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Chiral Separation of Salbutamol Enantiomers in Human Plasma

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A stereoselective and sensitive high performance liquid chromatography using fluorescence detector was examined for the determination of R(−) and S(+)-salbutamol in human plasma. Solid phase extraction method using silica as sorbent was used to extract salbutamol racemates from the plasma matrices. After fractionation and freeze-drying of the eluates containing salbutamol racemates, they were separated and quantified on a chiral stationary column. The detection limit of each enantiomer was 2 ng/ml in human plasma (S/N=3).

Key words: Enantiomer, Salbutamol, Solid phase extraction

INTRODUCTION

Salbutamol, 2-tert.-butylamino-1-(4-hydroxy-3-hydroxymethyl)phenylethanol (Fig. 1) , is a relatively selective adrenoceptor stimulant which is widely used as a therapeutic agent for bronchiral asthma and other forms of reactive airways diseases (Powell *et al.*, 1985). It is used clinically as a racemic mixture of two optical isomers, R(−) and S(+)-salbutamol. It is easily absorbed orally, but have low systemic availability due to extensive first-pass sulphation (Emn and Pekal, 1988; Bland *et al.*, 1990). Plasma protein binding of most β-agonists is negligible, the elimination half-life is relatively short and pharmacokinetics are independent of the dosage and duration of treatment. Differences in the pharmacokinetics of the enantiomers are evident. It is reported that the drug's agonistic activity resides mainly in the R(−) configuration, as is the case with other adrenoreceptor agonists (Morgan, 1990). Stereoselective disposition of enantiomers can result in different pharmacological profiles owing to the different rates of absorption, stereoselective presystemic metabolism, distribution and elimination. Therefore, there is a need for the evaluation of the pharmacokinetics and plasma concentration-effect relationships of the active isomer rather than the racemates (Ariens, 1984; Borgstom *et al.*, 1989; Chin *et al.*, 1989; Mehvar, 1989; Tan and Soldin, 1989).

Salbutamol is not readily extracted by general liquid phase extraction method, because salbutamol possesses one phenolic hydroxyl group and one aliphatic amino group. In order to do stereoisomeric separation and quantitation of R(−)- and S(+)-salbutamol, they were extracted from plasma by solid phase extraction method and separated as racemates using normal silica HPLC column. After the eluates containig salbutamol racemates were fractionated and freeze-dried, they were separated into each enantiomer on a chiral stationay phase column and quantified.

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MATERIALS AND METHODS

Materials

Salbutamol sulfate was acquired from Graxo Korea (batch No. 92310) and terbutaline sulfate from AB Draco. Chloroform, acetonitrile and methanol (HPLC Grade) were obtained from J.T.Baker Co. (USA). Ammonium phosphate (dibasic) was purchased from Shinyo Co. (Japan). 1,2 Dichloroethane and n-hexane ware purchased from Lab-Scan Co. (Ireland). Trifluoracetic acid was obtained from Aldrich Co. (German) and di-(2-ethylhexyl) phosphate (DEHP) from Sigma Co. (USA). Sep-pak cartridges (Silica 100 mg; part. No. 20520) were purchased from Waters Asso. Co. (USA).

Reagents and Standard Solution

Stock solutions of salbutamol and terbutaline(internal standard) were prepared in deionized distilled water each at the concentration of 1 μg/ml (as sulfate form). The stock solutions were stored at 4℃ where they were stable for at least five weeks. A stock salbutamol solution was used to prepare plasma standards containing 1, 2, 8, 15, 30, 50 ng/ml salbutamol. Pooled drug

free plasma were divided into 0.5 ml aliquots and stored at −20℃ until being assayed. These samples were used as controls for the assay of clinical samples and the stability study.

The mobile phase for achiral separation was 1.3 mM $(NH_4)_2HPO_4$ in methanol/water (65 : 35) with pH adjusted to 7.7 by 10% phosphoric acid. The solvent was filtered and degassed before use. The mobile phase for chiral separation was composed of hexane : 1,2 dichloroethane : methanol : trifluoracetic acid (240 : 140 : 25 : 1, v/v). Chloroform containing DEHP at 0.01 M was used as an ion-pairing solvent for dissolving the freeze-dried residues of the fractionated eluates from the achiral normal silica HPLC column.

Instruments

The high-performance liquid chromatographic system was equipped with Shimadzu (Kyoto, Japan) LC-9A pump, Shimadzu RF-535 fluorescence detector and Shimadzu CR-4AD data processor. Microsorb 80-125-C5 silica analytical column (Rainin. USA,4.0 mm I.D.× 30 cm) and a SUMICHIRAL OA-4700 chiral column (Osaka, Japan, 4.0 mm I.D×25 cm) were used. Speed-vac concentrator (Savant USA), vacuum pump (GAST, USA, Model ROA-P104-AA), deep-freezer (Scien. Temp. Corp, U.S.A) and freeze-dryer (Ilsan Engeneering Co., Korea) were used.

Plasma Samples

Drug-free blood was obtained from healthy human volunteers under no medication. Two tablets which contain 8 mg of salbutamol, were orally administrated to a healthy 24-year-old male volunteer. Blood samples were drained into Venoject heparin-containing tubes at the interval of 30 min and 1 hour for 12 hours.

Preparation of Samples

500 μl aliquot of a human plasma, standard, or quality control samples and 50 μl of internal standard (terbutaline) solution were well mixed. The solid-phase (silica) extraction columns were preconditioned by passing 1 ml of acetonitrile followed by 1 ml of deionized water under the low vacuum which was released immediatly after the solvents eluted from all the cartridge. 500 μl of the previously mixed plasma sample was transferred to the top of the preconditioned column and minimum vacuum was applied. Each column was then washed with 1ml of acetonitrile under the low vacuum until all the wash solvents were eluted from the cartridge. The columns were dried under the full vacuum for additional 5 min. Salbutamol and terbutaline were then eluted from the silica sorbent by passing 7 ml of methanol. The sample tubes containing the column effluents were dried by the Speed Vac concentrator.

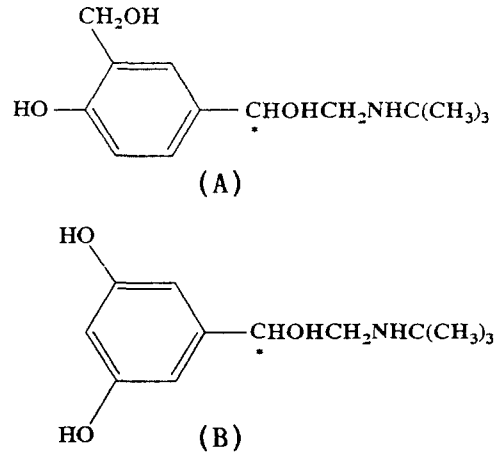

Fig. 1. Chemical structures of (A) salbutamol and (B) Terbutaline (internal standard).

The residues were reconstituted in 200 μl mobile phase and 100 μl was injected into the achiral HPLC system using silica column and monitored by fluorescence detector with excitation and emission wavelength set at 235 nm and 305 nm, respectively.

The eluate containing salbutamol racemates was collected, freeze-dried. The residue was dissolved in 200 μl of 0.01 M di-(2-ethylhexyl) phosphate and 100 μl of the resulting solution was injected into the chiral HPLC system.

Those fractions of the chiral column eluate containing the resolved enantiomers were collected and evaporated to dryness under the stream of nitrogen. Their direction of rotation (+ or −) was determined by measuring their cotton effect at 276-280 nm by a Jasco Model J-20 C Spectropolarimeter.

RESULTS

Chromatography

Fig. 2 shows typical chromatograms of salbutamol racemates extracted with Sep-pak silica cartridge from plasma. (A) and (B) chromatograms were from blank plasma and plasma spiked with 1 ng/500 μl salbutamol, repectively. Chromatogram C was from volunteer's plasma taken after the oral admination of 8.0 mg of salbutamol. The peaks corresponding to salbutamol and internal standard, terbutaline, were well resolved from co-eluting endogenous substances.

The retention times of salbutamol racemate and terbutaline were approximately 33 and 38 min under this condition, respectively. The chromatogram from blank plasma showed no interfering peaks at these retention times. The resolution and selectivity factor between salbutamol and terbutalin were 4.02 and 1.15, respectively.

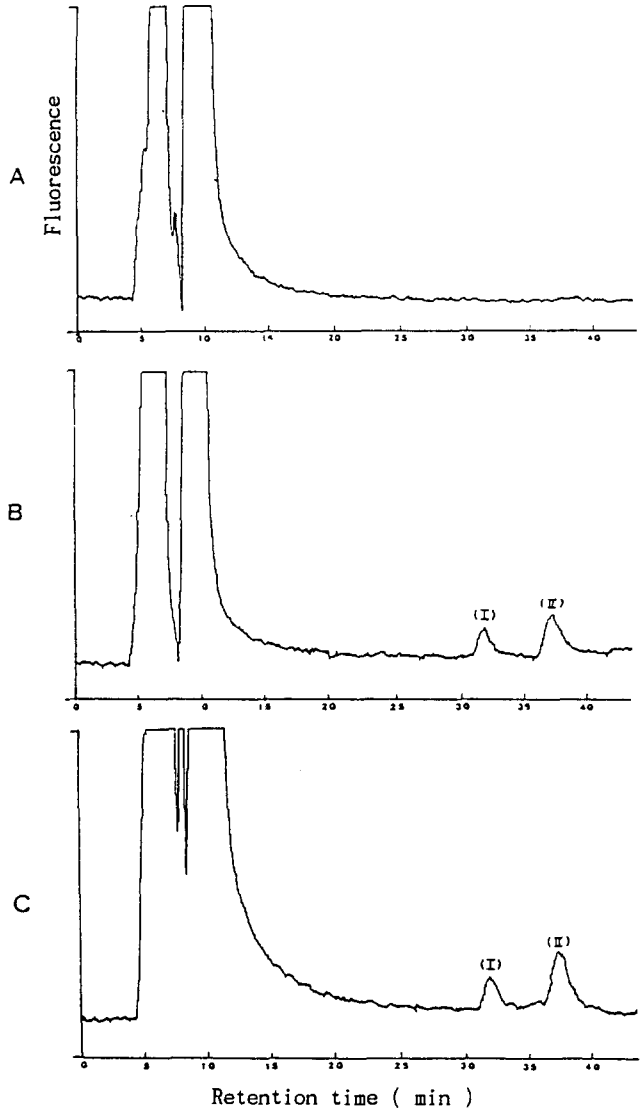

Fig. 2. Achiral HPLC chromatograms of (A) blank plasma. (B) plasma spiked with 1ng of salbutamol racemate and (C) human plasma sample taken at 0.5 h after an oral administration of two sabutamol sulfate tablets (8.0 mg).
Peaks: I=Intemal standard (terbutaline), II=Salbutamol

Fig. 3 shows a typical chromatogram of salbutamol enantiomers extracted from plasma. The resolution was 1.72 and selectivity factor was 1.12. R(−)-Salbutamol was found to elute faster than S(+)-Salbutamol from the chiral column.

Elution Curves and Recovery

The absolute recoveries of salbutamol and terbutaline were determinded by comparing the peak areas of the plasma standards extracted to those of standards at the equivalent concentrations.

Salbutamol and terbutaline are polar and hydrophilic compounds which do not partition readily into organic solvents from aqueous solution. We used thus solid phase extraction method to extract salbutamol from plasma samples.

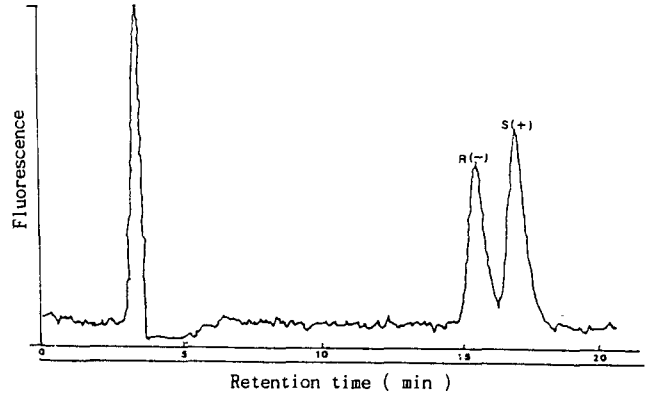

Fig. 3. Chromatogram of salbutamol enantiomers from the human plasma taken at 4 h after an oral administration of two sabutamol sulfate tablets (8.0 mg).

Table I. Recoveries of salbutamol racemates in pooled human plasma (n=5)

Salbutamol conentration (ng/ml)	percentage recovery (mean± S.D.)	Coefficient of variation (%)
2	79.3± 4.4	5.5
4	85.4± 6.5	7.6
16	82.1± 8.7	10.4
30	90.0± 3.3	3.7
60	82.4± 4.5	5.4
100	93.0± 4.9	5.3
Amount of internal standard (terbutaline) added		
100	81.7± 6.8	8.2

Table II. Recoveries of salbutamol enantiomers (n=5)

Racemic salbutamol concentration (ng/ml)	Percentage recovery (mean± S.D.)		Coefficient of variation (%)	
	(+)	(−)	(+)	(−)
16.0	84.4± 15.4	81.5± 15.8	18.3	19.4
30.0	85.9± 5.8	95.0± 5.4	6.7	5.7
60.0	80.2± 6.3	84.7± 6.0	7.8	7.1
100.0	94.2± 5.0	91.8± 5.0	5.3	5.5

The adequate methanol volume for the elution of salbutamol and terbutaline was found to be 7 ml.

Using this procedure, the average recovery of salbutamol at various concentrations was 85% and that of terbutaline 81.7% (Table I).

Table II lists rocoveries of enantiomers at the range from 8 ng to 50 ng/ml concentrations. They are calculated by the area ratio between the enantiomers. And the actual amount of each enantiomer can be calcula-

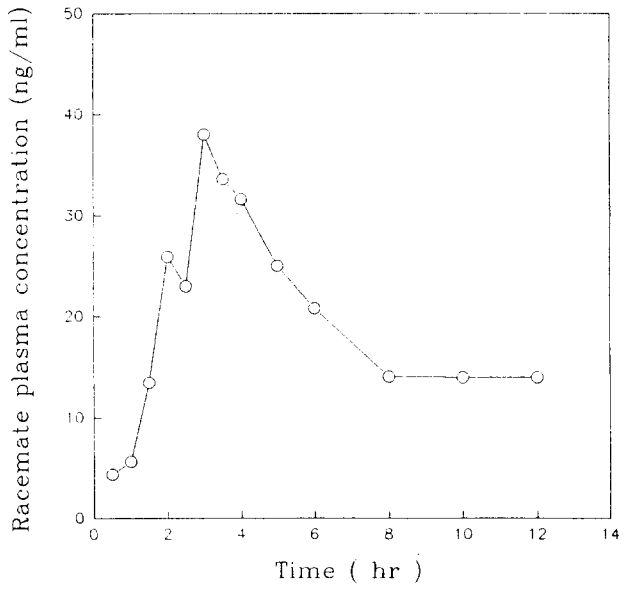

Fig. 4. Plasma concentration-time curve of salbutamol racemate from a volunteer after an oral administration of two sabutamol sulfate tablets (8.0 mg).

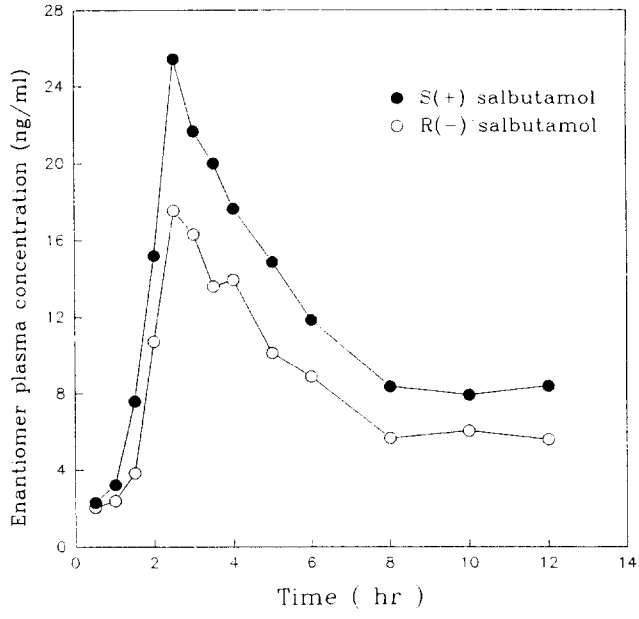

Fig. 5. Plasma concentration-time curves of R-(−) and S-(+) salbutamol enantiomers after an oral administration of two sabutamol sulfate tablets (8.0 mg).

ted if the total amount is known.

Linearity, Precision and Accuracy

Inter-day precision was determined by preparing standard curves using plasma containing salbutamol. The present method was found to be linear over the standard curve range from 2 to 100 ng/ml and the estimated intercepts did not differ signifficantly from the origin.

Table III. Inter-day precisions for the assay of salbutamol enantiomers (n=5)

Racemic salbutamol concentration (ng/ml)	Coefficient variation (%)	
	(+)	(−)
16.0	17.6	18.1
30.0	25.7	23.0
60.0	8.1	8.9
100.0	8.1	8.3

The regression equation of the calibration curve was Y=0.1235x+0.0247 (r=0.9973, n=5). The reproducibility of the method was assessed by statistical analysis of the daily standard curves. Inter-day precision was determined by analysing four replicates at each drug concentration. The relative standard deviations (RSD) were found to range from 3.3 to 8.6%.

Inter-day precision data for enantiomeric salbutamol assay was showed in Table III. Coefficient of variation was ranged from 8.1 to 25.7% at different concentration range.

Fig. 4 shows the plasma concentration-time curve of salbutamol racemate after the oral administration of two salbutamol sulfate tablets (8.0 mg) to a healthy volunteer. The maximum plasma concentration was about 44 ng/ml.

Fig. 5 shows the plasma concentration-time curves of salbutamol enantiomers after the oral administration of two salbutamol sulfate tablets (8.0 mg) to a healthy volunteer.

Detection Limit

With the achiral analysis set at a sensitivity of 1 mV full scale, the detection limit of salbutamol racemate taken as a signal-to-baseline noise ratio of 3 was judged to be 1 ng/ml.

In a chiral condition, the detection limit of salbutamol enantiomer was 2 ng/ml (S/N=3).

DISCUSSION

The present work describes a chiral HPLC separation method for the optical isomers of salbutamol, a bronchodilatory drug which is widely used for the treatment of asthma around the world. The established separation technique was employed to quantitatively determine the two salbutamol enantiomers extracted from human plasma after the oral administration of the racemic drug. The resolution achieved with the SUMICHIRAL OA-4700 column provided sufficiently good pharmacokinetic data. The detection limit of each isomer was about 2 ng/ml (S/N=3).

In conclusion, the relatively simple method for the determination of salbutamol enantiomer in plasma de-

scribed here has enough sensitivity for the pharmacokinetic study of salbutamol enantiomers after oral admination of salbutamol racemates to human.

ACKNOWLEDGEMENT

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