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Optimization of solid phase extraction clean up and validation of quantitative determination of carbazochrome sodium sulfonate in human plasma by liquid chromatography-electrospray ionization tandem mass spectrometry

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ABSTRACT

A mixed-mode anion exchange solid phase extraction (SPE) method for extraction and clean up of carbazochrome sodium sulfonate (CSS) and (1S)-(+)-10-camphorsulfonic acid (IS) was optimized for quantification by high-performance liquid chromatography/negative electrospray ionization mass spectrometry. The analytes were extracted from 1 mL of human plasma via SPE on Oasis® WAX cartridge. Chromatographic separation was achieved on a Zorbax SB-Aq (4.6 × 250 mm, 5 μm) column under an isocratic condition. Detection was performed using electrospray ionization in negative ion multiple reaction monitoring (MRM) mode. The deprotonated precursor to product ion transitions monitored for CSS and IS was at m/z 299.0 → 256.0 and m/z 230.9 → 79.8, respectively. The method was fully validated for its selectivity, sensitivity, precision, accuracy, recovery, matrix effect and stability. Linear range was 0.189–37.8 ng/mL with a high square regression coefficient (r = 0.9995). The intra-and inter-day precision (RSD, %) ranged from 0.95% to 4.17%, and the intra-and inter-day accuracy was between 95.03% and 105.9%. This method was successfully applied to a bioequivalence study of 90 mg CSS formulation in 18 healthy Chinese male subjects under fasting condition.

1. Introduction

Carbazochrome sodium sulfonate (1-methyl-6-oxo-2,3,5,6-tetrahydroindole-5-semicarbazono-2-sulfonic acid sodium salt three hydrate, CSS) is a capillary stabilizer and used clinically for the treatment of haemorrhage due to the fragility of capillaries [1–3]. It has been reported that CSS could attenuate the endothelial barrier dysfunction, which was induced by tryptase, thrombin and bradykinin without affecting the endothelial permeability enhanced by Ca^{2+} ionophores, such as ionomycin and A23187 or phorbol 12-myristate 13-acetate [4]. In addition, a randomized prospective study showed that using tranexamic acid and CSS with the drain-clamping method could significantly decrease blood loss after total knee arthroplasty without increasing the risk of asymptomatic deep venous thrombosis [5].

Since CSS is becoming more and more widely used for the treatment of hemorrhage, establishing a rapid and sensitive quantitative method for determination of CSS in biofluid is very important. However, until now, there were only two reports about its bioassay. Gan et al. [6] developed a fluorescence quenching method for the determination of CSS in biological fluids. Nevertheless, the method did not use internal standard for quantification, and demonstrates a low sensitivity (the best LLOQ was just 0.1 μg/mL of CSS-Tryptophan system). Song et al. [7] reported an LC-APCI-MS/MS method with an LLOQ of 0.5 ng/mL, and its application to a pharmacokinetic study of CSS. However, APCI was favored for the determination of less polar compounds, while ESI is widely used for the analysis of polar and ionic compounds [8]. Due to the physicochemical properties of CSS (polarity and ionization), ESI may be more suitable than APCI for optimal analysis. In addition, mixed-mode anion exchange cartridge may be better suited for the clean up and enrichment of compounds containing a sulfonic group and lead to reduced matrix interferences in ESI because of its combination of ion exchange and reversed-phase mechanisms.

Therefore, in this study, we designed a mixed-mode anion exchange solid phase extraction (SPE) coupled with LC-ESI-MS/MS method for the determination of CSS in human plasma samples. The method offers relatively higher sensitivity (LLOQ = 0.189 ng/mL)

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using smaller injection volume (5 μL) as compared to Song's method. The analytical method was fully validated and applied to a bioequivalence study in 18 healthy Chinese volunteers following a single oral dose of 90 mg carbazochrome sodium sulfonate.

2. Experimental

2.1. Chemicals and reagents

Carbazochrome sodium sulfonate (Batch No. 100366-200702, purity 84.6%) was supplied by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). (1S)-(+)-10-Camphorsulfonic acid used as internal standard (IS) (Batch No. S46146-238, purity 99%) was purchased from Sigma Company, Inc. (St. Louis, MO, USA).

Methanol (HPLC grade) was purchased from Merck Company, Inc. (Darmstadt, Germany), and formic acid (HPLC grade) and ammonium hydroxide (HPLC grade) from Sigma Company, Inc. (St. Louis, MO, USA). Water (HPLC grade) was distilled using a Milli-Q plot water purification system (Bedford, MA, USA). Oasis® WAX 1cc Cartridge 30 mg was obtained from Waters Company, Inc. (Milford, MA, USA).

2.2. Instruments

The HPLC was performed on an Agilent 1200 system equipped with a G1367C autosampler, a G1379B degasser, a G1316B thermostatted column and a G1312B binary pump (Agilent, Waldbronn, Germany). The HPLC system was coupled to an API 4000 triple-quadrupole mass spectrometer (Applied Biosystems, Concord, Ontario, Canada) via electrospray ionization interface for mass analysis and detection. Data acquisition was performed with Analyst 1.4.2 software (Applied Biosystems).

2.3. LC-ESI-MS/MS conditions

Separation was peformed on an Agilent Zorbax SB-Aq (4.6 × 250 mm, 5 μm) at a column temperature of 40 °C. An isocratic mobile phase consisting of methanol/0.2% formic acid in water (50:50, v/v) was used at a flow rate of 1 mL/min, with the injection volume of 5 μL. The autosampler was set at 10 °C. A primary flow rate of 1 mL/min was split to 500 μL/min using a T-piece. All measurements were carried out with the mass spectrometer operated in negative ion mode. The multiple reaction monitoring transitions were m/z 299.0 → 256.0 for CSS, and m/z 230.9 → 79.8 for IS. Other parameters were as follows: collision gas, curtain gas, ion source gas 1 and ion source gas 2 (nitrogen) 6, 15, 60, and 50ψ, respectively; dwell time 200 ms; ion spray voltage and temperature was −4500 V and 500 °C, respectively; declustering potential (DP) −85 V for CSS and −56 V for IS; collision energy −22 V for CSS and −40 V for IS; entrance potential (EP) −10 V for CSS and IS; collision exit potential (CXP) −7 V for CSS and IS. Unit resolution was used for both Q1 and Q3 mass detection.

2.4. Preparation of standard solution and quality control (QC) samples

A stock solution (0.756 mg/mL) of CSS was prepared in water and was further diluted with water to achieve standard working solutions at concentrations of 378.0, 189.0, 75.6, 37.8, 18.9, 7.56, 3.78, 1.89 ng/mL, and QC stock solutions: low (3.40 ng/mL), medium (34.0 ng/mL) and high (340 ng/mL). IS stock solution 1.18 mg/mL prepared in water was diluted with water to give a final concentration of 118 ng/mL.

The standard working solutions (100 μL) were used to spike blank plasma samples (1000 μL). The final concentrations of CSS standard calibration plasma samples were 37.8, 18.9, 7.56, 3.78, 1.89, 0.756, 0.378 and 0.189 ng/mL, respectively. The QC samples were also prepared in the same way by adding 100 μL diluted QC stock solutions into 1000 μL blank human plasma. The final concentrations of CSS in the low, medium and high QC plasma samples were 0.340 ng/mL, 3.40 ng/mL, 34.0 ng/mL, respectively.

2.5. Sample preparation and solid phase extraction procedure

For the isolation of the analyte from human plasma, 20 μL IS solution (188 ng/mL) and 100 μL water (supplementary volume) were added to 1 mL plasma sample and vortex mixed for 30 s. Extraction and clean up of human plasma samples were carried out by SPE according to the following procedure. The Oasis WAX (30 mg, 1cc) cartridges were conditioned with 1 mL methanol and equilibrated with 1 mL water. The plasma samples were applied to the cartridges. Following sample application, the cartridges were washed with 1 mL 2% formic acid water solution, and subsequently washed with 1 mL methanol. After drying, the analytes were eluted with 1 mL methanol containing 5% ammonium hydroxide. The eluate was evaporated to dryness at 40 °C using a gentle stream of nitrogen. The residue was reconstituted in 100 μL of the mobile phase, then vortex-mixed and centrifuged at 10,500 rpm for 10 min. A 5 μL aliquot of the resulting solution was injected onto the LC-ESI-MS/MS system for analysis.

2.6. Method validation

The method was validated prior to the analyses of human plasma samples according to the guidance of bioanalytical method validation [9]. The selectivity, linearity, precision, accuracy, sensitivity, matrix effect, recovery and stability of CSS in plasma sample were assessed and investigated.

To evaluate selectivity, drug-free plasma samples from 6 individuals were analyzed to check the presence of any interfering peaks at the elution times of both CSS and IS. The calibration curves were constructed using 8 standards ranging in concentration from 0.189 to 37.8 ng/mL (three standards for each level that were independently prepared). The validity of the linear regression equation was indicated by the correlation coefficient (r). The intra-day and inter-day precisions were evaluated by assessing QC samples at the following concentrations (n = 6): LLOQ (0.189 ng/mL), low (0.340 ng/mL), medium (3.40 ng/mL), and high (34.0 ng/mL). The relative standard deviation (RSD%) and accuracy (%) were calculated.

As proposed by Matuszewski et al. [10], matrix effect (ME) and recovery (RE) were examined in 6-fold replicates by comparing the peak areas of analytes between three different sample sets. In set 1, analytes were dissolved in matrix component-free reconstitution solvent. In set 2, analytes were prepared in plasma extracts originating from six different sources and spiked after extraction. In set 3, analytes were prepared in plasma from the same six different sources as in set 2, but the plasma samples were spiked before extraction. The ME and RE was calculated as follows:

$$\mathrm{ME}\,(\%) = \frac{\mathrm{Area}_{\mathrm{set2}}}{\mathrm{Area}_{\mathrm{set1}}}$$

$$\mathrm{RE}\,(\%) = \frac{\mathrm{Area}_{\mathrm{set3}}}{\mathrm{Area}_{\mathrm{set2}}}$$

Stability tests involved leaving the untreated plasma sample at ambient temperature for 8 h without light, placing the treated plasma sample in auto-sampler for 24 h, three freeze–thaw cycles from −20 to 25 °C, and storing for 145 days at −20 °C. Stability analysis was performed using three aliquots of each QC sample at three different concentrations (0.340 ng/mL, 3.40 ng/mL and 34.0 ng/mL).