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Quantitative determination and pharmacokinetic study of solamargine in rat plasma by liquid chromatography–mass spectrometry

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ABSTRACT

A sensitive and simple liquid chromatography–mass spectrometry (LC–MS) method has been developed and validated for the quantification of solamargine, a steroidal glycoalkaloid, in rat plasma. Vincristine was selected as the internal standard. Sample preparation involved simple liquid–liquid extraction by ethyl acetate with high efficiency. The chromatographical separation was performed on a Shimadzu C_{18} column (150 mm × 2.0 mm, 5 μm) with a gradient elution of acetonitrile and 0.02% (v/v) formic acid. The elutes were detected under positive electrospray ionization (ESI) and the target analytes quantified by selected ion monitoring (SIM) mode. The method was sensitive with the lowest limit of quantitation (LLOQ) at 0.5 ng/mL in 50 μL of rat plasma. Good linearity (r^2 = 0.9996) was obtained covering the concentration of 0.5–2000.0 ng/mL. The intra- and inter-day assay precision ranged from 2.87 to 3.60% and 0.52 to 6.81%, respectively. In addition, the stability, extraction recovery and matrix effect involved in the method were also validated. The practical utility of the aforementioned method was successfully confirmed in the pharmacokinetic evaluation of solamargine in Sprague-Dawley rats after intravenous administration.

1. Introduction

Steroidal glycoalkaloids represent a class of naturally occurring compounds possessing a variety of biological activities. Solamargine (Fig. 1), a steroid alkaloid glycoside existing in at least 100 *Solanum* species [1], was originally found to be toxic to herbivores and microbial pathogens [2]. Following pharmacological studies revealed that solamargine could exert inhibitory effects on tumor cells including human hepatoma cells [3], colon cancer cells [4] and lung cancer cells [5]. Importantly, combined anti-tumor therapy with solamargine could increase the susceptibility of lung cancer cells [6] and breast cancer cells [7] to chemotherapeutics and the synergistic action highlighted the unique anti-tumor efficacy of solamargine. Detailed study of the underlying molecular mechanisms has received widespread attention and yielded important discoveries [7–12]. In fact, Coramsine, a chemotherapeutic and immunomodulating agent with a 1:1 mixture of solasonine and solamargine, was demonstrated to exhibit strong anti-neoplastic activity in specific cancer cell lines, animals and humans, and its pre-clinical studies have been successfully completed in 2008 [http://en.wikipedia.org/wiki/Coramsine]. Taken together, these evidences strongly indicate that solamargine could become a promising candidate in the development pipeline of anti-tumor drugs.

For the further development of solamargine and better elucidation of its pharmacological efficacy, reliable analytical methodologies of solamargine in biological samples are warranted for characterizing its disposition and pharmacokinetic behaviors, thus providing essential information for drug-like assessment in the early stage of drug development. Currently, however, in contrast to the extensive pharmacological studies available, the quantitative method of solamargine is largely lacking. Due to the absence of a typical UV chromophore, detection of solamargine by a previously reported high-pressure liquid chromatography with UV detection (HPLC-UV) was compromised by poor sensitivity and repeatability [1]. A recently reported quantitative method for solamargine in plant, based on post chromatographic derivatization after separation through high-performance thin-layer chromatography (HPTLC), was feasible for the quality assessment of medicinal plants [13]. However, in light of the requirement for sensitive and accurate determination of solamargine in biosamples, this method could be compromised by its poor sensitivity. Liquid

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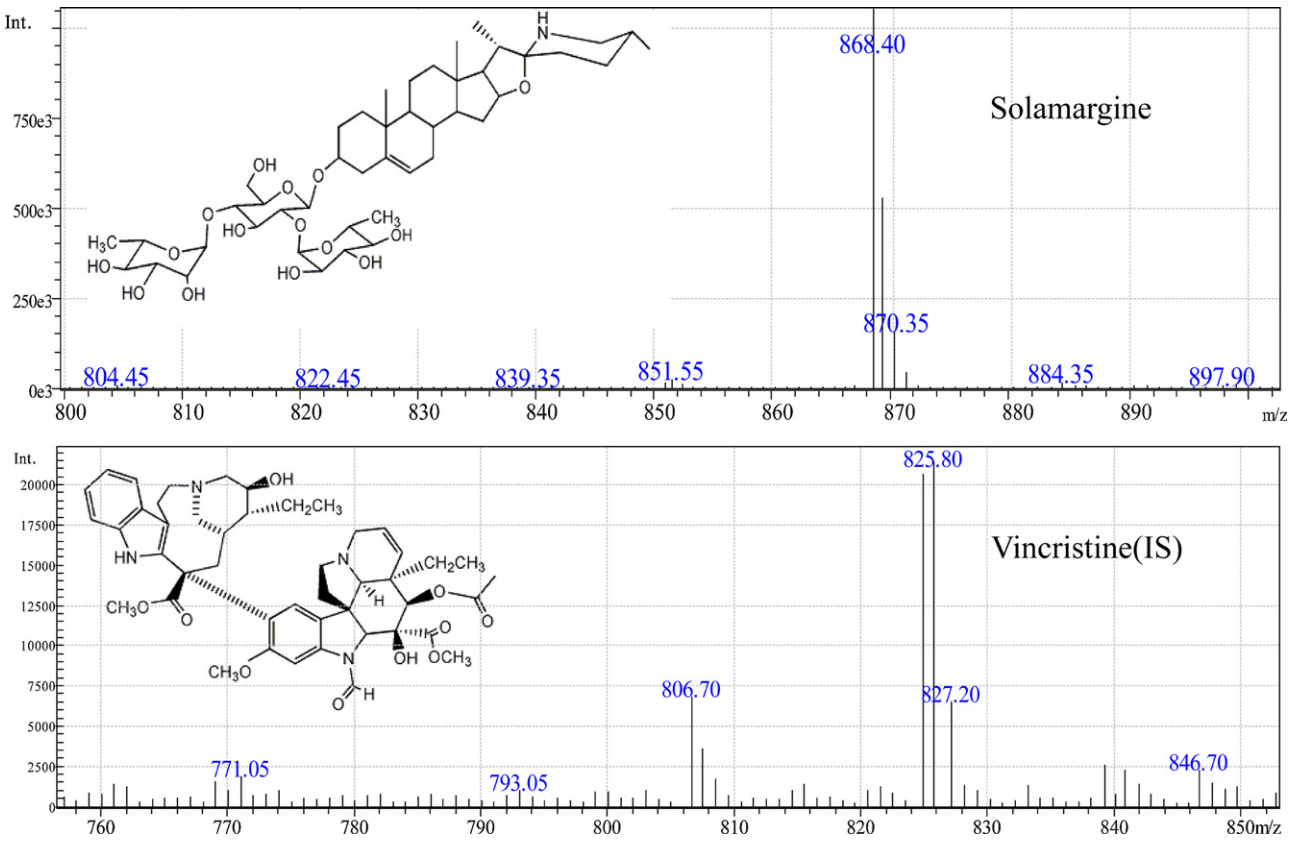

Fig. 1. Chemical structures and mass scan spectra for solamargine and vincristine (IS).

chromatography–mass spectrometry (LC–MS) proves a feasible alternative attributed to its good separation and detection capacity. Moreover, LC–MS based techniques have been extensively applied in the bioanalysis and pharmacokinetic studies of numerous drugs [14–16]. Notably, a LC–MS based method has been developed for the quantitative study of solamargine in the extract from a herbal medicine [17]. Nevertheless, it is conceivable that the sensitivity was far from the requirement of quantitative assay by biological samples. According to our knowledge, no LC–MS procedure for quantitative measurement of solamargine in biological samples has been reported in the literature at present. Herein, based on LC–MS, we aimed to develop a validated quantitative method for solamargine in rat plasma after a single-step liquid–liquid extraction (LLE) with ethyl acetate, and provided the first report on comprehensive pharmacokinetic parameters of solamargine in rats following intravenous administration. In the context of the extensive mechanistic research underway, this information holds promise for better elucidation and manipulation of the pharmacodynamic effects of solamargine in future studies.

2. Materials and methods

2.1. Chemicals and materials

Solamargine (purity > 95%) was supplied by Jiangsu Kanion Pharmaceutical Co., Ltd. (Jiangsu, China) and vincristine (internal standard) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC grade acetonitrile and methanol were purchased from Merck (Merck Company, Germany). Other chemicals were all of analytical grade. Ultrapure water was generated from the Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Instrumentation and chromatographic conditions

Liquid chromatography was performed on a Shimadzu LC-10AD HPLC system (Shimadzu, Kyoto, Japan) consisting of a LC-10AD pump, a DGU-14AM degasser, a SIL-HTc autosampler and a CTO-10 Avp column oven. The HPLC system was coupled to a Shimadzu LCMS-2010A quadrupole mass spectrometer by an electrospray ionization (ESI) interface. Data acquisition and processing were performed by Shimadzu LCMS Solution software (Version 2.04).

Chromatographic separation was carried out on a Shim-Pack VP-ODS C_{18} column (150 mm × 2.0 mm, 5 μm) with a gradient elution of the mobile phase system consisting of acetonitrile (A) and 0.02% formic acid (B). The elution progressed following the time program: 0.02–4.5 min, B% 15–70; 4.5–5.0 min, B% 70–15; 5.0–8.0 min, B% 15–15. The temperature of the column and auto-sampler was kept constant at 40 °C and 10 °C, respectively.

The tuning parameters for the ESI were set as follows: curved desolvation line (CDL) voltage 25.0 kV, probe voltage 4.5 kV, Q array voltage 60 V, RF 150 V, CDL temperature 250 °C, block temperature 200 °C. Spray gas and drying gas flow rate were set at 1.5 and 2.5 L/min, respectively. The $[M+H]^+$ ions of solamargine (m/z 868.4) and vincristine (m/z 825.8) were selected as ions for selected ion monitoring (SIM) as best sensitivity was found under the positive scanning mode (Fig. 1).

2.3. Preparation of stock and standard solutions

The stock solutions of solamargine and vincristine (IS) were separately prepared in methanol at concentrations of 1.0 mg/mL and 500.0 μg/mL. Immediately before use, serial dilution of the stock solution with methanol provided solamargine working solutions covering the concentration from

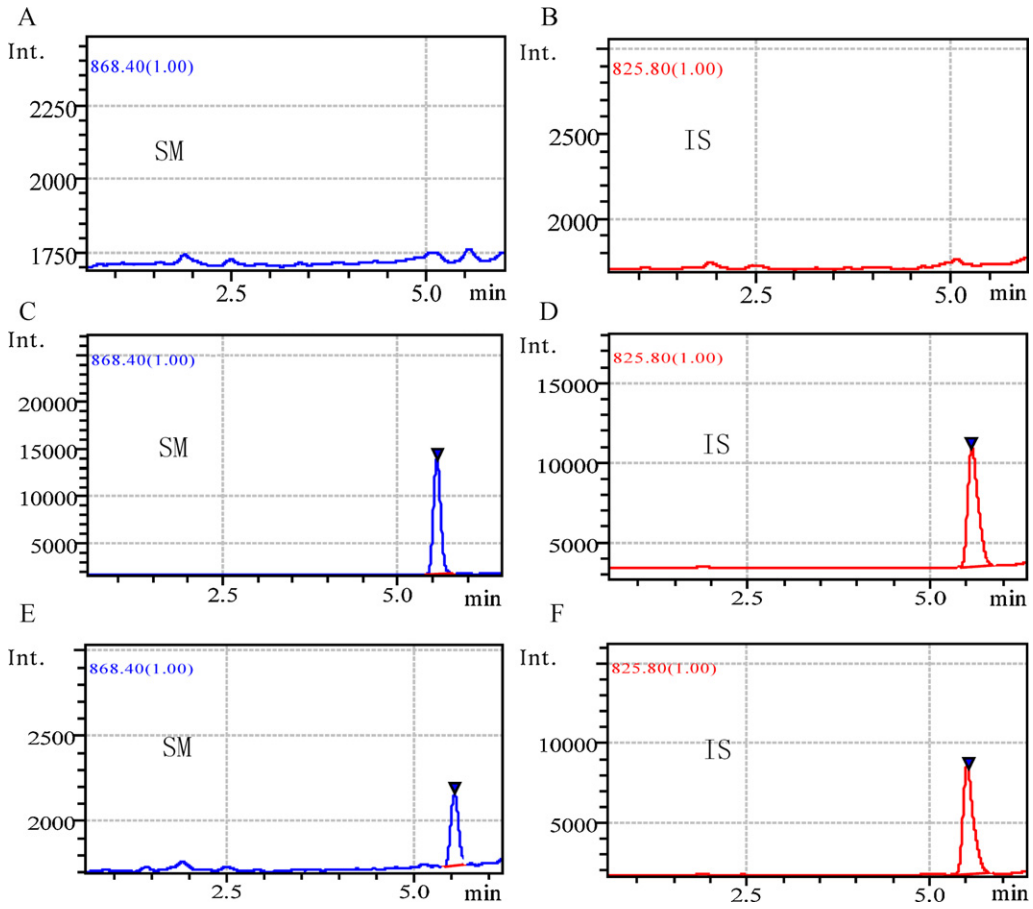

Fig. 2. Representative chromatograms of solamargine and vincristine in rat plasma: (A) blank plasma; (B) blank rat plasma spiked with solamargine (500.0 ng/mL) and vincristine (2.0 μg/mL); and (C) plasma at 10 min after intravenous administration of solamargine at 1.0 mg/kg. Retention time for solamargine and vincristine were 5.5 min and 5.7 min, respectively.

2.5 ng/mL to 10.0 μg/mL. The working solution of vincristine was diluted to 2.0 μg/mL. All the solutions were stored at 4 °C.

Five calibration samples were made by spiking 10 μL of solamargine and 10 μL of vincristine working solutions into 50 μL of blank rat plasma in an Eppendorf microcentrifuge polypropylene tube and the final concentrations of solamargine in the standard curve were at 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100.0, 200.0, 500.0, 1000.0 and 2000.0 ng/mL. As for the quality control (QC) sample preparation, 50 μL of blank rat plasma was individually spiked with 10 μL of solamargine and 10 μL of vincristine working solutions to generate theoretical concentrations of solamargine at low, medium and high concentrations: 1.0, 100.0 and 1000.0 ng/mL. The QC samples were frozen until analysis.

2.4. Sample preparation

Liquid–liquid extraction (LLE) by ethyl acetate was applied for the pretreatment of QC samples, calibration standards, and plasma samples. After thawing at room temperature, 50 μL of plasma sample was added with 10 μL aliquot of IS solution (2.0 μg/mL) and mixed. Then 1.0 mL of ethyl acetate was added and the mixture was vortexed for 5 min, followed by centrifugation at 6000 × *g* for 5 min. Then, 0.7 mL of the organic layer was transferred to another tube and evaporated to dryness in a rotary evaporator (Thermo Fisher Scientific, NJ, USA) at 45 °C. 200 μL of methanol was added to reconstitute the residue and 5.0 μL aliquot was injected for analysis.

2.5. Method validation

A thorough and complete method validation for determination of solamargine in rat plasma was carried out following the FDA guidelines [18]. The method was validated for specificity, sensitivity, matrix effect, recovery, linearity, precision, accuracy and stability.

2.5.1. Specificity

The specificity of the method was investigated by analyzing six different batches of drug-free rat plasma for the exclusion of any potential endogenous interference. They were compared to those plasma samples spiked with solamargine at known concentrations. All the plasma were pretreated and analyzed under the same procedure as described.

2.5.2. Matrix effect and recovery

The effects of rat plasma components on the ionization efficiency of solamargine and IS were evaluated by comparing the peak areas of the analytes spiked into blank extracted plasma with those of the counterparts prepared in methanol at three concentration levels. Extraction recovery tests were performed by making comparison between the peak areas of the sample prepared by plasma extraction and those of directly injected standards. The recovery of the IS was determined in a similar way.

2.5.3. Calibration curve

Calibration curves were prepared by spiking into blank plasma with working solutions to achieve final concentration at 0.5, 1.0, 2.0,

Table 1
Recovery and matrix effect of solamargine in rat plasma.

	Nominal concentration (ng/mL)	Solamargine	Vincristine (IS) (400 ng/mL)
Recovery, $n = 5$ (%)	1.0	95.1	81.7
	100.0	101.7	91.2
	1000.0	102.1	87.6
Matrix effect, $n = 5$	1.0	104.4	106.8
(%)	100.0	106.5	102.6
	1000.0	101.8	104.9

Table 2
Precision and accuracy validation results for solamargine determination ($n = 5$).

Concentration (ng/mL)	Intra-day		Inter-day	
	Precision (RSD%)	Accuracy (RE%)	Precision (RSD%)	Accuracy (RE%)
1.0	9.46	1.8	7.07	1.4
50.0	2.93	−3.1	2.24	−1.3
1800.0	1.86	0.4	2.21	0.2

5.0, 10.0, 20.0, 50.0, 100.0, 200.0, 500.0, 1000.0, and 2000.0 ng/mL. The peak area ratios of solamargine to IS were plotted with plasma concentrations to give the correlation coefficient and linear regression equation after weighed linear regression. Five calibration curves were performed in whole. The LLOQ was established as the lowest concentration of the calibration curve at which both the precision was within 20% and the accuracy was within 20% by means of the results from five replicates.

2.5.4. Precision and accuracy

Intra- and inter-day variations were chosen to validate the precision and accuracy of the assay by analyzing sample concentrations at 1.0, 50.0 and 1800.0 ng/mL. Five replicates were determined at each concentration level for the evaluation of intra-day variation, while five replicates at those three concentration levels determined over three consecutive days were analyzed to represent the inter-day variation. The precision of the method was expressed in terms of RSD%.

2.5.5. Stability

Spiked samples with solamargine at low, medium and high concentrations were used for stability validation under a variety of storage and handling conditions. Freeze–thaw stability was assessed over three freeze/thaw cycles. Short-term stability was determined by keeping the samples at room temperature for 6 h. Long-term stability was evaluated by analyzing samples stored at −20 °C for 30 days. Post-preparative stability was assessed by reanalyzing post-extraction samples kept in the autosampler at 10 °C for 24 h. The stability was acceptable when 85–115% of the initial analytes were found.

2.6. Application to a pharmacokinetic study in rats

Sprague-Dawley rats (male, weighing 200 ± 20 g) were obtained from the Laboratory Animal Center of Peking University Health Science Center (Beijing, China) and kept in an environmentally controlled breeding room for at least 3 days before experimentation. The rats were fasted overnight but with free access to water before the test. Animal welfare and experimental procedures were strictly in accordance with the guide for the care and use of laboratory animals (National Research Council of USA, 1996). After intravenously administering solamargine at 1.0, 2.0 and 4.0 mg/kg, 100 μL of heparinized blood samples were collected at 5, 10, 20, 40, 60, 120, 180, 240, 300, 360 and 480 min from the ophthalmic veins and immediately centrifuged at 2000 × g for 10 min. The supernatant plasma were collected and immediately frozen at −20 °C until analysis. The collected blood samples were pretreated with the aforementioned method (sample dilutions were made when necessary) and the concentrations of solamargine in each sample were finally determined based on the validated LC–ESI-MS method.

Pharmacokinetic parameters in rats were estimated by a compartmental method using DAS 2.0 software package (purchased from Wannan Medical College, China). The plasma concentration at 5 min ($C_{5\,min}$) was observed value with no interpolation. The area under concentration–time curve up to the last measured time point (AUC_{0-t}) was calculated by the trapezoidal rule. The AUC_{0-t} extrapolated to infinity ($AUC_{0-\infty}$) was generated by extrapolating the AUC_{0-t} to infinity using the elimination rate constant and the last measured concentration. The CL for intravenous dosing was estimated by dividing the administered dose by the $AUC_{0-\infty}$.

3. Results and discussion

3.1. Method development

A simple LLE method was utilized for extraction from plasma samples and, after comparison, ethyl acetate was chosen for its good extraction efficiency. Given the steroidal glycoalkaloid moiety in the molecular structure of solamargine, positive ESI mode was preferentially selected in the full scan spectrum. Addition of 0.02% formic acid to the mobile phase was found to facilitate optimum separation and enhance detection under the positive ionization mode. Under this condition, a predominant signal with m/z at 868.40 was observed and identified as $[M+H]^+$ for solamargine. In the determination of proper internal standard compound, we tested vincristine despite its structural difference to solamargine. Vincristine was finally selected as the internal standard due to its similar chromatographic behavior, ionization and extraction efficiency to solamargine under the same analytical condition. With a gradient curve, good separation and optimal detection response were achieved and the analytical process could be completed within 8.0 min.

3.2. Method validation

3.2.1. Specificity

Under the chromatographic conditions described above, the retention time for solamargine and vincristine were 5.5 min and 5.7 min, respectively. No apparent interferences were observed for solamargine or internal standard in blank rat plasma and rat plasma sample obtained after intravenous administration of solamargine (Fig. 2). These results supported the high specificity and selectivity of the method in the analysis of plasma samples.

Table 3
Stability of solamargine under different storage conditions ($n = 5$).

Storage conditions	Concentration (ng/mL)	Recovery (%)	RSD (%)
Three freeze–thaw cycles	1.25	97.9	6.0
	40.0	102.2	2.2
	800.0	100.0	1.8
Room temperature for 6 h	1.25	100.1	8.6
	40.0	102.0	1.5
	800.0	99.5	1.0
Freeze at −20 °C for 30 days	1.25	103.0	6.0
	40.0	103.3	1.7
	800.0	100.3	1.5
Autosampler (10 °C) for 24 h	1.25	100.5	3.5
	40.0	103.5	1.2
	800.0	100.3	2.0

3.2.2. Matrix effect and recovery

Determined at low, medium and high concentrations, no apparent ionization interference was found and the mean recovery rates were higher than 90%. The matrix effect and recovery values were summarized in Table 1 and proved to be satisfactory.

3.2.3. Linearity

The calibration curve showed good linearity over the range of 0.5–2000.0 ng/mL with correlation coefficient (r^2) exceeding 0.99. Over this concentration range, a typical calibration curve was $y = 2.300 \times 10^{-3}x + 8.000 \times 10^{-4}$ (weighed factor: $1/x$). The LLOQ for solamargine in rat plasma was 0.5 ng/mL as both the precision and accuracy were acceptable at this concentration (Fig. 3).

3.2.4. Precision and accuracy

Precision and accuracy were validated at three concentrations as shown in Table 2. The intra-day and inter-day variation, as well as the accuracy, were within the acceptable range, confirming that the current method was reproducible and accurate.

3.2.5. Stability

Stability of solamargine under various storage conditions was investigated at three different concentrations (Table 3). The results indicated that solamargine in the plasma was stable under the various conditions evaluated, thus the samples were stable during the whole process of analysis.

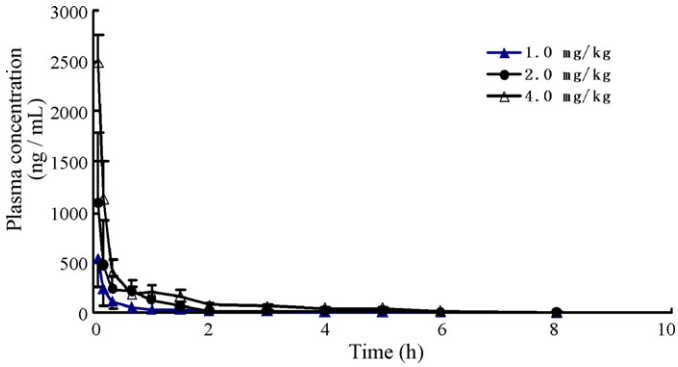

Fig. 4. Mean plasma concentration–time curve of solamargine after a single intravenous dose at 1.0, 2.0 and 4.0 mg/kg to rats (mean ± SD, $n = 5$).

3.3. Application to a pharmacokinetic study

The developed method was successfully applied to analyze rat plasma samples after intravenous injection of solamargine at 1.0, 2.0 and 4.0 mg/kg to rats, supporting its applicability to biosample assay. The mean plasma concentration–time profiles of solamargine after intravenous administration are shown in Fig. 4, and the pharmacokinetic parameters based on a compartmental method are summarized in Table 4. To date, report on the pharmacokinetic behavior of solamargine is not available. According to our research (data not shown), the pharmacokinetic properties of solamargine in rats were linear within the concentration range. As illustrated in the time–concentration profile, only trace amount of solamargine could be detected at 8 h after intravenous administration, suggesting that elimination or biotransformation of solamargine was relatively quick in rats. Given the potential clinical use as an anti-tumor agent, the pharmacokinetic profile of solamargine in human, especially cancer patients, warrants careful considerations.

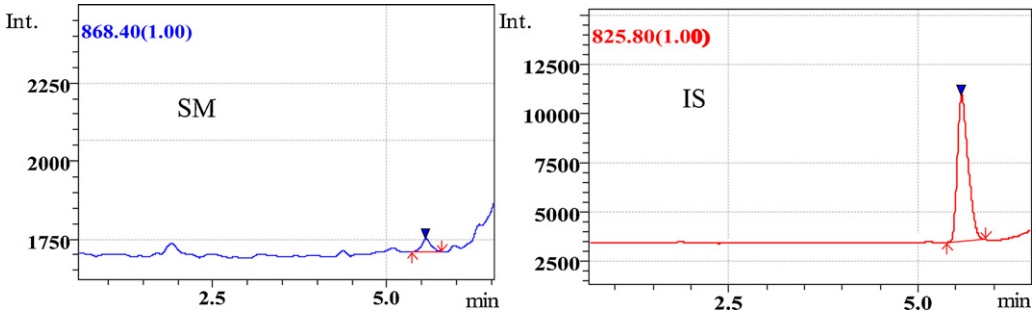

Fig. 3. Typical chromatograms of solamargine and vincristine at the level of LLOQ.

Table 4
Pharmacokinetic parameters after a single intravenous dose of 1.0, 2.0 and 4.0 mg/kg solamargine in rats ($n = 5$).

Parameters	Mean ± SD		
	1.0 mg/kg	2.0 mg/kg	4.0 mg/kg
$C_{5\,min}$ (ng/mL)	538.54 ± 278.79	1094.37 ± 694.87	2483.40 ± 272.89
Cl (L/kg h)	3.81 ± 0.68	4.10 ± 1.23	3.97 ± 0.44
Vd (L/kg)	20.16 ± 10.34	19.78 ± 12.55	15.68 ± 6.19
$t_{1/2}$ (h)	3.54 ± 1.50	3.29 ± 1.52	2.76 ± 1.10
AUC_{0-t} (ng h/mL)	242.41 ± 65.68	482.36 ± 138.07	947.74 ± 124.76
$AUC_{0-\infty}$ (ng h/mL)	270.50 ± 54.66	526.92 ± 161.72	1018.91 ± 117.26

4. Conclusion

In this work, we described a reliable quantitative method of solamargine in rat plasma which is essential for evaluating its pharmacokinetics profiles. For the first time, a sensitive and reliable LC–MS quantitative method was developed, validated and applied to pharmacokinetic profiling of solamargine in rats. The current method holds promise for facilitating the characterization of solamargine in other bio-samples. In addition, based on this validated method, the pharmacokinetic parameters of intravenously administered solamargine were calculated for the first time, providing valuable information for future development of solamargine in clinical studies.

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