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Determination of the optical purity of (R)-terbutaline by ^{1}H-NMR and RP-LC using chiral derivatizing agent, (S)-(−)-α-methylbenzyl isocyanate

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Abstract

A simple, convenient and precise ^{1}H-NMR and indirect HPLC methods were used for the determination of (S)-terbutaline in (R)-terbutaline. The enantiomers were converted to diastereomeric derivatives using (S)-(−)-α-methylbenzyl isocyanate and were successfully separated on an ODS column within 40 min with $R_S = 1.41$ and $\alpha = 1.09$. Interaction between chiral solutes by the formation of the diastereomeric complexes also led to differentiations of the ^{1}H-NMR spectra of enantiomers and optical purities were determined on the basis of the peak area of the enantiomeric amine proton resonance. The effect of various experimental parameter, such as reaction time, reaction temperature and concentration of chiral derivatizing agent on the derivatization reaction and composition of mobile phase on the ODS column is discussed. Validation data such as recovery, linearity and detection limit are also presented. The results from ^{1}H-NMR and RP-HPLC methods were compared with those from chiral HPLC method and no racemization was found during the experiments. NMR results had agreed with those obtained by indirect HPLC method and two methods could be used as a quality control method for the enantiomeric purity determination of (R)-terbutaline.

Keywords: Terbutaline; Optical purity; ^{1}H-NMR; (S)-(−)-α-methylbenzyl isocyanate

1. Introduction

Chiral separation is an important field in analytical chemistry especially so in pharmaceutical analysis because many compounds of pharmacological interest have one or more chiral centers and usually one of the enantiomers is more active

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than its antipode, which might even be inactive or have harmful side effects [1]. Currently, 88% of synthetic chiral drugs are sold as racemates [2] and it is, therefore, urgent to develop chiral separation methods for pharmacological investigation and drug quality control.

Various kinds of analytical methods have been developed for the determination of enantiomeric purity. HPLC has been the most generally used method and during the last decade capillary electrophoresis (CE) has been found to be a powerful tool for chiral separation because of its high efficiency and easy change of separation media compared with HPLC [3,4].

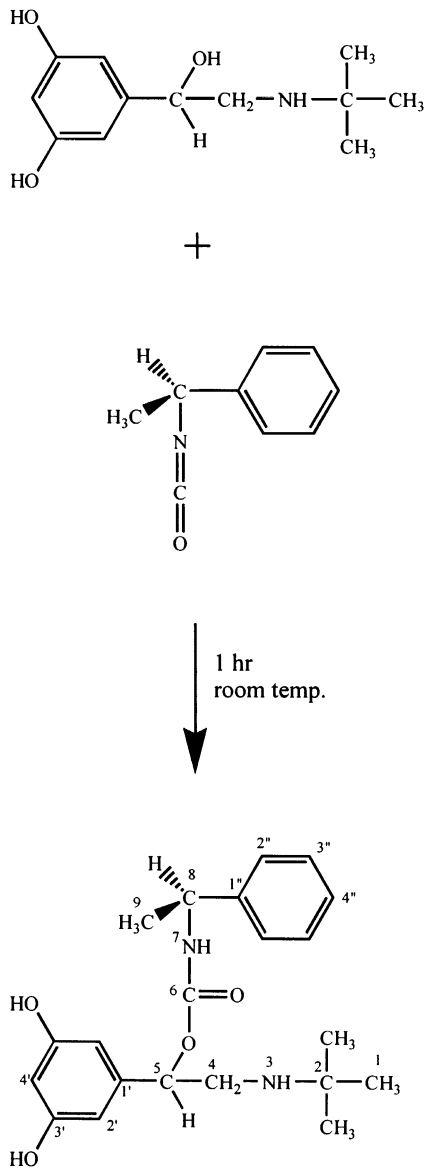

Fig. 1. Reaction of racemic terbutaline with (S)-MBIC.

At the same time, ^{1}H-NMR method has also been demonstrated to be effective for determining enantiomeric compositions, supplementing chiral chromatography and chiral capillary electrophoresis. Enantiomers can not be distinguished by ^{1}H-NMR without a chiral influence because enantiotopic nuclei show isochronous resonances. However, these nuclei become diastereotopic in a chiral environment and hence cause anisochronous resonances [5]. Recently, some general methods that facilitate the discrimination of enantiomers by ^{1}H-NMR have been reviewed [6,7]. They make use of chiral derivatizing agents (CDA), chiral lanthanide shift reagents (CLSR) and chiral solvating agents (CSA) for obtaining diastereotopic protons.

Terbutaline has been widely used as β_2-adrenergic bronchodilator and administered as racemic mixture. The agonistic effect of terbutaline resides predominantly in the enantiomers with (R)-configuration at the carbon atom related to the alcoholic hydroxy group [8].

There are some reports concerning the separation of terbutaline enantiomers [9–13]. In 1989, Walhagen and co-workers [9,10] reported chromatographic separation of enantiomers of terbutaline on a commercially available chiral column (Cyclobond I and Chiral-AGP). However, the separations shown were for racemic terbutaline. These methods were not validated for the quantitation of low levels of the (S)-enantiomer in (R)-terbutaline. In 1998, a paper was published on the determination of the enantiomeric purity of (−)-terbutaline using hydroxyethyl-β-cyclodextrin as a chiral selector in polyethylene glycol [11]. To date, there have been no published methods for the quantitation of terbutaline enantiomers by NMR techniques.

In this work, we describe a simple, specific and quantitative ^{1}H-NMR and reversed-phase HPLC methods for the determination of the enan-

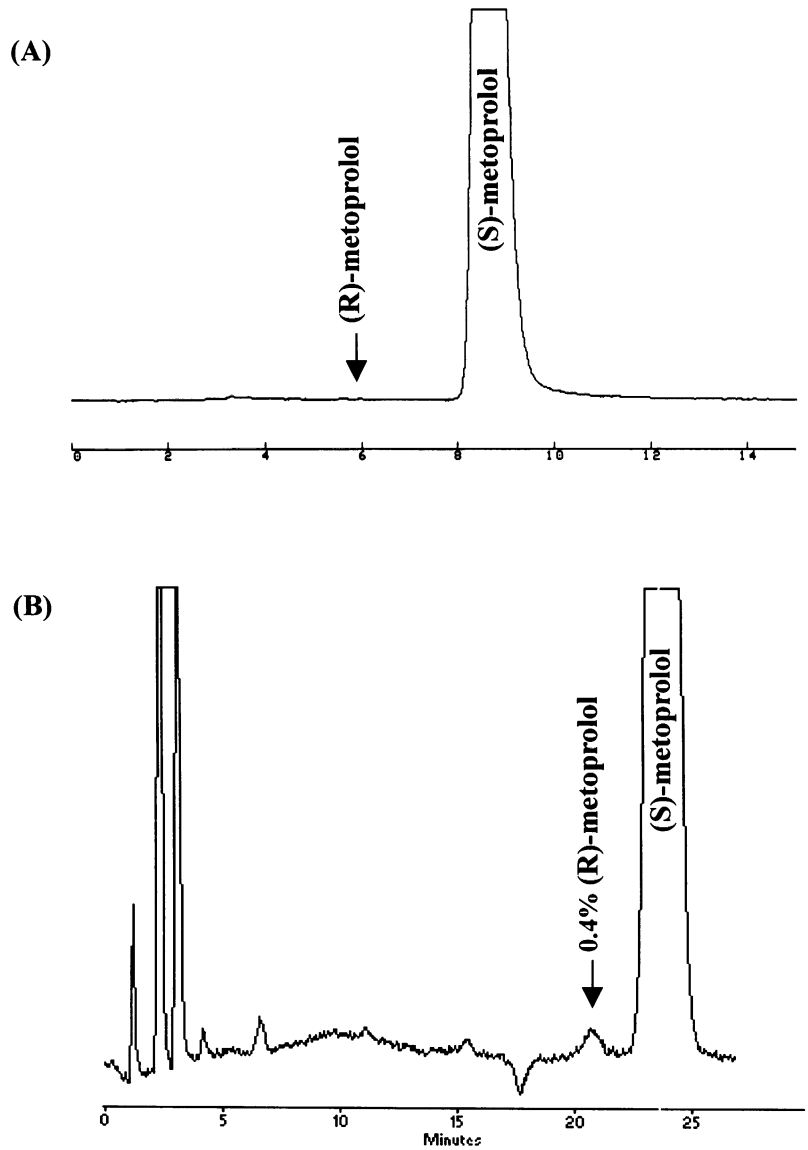

Fig. 2. Chromatograms of (A) enantiomerically pure (S)-terbutaline; and (B) (S)-terbutaline derivatives with (S)-MBIC. Chiral column: Chiralcel OD, 5 μm, 250 × 4.6 mm; chiral mobile phase: *n*-hexane-ethanol-isopropanol-diethylamine (88:6:6:0.25, v/v/v/v); achiral column: Inertsil ODS-3, 5 μm, 150 × 4.6 mm; achiral mobile phase: 63% methanol; flow rate: 1.0 ml/min; fluorescence detector: Ex 276 nm, Em 309 nm.

tiomeric composition of terbutaline. This method is based on the reaction of terbutaline with (S)-(−)-α-methylbenzyl isocyanate and the separation of the corresponding diastereomeric derivatives by ^{1}H-NMR and reversed-phase HPLC with an ODS column. These methods could be applied for the enantiomeric purity determination of (R)-terbutaline.

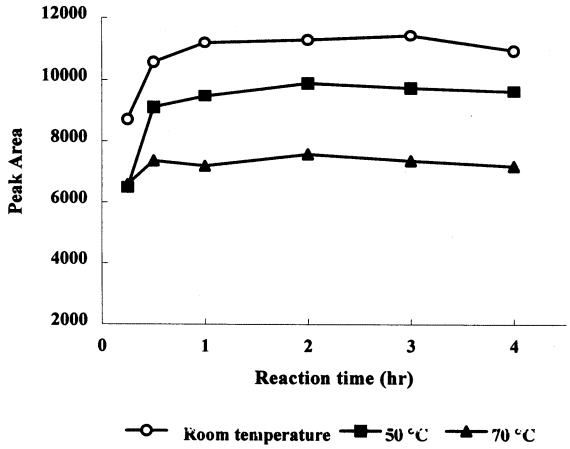

Fig. 3. Effect of reaction time and temperature on racemic terbutaline derivatives with (S)-MBIC.

2. Experimental

2.1. Materials

Racemic terbutaline sulfate, (S)-(−)-α-methylbenzyl isocyanate (>99%) and deuterodimethyl sulfoxide (DMSO-d_6, 99.9 atom% D) were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). The metoprolol tartrate was provided by Yuhan Corp. (Kunpo, Kyeonggi, Korea). (S)-(+)-terbutaline and (R)-(−)-terbutaline were prepared by semi-preparative HPLC using a Sumichiral OA-4900 chiral column (250 × 4.6 mm i.d., 5 μm, SCAS, Osaka, Japan) and *n*-hexane-1,2-dichloroethane-methanol-trifluoroacetic acid (240:140:15:1, v/v/v/v) as a mobile phase at the Department of Pharmacy, Kangwon National University (Kangwon, Korea). Metoprolol enantiomers were prepared by chromatographic resolution of racemic metoprolol tartrate on a Chiralcel OD column following a reported procedure [14]. Methanol, *n*-hexane, ethyl acetate and 1,2-dichloroethane as a HPLC grade and other reagents as an analytical grade were obtained from Duksan Pure Chemicals Co. (Ansan, Kyeonggi, Korea).

2.2. Apparatus

The HPLC system used in this study consisted of a Shimadzu (Kyoto, Japan) LC-9A pump, a Rheodyne Model 7725*i* injector and a Shimadzu RF-10AXL fluorescence detector with excitation/emission wavelengths of 276/306 nm. Data were acquired with a Shimadzu Chromatopac C-R4A data processor.

For the direct chiral separations, the chromatographic column used was Sumichiral OA-4900 (250 × 4.6 mm i.d., 5 μm, SCAS, Osaka, Japan).

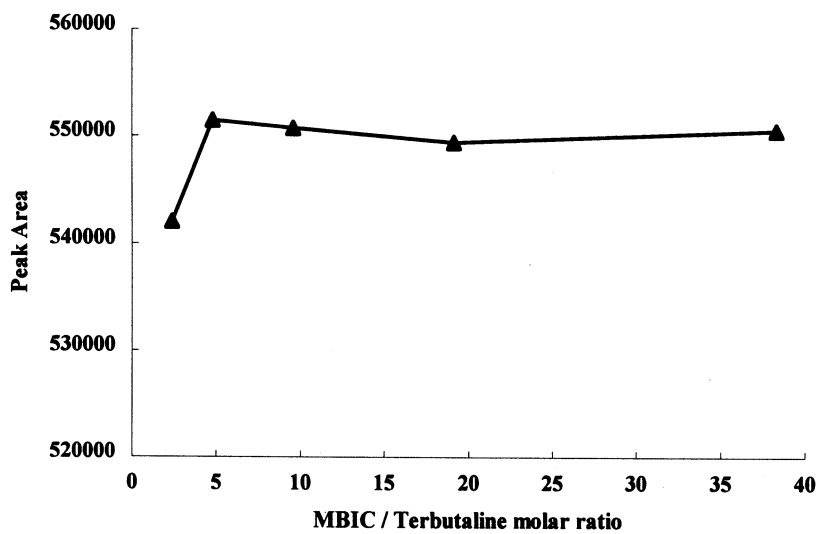

Fig. 4. Effect of (S)-MBIC concentration on the derivatization reaction.

Table 1
^{1}H-NMR assignments and chemical shift change ($\Delta\delta$) of racemic, (S)- and (R)-terbutaline derivatives induced by (S)-MBIC

Proton (^{1}H)	Chemical shift (δ)			Chemical shift change ($\lvert\delta_R$-$\delta_S\rvert$)
	Racemic-terbutaline	(S)-terbutaline	(R)-terbutaline	
H1, H9	1.327	1.328	1.330	0.002
H3	6.343 6.417	6.362	6.421	0.059
H4	3.221	3.227	3.219	0.008
H5	4.520	4.525	4.532	0.007
H7	7.467 7.516	7.471	7.516	0.045
H8	4.694	4.695	4.730	0.035
H2′	6.253	6.257	6.254	0.003
H3′-OH H4′-OH	9.226	9.223	9.228	0.005
H4′	6.115	6.119	6.112	0.007
H2″,3″,5″,6″	7.324	7.319	7.324	0.005
H4″	7.216	7.216	7.212	0.004

The mobile phase was *n*-hexane-ethyl acetate-methanol-trifluoroacetic acid (240:250:25:1, v/v/v/v). The HPLC separation of the diastereomers formed during derivatization was performed using a reversed-phase system. The chromatographic column used was Inertsil ODS-3 (150 × 4.6 mm i.d., 5 μm, GL science, Tokyo, Japan) and the mobile phase was 45% methanol in water. The systems were operated at ambient temperature with a flow rate of 1.0 ml/min.

All ^{1}H-NMR spectra were obtained using a Bruker AMX-500 (Milton, Ontario, Great Britain) spectrometer and were referenced to tetramethylsilane (TMS) taken as 0.00 ppm on the δ scale. The instrument was set to acquire at least 128 scans for a proton spectrum. The spectrum was processed using a line broadening of 0.3 Hz. Phasing and baseline correction were applied. Longitudinal (T_1) relaxation times were measured by the inversion recovery method.

2.3. Derivatization procedure

Solution of (S)- and (R)-terbutaline were prepared in DMSO-d_6 (10 mg/ml) and mixed to give samples with various ratios of the (S)- and (R)-enantiomers. The volumes of the sample were adjusted to 0.4 ml with DMSO-d_6.

For the direct chiral separation, a 20 μl of the sample was transferred to 2 ml vial. After the content was diluted to volume with methanol (the analytical concentration of the sample was approximately 0.1 mg/ml), a 2 μl volume of the sample was injected into the chiral HPLC system.

For the indirect chiral separation, 100 μl of (S)-(−)-α-methylbenzyl isocyanate ((S)-MBIC, 65 mg/ml in DMSO-d_6) were added to the mixture of (S)- and (R)-terbutaline (the analytical concentration of the sample was approximately 1 mg/500 μl). Then, the solution was vigorously shaken and allowed at stand at room temperature for 1.5 h. The molar ratio of terbutaline versus (S)-MBIC is ca. 1:10. After reaction, the samples were transferred to 5-mm NMR tubes and the proton spectrum was acquired, using the conditions described above. Finally, a 1 μl volume of the solution used in the NMR technique was injected into the achiral HPLC system. The reaction scheme is shown in Fig. 1.

2.4. Determination of the enantiomeric purity of (S)-MBIC

The optical purity of (S)-(−)-α-methylbenzyl isocyanate was obtained by comparing the enantiomeric purity of enantiomerically pure (S)-metoprolol before the derivatization reaction with that of (S)-metoprolol after the derivatization with (S)-MBIC.

The direct chiral separation of metoprolol was performed by chiral HPLC with Chiralcel OD column (250 × 4.6 mm i.d., 5 μm, Daicel, Tokyo,

Japan) using a mixture of *n*-hexane, ethanol, isopropanol and diethylamine (88:6:6:0.25, v/v/v/v) as a mobile phase [14]. The (R)-metoprolol was not detected (the detection limit is 0.03%). That is, enantiomeric purity of (S)-metoprolol used in this work was found to be more than 99.97%. The (S)-metoprolol was derivatized with (S)-MBIC according to the procedure described above. And then separation of the enantiomers as diastereomers was achieved by reversed-phase HPLC using Inertsil ODS-3 column and 63% methanol in water as a mobile phase. The optical purity of (S)-metoprolol obtained by reversed-phase HPLC was 99.6% ($n = 20$, C.V. = 0.04%). Typical chromatograms are shown in Fig. 2. As a result, enantiomeric purity of (S)-MBIC was found to 99.6%.

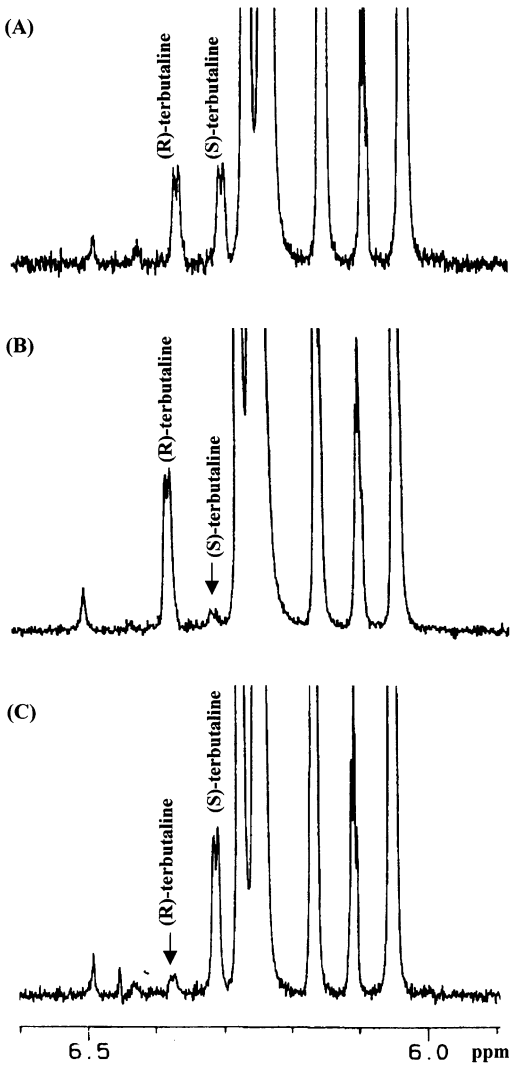

Fig. 5. Typical ^{1}H-NMR spectra of the amine region (δ 5.9–6.6) for (A) racemic terbutaline; (B) standard (R)-terbutaline spiked with 8% (S)-terbutaline; and (C) standard (S)-terbutaline spiked with 8% (R)-terbutaline after the derivatization with (S)-MBIC in DMSO-d_6 at 500 MHz.

3. Result

3.1. Effects of reaction time, temperature and reagent concentration

The effects of reaction time and temperature were investigated through peak areas of diastereomeric derivatives in the range 0.5–4 h at room temperature, 50 and 70°C. The result is shown in Fig. 3. As the reaction temperature was increased from room temperature to 50 or 70°C, the peak areas of diastereomers were decreased. In the course of a time study, the constant peak areas were obtained above 1 hr reaction. Considered to be sufficient time for reaction, 1.5 h and room temperature was adopted as the reaction time and temperature, respectively.

The effect of (S)-MBIC concentration on the derivatization was investigated in the range from 2 to 40 mM. The sample concentration was fixed at 1 mM. The result is shown in Fig. 4. The constant peak areas for racemic terbutaline derivatives, that is, completed reaction was obtained above 10 mM chiral reagent concentration.

3.2. NMR study

The spectra of enantiomers are identical in achiral solvent, and usually there is no difference between the spectrum of a racemic mixture and that of a single isomer. However, in some case interaction between chiral solutes can lead to differentiations of the spectra of enantiomers because of the formation of diastereomeric complexes.

Racemic, (R)- and (S)-terbutaline derivatives were prepared by the reaction (Fig. 1) and were

Table 2
Recoveries and Precision of (S)-terbutaline added to (R)-terbutaline ($n = 3$)

Theory content (%)	Found content (%)					
	Chiral HPLC		NMR		Reversed-phase HPLC	
	Average (%)	C.V. (%)	Average (%)	C.V. (%)	Average (%)	C.V. (%)
31.1	31.02	3.10	31.96	4.78	30.40	4.13
10.8	10.79	2.38	11.79	6.26	11.97	3.78
8.1	8.05	8.13	8.26	10.93	8.34	8.05
5.6	5.81	11.36	6.38	9.38	6.59	5.72
3.4	3.39	8.84	–	–	3.99	10.12

investigated by using nuclear magnetic resonance spectrometry. The ^{1}H-NMR spectra of terbutaline derivatives were assigned by inspection. The assignments are shown in Table 1, with the change in chemical shift ($\Delta\delta$) observed on the separation between signals arising from the individual enantiomers. Two sets of resonances were observed for almost each proton or group of equivalent protons of the diastereomeric derivatives of racemic terbutaline. Generally, up-field chemical shifts were observed in (S)-terbutaline compared with (R)-terbutaline. In amide (H7, –CO–NH–) and amine (H3, $-CH_2-NH-$) proton, the higher $|\delta_R - \delta_S|$ values of 0.045 and 0.059 ppm were obtained, respectively, and these results permitted more precise measurements of optical purity of (R)-terbutaline by NMR. However, when an excess of (S)-MBIC was used, the H7 (amide, –CO–NH–) signal was obscured by (S)-MBIC signals and could not be used for the determination of the optical purity of (R)-terbutaline. Thus the determination of the optical purity of (R)-terbutaline was performed on the H3 resonance, for which good differentiation between (R) and (S)-terbutaline observed.

Spectra of the amine region of the derivatives of racemic terbutaline, 8% (S)-terbutaline spiked in (R)-terbutaline and 8% (R)-terbutaline spiked in (S)-terbutaline are shown in Fig. 5.

Four synthetic mixtures of the (R)- and (S)-enantiomers of terbutaline, made up in the proportions shown in Table 2, were converted to the diastereomeric salts with (S)-MBIC and analyzed by the proposed method. A plot of the measured amine (H3) resonance peak area ratios for the two terbutaline enantiomers versus the known concentrations of the two was found to be linear over the range 5.6–31.1% ($r = 0.9997$) and the experimental results were found in good agreement with the known concentrations of each enantiomers in the synthetic mixtures. The recoveries of the (S)-terbutaline were 102.5–114.3%.

During the derivatization with (S)-MBIC, the possibility of racemization had to be established. For this purpose, before the derivatization with (S)-MBIC, enantiomeric purities of synthetic mixtures described above were determined on a Sumichiral OA-4900 chiral column and compared with results from NMR method. The enantiomeric purity determined by chiral HPLC was plotted against that determined by NMR. The slope and coefficient (r) of the plot was 1.02 ± 0.02 and 0.9996, respectively. The linearity of the lines with a slope near 1 showed that no racemization occurred during these experiments.

Under the conditions described above (128 scans on a 500-MHz instrument), the limit of detection was about 3.3% and the limit of quanti-

Table 3
Capacity factor, separation factor and resolution of terbutaline racemate using methanol mobile phase

Methanol content (%)	k' (+)	k' (−)	α	R_S
50	15.1	16.0	1.06	0.75
48	19.3	20.7	1.07	1.06
45	27.2	29.6	1.09	1.41
40	48.2	53.8	1.12	2.03

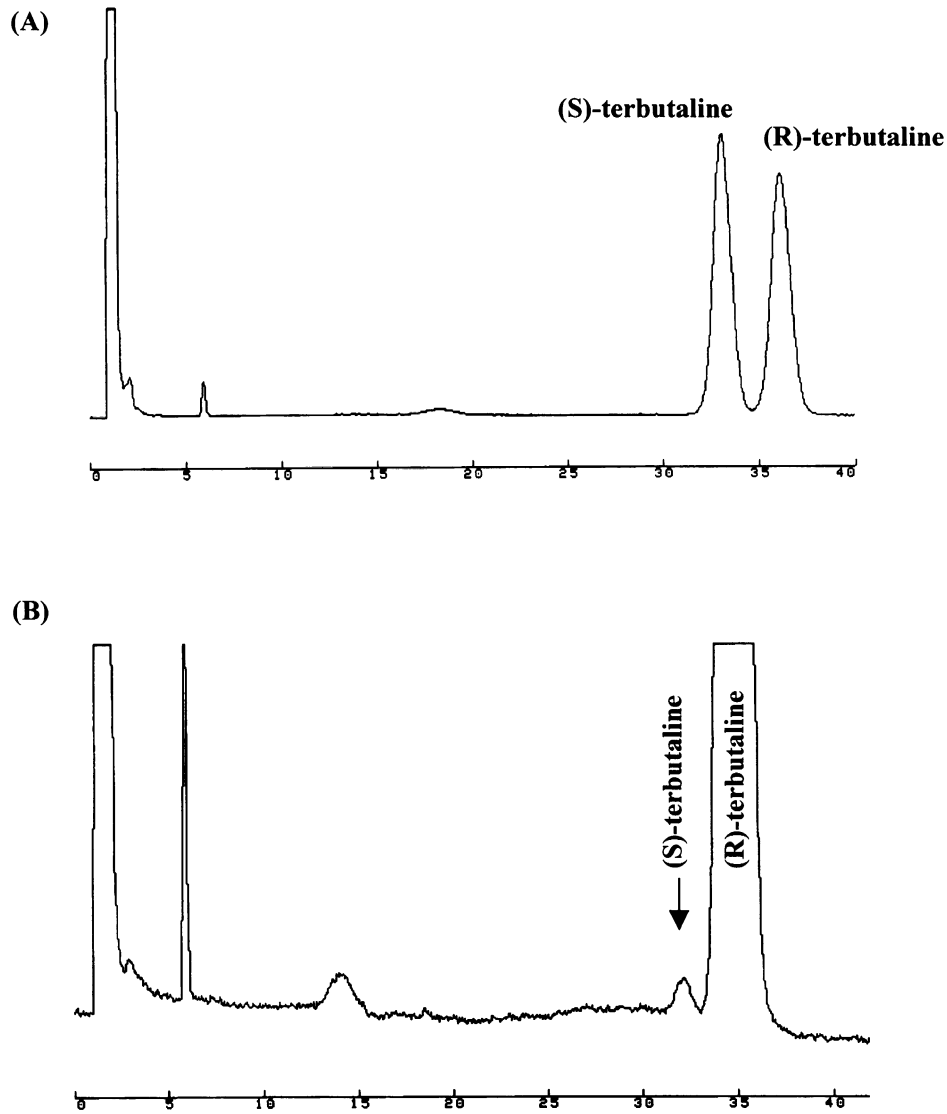

Fig. 6. Typical chromatograms of (A) a racemic terbutaline; and (B) standard (R)-terbutaline spiked with 0.1% (S)-terbutaline derivatives with (S)-MBIC. Column: Inertsil ODS-3, 5 μm, 150 × 4.6 mm; mobile phase: 43% methanol; flow rate: 1.0 ml/min; fluorescence detector: Ex 276 nm, Em 306 nm.

tation about 5.6%. However, this limit could be lowered by using a higher-field instrument, increasing the number of scans acquired or increasing sample amount.

Enantiomeric purity of (S)-MBIC (99.6%) did not give an important effect to the value of (S)-terbutaline in the range 5.6–31.1%.

3.3. HPLC study

Separation of the (S)-MBIC derivatized terbutaline racemate was investigated with the ODS column using a mobile phase of a water and an organic solvent such as acetonitrile or methanol. The enantiomers of terbutaline were well resolved

as their (S)-MBIC derivatives by reversed-phase HPLC. Baseline resolution was achieved in both organic solvents. Methanol was more effective than acetonitrile for the resolution of the diastereomers, as judged from the resolution values and analysis times. Capacity factors (k'), separation factor (α) and resolution (R_S) of corresponding diastereomers obtained with various methanol concentrations are summarized in Table 3 and a typical chromatogram using 45% methanol mobile phase is shown in Fig. 6, with a chromatogram of 0.1% (S)-terbutaline spiked in (R)-terbutaline. Under the chromatographic conditions described above, the (S)-enantiomer elutes before the (R)-enantiomer. Elution of the (S)-enantiomer prior to the (R)-enantiomer makes this method ideal for trace analysis of the (S)-enantiomer present in the (R)-enantiomer.

The accuracy and precision of the method were determined by replicated analysis of five samples of (S)-terbutaline added to (R)-terbutaline in the range 3.4–31.1%. The results obtained are shown in Table 2. The precision for each level was 3.78–10.12%. The theoretical concentration of impurity is plotted against the experimentally determined concentration. The equation of the line obtained was $y = 0.95x + 1.06$. The R.S.D. values (%) for the slope and intercept are 1.50 and 29.85, respectively. The correlation coefficient of the plot found to be more than 0.998, indicating good linearity.

The detection limit of the (S)-terbutaline at a signal-to-noise ratio of 3 was ca. 0.09%. However, the determination of samples of (S)-terbutaline added to (R)-terbutaline in the range below 3% could not be accepted because (S)-MBIC contained 0.4% of (R)-MBIC as an impurity.

In order to use the approach of chiral derivatization for the determination of the optical purity of a substance, the enantiomeric purity of the reagent itself has to be of high optical purity, compared with the level of the substance being investigated.

Practically, when sample solution added 1 and 0.5% of the (S)-terbutaline was treated according to the derivatization procedure (Section 2.3), values of 1.4 and 1.0% were found, respectively, indicating that the enantiomeric purity of (S)-MBIC give an important effect to the value of (S)-terbutaline. Therefore, a correction factor had to be used and the content of below 3% (S)-terbutaline in (R)-terbutaline was calculated again by the following equation:

$$\text{(S)} - \text{terbutaline content(\%)} = \frac{\text{Experimental(S)} - \text{terbutaline content(\%)} - 0.4}{0.992}$$

Almost 100% recoveries, that are 1.01 and 0.60%, respectively, were obtained.

The ruggedness of the method was determined by analyzing six independent sample preparations, by two analysts on two columns from different lots. The samples were analyzed using two different instruments, different mobile phase and different lots of reagent. The R.S.D. value of the method was 3.18%, indicating the ruggedness of the developed method.

The robustness of an analytical method is defined as a measure of its capacity to remain unaffected by small but deliberate variations in method parameter. The robustness of this method was tested by varying the concentrations of the drug and derivatizing reagent, varying in the reaction time and temperature, etc. It was found that varying the concentrations of the drug or reagent did not change the analytical result, as long as the molar amounts of reagent were more than five times of that of the drug. And it was necessary to wait at least 1 h after adding (S)-MBIC in order to ensure complete reaction. The DMSO-d_6 solution of the derivatives of terbutaline was found to be stable for at least 1 week.

The ^{1}H-NMR method for the determination of the optical purity of (R)-terbutaline after the derivatization with (S)-MBIC was compared with a reversed-phase HPLC method using an ODS column. The concentrations of impurity determined by RP-HPLC method were plotted against those determined by ^{1}H-NMR method and the equation of the line obtained was $y = 1.08x - 0.83$ ($r = 0.9999$). The R.S.D. values (%) for the slope and intercept are 0.93 and 22.84, respectively. These results show that reversed-phase HPLC and NMR methods are good agreement.

4. Conclusions

Derivatization of racemic terbutaline with (S)-MBIC leads to corresponding diastereomers that are separable by reversed-phase HPLC and NMR method. The derivatization was simple and completed in less than 1 h at room temperature. NMR method in the measurement of the optical purity of (R)-terbutaline was very convenient and precise and the NMR results had agreed with those obtained by HPLC method.

These results clearly demonstrate that these methods are quite satisfactory for use as a quality control method for the drug containing (R)-terbutaline.

However, enantiomeric purity of chiral derivatizing agent, (S)-MBIC, had to be considered for the quantitation of below 3% level of the (S)-enantiomer in (R)-terbutaline.

Acknowledgements

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