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Article

Development and Validation of UHPLC-MS/MS for Simultaneous Determination of Scutellarin and Salicylic Acid in Rat Plasma: Application to Herb-Drug Pharmacokinetic Interaction Study

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Dengzhanxixin injection (DI) product has been clinically proven to be effective for treating cardio-cerebrovascular disease. Furthermore, herb-drug interactions between the DI product and drugs that are commonly co-administered, such as aspirin, must be explored to ensure safe clinical use. A reliable ultra-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method was developed and validated for simultaneous determination of scutellarin and salicylic acid (SA) in rat plasma. The proposed method has been successfully applied to pharmacokinetic studies on analytes in rats after i.g. administration of aspirin alone, i.v. administration of DI alone or a combination of both (i.g.: 10 mg kg^{-1} aspirin, i.v.: 4 mL kg^{-1} DI). Both SA and scutellarin exhibited a two-compartment model pharmacokinetic profile. Aspirin did not change the concentration-time course for scutellarin, but DI decreased the peak plasma concentration and the area under the SA curve. This study indicates that co-administration of aspirin and DI can cause an apparent herb-drug pharmacokinetic interaction in rats.

Keywords: dengzhanxixin injection, scutellarin, aspirin, salicylic acid, pharmacokinetics, UHPLC-MS/MS

Introduction

Despite significant progress in preventing and treating cardio-cerebrovascular disease (CVD), it remains one of the leading causes of world-wide mortality. To treat CVD, traditional Chinese medicine (TCM) has been used in patients for many years. Dengzhanxixin injection (DI) is a well-known TCM preparation and has been commonly used in clinical practice; it is extracted from the Chinese herb *Erigeron breviscapus* (Vant.) Hand.-Mazz.[1] It is mainly used to treat cerebral infarction and sequelae, cerebral thrombus, coronary heart disease and angina pectoris.[2]

Scutellarin is the major active component in DI with various biological activities, such as antihypertensive, antiischemic and antiarrhythmic effects.[3-7] The potential scutellarin anticoagulation mechanism may include blocking the tissue-type plasminogen activator, epoprostenol and thrombomodulin.[8]

Aspirin is also referred to as acetylsalicylic acid and is currently often used for primary prevention of CVD.[9] After aspirin administration, it is rapidly hydrolyzed in the body to produce salicylic acid (SA), which is the major aspirin metabolite.[10-12]

Many TCM hospital prescriptions for treating certain CVDs contain DI and aspirin.[13,14] To our knowledge, no publication has attempted to simultaneously detect scutellarin and SA or study the pharmacokinetic interplay between DI and aspirin *in vivo*. Therefore, the rationality of combining the applications and the pharmacokinetic interaction between the two drugs must be investigated.

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Experimental

Reagents and materials

SA (purity > 99.9%), scutellarin (purity > 91.7%) and tinidazole (internal standard (IS), purity > 100.0%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products. The structures of the analytes are shown in Figure 1. DI (10 mL vial^{-1}, lot No. 20120740, containing 504.4 µg mL^{-1} scutellarin and expired before May 2015) was manufactured by the Yunnan Biovalley Dengzhanhua Pharmaceutical Group (Yunnan, China). Aspirin enteric-coated tablets (100 mg tablet^{-1}, lot No. 120502 and expired before April 2015) were manufactured by Shenyang Original Pharmaceutical Group (Shenyang, China). Methanol and acetonitrile (Merck, USA) were ultra-performance liquid chromatography (UHPLC) grade. The additional reagents were analytical reagent (AR) grade. The ultrapure water used for ultra-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) was from a Milli-Q water purification system (Millipore, USA).

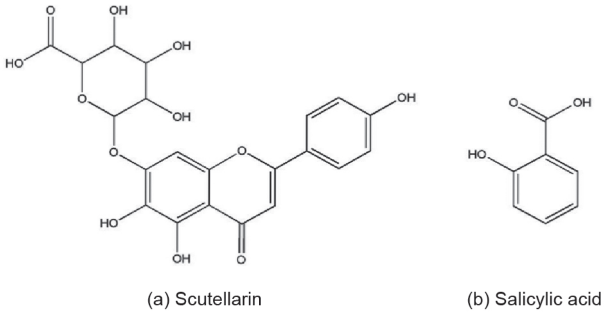
(a) Scutellarin (b) Salicylic acid

Figure 1. Chemical structures of (a) scutellarin and (b) SA.

Standard solution and plasma sample preparation

The standard stock solutions were prepared by dissolving SA (1024 µg mL^{-1}) and scutellarin (960 µg mL^{-1}) in methanol. Working solutions were prepared from the stock solutions through diluting in methanol until use. All working solutions were stored at 4 °C. An IS solution was diluted to the final concentration 1.20 µg mL^{-1} using methanol. The calibration standards and quality control (QC) samples were prepared by spiking blank plasma with combined working solutions. The following calibration standards were generated: 80, 160, 320, 640, 1280, 2560, 5120 ng mL^{-1} for SA and 15, 30, 60, 120, 240, 480, 960 ng mL^{-1} for scutellarin. QC samples were prepared at 160, 640, 2560 ng mL^{-1} for SA as well as 30, 120, 480 ng mL^{-1} for scutellarin. All the standard calibration samples and QC samples were stored at –20 °C.

UHPLC-MS/MS instrumentation and conditions

Analyses were performed with Agilent UHPLC 1290 system (Agilent, USA) consisting of a quaternary pump, an autosampler and an online degasser. The samples were chromatographically separated using an ACQUITY UPLC BEH C18 column (2.1 × 50 mm, 1.7 µm, 130 Å). The mobile phase consisted of methanol-2 mmol L^{-1} ammonium acetate (65:35; v/v) at the flow rate 0.2 mL min^{-1}. The autosampler temperature was maintained at 4 °C, and the injection volume was 5 µL. The total UHPLC run time was 2 min and the column temperature was maintained at 35 °C.

The analytes and IS were detected using an API 4000 tandem quadrupole mass spectrometer (Applied Biosystems, USA) with an electrospray ionization (ESI) interface in negative ion mode. Multiple reaction monitoring (MRM) was used to monitor precursor to product ion transition of *m/z* 137.0→93.0 for SA, *m/z* 461.1→285.0 for scutellarin and *m/z* 246.0→125.8 for IS. The product ion MS are shown in Figure 2. The analytical data were processed using Analyst software.[15]

For the analytes and IS, the source parameters were ion spray voltage, 4000 V; turbo heater temperature, 500 °C; collision activation dissociation, 6 psi; and curtain gas, 20 psi. The compound-dependent parameters, such as declustering potential and collision energy were optimized at 90 and 25 V for SA, 90 and 28 V for scutellarin and

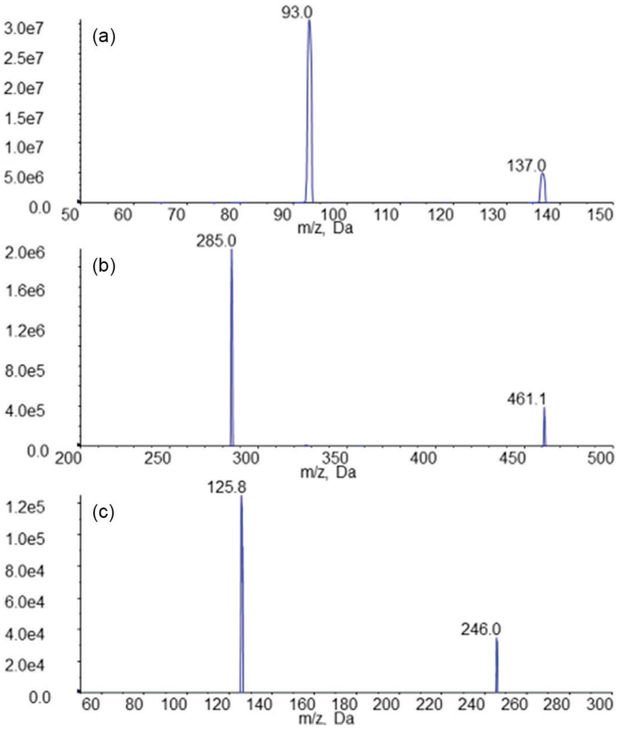

Figure 2. Product ion spectra of $[M - H]^-$ of (a) SA; (b) scutellarin and (c) IS.

25 and 15 V for IS, respectively. Quadrupole 1 and quadrupole 3 were maintained at unit resolution. The dwell time was 200 ms for both the analytes and IS.

Sample extraction procedure

An aliquot of 50 µL plasma, 50 µL 1 mol L^{-1} hydrochloric acid and 10 µL IS (1.20 µg mL^{-1}) were added to a 1.5 mL centrifuge tube. After vortex-mixing for 1 min, 800 µL ethyl acetate was added. Next, the tube was vigorously vortexed for 3 min followed by centrifugation for 10 min at 12000 rpm (13800 × g). 750 µL of supernatant was transferred into another tube and evaporated to dryness at 40 ºC under a gentle nitrogen stream. The residue was reconstituted with 100 µL mobile phase and vortexed for 1 min; next, it was centrifuged for 5 min at 12000 rpm. 5 µL supernatant was injected onto the UHPLC-MS/MS system for analysis.

Method validation

The method was validated for selectivity, sensitivity, carryover, matrix effect (ME), linearity, accuracy and precision, recovery, stability and dilution integrity.[16]

Specificity was assessed by comparing the total ion chromatography (TIC) of 6 blank plasma samples with the TIC of plasma samples spiked with scutellarin, SA and IS to detect potential interferences in the analyte and IS retention times. Further, 5 µL working solution was prepared in the mobile phase and injected to detect interference.

Carryover was evaluated by three injections of an upper limit of quantification (ULOQ) sample of the calibration curve, immediately followed by three injections of a blank plasma sample. Carryover was considered acceptable if the mean peak area counts of analytes and IS were not more than 20% for analytes, and 5% for IS, compared to the area counts in the lowest limit of quantification (LLOQ) sample.

ME was defined as the ion suppression/enhancement of the analyte ionization, which was evaluated by comparing the area response of the post-extraction blank plasma samples spiked with two analytes at three QC levels (A) to equivalent concentration standard solutions that were dried directly and reconstituted with the same mobile phase (B). The ratio (A/B × 100)% was used to evaluate the matrix effect. The same procedure was performed for the IS.

LLOQ was defined as the quantity that could be detected with a signal-to-noise ratio of 10 and was determined using five replicates with a precision less than 20% using the relative standard deviation (RSD) and accuracy between 80 and 120% for the spiked concentration.

The linearity for each analyte was assessed by analyzing the calibration curves from seven non-zero calibration sample concentrations in duplicate for five separate runs. Blank plasma samples were analyzed to discern interferences. The calibration curves were plotted using a weighted least squares linear regression method (weighting factor = $1/x^2$) through measuring the peak area ratio of the scutellarin or SA to the IS.

The scutellarin and SA recoveries were determined at three different concentration levels by comparing the extracted sample peak areas (spiked before extraction) with the standard solutions peak areas at the same concentration.

The intra-batch precision and accuracy were evaluated using five replicate analyses for scutellarin and SA at three QC levels on the same analytical run. The inter-batch precision and accuracy were calculated after repeated analysis in three different analytical runs. The concentrations were calculated using the calibration curve. The accuracy and precision were calculated and expressed as the % bias and relative standard deviation (% RSD), respectively.

The stabilities of scutellarin and SA in rat plasma were determined in triplicate through different storage conditions in the following ways: (*i*) stability of scutellarin and SA in rat plasma during sample preparation was assessed by detecting samples after storage for 6 h at room temperature; (*ii*) for freeze-thaw stability, the plasma samples were determined through three freeze (–20 °C)-thaw (room temperature) cycles; (*iii*) to evaluate the stability of the treated plasma samples in the auto-sampler, QC samples were prepared and placed in the auto-sampler at 4 °C for a period of 24 h, and then injected for analysis; (*iv*) the long-term stability was performed by assaying the plasma at –20 °C.

To confirm the ability to measure the diluted samples from beyond the ULOQ to within the calibration concentration range, plasma samples (n = 6) containing 12.8 and 2.4 µg mL^{-1} SA and scutellarin, respectively, were diluted to 640 and 120 ng mL^{-1} (20-fold) with blank plasma, measured and subsequently compared with nominal concentration.[17]

Pharmacokinetic study

The preclinical pharmacokinetic study was based on 18 Sprague-Dawley rats (male, weighting 300 ± 20 g, purchased from Experimental Animal Center of Nanjing University of Traditional Chinese Medicine, Jiangsu Province, license No. SCXK-2010-0033). The investigation was approved by the Animal Ethics Committee of the Nanjing University of Traditional Chinese Medicine with No. 13011. The animals were pathogen-free and acclimated for at least a week in their environmentally controlled quarters (24 ± 1 °C and 12/12 h light/dark cycle) with free access to standard chow and water. The rats were randomly divided into 3 groups with 6 rats *per* group: group 1 (intragastrical (i.g.) administration of aspirin at 10 mg kg^{-1}, single-i.g. aspirin); group 2 (intravenous (i.v.) administration of DI at 4 mL kg^{-1}, single-i.v. DI); group 3 (i.g. administration of aspirin at 10 mg kg^{-1} and i.v. administration of DI at 4 mL kg^{-1}, unite-i.g./i.v. aspirin and DI). Diet was prohibited for 12 h before the experiment, but water was taken freely. Blood samples of approximately 150 µL were collected into heparinized centrifuge tubes from the fossa orbitalis vein 0, 0.083, 0.167, 0.5, 0.75, 1, 2, 3, 4, 6, 10, 14 h after drug administration. Following centrifugation (4000 rpm for 10 min), the separated plasma samples were stored at −20 °C until analysis.

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated using Drug and Statistic (DAS) 3.0 pharmacokinetic software.[18] The pharmacokinetic parameters include compartment and statistic parameters.

The area under the plasma concentration-time curve (AUC) was calculated using the linear trapezoidal rule. The elimination rate constant (kel) was calculated using linear regression for the terminal points in the semi-log plot of plasma concentration *versus* time. The half-life of drug elimination during the terminal phase ($t_{1/2}$) was calculated using the formula $t_{1/2} = 0.693/\text{kel}$. The mean residence time (MRT) was calculated as AUMC/AUC; AUMC is the area under the first moment plasma concentration-time curve. The volume of distribution (Vd) for the central compartment was calculated as the dose/C_0; C_0 is the concentration measured immediately after administration. Plasma clearance (CL) was calculated as the dose/AUC.

The data are presented as mean ± SD. The pharmacokinetic data were compared through variance (Student's *t*-test), and statistically significant differences were $p < 0.05$ (SPSS statistical software package).[19]

Results and Discussion

Selectivity and sensitivity (LLOQ)

The liquid-liquid extraction methodology with mass spectrometry yielded good selectivity for the analytes and IS. Figure 3 shows the total ion chromatography (TIC) for the spiked plasma with two analytes (600 and 3200 ng mL^{-1} for scutellarin and SA, respectively) and IS (1.20 µg mL^{-1}) as well as the TIC for the group 3 plasma after a single

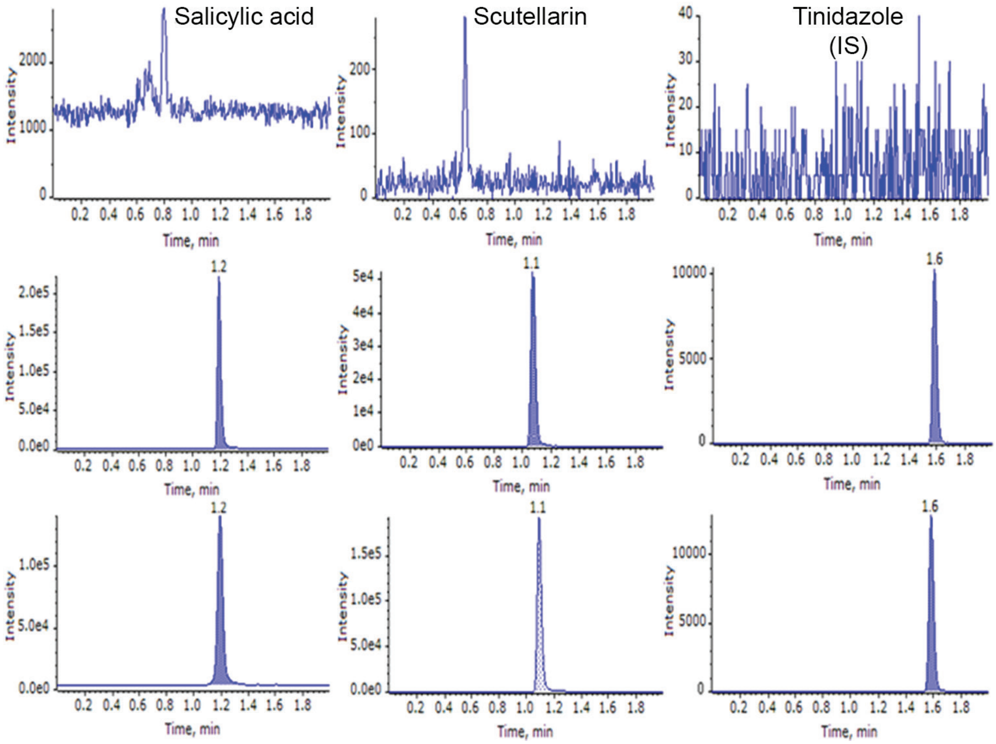

Figure 3. Chromatograms of salicylic acid (SA), scutellarin and tinidazole. Top, a blank rat plasma sample; middle, a blank plasma spiked with SA (3200 ng mL^{-1}), scutellarin (600 ng mL^{-1}) and IS (1.20 µg mL^{-1}); bottom, a rat plasma sample of group 3 after single dose at intervals of 2 h.

dose at 2-h intervals. The retention time was 1.1, 1.2 and 1.6 min for scutellarin, SA and IS, respectively. Endogenous interference was not detected in blank plasma samples at the retention time for the two analytes and IS.

In this case, the scutellarin and SA LLOQs were 15 and 80 ng mL^{-1}, respectively.

Carryover

No peak was observed at the retention times of analytes or IS in the chromatogram of a blank sample analyzed after the injection of ULOQ sample, indicating the absence of carryover.

Linearity and matrix effect

For SA, the linear regression equation for the mean of five calibration curves was $y = (1.2923 \pm 0.0089)x - (0.4981 \pm 0.0042)$, regression coefficient (r) = 0.9975. For scutellarin, the equation for the mean of five calibration curves was $y = (0.5368 \pm 0.0043)x - (0.1068 \pm 0.0046)$, r = 0.9985.

The ME represents the ion suppression in this method. The ME was consistent throughout the lots and did not affect quantitative analysis of the analyte and IS peaks. The results are shown in Table 1.

Table 1. Matrix effect of scutellarin, SA and tinidazole (IS)

Matrix	Concentration / (ng mL^{-1})	ME / %
Scutellarin (n = 3)	30	101.34 ± 7.28
	120	103.61 ± 5.83
	480	98.92 ± 6.99
SA (n = 3)	160	98.51 ± 5.78
	640	97.91 ± 4.14
	2560	97.85 ± 2.61
Tinidazole (n = 9)	120	83.22 ± 3.12

ME: matrix effect; SA: salicylic acid.

Recovery

The SA and scutellarin recoveries are shown in Table 2. The recoveries for the two analytes and IS were promoted by acidifying the plasma samples with hydrochloric acid; thus, 50 µL 1 mol L^{-1} hydrochloric acid was added to the plasma samples before extraction. The recoveries were compared after using different organic solvents as the extractant. Because the two analyte polarities varied, ethyl acetate was the extractant used to generate suitable recoveries for each analyte.

Table 2. Recoveries of scutellarin, SA and IS from rat plasma using ethyl acetate as extracting solvent

	Concentration / (ng mL^{-1})	Recovery (n = 5)
Scutellarin	30	97.24 ± 5.88
	120	99.01 ± 2.82
	480	98.12 ± 6.23
SA	160	88.54 ± 4.13
	640	90.62 ± 3.21
	2560	92.32 ± 3.71
Tinidazole	120	78.22 ± 3.67

SA: salicylic acid.

Accuracy and precision

An accuracy between 85 and 115% and a precision less than 15% were acceptable. The intra-batch and inter-batch precisions and accuracies for the rat plasma scutellarin and SA analyses are shown in Table 3; these data show that the UHPLC-MS/MS assay is excellent for quantitatively analyzing the two rat plasma analytes simultaneously.

Stability

Results of the stability (n = 3) are summarized in Table 4. Both scutellarin and SA were found to be stable in the plasma samples through three freeze-thaw cycles, and after being stored at –20 ºC for 20 days or being stored at room temperature for 6 h. No significant degradation was observed in concentration of scutellarin or SA when extracted plasma samples were kept at 4 ºC in the auto-sampler for up to 24 h.

Dilution integrity

The 1:20-diluted samples were analyzed, and the results demonstrated precision was estimated to be below 8.52%, and the accuracy was within ± 7.68%, both were no more than 15%. These results indicated that plasma samples whose concentration exceeded the ULOQ could be diluted to calibration range and reanalyzed.

Application

Plasma concentration-time courses for SA and scutellarin are shown in Figures 4a and 4b, respectively. The SA and scutellarin pharmacokinetics both fit best to a two-compartment open model. The SA and scutellarin pharmacokinetic parameters are summarized in Tables 5 and 6. The aspirin did not produce a statistically

Table 3. Intra- and inter-day accuracy and precision of scutellarin and SA assay in rat plasma

	Concentration / (ng mL^{-1})	Intra-day (overall mean, n = 5)			Inter-day (overall mean, n = 15)		
		Concentration found / (ng mL^{-1})	Accuracy / %	RSD / %	Concentration found / (ng mL^{-1})	Accuracy / %	RSD / %
Scutellarin	30	28.95	96.50	7.19	31.26	104.20	9.57
	120	119.72	99.77	4.25	118.78	98.98	5.32
	480	482.27	100.47	5.56	484.52	100.94	4.21
SA	160	158.28	98.93	5.41	162.63	101.64	7.36
	640	643.12	100.49	3.87	658.29	102.86	5.87
	2560	2568.26	100.32	5.76	2589.29	101.14	8.42

RSD: relative standard deviation; SA: salicylic acid.

Table 4. Stability of scutellarin and SA in rat plasma under different storage conditions (mean ± SD, n = 3)

Storage condition	Scutellarin			SA		
	Added / (ng mL^{-1})	Measured / (ng mL^{-1})	Deviation / %	Added / (ng mL^{-1})	Measured / (ng mL^{-1})	Deviation / %
Short-term stability	30	31.25 ± 1.03	4.17	160	150.65 ± 10.23	–5.84
	120	126.38 ± 6.79	5.32	640	662.72 ± 24.15	3.55
	480	461.47 ± 16.35	–3.86	2560	2689.21 ± 103.45	5.05
Three freeze-thaw cycles	30	32.13 ± 2.01	–7.1	160	148.44 ± 9.88	–7.23
	120	127.55 ± 7.71	6.29	640	672.65 ± 25.43	5.1
	480	472.71 ± 18.47	–1.52	2560	2705.43 ± 110.28	5.68
At 4 ºC in the auto-sampler for 24 h	30	32.04 ± 1.24	6.8	160	154.43 ± 8.97	–3.48
	120	127.16 ± 7.02	5.97	640	659.32 ± 23.39	3.02
	480	470.76 ± 15.95	–1.93	2560	2589.62 ± 108.55	1.16
Long-term stability	30	31.53 ± 1.56	5.1	160	150.22 ± 10.45	–6.11
	120	113.75 ± 6.45	–5.21	640	679.68 ± 25.65	6.2
	480	492.51 ± 17.36	2.61	2560	2743.15 ± 120.04	7.15

SA: salicylic acid.

Table 5. Pharmacokinetic parameters of SA after single administration of aspirin or in combination with DI in rats (n = 6)

Parameter	Group 1 (aspirin)	Group 3 (aspirin + DI)
V/F / (L kg^{-1})	0.252 ± 0.013	0.214 ± 0.115
$t_{1/2\beta}$ / h	4.368 ± 0.329	2.775 ± 0.619^a
CL/F / (L h kg^{-1})	0.040 ± 0.004	0.052 ± 0.017
T_{max} / h	0.75 ± 0	3 ± 0^a
C_{max} / (mg L^{-1})	40.509 ± 4.074	30.528 ± 1.487^a
AUC_{0-14} / (h mg L^{-1})	253.337 ± 11.816	191.749 ± 26.106^a
$AUC_{0-\infty}$ / (h mg L^{-1})	272.806 ± 5.915	199.179 ± 29.050^a
MRT_{0-14} / h	5.068 ± 0.287	5.377 ± 0.596
$MRT_{0-\infty}$ / h	6.113 ± 0.547	6.282 ± 1.157

^{a}Significantly different from group 1, $p < 0.05$. V: volume of distribution; F: fraction of the administered dose systemically available; $t_{1/2\beta}$: half-life of drug elimination during the terminal phase; CL: plasma clearance; T_{max}: time to reach C_{max}; C_{max}: maximum plasma concentration; $AUC_{0-\infty}$: area under the plasma concentration-time curve from zero to infinity; MRT: mean residence time.

significant effect on the scutellarin concentration-time profiles after a single dose; however, aspirin and DI co-administration yielded significant differences in the SA C_{max}, T_{max}, $AUC_{0-\infty}$ and $t_{1/2\beta}$, where C_{max} is the maximum plasma concentration; T_{max} is the time to reach C_{max}; $AUC_{0-\infty}$ is the area under the plasma concentration-time curve from zero to infinity; $t_{1/2\beta}$ is the half-life of drug elimination during the terminal phase. Compared with group 1, the SA C_{max}, $AUC_{0-\infty}$ and $t_{1/2\beta}$ in group 3 exhibited more decline. The SA T_{max} in group 3 was markedly greater than in group 1. Although the SA CL/F (F is the fraction of the administered dose systemically available) differed slightly between group 1 and group 3, we did not observe a significant difference. Based on the above results, we conclude that aspirin and DI exhibit an apparent herb-drug pharmacokinetic interaction, and the aspirin and DI combination noticeably altered SA absorption, distribution and disposition. The previous study showed that long-term administration

Table 6. Pharmacokinetic parameters of scutellarin after single administration of DI or in combination with aspirin in rats (n = 6)

Parameter	Group 2 (DI)	Group 3 (aspirin + DI)
V/F / (L kg^{-1})	10.920 ± 2.973	6.822 ± 0.175
$t_{1/2\beta}$ / h	4.458 ± 0.474	3.908 ± 0.513
CL/F / (L h kg^{-1})	0.726 ± 0.093	0.632 ± 0.042
C_{max} / (mg L^{-1})	22.736 ± 4.636	28.260 ± 1.326
$AUC_{0\text{-}14}$ / (h mg L^{-1})	12.923 ± 1.856	15.071 ± 0.771
$AUC_{0\text{-}\infty}$ / (h mg L^{-1})	16.312 ± 2.942	16.300 ± 0.634
$MRT_{0\text{-}14}$ / h	2.875 ± 0.490	2.429 ± 0.055
$MRT_{0\text{-}\infty}$ / h	9.873 ± 6.462	3.997 ± 0.571

V: volume of distribution; F: fraction of the administered dose systemically available; $t_{1/2\beta}$: half-life of drug elimination during the terminal phase; CL: plasma clearance; C_{max}: maximum plasma concentration; $AUC_{0\text{-}\infty}$: area under the plasma concentration-time curve from zero to infinity; MRT: mean residence time.

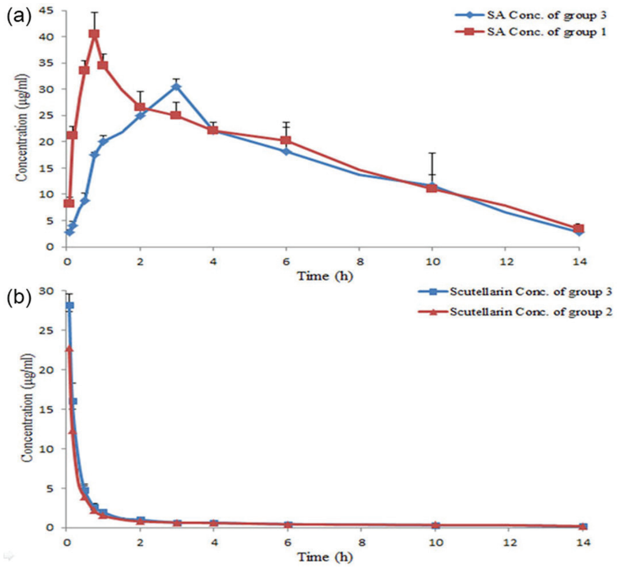

Figure 4. Mean plasma concentration of SA (a) or scutellarin (b) in rats after a single dose. (a) Plasma concentration-time SA profiles following i.g./i.v. administration of either aspirin alone or with DI; (b) plasma concentration-time scutellarin profiles following i.g./i.v. administration of either DI alone or with aspirin.

of aspirin may lead to SA accumulation *in vivo*.[20,21] Meanwhile, high concentration of SA has the certain toxic and side effect.[22,23] Therefore, combination of aspirin and DI decreased the plasma concentration of SA, which may contribute to reduce the toxic and side effect of SA.

Conclusions

A sensitive UHPLC-MS/MS method was successfully used to characterize the aspirin and DI herb-drug pharmacokinetic interaction in rats. The advantages of the method presented in this paper are the simple sample preparation and the simultaneous determination of both analytes within a short run time, which makes high throughput of sample analysis available. The small starting volume of plasma (50 μL) is especially important because of low blood volume available in a single rat for routine pharmacokinetic sample. In addition, this UHPLC-MS/MS with a simple liquid extraction can ultimately be applied to other preclinical and clinical biosamples (plasma, urine, tissue, etc.) for future pharmacokinetic studies.

Aspirin and DI co-administration appreciably decreased the SA C_{max}, $AUC_{0\text{-}\infty}$ and $t_{1/2\beta}$, and the T_{max} parameters increased significantly. However, the scutellarin pharmacokinetic parameters were not significantly affected by aspirin. Our animal study indicates that aspirin and DI co-administration can produce significant pharmacokinetic herb-drug interactions in rats.

Acknowledgments

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