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The bioavailability and excretion of an antitussive compound IAsp-N-Glc in rats by validated UPLC-MS/MS methods

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Abstract: IAsp-N-Glc is a potential antitussive agent that is first reported to be isolated from *Ginkgo Semen*, but the bioavailability and excretion of IAsp-N-Glc are unknown. Therefore, we carried out our study to obtain the bioavailability and excretion profiles of IAsp-N-Glc in rats. Rapid, specific, and reliable quantification methods for the measurement of IAsp-N-Glc in rat plasma and fecal samples by using ultra-high-performance liquid chromatography coupled with triple quadrupole mass spectrometry were developed and validated. A C_{18} column was used for the separation of IAsp-N-Glc and internal standards, and water (containing 0.1% formic acid) and acetonitrile were chosen as the mobile phase for the separation in the flow-gradient mode. In the ranges of 37.5-7500 ng/mL and 120-30000 ng/mL, the calibration curves of IAsp-N-Glc exhibited satisfactory linearity for plasma and fecal samples with each linear correlation coefficient higher than 0.99, respectively. The methods were reproducible and reliable. The analytes were stable, and no apparent matrix effects were observed. The bioanalytical methods were successfully used to study the pharmacokinetics and excretion of IAsp-N-Glc in rats. Oral administration of IAsp-N-Glc exhibited a low absolute oral bioavailability (1.83±0.09%), and 59.63±6.29% of IAsp-N-Glc was excreted in feces. This report is the first to describe the bioavailability and excretion of IAsp-N-Glc in rats and will lay the foundation for the in-depth study and drug development of IAsp-N-Glc.

Keywords: *Ginkgo Semen,* IAsp-N-Glc, pharmacokinetics, bioavailability, excretion

INTRODUCTION

Cough is a common clinical symptom worldwide, and it is a physiological reflex that removes foreign materials and secretions from the airways (Kuang *et al*., 2018, Nasra and Belvisi 2009). However, the severe cough would impact people's lives (Zhong *et al*., 2016), and many traditional Chinese medicines possess a potential antitussive effect. *Ginkgo Semen*, known as Baiguo in China, is a traditional Chinese herbal medicine, which is widely utilized for relieving cough and asthma over hundreds of years, and it could be an astringent for the lung to relieve asthma (Chinese Pharmacopoeia Commission, 2015).

(2-(1-((2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2*H*-pyran-2-yl)-1*H*-indol-3-yl) acetyl)-*L*-aspartic acid (IAsp-N-Glc, fig. 1A), which is first reported to be isolated from *Ginkgo Semen*, is a compound that exerts a potential antitussive effect (Zhang *et al*., 2020).

Pharmacokinetics plays a primary role in drug discovery and development and provides useful information associated with the potential efficacy and toxicity, and intelligent use of drugs (Elhennawy and Lin, 2017, Li *et al*., 2018, Zhang *et al*., 2020). The disposition information of IAsp-N-Glc *in vivo* could provide valuable data for its dosing regimen. The exposure and excretion information of IAsp-N-Glc could also be conducive to understand the relationship between pharmacological activity and its dose (Zhang *et al*., 2018, Zhang *et al*., 2020). However, information about the disposition of IAsp-N-Glc has never been reported, and assessments of the pharmacokinetics, absolute bioavailability, and excretion of IAsp-N-Glc were conducted in this study.

In our study, rapid, specific, and reliable quantification methods for the measurement of IAsp-N-Glc in rat plasma and fecal samples by using ultra-high-performance liquid chromatography coupled with triple quadrupole mass spectrometry (UPLC-MS/MS) were developed and validated. The validated methods were utilized to the pharmacokinetic study of IAsp-N-Glc in rats after the intravenous injection (i.v.) (1 mg/kg) and oral administration (15 mg/kg, 30 mg/kg, 60 mg/kg) of IAsp-N-Glc. The excretion profile of IAsp-N-Glc was also examined in rats after oral administration (15 mg/kg) of IAsp-N-Glc. This is the first report to describe the pharmacokinetics, absolute bioavailability, and excretion of IAsp-N-Glc. Our study will provide useful data for the further drug development of IAsp-N-Glc (Cui *et al*., 2018, Shen *et al*., 2018).

MATERIALS AND METHODS

Chemicals and reagents

IAsp-N-Glc and (2-(1-((2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2*H*-pyran-2-yl)-1*H*-indol-3-yl) acetyl)-*L*-glutamic acid (IGlu-N-Glc) with purity

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higher than 98% were prepared and verified in our laboratory using an established method. Isoquercitrin (purity higher than 98%) was provided by Jiangxi BaiCaoYuan Biological Technology Co. Ltd. (Nanchang, China). UPLC-grade methanol, water, and acetonitrile were purchased from Thermo Fisher Scientific (Pittsburgh, PA, USA). UPLC-grade formic acid was obtained from ROE Scientific Inc. (Newark, DE, USA). Isoquercitrin and IGlu-N-Glc were selected as the internal standard in this study. The chemical structures of IAsp-N-Glc, IGlu-N-Glc (internal standard1, IS1), and isoquercitrin (internal standard2, IS2) are displayed in fig. 1.

Experimental animals

Male Sprague-Dawley rats (weighing 250±20 g) were provided by SPF (Beijing) Biotechnology Co. Ltd. (Beijing, China). The animals were bred in the animal room for seven days of accommodation, and they had free access to normal water and food. The feeding condition was controlled on a 12 h dark/light cycle, the temperature of 25±2°C, and relative humidity of 50±5%. Before the experiments, the rats had fasted for 12 hours with water available ad libitum. The animal experiment protocols were approved by the Laboratory Animal Ethics Committee of the Institute of Basic Theory for Chinese Medicine, China Academy of Chinese Medicine Science.

Instruments and UPLC-MS/MS conditions

An Agilent 1290 chromatography system coupled with an Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies, USA) equipped with an electrospray ionization (ESI) source was applied to the sample determination. An Agilent ZORBAX Eclipse Plus C_{18} column (2.1 ×50 mm, 1.8 μm, Agilent Technologies, USA) was utilized to the chromatographic separation of IAsp-N-Glc and internal standards. The separation was carried out at 35℃, and the flow rate was 0.2 mL/min. The gradient elution was used for the separation of the analytes with the mobile phase consisted of water (containing 0.1% formic acid) (A) and acetonitrile (B). For plasma samples, the elution gradient was 5% B over 0-1 min, 5-30% B over 1-2 min, 30-80% B over 2-6 min, and 80% B over 6-8 min. Meanwhile, for fecal samples, the elution gradient was 5% B over 0-1 min, 5-20% B over 1-2 min, 20-80% B over 2-6 min, and 80% B over 6-8 min.

MS/MS operating conditions were optimized, and the optimized conditions were set as follows: gas temperature: 400 ℃, sheath gas temperature: 350 ℃, gas flow rate: 10 L/min, sheath gas flow rate: 11 L/min, nebulizer gas pressure: 45 psi, and capillary voltage: 3500 V. The analytes were measured by mass spectrometer by utilizing multiple reactions monitoring (MRM) mode. The concentrations of IAsp-N-Glc and isoquercitrin in rat plasma were determined by the mass spectrometer in positive mode, and the quantitative transitions were m/z 453.1→291.1 for IAsp-N-Glc and 465.2→303.1 for IS1. Similarly, the quantitative transitions were m/z 453.1→291.1 for IAsp-N-Glc and 465.2→303.1 for IS1, which was used for the determination of IAsp-N-Glc and IS2 concentrations in fecal samples by the mass spectrometer in negative mode. The MS spectra of the product ions are shown in fig. 2. The data was acquired with an Agilent MassHunter Workstation (Agilent Technologies, USA).

Preparation of standards and quality control (QC) samples

Standard solutions were prepared by dissolving IAsp-N-Glc, IS1, and IS2 in methanol to obtain concentrations of 0.15, 0.40, and 0.20 mg/mL, respectively. Then, the working solutions were prepared by serial diluting the standard solution with methanol. Standard solutions of IS1 and IS2 were diluted to final concentrations of 400 ng/mL and 8000 ng/mL with methanol. Working solutions were spiked into blank rat plasma and fecal samples to prepare calibration standards. The final calibration concentrations of IAsp-N-Glc in rat plasma were: 37.5, 75, 187.5, 375, 750, 1875, 3750, and 7500 ng/mL. The final calibration concentrations of IAsp-N-Glc in fecal samples were: 120, 300, 600, 1500, 3000, 6000, 15000, 30000 ng/mL. Similarly, the quality control (QC) samples were independently prepared at concentrations of 60, 600, and 6000 ng/mL in rat plasma

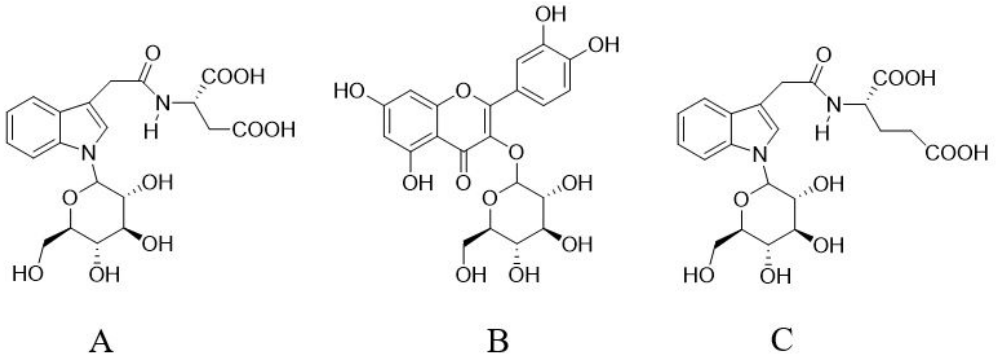

Fig. 1: Chemical structures of IAsp-N-Glc (A), isoquercitrin (B) (IS1), and IGlu-N-Glc (C) (IS2).

and 240, 2400, and 24000 ng/mL in fecal samples, respectively. All solutions are stored in a refrigerator at 4 ℃ before analysis.

Sample preparation

Rat plasma samples were taken out of the -20 ℃ condition and thawed at room temperature before analysis. In our study, a rat plasma sample (100 μL) and 20 μL of IS1 solution were spiked into a 5 mL centrifuge tube, respectively. After the vortex, 400 μL of methanol (containing 0.1% formic acid) was spiked into the tube for protein precipitation. The mixture was centrifuged at 12,000 rpm for 10 min at 4 ℃ after vortexed for 4 min. Finally, four μL supernatant was injected and analyzed (Chen *et al*., 2018).

Fecal samples were homogenized in a 0.9% NaCl solution (w/v), ultrasonically extracted for 15 min. After 5 min of centrifugation, the mixture was centrifuged at 6000 rpm for 10 min at 4℃ for obtaining the supernatant, and another new tube was used for the storage of the rat fecal supernatant. Then, a rat fecal supernatant (100 μL) and 20 μL of IS2 solution were spiked into a 5 mL centrifuge tube, respectively. After the vortex, 400 μL of methanol (containing 0.1% formic acid) was spiked into the tube for protein precipitation. The sample was centrifuged at 12,000 rpm for 10 min at 4 ℃ after vortexed for 4 min. Finally, four μL supernatant was injected and analyzed.

Method validation

The method was developed and validated in accordance with the guidelines published by the Food and Drug Administration (USA Department of Health and Human Services, 2013). The specificity, linearity, sensitivity, accuracy, precision, recovery, matrix effect, and stability were investigated by using QC samples.

Specificity

Typical chromatograms of blank biological samples from 6 individual rats were compared with chromatograms of the corresponding spiked blank biological samples and biological samples obtained after the administration of IAsp-N-Glc to investigate the specificity and exclude the influences of endogenous substances.

Linearity

Linearity was evaluated by assaying calibration standards at eight concentrations (Wang *et al*., 2018). The calibration curves were constructed by plotting the peak area ratios (y) of IAsp-N-Glc to ISs against the corresponding nominal concentration (x) using the least square weighted linear regression analysis, and $1/x^2$ was used as the weighting factor. The lower limit of quantification (LLOQ) was defined as the lowest concentration of the calibration curves with a signal-to-noise (S/N) ratio ≥10, and the accuracy and precision at LLOQ should be within ± 20%.

Precision and accuracy

The QC samples at three concentrations of IAsp-N-Glc were used for the determination of precision and accuracy. The intra-day precision and accuracy were measured QC samples (n=6) on a single day, and the inter-day precision and accuracy were assessed on three consecutive days. The precision and accuracy of IAsp-N-Glc were calculated as

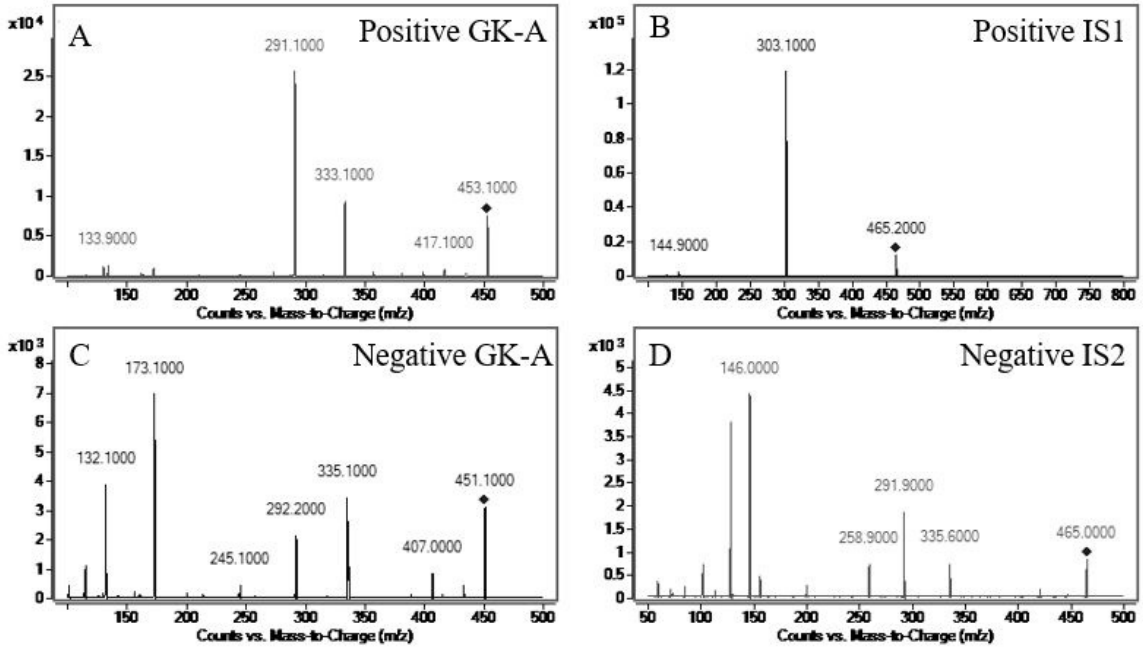

Fig. 2: $[M+H]^+$ product ion spectra of IAsp-N-Glc (A) and IS1 (B) and $[M-H]^-$ product ion spectra of IAsp-N-Glc (C) and IS2 (D).

relative standard deviation (RSD%) and the relative error to the nominal concentrations (RE%), respectively. The precision and accuracy were acceptable within ± 15%. For the LLOQ, the accuracy and precision should be within ± 20%.

Extraction recovery and matrix effect

The QC samples (n=6) at three concentrations were used for analyzing the extraction recoveries and matrix effects of the analytes. The recoveries of the analytes were assessed by comparing the peak area of the analytes in QC samples against the peak area of the analytes in post-treated biological samples spiked with the analytes. The matrix effects were evaluated by comparing the peak area of analytes in post-treated biological samples spiked against the analytes with the peak area of analytes in the corresponding standard solutions (Rashid *et al*., 2018).

Stability

The QC samples (n=6) at three concentrations were used to investigate the stability of the IAsp-N-Glc in different storage conditions. The short-term stability was assessed by keeping QC samples at room temperature for 6 h. The QC samples were conducted for three freeze-thaw cycles to evaluate the freeze-thaw stability. The QC samples were stored at -20°C for one month to evaluate the long-term stability. The post-preparative stability was determined after the extracted QC samples kept at 4°C for 24 h.

Pharmacokinetic study

Specific pathogen-free grade male SD rats were divided into four groups: six rats in the low-dose oral administration group (15 mg/kg), six rats in the middle-dose oral administration group (30 mg/kg), six rats in the high-dose oral administration group (60 mg/kg) and six rats in the intravenous (i.v.) administration group (1 mg/kg). IAsp-N-Glc was dissolved in 0.9% sodium chloride for oral administration and intravenous injection. Afterward, approximately 0.25 mL of blood samples were collected into EDTA-K2 tubes by puncture of the suborbital vein at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 h. Then, the harvested blood samples were centrifuged at 8000 rpm for 10 min at 4 °C. The supernatant of plasma was transferred into another clean tube and stored at -20 °C before analysis. The absolute bioavailability (F%) of IAsp-N-Glc was calculated using the following equation (Huang *et al*., 2016):

$$F\ (\%) = \frac{[AUC(0\text{-}\infty)oral \times Dose(i.v.)]}{[AUC(0\text{-}\infty)i.v. \times Dose(oral)]} \times 100\%$$

Excretion study

Six male rats were intragastrical administrated IAsp-N-Glc at a single dose of 15 mg/kg and housed in separate stainless-steel metabolism cages. Fecal samples were collected at 0-6, 6-12, 12-24, 24-36, 36-48, 48-60, and 60-72 h (Zhang *et al*., 2017).

STATISTICAL ANALYSIS

The pharmacokinetic parameters of IAsp-N-Glc were analyzed by using "Drug and Statistics 3.0" (DAS 3.0). Data of pharmacokinetic parameters were expressed as mean ± S.D.

RESULTS

Optimization of chromatography and mass spectrometry conditions

Various mobile phases, including acetonitrile, methanol, formic acid, and ammonium formate, were assessed to optimize analytical performance and conditions. The use of acetonitrile as the organic solvent produced a better separation than methanol. The peak shape could be improved, and the response could be increased by adding an appropriate amount of formic acid into the water, and 0.1% formic acid was selected finally. Ultimately, water (containing 0.1% formic acid) and acetonitrile were chosen as the mobile phase for gradient elution. Chromatographic separation could be achieved within 8 min.

IAsp-N-Glc and IS1 and IS2 were tested by mass spectrometer operating in both positive and negative ion modes for obtaining better MS parameters. For plasma samples, IAsp-N-Glc and IS1 displayed a better sensitivity in positive ionization mode, and for fecal samples, IAsp-N-Glc and IS2 exhibited a better response in negative ionization mode. Meanwhile, other parameters, including the gas temperature, fragmentor, and collision energy, were carefully optimized for the maximum response (Liu, *et al*.). The optimized mass parameters of the IAsp-N-Glc and internal standards (ISs) were presented in table 1.

Optimization of sample pretreatment steps

Acetonitrile, methanol, and acetone were used as protein precipitants for the pretreatment of biological samples. Meanwhile, chloroform and ethyl acetate were used as the extraction solvent for the pretreatment of biological samples. The method possessed the best recoveries and no obvious matrix effects of IAsp-N-Glc would be chosen for the pretreatment of biological samples (Elhennawy and Lin, 2017, Shen *et al*., 2018, Tian *et al*., 2017). Ultimately, methanol (containing 0.1% formic acid) was selected for pretreatment of biological samples because of its satisfactory extraction efficiency and acceptable matrix effects.

Method validation

Selectivity

Fig. 3-1 and fig. 3-2 present typical MS/MS chromatograms obtained from blank biological samples, biological samples spiked with IAsp-N-Glc and IS1 or IS2, and plasma and fecal samples collected 1.5 h and 12 h, respectively, after the administration of IAsp-N-Glc. At the retention time of IAsp-N-Glc, IS1, and IS2, there was no

Table 1: MS parameters for IAsp-N-Glc and ISs

Analytes	Precursor ion (m/z)	Product ion (m/z)	Fragmentor (V)	Collision energy (V)	Polarity
IAsp-N-Glc	453.1	291.1	60	7	Positive
isoquercitrin（IS1）	465.2	303.1	100	7	Positive
IAsp-N-Glc	451.1	173.1	105	19	Negative
IGlu-N-Glc（IS2）	465.0	146.0	115	23	Negative

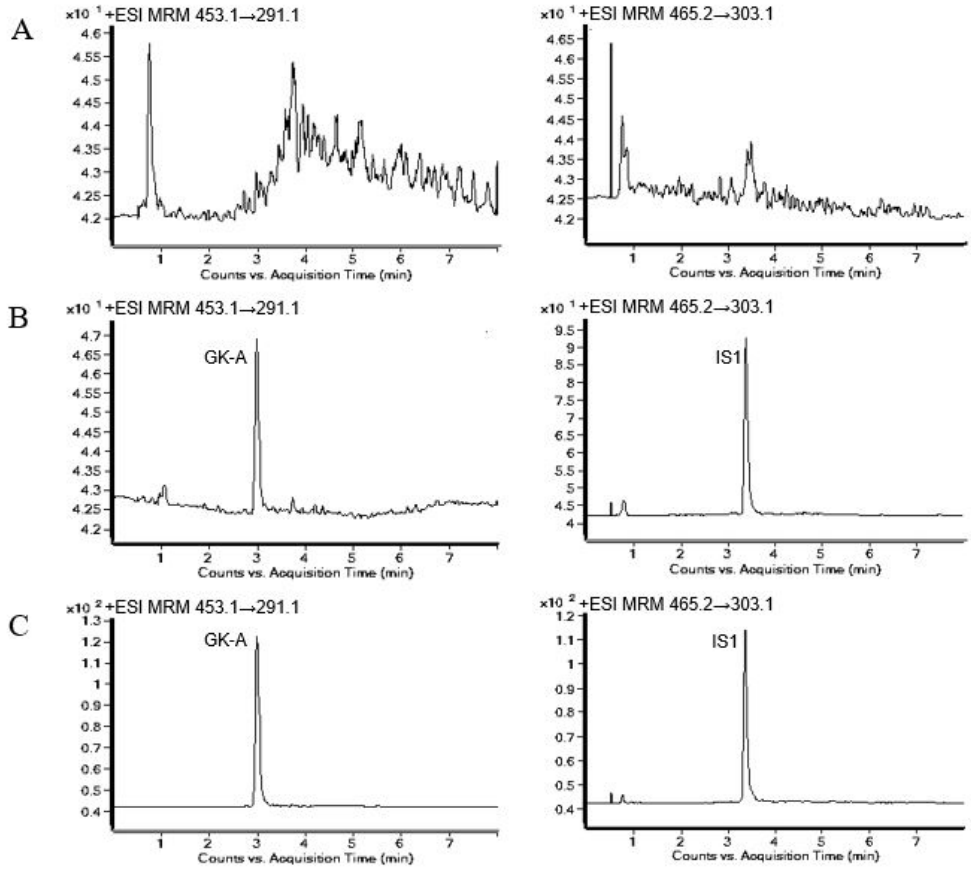

Fig. 3-1: Typical Chromatograms of IAsp-N-Glc and IS1 in rat plasma samples: (A) a blank plasma sample; (B) a blank plasma sample spiked with IAsp-N-Glc and IS1; (C) a rat plasma sample obtained 1.5 h after oral administration of IAsp-N-Glc.

apparent endogenous interference for the determination of the analytes, which suggested that the selectivity of the methods was satisfactory.

Linearity and LLOQ

In rat plasma, the typical regression equation for IAsp-N-Glc was y=0.554x-0.003 ranged from 37.5 ng/mL to 7500 ng/mL with a correlation coefficient (r^2) greater than 0.99, and the LLOQ was 37.5 ng/mL. In rat feces, the representative calibration curve for IAsp-N-Glc was y=0.355x+0.002 between 120 ng/mL and 30000 ng/mL with a correlation coefficient (r^2) higher than 0.99, and the LLOQ was 120 ng/mL.

Precision and accuracy

The results for precision and accuracy are summarized in table 2. For plasma samples, the intra-day precision (RSD%) was no more than 8.4% and the accuracy (RE%) between -13.0% and 4.6%. Similarly, the inter-day precision (RSD%) was within 7.6% and the accuracy (RE%) over the range of -6.0%-4.3%. For fecal samples, the intra-day precision (RSD%) was not greater than 3.4%, and the accuracy (RE%) ranged from 9.2% to 11.7%. Similarly, the inter-day precision (RSD%) was within 5.5%, and the accuracy (RE%) ranged from 1.0% to 4.4%. These results showed that the established methods were accurate and reproducible.

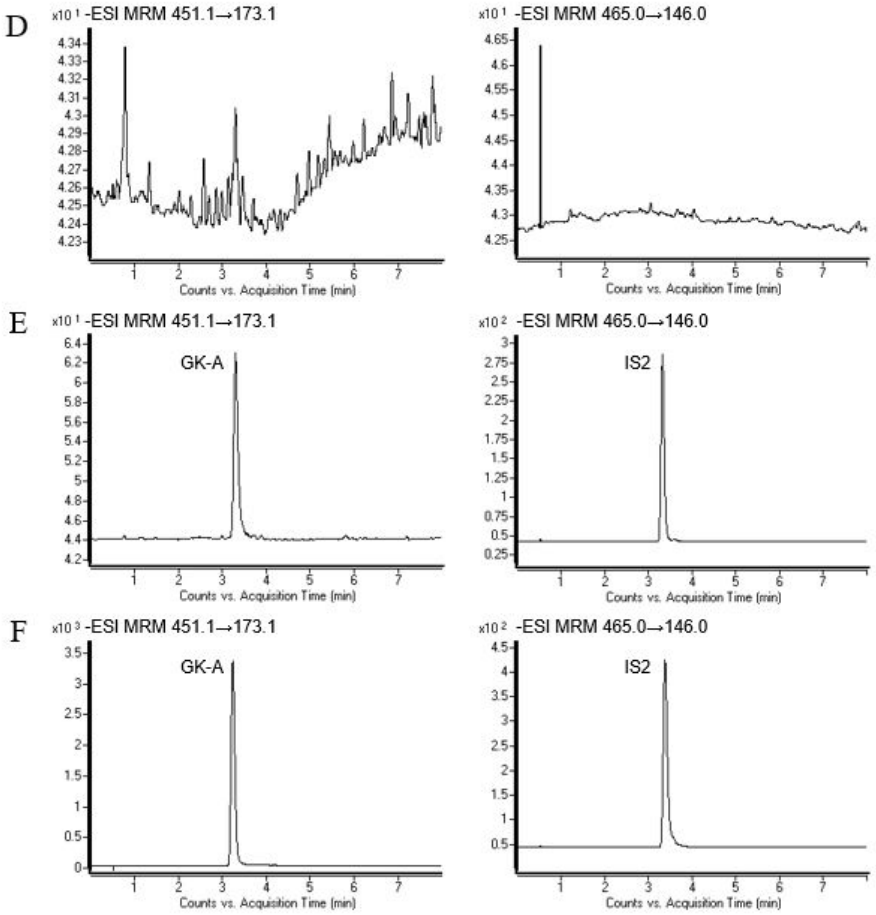

Fig. 3-2: Typical Chromatograms of IAsp-N-Glc and IS2 in rat feces samples: (D) a blank feces sample; (E) a blank feces sample spiked with IAsp-N-Glc and IS2; (F) a rat feces sample obtained 12 h after oral administration of IAsp-N-Glc.

Recovery and matrix effect

The results of extraction recovery and matrix effects are summarized in table 3. The extraction recovery of IAsp-N-Glc was between 91.5% and 93.2% in rat plasma samples and ranged from 92.1% to 99.0% in rat fecal samples. The matrix effect of IAsp-N-Glc was between 88.5% and 91.1% in rat plasma samples and ranged from 93.5% to 100.4% in rat fecal samples. These results showed that the extraction recoveries of IAsp-N-Glc were satisfactory, and no apparent matrix effects for the determination of IAsp-N-Glc in biological samples.

Stability

The stability data of IAsp-N-Glc under different storage conditions are displayed in table 4. According to the results, no apparent degradation of IAsp-N-Glc was observed, suggesting that IAsp-N-Glc was stable during storage and analysis.

Pharmacokinetics study

The established method was used to evaluate the pharmacokinetic study of IAsp-N-Glc in rat plasma after the oral and intravenous administration of IAsp-N-Glc. According to the measured concentration of IAsp-N-Glc in rat plasma, the mean plasma concentration-time curves were plotted and displayed in fig. 4. The main pharmacokinetic parameters of IAsp-N-Glc were analyzed by using DAS 3.0 with the non-compartmental model and summarized in table 5. The result indicated that the plasma concentration of IAsp-N-Glc was decreased and cleared from the circulation within 12 h after the intravenous administration. According to the results presented in table 5, we found that the maximum concentration (C_{max}) of IAsp-N-Glc in rat plasma after the oral administration were 237.242±78.119, 551.595±171.274, and 912.577±179.120 μg/mL at 1.667±0.683, 1.500±0.447, 1.500±0.447 h (T_{max}) for 15, 30, and 60 mg/kg dosing groups, respectively. Meanwhile, the AUC values for IAsp-N-Glc were 766.358±84.322, 1562.935±458.641, and 3432.168± 1001.544 μg/L·h for 15, 30, and 60 mg/kg oral administration dosing groups, showing an increasing trend as the dose increased. In addition, a dramatically lower clearance of the drug was observed following administration via the intravenous route (0.344±0.026, L/h/kg) than the following administration via the oral route (20.203±0.922, L/h/kg). The mean oral bioavailability (F)

Table 2: Precision and accuracy for IAsp-N-Glc in the QC samples of rat plasma and feces (n = 6).

Sample	QC concentration (ng/mL)	Intra-day		Inter-day	
		Accuracy RE (%)	Precision RSD (%)	Accuracy RE (%)	Precision RSD (%)
Plasma	60	-13.0	8.4	-6.0	7.6
	600	4.6	4.4	4.3	0.5
	6000	-0.1	3.6	0.8	2.9
Feces	240	10.8	3.4	4.4	3.8
	2400	9.2	1.0	1.0	5.4
	24000	11.7	2.6	1.1	5.5

Table 3: Recovery and matrix effect for IAsp-N-Glc in QC samples of rat plasma and feces (n = 6)

Sample	QC concentration (ng/mL)	Recovery (%)	RSD/%	Matrix effect (%)	RSD%
Plasma	60	93.2	6.1	91.1	4.3
	600	91.5	3.1	88.5	2.4
	6000	92.0	7.1	91.1	0
Feces	240	98.2	3.2	94.9	5.4
	2400	92.1	3.1	100.4	6.0
	24000	99.0	2.5	93.5	1.6

Table 4: Summary of the stability of IAsp-N-Glc in rat plasma and feces stored under different conditions (n = 6)

Sample	QC concen-tration (ng/mL)	Short-term stability		4°C for 24 h		automatic sampler for 24 h		Freeze-thaw stability		Long-term stability	
		Measured (ng/mL)	RSD %	Measured (ng/mL)	RSD %	Measured (ng/mL)	RSD %	Measured (ng/mL)	RSD %	Measured (ng/mL)	RSD %
Plasma	60	55.0	8.8	59.5	3.2	56.5	9.6	66.0	7.3	57.0	9.7
	600	622.0	3.6	625.5	3.7	632.5	5.3	637.0	6.4	572.5	5.5
	6000	5870.0	3.2	5595.0	3.1	6054.0	3.2	6383.0	3.8	5918.5	13.2
Feces	240	266.0	3.4	262.0	2.5	270.0	6.1	256.0	3.5	257.0	3.9
	2400	2622.0	1.0	2743.0	2.5	2610.0	1.9	2466.0	3.1	2495.0	1.0
	24000	26812.0	2.6	27411.0	1.8	25730.0	1.2	25258.0	2.0	24472.0	5.1

was calculated based on the AUC and dosage administered via the intravenous and oral routes. The bioavailability of IAsp-N-Glc was 1.83±0.09%. In conclusion, IAsp-N-Glc displayed a low bioavailability.

Excretion study

The concentrations of cumulatively excreted IAsp-N-Glc in feces were measured after the oral administration of IAsp-N-Glc (15 mg/kg) to rats, and the accumulative excretion ratio of IAsp-N-Glc by feces from 0 h to 72 h was shown in fig. 5. The cumulative excretion ratio of IAsp-N-Glc was 59.63±6.29% in feces within 72 h. Thus, the primary route of elimination of IAsp-N-Glc was excreted in the feces.

DISCUSSION

Ginkgo Semen has been widely used in China to relieve cough for hundreds of years. IAsp-N-Glc is first reported to be isolated from *Ginkgo Semen* and exerts an antitussive effect, as shown in our previous study. Information about the disposition of IAsp-N-Glc is essential for elucidating its therapeutic effectiveness. LC-MS/MS is a useful tool in pharmacokinetic research because of its selectivity and sensitivity (Jin *et al*., 2014). The aim of our study was to analyze the bioavailability and excretion of IAsp-N-Glc in rats by using UPLC-MS/MS. Plasma IAsp-N-Glc levels were determined in positive ion mode, and levels in fecal samples were determined in negative ion mode to obtain better separation and peak shapes. The pharmacokinetic parameters were calculated after the administration of IAsp-N-Glc in rats by intragastrical administration and intravenous injection. IAsp-N-Glc displayed poor oral bioavailability, and it was mostly excreted in rat feces. The absorption properties and metabolic characteristics of IAsp-N-Glc may directly influence the oral bioavailability, and further studies should be carried out to resolve these questions in the future. The modified structure of

Table 5: Pharmacokinetic parameters of IAsp-N-Glc in rat plasma

Parameters	Oral			Intravenous injection
	15 mg/kg (n=6)	30 mg/kg (n=6)	60 mg/kg (n=6)	1 mg/kg (n=6)
$AUC_{(0-t)}$ (μg/L·h)	766.357±84.322	1559.002±454.397	3348.778±971.554	2922.488±226.69
$AUC_{(0-\infty)}$ (μg//L·h)	766.358±84.322	1562.935±458.641	3432.168±1001.544	2922.488±226.69
$MRT_{(0-t)}$ (h)	2.541±0.351	2.477±0.202	3.217±0.367	0.779±0.168
$MRT_{(0-\infty)}$ (h)	2.541±0.351	2.501±0.239	3.503±0.800	0.779±0.168
$t_{1/2z}$ (h)	0.429±0.089	0.682±0.571	1.619±1.271	0.5±0.037
T_{max} (h)	1.667±0.683	1.500±0.447	1.500±0.447	-
Vz/F (L/kg)	12.118±2.382	19.12±12.238	39.459±27.212	0.23±0.008
CLz/F (L/h/kg)	19.781±2.27	21.483±9.64	19.346±8.049	0.344±0.026
C_{max} (μg/L)	237.242±78.119	551.595±171.274	912.577±179.120	2719.696±298.651
Absolute bioavailability (F)	1.75%	1.78 %	1.96%	-

Data were expressed as mean ± S.D.

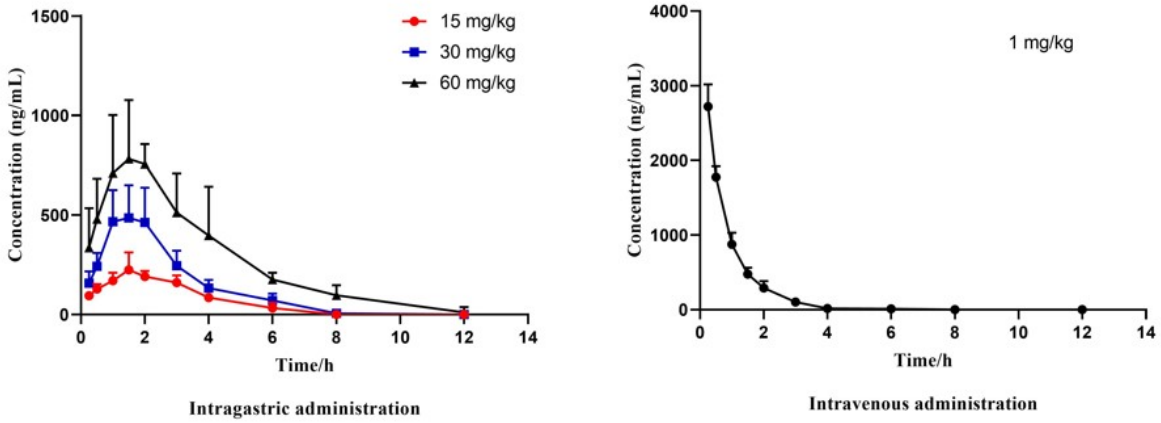

Fig. 4: Mean plasma concentration-time curves after oral (15, 30, and 60 mg/kg) and intravenous (1mg/kg) administration of IAsp-N-Glc in rats.

IAsp-N-Glc may achieve better bioavailability, and more related research is needed.

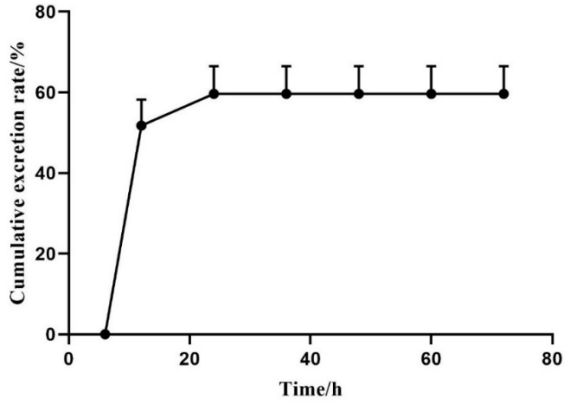

Fig. 5: Fecal excretion profile of IAsp-N-Glc (15 mg/kg) in rats after the oral administration.

CONCLUSIONS

The present study is the first report on the pharmacokinetics and excretion of IAsp-N-Glc. Rapid, sensitive, and selective UPLC-MS/MS methods were developed and validated to determine IAsp-N-Glc concentrations in rat plasma and fecal samples. The oral administration IAsp-N-Glc resulted in a poor absolute oral bioavailability 1.83±0.09%, and 59.63±6.29% of IAsp-N-Glc was excreted in the feces. According to the pharmacokinetic parameters of IAsp-N-Glc, IAsp-N-Glc was absorbed fast and eliminated rapidly in rats. IAsp-N-Glc was mostly excreted in the feces. This report provides essential disposition information for the further drug development of IAsp-N-Glc as a potential antitussive agent.

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