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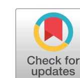
Original Article

Quantitative determination of monotropein in rat plasma and tissue by LC–MS/MS and its application to pharmacokinetic and tissue distribution studies



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ABSTRACT

A selective and sensitive liquid chromatography tandem with mass spectrometry was developed and validated for accurate determination of monotropein in rat plasma and tissues. All biological samples were prepared by simple protein precipitation method using catalpol as an internal standard. The analyte and internal standard were separated on a C_{18} analytical column with 2 min of run time, at flow rate of 0.5 ml/min. The detection was performed on a triple-quadrupole tandem mass spectrometer equipped with negative-ion electrospray ionization by selected-reaction monitoring of the transitions at *m*/*z* 389→147 for monotropein and *m*/*z* 361→169 for the internal standard. The calibration curves for plasma and tissue samples were linear over the concentration range of 4–2000 ng/ml, with a lower limit of quantification of 4 ng/ml. The method was successfully applied to a pharmacokinetic and tissue distribution study of monotropein in rats.

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Introduction

Monotropein (**1**) is an iridoid glycoside isolated from the root of *Morinda officinalis* F.C.How, Rubiaceae (Yang et al., 2016; Zhao et al., 2017a). Monotropein exhibits an evident anti-inflammatory effect that is mainly related to the inhibition of the expressions of inflammatory mediators *via* NF-κB inactivation (Shin et al., 2013). Furthermore, administration of monotropein exerts good bone protective effects by increasing bone mineral content and volume fraction, and improving bonemicro structure and biochemical properties in an ovariectomy mice model of osteoporosis (Zhang et al., 2016). Therefore, it may be a promising candidate for the prevention and treatment of osteoporosis. In addition, monotropein displays anti-apoptosis, antioxidant, antinociceptive and anti-catabolic activities *in vitro* (Choi et al., 2005; Wang et al., 2014, 2017; Zhu et al., 2016; Yang et al., 2017). As such, this compound has been extensively investigated. Therefore, it is important to establish analytical methods for the determination of monotropein in biological fluids and to study pharmacokinetics and tissue distribution in animals.

Several methods have been reported to analyze monotropein in plants and herbal prescriptions, including high-performance liquid chromatography (HPLC) (Yang et al., 2008; Liang et al., 2008; Zhao et al., 2017b), gas chromatography (Inouye et al., 1976), and HPLC coupled with mass spectrometry (Li et al., 2016a). Recently, an LC tandem with mass spectrometry (LC–MS/MS) method was reported to quantify monotropein and another iridoids glycoside deacetylasperulosidic acid in rats after consumption of *Morinda officinalis* extract (Li et al., 2016b), but the pharmacokinetic properties after given with pure monotropein still remain unknown so far. Here a sensitive and robust LC-MS/MS method was established and validated for the determination of monotropein in rat plasma and various tissues. The method was successfully applied to pharmacokinetics and tissue distribution of monotropein in rats after intragastric administration.

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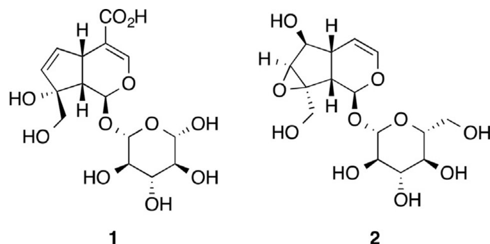
Materials and methods

Chemicals and reagents

Reference standards of monotropein (**1**) (purity 94.2%) and catalpol (**2**) (internal standard, IS; purity 98.1%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC-grade methanol and acetonitrile were purchased from Tedia Co. (Ohio OH, USA). Deionized water was prepared using a Milli-Q Purification System (Millipore, Bedford, MA, USA). All other reagents were of analytical reagent grade.



Instruments and chromatographic condition

The LC–MS/MS system was a Dionex Ultimate 3000 RSLC system equipped with a triple-quadrupole TSQ Vantage mass spectrometer (Thermo Scientific, San Jose, CA, USA). The stationary phase was a Thermo Scientific Hypersil GOLD C_{18} column (4.6 mm × 50 mm, 3.0 μm) with 40 °C column temperature. The mixture of methanol and water (50:50, v/v) was used as the mobile phase with an isocratic elution pattern at a flow rate of 0.5 ml/min. Mass spectral ionization, fragmentation and acquisition parameters were optimized by directly injecting monotropein and catalpol standard solutions in the negative ESI mode. Nitrogen was employed as the sheath and auxiliary gases at the pressures of 45 psi and 15 psi, respectively. Argon was employed as the collision gas. The data were acquired under selected-reaction monitoring (SRM) mode with precursor to product qualifier transition m/z 389→147 for monotropein at collision energy of 22 eV and m/z 361→169 for IS at collision energy of 20 eV, respectively (Fig. 1).

Preparation of standard and quality control (QC) samples

A stock solution of monotropein (**1**) (0.4 mg/ml) was prepared by dissolving 4 mg in 10 ml of methanol-water (50:50, v/v). A stock solution of catalpol (**2**, IS) was prepared in methanol-water at a concentration of 0.5 mg/ml and further diluted with methanol given the working solution at the concentration of 50 ng/ml. All stock solutions were stored at −60 °C until use. Calibration curves were prepared by spiking 10 μl of the appropriate standard solution with 190 μl of blank rat plasma or rat tissue homogenate. The effective calibration concentrations in all biological matrices were 4.0, 10, 20, 50, 200, 500, 1000 and 2000 ng/ml for monotropein. Another stock solution of monotropein (0.4 mg/ml) in methanol-water (50:50, v/v) was used to prepare QC samples. The QC samples were prepared in the same way as calibration standard at concentrations of 8 (low), 100 (medium) and 1600 ng/ml (high). 50 μl of the calibration standard and QC samples were then pipetted into plastic tubes and treated as '*Extraction of plasma and tissue samples*' section.

Extraction of plasma and tissue samples

Plasma or tissue homogenate (50 μl), IS (50 μl, 50 ng/ml) and acetonitrile (300 μl) were drawn into a plastic tube, vortexed for 3 min then centrifuged at 13,680 × *g* for 10 min. The organic layer was separated and evaporated to dryness at 40 °C under a nitrogen stream. The residue was reconstituted in 200 μl of the mobile phase and 5 μl was injected for LC–MS/MS analysis. Similarly, tissue homogenates were processed and analyzed by LC–MS/MS.

Method validation

The LC–MS/MS method was validated for carryover, selectivity, linearity, sensitivity, precision, accuracy, matrix effect, stability and extraction recovery in accordance with the Bioanalytical Method Validation of FDA (US Food and Drug Administration, 2001). Blank matrix samples obtained from six rats were screened to evaluate selectivity. Calibration curves were constructed by plotting the peak area ratios (y-axis) of the analyte to IS against the spiked concentrations (x-axis) and analyzed by weighted ($1/x^2$) least squares linear regression. The lower limit of quantification (LLOQ) was defined as the lowest concentration that yield a signal-to-noise ratio (S/N) greater than or equal to 10. Intra- and inter-day accuracy and precision were evaluated with five replicates at three QC levels on a single assay and on three consecutive validation days. Acceptable criteria were within 15% of precision and accuracy, except the LLOQ, which was within 20%. Recovery was determined by comparing peak areas of analyte in extracted QC samples with those of the spiked at corresponding concentrations to post-extracted blank matrix. Matrix effect was studied by comparing peak areas of analyte spiked in post-extracted blank matrix with those of spiked in post-extracted water. Stability was investigated under four different conditions as follows: short-term (ambient temperature for 6 h), long-term (−60 °C for 30 days), freeze and thaw (three cycles at −60 °C and room temperature), and post-preparation stability (autosampler at 4 °C for 12 h).

Stability study of monotropein in simulated gastric and intestinal fluids

Monotropein (**1**, 2000 ng/ml) was incubated in simulated gastric fluid (pH 1.2), and simulated intestinal fluid (pH 6.8) for 2 h in a shaking water bath (37 °C and 100 rpm). Organic content in the reaction mixture was kept within 0.5%. 100 μl of samples were taken at 0, 0.25, 0.5, 1.0, 1.5, and 2.0 h and quenched with 300 μl of acetonitrile containing 50 ng/ml IS (**2**). Samples were vortexed and centrifuged at 13,680 × *g* for 10 min. The organic layer was separated and evaporated to dryness at 40 °C under a nitrogen stream. The residue was reconstituted in the mobile phase and analyzed using the developed LC–MS/MS method.

Pharmacokinetic and tissue distribution studies

The animal study was approved by the Institutional Animal Ethical Committee of the First Hospital of Jilin University (Approval Number: 2017-123). For pharmacokinetic study, eighteen rats (220 ± 20 g) were randomly divided into three groups (six rats per group). Monotropein freshly prepared by suspending the required amounts in 0.5% sodium carboxymethyl cellulose (CMC-Na) solution was given to rats *via* intragastric administration at doses of 10, 20 and 40 mg/kg, respectively. Rats were fasted 12 h prior to the monotropein dose. Approximately 250 μl blood samples were collected into heparinized tubes from the tail vein into heparinized 1.5 ml polythene tubes at 0 (predose), 0.083, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12 and 24 h after intragastric administration. The samples were immediately centrifuged (860 × *g*, 10 min), then the supernatant layer was collected and stored at −60 °C until analysis. The pharmacokinetic parameters, including maximum plasma concentration (C_{max}), time reaching C_{max} (T_{max}), elimination half-life ($t_{1/2}$), area under the plasma concentration–time curve (AUC), and mean residence time (MRT) were calculated using DAS software (version 2.1, China State Drug Administration).

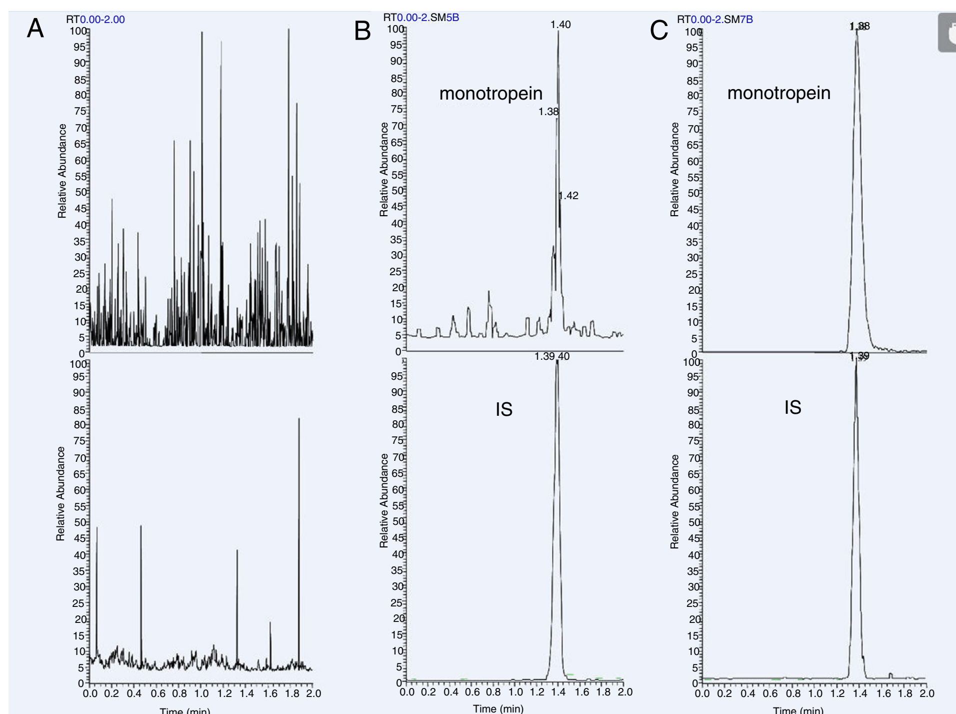


Fig. 1. Representative chromatograms of monotropein and IS in (A) blank plasma, (B) plasma spiked with monotropein and IS at LLOQ level and (C) a real plasma sample after oral administration of monotropein (20 mg/kg).

For tissue distribution study, twenty rats were randomly divided into four groups (n = 5/group). Rats were intragastrically administered 20 mg/kg monotropein that was suspended in 0.5% CMC-Na solution, then five rats were sacrificed for each 0.25, 1, 6 and 12 h time period. The heart, liver, spleen, lung, kidney, stomach, and small intestine were removed immediately, rinsed with saline and blotted dry. Small slices of tissues were individually homogenized in water (1:5, w/v) for LC–MS/MS analysis. All the remaining tissues were stored at −60 °C until further treatment.

Data analysis

Statistical analysis was performed using Origin 7.5 software for data calculating and mapping. All data were represented as mean ± standard deviation (mean ± SD).

Results and discussion

Optimization of mass spectrometry conditions

To optimize ESI conditions for monotropein (**1**) and IS (**2**), full-scan ESI mass spectra were achieved in the positive- and negative-ion modes. The results showed that ESI in the negative ions mode (ESI−) achieved higher sensitivity. Fig. 2 shows the product ion mass spectra of monotropein and IS in negative-ion mode. The major fragment ions observed in each product spectrum were at m/z 119, 135, 147, 165, 191 and 227 for monotropein. The m/z 147 fragment was dominant and therefore used for quantification. Similarly, the m/z 169 fragment was dominant for the IS and chosen for SRM scan. Finally, SRM was performed by monitoring the transitions of m/z 389→147 for monotropein at 22 eV and m/z 361→169 for IS at 20 eV.

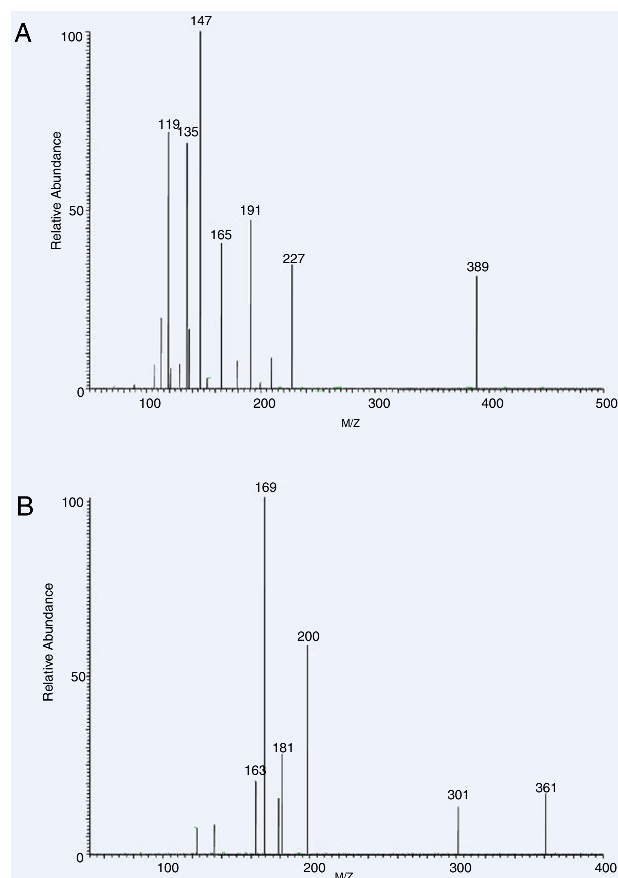


Fig. 2. Product ion mass spectra of monotropein (A) and IS (B) in negative ESI mode.

Table 1
Intra- and inter-day precision and accuracy, recovery and matrix effect for monotropein in rat plasma and tissues.

Biological samples	Nominal concentration (ng/ml)	Intra-day (n = 5)		Inter-day (n = 15)		Recovery (%)	Matrix effect (%)
		RSD (%)	RE (%)	RSD (%)	RE (%)		
Plasma	4.0	7.5	12.7	6.3	8.1	–	–
	8.0	1.6	−4.7	11.6	−0.7	95.2	102.9
	100	13.2	−5.4	7.7	−10.3	91.3	98.3
	1600	7.3	−0.8	13.8	2.0	91.4	97.1
Heart	4.0	5.2	14.0	2.0	11.2	–	–
	8.0	9.0	2.1	8.3	−8.2	94.0	92.5
	100	6.6	−3.1	14.2	−10.4	86.3	92.0
	1600	10.3	8.1	3.3	7.9	85.7	99.8
Liver	4.0	8.1	0.5	2.7	3.8	–	–
	8.0	0.5	−4.3	3.0	−3.2	83.3	100.3
	100	9.2	11.8	3.9	−12.2	90.5	101.4
	1600	14.1	8.2	12.8	6.3	85.1	105.2
Spleen	4.0	5.7	−2.3	0.6	8.9	–	–
	8.0	8.9	6.6	0.8	−6.3	93.7	93.7
	100	6.2	2.8	6.7	8.2	95.6	98.8
	1600	7.9	−14.0	4.3	−5.0	89.1	92.1
Lung	4.0	6.4	−2.1	6.7	−7.4	–	–
	8.0	5.2	−9.1	7.1	5.4	95.8	105.1
	100	13.6	−7.3	4.2	−12.0	87.4	97.1
	1600	8.3	8.8	2.3	8.4	94.1	97.0
Kidney	4.0	3.0	7.0	1.9	−1.2	–	–
	8.0	5.9	−9.9	7.2	6.4	91.5	103.9
	100	2.7	−11.2	2.7	4.3	92.8	96.0
	1600	3.9	−9.6	3.2	0.6	84.9	92.7
Stomach	4.0	10.8	9.2	13.5	7.1	–	–
	8.0	14.5	4.8	9.7	13.8	93.4	95.0
	100	10.2	1.5	4.6	−10.5	87.0	98.4
	1600	9.3	3.3	2.3	1.1	96.0	94.8
Intestine	4.0	2.3	1.5	4.9	−4.5	–	–
	8.0	12.1	−7.0	8.5	−8.1	87.2	98.4
	100	4.5	3.0	1.4	7.1	85.0	95.1
	1600	7.8	14.9	2.0	6.4	84.5	104.1

Method validation

Selectivity

The selectivity of the method was determined by screening six different sources of drug-free rat plasma. Typical SRM chromatograms of blank rat plasma, spiked plasma with the analyte at LLOQ level (4 ng/ml) and IS (50 ng/ml), and plasma samples from rats are shown in Fig. 1. Results indicated that no significant endogenous interference was observed for the determination of monotropein and IS in plasma and tissue homogenate.

Carryover, linearity and lower limit of quantification

The maximal peak area of carryover samples were ≤5.7% for analyte and 0.2% for IS (**2**) of the peak area at LLOQ, respectively. As a result, the carryover effect for monotropein and IS was not detectable with the chosen LC–MS/MS determination. A linear response in the peak area ratios was observed over a concentration range of 4–2000 ng/ml in rat plasma and tissues samples. The best linear fit and least-squares residuals for the calibration curve were achieved with a weighting factor of $1/x^2$. The coefficient of determination (r^2) obtained from plotting the ratio of analyte/IS peak area against the nominal concentration were greater than 0.99 in each run. The LLOQ of monotropein was determined to be 4 ng/ml (S/N ≥10) in all biological matrices, the precision and accuracy of LLOQ were less than 13.5% and within ±14.0%. The LC–MS/MS assay was sensitive enough for the pharmacokinetic and tissue distribution studies of monotropein after intragastric administration in rats.

Precision and accuracy

The intra- and inter-day precision and accuracy of monotropein in plasma and tissue homogenates at three QC concentrations were well within the acceptable limits. Results indicated that the developed method was accurate and precise for the quantification of monotropein in rat plasma as well as other tissue matrices over the established concentration ranges (Table 1).

Recovery and matrix effects

The extraction recovery and matrix effect for monotropein in different biological matrices are shown in Table 1. The recovery of monotropein in all biological matrices was consistent and reproducible. The matrix effect for monotropein in all biological matrices was considered as negligible for ion suppression or enhancement from all biological matrices.

Stability

Monotropein was found to be stable in plasma and tissue homogenates under different storage conditions that could be undergone during the entire experimental process. Results demonstrates that no significant loss was observed under the investigated storage conditions (Table 2). The deviations (RE%) of the calculated concentrations for three QC levels were within ±14.8% from their nominal concentration value. And the stability of stock solution was also excellent with RE% values ranged from −2.5% to 0.7% after storage at 4 °C for four weeks (Data not shown). Thus, the results were found to be within the acceptable limits during the entire validation.

Stability study of monotropein in simulated gastric and intestinal fluids

Results for stability were determined as percent parent remaining at different time points relative to the parent at 0 min. The

Table 2
Stability data for monotropein in tissue and plasma ($n = 5$).

Biological samples	Nominal concentration (ng/ml)	Short-term stability (ambient temperature for 6 h)		Long-term stability (at −60 °C for 30 days)		Three free–thaw stability		Post-preparation stability (autosampler at 4 °C for 12 h)	
		RE%	RSD%	RE%	RSD%	RE%	RSD%	RE%	RSD%
Plasma	8.0	−8.3	8.9	−7.8	8.5	9.2	7.8	9.8	8.5
	100	−3.5	6.5	7.1	2.5	−13.8	6.4	−3.3	9.4
	1600	11.2	11.0	−2.3	5.7	2.3	14.2	1.7	11.0
Heart	8.0	2.0	5.0	−4.8	6.9	6.9	7.5	−4.7	12.8
	100	1.8	3.5	−10.2	9.8	−9.5	1.6	−14.8	10.0
	1600	0.6	11.6	11.7	1.7	8.7	9.9	−8.2	3.5
Liver	8.0	−12.0	5.8	−2.6	13.0	−6.0	2.4	−5.6	4.6
	100	13.6	2.7	0.4	6.8	−0.5	3.0	11.0	9.5
	1600	8.0	1.6	−8.8	6.9	−13.2	12.2	7.9	1.7
Spleen	8.0	5.5	7.8	14.5	7.8	6.2	4.2	−4.3	7.9
	100	−4.2	12.2	10.1	5.2	14.4	3.5	−12.4	1.6
	1600	−5.0	12.8	−6.7	2.6	14.6	5.5	1.3	6.0
Lung	8.0	−5.1	6.6	3.9	3.1	12.5	6.3	7.7	2.8
	100	0.9	2.5	−4.4	0.7	4.2	3.2	−5.4	11.6
	1600	−5.5	7.7	−2.3	1.9	−9.1	7.3	2.1	1.5
Kidney	8.0	3.9	10.2	−9.8	10.5	5.2	12.9	10.0	1.9
	100	2.6	1.3	11.6	2.4	13.6	12.0	10.1	1.2
	1600	3.7	2.1	2.4	4.6	2.4	11.2	−5.8	3.1
Stomach	8.0	10.6	1.2	4.5	7.2	6.8	9.5	−6.6	11.6
	100	8.1	10.9	−3.9	2.5	7.9	13.0	7.0	3.0
	1600	−11.5	4.0	−11.7	11.0	−5.3	6.5	−8.8	4.1
Intestine	8.0	2.9	4.3	−12.6	9.6	4.9	8.7	12.4	6.4
	100	−6.0	8.9	3.5	11.6	−1.7	6.5	6.9	1.5
	1600	9.8	5.4	8.4	1.8	−0.8	2.5	6.5	6.6

Table 3
Pharmacokinetic parameters of after intragastric administration of monotropein to rats ($n = 6$).

Parameters	Unit	*p.o.*		
		10 mg/kg	20 mg/kg	40 mg/kg
C_{max}	ng/ml	112.1 ± 32.3	188.9 ± 37.9	327.6 ± 62.4
T_{max}	h	1.4 ± 0.4	1.3 ± 0.4	1.3 ± 0.5
$t_{1/2}$	h	2.9 ± 1.1	3.9 ± 1.6	4.5 ± 1.4
AUC_{0-t}	ng h/ml	547.9 ± 285.8	1114.0 ± 599.1	2086.6 ± 628.5
$AUC_{0-\infty}$	ng h/ml	629.8 ± 332.2	1217.8 ± 603.3	2164.1 ± 637.2
CL/F	l/h/kg	21.8 ± 14.6	22.0 ± 15.3	20.6 ± 8.6
V_d/F	l/kg	76.6 ± 25.3	101.4 ± 31.7	125.7 ± 38.1

remaining monotropein at different time points was calculated to be 95.3–98.9% amounts of the parent drug at 0 min, indicating that monotropein was stable in simulated gastric fluid (pH = 1.2) and simulated intestinal fluid (pH=6.8).

Pharmacokinetic study

The LC–MS/MS method was successfully applied to investigate the pharmacokinetics of monotropein following intragastric administration of a single dose (10, 20 and 40 mg/kg). The main pharmacokinetic parameters calculated using DAS software non-compartmental analysis are shown in Table 3. Mean plasma concentration-time curves ($n = 6$) are presented in Fig. 3. It can be seen that monotropein was rapidly absorbed after intragastric administration in rats, with the time reaching peak concentration (T_{max}) values ranging from 1.3 to 1.4 h and the elimination half-time ($t_{1/2}$) from 2.9 to 4.5 h among the different groups. The maximum plasma concentrations (C_{max}) of monotropein were about 112.1 to 327.6 ng/ml. The C_{max} and $AUC_{0\text{-}t}$ appeared linear over the dose ranges of 10–40 mg/kg.

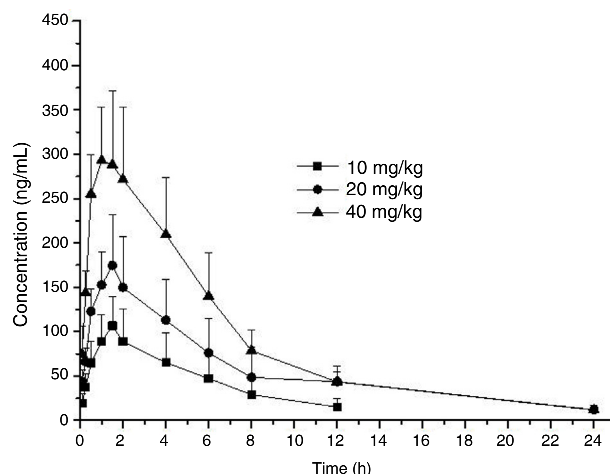


Fig. 3. Mean plasma concentration–time curves of monotropein after intragastric administration of monotropein in rats (mean ± SD, $n = 6$).

Tissue distribution study

The concentrations of monotropein in heart, liver, speen, lung, kidney, stomach and small intestine at the indicated times are shown in Fig. 4 following intragastric administration of monotropein (20 mg/kg) to rats. The results indicated that monotropein was distributed rapidly and widely in the tested tissues in the order of kidney > stomach > small intestine > liver > heart > lung > spleen, and eliminated quickly in which there was no long-term accumulation in the tested tissues. The levels of monotropein in spleen were much lower than those in other

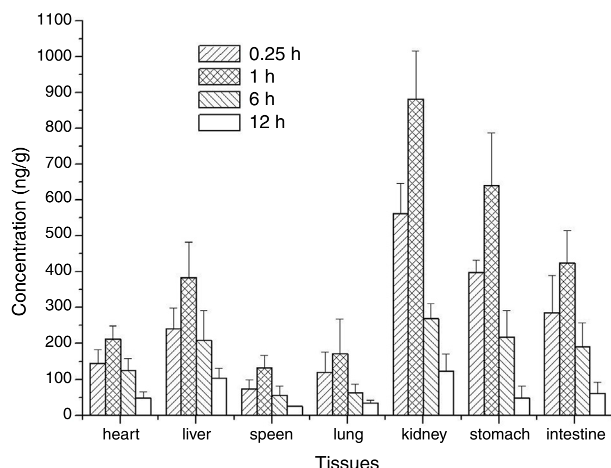


Fig. 4. Concentrations of monotropein in rat tissues at 0.25 h, 1 h, 6 h and 12 h after intragastric administration of 20 mg/kg monotropein in rats (mean ± SD, $n = 5$).

tissues. The high levels in kidney indicated that large quantity of monotropein was prone to be eliminated through kidney.

Conclusions

A simple and sensitive LC–MS/MS method for the determination of monotropein in rat plasma and tissues was developed at the first time. It was demonstrated that monotropein in rats was easily absorbed and moderately eliminated after intragastric administration. Tissue distribution showed that this compound could be distributed widely, and penetrated into all of the tissues tested. These pharmacokinetics and tissue distribution results provided useful information for the development of a drug containing monotropein, and for clinical rational use of *Morinda officinalis* preparations.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contributions

QZ and JW wrote the manuscript. HY and RL carried out the data analyses. XL and JW designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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