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Determination of Terbutaline Enantiomers in Human Urine by Capillary Electrophoresis Using Hydroxypropyl-β-cyclodextrin as a Chiral Selector

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A method for the determination of terbutaline enantiomers in human urine by capillary elctrophoresis has been developed. Optimum resolution was achieved using 50 mM phosphate buffer, pH 2.5, containing 15 mM of hydroxypropyl-β-cyclodextrin as a chiral selector. Urine samples were prepared by solid-phase extraction with Sep-pak silica, followed by CE. The assay was linear between 2-250 ng/mL ($R = 0.9998$ for (*S*)-(+)-terbutaline and $R = 0.9999$ for (*R*)-(−)-terbutaline) and detection limit was 0.8 ng/mL. The intra-day variation ranged between 6.3 and 14.5% in relation to the measured concentration and the inter-day variation was 8.2-20.1%. It has been applied to the determination of (*S*)-(+)-terbutaline and (*R*)-(−)-terbutaline in urine from healthy volunteer dosed with racemic terbutaline sulfate.

Key words: Terbutaline, Capillary electrophoresis, Chiral separation, Hydroxypropyl-β-cyclodextrin, Urine

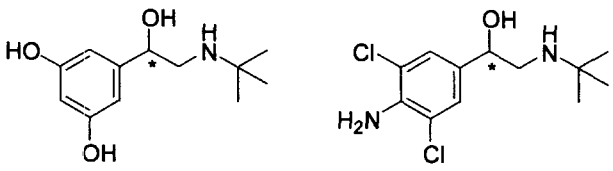
INTRODUCTION

Since the enantiomers of chiral drugs often present different pharmacological and toxicological properties, enantiomeric separations are particularly important in the pharmaceutical field. The determination of individual enantiomers is required for the control of the optical purity of bulk drug substances as well as in pharmacokinetic and clinical studies (Ariens, 1992).

Terbutaline, a sympaticomimetic drug-selective β_2-receptor agonist is used in the treatment of asthma and lung disease (Fig. 1). The drug is usually administered as a racemate, but studies have shown that only the (−)-enantiomer has the desired therapeutic pharmacological effect(Jeppson *et al.*, 1984). For that reason it is of great importance that the enantiomers of such molecules can be separated, especially for biomedical analysis.

Capillary electrophoresis has become an established powerful tool for enantiomeric separation of racemic drugs (Fanali *et al.*, 2001; Nishi, 1996; Verleysen and Sandra, 1998) due to its main advantage over HPLC and capillary gas chromatography, such as high separation efficiency, short analysis time, instrumental simplicity, low consumption of chiral selectors and reduced operation costs.

Basic enantiomers like terbutaline can be separated by capillary electrophoresis using cyclodextrins as a chiral selector (Vigh and Sokolowski, 1997; Guttman and Cooke, 1994). 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 gel (Boer and Ensing, 1998). The author reports that the addition of polyethylene glycol results in an increase in



Terbutaline Clenbuterol (internal standard)

Fig. 1. Structure of terbutaline and clenbuterol (internal standard). Chiral centers in these molecules are indicated by an asterisk.

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resolution, but at higher concentrations the baseline starts to fluctuate impairing quantitation of low concentrations of the individual enantiomers. In the previous report, we determined the terbutaline enantiomers in human urine by coupled achiral-chiral high-performance liquid chromatography (Kim *et al.*, 2001). No capillary electrophoretic method for the determination of terbutaline enantiomers in human body fluids has been described previously.

In this work, a simple but highly efficient capillary electrophoretic method was developed and validated for the quantitation of terbutaline enantiomers in human urine using hydroxypropyl-β-cyclodextrin as a chiral selector. The assay also was applied to the stereoselective pharmacokinetic studies of terbutaline.

MATERIALS AND METHODS

Chemicals

Phosphoric acid (85%), sodium dihydrogen phosphate and sodium hydroxide were of analytical grade from Duksan Pure Chemical Co. (Ansan, Kyeonggi, Korea). Methanol from Duksan Pure Chemical Co. was of HPLC grade. Hydroxypropyl-β-cyclodextrin (HP-β-CD) was obtained from Aldrich (Milwaukee, WI, USA). Terbutaline sulfate and clenbuterol hydrochloride were purchased from Sigma (Dorset, UK). Their structures are shown in Fig. 1. (*S*)-(+)-terbutaline and (*R*)-(−)-terbutaline were prepared from racemic terbutaline by semi-preparative chiral high-performance liquid chromatography (Kim *et al.*, 2001).

Electrophoresis equipment and operating conditions

All experiments were performed on a HP 3D CE instrument (Hewlett Packard, Waldbronn, Germany), comprising a diode-array detector and Chemstation software for data handling. The compounds were separated in an uncoated fused silica capillary (75 μm I.D., Hewlett Packard). The capillary length was 56.0 cm (to the detector 48.0 cm). Separation was carried out in 0.05M phosphate buffer of pH 2.5. 15 mM HP-β-CD was used as chiral selector. The applied voltage was 25 kV. The temperature was 20°C. Injection was performed at 20 kV for 10 s. Detection was at 205 nm. The capillaries were conditioned according to the following procedure before every run: water for 2 min; 0.1 M NaOH for 2 min; water for 2 min; background electrolyte solution (BGE) for 5 min; and finally with the chiral selector solution for 3 min prior to application of the analyte.

Sample preparation

A 10 μL volume of internal standard solution (clenbuterol HCl, 5 μg/mL, in water) was spiked into 2 mL urine. The mixture was vortex-mixed for 10 s and applied to a Sep-*pak* silica cartridge pre-conditioned with acetonitrile (1 mL) and water (1 mL). The cartridge was washed with water (1 mL) and then acetonitrile (2 mL). The analytes were eluted with 6 mL of methanol. The eluate was concentrated to dryness, and the residue was reconstituted in 0.2 mL of methanol for an assay sample.

Validation studies

Spiked urine samples were prepared by adding known amounts of racemic terbutaline and the internal standard (clenbuterol) to drug-free urine at six concentration levels (4, 10, 50, 100, 250 and 500 ng/mL) and used for evaluation of the linearity, accuracy and precision. Calibration curves according to the internal standard method were obtained by plotting concentration vs. peak-area ratios (area of each terbutaline enantiomer/total areas of clenbuterol enantiomers). The precision of the method was assessed by determining the intra-day assay coefficients of variation (C.V.) of the analysis (n = 6) of spiked urine samples. The C.V. and accuracy for inter-day assay were evaluated by analysis of samples at three concentrations (10, 100 and 500 ng/mL), repeated for three different days.

Application

This analytical method was applied to a pharmacokinetic study. Racemic terbutaline sulfate (5 mg) was administered once orally to a 25-year-old healthy male volunteer. He was free from other medication for the duration of the experiment. Urine samples were collected at various times over 48 h and stored at -20°C until analysis.

RESULTS AND DISCUSSION

Capillary electrophoresis

In the previous paper (Kim *et al.*, 2001) we have investigated the most optimal operating conditions, such as buffer composition, buffer pH and the concentration of HP-β-CD for the chiral separation of several β_2-agonists with uncoated fused silica capillaries. The enantiomers of terbutaline and clenbuterol were resolved best when 0.05 M phosphate buffer of pH 2.5 and 15 mM HP-β-CD was used. The enantiomeric migration order of terbutaline was determined by injected each enantiomer separately under the same CE conditions. And the elution order of the clenbuterol enantiomers was determined by Gausepohl (Gausepohl and Blaschke, 1998). For terbutaline and clenbuterol, (*R*)-form was migrated before the (*S*)-form. Under the chiral conditions, the migration times of (*R*)-(−)-clenbuterol, (*S*)-(+)-clenbuterol, (*R*)-(−)-terbutaline and (*S*)-(+)-terbutaline were 15.18, 15.70, 16.48 and 17.45 minutes, respectively. For the terbutaline enantiomers, the stereoselectivity (α) was 1.09 and the resolution factor (R_s) was 5.51. Fig. 2 shows chromatograms obtained from analysis of drug-free human urine and urine samples. No interferences with either ter-

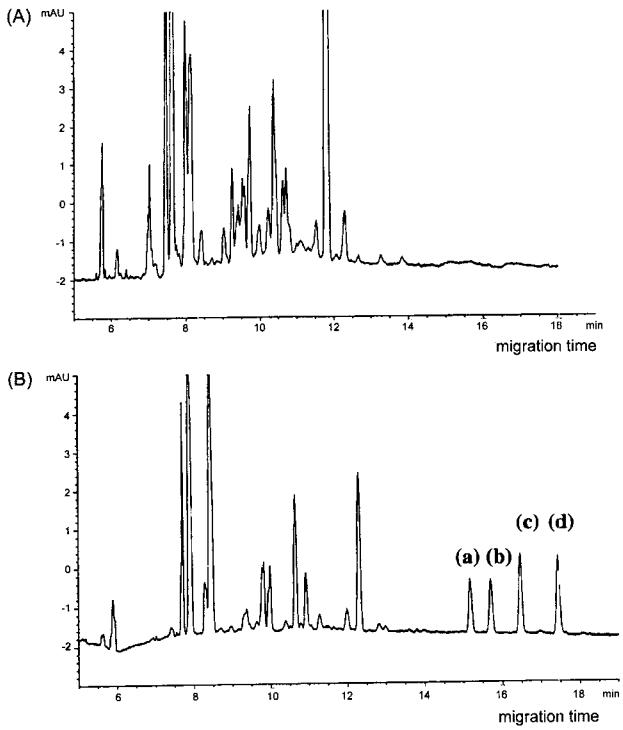

Fig. 2. Representative electropherogram of (A) blank urine and (B) 6.25-h urine sample after oral administration of 5mg terbutaline sulfate. Peak a; (*R*)-(−)-clenbuterol, Peak b; (*S*)-(+)-clenbuterol, Peak c; (*R*)-(−)-terbutaline (95.23 ng/mL), Peak d; (*S*)-(+)-terbutaline (91.48 ng/mL). Condition: BGE, 0.05 M phosphate buffer of pH 2.5 and 15 mM HP-β-CD; capillary, uncoated fused-silica (75 μm I.D.×56.0 cm, effective length 48 cm); applied voltage, 25 kV; detection, 205 nm; temperature, 20°C.

butaline enantiomers or the internal standard were detected from blank urine.

Linearity and limit of detection

The calibration curves were obtained by analyzing spiked urine samples. Calibration curves for each enantiomers showed good linearity in the concentration range 2-250 ng/mL in urine. The equation of the calibration line obtained for (*S*)-(+)-terbutaline is: Y = 0.0091X + 0.0045 and for (*R*)-(−)-terbutaline is: Y = 0.009X + 0.0045. The correlation coefficients of (*S*)-(+)-terbutaline and (*R*)-(−)-terbutaline were 0.9998 and 0.9999, respectively. The limit of detection, estimated under the described conditions at a signal-to-noise ratio of three was 0.8 ng/mL.

Accuracy and precision

Accuracy and precision of the method were determined by replicated analysis of blank human urine spiked with six concentrations of terbutaline enantiomers within the range 2-250 ng/mL. Six replicates of each concentration were analyzed on each of three separate days. The results obtained are shown in Table I and Table II. The intra-day

Table I. Intra-day precision for the (*S*)-(+)-terbutaline and (*R*)-(−)-terbutaline in human urine (n = 6)

Added Conc. (ng/mL)	(*S*)-(+)-terbutaline			(*R*)-(−)-terbutaline		
	Measured Conc. (ng/mL)	Accuracy (%)	C. V. (%)	Measured Conc. (ng/mL)	Accuracy (%)	C. V. (%)
2	2.0	99.4	14.5	2.0	99.3	12.9
5	5.2	102.9	6.8	5.2	104.3	10.5
10	10.6	106.2	8.5	10.6	105.6	9.5
50	48.8	97.6	7.8	49.4	98.8	7.0
125	127.9	102.3	7.7	127.6	102.1	6.3
250	247.6	99.1	6.5	248.7	99.5	7.5

Table II. Inter-day precision for the (*S*)-(+)-terbutaline and (*R*)-(−)-terbutaline in human urine (n = 3)

Added Conc. (ng/mL)	(*S*)-(+)-terbutaline			(*R*)-(−)-terbutaline		
	Measured Conc. (ng/mL)	Accuracy (%)	C. V. (%)	Measured Conc. (ng/mL)	Accuracy (%)	C. V. (%)
5	5.2	104.2	20.1	5.6	111.9	18.6
50	50.9	101.7	10.8	52.1	104.2	8.2
250	253.8	101.5	9.1	254.1	101.6	10.6

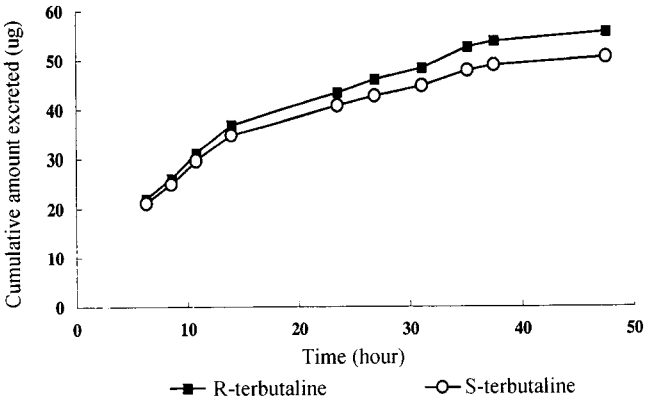

Fig. 3. Cumulative urinary excretion of terbutaline enantiomers following a single oral dose of 5mg terbutaline sulfate.

precision for each concentration was 6.5-14.5% for (*S*)-(+)-terbutaline and was 6.3-12.9% for (*R*)-(−)-terbutaline. The inter-day precision was 9.1-20.1% for (*S*)-(+)-terbutaline and was 8.2-18.6% for (*R*)-(−)-terbutaline. The accuracy, determined for each concentration, ranged from 97.6% to 111.9%.

Application

The method described was applied to a pharmacokinetic study of terbutaline enantiomers in human urine samples obtained after the oral administration of tablet containing 5 mg terbutaline sulfate. A typical urinary excretion data of terbutaline enantiomers from a healthy male volunteer is shown in Fig. 3. From the 0-48 h urine, the amounts of ter-

butaline enantiomers were determined. Unchanged terbutaline accounted for 2.49% of the dose and ((S)-(+)-terbutaline)/((*R*)-(−)-terbutaline) ratio was 0.91. The (*R*)-(−)-terbutaline had a higher excretion rate than (*S*)-(+)-terbutaline.

Capillary electrophoretic method was developed for the stereoselective assay of terbutaline in human urine using hydroxypropyl-β-cyclodextrin as a chiral selector and was applied to the determination of terbutaline enantiomers in urine after oral administration of racemic terbutaline sulfate to human. The present method is convenient and simple and has been fully validated and proved to be suitable for the stereoselective pharmacokinetic studies of terbutaline.

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