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Determination of the optical purity of indacrinone by proton nuclear magnetic resonance spectroscopy using chiral lanthanide chelates

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Abstract: A simple, specific and reliable ^{1}H-NMR spectroscopic method for the quantitative determination of the optical purity of indacrinone is described. After conversion of the *S*(+)- and *R*(−)-enantiomers to their methyl ester derivatives, they were coordinated with chiral europium(III) or praseodymium(III) shift reagents in CCl_4/C^2HCl_3 (2:1). The optical purity was calculated from the relative intensities of the enantiomeric resonance signals for the protons of the methyl groups at position C(2) of the indanone ring. Mean ±SD (n = 6) recoveries of *S*(+)-indacrinone from synthetic enantiomeric mixtures amounted to 99.75 ± 0.63% when using europium(III) and 100.01 ± 0.55% when using praseodymium(III).

Keywords: *Indacrinone; enantiomers; ^{1}H-NMR analysis; lanthanide shift reagents.*

Introduction

Long-term diuretic therapy is a widespread and well established clinical approach in the treatment of hypertension, edema, and congestive heart failure [1]. However, the search for a satisfactory diuretic has continued since most available agents also elevate the serum uric acid. A systematic study of the pharmacological profiles of (acylaryloxy)acetic acid analogues of ethacrynic acid has led to the discovery of a new class of so-called polyvalent diuretics [2, 3], of which indacrinone is the compound that seems to have received the greatest attention.

Indacrinone, (±)-[(6,7-dichloro-2-methyl-1-oxo-2-phenyl-5-indanyl)oxy]acetic acid, is a novel loop diuretic capable of exerting combined saluretic and uricosuric activities in laboratory animals and humans [2, 4]. In man, it is a more potent diuretic, has a more gradual onset, and shows a longer duration of action than furosemide [4]. The enantiomers of indacrinone show different pharmacological activities, with the kaliuretic, natriuretic, and diuretic activities residing principally in the *R*(−)-enantiomer, and the uricosuric activity being present principally in the S(+)-enantiomer [1, 3–7].

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Continuous use of the racemate results in hyperuricemia, possibly because the decrease in extracellular fluid volume that is associated with the potent natriuretic properties of the *R*(−)-enantiomer prevails over the inherent uricosuric activity of the *S*(+)-enantiomer [1].

Owing to the pharmacodynamic and pharmacokinetic characteristics of indacrinone, efforts have been made to alter the natural enantiomeric ratio of 1:1, to another that would enhance the uricosuric action, while maintaining the diuretic effect [1, 7, 8]. Thus, a 1:9 ratio of *R*(−) to *S*(+) in the mixture has been recommended for wide-scale use as a diuretic with good antihypertensive activity, favourable uric acid profile, and lack of hepatotoxicity [1]. An additional beneficial effect to be derived from raising the proportion of the *S*(+)-enantiomer is related to differences in the metabolic clearance rates of the enantiomers, since the *R*(−)-enantiomer possesses renal and plasma clearance values that are about 25-fold higher than those of the *S*(+)-enantiomer [8].

Determination of the enantiomeric composition of chiral drugs becomes an important task when a close correspondence is demonstrated between the chirality of the drug and its biological properties. This determination is especially significant for drugs whose pharmacological effects are dependent on enantiomeric compositions. In this regard, the use of chiral lanthanide nuclear magnetic shift reagents for inducing unequal enantiomeric frequency shifts in the proton nuclear magnetic resonance (^{1}H-NMR) spectra of chiral drugs has served as the basis for straightforward and reliable methods for measuring optical purities [9–14]. This report describes the development of a simple, selective, and accurate ^{1}H-NMR spectroscopic method with lanthanide shift reagents for the determination of the enantiomers of indacrinone in the presence of each other.

Experimental

Apparatus

All ^{1}H-NMR spectra were obtained with a 90 MHz EM-390 continuous wave spectrometer (Varian Instrument Group, Palo Alto, CA, USA) at an ambient probe temperature of 35 ± 1°C, and were referred to tetramethylsilane (TMS) at δ = 0.00 ppm.

Chemicals

TMS (99.9+%), obtained from Aldrich Chemical Co. (Milwaukee, WI, USA) was made free of tetrahydrofuran by consecutive washes with sulphuric acid and saturated potassium bicarbonate, distillation, and storage over molecular sieves, type 4A. The carbon tetrachloride (CCl_4) and deuterochloroform (C^2HCl_3; 99.5 atom % ^{2}H) were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA) and were stored over molecular sieves, type 4A. The shift reagents tris-[3-(heptafluoropropylhydroxymethylene)-d-camphorato]europium(III) and tris-[3-(heptafluoropropylhydroxymethylene)-d-camphorato]praseodymium(III), hereafter designated as Eu(hfc)$_3$ and Pr(hfc)$_3$, respectively, were stored over P_2O_5 in an evacuated desiccator or under a N_2 atmosphere. Samples of *S*(+)-, *R*(−)-, and (±)-indacrinone were a generous gift of Merck, Sharp & Dohme Research Laboratories (West Point, PA, USA).

Derivatization of samples

Synthetic mixtures of *S*(+)- and *R*(−)-indacrinone were prepared by accurately weighing the quantities of each enantiomer that are given in the accompanying tables.

These samples were converted to the corresponding methyl ester derivatives by either of the following methods:

(a) *Refluxing method.* The sample was dissolved in 40 ml of methanol, 2 ml of 12 M HCl was added, and the mixture refluxed for 1 h. The reaction mixture was evaporated to a small volume, transferred to a separatory funnel, and extracted with three 15-ml portions of ether. The ethereal extracts were combined, the solvent was evaporated to dryness under a stream of N_2, and the residue was dried at 50°C *in vacuo*.

(b) *Diazomethane treatment.* The sample was allowed to react with 3 ml of freshly made 0.25 M ethereal diazomethane for 5 min at room temperature, the solution was evaporated to dryness under a stream of N_2, and the residue dried at 50°C *in vacuo*. Solutions for ^{1}H-NMR studies were prepared by dissolving the residues of methyl esters in CCl_4/C^2HCl_3 (2:1) containing 1% (v/v) of TMS. These solutions were stored immediately in glass vials that were crimper-sealed with rubber septa and aluminium seals. Samples for analysis were withdrawn through the septa with the aid of a fixed needle, liquid-tight, dry microlitre syringe.

NMR lanthanide-induced shift studies

Runs were performed using a constant concentration of substrate and varying concentrations of lanthanide shift reagent. To obtain the required change in shift reagent to substrate molar ratio, the shift reagent was added first to a dry NMR tube, followed by an appropriate volume of substrate stock solution (the exact amount having been predetermined gravimetrically). The NMR tube was capped immediately, its contents were mixed by inversion, and then allowed to stand for 10 min. After recording the ^{1}H-NMR spectrum, the additions of substrate stock solution and the recordings were continued until at least one set of enantiomeric signals appeared well resolved from each other and from other resonances.

Determination of the enantiomeric purity

An accurately weighed quantity of indacrinone (18.3 mg) was transferred to a ground-glass, 100 ml round-bottom flask, methanol/12 M HCl (20:1), was added and mixed until dissolved. A condenser was attached, and the mixture was refluxed for 1 h. The reaction mixture was evaporated to a small volume, and extracted with three 15-ml portions of ether. The etheral extracts were combined and evaporated to dryness under a stream of N_2. The residue was dried at 50°C *in vacuo*. Alternatively, the esterification was carried out in a glass-stoppered flask with 3 ml of fresh 0.25 M ethereal diazomethane. The reaction was allowed to proceed for 5 min, the mixture evaporated to dryness under a stream of N_2, and the residue dried at 50°C *in vacuo*. The residue of methyl esters was dissolved in 0.5 ml of CCl_4/C^2HCl_3 (2:1), and without delay, using a dry fixed needle microlitre syringe, the solution was transferred to a NMR tube that contained 12.4 mg of $Eu(hfc)_3$ or 2.7 mg of $Pr(hfc)_3$, mixed by inversion, and allowed to stand for 10 min. The ^{1}H-NMR spectrum was recorded and the relative intensity of the resonance signals for the methyl protons at position C(2) of the indanone ring was measured. Taking the intensity of the resonance signals as being proportional to the total mole fraction, n, of a given enantiomer, the enantiomeric purity was calculated from

$$\frac{A_{(+)} + A_{(-)}}{A_{(+)} - A_{(-)}},$$

where $A_{(+)} > A_{(-)}$; $A_{(+)}$ is the peak area (or height) of the signal for the $R(+)$-enantiomer; and $A_{(-)}$ is the peak area (or height) of the signal for the $S(-)$-enantiomer. The percentage composition in the $R(+)$- and $S(-)$-enantiomers were obtained from

$$\frac{A_{(+)} \times 100}{A_{(+)} + A_{(-)}} \text{ and } \frac{A_{(-)} \times 100}{A_{(+)} + A_{(-)}},$$

respectively.

Results and Discussion

Conversion of the enantiomers of indacrinone to the methyl ester derivatives circumvented the problems associated with the low solubility of the parent compounds in relatively non-polar solvents, and with the instability of the lanthanide shift reagent in solutions of the carboxylic acid forms. Whereas the methyl esters were poorly soluble in CCl_4, they readily dissolved in a mixture of CCl_4/C^2HCl_3 (2:1). The ^{1}H-NMR spectrum of a mixture of enantiomeric indacrinone methyl esters in this solvent combination is presented in Fig. 1. The following resonances were noted: (a) singlet at 1.67 ppm, due to the methyl protons at position C(2) of the indanone ring; (b) characteristic AB pattern between 3.06 and 3.62 ppm, assigned to the two non-equivalent methylene protons at C(3); (c) singlet at 3.85 ppm, resulting from the ester methyl protons; (d) singlet at 4.83 ppm, due to the OCH_2 protons; (e) singlet at 6.78 ppm, ascribed to the aromatic proton at position C(5); and (f) signal at 7.28 ppm, arising from the five protons of the phenyl group at position C(2).

Owing to the electron withdrawing effect of their halogen atoms, fluorinated lanthanide chelates are strong Lewis acids. As such, they are capable of effective coordination with Lewis basic sites in an organic substrate as well as with poor Lewis

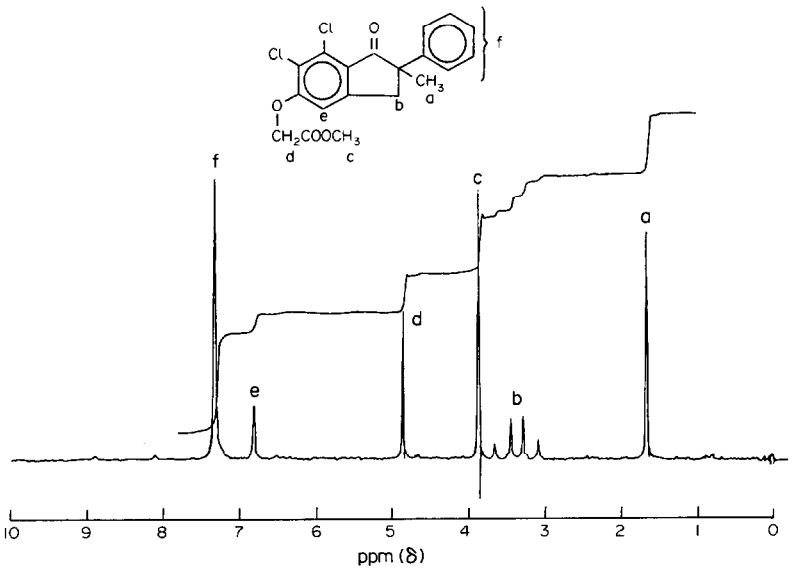

Figure 1
^{1}H-NMR spectrum of a mixture of $S(+)$- and $R(-)$-indacrinone methyl esters in CCl_4/C^2HCl_3 (2:1).

bases such as halogenated compounds [15, 16]. Of these bases, $Eu(hfc)_3$ and $Pr(hfc)_3$ were selected as the chiral shift reagents for the study.

Coordination of the methyl esters of indacrinone with the lanthanide ion may take place at three binding sites on the substrate, with the binding ability decreasing in the order ketone > ester > ether [17]. Accordingly, complexation to $Eu(hfc)_3$ at low shift reagent to substrate molar ratios will be expected to occur almost exclusively at the carbonyl group rather than at the poor electron donor aromatic ether group [18]. At higher molar ratios, complexation is expected to double or become even greater because of the competition between the ketone and ester groups for the lanthanide chelate, thus making the induced shifts additive.

Figures 2 and 3 represent selected ^{1}H-NMR spectra of a mixture of *S*(+)- and *R*(−)-

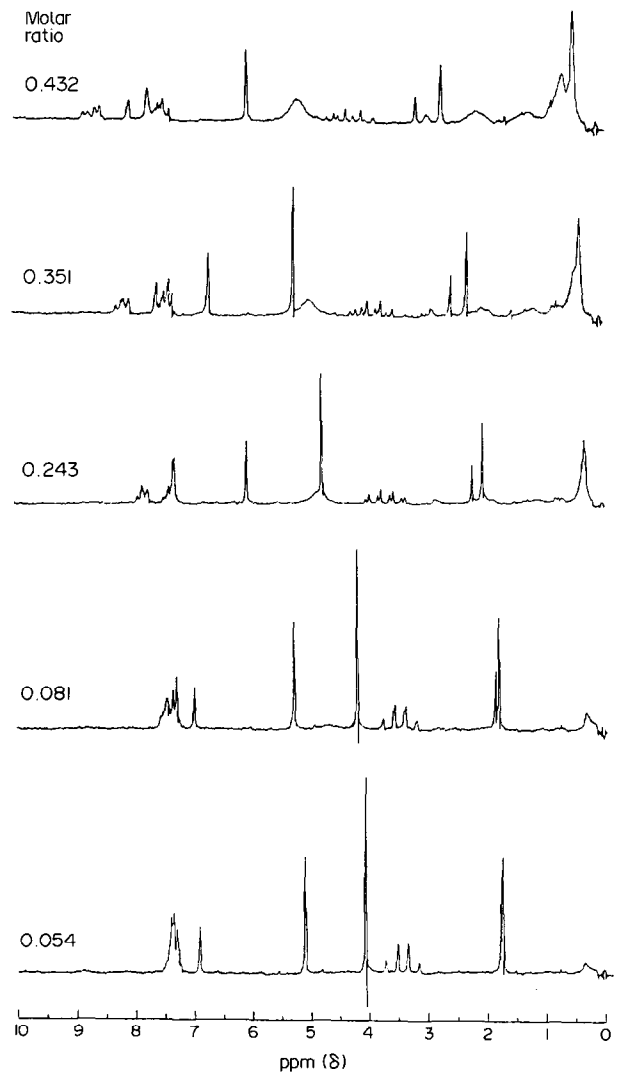

Figure 2
^{1}H-NMR spectra of a mixture of *S*(+)- and *R*(−)-indacrinone methyl esters, 0.1 M in CCl_4/C^2HCl_3 (2:1), after complexation with various molar equivalents of $Eu(hfc)_3$.

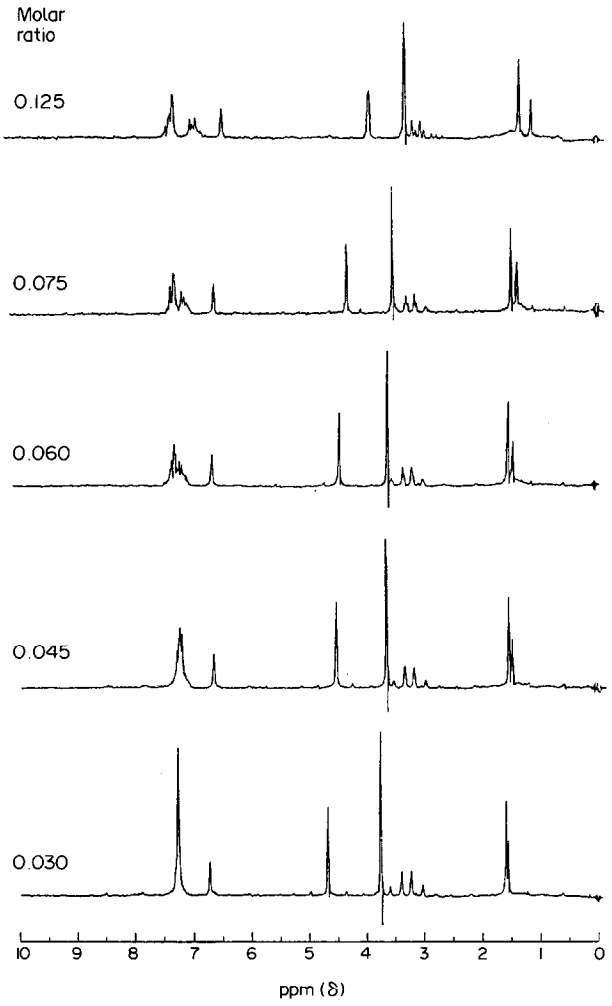

Figure 3
^{1}H-NMR spectra of a mixture of *S*(+)- and *R*(−)-indacrinone methyl esters, 0.1 M in CCl_4/C^2HCl_3 (2:1), after complexation with various molar equivalents of $Pr(hfc)_3$.

indacrinone methyl esters, 0.1 M in CCl_4/C^2HCl_3 (2:1), at various molar ratios of $Eu(hfc)_3$ and $Pr(hfc)_3$ to substrate. The $\Delta\delta$ increased as the molar ratio increased, which is to be expected from lanthanide shift reagents [19]. At a $Eu(hfc)_3$ to substrate molar ratio of 0.054 and a $Pr(hfc)_3$ to substrate molar ratio of 0.030, the enantiomeric methyl esters of indacrinone started to yield non-equivalent spectra, with the resonances of the methyl groups at position C(2) of the indanone ring starting to separate from each other. Complete resolution of the latter signals was attained when the molar equivalents of $Eu(hfc)_3$ and $Pr(hfc)_3$ were raised to 0.081 and 0.045, respectively. The monitoring of the $\Delta\Delta\delta$ values for the AB system due to the methylene protons at position C(3), the *ortho*-protons of the phenyl group at position C(2), and the phenyl proton of the indanone moiety indicated that they decrease as the distance between the proton in question and the chiral centre at position C(2) increases. At a $Eu(hfc)_3$ to substrate molar ratio of

0.243 the AB pattern for the methylene protons at C(3) became doubled. At a molar ratio of 0.432, a well resolved doublet was identified for each of the enantiomeric *ortho*-phenyl protons, with the signal for the ester methyl protons remaining a sharp singlet and the signals for the OCH_2 protons undergoing slight broadening. Based on the behaviour of the protons for the methyl group attached to C(2) and the ester methyl group, one can conclude that the more shifted signals do not necessarily undergo splitting, whereas those signals showing the least shifting will show a large splitting. Furthermore, the splitting was not proportional to the magnitude of the induced shift.

The effect of $Pr(hfc)_3$ on the ^1H-NMR spectrum of a mixture of *S*(+)- and *R*(−)-indacrinone methyl esters is shown in Fig. 3. In general, chemical shifts were in the low frequency direction. At comparable molar ratios, resolution of the enantiomeric signals for the methyl group at C(2) was slightly greater than that observed with the $Eu(hfc)_3$ analogue. Nevertheless, the increase in $\Delta\Delta\delta$ was counteracted by a reduction in spectral dispersion, due to the overlapping of the moderately shifted signals with strongly shifted ones, and by the interference by broad signals of the shift reagent at higher molar ratios. Of the two enantiomeric indacrinone methyl esters, the *R*(−)-enantiomer complexed to a greater extent than its *S*(+) counterpart since the former exhibited larger shifts with either $Pr(hfc)_3$ or $Eu(hfc)_3$ than the latter, and the sense of non-equivalence, but not necessarily the magnitude, was the same for all resolved signals. For the most part, the addition of a lanthanide chelate caused the $\Delta\delta$ for the methyl ester of *R*(−)-indacrinone to be larger than those of the *S*(+)-enantiomer.

The most suitable concentration of substrate for the analysis was 0.1 M, with higher concentrations resulting in signal broadening. Sample dilution was found to alter both $\Delta\delta$ and $\Delta\Delta\delta$ between enantiomers. The use of either $Eu(hfc)_3$ or $Pr(hfc)_3$ provided adequate resolution of the enantiomeric signals for quantitative purposes. From the values of $\Delta\delta$ and $\Delta\Delta\delta$ at various molar equivalents of lanthanide shift reagent listed in Tables 1 and 2,

Table 1
Shift data (ppm) for mixtures of *R*(−)- and *S*(+)-indacrinone methyl esters after complexation with various molar equivalents of $Eu(hfc)_3$*

	R(−)-enantiomer		*S*(+)-enantiomer		
Molar ratio	δ	Δδ	δ	Δδ	ΔΔδ
0.000	1.67	0.00	1.67	0.00	0.00
0.027	1.70	0.03	1.70	0.03	0.00
0.054	1.77	0.10	1.73	0.06	0.04
0.081	1.84	0.17	1.78	0.11	0.06
0.108	1.93	0.26	1.85	0.18	0.08
0.135	1.99	0.32	1.88	0.21	0.11
0.162	2.06	0.39	1.94	0.27	0.12
0.189	2.08	0.41	1.97	0.30	0.11
0.216	2.14	0.47	1.99	0.32	0.15
0.243	2.22	0.55	2.04	0.37	0.18
0.270	2.30	0.63	2.09	0.42	0.21
0.297	2.38	0.71	2.15	0.48	0.23
0.324	2.45	0.78	2.21	0.54	0.24
0.351	2.53	0.86	2.26	0.59	0.27
0.378	2.62	0.95	2.32	0.65	0.30
0.405	2.77	1.10	2.43	0.76	0.34
0.432	3.07	1.40	2.65	0.98	0.42

*Total concentration of drug of 0.1 M in CCl_4/C^2HCl_3 (2:1). Resonances of the methyl protons at position C(2) were used for the analyses.

Table 2
Shift data (ppm) for mixtures of *R*(−)- and *S*(+)-indacrinone methyl esters after complexation with various molar equivalents of $Pr(hfc)_3$*

Molar ratio	*R*(−)-enantiomer δ	*R*(−)-enantiomer Δδ	*S*(+)-enantiomer δ	*S*(+)-enantiomer Δδ	ΔΔδ
0.000	1.67	0.00	1.67	0.00	0.00
0.015	1.63	−0.04	1.63	−0.04	0.00
0.030	1.54	−0.13	1.58	−0.09	0.04
0.045	1.49	−0.18	1.55	−0.12	0.06
0.060	1.42	−0.25	1.50	−0.17	0.08
0.075	1.37	−0.30	1.47	−0.20	0.10
0.125	1.05	−0.62	1.25	−0.42	0.20
0.175	0.88	−0.79	1.13	−0.54	0.25
0.225	0.30	−0.47	0.80	−0.87	0.60

*Total concentration of drug of 0.1 M in CCl_4/C^2HCl_3 (2:1). Resonances of the methyl protons at position C(2) were used for the analyses.

it was determined that at a $Eu(hfc)_3$ to substrate molar ratio of 0.216 and a substrate concentration of 0.1 M in CCl_4/C^2HCl_3 (2:1), the singlet for the methyl protons at position C(2) became split into two sharp singlets, one at 2.14 ppm [*R*(−)-enantiomer] and the other at 1.99 ppm [*S*(+)-enantiomer], as shown in Fig. 4(A). Although $Pr(hfc)_3$ induced larger ΔΔδ for the same set of enantiomeric signals, only a molar ratio of 0.045 provided the resolution required for quantitative purposes, with higher molar ratios resulting in signal overlapping. Like $Eu(hfc)_3$, $Pr(hfc)_3$ also caused the enantiomeric signals of interest to split into two sharp singlets, one at 1.49 ppm [*R*(−)-enantiomer] and the other at 1.55 ppm [*S*(+)-enantiomer], as seen in Fig. 4(B).

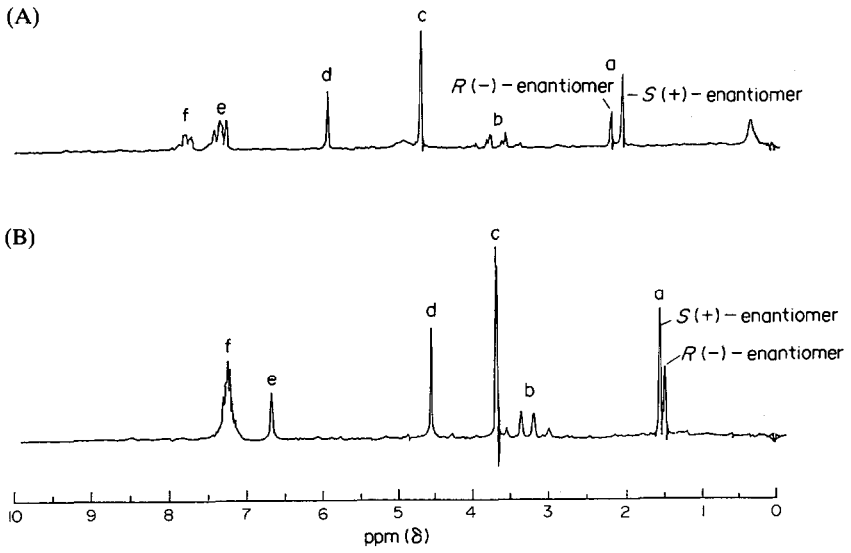

Figure 4
^1H-NMR spectrum of a mixture of *S*(+)- and *R*(−)-indacrinone methyl esters, 0.1 M in CCl_4/C^2HCl_3 (2:1), complexed with (A) 0.216 molar equivalents of $Eu(hfc)_3$ and (B) 0.045 molar equivalents of $Pr(hfc)_3$.

Table 3
Analysis of synthetic mixtures of *R*(−)- and *S*(+)-indacrinone by ^{1}H-NMR spectroscopy using Eu(hfc)$_3$ as chiral shift reagent*

Sample	*R*(−)-enantiomer (mg)	*S*(+)-enantiomer (mg)	*S*(+)-enantiomer (%) Added	Found	Rec.
1	111.9	1.9	1.67	1.65	98.80
2	20.0	8.4	29.58	29.55	99.90
3	17.2	11.2	39.44	39.25	99.52
4	9.7	18.7	65.75	65.62	99.80
5	8.1	20.3	71.48	72.02	100.76
6	4.2	24.2	85.21	84.95	99.69
Mean					99.75
SD					0.63

*Total concentration of drug of 0.1 M in CCl_4/C^2HCl_3 (2:1) and lanthanide shift reagent to substrate molar ratio of 0.216.

Table 4
Analysis of synthetic mixtures of *R*(−)- and *S*(+)-indacrinone by ^{1}H-NMR spectroscopy using Pr(hfc)$_3$ as chiral shift reagent*

Sample	*R*(−)-enantiomer (mg)	*S*(+)-enantiomer (mg)	*S*(+)-enantiomer (%) Added	Found	Rec.
1	22.7	5.7	20.07	20.12	100.25
2	21.5	6.9	24.30	24.23	99.71
3	18.2	10.2	35.92	35.58	99.05
4	13.7	14.7	51.76	51.82	100.12
5	10.2	18.2	64.08	64.25	100.27
6	7.5	20.9	73.59	74.05	100.63
Mean					100.01
SD					0.55

*Total concentration of drug of 0.1 M in CCl_4/C^2HCl_3 (2:1) and lanthanide shift reagent to substrate molar ratio of 0.045.

The proposed method was applied to six synthetic mixtures of *R*(−)- and *S*(+)-indacrinone prepared in the proportions listed in Tables 3 and 4. Using the recommended experimental conditions, the mean recovery values of *S*(+)-indacrinone were found to be in excellent agreement with the expected values (Tables 3 and 4), with as little as 1.6% of this enantiomer being detectable in an enantiomeric mixture.

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