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Validation of HPLC analysis method of a novel antihypertensive agent MS23 in rat plasma

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Abstract

MS23 is a vasodilator with unique dual action pharmacological profile to inhibit type 4 PDE and antagonize L-type calcium channels. We validated an analytical protocol for MS23 in rat plasma using high performance liquid chromatography (HPLC). A C18 column and a phosphate/acetonitrite buffer were used for chromatographic separation. UV detection was performed at 307 nm. The calibration curve for MS23 was linear in the range from 50 to 10,000 ng/ml. The limit of quantification (LOQ) was 50 ng/ml. The results demonstrate that the method has linearity ($R = 0.9989$), specificity, and acceptable precision/accuracy. This method is simple, economic, and sufficient for in vivo pharmacokinetic studies on the compound.

Keywords: Validation; MS23; Antihypertensive; PDE4 inhibitor; Dual action; Calcium channel antagonist

1. Introduction

Blood pressure control is crucial in reducing the morbidity and mortality associated with cardiovascular diseases, such as stroke and congestive heart failure [1]. Among clinically used antihypertensives, approximately two thirds of them ultimately lower the blood pressure through vasodilation. Vascular tone is regulated by multiple signaling pathways [2,3], and the cyclic nucleotides (cAMP and cGMP) are potent regulators of vascular smooth muscle relaxation [4–7]. Phosphodiesterases (PDEs) hydrolyze cAMP and cGMP [8]. Inhibition of PDE has been established as an effective and reliable approach to increase intracellular cAMP and/or cGMP [4–6,9,10]. Among the 11 subtypes of PDEs, PDE3, PDE4 and PDE5 are the major targets associated with vasodilation [11,12]. As suggested recently, selective inhibition of PDE4 could be a promising therapy for hypertensive patients [10].

MS23 was reported as a dual action agent that selectively inhibits PDE4 and antagonizes L-type calcium channels [13,14]. This compound could be developed as a preferable antihypertensive drug by enhancing the blood pressure-lowering efficacy and reducing the adverse response associated with targeting each individual mechanism [10].

There is no reported analytical method available for quantifying the concentration of MS23 in biological fluids, such as plasma. Therefore, we developed an analytical protocol to measure MS23 in rat plasma. MS23 has a pyrrole ring structure that possesses a characteristic UV absorption peak at 307 nm (as shown in Fig. 1). This property of MS23 warrants the use of high performance liquid chromatographic (HPLC) method to quantify the compound in biological fluids. The validation study indicates that the protocol is appropriate for in vivo evaluating the pharmacokinetics of MS23 in rats or human subjects.

2. Methods

2.1. Chemicals and reagents

Rat plasma, potassium phosphate dibasic, acetonitrile, methanol were purchased from Fisher Scientific (Pittsburgh, PA, USA), sodium hydroxide from Sigma (St. Louis, MO, USA), perchloric acid (70%) from EM science (Darmstadt, Germany). All solvents were of HPLC grade. MS23 and internal standard S22 were synthesized in our laboratory and characterized with H-NMR, C13-NMR and high-resolution mass spectrometry.

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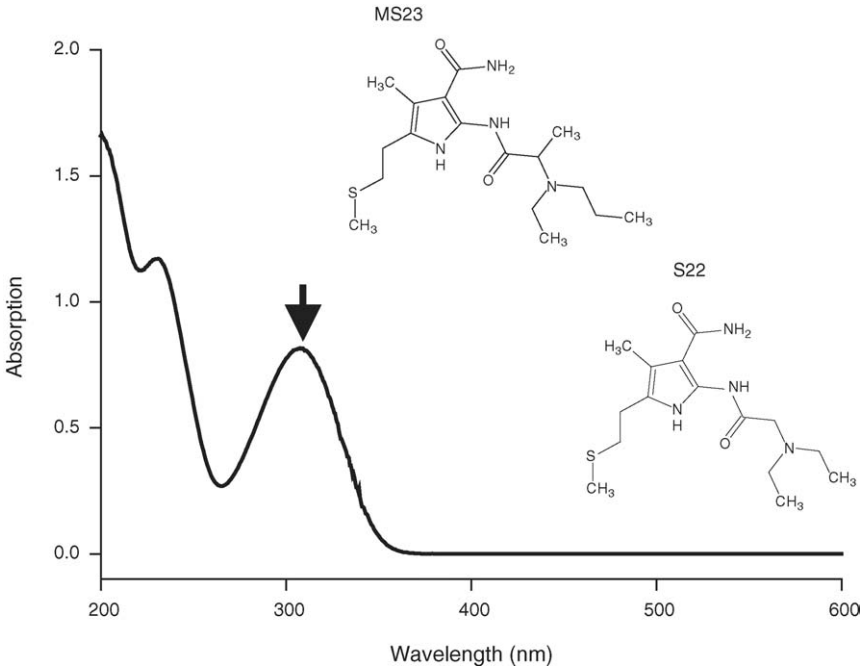

Fig. 1. UV absorption spectrum of MS23. The arrow indicates the MS23 specific UV absorption peak at 307 nM. Method: MS23 was dissolved in aqueous solution at a concentration of 30 μM. The solution was scanned with Lambda EZ210 UV–vis Spectrophotometer (Perkin-Elmer, Norwalk, CT) in a quartz cuvette cell and absorption spectrum was generated after subtraction of that of water in the same cuvette cell. The scanning starts from 600 nm and ends till 200 nm with scanning velocity 800 nm/min. S22 has similar UV absorption spectrum. The chemical structures of MS23 and S22 are illustrated in the insets.

2.2. Apparatus

The analyses were carried out using a System Gold HPLC system (Beckman Coulter, USA) equipped with a 125 solvent module, a 166 UV detector and a 507 autosampler. A compound-specific wavelength of 307 nm was chosen for the detection of MS23. The separation was carried out using a Pinnacle II 5 μm C18 column (150 mm × 4.6 mm) proceeded by a Pinnacle II C18 guard column (4.0 mm × 10 mm) (Resteck, PA).

2.3. Mobile phase

An isocratic HPLC mobile phase consisted of 25% acetonitrile and 75% phosphate buffer containing 6.8 g potassium phosphate monobasic and 150 μl 85% phosphoric acid in 1 liter volume (pH 3.47). The mobile phase was made fresh daily and filtered with NL17 polyamide membrane filter (0.45 μm, Schleicher & Schuell, Germany) and degassed before use. All the assays were performed at room temperature and a flow rate of 1 ml/min.

2.4. Sample preparation

MS23 was isolated and purified from reconstituted samples of rat plasma by a solid-phase extraction method using C18-HD Empore High Performance Extraction Disk Cartridges (3M, MN, USA) prepared immediately before sample processing. Methanol (0.4 ml) and deionized distilled H_2O (0.4 ml, DD-H_2O) were used sequentially to condition the cartridges by applying positive pressure. Residual DD-H_2O was left to keep the surface of the disk wet. Samples (100 μl) with known or unknown MS23 concentration were mixed with 100 μl of internal standard solution (500 ng/ml S22) and 400 μl of 7% perchloric acid. After vortex-mixing and sonication (15 s), the mixture was centrifuged for 5 min at 12,000 × *g*. Then, 550 μl of supernatant was mixed with 230 μl 2M NaOH, loaded, and pushed through the cartridge by positive pressure. 0.5 ml DD-H_2O and 0.3 ml of 20% acetonitrile was added to rinse and remove the non-specifically bound substances. Finally MS23 and internal standard were eluted by 150 μl methanol twice into a collecting micro-centrifuge tube and dried under streams of nitrogen gas at room temperature inside a fume hood. The dried samples were resuspended in 120 μl of mobile phase and 50 μl was subsequently injected for quantification analysis.

2.5. Validation

MS23 was added to blank rat plasma to achieve a range of concentrations 20, 50, 100, 200, 400, 1000, 2000, 5000, 10,000 ng/ml, respectively, and extracted using the protocol described above. The standard calibration curves used to quantify the MS23 concentration in a given sample were constructed with the spike area ratios of MS23 and the internal standard. The linearity, intra- and interday precision, accuracy, recovery and stability were validated to show the reliability of the analytical method [15,16]. Standard calibration curves were fitted using linear regression (Origin 5.0, Microcal Software Inc., MA, USA). Intraday precision was defined as relative standard deviation (R.S.D.) calculated from the values measured from five samples at concentrations of 50, 500 and 5000 ng/ml, respectively, in one day ($n = 5$). Intraday accuracy was defined as relative value on the same measurements of intraday precision.