions in terms of substrate concentration and lanthanide chelate to substrate molar ratio led to two sets of signals of utility for quantitative purposes. Analysis of synthetic enantiomeric mixtures by the proposed method demonstrated excellent agreement between the assay results and the known masses of each enantiomer present in the mixture samples. The average $\pm$ S.D. recovery values were 99.39 $\pm$ 0.92 and 99.42 $\pm$ 0.68% ($n = 10$) of (*S*)-(+)-ibuprofen depending on whether the quantitation was based on the α-methyl protons or ester methyl protons, respectively. © 1997 Elsevier Science B.V.

Keywords: Ibuprofen; Optical purity; Proton nuclear magnetic resonance spectroscopic analysis; Lanthanide chelate

1. Introduction

Ibuprofen is a nonsteroidal anti-inflammatory agent that belongs to the class of α-arylpropionic acids [1]. Ibuprofen exhibits optical isomerism because of a chiral carbon atom on the propionic acid side chain. As with other compounds in this group, the anti-inflammatory activity of ibuprofen resides only in the *S*-(+)-enantiomer [2]. Anti-inflammatory drugs such as ibuprofen exhibit enantioselectivity in their distribution due to their specific interaction with chiral bio-molecules. The *R*-form of ibuprofen selectively binds to adipose tissue [3]. In addition, the racemic modification is known to undergo stereospecific metabolic chiral inversion, a unique bio-transformation whereby inactive *R*-(-)-enantiomer slowly converts into the pharmacologically active *S*-(+)-antipode [4–6].

The conventional approach for the determination of optical purity of chiral drugs is based on the measurement of optical rotation of the enantiomer, enantiomeric mixture, or racemic mixture in solution [7]. However, optical rotations may not conform to actual enantiomeric compositions

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[8] particularly when the sample contains traces of impurities which may or may not be optically active. Disadvantages of polarimetry for measuring optical purity include (a) the need for the molecule to exhibit a medium to high optical rotatory power to permit the accurate determination of small differences in enantiomeric excess, (b) the need to isolate the chiral drug in pure form and without accidental enantiomeric enrichment, (c) the need to know with certainty the absolute rotation of the pure enantiomer, (d) the relatively large sample required, and (e) the dependence of the accuracy of the determination on such factors as temperature, solvent, and the presence of impurities. Indirect methods such as isotopic dilution, kinetic resolution, enzymatic assay, and microcalorimetric techniques are considered to be experimentally cumbersome [9].

The stereoselective chromatographic separation of the enantiomers of ibuprofen and of related arylacetic acids with analgesic and anti-inflammatory actions has been the subject of several reports [10–18]. For example, the enantiomers of ibuprofen [10] and indoprofen [11] have been determined as the α-methylbenzylamide derivatives by gas chromatography (GC), and the same type of derivative has been used for the analysis of benoxaprofen [12] and carprofen [13] by liquid chromatography (LC). In addition, enantiomers of ibuprofen have been analyzed as a pair of diastereomers by liquid chromatography on achiral columns after acid-catalyzed esterification with *S*-(+)-2-octanol [14] or after amide formation with an optically active reagent like (−)-1-(4-dimethylamino-1-naphthyl)ethylamine [15], *S*-(−)-1-phenylethylamine [16] or L-leucine [17]. Although chiral derivatization to a mixture of diastereomers can lead to a most satisfactory enantioselective resolution, this approach is susceptible to drawbacks such as the possibility of partial racemization during the derivatization reaction [10,12] or kinetic resolution due to: (1) differences in diastereomeric transition states [14,18]; (2) the likelihood of introducing systematic error upon the use of enantiomerically impure chiral derivatizing reagent [14,19]; (3) long analysis times [19]; (4) the necessity for multiple procedural steps that may result in sample losses; and (5) the reliance on samples of the pure enantiomers for use as reference standards during quantitation steps.

Because of the on going trends in the pharmaceutical industry, many racemic drugs including ibuprofen are remanufactured in a single enantiomeric form. The purpose of the proposed NMR method using a chiral lanthanide shift complex is to serve as a means of establishing the level of (*R*)-(−)-enantiomer present as impurity in (*S*)-(+)-ibuprofen. In this manner, the enantiomeric impurity can be measured directly, with a high degree of enantioselectivity and without reliance on pure enantiomeric standards.

2. Experimental

2.1. Apparatus

All ^{1}H NMR spectra were obtained with a Varian EM-390 spectrometer (Varian Associate, Sunnyvale, CA) operating at a probe temperature of 35 ± 1°C and were referenced to chloroform taken as 7.27 ppm on the δ scale.

2.2. Samples

The *S*-(+)- and the *R*-(−)-2-(4-isobutyl phenyl)propionic acid enantiomers were generously supplied by the manufacturer (Upjohn, Kalamazoo, MI). The optical purity of the samples was checked by polarimetry and the proposed method.

2.3. Chemicals

TMS (>99.9%), carbon tetrachloride (CCl_4, >99.9%), and tris-[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III) ($Eu(hfc)_3$, 98%), were purchased from Aldrich (Milwaukee, WI). CCl_4 was distilled prior to use, and stored over type 4A molecular sieves (Aldrich). TMS was washed with concentrated sulfuric acid followed by saturated potassium bicarbonate solution, distilled, and stored over type 4A molecular sieves. $Eu(hfc)_3$ was stored over P_2O_5 under a dry N_2 atmosphere. To minimize the

Table 1
Determination of the enantiomeric composition of synthetic mixtures of (R)-(−)- and (S)-(+)-ibuprofen by ^{1}H NMR spectroscopy with chiral Eu(hfc)$_3$[a]

Sample No.	(*R*)-(−) form (mg)	(*S*)-(+) form (mg)	Amount of (*S*)-(+)-ibuprofen (%)				
			Added[a]	Found		Recovery[b]	
				C–CH_3	CO_2CH_3	C–CH_3	CO_2CH_3
1	1.62	9.93	85.97	84.15	84.39	97.88	98.16
2	2.27	9.25	80.30	80.01	80.07	99.63	99.71
3	3.01	8.53	73.92	73.39	73.28	99.28	99.15
4	4.08	7.45	64.61	64.85	64.42	100.37	99.71
5	4.55	6.95	60.43	60.33	60.21	99.83	99.64
6	6.10	5.39	46.19	46.63	46.72	99.40	99.59
7	6.75	4.78	41.46	41.75	41.65	100.70	100.46
8	7.93	3.55	30.92	30.81	30.88	99.64	99.87
9	9.11	2.35	20.51	20.35	20.41	99.22	99.51
10	113.31	2.22	1.92	1.99	1.89	97.92	98.44
Mean						99.39	99.42
S.D.						0.92	0.68

[a] The total concentration of drug was approximately 0.1 M in CCl_4 (this was made by applying the appropriate dilution or weighing the accurate amounts); the concentration of Eu(hfc)$_3$ was approximately 0.1 M.
[b] Amounts recovered were calculated from (amount found × 100)/amount added, where amount found, mg(*S*)-(+)-enantiomer, was calculated from: $[A_{(S)\text{-}(-)} \times \text{mg taken}/[A_{(R)\text{-}(-)} + A_{(S)\text{-}(+)}]$.

possibility of contamination by ambient moisture or air, all work with Eu(hfc)$_3$ was conducted within a soft plastic glove box (AtmosBag®, Aldrich)

2.4. Preparation of samples

Synthetic mixtures of *S*-(+)- and *R*-(−)-enantiomers were prepared by accurately weighing the quantities of each enantiomer that are listed in Table 1. These samples were first converted to the corresponding methyl esters by either of the following methods.

2.4.1. Refluxing method

The sample was dissolved in 40 ml methanol, mixed with 1 ml 12 M hydrochloric acid, and refluxed for 1 h. The reaction mixture was evaporated to a small volume under reduced pressure, transferred to a separator, and extracted with three 15 ml portions of ether. The ethereal extracts were combined, the solvent was evaporated to dryness under a stream of dry nitrogen, and the residue was dried at 50°C in vacuo.

2.4.2. Diazomethane treatment

The sample was allowed to react with 30 ml freshly prepared 0.25 M ethereal diazomethane for 5 min at room temperature. The solution was then evaporated to dryness under a stream of dry nitrogen and the residue was dried to a constant weight in vacuo at 50°C.

Solutions for the ^{1}H-NMR studies were prepared by dissolving the accurately weighed residue of the methyl esters in CCl_4 containing 0.1% (v/v) of $CHCl_3$. These solutions were immediately stored in glass vials that were crimper-sealed with Teflon coated rubber septa and aluminum seals. Samples for analysis were withdrawn through the septa by means of a fixed needle, liquid-tight, dry microliter syringe.

2.5. NMR studies of lanthanide-induced shifts

The required changes in lanthanide shift chelate to substrate (*L*/*S*) molar ratios were obtained by first adding the shift chelate to a dry NMR tube, followed by an appropriate aliquot of substrate stock solution (the exact amount having been

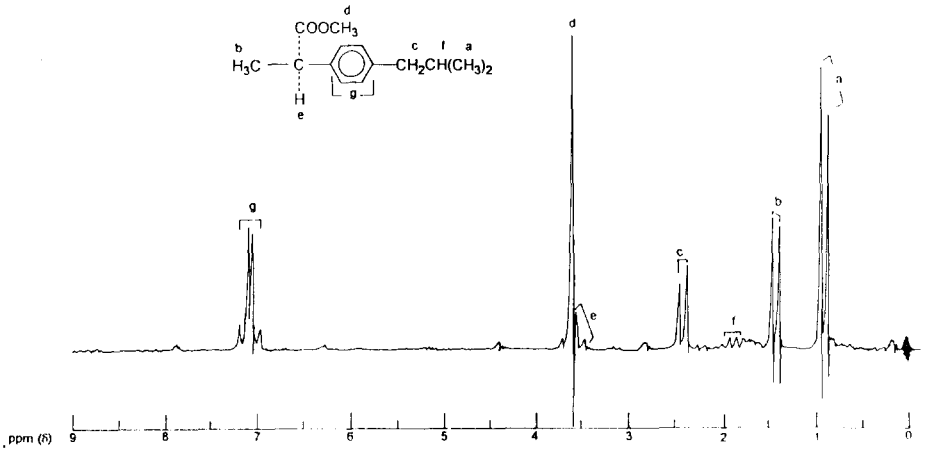

Fig. 1. The ^{1}H NMR spectrum of S-(−)- and R-(+)-ibuprofen methyl ester in CCl_4

determined gravimetrically). The NMR tube was capped immediately, its contents were mixed by inversion, allowed to stand for 10 min, and then placed in the spectrometer to obtain the ^{1}H-NMR spectrum. To the same tube, a second aliquot of the substrate stock solution was added, and the spectrum was obtained once more. The additions and spectral recordings were repeated until an appropriate number of spectra were available for properly defining the effects of the molar ratio of L/S on the enantiomeric spectral lines.

2.6. Determination of enantiomeric composition

A quantity of ibuprofen sample (approximately 11.0 mg) was converted to methyl ester as described in Section 2.4. The dry residue was dissolved in 0.5 ml CCl_4 containing 0.1% (v/v) $CHCl_3$, and the solution was transferred to a dry NMR tube containing approximately 60.0 mg of $Eu(hfc)_3$. The tube was capped, inverted several times to effect solution, allowed to stand for 10 min, and then used to obtain the ^{1}H-NMR spectrum. The relative intensities of the resonance signals for the enantiomeric ester methyl protons (singlets) at 9.43 ppm [S(+)-enantiomer] and 9.62 ppm [R(−)-enantiomer] or, alternatively, the enantiomeric signals for the α-methyl protons doublet at 6.33 ppm [S(+)-enantiomer] and 6.05 ppm [R(−)-enantiomer] were measured and used to calculate the percentage of each enantiomer in the sample taken from the following equation:

$$\%S(+)\text{-enantiomer} = [A_{S(+)} \times 100]/[A_{(S+)} + A_{R(-)}], \quad \textit{and}$$

$$\%R(-)\text{-enantiomer} = [A_{R(-)} \times 100]/[A_{S(+)} + A_{R(-)}]$$

where $A_{S(+)}$ is the integral value of the resonance signal for the S(+)-enantiomer, and $A_{R(-)}$ is the integral value of the resonance signal for the R(−)-enantiomer.

3. Results and discussion

Because of the known instability of complexes of a lanthanide shift chelate with substrates containing carboxyl groups [20,21] the enantiomers of ibuprofen were first converted into the methyl ester derivatives. Esterification with either methanolic hydrochloric acid or ethereal diazomethane was rapid and quantitative and afforded a product that did not require further purification. Ester groups demonstrate enhanced coordinating ability by virtue of their appreciable Lewis basicity and their minimal steric hindrance [22–24].

The ^{1}H NMR spectrum of S-(−)- and R-(+)-ibuprofen methyl ester in CCl_4 shown in Fig. 1 displayed the following resonances: (a) a doublet at 0.93 ppm representing the methyl protons of the isobutyl group; (b) a doublet at 1.47 ppm,

ascribed to the α-methyl protons; (c) a doublet at 2.43 ppm, originating from the methylene adjacent to the phenyl group; (d) singlet at 3.61 ppm, due to the methyl ester protons; (e) a multiplet centered at about 1.87 ppm, arising from the methine proton of the isobutyl moiety; (f) a quartet centered at 3.59 ppm, due to the α-methine proton; and (g) the A_2B_2 pattern centered at about 7.08 ppm, resulting from the four phenyl protons.

The effect of varying the shift chelate to substrate molar ratio on the separation of the enantiomeric signals was studied systematically with a mixture of *S*-(+)- and *R*-(−)-enantiomers, total drug content 0.1 M in CCl_4. The complexation with $Eu(hfc)_3$ caused the resonances of ibuprofen methyl ester to undergo substantial downfield shifts from their original positions in the uncomplexed spectrum, as would be expected from the pseudocontact interaction of Eu(III) with the rapidly exchanging mixture of the coordinated and free forms of ibuprofen methyl ester. The magnitude of the lanthanide-induced shift was sensitive to the distance and orientation of the resonating nucleus with respect to the paramagnetic center, with the induced shift ($\Delta\delta$) for the resonance frequency of a given proton decreasing as the distance increases between the proton in question and the carbonyl of the ester group, the obvious coordination site. Accordingly the largest induced shift was observed for the methine proton geminal to the carbonyl of the ester group and the smallest induced shift was observed for the methyl protons of the isobutyl moiety. The induced downfield shift increased with increasing shift chelate to substrate molar ratio up to a point, and then tended to level out at higher ratios, as would be expected from complexation of a monobasic functionality by a lanthanide shift chelate. Line broadening was slight, increasing with increasing shift, but it was not serious up to a shift chelate to substrate molar ratio of two.

The magnitude of the enantiomeric shift difference ($\Delta\Delta\delta$) for the α-methine, α-methyl, and methyl ester protons of *S*-(+)- and *R*-(−)-ibuprofen methyl ester varied with the changes in *L*/*S* molar ratios. Interestingly, the signal for the α-methyl protons of the *S*-(+)-enantiomer was shifted to a greater extent than that of the *R*-(−)-enantiomer, whereas the reverse situation was noted for the enantiomeric ester methyl signals. Such differences in the sense of nonequivalence are probably a reflection of differences in the geometries of the complexes formed. They clearly demonstrate that the $\Delta\Delta\delta$ values are not simply the result of differences in equilibrium constants but also of differing structural and conformational features for each of the enantiomeric-shift chelate complexes. However, in the light of the present results, it is not possible to establish their relative contributions to enantiomeric shift differences.

Since NMR spectroscopy provides a weighed time-average view of a dynamic process, then, a less than enantiomerically pure chiral lanthanide shift chelate will only affect the position but not the relative size of the bands stemming from the particular enantiomer [21]. Accordingly, using more enantiomerically pure chiral lanthanide shift chelate than the one used, will contribute only a negligible effect to the magnitude induced shift and the induced shift differences.

Table 2 summarizes the shift data for the α-methyl doublets, and methyl ester singlets for the (*S*)-(+)- and (*R*)-(−)-ibuprofen methyl esters in CCl_4 at various $Eu(hfc)_3$ to substrate molar ratios and substrate concentrations of approximately 0.1 M. The degree of nonequivalence of the α-methyl doublets, and methyl ester singlets was sufficiently large and well separated from other signals to permit their use in the direct quantitative determination of the enantiomers. Resolution was optimal at *L*/*S* ratio of about 1 in CCl_4 which corresponded to concentrations of substrate and $Eu(hfc)_3$ of 0.1 M each. Under these conditions, the enantiomeric α-methyl protons of the (*S*)-(+)- and (*R*)-(−)-enantiomers each resonated as a doublet centered at 6.33 ppm and 6.05 ppm, respectively, whereas the enantiomeric ester-methyl signals of the (*S*)-(+)- and (*R*)-(−)-enantiomers each appeared as a singlet at 9.43 ppm and 9.62 ppm, respectively, as shown in Fig. 2. Both sets of signals were found to be suitable for the quantitative determination of the enantiomeric composition of samples of ibuprofen based on the measurement of relative intensities.

Table 2
Shift data for the $-CCH_3$ and $-CO_2CH_3$ signals of a mixture of (*S*)-(+)- and (*R*)-(−)-ibuprofen methyl esters after complexation with various molar ratios of $Eu(hfc)_3$

$Eu(hfc)_3$: substrate	$-CCH_3$					$-CO_2CH_3$				
	(*S*)-(+)		(*R*)-(−)			(*S*)-(+)		(*R*)-(−)		
	δ	$\Delta\delta$	δ	$\Delta\delta$	$\Delta\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	$\Delta\Delta\delta$
0.00	1.47	0.00	1.47	0.00	0.00	3.61	0.00	3.61	0.00	0.00
0.44	3.70	2.23	3.60	2.13	0.10	6.15	2.54	9.21	2.60	0.06
0.48	4.24	2.77	4.12	2.60	0.12	6.81	3.20	6.90	3.29	0.09
0.53	4.46	2.99	4.32	2.85	0.14	6.86	3.25	6.95	3.34	0.09
0.59	4.68	3.21	4.52	3.05	0.16	7.36	3.75	7,46	3.85	0.10
0.67	4.97	3.50	4.79	3.32	0.18	7.72	4.11	7.84	4.23	0.12
0.76	5.47	4.00	4.27	3.80	0.20	8.25	4.64	8.38	4.77	0.13
0.91	5.94	4.47	5.69	4.22	0.25	8.97	5.36	9.13	5.52	0.16
1.01	6.33	4.86	6.05	4.58	0.28	9.43	5.82	9.62	6.01	0.19
1.09	6.38	4.91	6.11	4.64	0.27	9.49	5.88	9.66	6.05	0.17
1.18	6.56	5.09	6.29	4.82	0.27	9.72	6.11	9.89	6.28	0.17

The level of impurity, as amount of (*R*)-(−)-enantiomer in mixtures of both (*S*)-(+)- and (*R*)-(−)-ibuprofen, was varied over the range shown in Table 1, (i.e. 1.92–85.97%). After the conversion to the methyl esters, each sample was mixed with specific amounts of $Eu(hfc)_3$, and dissolved in CCl_4 to obtain solutions 0.1 M in total substrate concentration and 0.1 M in shift chelate. From the relative intensities of the enantiomeric α-methyl or ester methyl proton signals, the enantiomeric compositions were readily calculated. The analytical results were found to be in close agreement whether they were based on the integral of the α-methyl or ester methyl proton signals. The absence of any racemization has been evidenced from the closeness of the analytical results obtained using the proposed method and the true values calculated from the masses of the enantiomers in the mixtures. At the same time, they were indicative of the good accuracy of the

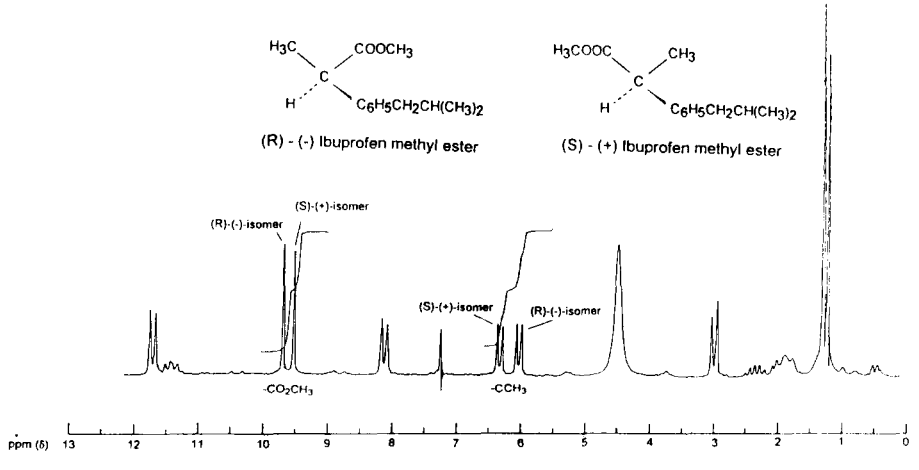

Fig. 2. The enantiomeric α-methyl protons of the (*S*)-(+)- and (*R*)-(−)-enantiomers each resonate as a doublet centered at 6.33 ppm and 6.05 ppm, respectively, whereas the enantiomeric ester-methyl signals of the (*S*)-(+)- and (*R*)-(−)-enantiomers each appear as a singlet at 9.43 ppm and 9.62 ppm, respectively.

method. The mean ± S.D. recovery values were 99.39 ± 0.92 and 99.42 ± 0.68% of (*S*)-(+)-ibuprofen, depending on whether the quantitation was based on the α-methyl or ester methyl proton signals, respectively. With the instrument used, levels of impurity of about 1.92% of (*R*)-(−)-enantiomer in the (*S*)-(+)-enantiomer were detected, which will be much less if one were to use a higher-field instrument, for example 300-500MHz, in which case the level is expected to be well below 0.1% with similar accuracy.

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