Short communication

Determination of mangiferin in rat plasma by liquid–liquid extraction with UPLC–MS/MS

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ABSTRACT

An ultra-performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) method employing electrospray ionization (ESI) has been developed for the determination of mangiferin in rat plasma using diphenhydramine as the internal standard (IS). Liquid–liquid extraction (LLE) was used for sample preparation and the analysis was achieved with gradient elution on C_{18} reversed phase column. The method was validated over the concentration range 0.02–5.0 µg/mL for oral administration and 0.4–100 µg/mL for intravenous administration. The intra-day and inter-day precision of mangiferin expressed as RSD < 15% and the accuracy (RE) did not exceed 15%. This validated method is a novel technique for sample preparation and quantitation, which was successfully applied to estimate the bioavailability of mangiferin.

1. Introduction

Mangiferin, 1,3,6,7-tetrahydroxyxanthone-C-2-β-ᴅ-glucoside, is a natural glucosyl xanthone from the Chinese medicinal herb, *Mangifera indica* [1], and it has also been isolated from other medical plants. Researchers have reported that mangiferin exhibits a variety of pharmacological effects including antibacterial [2], immuno-modulatary [3] and anticancer [4]. Recently, more attention has been paid to the antidiabetic activity of mangiferin [5–7] and the results suggest that mangiferin is a promising oral treatment for diabetes. However, the clinical application of the drug is greatly restricted due to its poor absorption. So, the quantitation of mangiferin in biological samples is a vital part of the drug development and a fast and reliable bioanalytical method is required.

Previous methods for the determination of mangiferin in biological fluids were mainly based on HPLC-UV [8–10]. These techniques were inadequate for pharmacokinetic studies due to the need for large volumes of biological samples, the long chromatographic run time and low sensitivity. Suryawanshi et al. [11] developed an LC–MS/MS based method for simultaneous analysis of mangiferin and other glycosides in rat plasma. The method was sensitive (3.13 ng/mL) and the sample treatment time was short. However, the chromatographic run time was long (12 min) and the concentration range for mangiferin was restricted (3.13–200 ng/mL). To date, no studies have been reported regarding estimation of the oral

bioavailability of mangiferin. To carry out a bioavailability study on mangiferin and screen formulations later, a fast, sensitive, robust and wide linear determining range method to quantify mangiferin in rat plasma was developed and validated.

2. Experimental

2.1. Reagents and chemicals

Mangiferin (90%, purity) was purchased from Nanjing Sulang Medical Technology Development Co. Ltd. (Nanjing, China). Diphenhydramine was provided by the Department of Analytical Chemistry of Shenyang Pharmaceutical University (Shenyang, China). A pure reference standard (>99%, purity) of mangiferin was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile and formic acid (HPLC grade) were purchased from Dikma Company (Richmond, Hill, NY, USA). All other reagents were of analytical grade.

2.2. Calibration standards and quality control (QC) samples

Stock solutions were prepared with methanol at concentrations of 250 µg/mL for mangiferin and 0.5 ng/mL for IS. Standard solutions of mangiferin were serially diluted with methanol to concentrations of 200, 100, 50, 25, 20, 10, 5, 2, 1, 0.5, 0.2 and 0.1 µg/mL and the IS working solutions were diluted to obtain concentrations of 0.25 and 0.025 ng/mL. All working solutions were stored in a refrigerator ($-20\,°C$).

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Calibration curves for oral administration were prepared by adding 20 μL mangiferin standard solutions (0.1–25 μg/mL) and 20 μL IS working solution (0.025 ng/mL) to test-tubes. After evaporation to dryness, 100 μL pooled blank plasma was added to give plasma concentrations in the range 0.02–5.0 μg/mL. Calibration curves for intravenous administration were prepared by adding 20 μL mangiferin working solution (1–250 μg/mL) and 20 μL IS working solution (0.25 ng/mL) to test-tubes. After evaporation to dryness, 50 μL pooled blank plasma was added to give plasma concentrations in the range 0.4–100 μg/mL. The QC samples were prepared at concentrations of 0.04, 0.4 and 4.0 μg/mL for samples obtained after oral administration and 0.8, 8.0 and 80 μg/mL for samples obtained after intravenous administration.

2.3. Sample preparation

Plasma samples were kept in plastic vials at −20 °C until analysis. For samples obtained after oral administration, 100 μL of the plasma sample was mixed with 20 μL IS solution (0.025 ng/mL). For samples after intravenous administration, 20 μL IS solution (0.25 ng/mL) and 50 μL of the plasma sample were added. Subsequently, 50 μL aqueous hydrochloric acid solution (1 mol/mL) and 2 mL acetoacetate–n-butanol–isopropanol (24:5:1,v/v/v) were added and vortexed for 10 min, followed by centrifugation at 5000 rpm for 10 min. The organic layer was transferred to another tube and dried at 60 °C. The residue was reconstituted in 0.4 mL (for samples after oral administration) or 2 mL (for samples after intravenous administration) mobile phase and centrifugated at 12,000 rpm for 10 min. A 5 μL aliquot of each supernatant was injected into the UPLC–MS/MS system for analysis.

2.4. Instrumentation

Chromatography was performed on an ACQUITY™ UPLC system (Waters Corp., Milford, MA, USA) with a conditioned autosampler at 4 °C and separation was performed at 35 °C using an ACQUITY UPLC™ BEH C_{18} column (50 mm × 2.1 mm, 1.7 μm, Waters Corp., USA). The analysis was achieved with gradient elution using (A) acetonitrile and (B) water (containing 0.1% formic acid) as the mobile phase. The elution started with 90%B then the composition was linearly changed to 30%B over 0.5 min and maintained at the level for 1.4 min. Finally, the composition was returned to the initial composition over 0.1 min and maintained for 1.0 min. Mass spectrometric detection was performed on a Waters ACQUITY™ TQD triple-quadrupole tandem mass spectrometer (Waters Corp., Manchester, UK) in positive ESI mode. Nitrogen was used as the desolvation gas (550 L h^{-1}) and cone gas (50 L h^{-1}). For collision-induced dissociation, argon was used as the collision gas at a flow rate of 0.20 mL min^{-1}. The cone voltage and collision energy were set at 32 V and 30 eV for mangiferin, and 30 V and 15 eV for IS. The other parameters were as follows: capillary voltage, 2.8 kV; source temperature, 100 °C; desolvation temperature, 400 °C. Quantitation was performed using the MRM of the transitions of m/z 423.1 → 272.9 for mangiferin and m/z 256.1 → 166.9 for IS.

2.5. Bioanalytical method validation

The intra-day accuracy and precision were assessed by determining QC samples using six replicates on the same day while the inter-day accuracy and precision were evaluated by analysis of three batches on different validation days. The precision was calculated as the RSD and the accuracy was expressed as the relative error (RE), i.e. (calculated concentration − nominal concentration)/(nominal concentration) × 100%. In addition, the accuracy

was required to be within ± 15%, and the precision should not exceed 15%.

Recoveries at three QC levels were determined by comparing the peak areas of extracted plasma standards with the peak areas of post-extraction plasma blanks spiked with equivalent concentrations using six replicates. The matrix effect was investigated at three QC levels using six replicates by comparing the peak areas of spike-after-extraction samples with neat standard solutions.

The stability of mangiferin in rat plasma was studied at four concentrations (0.04, 4.0, 0.8 and 80 μg/mL) using three replicates with regard to short term, storage term and freeze–thaw stability. All stability studies were evaluated by comparisons of plasma samples stored under different conditions with freshly prepared samples. The short term stability was assessed by analyzing plasma samples left at room temperature for 5 h and kept in an autosampler at 10 °C for 6 h. The storage stability was evaluated by keeping plasma at −20 °C for 15 days and the freeze–thaw stability was investigated after three freeze (−20 °C)–thaw (room temperature) cycles.

2.6. Bioavailability study

Twelve male Wistar rats (180–200 g) were randomly divided into two equal groups. One group was given mangiferin orally at a dose of 25 mg/kg, while the other group was given it at a dose of 10 mg/kg by intravenous administration. The oral and intravenous solutions were both prepared in phosphate buffer pH 8.0 (nearly isosmotic with normal saline). Blood samples (0.3 mL) were withdrawn from the rats vein at 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 10 and 12 h after oral administration and 0, 0.083, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6 and 8 h after intravenous administration.

3. Results and discussion

3.1. Mass spectrometry and liquid chromatography

Fig. 1 shows the product ion spectra of [M+H]$^+$ ions from mangiferin and IS. As reported by Satyendra et al., the [M+H]$^+$ ion at m/z 423 offered fragment ion at m/z 303 corresponding to the loss of 120 mass units due to fragmentation of the C-glycosidic unit [11]. Following the loss of a neutral unit [–CH$_2$O] a fragment ion was obtained at m/z 273. The retention times were 1.16 min for mangiferin and 1.68 min for IS, and the total chromatographic run time was 3.0 min.

3.2. Sample clean up and IS

In our initial studies, protein precipitation with acetonitrile [11] was tried, however, there was ion suppression and a lower recovery (<50%) was obtained when the plasma concentrations exceeded 1000 ng/mL. Therefore, LLE was used for plasma samples, offering a relatively "clean" sample. As shown in Fig. 1, the molecule of mangiferin contains a tricyclic aromatic ring. The planar molecular structure resulted in strong intermolecular binding forces, which possibly explains its poor solubility in many nonpolar solvents. In this study, samples of mangiferin and IS were successfully extracted from plasma using acetoacetate:n-butanol (water saturation):isopropanol (24:5:1, v/v/v). Meanwhile, mangiferin is a weak organic acid, and adding 1 mol/mL HCl to the sample prior to extraction maximized the conversion of mangiferin to its unionized form. Initially, baicalin was selected as an IS because it has a similar structural nucleus to mangiferin, but it was not stable during the evaporating process. So, the chemical reference standard, diphenhydramine, was also tried and used throughout the investigations because it remained stable during the evaporation process.
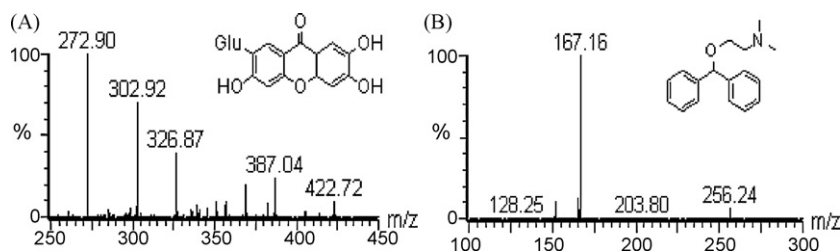
Fig. 1. The product ion spectra of [M+H]$^+$ ions from mangiferin (A) and IS (B).
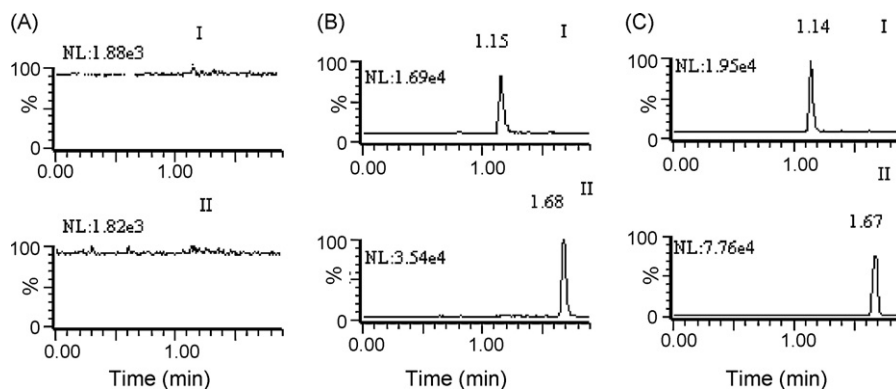

Fig. 2. Chromatograms of mangiferin (I) and IS (II) in Wistar rat plasma. (A) Blank plasma sample, (B) QC sample at a plasma concentration of mangiferin 0.4 µg/mL and (C) plasma sample 1 h after intravenous administration of mangiferin 10 mg/kg to a Wistar rat.

Table 1
Accuracy and precision of the analysis of mangiferin in rat plasma.

Added concentration (ng/mL)	Detected concentration (ng/mL)	Intra-day RSD (%)	Inter-day RSD (%)	RE (%)
40	39.0	9.7	8.9	−2.5
400	374.5	7.3	6.2	−6.4
4000	4002.6	4.8	9.2	0.1
800	864.4	8.7	8.0	8.1
8000	8384.2	11.1	9.1	4.8
80000	88546.8	2.5	2.6	10.7

3.3. Method validation

The linearity of the calibration curves was estimated by linear least-square regression using $1/x^2$ as a weight factor. Typical equations of the calibration curves were: $y = 4.75 \times 10^{-5}x + 4.9 \times 10^{-4}$, $r = 0.993$ (oral administration); $y = 1.76 \times 10^{-6}x - 4.0 \times 10^{-4}$, $r = 0.995$ (intravenous administration). Where y represents the ratio of the mangiferin peak area to that of the IS and x represents the plasma concentration. The LLOQ (0.02 and 0.4 µg/mL) was defined as the lowest concentration on the calibration curves. The precision and accuracy were 11.3% and 7.0% at 0.02 µg/mL, and 2.4% and 12.1% at 0.4 µg/mL, respectively.

The selectivity was evaluated by comparing the chromatograms of blank rat plasmas, QC samples and plasma samples, and these are shown in Fig. 2. No interfering peaks were observed. The intra-day and inter-day accuracy and precision were assessed and the results are shown in Table 1.

The recoveries of the analyte from rat plasma were $75.0 \pm 7.2\%$, $86.5 \pm 6.4\%$ and $78.9\% \pm 2.3\%$ at concentrations of 0.04, 0.4 and 4.0 µg/mL while the values were $71.0 \pm 3.8\%$, $84.6 \pm 9.7\%$ and $81.4\% \pm 9.1\%$ at concentrations of 0.8, 8.0 and 80 µg/mL, respectively. The recoveries of IS exceeded 60%. The matrix effect was determined and the results were in range of 85–115%.

The stability experiments demonstrated that mangiferin was stable after 5.0 h at room temperature ($|RE| \leq 9.8$), 6.0 h at 10 °C in an autosampler ($|RE| \leq 7.6$), 15 days storage at −20 °C ($|RE| \leq 7.9$) and three freeze–thaw-cycles in rat plasma ($|RE| \leq 8.6$). The precision (RSD) did not exceed 8.5%.

3.4. Pharmacokinetic study

The validated analytical method was successfully applied to an oral bioavailability study. The pharmacokinetic parameters were calculated applying a non-compartmental description of the observed data using drug and statistics (DAS) software, version 2.0 (Shanghai, China). The oral bioavailability was calculated:
$$\frac{AUC(0\rightarrow t)_{oral} \times 10}{AUC(0\rightarrow t)_{iv} \times 25} \times 100\%$$

The main pharmacokinetic parameters were expressed as mean ± SD (shown in Table 2). The oral bioavailability was 1.2%.

Table 2
Pharmacokinetic parameters of mangiferin after oral and intravenous administration to rats.

Parameter	Oral administration (n = 6)	Intravenous administration (n = 6)
$AUC_{(0-t)}$ (µg/L h)	1855.0 ± 887.7	61184.1 ± 22471.4
$AUC_{(0-\infty)}$ (µg/L h)	2036.2 ± 942.2	62065.2 ± 23013.2
$T_{1/2}$ (h)	3.2 ± 0.6	0.9 ± 0.4
Tmax (h)	2.5 ± 0.8	0.083
MRT_{0-t} (h)	4.3 ± 0.5	1.3 ± 0.2
Cmax (µg/L)	301.3 ± 133.0	67798.3 ± 31235.9
CLz (L/h kg)	(13.95 ± 4.64) × F	0.15 ± 0.04

F represents the bioavailability of mangiferin.

Therefore, chemical and pharmaceutical methods to improve the oral bioavailability of mangiferin will be an important subject for further studies.

4. Conclusion

A new UPLC–MS/MS method with LLE for sample preparation has been employed for the analysis of biological samples. The method is suitable for preclinical pharmacokinetic studies of mangiferin due to its short chromatographic time, small sample volume and low injection volume. The sensitivity of the method is sufficient to carry out bioavailability studies on mangiferin.

References

[1] A. Schieber, N. Berardini, R. Carle, Identification of flavonol and xanthone glycosides from mango (*Mangifera indica* L. Cv. "Tommy Atkins") peels by high-performance liquid chromatography–electrospray ionization mass spectrometry, J. Agric. Food Chem. 51 (2003) 5006–5011.

[2] I. Bairy, S. Reeja, Siddharth, P.S. Rao, Evaluation of antibacterial activity of *Mangifera indica* on anaerobic dental microglora based on in vivo studies, Indian J. Pathol. Microbiol. 45 (2002) 307–310.

[3] N. Makare, S. Bodhankar, V. Rangari, Immunomodulatory activity of alcoholic extract of *Mangifera indica* L. in mice, J. Ethnopharmacol. 78 (2001) 133–137.

[4] N. Yoshimi, K. Matsunaga, M. Katayama, The inhibitory effects of mangiferin, a naturally occurring glucosylxanthone, in bowel carcinogenesis of male F344 rats, Cancer Lett. 163 (2001) 163–170.

[5] T. Miura, H. Ichiki, Antidiabetic activity of a xanthone compound, mangiferin, Phytomedicine 8 (2001) 85–87.

[6] T. Miura, H. Ichiki, N. Iwamoto, Antidiabetic activity of the rhizoma of Anemarrhena asphodeloides and active components, mangiferin and its glucoside, Biol. Pharm. Bull. 24 (2001) 1009–1011.

[7] H. Ichiki, T. Miura, M. Kubo, New antidiabetic compounds, mangiferin and its glucoside, Biol. Pharm. Bull. 21 (1998) 1389–1390.

[8] Y.L. Li, K.S. Bi, RP-HPLC determination and pharmacokinetic study of mangiferin in rat plasma after taking traditional Chinese medicinal-preparation: Zhimu decoction, Chromatographia 57 (2003) 767–770.

[9] Y.L. Li, Y.J. Sui, Y.H. Dai, LC Determination and pharmacokinetics study of mangiferin in rat plasma and tissues, Chromatographia 67 (2008) 957–960.

[10] H. Wang, G. Ye, Y.H. Tang, High-performance liquid chromatographic method for the determination of mangiferin in rat plasma and urine, Biomed. Chromatogr. 20 (2006) 1304–1308.

[11] S. Suryawanshi, R.K. Asthana, R.C. Gupta, Simultaneous estimation of mangiferin and four secoiridoid glycosides in rat plasma using liquid chromatography tandem mass spectrometry and its application to pharmacokinetic study of herbal preparation, J. Chromatogr. B 858 (2007) 211–219.