Pharmaceutica Analytica Acta
ISSN: 2153-2435

Lin et al., Pharm Anal Acta 2015, 6:6
DOI: 10.4172/2153-2435.1000383

Research Article Open Access

Determination of Sodium Tanshinone Iia Sulfonate in Rat Plasma by High Performance Liquid Chromatography and its Application to Pharmacokinetics Studies

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Abstract

A rapid high performance liquid chromatography (HPLC) method for the determination of sodium tanshinone IIA sulfonate (STS) in rat plasma is developed and validated. Sample preparation is accomplished through protein precipitation with methanol. The analyte and internal standard tanshinone I sulfonate are separated on an Agilent Extend C18 analytical column, using a mixture of methanol and triethylamine (0.1) (60:40, v/v) as the mobile phase. The low limit of quantification is 0.2 μg/mL, and the method is linear over the concentration range of 0.2-50 μg/mL for STS. The within- and between-run precisions (RSD) are within 5.8, mean extraction yields are always higher than 93.40, and it proves to be stable during all sample storing, preparation and analytic procedures. The method is successfully applied to a pharmacokinetic study after an intravenous administration of STS to rats at a dose of 6 mg/kg and pharmacokinetics is described by a three-compartment open model.

Keywords: Tanshinone IIA sulfonate; High performance liquid chromatography; Pharmacokinetics

Introduction

Tanshinone IIA is the main lipophilic component of Salvia miltiorrhiza (Danshen, in China), a widely used Chinese herbal medicine. Tanshinone IIA possesses a variety of pharmacological activities such as protection against myocardial ischemia, anti-inflammatory, antioxidant, protective effects on hepatic fibrosis, antitumor and neuroprotective activity [1-7]. But due to its poor water solubility, the clinical use of Tanshinone IIA is limited. After salification procedure, we got tanshinone IIA sulfonate (STS), a water-soluble derivative of tanshinone IIA, which also has a broad range of pharmaceutical effects [8-12]. The chemical structure of STS is illustrated in Figure 1.

STS quantification in plasma samples is required for pharmacokinetic studies. However, few articles dealing with its determination in biological samples have been reported. A high performance liquid chromatography (HPLC) method with ultraviolet (UV) detector were described to the assay of STS in mouse plasma, but the existing assays required mobile phase containing ion-paired reagents to reduce the peak tailing and improve the peak shape [13]. The use of ion-paired agents resulted in prolonged column equilibration time and complex mobile phase preparation. Another method with gradient elution also need more preparation time [14]. Especially, those HPLC methods used at least 0.4 mL of blood for the determination of STS and this amount of blood was too much for rats and was not suitable for the assay of STS in plasma during the pharmacokinetic study.

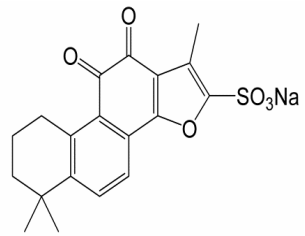

Figure 1: Chemical structure of STS.

Recently, the liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed to determined the concentrations of STS in rat plasma, but the method required LC-MS/MS equipment which might not be available in many laboratories [15-17]. The cost of experiment is more expensive, while there are more factors affecting the determination than the HPLC method. In this study, we report here a simple and sensitive HPLC method with ultraviolet detector for the determination of STS in rat plasma. The mobile phase composed of methanol and triethylamine (0.1) (60:40, v/v), without addition of ion-pairing agents. The peaks of STS and the IS were neat, symmetric and well separated. With UV detector, the analysis of STS in rat plasma could be achieved using only 0.1 mL of blood.

The sample pre-treatment procedure was different from the ones reported in the literature. Ethyl acetate was added without hydrochloric acid and the organic layer was removed to get rid of the liposoluble impurities. Then good extraction yields of STS were accomplished through protein precipitation procedure with methanol. The sample that has got rid of the liposoluble impurities was neater than the ones dealt with protein precipitation directly. It could protect the column and instrument effectively. The mean extraction yields were always higher than 93.40, which was much higher than the Liquid-liquid extraction described in the LC-MS/MS method [15-17]. The LOQ was 0.2 μg/mL, which was totally sensitive enough for the pharmacokinetic studies after intravenous administration of STS to rats at a dose of 6 mg/kg.

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Received May 21, 2015; **Accepted** June 23, 2015; **Published** June 25, 2015

Citation: Lin G, Jian J, Yuliang R (2015) Determination of Sodium Tanshinone Iia Sulfonate in Rat Plasma by High Performance Liquid Chromatography and its Application to Pharmacokinetics Studies. Pharm Anal Acta 6: 383. doi:10.4172/21532435.1000383

Experimental

Materials and reagents

Authentic standard of STS (98.1 purity) and sodium tanshinone I sulfonate (98.5 purity, the internal standard, IS) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). Methanol (HPLC-grade) was purchased from Tedia Company (Fairfield, USA). Triethylamine, ethyl acetate, phosphonic acid were all of analytical grade and purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China).

Stock solutions of the analyte and the IS (1 mg/mL) were prepared by dissolving suitable amounts of each reference compound in methanol, stored at 4°C. A series of standard working solutions and internal standard working solution (50 μg/mL) were obtained by diluting stock solutions with methanol. The quality control (QC) samples were separately prepared in blank plasma at the concentrations of 0.5, 5 and 40 μg/mL for STS, respectively.

Instrument and chromatographic conditions

All analysis were performed on an Agilent HPLC system (Series 1200, Agilent, USA) which equipped with a 1200 quaternary pump, a vacuum degasser, an autosampler, a controller module and a UV detector set at 271 nm. The chromatography data were recorded and processed with Chemstation software. Separations were obtained on an Agilent Extend C18 column (4.6 mm × 150 mm, 5 μm) kept at 25°C. A pre-column treatment of the same constituents was employed. The mobile phase was a mixture of methanol and triethylamine (0.1) (60:40, v/v), and the pH was adjusted to 3.5 with phosphonic acid. The flow rate was 1 mL/min and the injection volume was 20 μL.

Sample preparation

Briefly, to an aliquot of 100 μL of plasma sample in a disposable Eppendorf tube, 10 μL of the internal standard working solution (50 μg/mL) was added and mixed vigorously. Then 200 μL of ethyl acetate was added and vortex-mixed for 1 min, followed by centrifugation at 16000 rpm for 5 min. After the upper organic layer was removed, to get rid of the liposoluble impurities, 200 μL of methanol was added and vortex-mixed for 1 min in order to get rid of the protein and then the sample was centrifuged at 16000 rpm for 5 min. The resultant supernatant was used in the assay.

Method validation

Selectivity: Blank plasma samples from six different rats were subjected to the sample preparation procedure and injected into the HPLC. The resulting chromatograms were checked for possible interference from endogenous compounds.

Calibration Curves

Aliquots of 10 μL of analyte standard solutions (prepared daily) at eight different concentrations and 10 μL of the IS at a constant concentration (50 μg/mL) were added to 100 μL of blank plasma. The resulting mixture was subjected to the previously described sample preparation procedure and injected into the HPLC. The procedure was carried out in triplicate for each concentration. The analyte/IS peak area ratios (pure numbers) obtained were plotted against the corresponding concentrations of the analytes (expressed as μg/mL) and the calibration curves was set up by means of the least square method. The limit of quantification (LOQ) was defined as the lowest concentration at which both precision and accuracy were less than or equal to 20 with the ratio of signal-to-noise more than 10 (S/N>10).

Extraction yield (absolute recovery)

The procedure was the same as that described under "Calibration Curve" above, except the points were at three different concentrations (0.5, 5, 40 μg/mL, n=5). The analyte/IS peak area ratios were compared to those obtained by injecting standard solutions at the same theoretical concentrations and the extraction yield values were calculated.

Precision and accuracy

The assays described under "Extraction yield" were repeated five times within the same day to obtain within-run precision and five times over three different days to obtain between-run precision, both expressed as RSD values. The accuracy was also assessed by determining QC samples at three concentration levels and expressed by (mean observed concentration) / (spiked concentration) ×100.

Stability

The stability of STS in rat plasma was investigated by analyzing QC samples at concentrations of 0.5, 5 and 40 μg/mL at room temperature (approximately 25-28 °C) for 8h and for three freeze- thaw cycles at -20°C. Stability of the processed sample was determined by keeping the samples in an autosampler at 4°C for 24 h.

Application to Pharmacokinetics Study of STS

Six Sprague-Dawley rats (180 g to 220 g) were administered intravenously with STS at a dose of 6 mg/kg (dissolved in glucose parenteral solution (5, w/v). About 0.25 mL of blood via the angular oculi vein was collected into a heparinized tube at pre-dosing, and at 1, 3, 6, 10, 15, 20, 25, 30, 40, 60 min after administration. After the blood was centrifuged at 3500 rpm for 5 min, the plasma was rapidly collected and stored at -20°C until analysis. Pharmacokinetic analysis of STS concentration in rat plasma samples were performed using Drug and Statistic (DAS) version 2.0 pharmacokinetic software (Shanghai University of T.C.M, China).

Results and Discussion

Choice of the chromatographic conditions

It is critical to optimize chromatographic conditions to obtain good selectivity, high sensitivity, quick speed and symmetrical peak shape. Since STS is a hydrophilic ionic substance and weak acid, we added triethylamine to the mobile phase and adjusted the pH with phosphonic acid to reduce the peak tailing and improve the peak shape. We tried pH 7.0, 5.0, 4.0, 3.5 and 3.0, and found that when pH was adjusted to 3.5, the retention times are appropriate and the peaks are neat, symmetric and well separated.

Development of the Sample Pre-Treatment Procedure

STS was a water-soluble derivative of tanshinone IIA, designed to extend the clinical application of tanshinone IIA. In the literature, protein precipitation was used commonly due to the high polarity of STS, which was simple and fast [13].

In some other study, liquid-liquid extraction (LLE) method was used as the sample pre-treatment procedure [15]. Before LLE, hydrochloric acid was added to adjust the pH appropriately in order to change STS to tanshinone IIA. Tanshinone IIA possesses higher lipophilicity than STS, and it was supposed to be extracted by the organic solvent. After LLE, the organic layer was transferred and evaporated to dryness, which took more steps and was time-consuming, compared with protein precipitation. Meanwhile, as we know, besides targeted

drugs, LLE may extract numerous interfering substances from blood or plasma, like triglycerides (TG). It has been found, TG levels in whole blood noticeably influence the drug recovery to variable extents, when using LLE as the sample pretreatment method [18].

In this study, LLE was tested firstly for the sample pre-treatment. Ethyl acetate was added to plasma sample and the upper organic layer was transferred and evaporated to dryness under nitrogen gas at 40°C. The residue was reconstituted with mobile phase and injected into the HPLC system. The chromatogram showed that neither analytes or the IS was extracted by ethyl acetate. So ethyl acetate was used without addition of hydrochloric acid to get rid of the liposoluble impurities. LLE was then followed by protein precipitation procedure, and different solvents have been tried. The results showed that, after treated with acetone, there were interferences in the samples. After treated with ethanol, the shape of the peak was not good. Methanol and acetonitrile both showed good results, and considering acetonitrile was more toxic, we chose methanol as the precipitation solvent.

Thus, both protein precipitation and LLE were utilized in this study. This sample pre-treatment method had several advantages with respect to the previously published method which also used protein precipitation for the measurement of STS. The procedure provided certain improvement on sensitivity and reliability. Meanwhile, getting rid of the liposoluble impurities by LLE would make the sample neater than the one dealt with protein precipitation directly, and it could also protect the column and instrument effectively from the contamination of the impurities. So the sample pre-treatment procedure of this study may provide a new method to extract water-soluble drugs from plasma sample.

Figure 2A reports the chromatogram of six blank plasma samples, while Figure 2B reports the chromatogram of blank plasma sample spiked with a known amount of STS and the IS. Figure 2C reports the chromatogram of rat plasma sample at 6 min after intravenous administration with 6 mg/kg of STS. No interference can be detected near the retention times of the compounds of interest; furthermore, peak shapes and resolution are good.

Method Validation

Selectivity was evaluated by injecting into the HPLC six blank plasma samples and there were no peaks from endogenous compounds observed at the retention times of the analyte and the IS. Therefore, the method has demonstrated to be selective.

Eight-point calibration curves were set up over the concentration range of 0.2-50 μg/mL. Good linearity (r^2 = 0.9999) was obtained, with a limit of quantitation of 0.2 μg/mL. The linearity equation was: y=0.169 x+ 0.039, where x is the STS concentration, expressed as μg/mL, and y is the STS/IS peak area ratio (a pure number).

Extraction yield (absolute recovery) was assessed by determining QC samples at three concentration levels (0.5, 5, 40 μg/mL). The results of these assays are reported in Table 1. Mean extraction yields were always higher than 93.40.

The results of the precision and accuracy assays are reported in Table 2. RSD values were 2.6 or less for within-run precision and 5.8 or less for between-run precision. Mean recovery values also were always higher than 87.67. Thus, the results demonstrated that the values were within the acceptable range and the method was accurate and precise.

Results of the stability evaluation are shown in detail in Table 3. The results indicated that the analytes were stable in the autosampler for at

Spiked (μg/mL)	Recovery (%) (mean ± SD)	RSD (%)
0.5	99.49 ± 4.75	4.8
5	95.60 ± 1.60	1.7
40	93.40 ± 2.35	2.5

Table 1: Extraction recovery of STS in rat plasma (n=5).

Spiked (μg/mL)	Found (mean ± SD, μg/mL)	RSD (%)	Accuracy (%)
within-run			
0.5	0.44 ± 0.011	2.6	87.67
5	5.05 ± 0.088	1.7	100.95
40	39.78 ± 1.01	2.5	99.45
between-run			
0.5	0.45 ± 0.026	5.8	90.04
5	5.19 ± 0.21	4.0	103.85
40	40.79 ± 1.44	3.5	101.98

Table 2: Within- and between-run precision and accuracy for the measurement of STS in rat plasma (n=5).

Spiked	Found (mean ± SD, μg/mL)		
(μg/mL)	Freeze–thaw for three cycles	Room temperature for 8 h	Autosampler for 24h
0.5	0.43 ± 0.01	0.56 ± 0.10	0.54 ± 0.08
5	5.22 ± 0.07	4.93 ± 0.18	4.91 ± 0.12
40	39.39 ± 1.39	39.28 ± 1.40	40.37 ± 1.40

Table 3: Stability test of STS during the storing and preparation procedures (n=5).

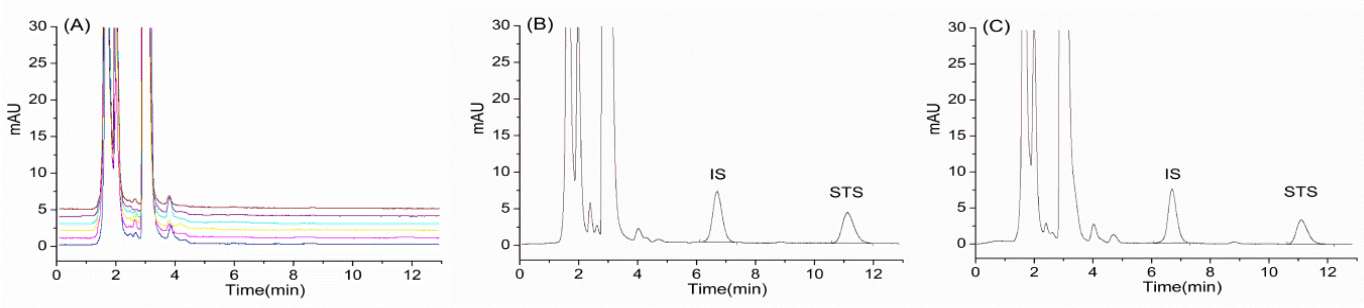

Figure 2: Typical chromatograms obtained from HPLC analysis of six different rats. (A) Chromatogram of blank rat plasma; (B) Chromatogram of blank mouse plasma spiked with 5 μg/mL STS and IS; (C) Chromatogram of rat plasma sample at 6 min after intravenous administration of STS at a dose of 6 mg/kg.

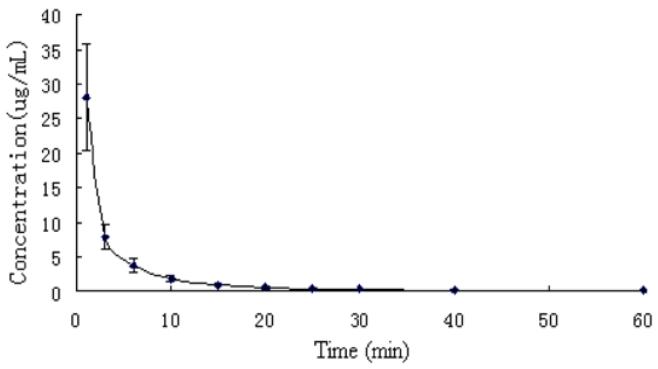

Figure 3: Plasma concentrations versus time profile after an intravenous administration of STS at a dose of 6 mg/kg.

Parameters	Mean	SD
C_{max} (μg/mL)	28.07	7.52
$t_{1/2\alpha}$ (min)	0.55	0.17
$t_{1/2\beta}$ (min)	3.33	0.69
$t_{1/2\gamma}$ (min)	26.57	24.10
V1 (l/kg)	0.083	0.046
CL (L/min/kg)	0.041	0.009
$AUC_{(0-60)}$(mg•min/L)	115.10	20.49
$AUC_{(0-\infty)}$(mg•min/L)	121.36	25.70

C_{max}: the maximum plasma concentration; $t_{1/2\alpha}$: Half-life of rapid distribution phase; $t_{1/2\beta}$: half-life of slow distribution phase; $t_{1/2\gamma}$: half-life of elimination phase; V1: apparent volumes of distribution of the central compartments; CL: total body clearance; AUC: area under the plasma concentration vs. time curve.

Table 4: Pharmacokinetic parameters of STS followed after a single intravenous administration of STS at a dose of 6 mg/kg (n = 6).

least 24 h. STS in rat plasma was stable after three freeze–thaw cycles and STS in plasma extract was stable at room temperature for 8 h.

Pharmacokinetic Analysis

The validated methods have been successfully applied to pharmacokinetic studies of STS in rat plasma after intravenous administration. Mean plasma concentration-time curve of STS, after intravenous administration at a dosage of 6 mg/kg to six rats is shown in Figure 3. Pharmacokinetics could be described by a three-compartment open model using Drug and Statistic (DAS) version 2.0 pharmacokinetic software and the main pharmacokinetic parameters are shown in Table 4.

Conclusion

The HPLC method presented here for the analysis of STS was feasible and rapid. The protein precipitation offered a fast sample preparation protocol compared to liquid-liquid extraction [14]. Sample pre-treatment was simple and gave good extraction yields and satisfactory precision. The chromatographic conditions were simpler and the LOQ was lower than the previously reported ion-pair reversed-phase HPLC method [13]. And the method was successfully applied to the pharmacokinetic studies after intravenous administration of STS to rats at a dose of 6 mg/kg.

References

1. Yagi A, Okamura N, Tanonaka K, Takeo S (1994) Effects of tanshinone VI derivatives on post-hypoxic contractile dysfunction of perfused rat hearts. Planta Med 60: 405-409.
2. Jang SI, Jeong SI, Kim KJ, Kim HJ, Yu HH, et al. (2003) Tanshinone IIA from Salvia miltiorrhiza inhibits inducible nitric oxide synthase expression and production of TNF-alpha, IL-1beta and IL-6 in activated RAW 264.7 cells. Planta Med 69: 1057-1059.
3. Cao EH, Liu XQ, Wang JJ, Xu NF (1996) Effect of natural antioxidant tanshinone II-A on DNA damage by lipid peroxidation in liver cells. Free Radic Biol Med 20: 801-806.
4. Niu XL, Ichimori K, Yang X, Hirota Y, Hoshiai K, et al. (2000) Tanshinone II-A inhibits low density lipoprotein oxidation in vitro. Free Radic Res 33: 305-312.
5. Liu Y, Wang X, Liu Y (2003) Protective effects of tanshinone IIA on injured primary cultured rat hepatocytes induced by CCl4. Zhong Yao Cai 26: 415-417.
6. Yuan SL, Wang XJ, Wei YQ (2003) Anticancer effect of tanshinone and its mechanisms. Ai Zheng 22: 1363-1366.
7. Lam BY, Lo AC, Sun X, Luo HW, Chung SK, et al. (2003) Neuroprotective effects of tanshinones intransient focal cerebral ischemia in mice. Phytomedicine 10: 286-291.
8. Yang L, Zou X, Liang Q, Chen H, Feng J, et al. (2007) Sodium tanshinone IIA sulfonate depresses angiotensin II- induced cardiomyocyte hypertrophy through MEK/ERK pathway. Exp Mol Med 39: 65-73.
9. Xiao Y, Qing WX, Lan MS, Ying CB (2006) Sodium tanshinone IIA sulfonate derived from Slavia miltiorrhiza Bunge up-regulate the expression of prolactin releasing peptide (PrRP) in the medulla oblongata in ovariectomized rats. Biochem Pharmacol 72: 582-587.
10. Zhou G, Jiang W, Zhao Y, Ma G, Xin W, et al. (2003) Sodium tanshinone IIA sulfonate mediates electron transfer reaction in rat heart mitochondria. Biochem Pharmacol 65: 51-57.
11. Takahashi K, Ouyang X, Komatsu K, Nakamura N, Hattori M, et al. (2002) Sodium tanshinone IIA sulfonate derived from Danshen (Salvia miltiorrhiza) attenuates hypertrophy induced by angiotensin II in cultured neonatal rat cardiac cells. Biochem Pharmacol 64: 745-749.
12. Wu TW, Zeng LH, Fung KP, Wu J, Pang H, et al. (1993) Effect of sodium tanshinone IIA sulfonate in the rabbit myocardium and on human cardiomyocytes and vascular endothelial cells. Biochem Pharmacol 46: 2327-2332.
13. Mao SJ, Jin H, Bi YQ, Liang Z, Li H, et al. (2007) Ion-pair reversed-phase HPLC method for determination of sodium tanshinone IIA sulfonate in biological samples and its pharmacokinetics and biodistribution in mice. Chem Pharm Bull 55: 753-756.
14. Li X, Li X, Wang L, Li Y, Xu Y, et al. (2007) Simultaneous determination of danshensu, ferulic acid, cryptotanshinone and tanshinone IIA in rabbit plasma by HPLC and their pharmacokinetic application in danxiongfang. J Pharm Biomed Anal 44: 1106-1112.
15. Hao H, Wang G, Cui N, Li J, Ding Z (2007) Determination of sodium tanshinone IIA sulfonate in plasma by liquid chromatography-electrospray ionisation-tandem mass spectrometry. Biomed Chromatogr 21: 1172-1179.
16. Liu Y, Li X, Li Y, Wang L, Xue M (2010) Simultaneous determination of danshensu, rosmarinic acid, cryptotanshinone, tanshinone IIA, tanshinone I and dihydrotanshinone I by liquid chromatographic-mass spectrometry and the application to pharmacokinetics in rats. J Pharm Biomed Anal 53: 698-704.
17. Song M, Hang TJ, Zhang Z, Chen HY (2007) Effects of the coexisting diterpenoid tanshinones on the pharmacokinetics of cryptotanshinone and tanshinone IIA in rat. Eur J Pharm Sci 32: 247-253.
18. Huang ZB, Yu T, Guo L, Lin Z, Zhao ZQ, et al. (2015) Effects of triglycerides levels in human whole blood on the extraction of 19 commonly used drugs using liquid-liquid extraction and gas chromatography-mass spectrometry. Toxicol Rep 2: 785-791.