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Short communication

A validated chiral liquid chromatographic method for the enantiomeric separation of safinamide mesilate, a new anti-Parkinson drug

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ABSTRACT

A enantioselective reversed-phase high performance liquid chromatographic method was developed for the enantiomeric resolution of safinamide mesilate, 2(*S*)-[4-(3-fluorobenzyloxy)benzylamino] propionamide methanesulfonate, a neuroprotectant with antiparkinsonian and anticonvulsant activity for the treatment of Parkinson disease. The enantiomers of safinamide mesilate were baseline resolved on a Chiralcel OD-RH (150 mm × 4.6 mm, 5 μm) column using a mobile phase system containing 300 mM sodium di-hydrogen phosphate buffer (pH 3.0):methanol:acetonitrile (65:25:10, v/v/v). The resolution between the enantiomers was not less than 3.0. The pH value of buffer solution in the mobile phase has played a key role in enhancing chromatographic efficiency and resolution between the enantiomers. The developed method was validated and proved to be robust. The limit of detection and limit of quantification of (*R*)-enantiomer were found to be 15 and 50 ng/mL, respectively, for 20 μL injection volume. The percentage recovery of (*R*)-enantiomer was ranged from 94.2 to 103.7 in bulk drug samples of safinamide mesilate. The sample solution and mobile phase were found to be stable at least for 48 h. The final optimized method was successfully applied to separate (*R*)-enantiomer from safinamide mesilate and was proven to be reproducible and accurate for the quantitative determination of (*R*)-enantiomer in bulk drugs.

1. Introduction

Since the discovery of difference between thalidomide enantiomers in pharmacological and toxicologic actions, discrimination of optical isomers has been one of the major subjects in the field of pharmacy, because optical purities of substrates with asymmetries are critical for the evaluation of their biological activities [1]. Enantiomers of racemic drugs often show different behaviors in pharmacological action and metabolic process. Often one enatiomer is active while the other can be non-active, poorly active or toxic. The pharmaceutical industry has raised its emphasis on the generation of enantiomerically pure compounds before undertaking pharmacokinetic, metabolic, physiological and toxicological evaluation in the search for drugs with greater therapeutic benefits and low toxicity [2,3].

High performance liquid chromatography (HPLC) is playing a more and more important role for the resolution of drug enantiomers in the field of pharmaceutical industry [4]. However, the development of the methods for the quantitative analysis of chiral compounds and for the assessment of enantiomeric purity is extremely challenging, because the same physical and chemical properties of the two enantiomers make discriminating and separating them very difficult. Recently, many chromatographic methods have been reported describing the use of chiral stationary phases in conjunction with HPLC, as ways to separate and thereby individually quantitate the enantiomers of an enantiomeric pair [5–8].

Safinamide mesilate, 2(*S*)-[4-(3-fluorobenzyloxy)benzylamino] propionamide methanesulfonate, as a neuroprotectant with antiparkinsonian and anticonvulsant activity for the treatment of Parkinson disease, is a novel sodium and calcium channel blocker endowed with selective and reversible inhibition of monoamino oxidase type B (MAO-B) [9].

Safinamide mesilate has been evaluated in preclinical and clinical studies [10–12]. It is prepared from (*S*)-alaninamide as a single enantiomer [13], while the (*R*)-enantiomer is the undesired enantiomer, which can be present as a chiral impurity. In the literature, three methods were reported for the bioassay of safinamide in biological fluids of humans and various animals, using the tandem mass spectrometry (LC–MS–MS) system and high performance liquid chromatography with fluorimetric detection (HPLC-FD) [14]. However, there is no reference for the enantiomeric separation of safinamide mesilate enantiomers in bulk drugs using HPLC. So it is essential to establish an effective method to analyze the

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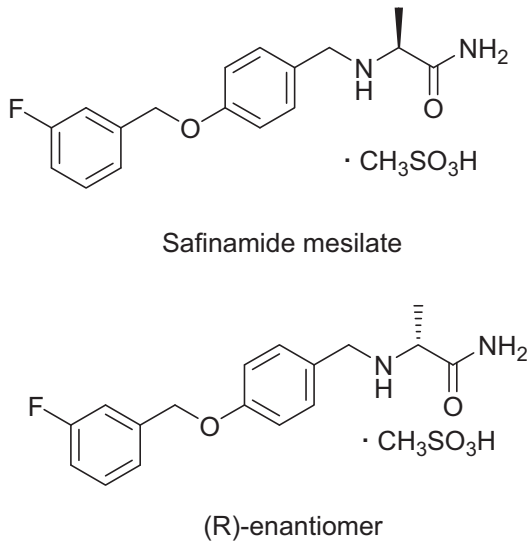

Fig. 1. Chemical structures of safinamide mesilate and (*R*)-enantiomer.

enantiomers of safinamide mesilate, and our report can be an element task for further researches. The chemical structures of safinamide mesilate and (*R*)-enantiomer are shown in Fig. 1, and (*R*)-enantiomer from safinamide mesilate may be at low level because of little (*R*)-alaninamide existing in starting material or racemization in synthesis.

This paper describes a chiral HPLC method for the enantiomeric separation of safinamide mesilate enantiomers using a modified cellulose based chiral stationary phase, Chiralpak OD-RH. The aim of this work was to optimize the chromatographic conditions in terms of temperature, pH value of buffer solution and mobile phase composition in order to separate and identify the enantiomers of safinamide mesilate. The developed HPLC method was reproducible and accurate for the quantitative determination of (*R*)-enantiomer in safinamide mesilate.

2. Experimental

2.1. Chemicals

Safinamide mesilate was kindly provided by Shijiazhaung Pharma Group NBP Pharmaceutical Ltd. (Shijiazhaung, China). (*R*)-enantiomer (the undesired enantiomer) was prepared in our laboratory. HPLC-grade methanol and acetonitrile were purchased from TEDIA (USA). Sodium di-hydrogen phosphate dihydrate, ortho-phosphoric acid and sodium hydroxide were purchased from Alfa Aesar (Tianjin, China). HPLC water from Heal Force system (Beijing, China) was used.

2.2. Columns

Preliminary column screening involved protein-based Chiral HPLC Columns, namely: Chiral AGP (150 mm × 4.0 mm, 5 μm), Chiral HSA (150 mm × 4.0 mm, 5 μm), Chiral CBH (150 mm × 4.0 mm, 5 μm) of Chromtech and then Chiralpak AD-RH (150 mm × 4.6 mm, 5 μm), Chiralcel OJ-RH (150 mm × 4.6 mm, 5 μm) of Daicel were also employed. The column used in the major method development activities was a modified cellulose based chiral column: Chiralcel OD-RH (150 mm × 4.6 mm, 5 μm, Daicel, Japan) column.

2.3. Chromatography

Chromatography was carried out using Agilent Technologies 1200 series instrument (USA) equipped with column oven, UV detector, and the data was processed using a computer program (Chemstation). The chromatographic conditions were optimized using a chiral stationary phase, Chiralcel OD-RH column (150 mm × 4.6 mm, 5 μm, Daicel, Japan). The isocratic mobile phase composition was a mixture of 300 mM sodium di-hydrogen phosphate buffer (pH 3.0):methanol:acetonitrile (65:25:10, v/v/v), which was pumped at a flow rate of 0.5 mL/min. The temperature of the column was maintained at 25 °C, and the eluant was monitored at a wavelength of 224 nm. The injection volume was 20 μL.

2.4. Sample preparation

Stock solutions of safinamide mesilate (1.0 mg/mL) and (*R*)-enantiomer (1.0 mg/mL) were prepared by dissolving the appropriate amount of the substances in methanol. The analyte concentration of safinamide mesilate was fixed as 50 μg/mL. Safinamide solutions spiked with low levels of (*R*)-enantiomer were prepared by transferring calculated amount of undesired enantiomer stock solution with pipette into the calculated amount of safinamide mesilate stock solution, and then the solution was added to volume with mobile phase and mixed well.

2.5. Validation of the method

2.5.1. Method reproducibility

Method reproducibility was determined by measuring repeatability and intermediate precision of retention times and peak areas for each enantiomer. The repeatability of the method was determined by analyzing six replicate injections containing safinamide mesilate (50 μg/mL) spiked with (*R*)-enantiomer (0.6%, 300 ng/mL). The intermediate precision was determined over 3 days by performing six successive injections each day.

2.5.2. Limit of detection and limit of quantification of (R)-enantiomer

The limit of detection (LOD), defined as lowest concentration of analyte that can be clearly detected above the base line signal, is estimated as 3 times the signal to noise ratio [15]. The limit of quantitation (LOQ), defined as lowest concentration of analyte that can be quantified with suitable precision and accuracy, is estimated as 10 times the signal to noise ratio [15]. LOD and LOQ were achieved by injecting a series of dilute solutions of (*R*)-enantiomer. The precision of the developed chiral method for (*R*)-enantiomer was checked by analyzing six test solutions of (*R*)-enantiomer prepared at the LOQ level and calculating the percentage relative standard deviation of area.

2.5.3. Linearity of (R)-enantiomer

Detector response linearity was assessed by preparing six calibration sample solutions of (*R*)-enantiomer covering from 50 ng/mL (LOQ) to 600 ng/mL (50, 100, 150, 250, 400 and 600 ng/mL) in mobile phase. Regression curve was obtained by plotting peak area versus concentration, using the least squares method. Linearity was checked for three consecutive days in the same concentration range from the same stock solution. The percentage relative standard deviation of the slope and *Y*-intercept of the calibration curve was calculated.

2.5.4. Quantification of (R)-enantiomer in bulk sample

The safinamide mesilate bulk sample, provided by Shijiazhaung Pharma Group NBP Pharmaceutical Ltd., showed the absence of