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Simultaneous Quantification of Salvianolic Acid B and Tanshinone IIA of Salvia Tropolone Tablets by UPLC-MRM-MS/MS for Pharmacokinetic Studies

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Summary. A rapid and sensitive ultraperformance liquid chromatography-multiple reaction monitoring-multi-stage/mass spectrometry (UPLC-MRM-MS/MS) method has been developed for simultaneous quantification of salvianolic acid B and tanshinone IIA of salvia tropolone tablets in dog plasma. This was achieved by performing quantification using the MRM acquisition with two channels of MRM-MS/MS and MS full scan for more accuracy qualitative results, and the fragmentation transitions of *m/z* 295→249, 191 for tanshinone IIA and *m/z* 297→279, 251 for IS in positive mode, *m/z* 717→519, 321 for salvianolic acid B and *m/z* 295→267, 239 for IS in negative mode were selected. The UPLC separation was achieved within 3 min in a single UPLC run. Linear calibration curves were obtained over the concentration range of 10 pg/mL^{-1} ng/mL for tanshinone IIA and 100 pg/mL^{-1} for salvianolic acid B. Lower limit of quantitation (LLOQ) was 10 pg/mL and 100 pg/mL for tanshinone IIA and salvianolic acid B, respectively. The inter-day and intra-day precision (relative standard deviation, RSD) in all samples were less than 8.21%, and the recoveries were over 85.9% for both tanshinone IIA and salvianolic acid B. The two channels of MRM with MS full scan approach could provide both qualitative and quantitative results without the need for repetitive analyses and resulted in the reduction of further confirmation experiments and analytical time. The pharmacokinetic study of the two active components of salvia tropolone tablets following oral gavage administration of dogs was thus explored with this method.

Key Words: salvia tropolone tablets, tanshinone IIA, salvianolic acid B, UPLC-MRM-MS/MS, pharmacokinetic study

Introduction

Salvia miltiorrhiza is a well-known traditional Chinese medicine, known as "danshen" in China, which has been widely used for the treatment of angina, myocardial ischemia, menstrual disorders, menostasis, coronary heart disease, and other cardiovascular diseases [1–4]. Previous studies demon-

strated that the active components of *S. miltiorrhiza* can be classified into water-soluble and lipid-soluble components [5]. Salvianolic acid B (water-soluble) and tanshinone IIA (lipid-soluble) were found to possess intensive pharmacological activities and thus have been recognized as marker components for *S. miltiorrhiza* in Chinese Pharmacopeia (2005, edition). Salvianolic acid B was found to have anti-inflammatory, anti-fibrogenic, skip flap ischemia–reperfusion attenuation, atherosclerosis reduction, and cyclooxygenase-2 attenuation properties [6–10], in addition to being an efficient radical scavenger and antioxidant [11]. Likewise, tanshinone IIA is also characterized by anti-inflammation activity, anti-oxidation, and cardio-protection, as well as neuro-protection, anticancer and diabetic nephropathy prevention [12–17]. Remarkably, both salvianolic acid B and tanshinone IIA were also found to inhibit atherogenesis hyperlipid activity [18].

Salvia tropolone tablets prepared from extract of *S. miltiorrhiza* (the main ingredients and respective amount were salvia total ketone ≥ 8.0 mg, tanshinone IIA ≥ 2.5 mg, salvianolic acids ≥ 50 mg, salvianolic acid B ≥ 30 mg) has attained phase I clinical approval in China since 2009. However, pharmacokinetic information available regarding the absorption, distribution, metabolism, and excretion of salvia tropolone tablets following oral gavage administration to animals has been limited. Until now, many analytical methods were reported to detect either the water-soluble components or the lipid-soluble components in biological samples, such as high-performance liquid chromatography-ultraviolet (HPLC-UV) [19] and HPLC-electrospray ionization/mass spectrometry (ESI/MS) methods [20–23]. Although several methods were reported to simultaneously determine water-soluble and lipid-soluble components in plasma [24–26], these were unfortunately time-consuming, lacked sensitivity, and had a limitation on the number of different MS scans that could be processed in a single LC run. Though the high selectivity of MS can be achieved, especially in using tandem mass spectrometric methods, such as one channel of MRM, it is still a challenge to determine analytes at trace levels. The target analyte may be also co-detected with matrix interferences; moreover, the determination of trace levels of analyte in biological sample can be very problematic, requiring a more sensitive method and enhanced LC-MS/MS data acquisition capability for quantitative analysis.

In this paper, we firstly introduced two channels of MRM with MS full scan to confirm the qualitative results, which could provide simultaneous quantifications of two different characteristic components in positive and negative modes in one single run. This method was applied to rapid pharmacokinetic studies of major components of salvia tropolone tablets. Details

of the method development, validation, and pharmacokinetic study are also described.

Materials and Methods

Chemicals and Materials

Tanshinone IIA, salvianolic acid B, and an internal standard (cryptotanshinone, IS) were purchased from the National Institute for Control of Pharmaceuticals and Biological Products (Beijing, China). Salvia tropolone tablets were supported by Beijing Botanitown Medical Technology Co., Ltd. (Beijing, China). Acetonitrile of HPLC grade was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Deionized water was purified using a Milli-Q system (Millipore, Milford, MA, USA). The other chemicals, reagents, and solvents used were all of chromatographic grade.

Instrument and Chromatography Conditions

Ultraperformance liquid chromatography was performed on an ACQUITY™ ultraperformance liquid chromatography (UPLC) system (Waters Corporation, Milford, MA, USA), equipped with a binary solvent delivery system and an autosampler. The chromatographic separation was achieved on a Waters ACQUITY BEH C_{18} column (50 mm × 2.1 mm, 1.7 μm; Waters, Wexford, Ireland) protected by a pre-column with a gradient elution using a mobile phase composed of 0.1% formic acid in both water (A) and acetonitrile (B). The gradients were as follows: 0–0.3 min: 5% B; 0.3–1.5 min: 5–95% B; 1.5–2.5 min: 95% B; 2.5–3.0 min: 95–5% B. The temperature of the column was maintained at 35°C, and the flow rate was set at 0.4 mL/min. The injection volume was 2 μL using the full loop mode for sample injection.

Mass spectrometric detection was operated on a Waters Xevo TQ-S (tandem quadrupole mass spectrometer, Micromass MS Technologies, Manchester, UK) using an electrospray ionization source. The desolvation gas was set to 1000 L/h at a temperature of 500°C, and a source temperature of 150°C. The capillary voltage and extractor voltage were set to 0.75 KV and 3 V, respectively. The collision gas flow was set at 0.15 mL/min. Two channels of MRM were performed in a single UPLC run. The following precursors to product ion transitions were selected for MRM scans: for tanshinone IIA, $[M+H]^+$ at *m/z* 295→249, 295→191; for salvianolic acid B $[M-H]^-$ at *m/z* 717→519, 717→321; and for IS $[M+H]^+$ at *m/z* 297→279, 297→251 $[M-H]^-$ at *m/z* 295→267, 295→239. Two MS full scans in both positive and negative modes were enabled followed by MRM, and the MS scan range was from *m/z* 50 to 800. All data collected in centroid mode were ac-

quired and processed using MassLynx™ V4.1 software with the TargetLynx™ V4.1 program (Waters Corporation, Milford, Massachusetts, USA). Details of the MS parameters are listed in *Table I*.

Table I. UPLC–ESI–MS/MS conditions

Compound name	Parent (*m/z*)	Daughter (*m/z*)	Cone voltage (V)	Collision energy (V)
Tanshinone IIA	295[M + H]$^+$	249 191	24 24	20 38
Salvianolic acid B	717[M – H]$^-$	519 321	42 42	18 30
Cryptotanshinone	297[M + H]$^+$	279 251	35 35	30 43
(Internal standard)	295[M – H]$^-$	267 239	35 35	35 43

Preparation of Standard and Quality Control Samples

The standard stock solutions of tanshinone IIA and salvianolic acid B were prepared separately by dissolving 1 mg of them in 1 mL acetonitrile, and stocked in a 1.5-mL centrifuge tube at 4°C. A series of standard working solutions at appropriate concentrations were diluted with acetonitrile. The IS stock solution of 1 mg/mL was prepared in acetonitrile, and 10 μg/mL was applied to each working solution and samples.

Calibration curve samples were prepared separately using the set of stock solutions within the concentration range of linearity and were evaporated in tubes under gentle stream of nitrogen at 50°C, and then diluted with blank dog plasma. All the quality control (QC) samples (for tanshinone IIA at low (30 pg/mL, LQC), medium (300 pg/mL, MQC), high (900pg/mL, HQC); for salvianolic acid B at low (300 pg/mL, LQC), medium (3000 pg/mL, MQC), high (90,000 pg/mL, HQC) were prepared in the same way as the calibration standards before analysis.

Samples Preparation

A 100-μL aliquot of dog plasma and 10 μL IS were mixed with 300 μL of acetonitrile with 0.1% formic acid in an OSTRO ninety-six well plate; the OSTRO 96-well plate design uses an in-well protein precipitation in combination with a single, rapid, pass-through method that provides consistent, high extraction recoveries for acids, bases, and neutrals. It is more simplified, there is more removal of phospholipids, and better enhances the ionization efficiency. An additional collection plate was fitted to prevent cross

contamination. The solutions were mixed thoroughly by aspiration three times with a pipette, followed by filtration onto the collection plate with positive pressure in 5 min. From the collection plate, a 2-μL aliquot was directly injected into the UPLC-ESI-MS/MS system for analysis.

Method Validation

This method was validated by studying the selectivity, linearity, precision, accuracy, recovery, matrix effect, and stability.

Linearity and Selectivity

To investigate whether the presence of endogenous constituents would interfere with the assay, six blank plasma samples were analyzed to detect any potential interference for co-eluting with analytes and IS. Chromatographic peaks of the analytes and IS were identified by comparing their retention time and MRM responses with those of authentic standards. Calibration standards of tanshinone IIA (six non-zero standards of the analyte, 10, 50, 100, 200, 500, and 1000 pg/mL) and salvianolic acid B (six non-zero standards of the analyte, 0.1, 1, 10, 100, 500, 1000 ng/mL) were prepared in control plasma and analyzed. Linearity of the calibration curve was obtained by plotting the peak area ratio of analyte to the signal area of IS acquired from the MRM as ordinate variables (y) versus the nominal concentrations of analyte (x) forming a linear regression. LLOD was considered the lowest concentration producing a signal-to-noise (S/N) ratio of 3 and lower limit of quantitation (LLOQ) was defined as the lowest measured concentration producing a signal-to-noise (S/N) ratio of 10.

Precision and Accuracy

The intra-day precision and accuracy were determined by analyzing the QC samples within the same day at different times. The inter-day precision and accuracy were determined by analyzing the QC samples over six consecutive days. The precision and accuracy were evaluated as the relative standard deviation of the mean values.

Extraction Recovery and Matrix Effect

The extraction recovery of tanshinone IIA and salvianolic acid B was determined by comparing the peak-areas of the pre-extraction QC samples (at three concentrations) to post-extraction QC samples. The matrix effect was

calculated by comparing the peak areas of the post-extraction QC samples to the stocked standard solutions as described by Matuszewski et al. [28].

Stability

The stability of tanshinone IIA and salvianolic acid B in dog plasma were examined by analyzing three concentrations of the standard solutions in the blank dog plasma in triplicate. These samples were stored at −20°C for 2 months and at ambient temperature for 4 h to evaluate long-term and short-term stabilities, respectively. Freeze to thaw stability was tested following three freeze–thaw cycles at temperature ranging from −20°C to ambient. Stability of the processed samples was assessed by reinjecting the samples after 24 h in an autosample at 4°C.

Animal Pharmacokinetic Study

Male beagle dogs, 7–8 kg, were obtained from the Sichuan Academy of Medical Sciences, Institute of Experimental Animals, (Chengdu, China). Experiments were carried out in accordance with the guidelines for Animal Experimentation established by the Pharmacology and Toxicology Research Institute of Chengdu and the Baikang Pharmaceutical Industry (Chengdu, China). The protocol was approved by the Animal Ethics Committee of the Chengdu Institute of Biology. After a quarantine period, dogs were fed and given 3 days to acclimate to the lab environment. Each dog in the first group was given a single oral gavage dosage of 160 mg/kg of the salvia tropolone tablets. Blood samples (1 mL) were withdrawn via the foreleg vein into 1.5 mL heparinized tubes at 7.5, 15, 30 min, 1, 1.5, 2, 4, 6, 8, and 10 h after the oral gavage administration. Each dog in a second group was administered a blank dosage. Blood was withdrawn via the same method as the first group. Plasma was immediately separated by centrifugation at 3500 rpm for 10 min and stored at −20°C before analysis.

The UPLC-MRM-MS/MS procedure was applied to simultaneously investigate the plasma concentration-time profiles of tanshinone IIA and salvianolic acid B in animals. The pharmacokinetic model and parameters were calculated by the practical pharmacokinetic program-version 87 (3P87), edited by the Committee of the Mathematics Pharmacology and the Chinese Society of Pharmacology (Beijing, China). The compartment model was established using the survival square sum (SUM), the Akaike's information criterion (AIC) and the fitted degree (r^2) methods.

Results and Discussions

Optimization of Chromatographic and Mass Spectrometric Conditions

In the current study, UPLC with MS detection was selected as the method of choice for assaying metabolites of salvia tropolone tablets in complex biological samples. A major challenge was to develop a simple and reproducible method that can quantify targeted analytes with high sensitivity and reproducibility. The UPLC separation was achieved on a C_{18} column within 3 min in a single UPLC run, the MS spectrometric parameters, such as collision energy, capillary voltage, and cone voltage were optimized to obtain the maximum sensitivities for the analytes and internal standard.

Coupled MRM and MS Full Scan Method

In order to avoid interference components from matrix effects, quantification was done by MRM in two channels of the protonated tanshinone IIA and IS, and deprotonated salvianolic acid B and IS. Two modes of MS full scan were also enabled to confirm the qualitative results, since the new Xevo TQ-S tandem quadrupole MS system provides a significant increase in

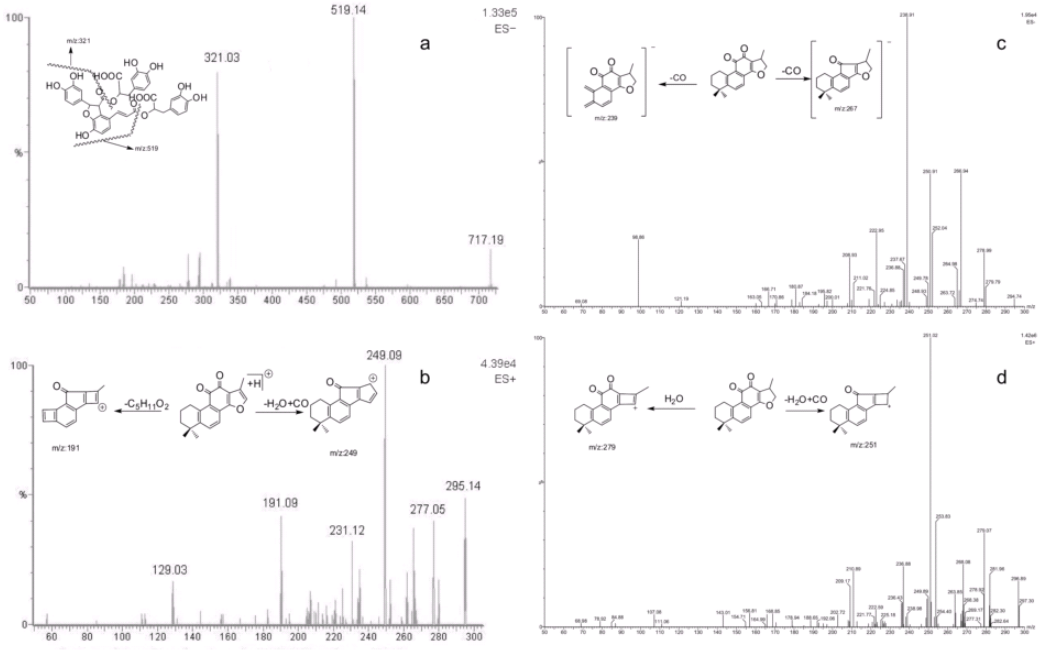

Fig. 1. Main product ions assigned to two standard compounds salvianolic acid B (a); tanshinone IIA (b); and internal standard (c–d).

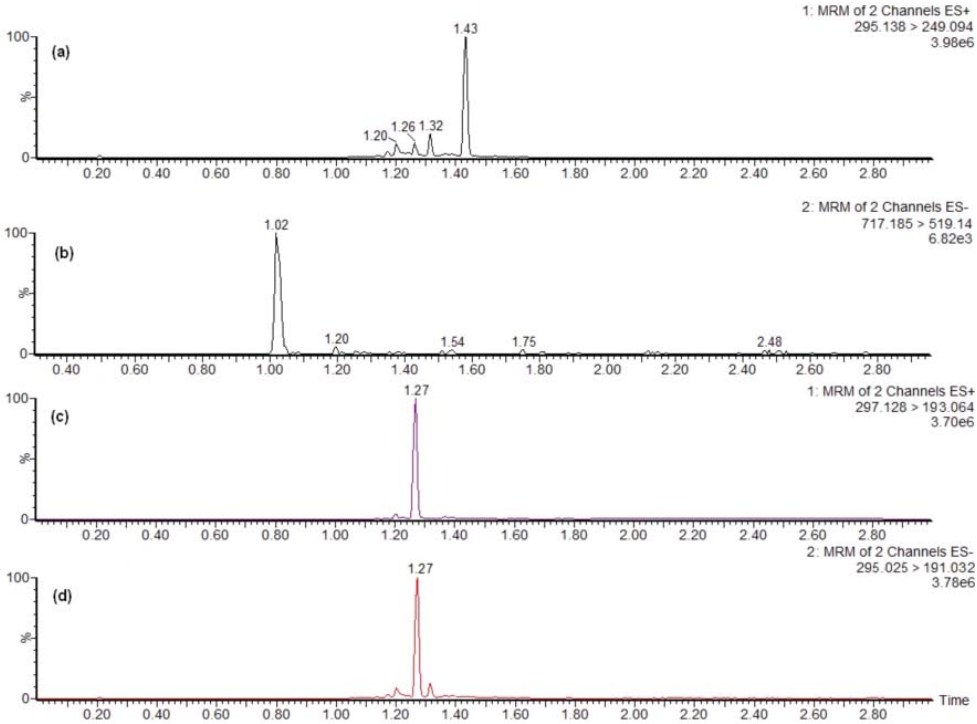

Fig. 2. Representative MRM chromatograms of tanshinone IIA (a), salvianolic acid B (b), internal standard (c–d) spiked in dog plasma.

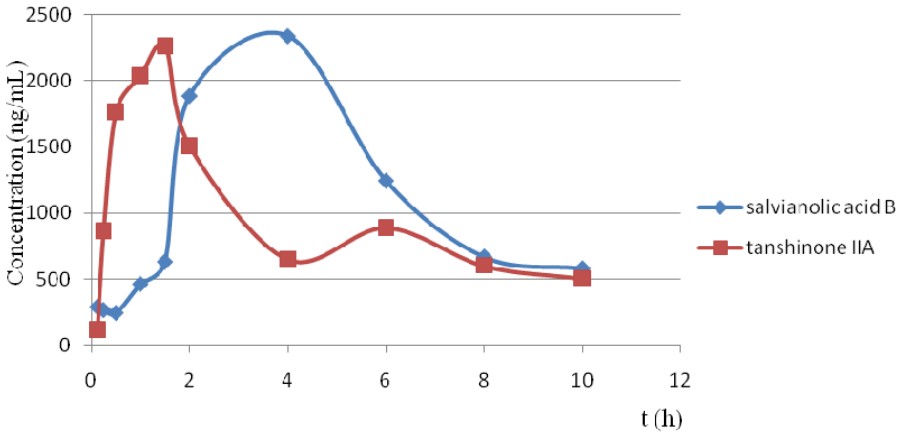

Fig. 3. Pharmacokinetic study profiles of salvianolic acid B and tashinone IIA in dog plasma

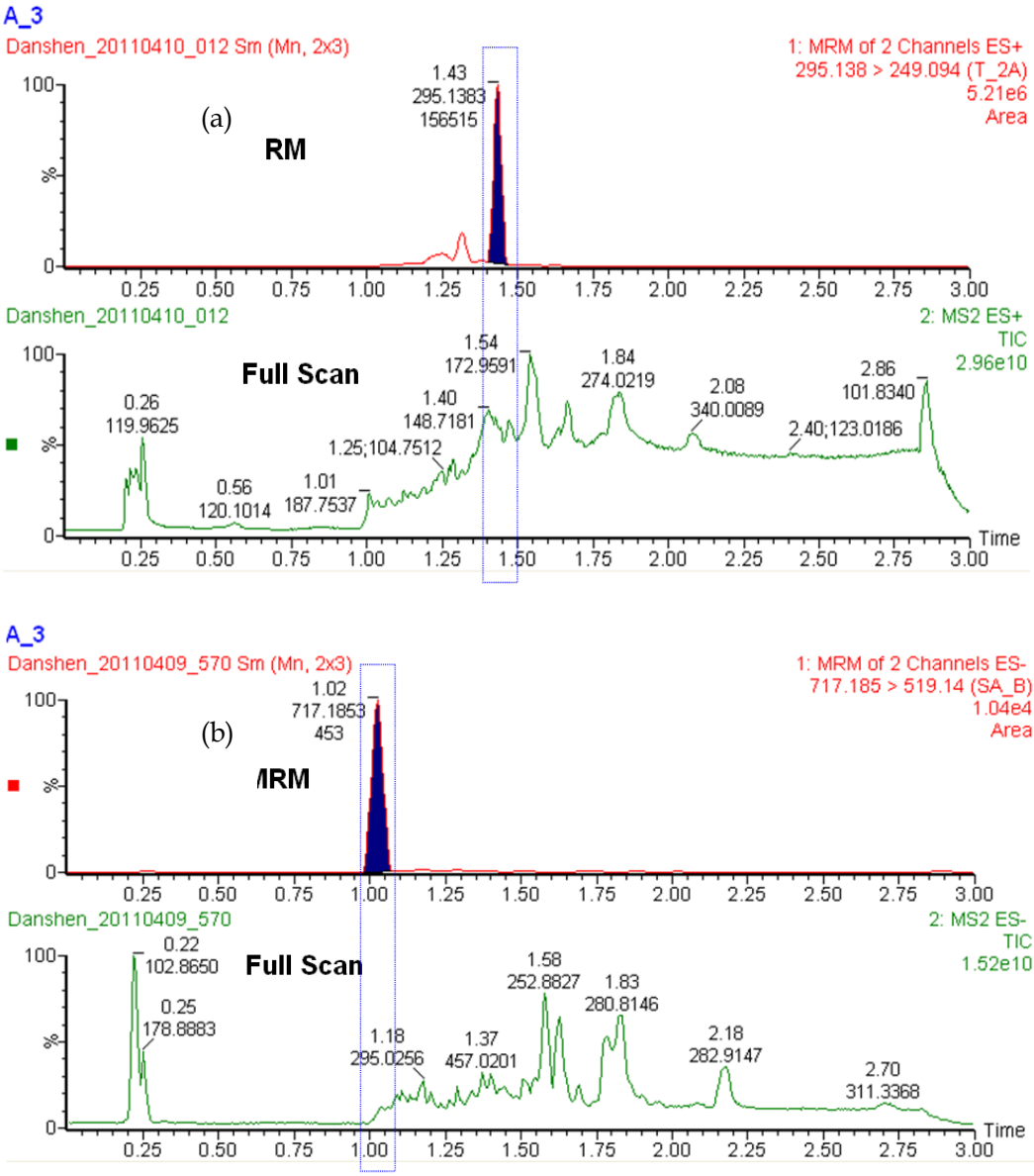

Fig. 4. The chromatograms of tashinone IIA (a), and salvianolic acid B (b) in plasma samples with two channels of MRM and their MS full scans.

full-scan sensitivity than previously achievable on a tandem quadrupole MS instrument. The fragmentations of tanshinone IIA, salvianolic acid B and IS were clearly investigated by infusing individual standard solutions into the mass spectrometer, which used a fluidics system at a flow rate of 10 μL/min.

The MS/MS product ion spectra of tanshinone IIA, salvianolic acid B, and internal standard are shown in *Fig. 1*. The proposed product ions used for MRM were indicated. The most abundant product ions for tanshinone IIA were observed at *m/z* 249 and 191. The 295→249 transition showed nearly twice the sensitivity of transition 295→191 with significant reduction in background noise. For salvianolic acid B, product ions at *m/z* 519 and 321 were observed, and the *m/z* 717→519 transition showed better tracking than *m/z* 717→321 transition in terms of precision and accuracy. Considering all these factors, the transition 295→249 for tanshinone IIA and 717→519 for salvianolic acid B were selected for the quantitative UPLC-MS/MS analysis. *Fig. 2a–d* showed the MRM chromatograms for the quantification oftanshinone IIA at *m/z* 295→249 with IS at *m/z* 297→279 in positive mode, and salvianolic acid B at *m/z* 717→519 with IS at *m/z* 295→267 in negative mode. Confirmation was achieved by MS full scans *(Fig. 4a, b)*, which were switched from MRM to MS scan in both positive and negative modes. The MS spectra were compared with those of authentic tanshinone IIA and salvianolic acid B, which could be used to unambiguously identify the peaks in biological samples.

Method Validation

Selectivity, Linearity, and Sensitivity

Under the optimized UPLC-MRM-MS/MS conditions, tanshinone IIA, salvianolic acid B and IS were separated within 3 min. Their retention times were 1.43, 1.02, and 1.27 min, respectively. Due to high selectivity of the two channels of MRM detection, no interference was observed.

Calibration curves were linear over the concentration range of 10 pg/mL–1 ng/mL for tanshinone IIA and 100 pg/mL–1 μg/mL for salvianolic acid B, with the corresponding linear regression equations of $y = 68.4581x + 646.152$ ($n = 3$, the correlation coefficient (r) = 0.9991) and $y = 0.0685 \times -12.0393$ ($n = 3$, $r = 0.9972$), where y is the peak area ratio of the analyte to IS, and x is the concentration of the analyte.

The LLOQ for tanshinone IIA and salvianolic acid B in dog plasma were 10 and 100 pg/mL, respectively, which were sufficient for pharmacokinetic study of the two components in dogs. At the concentration levels, the precision and accuracy at LLOQ were 2.12% and 1.05%, respectively, for tanshinone IIA, and 5.45% and 3.98%, respectively, for salvianolic acid B. Therefore, it is sensitive enough for the investigation of pharmacokinetic profiles of tanshinone IIA and salvianolic acid B in young beagle dogs.

Precision and Accuracy

Table II summarizes the precision and accuracy of the method from assaying the QC samples in six consecutive batches for tanshinone IIA and salvianolic acid B. The intra- and inter-day precisions were measured to be within 6.79% and 8.21%, and the accuracy was within 4.75% and 4.88%, indicating acceptable accuracy and precision of the proposed method.

Table II. Intra-day and inter-day precision, recovery and matrix effect of tanshinone IIA and salvianolic acid B in dog plasma

Compounds	Concentration added (pg/mL)	Intra-day (n = 6)			Inter-day (n = 6)			Recovery (n = 5)	Matrix effect
		Measured concentration (mean ± SD, pg/mL)	Precision (RSD%)	Accuracy (RE%[a])	Measured concentration (mean ± SD, pg/mL)	Precision (RSD%)	Accuracy (RE%[a])	(mean ± SD %)	(%, n = 5)
Tanshinone IIA	30	30.01 ± 0.28	6.79	4.75	30.97 ± 0.61	7.88	−1.75	91.2 ± 7.8	96.8 ± 7.3
	300	300.41 ± 1.19	5.83	2.05	300.39 ± 1.30	6.87	1.95	89.1 ± 4.7	88.9 ± 8.6
	900	900.23 ± 1.23	2.01	2.02	900.35 ± 1.55	5.02	2.31	86.4 ± 10.1	92.3 ± 10.2
Salvianolic acid B	300	300.05 ± 1.52	3.48	2.57	300.05 ± 0.45	8.21	−2.96	96.2 ± 6.9	92.9 ± 10.8
	3000	3000.27 ± 1.04	5.39	4.01	3000.26 ± 1.73	5.79	4.88	85.9 ± 10.3	83.8 ± 12.7
	90, 000	90000.19 ± 2.31	4.76	3.82	90000.39 ± 2.08	7.95	3.64	87.0 ± 11.5	84.7 ± 9.3

[a]RE is expressed as [(mean measured concentration) / (spiked concentration) − 1]

Recovery and Matrix Effects

The recoveries of tanshinone IIA added to dog plasma at concentrations of 30, 300, and 900 pg/mL were determined to be 91.2 ± 7.8%, 89.1 ± 4.7%, and 86.4 ± 10.1%, while recoveries of salvianolic acid B at three QC levels were 96.2 ± 6.9%, 85.9 ± 10.3%, and 87.0 ± 11.5%. The recoveries of IS were 93.2 ± 8.7% and 86.3± 6.5% in positive and negative MRM mode, respectively, which met the requirements of analysis.

The matrix effect evaluated for tanshinone IIA was 96.8%, 88.9%, and 92.3% [with the relative standard deviation (RSD) % of 7.3%, 8.6%, and 10.2%] at the three QC concentration levels, along with the values of 92.9%, 83.8%, and 84.7% (with the RSD % of 10.8%, 12.7%, and 9.3%) for salvianolic acid B. In addition, the matrix effect for IS was 90.5% and 102.6% in positive and negative MRM modes. These results indicate that the endogenous sub-

stances in this sample matrix show no significant effects on quantification of both analytes and IS. The data are shown in *Table II*.

Stability

The stabilities of tanshinone IIA and salvianolic acid B in dog plasma under different conditions were evaluated *(Table III)*. No significant degradation was observed for 2 months at −20°C (long-term stability), for 4 h at ambient temperature (short-term stability) during three freeze–thaw cycles. Post-extraction samples kept in the autosampler at 4°C for 24 h were also stable.

Table III. Stabilities of tanshinone IIA and salvianolic acid B in dog plasma

Stability	Remaining of tanshinone IIA (%)			Remaining of salvianolic acid B (%)		
	30 pg/mL	300 pg/mL	900 pg/mL	300 pg/mL	3000 pg/mL	90,000 pg/mL
Long-term stability	102.4 ± 2.8	104.3 ± 3.9	98.9 ± 4.1	95.8 ± 3.7	97.9 ± 2.9	93.3 ± 5.2
Short-term stability	97.3 ± 4.6	94.5 ± 2.2	93.1 ± 3.7	102.7 ± 6.8	105.3 ± 5.2	98.5 ± 6.0
Freeze–thaw stability	102.3 ± 1.8	105.3 ± 5.9	101.4 ± 4.3	102.0 ± 3.9	97.6 ± 4.5	103.1 ± 5.8
Processed sample stability	102.7 ± 4.9	94.7 ± 5.2	101.8 ± 6.1	94.3 ± 6.0	101.8 ± 3.7	97.3 ± 4.3

Application in Pharmacokinetic Study

The developed UPLC-MRM-MS/MS method allowed a reliable and sensitive simultaneous quantification of tanshinone IIA and salvianolic acid B in dog plasma and was successfully applied for studying the pharmacokinetics of the two components from salvia tropolone tablets following a single oral gavage administration to dogs. The concentrations of tanshinone IIA and salvianolic acid B in dog plasma samples were quantified after oral gavage administration of salvia tropolone tablets (160 mg/kg). Six groups of dog plasma samples were assayed to construct concentration-time profiles of tanshinone IIA and salvianolic acid B, and the mean concentration-time profile is shown in *Fig. 3*. The representative chromatograms of tashinone IIA and salvianolic acid B in plasma samples with two channels of MRM, and their MS full scans are shown in *Fig. 4a–b*. The pharmacokinetic parameters of tanshinone IIA and salvianolic acid B in dogs are listed in *Table IV*. Though studies on the pharmacokinetics of tanshinone IIA or salvianolic acid B have been reported of rat plasma [19, 22, 25–26], rabbit plasma [24, 27], and dog plasma [29–30], there is no information reported on both of the

pharmacokinetics of tanshinone IIA and salvianolic acid B in dog plasma. Although the main data for tanshinone IIA and salvianolic acid B were statistically consistent with those in these paper, there are discrepancies in the pharmacokinetic data, possibly because of drug formulation differences or animal species differences. The results indicate that the plasma drug concentration-time data of tanshinone IIA and salvianolic acid B are both best fitted to a two compartment open model, and some differences are found in the distribution and elimination between tanshinone IIA and salvianolic acid B. The plasma concentration of the two components declined rapidly after 2 h of oral administration, and after 4 h, the concentrations declined slowly. Moreover, salvianolic acid B was eliminated significantly slower than tanshinone IIA, which favors quick and long-term efficacy.

Table IV. Estimated pharmacokinetic parameters of tanshinone IIA and salvianolic acid B in dog plasma after intravenous administration of danshen extract

	tanshinone IIA	salvianolic acid B
$t_{1/2в}$	3.961 ± 1.225	5.654 ± 4.22
Vl/F (L/kg)	28.13 ±3.256	37.28 ± 2.846
Cl/F (L/h/kg)	12.59 ± 2.596	17.75 ± 2.439
$AUC_{(0-\infty)}$ (μg/L/h)	12712.61 ± 8.883	9886.52 ± 7.33
K_{10} (1/h)	0.447 ± 0.058	0.323 ± 0.167
K_{12}(1/h)	0.198 ± 0.046	0.213 ± 0.05987
K_{21} (1/h)	0.611 ± 0.456	0.292 ± 0.039
C_{max} (μg/L)	1719.33 ± 6.789	2571.44 ± 7.779

$t_{1/2в}$: elimination half-life in a two-compartment model; Vl/F: apparent volume of distribution; Cl/F: clearance; $AUC_{(0-\infty)}$: the area under the plasma concentration–time curve from 0 h to infinity; K_{10}: elimination of the apparent first-order rate constant from the central chamber; K_{12}: the rate constant from the central compartment to the periphery compartment; K_{21}: the rate constant from the peripheral compartment to the central compartment; C_{max}: maximum plasma concentration.

Conclusions

A new analytical method to simultaneously perform quantitative and qualitative analysis of trace amount of the water-soluble component and lipid-soluble component in dog plasma with a TQ-S system was developed. This was achieved by combining two channels of MRM acquisitions in two modes and the MS full scans within the same UPLC run. The whole method

was proven to be rapid, sensitive, and selective. It validates a reliable quantification of two active constituents, tanshinone IIA and salvianolic acid B in dog plasma, and the pharmacokinetic study of the two active components of salvia tropolone tablets following oral gavage administration would be a suitable reference in clinical application. The proposed method also has the potential to be utilized as the ideal platform for simultaneously acquiring useful qualitative and quantitative information from complex matrices in the field of pharmacokinetics.

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