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Rapid chiral separation of racemic cetirizine in human plasma using subcritical fluid chromatography-tandem mass spectrometry

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ABSTRACT

A method for fast chiral separation of cetirizine and quantitation of levocetirizine in human plasma using subcritical fluid chromatography with tandem mass spectrometry was developed and validated. The chromatographic separation was performed using a Chiralpak IE column (2.1 mm × 150 mm, 5 μm) with an isocratic elution of CO_2/organic modifier (55/45, v/v) at a flow rate of 0.85 mL/min. The organic modifier was composed of water/methanol (5/95, v/v). The makeup flow was optimized at water/methanol (10/90, v/v) and 0.2 mL/min. The most influential parameters on the separation of cetirizine affecting resolution, retention time and sensitivity were selected by fractional factorial design. The 3 selected factors were optimized by response surface methodology. Tandem mass spectrometry was used at electrospray ionization, positive ion mode, and multiple-reaction monitoring mode. Isotope-labeled cetirizine-d_4 was used as the internal standard. The sample preparation of human plasma was conducted by solid phase extraction of hydrophilic-lipophilic balance (HLB) type. The developed method was validated for selectivity, linearity, precision, accuracy, recovery, limit of quantitation (LOQ), and limit of detection (LOD). The real human plasma samples were analyzed and the pharmacokinetic results were compared with results of previous research. The developed method was found to be reliable based on the similarity between the results of the current and previous methods. The chiral separation for cetirizine and economic feasibility were compared with those of previous studies using normal phase-HPLC or reversed phase-HPLC. The established analytical method could be successfully applied to pharmacokinetic study with reduction in the analysis time and costs.

1. Introduction

Enantiomers of chiral drugs can possess entirely different efficacies and toxicities [1,2]. The biological activities and enantiomeric purity of each enantiomer should be demonstrated according to policy statements of drug authorities, e.g. of the US Food and Drug Administration [3]. Pharmacokinetic studies of single enantiomers or racemic drugs are also important in drug development [4]. For these reasons, development of analytical methods for the separation of enantiomeric drugs is needed.

High performance liquid chromatography (HPLC), capillary electrophoresis (CE), and supercritical fluid chromatography (SFC) have been powerful techniques for the separation of enantiomeric drugs [5–7]. Mass spectrometry can selectively detect the target compound in complex matrices, but it cannot distinguish structural isomers as enantiomers or diasteromers without separation. Thus, the separation of enantiomers was performed by converting into diastereomers by derivatisation or addition of additives (e.g. β-cyclodextrine) to the mobile phase in HPLC [8,9]. Chiral separation has been made easier by the development of the chiral stationary phase. HPLC often needs a relatively long analysis time for sufficient resolution of enantiomers [10]. In this respect, the use of Supercritical Fluid Chromatography (SFC) for rapid and orthogonal chiral analysis has been growing. Supercritical fluid has intermediate properties between liquid and gas and has a low viscosity, high diffusivity, and density close to those of liquid [11–13]. These properties enable fast and highly efficient analysis in supercritical fluid chromatography [14].

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Bioanalysis needs a robust system that can endure an environment of many injection stresses and high throughput assay in the clinical area. SFC was recently improved in terms of reliability and ruggedness due to an improvement in regulation of back-pressure [15]. Interest in the applicability of SFC has increased with its improvement in the areas of pharmacy and food analysis. The separation of enantiomers is one of the successful fields of application for SFC [16]. It has been used for rapid determination of pharmaceutical chiral compounds in biological fluids such as plasma, whole blood, and urine. The advantages of SFC are improved resolution and peak shape in a short analysis time and rapid method development compared to HPLC [12]. In this study, we used mobile phase as subcritical state in the chromatography system. Subcritical state is organic modifier (e.g. methanol)-rich state in CO_2-organic modifier mixture. The supercritical fluid is gradually changed to subcritical fluid according to increase of organic modifier in mobile phase [17].

Cetirizine is a second generation anti-histamine, carboxylated metabolite of hydroxyzine that has been improved in terms of shortcomings such as somnolence, anti-cholinergic effects, and cardiotoxicity. Cetirizine has two enantiomers due to the inclusion of one chiral center in the chemical structure, and these enantiomers have different pharmacological effects. Levocetirizine (R-form) is the active enantiomer and has a potent anti-histamine effect. It has a two-fold higher affinity for the H_1-receptor than does the racemic mixture and a 30-fold higher affinity than is seen for dextrocetirizine. Also, levocetirizine has a long half-life time in the living body due to its slower metabolism rate and also shows higher bioavailability than racemic cetirizine [18]. In earlier studies using ^{14}C-labeled levocetirizine, no racemization of levocetirizine in the living body or buffer solution (pH 7.4) was observed, demonstrating that levocetirizine is stable for racemization [19,20].

Studies of chiral separation for cetirizine were carried out in pharmaceutical formulations or biological samples like plasma and urine using HPLC-UV [21–26], LC–MS/MS [27,28], and CE [29–31]. Chiral stationary phases for HPLC systems mainly used Chiralcel OD-H or OD-R of polysaccharide type and α_1-acid glycoprotein of the glycoprotein type column. Toribio et al. [32] separated cetirizine enantiomers using SFC-UV with a Chiralpak AD column.

Design of experiment (DOE) is a statistical method for screening and optimization of factors affected in a system [33]. DOE can minimize the number of tests required, thus it saves time for developing the analytical method. DOE has several designs including screening design and response surface design. Fractional factorial design in screening design is used to select significant factors and to observe the tendencies of results. Box–Behnken design in response surface design enables the simultaneous investigation of different levels of variables that influence the set of responses.

In the present study, we developed and validated a rapid and reliable method for chiral separation of racemic cetirizine and determination of levocetirizine in human plasma using subcritical fluid chromatography with tandem mass spectrometry. The most influential chromatographic parameters of the developed method were selected by fractional factorial design and optimized by Box–Behnken design.

2. Experimental

2.1. Chemicals and reagents

The standards of racemic cetirizine dihydrochloride and levocetirizine dihydrochloride were purchased from Sigma–Aldrich (St. Louis, MO, USA) and International Scientific Standard (Gangwondo, Korea), respectively. Racemic cetirizine-d_4, used for an internal standard (IS), was purchased from CDN ISOTOPE (Quebec, Canada). HPLC-grade solvents (acetonitrile, methanol, and water) were purchased from JT Baker (Phillipsburg, NJ, USA). HPLC-grade formic acid (FA), acetic acid (AA), trifluoroacetic acid (TFA), triethylamine (TEA), ethanolamine (EA), diethylamine (DA), ethylenediamine (ED), butylamine (BA), and ammonium formate were purchased from Sigma–Aldrich. Control plasma was purchased from BioChemed Services (Winchester, UK).

The human plasma samples used for pharmacokinetic study were obtained by administrating a single dose of levocetirizine (2.5 mg) or racemic cetirizine (5 mg) to four healthy Korean volunteers. Blood samples were collected at pre-dose (0 h), 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24 and 36 h post-dose. The plasma samples were stored at −80 °C until analysis.

2.2. Sample preparation

The 200 μL of human plasma samples were spiked with 20 μL of cetirizine-d_4 (100 ng/mL) for IS. 1.8 mL of 20 mM ammonium formate buffer (adjusted to pH 3.0 using formic acid) was added to plasma samples and then subjected to vortex mixing. This solution was loaded into an HLB (hydrophilic-lipophilic balance) cartridge that was conditioned with 1 mL of methanol and 1 mL of 20 mM ammonium formate buffer (pH 3.0), sequentially. After washing with 1 mL of 20 mM ammonium formate buffer (pH 3.0)/methanol (40/60, v/v), a solid phase extraction (SPE) cartridge was dried using vacuum for 30 s. Elution was conducted by adding 500 μL of methanol to the cartridge, twice. After the eluted solution was evaporated under nitrogen stream at 40 °C, the residue was reconstituted in 200 μL of methanol and then 5 μL of aliquot was injected into the subcritical fluid chromatography-tandem mass spectrometry system.

2.3. Chromatography operation conditions

The subcritical fluid chromatograph used was a 1260 Infinity Analytical SFC system from Agilent Technologies (Santa Clara, CA, USA) equipped with solvent delivery pump, autosampler, column oven, UV detector, makeup flow pump, and a back-pressure regulator (BPR). The mobile phase was composed of CO_2 and methanol containing 5% water (55/45, v/v) at a 0.85 mL/min of flow rate. The makeup flow solution was methanol containing 10% water at 0.2 mL/min of flow rate. The injection volume was 5 μL and the column temperature was maintained at 50 °C. The chiral columns compared were Chiralpak IA, IB, IC, ID, IE, and IF from Daicel (Tokyo, Japan) and Chirex 3001 and 3005 from Phenomenex (West Palm Beach, FL, USA). All columns were 250 mm × 4.6 mm, 5 μm. The final applied column was Chiralpak IE (150 mm × 2.1 mm, 5 μm). Mobile phase temperature between chromatography and mass spectrometry was kept at 120 °C. The back-pressure of the chromatographic system was maintained at 140 bar using BPR.

To perform the design of experiment (DOE), 5 ng/mL of racemic cetirizine standard was used and the every experiment was once performed. The Pareto chart for the factors was made using the results of the experiments and statistics program. The software used for DOE was Design-Expert® (Ver. 9, Stat-Ease Inc., MN, USA).

2.4. Mass spectrometry operation conditions

Chiral detection was performed using a triple-quadrupole mass spectrometer, which was an LCMS-8040 from Shimadzu (Kyoto, Japan). The mass spectrometer was equipped with electrospray ionization (ESI) for an ion source at 3.5 kV of interface voltage. MS/MS detection was applied in positive ion mode and multiple-reaction monitoring (MRM) mode. The parameters of the mass spectrometer including desolvation line, heat block temperature, nebulizing gas, and drying gas were optimized at 300 °C,