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 (r² = 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.

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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 Ca²⁺ 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 bioasay. 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).
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 performed 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 50%, respectively; dwell time 200 μs; 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 mg/mL, and QC stock solutions: low (3.40 mg/mL), medium (34.0 mg/mL) and high (340 mg/mL). IS stock solution 1.18 mg/mL prepared in water was diluted with water to give a final concentration of 118 mg/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, and 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): LOQ (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:

\[
\text{ME(%) = } \frac{\text{Area}_{\text{set2}}}{\text{Area}_{\text{set1}}} \\
\text{RE(%) = } \frac{\text{Area}_{\text{set3}}}{\text{Area}_{\text{set2}}}
\]

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).
2.7. Application of the assay

The method described in this paper was applied to a bioequivalence study of two oral formulations of CSS (Test formulation, a 10 mg carboxazome sodium sulfonate dispersible tablet from a Chinese company; reference formulation, and a 10 mg carboxazome sodium sulfonate tablet produced by Jiangsu Wuzhong Industrial Co., LTD, China). The clinical bioequivalence study was approved by the Ethics Committee of the first Affiliated Hospital of Zhejiang University. All volunteers gave written informed consent to participate in the study according to the principles of the Declaration of Helsinki. Eighteen healthy Chinese volunteers were selected for the study. The study followed a single dose, two-way randomized crossover design with a 1-week washout period between doses. Heparinized blood samples were collected at the following times: before (0 h) and at 0.167, 0.333, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, and 12.0 h after dosing. The blood samples were centrifuged at 4000 rpm for 10 min, and plasma samples were separated and stored at −20 °C until analyzed.

3. Results and discussion

3.1. Method development

3.1.1. Optimization of LC-ESI-MS/MS condition

To get optimal analytical conditions for this assay, mass spectrometer settings and chromatographic conditions were investigated. Firstly, mass spectrometer parameters were derived from analyte infusion experiments using a syringe pump (typical concentration was 189 ng/mL and 118 ng/mL in water for CSS and IS, respectively). Both APCI and ESI sources with positive and negative ionization mode have been tested for the determination. The results revealed that both CSS and IS were more sensitive in negative ionization mode with ESI interface, which was different with Song's study [7]. The deprotonated precursor ion of CSS at m/z 299.0 was abundantly generated in the negative ESI mode. Thereafter, the loss of an HNnH2=O portion (43 Da) gave a product ion at m/z 256.0 and with a further loss of H2SO3 group (82 Da) led to a product ion of m/z 174.0, corresponding neutral loss of N2 (28 Da) to a product ion of m/z 146.0 at the collision energy of 20 eV. For IS, the prominent stable product ion was m/z 80.0 [M−1][+] at the collision energy of 40 eV. The product ion mass spectra and proposed ESI-MS1/MS fragmentation processes of CSS and IS were shown in Figs. 1 and 2. Based on the Q1 and product ion scan, the MRM ion reactions were: m/z 299.0 → 256.0 and 299.0 → 80.9. Both ion reactions were evaluated for the quantitation of CSS in human plasma. Endogenous interferences were obvious, when the ion combination of m/z 299.0 → 80.9 was chosen for CSS. The best specificity and sensitivity were obtained with MRM of m/z 299.0 → 256.0 for CSS. Therefore, three MRM transitions — two for CSS (quantifier, m/z 299.0 → 256.0; qualifier, m/z 299.0 → 80.9) and one for the IS (m/z 230.9 → 79.8) were chosen. The DP, CE, CXP for each transition and ion source parameters were also optimized; the final parameters are shown in Section 2.3.

To optimize the chromatography, the stationary phase, the composition of the mobile phase and the column temperature were all investigated, so as to achieve an optimal peak shape and good separation from the void volume. Because of their polarity and acidity properties, various available columns of different lengths and bonded phases (Zorbax SB-C18, Zorbax SB-Aq, Hypersil GOLD Aq and Atlantis T3) were carefully evaluated. As a result, Zorbax SB-Aq column was chosen in the present study for its high efficiency and peak symmetry. Different mobile phases (methanol–water and acetonitrile–water with different additives, such as formic acid and ammonium formate) were examined to obtain efficient chromatography and relatively short run time for CSS and IS. It was found that the addition of formic acid could remarkably improve the peak symmetry and ionization of CSS and IS. When methanol was used as the organic phase, the peak of CSS was further improved. Therefore, the mobile phase was selected as methanol: 0.2% formic acid in water 50:50 (v/v) to achieve better separation and less interference from other components in the plasma (Fig. 3). The observed retention times for CSS and IS were about 5.0 and 7.4 min, respectively.

3.1.2. Optimization of SPE process

Initially, sample preparation by protein precipitation was selected. However, after protein precipitation treatment, the response was much lower than that of the sample in mobile phase. Although special attention has been paid to optimization of the types of extraction solvents, the phenomenon of ion suppression still could not be eliminated. Because CSS and IS were extremely water-soluble and easily dissociated Na+ or H+ ions to become negative ions containing a sulfonic group, liquid–liquid extraction (LLE) with water-immiscible organic solvents was not effective for their purification. Therefore, anion exchange SPE cartridges were considered. Then, different cartridges (strong anion exchange sorbent–MAX, and weak anion exchange sorbent–WAX) and corresponding SPE protocols including wash and elute solutions were optimized. Analytes were washed with 2% formic acid, followed by 100% methanol, and
eluted with methanol containing 5% NH₄OH for WAX cartridges, while analytes were washed with 5% NH₄OH, followed by 100% methanol, and eluted with methanol containing 2% formic acid for MAX cartridges. The results of ME (%) and RE (%) using SPE, protein precipitation and LLE sample pretreatment methods are shown in Fig. 4. Compared to protein precipitation extraction, ion suppression was obviously improved using anion exchange SPE (ME: >88.50% for WAX, and >94.63% for MAX), because ion exchange SPE

![Diagram of ESI-MS/MS fragmentation processes for CSS (a) and IS (b).](image)

**Fig. 2.** Proposed ESI-MS/MS fragmentation processes for CSS (a) and IS (b).

![MRM chromatograms of CSS and IS obtained from human plasma samples: (A) blank plasma; (B) blank plasma spiked with standard solution (LLOQ); (C) plasma sample from a healthy subject 0.167 h after oral administration of 90 mg CSS with concentration of 0.671 ng/mL.](image)

**Fig. 3.** MRM chromatograms of CSS and IS obtained from human plasma samples: (A) blank plasma; (B) blank plasma spiked with standard solution (LLOQ); (C) plasma sample from a healthy subject 0.167 h after oral administration of 90 mg CSS with concentration of 0.671 ng/mL.
could remove most of the disruptors. Meanwhile, RE (%) of CSS and IS by WAX cartridge was much higher than that by MAX cartridge (CSS: 92.07% versus 11.60%, IS: 91.00% versus 8.07%). Therefore, in this study, we used WAX SPE for the sample pretreatment.

3.2. Method validation

3.2.1. Selectivity

The typical MRM chromatograms of mixed blank plasma from six drug-free individuals, a spiked plasma sample with CSS at LLOQ and IS, and a plasma sample from a healthy volunteer 0.167 h after an oral administration are shown in Fig. 3. The results indicated that there was no apparent endogenous interference for the determination of CSS.

3.2.2. Linearity of calibration curves and lower limit of quantification (LLOQ)

The standard calibration curve for spiked human plasma containing CSS was linear over the range 0.189–37.8 ng/mL. Good linearity was observed for the analyte using a weighted (1/x) least squares linear regression analysis with a coefficient of determination (r) of 0.9995 ± 0.0004 (n = 3). Typical equation for the calibration curve was as follows: Y = (0.0500 ± 0.0041)x + (0.0036 ± 0.0006) (n = 3), where “X” represents the plasma concentration of CSS (ng/mL) and “Y” represents the ratios of CSS peak area to that of IS.

The LLOQ under the optimized conditions was 0.189 ng/mL for CSS, which was judged from the fact that the precision and accuracy were less than 20% (Table 1) and the S/N ratio higher than 10. The LLOQ is sufficient for the bioequivalence study of CSS following an oral administration.

3.2.3. Precision and accuracy

QC samples at three concentration levels were calculated over three validation runs (once a day). Six replicates of each QC level were determined in each run. Table 1 summarizes the intra-day and inter-day precision and accuracy for CSS. In this assay, the intra-day precision expressed by relative standard deviation (RSD) was no more than 3.62% for all tested concentrations (0.340, 3.40, and 34.0 ng/mL), the inter-day precision was less than 4.17%. And the accuracies were between 95.03% and 105.9%. The above values were within the acceptable range, which demonstrated the good stability and repeatability of this described method.

3.2.4. Matrix effect and recovery

To evaluate the possibility of ion suppression or enhancement in the present experiments, chromatographic peak areas of CSS from the spiked after extraction samples at low, medium and high concentration levels were compared to those obtained from the standard solution at the same concentrations in neat solvent. The percent nominal concentrations and corresponding standard deviation (SD) determined were 90.04 ± 4.28%, 88.98 ± 1.96%, and 89.56 ± 1.63% at 0.340, 3.40, and 34.0 ng/mL for CSS, respectively. The same evaluation was performed for the IS and the percent nominal concentration was 107.8 ± 1.57%. The results indicated that ion suppression from plasma matrix was consistent for this analytical method and would not interfere the measurement of the analyte.

The mean recovery of CSS by the SPE extraction was 98.90 ± 4.70%, 91.24 ± 2.23%, and 91.90 ± 3.29% at concentrations of 0.340, 3.40, and 34.0 ng/mL, respectively. Mean recovery for IS was 90.82 ± 0.41% (shown in Table 2).

3.2.5. Stability

The stability experiment was performed by using QC samples. The analyte was shown to be stable in human plasma with a reduction of less than 10% at room temperature for 8 h. In the auto-sampler (10 °C), post-preparative extracted samples were also stable with no obvious reduction (<8% for 24 h). After storage at −20 °C for 145 days, a reduction of less than 9% was found in human plasma. The analyte was found to be stable after three freeze-thaw cycles with a reduction of less than 8% (Table 3).

3.3. Application

The validated method was applied to a bioequivalence study of CSS in 18 healthy Chinese subjects, who received 90 mg of both the test and reference formulations under fasting conditions. The method was simple and sensitive enough to determine the concentration up to 12 h post-dose. The precision and accuracy of the calibration and QC samples were well within the acceptable limits.
Table 1  
Intra-day and inter-day precision and accuracy of CSS in human plasma.

<table>
<thead>
<tr>
<th>QC levels</th>
<th>Concentration (ng/mL)</th>
<th>Mean concentration found (ng/mL)</th>
<th>Precision (RSD%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-day (n = 6)</td>
<td>LLOQ 0.189</td>
<td>0.178</td>
<td>3.40</td>
<td>94.06</td>
</tr>
<tr>
<td></td>
<td>L 0.340</td>
<td>0.323</td>
<td>3.62</td>
<td>95.03</td>
</tr>
<tr>
<td></td>
<td>M 3.40</td>
<td>3.55</td>
<td>2.81</td>
<td>104.3</td>
</tr>
<tr>
<td></td>
<td>H 34.0</td>
<td>36.0</td>
<td>0.95</td>
<td>105.9</td>
</tr>
<tr>
<td>Inter-day (3 days, n = 6)</td>
<td>L 0.340</td>
<td>0.329</td>
<td>4.17</td>
<td>96.57</td>
</tr>
<tr>
<td></td>
<td>M 3.40</td>
<td>3.51</td>
<td>2.74</td>
<td>103.3</td>
</tr>
<tr>
<td></td>
<td>H 34.0</td>
<td>35.3</td>
<td>2.07</td>
<td>103.8</td>
</tr>
</tbody>
</table>

Table 2  
Matrix effect and recovery for CSS and CSA in human plasma (n = 6, %).

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>Matrix effect (%)</th>
<th>Recovery (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>CSS</td>
<td>0.340</td>
<td>90.04 ± 4.28</td>
</tr>
<tr>
<td></td>
<td>3.40</td>
<td>88.98 ± 1.96</td>
</tr>
<tr>
<td>IS</td>
<td>34.0</td>
<td>89.56 ± 1.63</td>
</tr>
<tr>
<td></td>
<td>11.8</td>
<td>107.8 ± 1.57</td>
</tr>
</tbody>
</table>

Table 3  
Summary of the stability of CSS in human plasma on different conditions (n = 3).

<table>
<thead>
<tr>
<th>Stability conditions</th>
<th>Concentration (ng/mL)</th>
<th>Calculated concentration Mean ± SD (ng/mL)</th>
<th>Accuracy%</th>
<th>RSD%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-term (8 h, room temperature)</td>
<td>0.340</td>
<td>0.320 ± 0.003</td>
<td>93.96</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>3.40</td>
<td>3.36 ± 0.01</td>
<td>98.67</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>34.0</td>
<td>34.2 ± 0.26</td>
<td>100.4</td>
<td>0.75</td>
</tr>
<tr>
<td>Long-term (145 days, −20 °C)</td>
<td>0.340</td>
<td>0.317 ± 0.010</td>
<td>93.16</td>
<td>3.30</td>
</tr>
<tr>
<td></td>
<td>3.40</td>
<td>3.34 ± 0.22</td>
<td>98.23</td>
<td>6.65</td>
</tr>
<tr>
<td></td>
<td>34.0</td>
<td>34.1 ± 2.12</td>
<td>100.2</td>
<td>4.97</td>
</tr>
<tr>
<td>Auto sampler (24 h, 10 °C)</td>
<td>0.340</td>
<td>0.322 ± 0.020</td>
<td>94.65</td>
<td>6.13</td>
</tr>
<tr>
<td></td>
<td>3.40</td>
<td>3.51 ± 0.09</td>
<td>103.3</td>
<td>2.43</td>
</tr>
<tr>
<td></td>
<td>34.0</td>
<td>35.3 ± 0.90</td>
<td>103.7</td>
<td>2.57</td>
</tr>
<tr>
<td>Three freeze–thaw cycles (from 25 °C to −20 °C)</td>
<td>0.340</td>
<td>0.322 ± 0.002</td>
<td>94.46</td>
<td>0.67</td>
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<tr>
<td></td>
<td>3.40</td>
<td>3.50 ± 0.06</td>
<td>102.8</td>
<td>1.85</td>
</tr>
<tr>
<td></td>
<td>34.0</td>
<td>35.1 ± 1.16</td>
<td>103.4</td>
<td>3.31</td>
</tr>
</tbody>
</table>

The mean ± SD (n = 18) plasma concentration versus time profiles for CSS are depicted in Fig. 5 and the main pharmacokinetic parameters for CSS are summarized in Table 4.

4. Conclusion

An anion exchange SPE coupled with LC-ESI-MS/MS method for the quantification of CSS in human plasma was developed. Different ion exchange SPE cartridges were carefully optimized. Method validation has been demonstrated by a variety of tests for selectivity, linearity, sensitivity, precision, matrix effect, recovery and stability. This method has been shown to be suitable for the bioanalysis of CSS in a pharmacokinetics and bioequivalence study because of its high sensitivity and accuracy.

Acknowledgments

This research was funded by the Key Technologies R&D Program of 12th Five-year Plan of China (No. 2011ZX09302-003-03), and Zhejiang traditional Chinese medicine research fund projects (No. 2010ZB068).

References

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