Journal of Pharmaceutical and Biomedical Analysis 54 (2011) 1187–1191

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Determination of S-propargyl-cysteine in rat plasma by mixed-mode reversed-phase and cation-exchange HPLC–MS/MS method and its application to pharmacokinetic studies

Yuanting Zheng[a], Hongrui Liu[b,1], Guo Ma[a], Ping Yang[c], Lin Zhang[a], Yi Gu[d], Qing Zhu[b], Tengfei Shao[a], Peng Zhang[a], Yizhun Zhu[b,**], Weimin Cai[a,*]

[a] Department of Clinical Pharmacy, School of Pharmacy, Fudan University, Shanghai, China
[b] Department of Pharmacology, School of Pharmacy and Institute of Biomedical Sciences, Fudan University, Shanghai, China
[c] Analytical and Testing Center, School of Pharmacy, Fudan University, Shanghai, China
[d] Drug Metabolism and Pharmacokinetics, Hutchison Medipharma Ltd., Shanghai, China

ARTICLE INFO

Article history:
Received 24 June 2010
Received in revised form
11 November 2010
Accepted 22 November 2010
Available online 30 November 2010

Keywords:
S-propargyl-cysteine
HPLC–MS/MS
Method validation
Rat
Pharmacokinetics

ABSTRACT

A simple, fast and sensitive mixed-mode reversed-phase and cation-exchange HPLC–MS/MS method for the quantification of S-propargyl-cysteine (SPRC), a novel cardioprotective agent, has been developed and validated for preclinical studies. Chromatographic separation of SPRC and its internal standard (IS) was performed using a commercial analytical column which contained both C18 bonded silica particles and sulfonic acid cation-exchange particles. The optimized mobile phase was composed of acetonitrile/ammonium acetate buffer (10 mM, pH 4): 30/70 (v/v). Quantification was conducted by multiple reaction monitoring (MRM) of the transitions of *m/z* 160.0 → 143.0 for SPRC and 178.1 → 160.9 for S-butyl-cysteine (IS). The assay utilized methanol to achieve a simple and fast deproteinization. The lower limit of quantification (LLOQ) was 0.6 μg/mL (diluted with 50-fold of methanol) using 20 μL rat plasma. The assay was linear over a range from 0.6 to 159 μg/mL, with intra- and inter-batch accuracy (as relative error) less than ±5% and precision (as relative standard deviation) less than 10%. Using the validated assay, the pharmacokinetic properties of SPRC in rats were investigated. SPRC exhibits linear pharmacokinetics after oral or intravenous administration in rats. The bioavailability after oral administration at 25, 75, and 225 mg/kg was 96.6%, 97.0%, and 94.7%, respectively.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

S-propargyl-cysteine (SPRC, Fig. 1A), a new sulfur-containing amino acid derivative, is a novel cardioprotective agent in the preclinical stage. It is the analog of s-allylcysteine (SAC), which is derived from aged garlic and has been reported to have antioxidant, anti-cancer, anti-hepatopathic, and neurotrophic properties [1–4]. Previous studies demonstrate that SPRC has better cardioprotective effects than SAC via its H_2S-related pathway [5,6], and show that it has become a promising candidate to treat cardiovascular diseases.

Like most amino acids, SPRC has poor UV absorption and fluorescent properties. In addition, its high polarity makes it difficult to extract from biological matrices and may cause it to elute among endogenous, polar substances with ordinary reversed-phase chromatography. Many published methods of amino acids and their derivatives required pre-column derivatization (with phenyl isothiocyanate, o-phthalaldehyde, and dabsyl chloride) to achieve a suitable chromatographic behavior and sensitivity using HPLC–UV or HPLC–FLU [7–9]. Unfortunately, these methods often have a complicated, time-consuming sample pre-treatment and a long run time to eliminate possible interference from endogenous amino acids in biological matrices. Ion-exchange chromatography methods following post-column derivatization have also been reported to determine amino acid derivatives in biological matrices, but these need an additional derivatization apparatus which limited their extensive use [1]. Rosen et al. reported a relative sensitive HPLC–MS method to determine SAC [8], but a pre-column derivatization was still needed to improve its retention on a C18 column.

The present paper reports the development of a simple, fast, and sensitive method by using mixed reversed-phase and cation-exchange HPLC–MS/MS for the measurement of SPRC in rat plasma. Mixed-mode reversed-phase and cation-exchange HPLC combines the advantage of reversed phase chromatography and

* Corresponding author. Tel.: +86 021 51980023; fax: +86 021 51980001.
** Corresponding author. Tel.: +86 021 51980018; fax: +86 021 51980008.
E-mail addresses: HTzhuyz@fudan.edu.cn (Y. Zhu), weimincai@hotmail.com (W. Cai).
[1] [1]Co-first author.

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.11.027

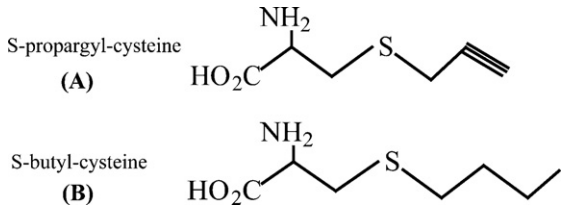

Fig. 1. Chemical structure of SPRC (A) and the internal standard S-butyl-cysteine (B).

ion-exchange chromatography, which is suitable to separate a mixture of polar and ionizable compounds [10,11]. We found that it was useful to separate the amino acid derivative SPRC from endogenous amino acid interference.

2. Material and methods

2.1. Chemicals and reagents

The reference substances of SPRC (>99% pure by HPLC) and internal standard (IS) S-butyl-cysteine (>99% pure by HPLC, Fig. 1B) were synthesized as mentioned before [5,6]. HPLC grade acetonitrile and methanol were from Fisher Scientific (Pittsburgh, PA, USA). Other reagents used had the highest purity commercially available. Blank Sprague–Dawley rat plasma was obtained from healthy, drug-free rats, and stored at −70 °C.

2.2. Animals

Sprague–Dawley rats (body weight of 200 ± 20 g, half male and half female) were obtained from Sino-British Sipper/BK Lab Animal Ltd. (Shanghai, China). Animal experiments were carried out in accordance with the Guidelines for Animal Experimentation of Fudan University (Shanghai, China) and the protocol was approved by the Animal Ethics Committee of this institution.

2.3. Instrumentation

Chromatographic analysis was performed on an Agilent 1200 Series HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with an autosampler, a vacuum degasser unit, and a binary pump. The MS/MS detection was conducted using an API 4000 triple-quadrupole spectrometer (Applied Biosystems/MDS Sciex, Concord, ON, Canada) with a Turbo Ion Spray (TIS) interface. Data acquisition and processing was performed by Analyst 1.5 software (Applied Biosystems/MDS Sciex, Concord, ON, Canada).

2.4. Chromatographic separations

Chromatographic separation of the analyte from potentially interfering substances was achieved at ambient temperature on

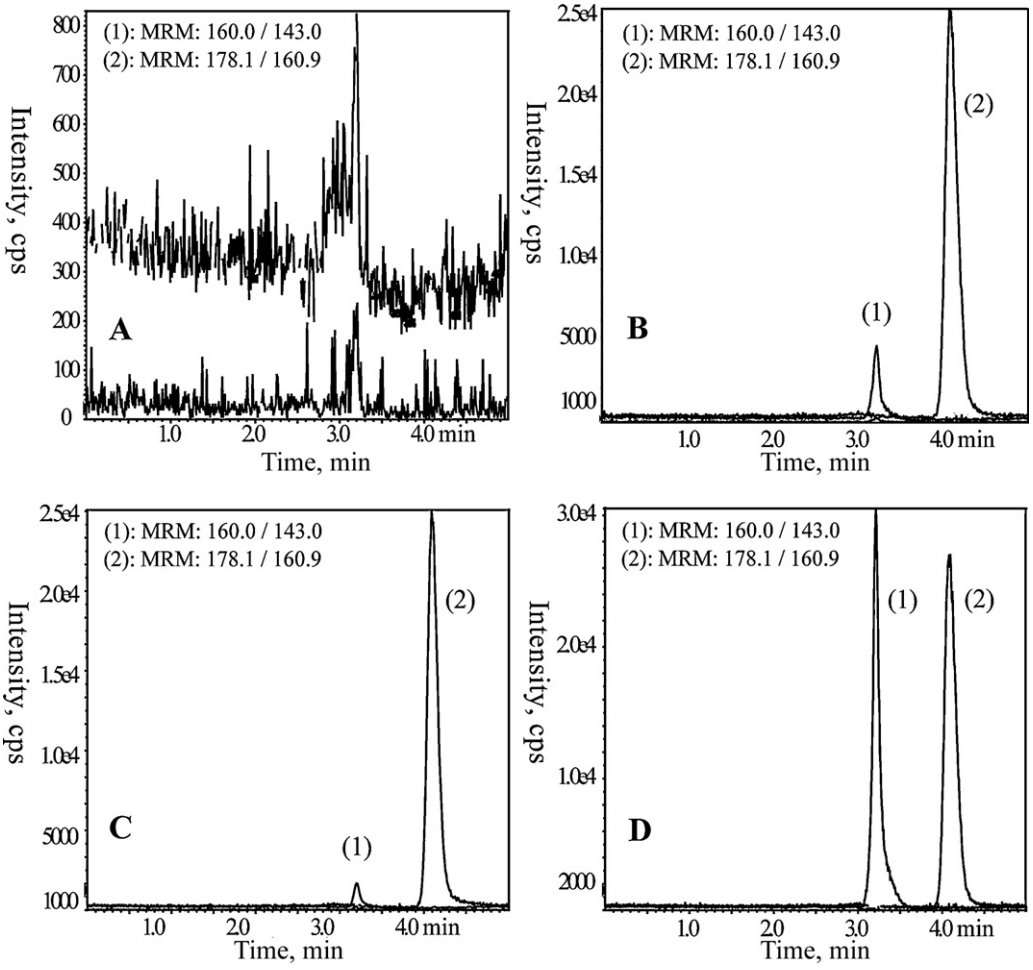

Fig. 2. Representative multiple reaction monitoring (MRM) chromatograms of SPRC (1) and S-butyl-cysteine (IS, 2): (A) a blank rat plasma; (B) standard solution of SPRC (2.5 μg/mL) and IS (0.44 μg/mL) injected directly; (C) rat plasma spiked with the analyte (the LLOQ at 0.6 μg/mL) and IS (0.44 μg/mL); (D) a plasma sample (calculated to be 49.3 μg/mL) 1 h after oral administration of 75 mg/kg SPRC in rat.

a CAPCELL PAK CR 1:4 (150 mm × 4.6 mm i.d. with 5 μm particles) column (Shiseido, Japan). The mobile phase was composed of acetonitrile/ammonium acetate buffer (pH 4.0; 10 mM): 30/70 (v/v), and was delivered isocratically at a flow rate of 0.5 mL/min.

2.5. Mass spectrometric conditions

The mass spectrometer was operated in positive ion mode. Quantification was performed using MRM of the transitions of *m/z* 160.0 → 143.0 for SPRC and *m/z* 178.1 → 160.9 for S-butyl-cysteine (IS). The optimal declustering potential (DP), entrance potential (EP), collision energy (CE) and collision-cell exit potential (CXP) for SPRC were 45 V, 15 V, 19 V, and 5 V, respectively. Such compound specific parameters for the IS were 42 V (DP), 15 V (EP), 16 V (CE), and 5 V (CXP), respectively.

2.6. Preparation of standards and quality control samples

A stock solution of SPRC was accurately prepared in deionized water at 3.18 mg/mL, and then stored at −70 °C. The calibration standards were prepared by spiking 10 μL of the appropriate dilution to 90 μL of blank rat plasma to the concentrations of 0.6, 1.2, 2.5, 5.0, 9.9, 19.9, 39.8, 79.5, and 159 μg/mL. The quality control (QC) samples used in the validation and in the plasma level determinations were prepared in the same manner, using a separately weighed stock solution. The nominal plasma concentrations of QC samples were 1.2, 9.9, and 79.5 μg/mL. S-butyl-cysteine was prepared as a 0.18 mg/mL solution in deionized water and stored at −70 °C.

2.7. Sample pretreatment

An aliquot of 20 μL of the samples were precipitated with 1 mL of methanol containing 0.44 μg/mL internal standard, and mixed vigorously for 1 min on a vortex-mixer, followed by centrifugation (MicroCL 17R, Thermo IEC, USA) at 12,000 rpm for 10 min at 4 °C. A volume of 5 μL was injected into the LC–MS/MS instruments using an autosampler operating at room temperature.

2.8. Method validation

The developed method was validated according to the Guidelines for Bioanalytical Method Validation of the Food and Drug Administration (FDA) [12].

Selectivity was evaluated by analyzing blank rat plasma samples obtained from six different rats. Linearity was assessed by construction of five independent calibration standards ranging from 0.6 to 159 μg/mL based on peak area ratios of SPRC/IS on five separate days. The lower limit of quantification (LLOQ) was evaluated singularly in five separate batches with a nominal concentration of 0.6 μg/mL.

The precision and accuracy were determined by analyzing five replicates of spiked samples at each level on three separate batches. Extraction recovery of the analyte was determined by comparing the peak areas of QC samples to those of post-extraction blank plasma samples spiked with equivalent amounts of analyte. Matrix effects were also evaluated by comparing the peak areas of post-extraction blank plasma samples spiked with analyte to the peak areas of direct injection of diluted stock solution at the same concentrations. Five different lots of plasma were used to assess the matrix effects and the possibility of ion suppression or enhancement for SPRC [13]. The stabilities of SPRC in rat plasma were investigated during a variety of storage and process conditions, including short-term stability (at room temperature for 8 h), freeze and thaw stability (three freeze at −70 °C/thaw at 37 °C cycles), long-term stability (at −70 °C for 2 months) and post-preparative stability (kept in the autosampler for 24 h).

To test whether the dilution of these samples before a subsequent analysis would affect the accuracy and precision of the drug determination, rat plasma samples containing 318 μg/mL of SPRC were diluted five fold with drug-free rat plasma, then analyzed in five replicates.

2.9. Pharmacokinetics application

Rats were fasted for 12 h before dosing and 3 h afterwards, with free access to water. 36 rats were randomly grouped in two major groups: intravenous (*i.v.*) and intragastric (*i.g.*) administration, with three sub-divisions for three dosages in each major group. The *i.v.* dosages of SPRC were 25, 75, and 225 mg/kg, prepared in isotonic saline. The *i.g.* dosages were also 25, 75, and 225 mg/kg, which were made by dissolving the SPRC powder in deionized water.

100 μL blood samples were collected by tail vein puncture at 0.08, 0.17, 0.33, 0.5, 1, 2, 4, 6, 8, 10, 12, 14, 16, and 24 h after *i.g.* administration, and at 0.03, 0.08, 0.17, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 10, 12, and 14 h after *i.v.* administration.

Pharmacokinetic parameters were calculated with DAS 2.0 software (Mathematical Pharmacology Professional Committee of China, Shanghai, China). Absolute bioavailability (*F*) was determined for each dose according to the following equation: $F(\%) = AUC_{0-t\,(i.g.)}/AUC_{0-t\,(i.v.)} \times 100$ (AUC_{0-t}, estimated using linear trapezoidal method). Log-transformed date for C_{max}/dose and AUC_{0-t}/dose were analyzed using one-way ANOVA regression model to test dose proportionality. T_{max} was analyzed using non-parametric test (Kruskal–Wallis test).

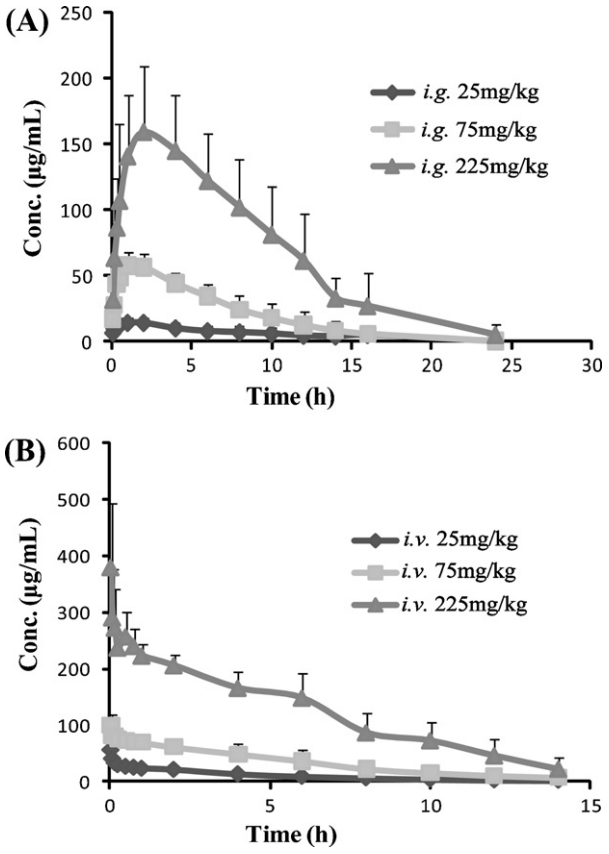

Fig. 3. Mean plasma SPRC concentration-time curves (n = 6): (A) *i.g.* administration of SPRC at 25, 75, and 225 mg/kg; and (B) *i.v.* administration of SPRC at 25, 75, and 225 mg/kg.

Table 1
Intra- and inter-batch precision and accuracy for determination of SPRC in rat plasma (n = 3 batches, 5 replicates per batch).

Nominal concentration (μg/mL)	Intra-batch			Inter-assay		
	Measured concentration (mean ± S.D.) (μg/mL)	RSD (%)	RE (%)	Measured concentration (mean ± S.D.)(μg/mL)	RSD (%)	RE (%)
1.2	1.2 ± 0.1	6.5	0.3	1.2 ± 0.1	7.5	−2.9
9.9	9.6 ± 0.6	6.6	−3.0	9.9 ± 0.5	4.7	0.1
79.5	82.3 ± 3.5	4.3	3.5	77.6 ± 4.4	5.7	−2.4

Table 2
Stabilities of SPRC in rat plasma under different conditions (n = 5).

Storage condition	1.2 μg/mL		9.9 μg/mL		79.5 μg/mL	
	Remaining (%)	RSD (%)	Remaining (%)	RSD (%)	Remaining (%)	RSD (%)
Short-term stability	103.8	4.7	104.0	4.9	101.9	2.7
Freeze and thaw stability	90.0	9.3	98.0	3.6	109.3	14.7
Long-term stability	109.9	11.3	110.4	6.1	114.8	3.1
Post-preparative stability	91.9	11.2	96.5	4.7	97.2	3.2

3. Results and discussion

3.1. Method optimization

The separation selectivity provided by RP-HPLC is limited to the hydrophobicity-based resolution of relatively nonpolar sample components. Because of its high hydrophilicity, SPRC were not retained on a reversed-phase column. For the development of this method, a mixed reversed-phase and cation-exchange analytical column was adopted to obtain enough retention of SPRC with a mobile phase of acetonitrile-ammonium acetate buffer. Based on ammonium acetate buffer, the influence of buffer concentrations and pH were investigated. As a result, 10 mM ammonium acetate (pH 4.0) was chosen for the mobile phase composition, which can lead to a good peak shape and signal intensity for the analyte. With the mobile phase composed of acetonitrile/ammonium acetate buffer (pH 4.0; 10 mM): 30/70 (v/v), the retention times of SPRC and IS were 3.21 ± 0.05 min (n = 10) and 4.12 ± 0.05 min (n = 10), respectively. This column was used successfully for the separation of the polar molecules that were weakly retained in the ordinary reversed-phase chromatography columns.

3.2. Method validation

Typical chromatograms obtained in rat plasma are shown in Fig. 2. No potential interfering peaks from endogenous matrix components were observed at the retention times of SPRC and IS.

The assay was linear over a concentration range from 0.6 to 159 μg/mL for SPRC. The regression model was evaluated using an analysis of variance (ANOVA) and a lack-of-fit test. The results revealed that the linear regression model fits the calibration data well. The mean equation for the standard curves in rat plasma was $y = 0.0572\ (\pm 0.0025) + 0.0616\ (\pm 0.0019) \times x$, and the coefficients of determination (R^2) were greater than 0.99. For each point on the calibration curves for SPRC, the concentrations back-calculated from the equation of the regression analysis were within 15% deviation from the nominal values (date not shown). The present method offered an LLOQ of 0.6 μg/mL with an accuracy of 6.2% and a precision of 8.9%.

Table 1 summarizes the intra- and inter-batch precision and accuracy for SPRC. Intra- and inter-batch precision and accuracy for all the concentrations were less than 10%.

The mean extraction recoveries of SPRC at three different concentrations were 96.4 ± 8.2%, 98.8 ± 2.6%, and 101.0 ± 2.5%, respectively. The mean matrix effects were 76.6 ± 4.2%, 76.1 ± 1.6%, and 78.3 ± 3.1%. In addition, we evaluated the variability of matrix effects in samples from six different individuals, and the variability in matrix effects (measured by RSD) was 5.5%, 2.1%, 4.0% at three different concentrations, which indicated the matrix effects were acceptable and wouldn't impact the reproducibility of analysis [13]. The mean extraction recovery and matrix effect of IS was 103.3 ± 5.2% and 97.6 ± 4.2%.

SPRC was determined to be stable under different temperature and storage conditions. All the samples evaluated showed no obvious degradation (less than 10%) of SPRC in plasma (Table 2).

Table 3
Pharmacokinetic parameters of SPRC in rats (mean ± SD, n = 6).

Parameters	*i.v.*			*i.g.*		
Dose(mg/kg)	25	75	225	25	75	225
C_{max} (μg/mL)	–	–	–	17.1 ± 2.0	59.0 ± 9.9	167.0 ± 45.5
$C_{1.8\,min}$ (μg/mL)	57.4 ± 28.7	96.9 ± 18.5	388.4 ± 105.6	–	–	–
T_{max} (h)	–	–	–	1.1 ± 0.5	1.3 ± 0.5	2.5 ± 1.8
$T_{1/2}$ (h)	3.1 ± 0.8	2.8 ± 1.9	2.8 ± 1.5	4.3 ± 0.9	3.0 ± 0.9	3.1 ± 1.0
MRT (h)	4.5 ± 1.0	5.1 ± 2.5	5.4 ± 1.6	7.0 ± 2.1	5.9 ± 1.5	7.1 ± 1.7
CL (ml/min/kg)	2.9 ± 1.0	2.8 ± 1.2	2.2 ± 0.6	3.2 ± 1.2	2.8 ± 0.8	2.5 ± 0.9
$V_{d,area}$ (l/kg)	0.75 ± 0.10	0.55 ± 0.11	0.49 ± 0.16	1.11 ± 0.21	0.73 ± 0.27	0.63 ± 0.17
AUC_{0-t} (mg/l h)	141.8 ± 34.2	471.4 ± 175.5	1692.9 ± 368.3	137.0 ± 63.8	457.1 ± 137.2	1603.8 ± 589.3
$AUC_{0-\infty}$ (mg/l h)	151.6 ± 39.1	518.8 ± 245.3	1828.2 ± 499.1	142.7 ± 65.2	468.4 ± 136.8	1656.2 ± 627.5
F (%)	–	–	–	96.6	97.0	94.7

C_{max}, maximum plasma concentration; T_{max}, time to maximum plasma concentration; $T_{1/2}$, elimination half life; MRT, mean residence time; CL, clearance; $V_{d,area}$, volume of distribution; AUC_{0-t}, area under the plasma concentration–time curve from time zero to time t; $AUC_{0-\infty}$, area under the plasma concentration–time curve from time zero infinity; F, bioavailability.

After dilution (1:5), the accuracy (measured as RE) compared with the nominal concentration was −2.8%, and the precision (measured as RSD) was 10.1%. This indicates the plasma samples containing SPRC above the high level of calibration curve can be diluted with blank matrix prior to sample pretreatment.

3.3. Pharmacokinetic study

Fig. 3 showed the plasma concentration-time-course profiles of SPRC after single *i.g.* and *i.v.* administrations to rats. The pharmacokinetic parameters are summarized in Table 3. Log transformed C_{max}/Dose and AUC_{0-t}/Dose were not different between dose levels after a single *i.g.* or *i.v.* administration ($p > 0.05$), and the mean T_{max} were also found to be independent of dose ($p > 0.05$), indicating a kinetic linearity.

After oral administration, SPRC was rapidly absorbed and the maximum plasma concentration (C_{max}) appeared at 1.1 ± 0.5, 1.3 ± 0.5, and 2.5 ± 1.8 h following 25, 75, and 225 mg/kg administration, respectively. The absolute bioavailability of oral SPRC was calculated to be 96.6%, 97.0%, and 94.7%, respectively.

4. Conclusion

A simple, fast mixed-mode reversed-phase and cation-exchange LC–MS/MS assay for the quantification of SPRC in rat plasma has been successfully developed and validated. The proposed method has been successfully applied to the pharmacokinetic studies of SPRC in rats. SPRC exhibits linear pharmacokinetics over a 25–225 mg/kg dose range, and its oral bioavailability was high, above 90% of the administered dose.

Acknowledgements

We sincerely appreciate Jessica Wright (Albany College of Pharmacy and Health Sciences, Albany, NY) for the language correction, and we also thank Jingping Shao and Xiuzhi Zhang (Fudan-Zhangjiang new drug R&D joint Platform, Shanghai, China) for the assistance in the LC–MS/MS analysis.

The project was supported by National Drug Innovative Program (2009ZX09301-011) and Traditional Chinese Medicine Modernization (10DZ1972100).

References

[1] Y. Kodera, A. Suzuki, O. Imada, S. Kasuga, I. Sumioka, A. Kanezawa, N. Taru, M. Fujikawa, S. Nagae, K. Masamoto, K. Maeshige, K. Ono, Physical, chemical, and biological properties of S-allylcysteine, an amino acid derived from garlic, J. Agric. Food Chem. 50 (2002) 622–632.
[2] S. Hatono, A. Jimenez, M. Wargovich, Chemopreventive effect of S-allylcysteine and its relationship to the detoxification enzyme glutathione S-transferase, Carcinogenesis 17 (1996) 1041–1044.
[3] F. Pérez-Severiano, M. Rodríguez-Pérez, J. Pedraza-Chaverrí, P. Maldonado, O. Medina-Campos, A. Ortíz-Plata, A. Sánchez-García, J. Villeda-Hernández, S. Galván-Arzate, P. Aguilera, A. Santamaría, S-Allylcysteine, a garlic-derived antioxidant, ameliorates quinolinic acid-induced neurotoxicity and oxidative damage in rats, Neurochem. Int. 45 (2004) 1175–1183.
[4] B.H.S. Lau, Suppression of LDL oxidation by garlic, J. Nutr. 131 (2001) 985S–988S.
[5] Q. Wang, H.R. Liu, Q. Mu, P. Rose, Y.Z. Zhu, S-propargyl-cysteine protects both adult rat hearts and neonatal cardiomyocytes from ischemia/hypoxia injury: the contribution of the hydrogen sulfide-mediated pathway, J. Cardiovasc. Pharmacol. 54 (2009) 139–146.
[6] Q. Wang, X.L. Wang, H.R. Liu, P. Rose, Y.Z. Zhu, Protective effects of cysteine analogues on acute myocardial ischemia: Novel modulators of endogenous H_2S production, Antioxid. Redox Signal. 12 (2010) 1155–1165.
[7] M.P. Ibolya, Derivatization and chromatographic behavior of the o-phthaldialdehyde amino acid derivatives obtained with various SH-group-containing additives, J. Chromatogr. A 913 (2001) 283–302.
[8] L. Bosch, A. Alegría, R. Farré, Application of the 6-aminoquinolyl-N-hydroxysccinimidyl carbamate (AQC) reagent to the RP-HPLC determination of amino acids in infant foods, J. Chromatogr. B 831 (2006) 176–183.
[9] C.K. Yan, F.D. Zeng, Pharmacokinetics and tissue distribution of S-allylcysteine in rats, Asian J. Drug Metab. Pharmacokinet. 5 (2005) 61–69.
[10] M. Washe, M.T. Kelly, M.R. Smyth, H. Ritchie, Retention studies on mixed-mode columns in high-performance liquid chromatography, J. Chromatogr. A 708 (1995) 31–40.
[11] J.T. Eleveld, H.A. Claessens, J.L. Ammerdorffer, A.M. van Herk, C.A. Cramers, Evaluation of mixed-mode stationary phases in liquid chromatography for the separation of charged and uncharged oligomer-like model compounds, J. Chromatogr. A 677 (1994) 211–227.
[12] Food Drug Administration, Guidance for Industry: Bioanalytical Method Validation, US Department of Health and Human Services, FDA, Center for Drug Evaluation and Research, Rockville, MD, 2001.
[13] C.T. Viswanathan, S. Bansal, B. Booth, A.J. Destefano, M.J. Rose, J. Sailstad, V.P. Shah, J.P. Skelly, P.G. Swann, R. Weiner, Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays, Pharm. Res. 24 (2007) 1962–1973.