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Enantioselective analysis of pheniramine in rat using large volume sample stacking or cation-selective exhaustive injection and sweeping coupled with cyclodextrin modified electrokinetic chromatography

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ABSTRACT

For the aim of simultaneously performing the enantioseparation and determination of the trace enantiomers in plasma samples, enantioseparation by HPLC using five kinds of chiral stationary phases were initially investigated. But unfortunately, enantioseparation could not be detected in reversed mobile phase mode with all the five columns. For this reason, two simple, economical and highly efficient online preconcentration methods, large volume sample stacking and sweeping (LVSS-sweeping) and cation-selective exhaustive injection and sweeping (CSEI-sweeping) both followed by the cyclodextrin modified electrokinetic chromatography (CDEKC) were examined in the present work. Parameters affecting the enantioseparation and enhancement efficiency of these two injection modes were monitored in detail, and migration order of the two enantiomers was identified by circular dichroism (CD) and HPLC. Upon optimization, two enantiomers were best separated with the improvement of sensitivity reaching 160-fold and 4000-fold respectively for LVSS-sweeping and CSEI-sweeping comparing with the normal CDEKC separation. Then the optimal condition of CSEI-sweeping-CDEKC was validated and showed high sensitivity (10 ng/mL for lower limit of quantification, LLOQ), satisfactory accuracy (96.8–111.6%) and precision (relative standard deviation, RSD within 9.4%). This demonstrated it to be a suitable strategy for the rapid enantioselective determination and quantitative analysis of pheniramine enantiomers in plasma samples. Therefore, the method was further applied in the enantiomeric analysis of pheniramine in rat pharmacokinetics and plasma protein binding investigations. Stereoselectivity in pharmacokinetics as well as plasma protein binding were observed, suggesting that the stereoselective protein binding might be responsible for the stereoselectivity in pharmacokinetics.

1. Introduction

Many pharmaceutical compounds possess an asymmetric center that is responsible for the optical activity of the drug. The pharmacological activities of such compounds are mostly restricted to one enantiomer [1]. Pheniramine (N-[3-phenyl-3-(2-pyridyl)propyl]-N,N-dimethylamine) just happened to be this certain kind of drugs. It is a highly potent first generation histamine H_1-receptor antagonist, which is still widely used in combination with other drugs for the clinical treatment of common cold, allergies and respiratory infections [2,3], and its activity is predominantly attributed to the dextrorotary S-enantiomer [4]. Considering of this, there is a requirement for its enantioselective monitoring.

Several analytical methods have been reported for the qualitative and quantitative determination of pheniramine in various matrices, including pharmaceutical preparations [5] and biological fluids [6]. Among them, capillary electrophoresis (CE) methods were most frequently used. A simple CE partial-filling technique with hydroxypropyl-β-cyclodextrin as chiral selector for the enantioseparation of pheniramine was presented by Phatthiyaphaibun [4]. A carboxyethyl-β-cyclodextrin mediated capillary zone electrophoresis system was developed by Peter Mikuš for the enantioseparation and quantitation of pheniramine enantiomers in pharmaceutical formations [3]. The application of capillary zone electrophoresis online coupled with capillary isotachophoresis and diode array detector to the identification and determination of pheniramine enantiomers and their metabolites

Abbreviations: BGE, background electrolyte; CD, circular dichroism; CDEKC, cyclodextrin modified electrokinetic chromatography; CSEI, cation-selective exhaustive injection; EOF, electroosmotic flow; HCB, high conductivity buffer; LCB, low conductivity buffer; LVSS, large volume sample stacking; S-β-CD, sulfated-β-cyclodextrin

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presented in urine samples were shown by Marák et al. [6]. And Pieštanský et al. proposed a multidimensional analytical approach, isotachophoresis-chiral capillary zone electrophoresis and triple quadrupole mass spectrometry for the ultra-trace determination of pheniramine enantiomers in human urine samples [7].

The enantioselective determinations of biologically active compounds like drugs surely have great significance in pharmaceutical and clinical research, especially in pharmacokinetic studies [8,9]. But to our knowledge, no method for the stereoselective pharmacokinetic or plasma protein binding study of the pheniramine enantiomers has been reported. As known, the analyses of target analytes presented in biological samples are complicated, which is mainly due to their very low concentration levels, so methods with high efficiencies are obviously required. Generally, high-performance liquid chromatography combined with mass spectrometry (HPLC-MS) is considered the preferred method. However, the normal phase separation mode was improper of being combined with a mass spectrometry detector because of the high proportion of n-hexane contained in the mobile phases [10,11]. As a consequence, the detection sensitivity of drugs that cannot be separated in the reverse phase HPLC was greatly restricted, and CE online preconcentration methods was thus considered.

Though emerged as a promising analytical technique of short analysis time and high resolution efficiency, the lack of sensitivity when coupled with UV detection indeed restricted the application in pharmacokinetic studies of CE. In this case, integration of an online preconcentration strategy becomes requisite to enhance the sensitivity of CE methods. According to the literatures, numerous online enrichment strategies have been developed, mainly via the conversion of a long injected sample zone into a narrow band inside the capillary [12]. The most widespread used preconcentration techniques that could achieve this aim are sample stacking and sweeping methods, including different modes such as large volume sample stacking (LVSS) [13], field-amplified sample stacking [14], cation-selective exhaustive injection (CSEI) [15], and/or sweeping [16]. And when combining the advantages of these two methods, the sample enrichment efficiency could be very high.

In this study, we focus our attention on the development of a sensitive and enantioselective online preconcentration method for stereoselective pharmacokinetic study of pheniramine enantiomers in rats. Considering of the failure in our preliminary separation by HPLC at reversed phase, cyclodextrin modified electrokinetic chromatography (CDEKC) has been employed and sulfated-β-cyclodextrin (S-β-CD) was used as the chiral pseudostationary phase [17]. Two highly efficient injection modes, LVSS-sweeping and CSEI-sweeping were evaluated. The influences of experimental conditions on these two methods were investigated. On comparing the detection sensitivity, CSEI-sweeping in combination with CDEKC was chosen to be validated and further applied to the chiral determination of pheniramine enantiomers in rat plasma samples. The mean plasma concentration-time profiles and major pharmacokinetic parameters of two enantiomers were successfully obtained after an oral administration of racemic pheniramine to rats. Stereoselectivity was observed with higher plasma concentration of the S-pheniramine than that of the R-pheniramine. On this basis, to provide an explanation for the stereoselective pharmacokinetics, binding of pheniramine enantiomers to rat plasma protein was investigated by equilibrium dialysis method.

2. Experimental

2.1. Chemicals and reagents

Pheniramine, (+)-brompheniramine, and S-β-CD (degree of substitution, 12–15) were purchased from Sigma-Aldrich (St. Louis, MO, USA). R-pheniramine was obtained from analysis chemistry laboratory, Shenyang Pharmaceutical University (Shenyang, China). Sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3) and sodium dihydrogen phosphate (NaH_2PO_4) were purchased form Tianjin Bodi Chemical Holding Co. Ltd (Tianjin, China). Chromatographic grade phosphoric acid, diethylether, methanol, acetonitrile, isopropanol, n-hexane, formic acid, diethylamine and tetrahydrofuran were obtained from Tianjin Concord Chemical Holding Co. Ltd (Tianjin, China). Semipermeable membranes (MD 25, 14,000 molecular weight cut off) for equilibrium dialysis were purchased from Viskase (Darien, IL, USA). Ultrapure water was used throughout the experiment and all solutions were passed through 0.22 μm pore size filters.

2.2. Apparatus

CE experiments were performed by a Beckman P/ACE^{TM} MDQ capillary electrophoresis system (Beckman, Fullerton, CA, USA) equipped with a diode-array detector. The wavelength employed for the determination of target compounds was 210 nm. Data were collected using 32 Karat 8.0 Software (Beckman, Fullerton, CA, USA). The temperature for the separation was maintained at 25 °C using a capillary cartridge coolant (Beckman, Fullerton, CA, USA).

The HPLC experiments were performed on a Shimadzu LC-10A HPLC system (Shimadzu, Japan) equipped with a LC-10AT pump and a SPD-10A UV–vis Detector. Data were collected on a computer running with Sepu 3000. Chiralpak IA (250 mm × 4.6 mm I.D., 5 μm), Chiralpak IB (250 mm × 4.6 mm I.D., 5 μm), Chiralpak IC (250 mm × 4.6 mm I.D., 5 μm), Chiralpak ID (250 mm × 4.6 mm I.D., 5 μm), and Chiralcel OD-RH (150 mm × 4.6 mm I.D., 5 μm) column were tested. All the five columns were purchased from Daicel Chemical Industries (Tokyo, Japan).

All pH measurements were carried out with a pH meter (Bante PHS-3BW, Shanghai, China). A centrifuge (Anting TGL-16G Shanghai, China) and a vortex (XK96-A, Jiangsu, China) were also used.

The pharmacokinetic parameters were calculated using DAS Software (Version 2.0, China) and compared by Student's *t*-test. Differences in parameters were considered as statistical significance at $P < 0.05$.

2.3. CE procedures

Sample analysis was performed in an uncoated fused-silica capillary column (Hebei Optical Fiber, China) with a total length of 50 cm (effective length 40 cm) × 50 μm I.D. New capillary was preconditioned for the first time by sequentially flushing with methanol (10 min), 1 M NaOH (30 min), 0.1 M NaOH (30 min) and water (30 min) at 20 psi. Every day, before starting the experiments, the capillary was rinsed successively at 20 psi with 0.1 M NaOH for 10 min, then water for 10 min, and conditioned with background electrolyte (BGE) for 20 min.

Subsequently, in LVSS-sweeping-CDEKC mode, phosphate buffer similar to BGE but devoid S-β-CD (non-CD-BGE, 70 mM, pH 2.5) was loaded into the capillary and then the large volume of the samples, diluted in 10 mM phosphate buffer (pH 5), was pressure-injected into the capillary at 10 psi for 20 s to enhance sensitivity. After sample injection, the inlet and outlet reservoirs were replaced with BGE (70 mM, pH 2.5) containing 30 mg/mL S-β-CD. The reverse polarity of −15 kV was applied, thus the stacking and separation were performed.

And in CSEI-sweeping-CDEKC mode, the capillary was first filled with low conductivity buffer (LCB, 30 mM phosphate buffer, pH 3) for 8 min at 20 psi. Afterwards, a high conductivity buffer (HCB, 200 mM phosphate buffer, pH 3) zone was introduced into the capillary by hydrodynamic injection at 5 psi for 0.6 min. After capillary conditioning, samples were electrokinetically injected at 10 kV (normal mode) for 99.9 s. Electrophoretic separation was performed under −20 kV with a BGE of 30 mM phosphate buffer and 30 mg/mL S-β-CD at both ends of the capillary.

For comparison, normal CDEKC was also carried out at pH 3 with a BGE of 30 mM phosphate buffer containing 20 mg/mL S-β-CD. Samples were injected using a 3 s hydrodynamic injection at 0.5 psi, followed by