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# Quantification of mangiferin in streptozotocin-induced diabetic rat plasma using UPLC–MS/MS: Pharmacokinetic assessment of *Gentiana rhodantha* extract after oral administration

**Ying Zhao[1] and Jian-Wu Li[2]***
[1]Department of Pharmacy, Shaanxi Provincial People's Hospital, Xi'an, Shaanxi, China
[2]Department of Burns And Plastic Surgery, Shaanxi Provincial People's Hospital, Xi'an, Shaanxi, China

**Abstract**: Mangiferin, a key bioactive constituent in *Gentiana rhodantha*, has a favorable impact on reducing blood sugar. A selective and sensitive UPLC MS/MS approach was developed for determining mangiferin in diabetic rats. Employing acetonitrile protein precipitation, chromatographic separation utilized a 2.1×50 mm, 3.5 μm $C_{18}$ column with a mobile phase of 0.1% formic acid aqueous and 5 mM ammonium acetate (A, 45%) and acetonitrile (B, 55%) at a 0.5 mL $min^{-1}$ flow rate. Quantification, employing the multiple reaction monitoring (MRM) mode, focused on precursor-to-product ion transitions at m/z 447.1→271.1 for baicalin m/z and 421.0→301.0 for mangiferin. Calibration curves demonstrated linearity in the 1.00~100 ng/mL range, with a lower quantification limit for rat plasma set at 1.00 ng/mL. Inter- and intra-day accuracies spanned -9.1% to 8.5%, and mangiferin mean recovery varied from 82.3% to 86.7%. The adeptly utilized UPLC-MS/MS approach facilitated the exploration of mangiferin pharmacokinetics in diabetic rats.

**Keywords**: *Gentiana rhodantha*; mangiferin, diabetes mellitus, antihyperglycemic effect, pharmacokinetics.

## INTRODUCTION

Diabetes, a persistent metabolic disorder (Wang *et al.*, 2022; Refardt *et al.*, 2020), arises from insufficient endogenous insulin in type-1 diabetes or impaired insulin function in type-2 diabetes (T2DM) (Majety *et al.*, 2023). Notably, Damanik and Yunir (2021) report that 90% of diabetes cases are T2DM. In 2014, World Health Organization data revealed over 422 million adults globally grappling with diabetes mellitus (DM), marking an upward trend (Lovic *et al*., 2020). Current DM therapies, including insulin and oral agents, face challenges such as secondary failure and adverse effects(Sun *et al.*, 2021; Darenskaya *et al.*, 2021), making T2DM prevention a pivotal clinical challenge.

Traditional Chinese medicine (TCM) shows promise in treating DM and its complications, with clinical efficacy highlighted by Kavaz *et al*. (2018, 2021). Network pharmacology research by Zhang *et al*. (2022) underscores TCM's systemic effects on the diabetes disease network, offering potential personalized treatments based on pathological features (Liu *et al.*, 2023). These findings emphasize TCM's potential in diabetes treatment.

*Gentiana rhodantha*, a significant herb in Chinese and ethnic medicines, is widely used in Southwest China for T2DM (Zhang *et al.*, 2023). Mangiferin, a key compound, effectively lowers blood sugar (Wang *et al.*, 2022). However, the anti-diabetic mechanism and pharmacokinetics of *Gentiana rhodantha* in T2DM rats remain unclear. This study aimed to establish a sensitive analytical method for understanding mangiferin's pharmacokinetics in diabetic rats.

**Corresponding author:* e-mail: 545078642@qq.com

## MATERIALS AND METHODS

### *Materials, reagents and kits*

*Gentiana rhodantha* was provided by Tongrentang Pharmacy (Beijing, China). The China Institute for Food and Drug Control supplied mangiferin (Lot No. 111607-201704), characterized by a purity exceeding 98% through HPLC. Streptozotocin (STZ) was supplied by Sigma Chemical Co. (St. Louis, MO, USA). Kits for glucose, HDL, LDL, and TG were obtained from Huili Biotech Co., LTD. (Changchun, China). Kemiou Chemical Reagents Co. (Tianjin, China) and Concord Technology (Tianjin, China) provided HPLC-grade formic acid and acetonitrile (CAN), respectively.

### *Gentiana rhodantha component preparation*

Utilizing a water-to-*Gentiana rhodantha* (whole grass) ratio of 5-fold (w/v), 100 g of the herb underwent a 1-h boiling process, which was repeated twice. Following filtration, the combined filtrates from both batches were evaporated, yielding *Gentiana rhodantha* extract at 7.13% (w/w).

### *Induction of diabetes by STZ*

Streptozotocin induced DM in male SD rats. STZ was injected via intraperitoneal injection at 40 mg/kg to induce stable diabetes, following a fasting period of at least 12 h. A reagent kit was used to determine fasted plasma glucose (FPG) levels 9 days post-STZ injection. Rats with FPG levels exceeding 11.1 mM qualified as diabetic rats were included in the subsequent experiments

(Zarin *et al.*, 2019). The T2DM rats were assigned to three groups randomly: positive group (diabetic group treated with metformin at 0.5 g/kg), *Gentiana rhodantha* group (diabetic group treated with *Gentiana rhodantha* at 9 g/kg), and diabetic model group.

***Pharmacokinetic studies of Gentiana rhodantha***

Retro-orbital bleeding was performed to collect blood samples from each rat before dosing and at specific time intervals (0.083, 0.17, 0.25, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, and 24.0 h) post-dosing into heparinized Eppendorf tubes on ice. Following collection, these tubes underwent a 10-min centrifugation at 13,000 rpm.

***Sample preparation and UPLC–MS/MS conditions***

Combining 50 µL of rat plasma with a 0.1 µg/mL baicalin internal standard (IS) solution initiated a 1-min vortexing process. Protein precipitation followed, with the addition of 150 µL acetonitrile, a 2-min vortex-mixing session, and centrifugation at 12,000 rpm for 10 min. The resulting supernatant was collected and subjected to an additional 5-min centrifugation at 12,000 rpm. UPLC-MS/MS analysis was performed using 5 µL of each sample.

The UPLC-MS/MS, featured an API 4000 mass spectrometer and utilized the Analyst 1.4.2 data processing system (AB SCIEX, USA). Chromatographic separation was performed on an Agilent ZORBAX-SB $C_{18}$ column (2.1 × 50 mm, 3.5 µm). With a flow rate of 0.5 mL $min^{-1}$, ion source temperature maintained at 550 °C, electrospray voltage set at 5.5 kV, employing a mobile phase of 0.1% formic acid aqueous and 5 mM ammonium acetate (A, 45%) and acetonitrile (B, 55%) , the analysis was conducted in a selected reaction monitoring mode.

***Validation of UPLC−MS/MS analytical method***

The method was validated as described previously (Zhao *et al.*, 2016) regarding specificity, linearity, sensitivity, precision, accuracy, recovery and matrix effect. Long-term stability was determined after storing QC samples at -20 °C for 30 days, freeze-thaw stability after three cycles, bench-top stability by keeping QC samples at 25 °C for 8h and post-preparative stability by placing processed QC samples in an autosampler (20 °C) for up to 24 h.

***Ethical approval***

Animal experiments at Gansu University of Traditional Chinese Medicine followed approved protocols by the Animal Ethics Committee [SYCK (Gan) 2015-0005].

## STATISTICAL ANALYSIS

Data are expressed as mean ± standard deviation (SD). An analysis of mean differences was conducted using the One-Way ANOVA test via SPSS 16.0 software. $P<0.01$ was considered significant difference.

## RESULTS

***Impact of Gentiana rhodantha on blood glucose levels in diabetic rat***

Blood glucose levels noticeably rose in STZ-induced diabetic rats compared to normal rats, during the experiment. *Gentiana rhodantha* administration demonstrated effective reduction of blood glucose levels on the 21st day, decreasing them from 16.5 to 8.20 mM (fig. 1).

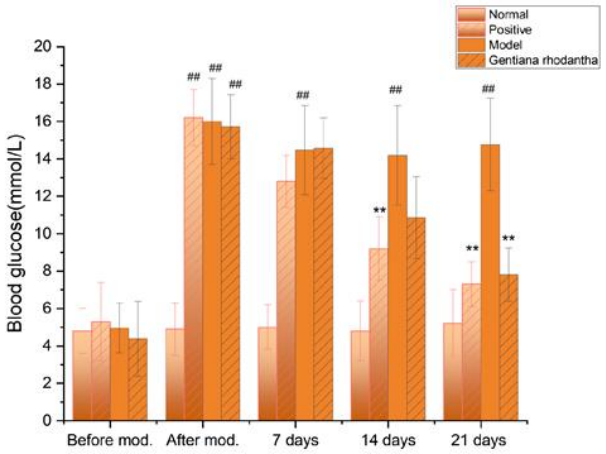


**Fig. 1**: Impact of *Gentiana rhodantha* on fasted blood glucose levels in diabetic rats. Data are presented as mean±SD (n=8). $^{\#\#}P<0.01$, compared to normal group; $^{**}P<0.01$, compared to the model group.

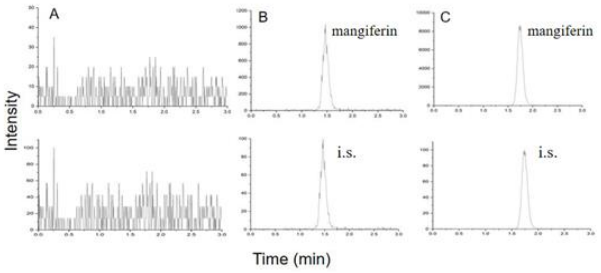


**Fig. 2**: SRM chromatograms of mangiferin and I.S. in rat plasma: (A) blank rat plasma; (B) blank plasma spiked with mangiferin and I.S.; (C) rat plasma 4 h after oral *Gentiana rhodantha* administration.

***Influence of Gentiana rhodantha on lipid levels in diabetic rat***

Rats displayed a significant reduction in LDL and TG levels when compared to the diabetic model group, after 21 days of administering *Gentiana rhodantha* (table 1). Specifically, TG levels decreased by 32.4%, and LDL levels exhibited a noteworthy decline of 44.0%. On the other hand, as can be seen in table 1, HDL-C levels in the serum returned to normal levels following the 21-day *Gentiana rhodantha* intervention. These results substantiate the efficacy of *Gentiana rhodantha* in modulating lipid metabolism in diabetic rats.

***Development of UPLC−MS/MS analytical method***

Ionization mode was optimized by a comprehensive analysis of MS fragmentation patterns for mangiferin and the internal standard. Positive ionization mode successfully attained satisfactory MS sensitivity for all analytes. Quantification utilized MRM, focusing on precursor-to-product ion transitions at m/z 421.0 → 301.0 for mangiferin and m/z 447.1 → 271.1 for baicalin. Baicalin was used as the IS due to its non-interaction with mangiferin and its stable signal intensity.

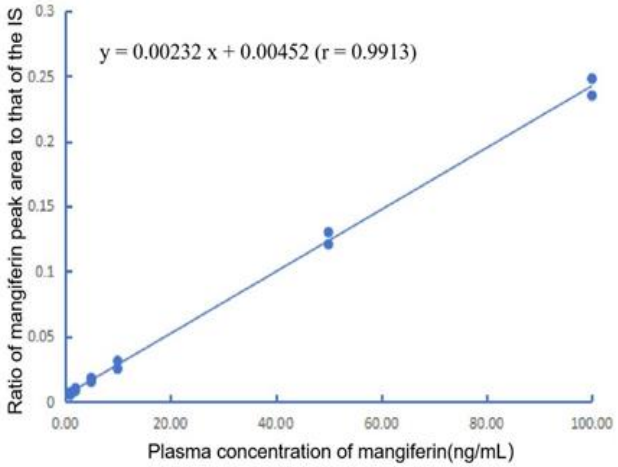


**Fig. 3**: Calibration curve of mangiferin in plasma

Comparative assessment of standard organic solvents in HPLC, specifically methanol and ACN, revealed ACN's superior effectiveness in compound separation. Consequently, ACN was adopted as the mobile phase. In the aqueous phase, the addition of 5mM ammonium acetate with 0.1% formic acid displayed optimal ionization efficiency for mangiferin.

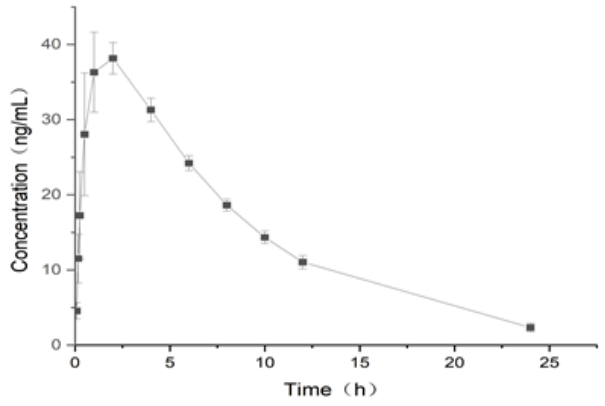


**Fig. 4**: Mangiferin plasma concentration–time profiles after a single oral dose of 9 g/kg *Gentiana rhodantha* in diabetic rats (n = 6, mean±SD)

***Validation of UPLC−MS/MS analytical method***

No interference emerged from endogenous substances at mangiferin retention times (fig. 2). This observation underscores the UPLC–MS/MS conditions' robust selectivity for the analyte.

The calibration curve was shown in fig. 3. Mangiferin's lower limit of quantitation (LLOQ) was established at 1.0ng/mL, with all back-calculated standard points exhibiting deviations below 13.6%, confirming strong linearity.

Inter- and intra-day precisions, expressed as RSDs, for the investigated compound consistently remained below 15%. Accuracies within inter- and intra-day ranges were -9.1% to 8.5%, as detailed in table 2.

Mangiferin mean recovery ranged from 82.3% to 86.7% (table 3). The mean matrix effects of mangiferin were ranged from 92.5% to 93.4%. The IS demonstrated a extraction recovery and mean matrix effect of 81.0% and 93.1%, respectively. These values align with acceptable standards, confirming the reliability and reproducibility of the mangiferin determination method in STZ-induced diabetic rat plasma.

As shown in table 4, stability analysis demonstrated no remarkable degradation under all tested conditions.

***Pharmacokinetic study***

Utilizing the validated UPLC–MS/MS approach, we conducted pharmacokinetic analysis post *Gentiana rhodantha* extract oral administration in male rats (n = 6). fig. 4 displays profiles detailing the mean mangiferin concentration in plasma versus time. Employing the non-compartment model, we calculated key pharmacokinetic parameters (mean residence time (MRT), area under concentration-time curve (AUC), time to reach maximum concentrations (Tmax), maximum plasma concentration (Cmax), and half-time ($T_{1/2}$)) , presented in table 5. This study represents the first exploration of mangiferin pharmacokinetic parameters in *Gentiana rhodantha*.

## DISCUSSION

Traditional Chinese medicine, with a long history, offers distinct advantages in diagnosing and treating T2DM (Li *et al*., 2021). Although widely applied in Southwest China for T2DM treatment, *Gentiana rhodantha*'s antihyperglycemic activity remains unstudied. To address this, we employed STZ-induced diabetic rats, a validated model reflecting human diabetes pathophysiology due to STZ selectively targeting pancreatic β-cells (Yin *et al.*, 2023). The study aimed to assess *Gentiana rhodantha*'s antidiabetic properties, utilizing metformin as a positive control for hypoglycemic activity (Foretz *et al.*, 2023). *Gentiana rhodantha* treatment significantly achieved the primary therapeutic goal of diabetes, reducing blood glucose levels and providing direct evidence of its hypoglycemic effect.

**Table 1**: Effects of treatments on TG, HDL-C and LDL-C in diabetic rats

| Groups | TG (mmol/L) | HDL-C (mmol/L) | LDL-C (mmol/L) |
|---|---|---|---|
| Normal group | 0.30±0.04 | 0.93±0.18 | 2.24±0.63 |
| Model group | 0.68±0.34## | 0.69±0.23# | 2.59±0.34# |
| Positive group | 1.23±0.50* | 0.79±0.22* | 2.32±0.71* |
| *Gentiana rhodantha* group | 0.46±0.12** | 0.83±0.20* | 1.45±0.13** |

Values (mean ± SD) for 8 rats / group; # $p < 0.05$ vs. normal control; ## $p < 0.01$ vs. normal control; *$p < 0.05$ vs. model group; **$p < 0.01$ vs. model group

**Table 2**: Inter-day and intra-day accuracies and precisions of mangiferin in QC samples prepared in rat plasma (n = 3 days).

| Analyte | Spiked concentration (ng/ml) | Intra-day precision RSD (%) | Intra-day accuracy RE (%) | Inter-day precision RSD (%) | Inter-day accuracy RE (%) |
|---|---|---|---|---|---|
| Mangiferin | 2.0 | 6.7 | -9.1 | 5.9 | 4.7 |
| | 10 | 4.7 | 8.5 | 6.1 | -9.8 |
| | 80 | 5.6 | 3.3 | 4.3 | 3.2 |

**Table 3**: Mangiferin and I.S. extraction recovery and matrix effect in rat plasma (n = 6).

| Analyte | Spiked concentration (ng/ml) | Recovery (%) | RSD (%) | Matrix effect (%) | RSD (%) |
|---|---|---|---|---|---|
| Mangiferin | 2.0 | 82.3± 3.5 | 4.3 | 92.5 ± 6.1 | 6.6 |
| | 10 | 86.7± 4.3 | 5.0 | 92.8 ± 5.6 | 6.1 |
| | 80 | 82.9±4.2 | 5.1 | 93.4 ± 4.7 | 5.1 |
| IS | 100 | 81.0± 3.1 | 3.9 | 93.1± 5.2 | 5.6 |

**Table 4**: Stability of mangiferin in rat plasma (mean ± SD, n = 3).

| Concentration (ng/ml) | mangiferin | | |
|---|---|---|---|
| | 2.0 | 10 | 80 |
| Post-preparative stability | | | |
| Found (ng/ml) | 1.80±0.23 | 9.27±0.98 | 72.31±4.25 |
| RSD (%) | 12.8 | 10.6 | 5.9 |
| RE (%) | -10.0 | -7.3 | -9.6 |
| Bench-top stability | | | |
| Found (ng/ml) | 1.85±0.15 | 9.32±0.76 | 78.25±3.68 |
| RSD (%) | 8.1 | 8.2 | 4.7 |
| RE (%) | -7.5 | -6.8 | -2.2 |
| Freeze–thaw stability | | | |
| Found (ng/ml) | 2.15±0.19 | 10.85±1.07 | 83.10±6.12 |
| RSD (%) | 8.9 | 9.9 | 7.4 |
| RE (%) | 7.5 | 8.5 | 3.9 |
| long-term stability | | | |
| Found (ng/ml) | 1.90±0.21 | 9.87±0.75 | 82.50±4.26 |
| RSD (%) | 11.1 | 7.6 | 5.2 |
| RE (%) | -5.0 | -1.3 | 3.2 |

**Table 5**: Pharmacokinetic parameters of mangiferin after *Gentiana rhodantha* administration (mean ± SD, n = 6).

| $T_{1/2}$ (h) | Tmax (h) | Cmax (µg/L) | AUC (0-t) (µg/L*h) | AUC (0-∞) (µg/L*h) | MRT (0-t) (h) | MRT (0∞) (h) | CLz/F (L/h/kg) |
|---|---|---|---|---|---|---|---|
| 5.369±1.5 | 1±0.00 | 45.817±3.1 | 369.309±12.76 | 387.802±17.98 | 6.662±0.31 | 7.887±0.92 | 51.667±2.44 |

Elevated plasma fatty acid contents in individuals with pre-diabetes and diabetes have been documented (Khan *et al.*, 2019). The TG/HDL-C ratio, linked to insulin resistance, metabolic syndrome, and obesity (Belladelli *et*

*al.*, 2022), offers a valuable tool for understanding disease pathology and monitoring pharmacological therapy. *Gentiana rhodantha* exhibited a reduction in LDL-C and TG levels and an augment in HDL-C levels, enhancing overall health and reinforcing its hypoglycemic potential.

The UPLC−MS/MS approach, validated per FDA guidelines for bioanalytical method validation, employed protein precipitation (PPT) and liquid-liquid extraction (LLE) for sample pre-treatment. The PPT method demonstrated satisfactory extraction efficiency for mangiferin and IS, ultimately selected for preparation of samples. This efficient method shows promise for pharmacokinetic studies of *Gentiana rhodantha* mangiferin in STZ-induced diabetic rats.

Mangiferin absorption from the rat gastrointestinal tract was detected at 0.083 h in plasma post oral administration of *Gentiana rhodantha* extract. The maximum concentration ($T_{max}$) occurred at 1.00 h, with blood concentration surpassing that of mangiferin alone, possibly due to synergistic interactions among TCM ingredients.

## CONCLUSION

*Gentiana rhodantha* demonstrates potential anti-hyperglycemic effects in diabetic rats induced by STZ, resulting in reduced levels of blood glucose and lipid metabolism modulation. A UPLC–MS/MS method for quantification of mangiferin in diabetic rat plasma was established. This analytical approach, known for its selectivity and reliability, was successfully applied for the first time to assess mangiferin pharmacokinetics post oral *Gentiana rhodantha* administration. The resulting pharmacokinetic profile of mangiferin in *Gentiana rhodantha*-treated rats serves as a fundamental aspect in understanding the plant medicine's metabolic mechanisms.

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