ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
24 (2000) 189–196

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Optimization of enantiomeric separation for quantitative determination of the chiral drug propranolol by ^{1}H-NMR spectroscopy utilizing a chiral solvating agent

George M. Hanna [a,*], Frederick E. Evans [b]

[a] *Food and Drug Administration, Northeast Regional Laboratory, 158-15 Liberty Avenue, Jamaica, NY 11433-1034, USA*
[b] *Food and Drug Administration, National Center for Toxicological Research, 3900 NCTR Drive, Jefferson, AR 72079-9502, USA*

Received 12 May 2000; accepted 27 May 2000

Abstract

High-field ^{1}H-NMR methodology for enantiomeric composition determination of the chiral drug propranolol utilizing a chiral solvating agent is reported. Optimal experimental conditions for the resolution of enantiomers were determined by studying the interaction of substrate concentration, chiral solvating agent concentration and temperature. The success of the method is based on the selection of a chiral solvating agent that has the following two characteristics. First, it possesses functional groups that are complimentary to those of the chiral substrate for significant interaction to occur. Second, it has a group of diamagnetic anisotropy near its stereogenic center for translating spatial environments of solute nuclei into different magnetic environments that are measurable by NMR spectroscopy. Optical purities were determined on the basis of the intensities of the methyl proton resonances. The analysis of synthetic enantiomeric mixtures of propranolol by the proposed NMR method resulted in assay values, which agreed closely with the known quantities of each enantiomer in the mixtures tested. The mean $\pm$ SD recovery values for the (*R*)-(+)-enantiomer was $100.0 \pm 0.6\%$ of added antipode ($n = 7$).

Keywords: Propranolol; ^{1}H-NMR spectroscopy; Chiral solvating agent; Enantiomers; Dastereoimeric solvates; Absolute configuration; Optical purity

1. Introduction

Propranolol [1-isopropylamino-3-(1-naphthoxy)-2-propanol] is an important β-adrenergic blocking agent which has gained widespread usage in the treatment of angina pectoris, cardiac dysrhythmias and hypertension. The pharmacological properties of the enantiomers of propranolol are quite different, and the β-adrenergic blocking activity resides in the (*S*)-(−) isomer [1–5] while the (*R*)-(+)-enantiomer has only a membrane stabilizing effect [2]. Further, the hepatic oxidation of propranolol is highly stereospecific [3]. It is therefore important to have a

* Corresponding author.

PII: S0731-7085(00)00386-1

method for the precise and accurate determination of the enantiomeric purity.

Methods proposed for the resolution of enantiomeric pairs of β-adrenergic blocking agents have varied depending on the purpose sought. On a preparative scale, the enantiomers of propranolol have been separated by multiple recrystalizations of the di (*p*-toluoyl) tartaric acid salts [6]. On an analytical scale, the frequently used approach appears to be derivatization of the drug with a chiral reagent, followed by chromatographic resolution of the resulting diastereomers. Using gas chromatography (GC) the propranolol enantiomers have been resolved as the derivatives formed with *N*-trifluoroacetyl-*S*-prolyl chloride [7] or with (*S*)-(−)-1-phenylethyl isocyanate [8]. Liquid chromatography (HPLC) has been also used for the resolution of propranolol, employing *N*-trifluoroacetyl-*S*-prolyl chloride [9,10], (*S*)-(−)-1-phenylethyl isocyanate [10], *tert*-butoxycarbonyl-*L*-analine anhydride (10), or *tert*-butoxycarbonyl-*L*-leucine anhydride [11] as the chiral derivatizing reagent. But the accuracy of these methods was mainly dependent on the purity of the derivatizing agent. Although extensively used, chiral reagents are susceptible to problems such as racemization and instability during usage or storage [7,10,12,13].

Different types of chiral stationary phases have been used for the resolution of β-blockers. Nevertheless, the prior derivatization of the amine function to amide, urea, or carbamate was required in order to reduce its basicity and shorten elution times. In that way, Pirkle et al. [14] resolved propranolol enantiomers as the lauryl amide derivative, Wainer et al. [15] assayed propranolol enantiomers as oxazolidin-2-one derivatives in human serum, and Yang et al. [16] carried out enantiomeric separation of a number of β-amino alcohols as α-naphthylurea derivatives. Protein-based chiral stationary phases have allowed the enantiomeric separation of β-blockers including propranolol [17–20]. But prior derivation was required; using α-acid glycoprotein [17,18], bovine serum albumin [19], or ovomucoid [20] derived chiral stationary phases.

Although all of the HPLC methods give good separation of the enantiomers of propranolol and other β-blockers, they are laborious as well as susceptible to sample losses. The enantiomers of a number of β-blockers have been resolved without resorting to derivatization [21,22]. Although direct, these methods require the use of the expensive pure enantiomers as reference standards.

The purpose of this paper is to describe a ^{1}H-NMR method for the direct determination of the optical purity of propranolol in which the chiral recognition and the required resolution of the enantiomeric resonances will be accomplished through interactions of the enantiomeric mixture with a chiral solvating agent.

2. Experimental

2.1. Apparatus

^{1}H-NMR spectra were obtained on an AM-500 spectrometer (Brüker Instruments, Inc., Billerica, MA, USA). The ^{1}H-NMR spectra were obtained under the following conditions: acquisition time, 4.65 s; data point resolution, 0.215 Hz/point; pulse width, 900; relaxation delay, 10 s; number of scan, 32; window function, none. Chemical shifts were referred to $CHCL_3$ (7.26 ppm).

2.2. Chemicals

Deuterochloroform ($CDCl_3$, 99.8 atom% D, stabilized with Ag foil), and (*R*)-(−)- and (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl) ethanol (TFAE, > 98%) were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA).

2.3. Samples

The *S*-(−)- and the *R*-(+)-propranolol hydrochloride enantiomers were obtained from Aldrich Chemical Co. or from Ayerst Laboratories Inc. (Rouses Point, NY, USA). The optical purity of the samples was checked by the proposed method.

2.4. Sample preparation

The hydrochloride salt of propranolol was converted to the free-base form as follows: a quantity

of the drug, accurately weighed, was dissolved to the extent possible in 1.5 ml of D_2O. One drop of 1.5 M sodium deuteroxide was added to $CDCl_3$ (0.75 ml). Both solutions were bubbled with argon and then combined. The $CDCl_3$ layer was removed using a separatory funnel. A second extraction was performed and $CDCl_3$ fractions were combined and evaporated to dryness. The sample was dried in vacuo at 50°C for approximately 45 min and weighed.

2.5. Optimization

Practical optimum experimental conditions for the determination of the enantiomeric composition were explored by observing (a) the effect of varying the chiral solvating agent to substrate molar ratio, and (b), the effect of temperature on the chemical shifts of the methyl protons. The required changes in chiral solvating agent to substrate molar ratios were obtained by first preparing stock solutions of propranolol in $CDCl_3$ (50 mg/ml) and (*S*)-(+)-TFAE (140 mg/ml). A total of 0.2 ml of propranolol (10 mg) solution and the appropriate amount of (*S*)-(+)-TFAE solution were added to a 5 mm NMR tube. The final volume was adjusted with $CDCl_3$ to 0.75 ml. The NMR tube was capped with a Teflon cap; 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.

The additions and spectral recording were repeated until an appropriate number of spectra were available for properly defining the effects of molar ratio of chiral solvating agent/substrate on the enantiomeric spectral lines.

2.6. Determination of enantiomeric purity

A quantity of propranolol hydrochloride sample (approximately 10.0 mg) was converted to the free-base as described in Section 2.4. The dry residue was dissolved in 0.5 ml $CDCl_3$, and the solution was transferred to a dry NMR tube containing approximately 42 mg of TFAE. The final volume was adjusted to 0.75 ml and then 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 intensities of enantiomeric methyl proton signals centered at ca. 0.89 ppm and ca. 0.96 ppm corresponding to *S*-(−)- and *R*-(+)-propranolol, respectively were measured and the percentage of each enantiomer was calculated based on the contribution of each resonance to the sum of both resonances as follows:

$$\%(S)\text{-}(-)\text{-enantiomer} = \frac{100 \times A_{(-)}}{A_{(-)} + A_{(+)}}$$

$$\%(R)\text{-}(+)\text{-enantiomer} = \frac{100 \times A_{(+)}}{A_{(+)} + A_{(-)}}$$

where $A_{(+)}$ = area the resonance signal for the methyl protons of the (*R*)-(+)-enantiomer, and $A_{(-)}$ = area of the resonance signal for the methyl protons of the (*S*)-(−)-enantiomer. A correction was made for spinning side bands and ^{13}C satellites.

3. Results and discussion

Certain structural features are required when selecting an effective chiral solvating agent. TFAE is ideally suited as a chiral solvating agent for the determination of enantiomeric purity of propranolol. First, the trifluoromethyl group of TFAE does not obscure propranolol resonances of interest. Second, it features the functionalities that are complementary to those of propranolol. Due to the close proximity to the trifluoromethyl group, the carbinol group of TFAE is a sufficiently acidic function to interact strongly with a hydrogen bond receptor in the propranolol enantiomers. While, the carbinyl hydrogen of TFAE is also slightly acidic because of the electronegative character and the inductive effect of the electron withdrawing groups directly attached to the stereogenic center and, thus, amenable to additional interaction with a secondary site in propranolol enantiomers. The primary and the secondary basic sites in propranolol are the amino nitrogen and the hydroxyl oxygen atoms, respectively. Consequently, two such points of interactions will engender greater populations of specific, short-lived, chelate-like, diastereomeric solvated conformers. Third, it possesses a group of high

diamagnetic anisotropy in the vicinity of its stereogenic center, namely the anthryl moiety, which is able to translate different average spatial environments around the enantiomeric solute nuclei into spectral lines with different chemical shifts. This effect is expected to be substantial in view of the two possible spatial orientation the anthryl moiety can adopt relative to the functional groups under its influence.

Fig. 1(a) shows the upfield region of the ^{1}H-NMR spectrum of a mixture of *S*-(−)- and *R*-(+)-propranolol in $CDCl_3$. The doublet centered at ca. 1.11 ppm represents the unresolved resonance signals for the enantiomeric methyl protons. Fig. 1(b) shows the corresponding upfield region spectrum of the diastereomeric solvates of propranolol enantiomers and which is enriched in the *R*-(+)-isomer. The diastereomeric solvates are formed upon the interaction with *S*-(+)-TFAE. The signals for the methyl protons are resolved into two sets of two doublets. The upfield set of the doublets centered at ca. 0.88 ppm, and ca. 0.91 ppm are assigned to the two methyl protons of *S*-(−)-propranolol and the downfield set of doublets centered at ca. 0.95 ppm, and ca. 0.98 ppm are assigned to the corresponding methyl protons of *R*-(+)-propranolol. The relative positions are reversed if *R*-(−)-TFAE is used instead.

The effect of varying the chiral solvating agent to substrate molar ratio on the separation of enantiomeric signals was studied systematically with a mixture of *S*-(−)- and *R*-(+)-enantiomers, total drug content 0.04 M in $CDCl_3$. The induced upfield shift increased with increasing solvating agent to substrate molar ratio up to a point, and then tended to level out at higher ratios. The enantiomeric shift difference for the methyl protons signals of *S*-(−)- and *R*-(+)-propranolol diastereomeric solvates increased in parallel fashion to the increase in solvating agent-substrate molar ratio until molar ratio of 3.5, after which a smaller increase is observed.

Enantiomeric spectral resolution was found also to increase by increasing sample dilution as a result of diminishing sample viscosity. However, nonequivalence was found to decrease with increased dilution of chiral solvating agent at constant molar ratio. Nonequivalence arises under these conditions only from spectral differences in the diastereomeric solvates, not from different degrees of association of solute enantiomers with chiral solvating agent, since no diminution of nonequivalence is observed even at high chiral

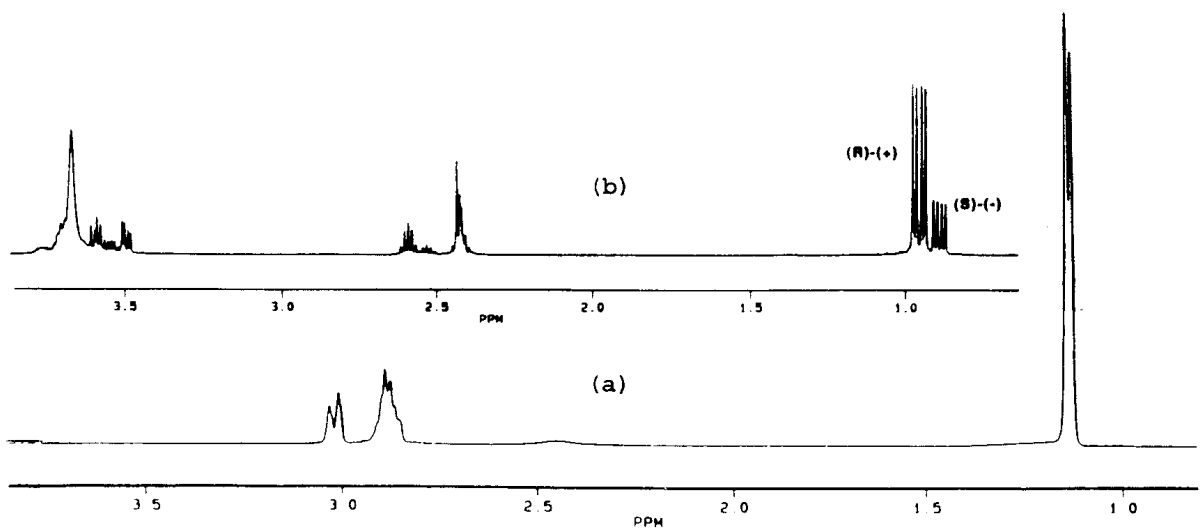

Fig. 1. ^{1}H-NMR spectrum (upfield region) of a mixture of (*R*)-(+)- and (*S*)-(−)-propranolol (combined concentration of ca. 0.04 M) in $CDCl_3$ at ca. 30°C: (a) without chiral solvating agent; and (b) with (*S*)-(+)-TFAE (ca. 0.15 M).

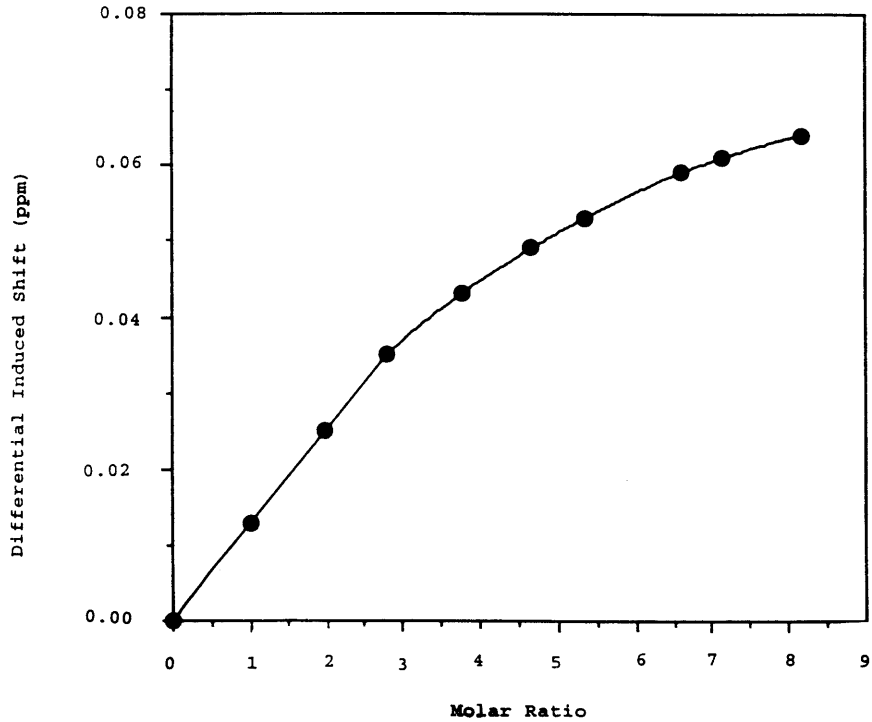

Fig. 2. Plot of differential induced shift ($\Delta\Delta\delta$) for the methyl protons of (*R*)-(+)- and (*S*)-(−)-propranolol diastereomeric solvates (ca. 0.04 M) in $CDCl_3$ at 30°C versus (*R*)-(+)-TFAE to substrate molar ratios.

solvating agent concentrations. Obviously, this occurs when the solute is completely solvated by the chiral solvating agent, and solvent appears not to interfere with solute-chiral solvating agent interactions. In these instances, solute-solute interactions were kept to a minimum by the combined use of an excess of solvating agent with a concentration of solute that was just enough to produce adequate strength. At molar ratio of 3.75, and propranolol concentration of 0.04 M, spectral nonequivalence among enantiomeric signals is found to reflect structural differences inherent to each diastereomeric solvate. Fig. 2 shows the plot of enantiomeric shift difference ($\Delta\Delta\delta$) for the methyl protons of *S*-(−)- and *R*-(+)-propranolol diastereomeric solvates versus *R*-(−)-TFAE to substrate molar ratio.

Since NMR spectroscopy provides a weighed time-average view of a dynamic process, then, a less than enantiomerically pure solvating agent will only affect the position but not the relative size of the bands stemming from the particular enantiomer [23]. Nonequivalence magnitude is apparently dependent on the enantiomeric purity of the solvating. Accordingly, using more enantiomerically pure chiral solvating agent than the one used, $>98\%$, will contribute only a negligible effect to the magnitude induced shift and the induced shift differences.

The effect of varying the temperature on the enantiomeric separation of the methyl protons resonances was evaluated with a mixture of *S*-(−)- and *R*-(+)-enantiomers, total drug content 0.04 M and *S*-(+)- TFAE to substrate molar ratio of ca. 3.75 in $CDCl_3$. Fig. 3 illustrates that the induced upfield shift and the differential induced shift for the methyl protons of *S*-(−)- and *R*-(+)-propranolol diastereomeric solvates increase with decreasing the temperature. Fig. 4 shows a plot of differential induced shift ($\Delta\Delta\delta$) for the methyl protons of *S*-(−)- and *R*-(+)-propranolol diastereomeric solvates with *S*-(+)-

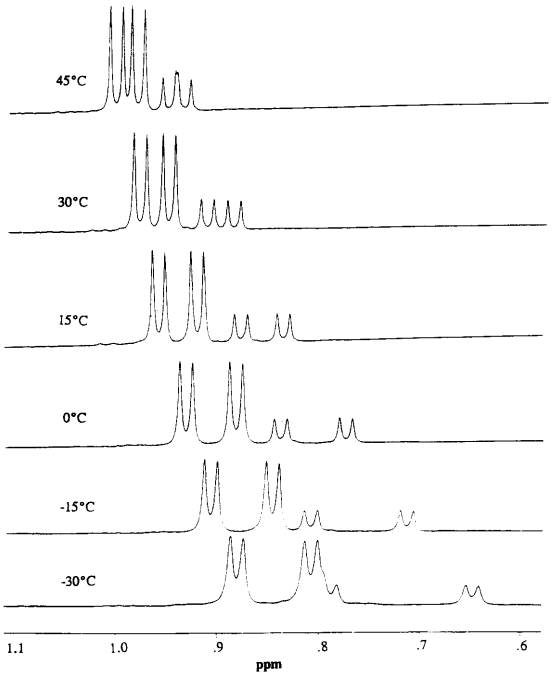

Fig. 3. ^{1}H-NMR spectrum of the methyl protons of a mixture of the diastereomeric solvates of (*R*)-(+)- and (*S*)-(−)-propranolol (combined concentration of ca. 0.04 M) with (*S*)-(+)-TFAE (ca. 0.15 M) in $CDCl_3$ at various temperatures.

TFAE in $CDCl_3$ versus temperature. Temperature reduction must have increased the intrinsic spectral difference of the diastereomeric solvates, by increasing the populations of the specific conformations that give rise to the nonequivalence.

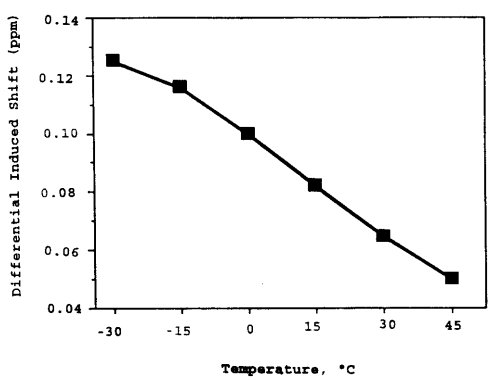

Fig. 4. Plot of differential induced shift (ΔΔδ) for the methyl protons of (*R*)-(+)- and (*S*)-(−)-propranolol diastereomeric solvates (combined concentration ca. 0.04 M) and with (*S*)-(+)-TFAE (ca. 0.15 M) in $CDCl_3$ at various temperatures.

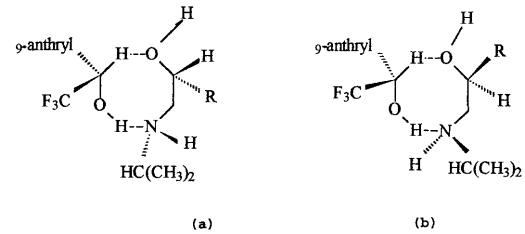

Fig. 5. Most abundant conformers of solvated diastereomers of (*S*)-(+)-TFAE and enantiomers of propranolol: (a) with (*S*)-(+) enantiomer; and (b) (*R*)-(−) enantiomer.

The advantages of using ^{1}H-NMR spectroscopy for measuring enantiomeric purity lie in the ubiquity and high sensitivity of ^{1}H nucleus and in the fact that relative signal intensities directly reflect the relative number of resonating nuclei and hence relative enantiomeric populations. The enantiomeric purity determination by this method is absolute in the sense that no reference to a standard of known optical purity is required. Nonequivalence sense for the chiral solvating agent-solute combination depends mainly on the configuration of each component; reversed senses of nonequivalence were observed for the combination of (*R*)-(−)-TFAE and propranolol. The chemical shifts were of the same magnitude, but their assignments were exchanged when going from one TFAE enantiomer to the other. As can be seen in Fig. 5(a, b) it is the spatial orientation of the methyl groups of each of the solvates that will determine if these groups will be shielded or less shielded by the anthryl group of the chiral solvating agent. For example, the chemical shift of the methyl groups will occur at higher field if these groups are located above or below the anthryl current, in other words, *cis*- to the anthryl moiety (as in (*S*)-(−)-propranolol, Fig. 5(a)). In contrast, the resonance signals for the methyl groups will appear at lower field when these groups are oriented with the plane of anthryl group of TFAE, that is *trans*- to the anthryl substituent (as in (*R*)-(+)-propranolol, Fig. 5(b)). Accordingly, the methyl resonances should appear at higher field in the solvated diastereomers derived from (*S*)-(−)-propranolol and (*S*)-(+)-TFAE than in solvated diastereomers made from (*R*)-(+)-propranolol and (*S*)-(+)-TFAE.

Table 1
Results of assay of synthetic mixtures of (R)-(+)- and (S)-(−)-propranolol by ^{1}H-NMR spectroscopy with chiral solvating agent[a]

Mixture number	Isomer (mg)	Isomer (mg)	Amount of (*R*)-(+)-propranolol (%)		
			(S)-(−) Added	(R)-(+) Found	Recovery[b]
1	0	6.77	100.0	99.8	99.8
2	0.015	6.77	99.8	99.4	99.6
3	0.045	6.77	99.3	98.9	99.6
4	0.089	6.77	98.7	98.1	99.4
5	0.425	6.77	94.1	94.1	100.0
6	0.836	6.77	89.0	89.3	100.3
7	1.75	6.77	79.5	80.5	100.3
Average					100.0
SD					0.6

[a] The total concentration of drug was ca. 0.04 M in $CDCl_3$; the concentration of TFAE was ca. 0.15 M.
[b] Amounts recovered were calculated from: (amount found × 100)/amount added; where amount found, mg(*R*)-(+)-enantiomer, was calculated from: $(A_{(R)-(+)} \times \text{mg taken}/[A_{(R)-(+)} + A_{(S)-(-)})$.

Seven mixtures of (*S*)-(−)- and (R)-(+)-propranolol, made up in proportions shown in Table 1, were mixed with the specific amounts of chiral solvating agent, and dissolved in $CDCl_3$, to yield solutions with ca. 0.04 M solute concentration and a TFAE: solute molar ratio of ca. 3.75. Enantiomeric composition or purity was calculated from the intensities of the resonances for the methyl protons as illustrated in Fig. 6. The assay values agreed very well with the known values.

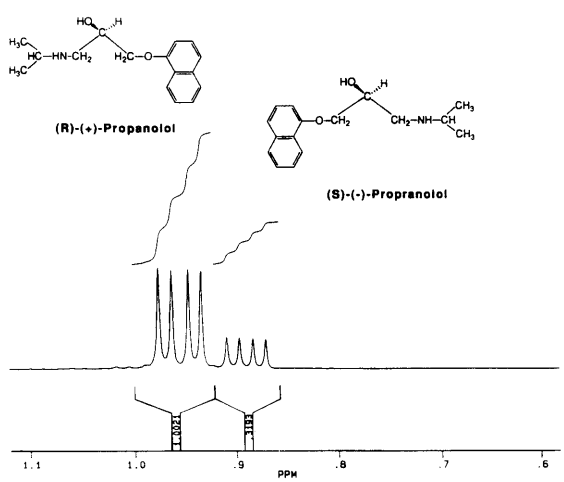

Fig. 6. ^{1}H-NMR spectrum of the methyl protons of the diastereomeric solvates of (*R*)-(+)- and (*S*)-(−)-propranolol (combined concentration of ca. 0.04 M) with (*S*)-(+)-TFAE (ca. 0.15 M) in $CDCl_3$ at 30°C.

Average recovery ± SD for the (*S*)-(−)-enantiomer was 100.0 + 0.6%.

References

[1] L.T. Potter, J. Pharmacol. Exp. Ther. 155 (1967) 91.
[2] A.M. Barrett, V.A. Cullum, Br. J. Pharmacol. 34 (1968) 43.
[3] T. Walle, U.K. Walle, M.J. Wilson, T.C. Fagan, T.E. Gafney, Br. J. Clin. Pharmacol. 18 (1984) 741.
[4] K.H. Rahn, A. Hawlina, F. Kersting, G. Planz, Naunyn–Schmiedeberg's Arch. Pharmacol. 286 (1974) 319.
[5] R. Howe, R.G. Shanks, Nature (London) 210 (1966) 1336.
[6] Y. Yost, J.L. Holtzman, J. Pharm. Sci. 16 (1979) 1181–1182.
[7] S. Caccia, C. Chiabrando, P. DePonte, R. Fanelli, J. Chromatogr. Sci. 16 (1978) 543.
[8] J.A. Thompson, J.L. Holtzman, M. Tsuru, C.L. Lerman, J. Chromatogr. 238 (1982) 470.
[9] J. Hermansson, C. von Bahr, J. Chromatogr. 221 (1980) 109.
[10] B. Silber, S. Riegelman, J. Pharmacol. Exp. Ther. 215 (1980) 643.
[11] J. Hermansson, Acta Pharm. Suec. 19 (1982) 11.
[12] G. Manius, R. Tscherne, J. Chromatogr. Sci. 17 (1979) 322.
[13] J. Gal, J. Pharm. Sci. 66 (1977) 169.
[14] W.H. Pirkle, J.M. Finn, J.L. Schreiner, B.C. Hamper, J. Am. Chem. Soc. 103 (1981) 3964–3966.
[15] I.W. Wainer, T.D. Doyle, K.H. Donn, J.R. Powell, J. Chromatogr. 306 (1984) 405–411.
[16] Q. Yang, Z.P. Sun, D.K. Ling, J. Chromatogr. 447 (1988) 208–211.

[17] J. Hermansson, J. Chromatogr. 325 (1985) 379–384.
[18] M. Enquist, J. Hermansson, Chirality 1 (1989) 209–215.
[19] E. Küsters, D. Giron, J. High Res. Chromatogr. Commun. 9 (1986) 531–533.
[20] J. Haginaka, J. Wakai, K. Takahashi, H. Yasuda, T. Katagi, Chromatographia 29 (1990) 587–592.
[21] Y. Okamoto, M. Kawashima, R. Aburatani, K. Hatada, T. Nishiyama, M. Masuda, Chem. Lett. (1986) 1237–1240.
[22] A.M. Krstulovic, M.H. Fouchet, J.T. Bruke, G. Gillet, A. Durand, J. Chromatogr. 452 (1988) 477–483.
[23] W.H. Pirkle, S.D. Beare, R.L. Muntz, Tedrahedron Lett. (1974) 2295–2298.