Quantitative determination of mogroside V in rat plasma by LC-MS/MS and its application to a pharmacokinetic study

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Abstract: Mogroside V is the most abundant (approximately 0.50%) cucurbitane-type triterpene glycoside in *Siraitia grosvenorii* and exhibits significant antitussive, expectorant, anti-carcinogenic, and anti-inflammatory effects. A sensitive, robust and selective liquid chromatography tandem with mass spectrometry (LC-MS/MS) was developed and validated for the determination and pharmacokinetic investigation of mogroside V in rat plasma. Samples were prepared through an one-step deproteinization procedure with 250 μL of methanol to a 75-μL plasma sample. Plasma samples were effectively separated on a Shiseido Capcell Pak UG120 C_{18} column (2.0 × 50mm, 3.0μm) using a mobile phase consisting of methanol: water (60:40, v/v) with an isocratic elution program. The running time for each sample was 7.0 min and the elution times of mogroside V and IS were 2.0 and 4.8 min, respectively. The detection relied on a triple-quadrupole tandem with mass spectrometer equipped with negative-ion electrospray ionization interface by selected-reaction monitoring (SRM) of the transitions at *m/z* 1285.6 → 1123.7 for mogroside V and *m/z* 1089.6 → 649.6 for IS. The calibration curve was linear over the range of 96.0–96000ng/mL with a limit of quantitation (LOQ) of 96.0ng/mL. Intra-day and inter-day precisions were both <10.1%. Mean recovery and matrix effect of mogroside V in plasma were in the range of 91.3-95.7% and 98.2-105.0%, respectively. This method was successfully applied in the pharmacokinetic study of mogroside V after intravenous or intraperitoneal administration of 1.12mg/kg mogroside V in rats.

Keywords: Mogroside V, LC-MS/MS, Pharmacokinetic study.

INTRODUCTION

The fruit of *Siraitia grosvenorii* (Luo-han-guo) is officially listed in the Chinese Pharmacopoeia due to the proven therapeutic effects on lung congestion, rheumatic arthritis, colds, sore throats, and constipation (State Pharmacopoeia Commission of P.R. China, 2010). Furthermore, the *S. grosvenorii* fruit is commonly used as a non-caloric sweetener for food and beverages. Modern pharmacological studies indicate that *S. grosvenorii* extracts exhibit anti-oxidative, antibacterial, anti-inflammatory, glucose-lowering, neuroprotective, hypoglycemic and anti-lipidemic effects (Chen *et al.,* 2011; Qi *et al.,* 2008; Takasaki *et al.,* 2003; Pan *et al.,* 2009; Song *et al.,* 2006; Zheng *et al.,* 2011). The main chemical substances found in *S. grosvenorii* are cucurbitane-type triterpene glycosides known as mogrosides (Chaturvedula and Prakash, 2011; Jin and Lee, 2012), among which mogroside V is the most abundant (approximately 0.50%) and most bioactive. Mogroside V exhibits significant antitussive, expectorant, anti-carcinogenic, and anti-inflammatory effects (Liu *et al.,* 2007; Shi *et al.,* 2014). In addition, mogroside V can stimulate insulin secretion in rat insulinoma cells, which suggests that mogroside V is beneficial for the treatment of diabetes (Zhou *et al.,* 2009). Thus, the development of sensitive analytical methods for the quantification of mogroside V in biological fluids is necessary for further *in vivo* evaluation.

Several analytical methods exist for the determination of mogroside V in the herbs or biological samples. The majority of these methods involve high-performance liquid chromatography (HPLC) (Deng *et al.,* 2013; Li *et al.,* 2007; Lu *et al.,* 2012; Zhang *et al.,* 2011), liquid chromatography-mass spectrometry (LC-MS) (Murata *et al.,* 2010), ultra-high performance liquid chromatography/ quadrupole time-of- flight mass spectrometry (UHPLC/ QTOF-MS) (Zhang *et al.,* 2012), and HPLC tandem with ion trap TOF multistage mass spectrometry (HPLC-IT-TOF-MS^n) (Xu *et al*., 2015). One study reported the distribution and metabolism of mogroside V in rats (Xu *et al*., 2015); however, to date, the pharmacokinetic properties of mogroside V remain unknown and uncharacterized. In the present study, a UHPLC tandem with mass spectrometry (UPLC–MS/MS) method was developed for the determination of mogroside V in rat plasma. The newly developed method was successfully applied in the pharmacokinetic study after intraperitoneal or intravenous administration of mogroside V in rats.

MATERIALS AND METHODS

Reagents and chemicals

Mogroside V (fig. 1, purity > 98%) and polygalasaponin F (internal standard, IS, purity >98%) were purchased from the National Institutes for Food & Drug Control (Beijing, China). HPLC grade of methanol was purchased from Merck Company (Darmstadt, Germany). Purified water

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was supplied by Wahaha Group Co., Ltd. (Hangzhou, China).

Liquid chromatographic and mass spectrometric conditions

Chromatographic analysis was achieved on a Shimadzu SIL-20 AC HT system (Shimadzu, Kyoto, Japan). Separation of the analyte and IS was performed on a Shiseido Capcell Pak UG120 C_{18} column (2.0 × 50 mm, 3.0 μm; Shiseido, Tokyo, Japan) using a mobile phase consisting of methanol: water (60:40, v/v). The flow rate was kept at 0.5mL/min with 50% flow split after post-column elution. Flow was directed to the ion spray interface.

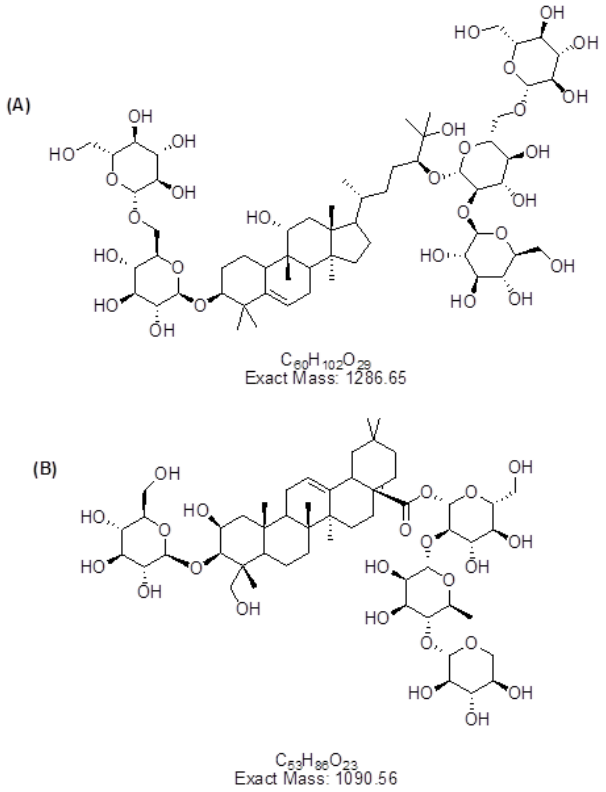

Fig. 1: The chemical structures of mogroside V (A) and IS (B).

Mass spectrometric detection was carried out on a triple-quadrupole mass spectrometer that was equipped with an electrospray ionization (ESI) and operated in negative ionization mode (Finnigan TSQ Quantum Ultra, Thermo Scientific, San Jose, CA, USA). The transitions monitored were 1285.6 → 1123.7 for mogroside V and *m/z* 1089.6 → 649.6 for IS, respectively. Collision energy was set at 47 and 50 eV for mogroside V and IS, respectively. Tube lens was set at 240 and 236 eV for mogroside V and IS, respectively. Selected-reaction monitoring (SRM) mode was used for data acquisition. The capillary temperature was set at 350 ºC, and the ion spray needle voltage was adjusted to 3500 V. Nitrogen was used as sheath (35Arb) and auxiliary gas (10Arb) for nebulization and desolvation. Argon was used as collision gas (1.5mTorr) for collision-induced dissociation.

Preparation of standards and quality control samples

Stock solutions of mogroside V and polygalasaponin F were both prepared in methanol at concentrations of 2.4 and 1.2 mg/mL, respectively. Stock solution of mogroside V was then serially diluted with methanol to give working standard solutions at desired concentrations. The IS solution was prepared by dilution with methanol to 1.2 μg/mL. In addition, an appropriate amount of mogroside V was dissolved in methanol to make a concentration of 2.4 mg/mL and use as quality control (QC) samples. All the solutions were stored at –20ºC and brought to room temperature before use.

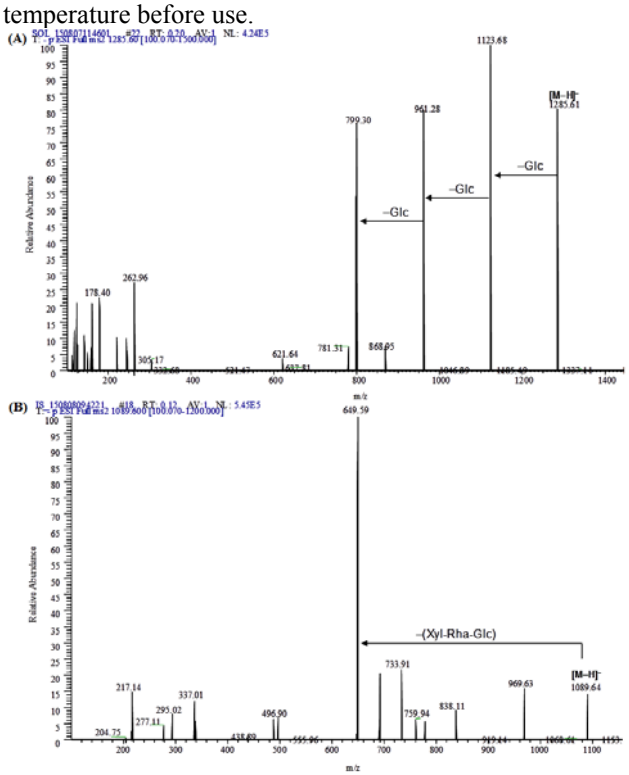

Fig. 2: The product ion mass spectra of mogroside V (A) and IS (B).

Calibration standards were prepared daily by spiking 10 μL of blank plasma with 190μL of standard working solutions to yield the final concentrations of 96.0, 384, 960, 3840, 9600, 38400 and 96000ng/mL. QC samples were prepared according to the procedure for the standard sample preparation and stored at -20ºC. The low, medium, and high concentrations of QC samples were 192, 1920, and 76800ng/mL, respectively.

Sample preparation

All frozen plasma samples were thawed at room temperature (25ºC) and homogenized through vortexing. 250μL of methanol containing IS (1.2μg/mL) was added to 75μL plasma. The mixture was vortexed for 1 min and centrifuged at 15,000 × *g* for 5min to separate protein.

Table 1: Precision, accuracy and matrix effect of mogroside V in rat plasma (n = 6).

Analyte	Concentration (ng/mL)	CV (%)		Accuracy (%)		Matrix effect (%)
		Intra-day	Inter-day	Intra-day	Inter-day	
Mogroside V	96.0	6.3	10.1	93.5	98.0	105.0
	192	4.7	5.1	95.0	101.4	98.2
	1920	9.2	7.5	104.6	110.7	101.8
	76800	3.3	6.9	93.1	96.2	98.8

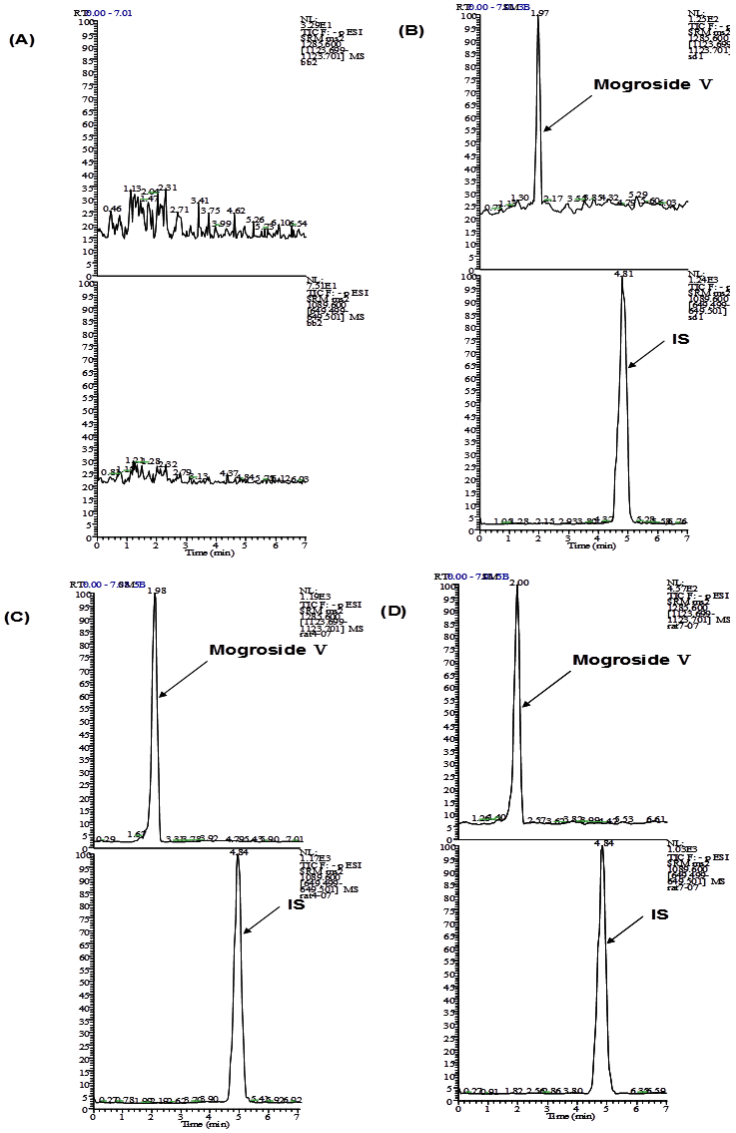

Fig. 3: Representative chromatograms of: (A) blank rat plasma, (B) blank rat plasma spiked with mogroside V at the lower limit of quantitation (96.0ng/mL), (C) a representative plasma sample 3 h after intravenous administration, and (D) a representative plasma sample 3 h after intraperitoneal administration

The supernatant was transferred to a clean centrifuge tube and evaporated to dryness under a stream of nitrogen. The residue was reconstituted in 100µL of methanol: water (60:40, v/v) with vortexing for 1min and centrifuged for 5min. A 4 µL aliquot was injected into the LC–MS/MS system for analysis.

Specificity, sensitivity and linearity

The specificity of the method was evaluated by comparing the chromatograms of six different sources of blank plasma with corresponding spiked plasma samples. The limit of quantification (LOQ) was defined as the lowest concentration level that produced an accuracy of

Table 2: Recovery data of mogroside V from rat plasma at three QC concentrations (n=6).

	Nominal concentration (ng/mL)		
	192	1920	76800
Recovery (%)	91.3	95.7	94.8
SD	7.6	2.1	10.3
Precision (CV%)	8.3	2.2	10.9

Table 3: Summary of stability of mogroside V under various storage conditions (n = 5).

Condition	Concentration (ng/mL)		CV (%)	Accuracy (%)
	Nominal	Found		
Ambient, 6h	192	202.3±12.7	6.3	105.4
	1920	1797.9±102.4	5.7	93.6
	76800	73158.1±1757.3	2.4	95.3
–20ºC, 43 days	192	184.4±22.0	11.9	96.0
	1920	1935.6±65.3	3.4	100.8
	76800	82337.3±632.4	0.8	107.2
Three freeze-thaw cycles	192	187.6±3.1	1.6	97.7
	1920	2072.8±36.0	1.7	108.0
	76800	69992.4±9393.7	13.4	91.1
Autosample, 12ºC, 12h	192	175.3±8.7	5.0	91.3
	1920	1950.0±157.1	8.1	101.6
	76800	79825.9±2497.8	3.1	103.9

Table 4: The pharmacokinetic parameters of mogroside V in rats after intravenous or intraperitoneal administration at 1.12 mg/kg (n = 6, mean ± SD).

Parameters	Intravenous	Intraperitoneal
$AUC_{(0-t)}$ (mg h/L)	61.26±7.00	9.12±0.64
$AUC_{(0-\infty)}$ (mg h/L)	61.69±7.24	9.43±0.61
$MRT_{(0-t)}$ (h)	1.86±0.33	2.67±0.17
$t_{1/2}$ (h)	1.53±0.36	1.45±0.13
T_{max} (h)	/	1.40±0.55
C_{max} (μg/mL)	36.30±2.26	2.72±0.25
V_Z (L/kg)	0.04±0.01	0.12±0.01

80-120% and precision of ≤20% during the method development. Seven non-zero calibration standards were analyzed and fitted in duplicates in 3 separate validation days.

Accuracy and precision

The inter- and intra-day accuracy and precision were evaluated from the assay analysis of six replicates of LOQs and QCs at concentrations of 96.0, 192, 1920 and 76800 ng/mL on three validation days. The precision was expressed as the coefficient of variation (CV). The CV value of ≤15% was considered to be acceptable at all concentration levels except the LOQ, for which a CV of ≤20% was considered acceptable.

Recovery and matrix effect

The extraction recovery of mogroside V and IS was examined by comparing the peak areas of the extracted plasma samples from QC samples with those obtained from the direct injection of the standard solutions at same concentrations (n = 6). The matrix effect was evaluated by comparing the peak areas of analyte spiked into the extracted plasma with those of the standard solutions spiked with the same concentration of mogroside V in the mobile phase.

Stability

The stability was investigated by five replicates of QC samples. Plasma samples were stored for 6h at room temperature and stored at -20ºC for 43 days to evaluate short-term and long-term stability, respectively. The freeze-thaw stability was investigated by analyzing the QC samples undergone three freeze (-20ºC) and thaw (room temperature) cycles. The processed QC samples placed in the autosampler at 12ºC for 12h were evaluated for the post-preparative stability.

Pharmacokinetic study

Twelve male Wistar rats (220±20g) were randomly divided into 2 groups. One group was given

intraperitoneal (i.p.) injection of mogroside V and the other group was given intravenous (i.v.) administration of the compound. Blood samples (approximately 250μL) were withdrawn via oculi chorioideae vein and collected into heparinized tubes at 0, 0.083, 0.167, 0.33, 0.67, 1, 2, 3, 4, 6, 8 and 12 h after administration of 1.12 mg/kg mogroside V dissolved in normal saline. All blood samples were immediately centrifuged at 4000 rpm for 10 min and stored at -20ºC until analysis. The pharmacokinetic parameters were calculated with the DAS package (Version 2.1.1, Shanghai, China) according to non-compartmental model.

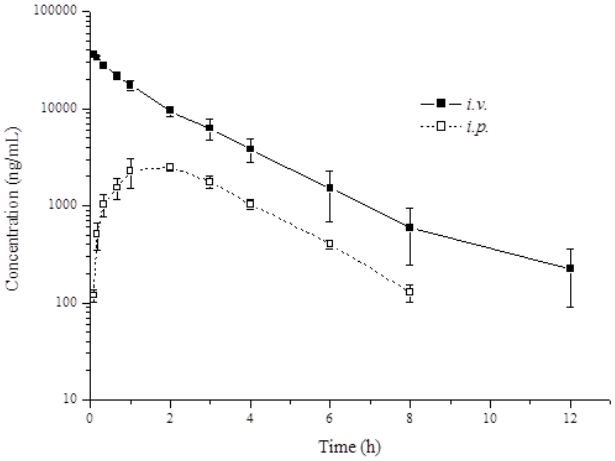

Fig. 4: Mean concentration time profiles of mogroside V in rats after intravenous (i.v.) or intraperitoneal (i.p.) administration at .1.12 mg/kg (mean ± SD, n = 6).

RESULTS

Optimization of mass spectrometric conditions

The product ion at *m/z* 1123.7 was observed to be the most abundant ion for the deprotonated quasi-molecular ion $[M–H]^-$ at *m/z* 1285.6 of mogroside V, which indicated the loss of one glucose moiety of mogroside V (fig. 2A). Thus, SRM transition *m/z* 1285.6→1123.7 was selected for the quantification of mogroside V. Similarly, SRM transition *m/z* 1089.6→ 649.6 was selected for IS, in which the product ion at *m/z* 649.6 was the most abundant ion of the deprotonated quasi-molecular ion $[M–H]^-$ at *m/z* 1089.6 (fig. 2B), indicating the loss of one xylose-rhamnose and one glucose moiety.

Specificity, sensitivity, linearity

Specificity was evaluated by comparing the chromatograms of blank plasma of six different rats with those of the corresponding spiked plasma samples. As shown in fig. 3, there was no interfering peak at the retention times of mogroside V and IS.

The sensitivity was evaluated by the response analysis of six replicates of LOQ samples on three validation days. And the signal-to-noise ratio of higher than 10 was observed as shown in fig. 3.

All the calibration standards within the concentration range of 96.0-96000ng/mL were analyzed for evaluating the linearity with a correlation coefficient (r) ≥ 0.995. The mean linear equation obtained for mogroside V was y = (0.00058±0.00002) x + (0.02598±0.01402), where y presents the peak area ratio of analyte/IS and x is the analyte's concentration. The accuracy and CV% for the calibration standards ranged from 94.8% to 103.0% and 3.7% to 6.2%, respectively.

Precision and accuracy

The precision of the method was investigated by determining CV% for LOQs and QCs on three validation days (table 1). Intra-day precision was less than 9.2%, and the inter-day precision was less than 10.1%. Intra-day and inter-day accuracy of the method ranged from 96.2 to 105.0%.

Matrix effect and carryover

The matrix effect for mogroside V at concentrations of 96.0, 192, 1920, and 76800ng/mL in rat plasma was measured to be 105.0, 98.2, 101.8 and 98.8% (n=6), respectively (table 1). The matrix effect for the IS (1.2 μg/mL) was measured to be 92.9% (n=6). As a result, the matrix effect of the analyte and IS from rat plasma was considered negligible with this method. The chromatograms for mogroside V and IS showed no significant peaks in the blank samples that were injected after three consecutive tests of the highest concentration standard samples; therefore, no carryover effect was observed in the present study.

Recovery and stability

The recovery of mogroside V from the low, medium and high QC samples ranged from 91.3% to 95.7% with a maximum CV of 10.9% (table 2), indicating the simple protein precipitation procedure gave reproducible recovery for the analyte and IS.

Stability tests were performed at the three QC levels (n = 5) under different storage conditions described above. Results are summarized in table 3. The room temperature, long-term (43 days), freeze-thaw and autosampler stability indicated that the analyte was stable since the accuracy (91.1–108.0%) and CVs (0.8–13.4%) of the mean test responses were within acceptable criteria in all stability tests.

Application to a pharmacokinetic study

The method was successfully applied in the determination of plasma concentrations of mogroside V in rats after i.p. and i.v. administration. Plasma concentration–time curves are presented in fig. 4 and the main pharmacokinetic parameters of mogroside V are summarized in table 4. After i.v. administration, the mean area under the plasma concentration–time curve from time zero to the last measurable plasma concentration point ($AUC_{(0–t)}$) and the

mean area under the plasma concentration-time curve from time zero to time infinity ($AUC_{(0-\infty)}$) values were 61.26±7.00 and 61.69±7.24 mg h/L, respectively. After i.p. administration at 1.12 mg/kg, mogroside V reached a maximum plasma concentration (C_{max}) of 2.72±0.25 µg/mL at a peak time (T_{max}) of 1.40±0.55 h. The mean $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ values were 9.12±0.64, and 9.43± 0.61 mg h/L, respectively. Half-life ($t_{1/2}$) was determined to be 1.45 and 1.53h for i.p. and i.v. administration, respectively, with no statistical significant difference. The apparent volumes of distribution (V_Z) at terminal phase were 0.04 and 0.12 L/kg for i.v. and i.p. administration, respectively. The elimination performance and characteristic distribution of mogroside V could be explained by (1) limited membrane permeability or (2) extensive biliary excretion through active transport. As a result, the above mentioned conditions may lead to a short $t_{1/2}$, low systemic exposure, and small V_Z.

DISCUSSION

Polygalasaponin F was selected as the internal standard because of similar chemical structure, mass spectrometric behavior, and recovery to mogroside V. LC-MS/MS conditions should be further optimized for the determination of the analyte and IS. MS spectra of mogroside V and IS were analyzed in negative ion mode because the intensity was lower when positive ion mode was used to test the analytes. SRM mode was used because of higher specificity and sensitivity relative to the selection-ion monitoring scan mode.

For the mobile phase composition, methanol and acetonitrile were tested as the organic phase, and results showed that a methanol-based mobile phase could generate high response for both mogroside V and IS. Furthermore, improvement in peak shape with proper retention time was observed. Addition of 0.01%, 0.05%, or 0.1% formic acid or acetic acid considerably weakened the intensity of the analytes; thus, a mobile phase consisting of methanol:water (60:40, v/v) without any additives was finally selected.

CONCLUSION

In the present study, an LC-MS/MS method for the determination of mogroside V in rat plasma was reported. The novel method was successfully validated and observed to be rapid, sensitive, and selective. A simple protein precipitation procedure made the method readily applicable for large sample batches. The LC-MS/MS method was successfully applied to a pharmacokinetic study of mogroside V after intravenous or intraperitoneal administration of a single dose of 1.12 mg/kg to rats. The pharmacokinetic profile of mogroside V in rats was characterized for the first time.

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