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Simultaneous quantification of psoralen and isopsoralen in rat plasma by ultra-performance liquid chromatography/tandem mass spectrometry and its application to a pharmacokinetic study after oral administration of Haigou Pill

Yuan Gu[a,b], Duanyun Si[b], Jing Gao[b], Yong Zeng[b], Changxiao Liu[b,*]

[a] School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China
[b] Tianjin State Key Laboratory of Pharmacokinetics and Pharmacodynamics, Tianjin Institute of Pharmaceutical Research, Tianjin 300193, China

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ABSTRACT

A rapid, specific and sensitive ultra-performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS) method has been established for simultaneous quantitation of psoralen and isopsoralen in rat plasma. Plasma samples were pretreated by direct protein precipitation with acetonitrile. Chromatographic separations were performed on an ACQUITY UPLC BEH C_{18} column (50 mm × 2.1 mm, i.d., 1.7 μm) at 35 °C with a linear gradient of acetonitrile and 0.1% formic acid in water at a flow rate of 0.3 mL/min. The two isomers were satisfactorily separated (R = 1.7) with a runtime of 4 min. Psoralen, isopsoralen, and the internal standard (IS) furazolidone were ionized with an APCI source operated in positive ion mode. The MS/MS transitions used for monitoring were at m/z 187.0 → 130.9 for psoralen and isopsoralen, and m/z 225.9 → 121.9 for IS. Calibration curve was linear over the concentration range of 1–500 ng/mL with the lower limit of quantitation of 1 ng/mL for both isomers. The mean extraction recoveries were 78.5 ± 6.7% and 81.9 ± 8.0% for psoralen and isopsoralen, respectively. The intra- and inter-day precisions were less than 5.6% and 5.2%, and the accuracy was within ±2.1% for both isomers. No matrix effect was observed in this method. Psoralen and isopsoralen were stable during all storage, pretreatment and analytical periods. The validated method has been successfully applied to a pharmacokinetic study of psoralen and isopsoralen after oral administration of Haigou Pill to rats.

1. Introduction

Psoralen and isopsoralen, characterized as furocoumarin compounds, are the bioactive constituents of the herbaceous plant of Leguminosae such as *Psoralea corylifolia* L. They are widely used for the treatment of various skin diseases such as psoriasis, vitiligo and chronic graft-versus-host, and are also proved to possess anticancer, cytotoxic, antibacterial, cardiac, diaphoretic, diuretic, stimulant, aphrodisiac and tonic effects [1–8]. Many formulations containing psoralen and isopsoralen have been clinically used. However, little information is available about the pharmacokinetic profiles of both furocoumarin isomers.

A validated bioanalytical method is necessary to support any pharmacokinetic studies. There were several reports about the simultaneous determination of psoralen and isopsoralen in plants or crude formulation by high performance liquid chromatography with ultraviolet detection (HPLC-UV) methods [9–12]. However, these published HPLC methods with conventional chromatographic columns (250 mm × 4.6 mm, i.d., 5 μm) are hindered for pharmacokinetic study which involve in large number of sample determination by low sensitivity (≥40 ng/mL), long chromatographic runtimes (20–30 min) and low throughput. Furthermore, multiple extraction and large plasma volume are required to reach the low quantification limit in biological matrices by the UV detection.

Mass spectrometry (MS) is considered specific and sensitive for most drugs and drug metabolites, compared with other detection techniques including UV and fluorescence. Ultra-performance liquid chromatography (UPLC) using a sub-2 μm particle column, as recently developed technology, could provide faster analysis, higher sample throughput, better sensitivity and superior chromatographic resolution than conventional LC methods [13–16]. UPLC coupled to a triple quadrupole mass spectrometer capable of performing high-speed data acquisition operated in the multiple-reaction monitoring (MRM) mode (UPLC/MS/MS) is a powerful approach to significantly improve peak resolution, sensitivity and speed of analysis [17]. In the present study, a rapid and sensitive UPLC/MS/MS method was developed and validated for the simultaneous determination of psoralen and its isomer isopsoralen in rat plasma. The method used a small sample volume (100 μL) with a

* Corresponding author. Tel.: +86 22 2300 6283; fax: +86 22 2300 6283.
E-mail addresses: tjpks@163.com, ddysi@sohu.com (C. Liu).

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simple protein precipitation for sample preparation and had good chromatographic resolution ($R = 1.7$) with a run time of 4 min, and 1 ng/mL of quantitation limit for both analytes. Haigou Pill is a compound formulation prepared by traditional Chinese medicine (TCM), containing psoralen and isopsoralen, which has been clinically used in Hong Kong, China and several Southeast Asia countries. We successfully applied the newly established method to a pharmacokinetic study of psoralen and isopsoralen in rats after oral administration of Haigou Pill (1.6 g/kg).

2. Experimental

2.1. Chemicals and reagents

Haigou Pill (LOT 070203) was supplied by Bright Future Pharmaceuticals Factory (Hong Kong, China). Psoralen, isopsoralen and furazolidone (IS) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile (HPLC grade) was purchased from Fisher Scientific (Fair Lawn, NJ, USA) and formic acid (HPLC grade) was from Tianjin Concord Tech Reagent Co., Ltd. (Tianjin, China). All other reagents were of analytical grade. Double distilled water, prepared by a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA) was used for all aqueous solutions throughout the experiment.

2.2. Chromatography conditions

Chromatographic separations were performed on an ACQUITY UPLC BEH C_{18} column (50 mm × 2.1 mm, i.d., 1.7 μm, Waters Corp., Milford, MA, USA) using an ACQUITY^{TM} UPLC system equipped with a binary pump (high-pressure solvent delivery system), a vacuum degasser and an autosampler (Waters Corp., Milford, MA, USA). The VanGuard Pre-Column (5 mm × 2.1 mm, i.d., 1.7 μm, Waters Corp., Milford, MA, USA) was used to protect UPLC column performance. The column oven was maintained at 35 °C and eluted with a linear gradient of A (acetonitrile containing 0.1% formic acid, v/v) and B (water containing 0.1% formic acid, v/v) at a flow rate of 0.3 mL/min. The gradient proportion of A linearly increased from 22% to 37% over 3.0 min and then was rapidly returned to 22% to re-equilibrate 1.0 min prior to the next injection. The samples were kept at 10 °C in the autosampler. The column eluent was directed to a mass spectrometer for detection.

2.3. Mass spectrometry conditions

The mass spectrometry was performed on a Waters ACQUITY TQD^{TM} triple quadrupole system with a Z-spray ionization source (Waters Corp., Milford, MA, USA). MassLynx v.4.1 software was used for data acquisition and analysis (Waters Corp., Milford, MA, USA). Ionization was operated using an atmospheric pressure chemical ionization (APCI) source in positive ion mode. The ionization source parameters were following: corona current 3 μA, cone voltage 40 V for analytes and 35 V for IS, source temperature 110 °C, desolvation temperature 450 °C, desolvation gas flow 600 L/h, cone gas flow 30 L/h. Quantification was performed in multiple-reaction monitoring (MRM) mode with specific ion transitions of protonated precursor ion to product ion at m/z 187.0 → 130.9 for both psoralen and isopsoralen, and at m/z 225.9 → 121.9 for IS, respectively. The optimized collision energy was 26 eV for psoralen and isopsoralen, and 20 eV for IS, respectively. Nitrogen was used as the desolvation and cone gas, and argon was used as the collision gas. Dwell time of 0.1 s per channel was used when acquiring MRM data.

2.4. Preparation of standards and quality control (QC) samples

The stock solutions of psoralen, isopsoralen and IS were dissolved in acetonitrile at 1.0 mg/mL, respectively. A series of standard working solutions containing binary mixtures of psoralen and isopsoralen were prepared by mixing and further serially diluting the stock standard solutions with acetonitrile at the concentrations of 10, 20, 100, 500, 1000, 2000 and 5000 ng/mL. The IS working solution was also prepared by diluting the IS stock solution with acetonitrile to get the concentration of 500 ng/mL. All the solutions were kept with brown volumetric flask at 4 °C.

The calibration standard samples were prepared by spiking 10 μL working standard solutions to 100 μL blank rat plasma. The concentration levels of psoralen and isopsoralen in plasma ranged from 1 to 500 ng/mL. Quality control (QC) samples were similarly prepared at low, medium and high levels (2, 50, 200 ng/mL). All the spiked plasma samples were then treated according to sample preparation procedure. Both the calibration standard samples and the QC samples were applied in the method validation and the pharmacokinetic study.

2.5. Sample preparation

All psoralen and isopsoralen samples were protected from light exposure. Rat plasma sample was performed by direct protein precipitation with acetonitrile. An aliquot of 100 μL incurred plasma samples and 10 μL acetonitrile (or 100 μL blank plasma samples and 10 μL working solutions) were added with 300 μL IS working solution (500 ng/mL) in polypropylene tubes. Subsequently, the tubes were vigorously vortex-mixed for 30 s to precipitate plasma proteins, centrifuged for 5 min at 6000 × g. An aliquot of 300 μL of the upper organic layers was transferred to glass tubes and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was reconstituted in 100 μL of the initial mobile phase, briefly vortex-mixed and centrifuged again for 5 min at 6000 × g. The supernatant was transferred to a injection vial and then 5 μL aliquots of the supernatant were injected for UPLC/MS/MS analyses.

2.6. Method validation

2.6.1. Selectivity

Six different rat blank plasma samples were prepared and analyzed to investigate potential interferences of endogenous compounds co-eluting with analytes and IS. The chromatogram of blank plasma samples was compared with those of plasma samples spiked with analytes and IS and plasma sample after oral doses of Haigou Pill. Chromatographic peaks of analytes and IS were identified on the basis of their retention times and MRM responses.

2.6.2. Matrix effect

The matrix effect was defined as the ion suppression/enhancement on the ionization of analytes. The matrix effect was evaluated by comparing the corresponding peak areas of the post-extraction spiked samples to those of the standard solutions evaporated directly and reconstituted in mobile phase. Experiments were performed at the three QC levels, in triplicate. If the ratio is less than 85% or more than 115%, the matrix effect is implied [18].

2.6.3. Linearity

Calibration curves, over a linear range from 1 to 500 ng/mL, were prepared and assayed in triplicate on three consecutive days. Calibration graphs were constructed based on the peak area ratio (analyte/IS) versus the spiked concentrations by least square linear regression analysis with a weighting factor of $1/x^2$. Deviations of these back-calculated concentrations from the spiked

concentrations were set within ±15% (±20% for the lower limit of quantitation). The correlation coefficient and deviation reflected the assay performance over the concentration range.

2.6.4. Lower limit of quantitation

The lower limit of quantitation (LLOQ) of the assay was assessed as the lowest concentration on the calibration curve that can be quantitatively determined with acceptable precision less than 20% and accuracy within ±20%. The LLOQ was established based on six replicates on three consecutive days.

2.6.5. Precision and accuracy

Precision and accuracy were assessed by determining six replicates of the low, medium and high QC samples (2, 50, 200 ng/mL) on three consecutive days. The precision was expressed as the relative standard deviation (RSD) and the accuracy was described as the relative error (RE). The acceptable intra- and inter-day precision is required to be less than 15% and the acceptable accuracy is required to be within ±15% for all QC samples.

2.6.6. Recovery

The extraction recoveries of psoralen and isopsoralen were estimated by comparing the peak areas of blank plasma samples spiked with analyte before extraction with those of the post-extraction spiked samples at the same concentrations. Experiments were performed at the three QC concentration levels, in triplicate.

2.6.7. Stability

The stability of analytes in rat plasma was investigated by comparing the measured concentrations of triplicate low, medium and high QC samples with the spiked concentrations under the following four storage conditions: (1) stability of analytes in plasma during sample preparation was assessed by detecting samples after storage for 6 h at room temperature. (2) For freeze–thaw stability, the plasma samples were determined through three freeze (−20 °C)–thaw (room temperature) cycles. (3) To evaluate the stability of the treated plasma samples in the autosampler, QC samples were prepared and placed in the autosampler at 10 °C for a period of 12 h, and then injected for analysis. (4) The long-term stability was performed by assaying the plasma samples after 30 days of storage at −20 °C. All the samples were analyzed together with calibration curves that were freshly prepared. The analyte was considered stable when the percentage deviation was within ±15%.

2.6.8. Application to pharmacokinetic study

Six male Wistar rats (200 ± 10 g) were purchased from Institute of Radiation Medicine, Chinese Academy of Medical Sciences, Tianjin, China. Rats were housed in a clean room at a temperature of 23 ± 1 °C with a 12 h light–dark cycle and access to food and water *ad libitum* for 7 days before the experiment. Before orally administered the rats were fasted for 12 h but with access to water, and were further fasted for 2 h after administration. The ground Haigou Pill was suspended in 0.5% carboxymethyl cellulose sodium (CMC-Na) aqueous solution as a 320 mg (pill)/mL suspension and was orally administered to male Wistar rats (Haigou Pill powder 1.6 g/kg body weight, containing 2.82 mg psoralen and 2.138 mg isopsoralen/kg body weight) by gavage. Blood samples of 200 μL were collected in heparin containing tubes from the epicanthic veins of rats before drug administration (0 h) and at 0.167, 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 h after oral administration. The blood samples were immediately centrifuged at 6000 × *g* for 5 min. The plasma samples were stored at −20 °C until analysis. All the experimental procedures were approved and performed according to the guidelines of the Institutional Animal Care and Use Committee of the Tianjin Institute of Pharmaceutical Research. The pharmacokinetic parameters were calculated for each subject by the DAS 2.0 software (Mathematical Pharmacology Professional Committee of China, Shanghai, China). The maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were directly obtained from the experimental data. The elimination rate constant (K_e) was calculated by linear regression of the terminal semi-log plot of plasma concentration versus time, and $t_{1/2}$ was calculated as $0.693/K_e$. The area under the curve (AUC_{0-t}) was calculated using the linear trapezoidal rule from zero to the last plasma drug concentration. $AUC_{0-\infty}$ was calculated as $AUC_{0-t} + C_t/K_e$, where C_t is the last detectable plasma concentration.

3. Results and discussion

3.1. Chromatographic conditions

Isomers which could have the same parent ion and daughter ion are generally not distinguishable by the mass spectrometry. Therefore, chromatographic separation has become a critical step for determination of isomers. Most published HPLC methods for the chromatographic separation of isomers require 20–30 min gradients with standard chromatographic columns (250 mm × 4.6 mm, i.d., 5 μm). In this assay, we had initial attempted to perform conventional HPLC-UV method with standard C_{18} column for determination of isomers in run time of 20 min before UPLC/MS/MS was implemented. Although this method was acceptable, it clearly needed to be improved due to incomplete isomer separation, long runtime and poor sensitivity, which was therefore not suitable for high sample throughput in bioanalysis. UPLC with sub-2 μm particle column is a new chromatographic separation technology that has gained popularity in analytical chemistry. According to the Van Deemter equation, as the particle size decreases to less than 2.5 μm, there is a significant gain in chromatographic efficiency without compromising higher flow rates or higher linear velocities [19]. Thus, UPLC takes full advantage of chromatographic principles to achieve separations on column packed with much smaller particles (1.7 μm), with superior resolution, faster analysis times and better signal-to-noise ratios than traditional HPLC.

The mobile-phase composition and pH played important roles in obtaining appropriate retention time, higher chromatographic resolution and optimal ionization. The mobile phase consisting of A (acetonitrile containing 0.1% formic acid, v/v) and B (water containing 0.1% formic acid, v/v) with a linear gradient elution was proved to be optimal for this study. As the mobile-phase modifier, 0.1% formic acid promoted the protonation in APCI positive mode than 10 mM ammonium formate. The use of acetonitrile provided lower background noise and symmetric chromatographic peaks than methanol which produced tailing peak. Isocratic and linear gradient elution were both investigated, however, gradient elution combined with the sub-2 μm particle column particles was found to produce narrower (peak width about 0.1 min) peaks, proper retention time, better chromatographic resolution ($R = 1.7$) and higher sensitivity. Furthermore, the column with sub-2 μm particles was more likely to be blocked and, therefore, gradient elution could prolong the column life [20]. Peak width was about 0.1 min with the UPLC BEH column, which resulted in an increased signal-to-noise ratio. Both isomers and IS were rapidly eluted with retention times less than 3.0 min. Fast analysis time without the loss of resolution is a consequence of small particles resulting in superior chromatographic efficiency, higher linear velocities, rapid column re-equilibration and minimal baseline drift, which may meet the requirement for high sample throughput in bioanalysis.

3.2. Mass spectrometric conditions

The advantages of UPLC system were strengthened by coupling to a triple quadrupole mass spectrometer, which provided superior selectivity and sensitivity. The two most-common soft ionization techniques used for LC–MS interfaces are electrospray ionization (ESI) and APCI. APCI produces singly charged protonated in positive ion mode for a broad range of nonvolatile substances. Reducing matrix effects are a challenge in LC–MS quantitative analyses, especially when ESI is chosen as the interface which has been reported to be more likely to produce such kind of effects [18,21]. APCI with minimal matrix effects provided better ionization of psoralen and isopsoralen than ESI under positive ion mode in our preliminary experiments. The MRM scan mode has the excellent advantage of structurally specific filtering. Therefore, the signal-to-noise ratio of using the MRM scan mode is significantly superior to that of using the selected ion recording (SIR) scan mode. Cone voltage and collision energy were optimized for each analyte by infusion of 500 ng/mL of standard solutions with the flow rate of 10 μL/min via a syringe pump. The full-scan positive ion scans of isomers and IS formed the protonated molecular ions ($[M+H]^+$) of *m*/*z* 187.0 and *m*/*z* 225.9, and then, after fragmentation in the collision cell, the most abundant and stable daughter ions (Fig. 1) were at *m*/*z* 130.9 for isomers and at *m*/*z* 121.9 for IS, respectively. To obtain acceptable chromatographic peak statistics (12–20 points per peak) which affects the data quality for quantification, 0.1 s dwell time was set for each reaction channel without significant losses in sensitivity and precision.

3.3. Internal standard

For selecting the ideal internal standard, a similar processing method and a suitable retention time are of significant importance. The isotope labeled internal standard is the most ideal selection. However, it is not always commercially available and is usually more expensive than the analogues. In our laboratory, no furocoumarin substances were available, and then several other compounds such as metronidazole, diphenhydramine, brodimoprim and furazolidone were investigated. Furazolidone was finally adopted as internal standard because of similarity to the analytes in mass spectrometric behaviours and sample preparation procedure.

3.4. Sample preparation

A number of samples need to be analyzed in pharmacokinetic study. Thus, a simple, rapid and convenient sample preparation is necessary and critical. Protein precipitation was used for the sample preparation after comparing the liquid–liquid extraction (LLE) with *n*-hexane-dichloromethane (2:1) and ethyl acetate. The protein precipitation with much simpler and less time-consuming procedure had very similar extraction recoveries with LLE in the study. Acetonitrile producing higher recoveries was chosen as precipitation agent instead of methanol. The protein precipitation is more advisable and advantageous in the present work, because it could not only ensure adequate recovery and high sensitivity, but also perform simple.

3.5. Method validation

3.5.1. Selectivity and specificity

Assay selectivity is the ability to distinguish and determine the analytes in the presence of endogenous compounds. Representative chromatograms of blank plasma, spiked plasma and incurred plasma (rat plasma after oral administration of Haigou Pill) are presented in Fig. 2. The retention times were 1.09, 2.56 and 2.73 min for

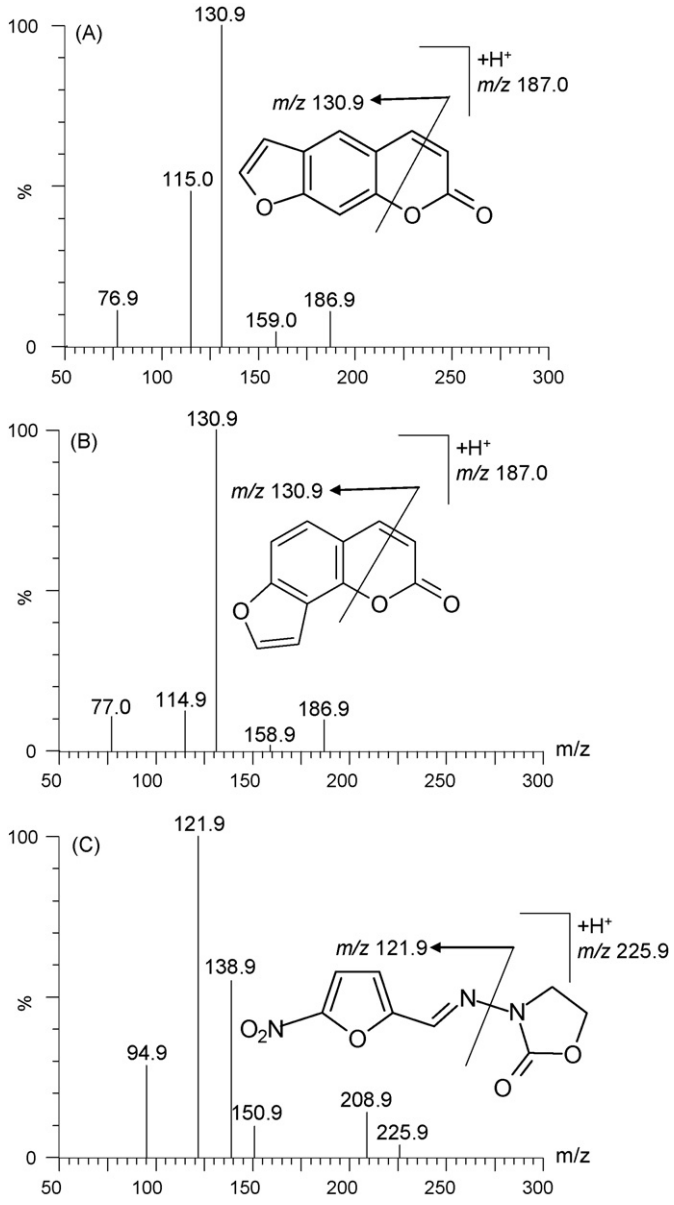

Fig. 1. Product ion mass spectra of $[M+H]^+$ of (A) psoralen, (B) isopsoralen and (C) furazolidone (IS).

IS, psoralen and isopsoralen, respectively. As shown in the figures, there was no significant endogenous substance that could interfere with the analytes and IS, and a stable baseline was maintained throughout.

3.5.2. Matrix effect

Matrix effect can suppress or enhance ionization of the analytes caused by co-eluting endogenous substances in biological matrices [18,22,23] and therefore affects the sensitivity and accuracy of the method. The ratios (peak areas) are between 91.0% and 96.7% for psoralen and between 88.2% and 98.7% for isopsoralen at three QC levels, respectively, indicating that no endogenous substances decreased or increased the response of the analytes under the present chromatographic, mass spectrometric and extraction conditions.

3.5.3. Linearity and lower limit of quantitation

The calibration curves were ranged from 1 to 500 ng/mL for each isomers, which was selected according to the concentrations

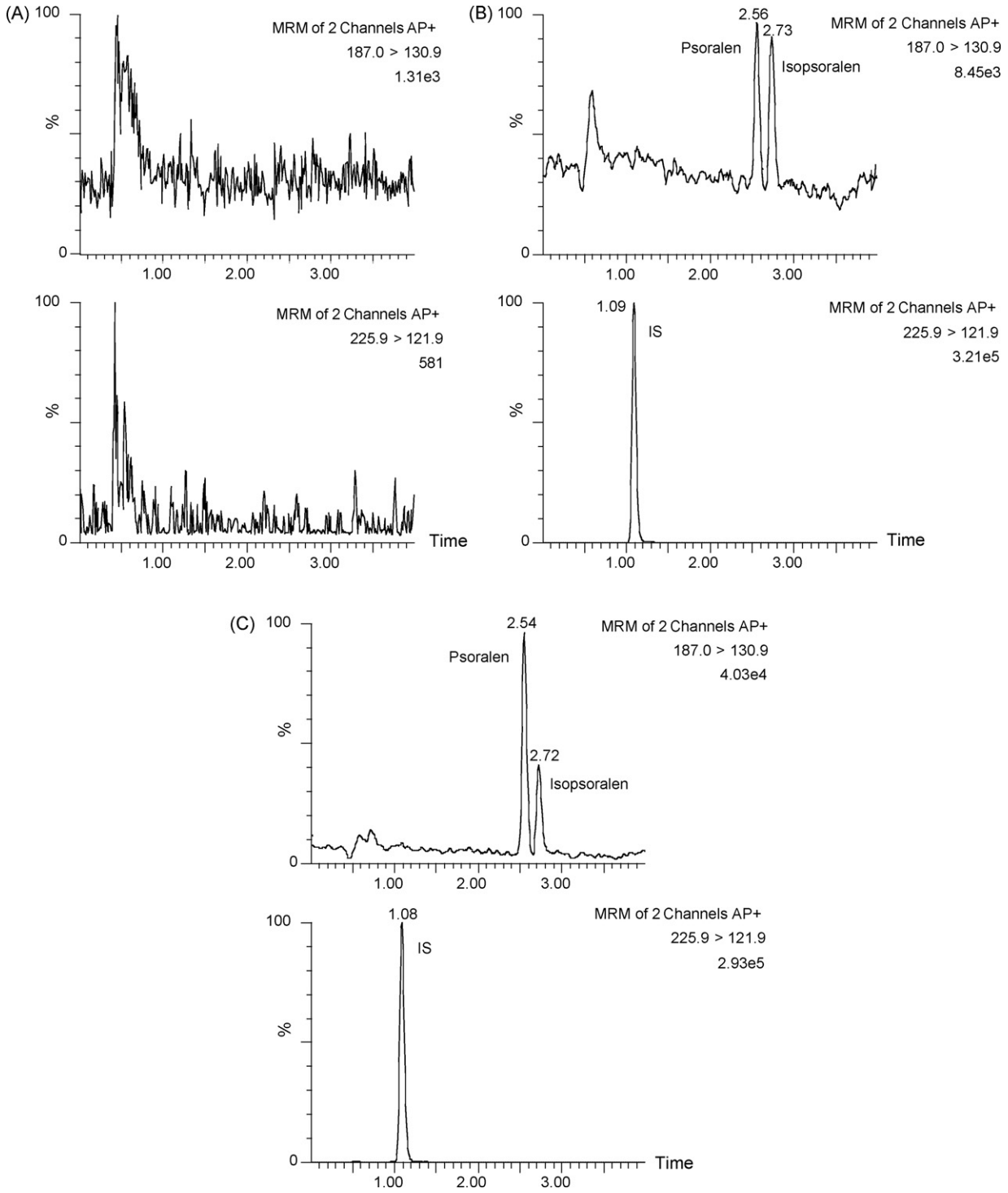

Fig. 2. Typical MRM chromatograms of psoralen, isopsoralen and IS: (A) a blank rat plasma sample; (B) a blank plasma spiked with psoralen and isopsoralen (LLOQ 1 ng/mL) and IS (500 ng/mL); and (C) a rat plasma sample from 0.17 h after oral administration of 1.6 g/kg Haigou Pill spiked with IS.

anticipated in the incurred samples. The calibration curves showed excellent linearity with the correlation coefficient (r^2) being >0.9962 and with the observed deviation within ±8.9% for all calibration concentrations. The typical regression equations obtained by least squared regression were $y = 0.007157x - 0.0008701$, $r^2 = 0.9976$ (psoralen) and $y = 0.005840x + 0.003997$, $r^2 = 0.9981$ (isopsoralen), respectively.

The LLOQ of both isomers was 1 ng/mL with the intra- and inter-day precisions were less than 3.8% and 4.2%, and the accuracy was within ±3.2% (Table 1), which was sufficient for pharmacokinetic studies of psoralen and isopsoralen in rat.

3.5.4. Precision and accuracy

QC samples at three concentrations were analyzed in six replicates for determining the accuracy and precision of this assay. As shown in Table 1, the intra- and inter-day precisions were less than 5.6% and 5.2%, and the accuracy was within ±2.1% for both isomers. All within the acceptable limits indicated that the present method has a satisfactory accuracy and precision.

Table 1
Precision and accuracy of psoralen and isopsoralen in rat plasma ($n = 6$).

Compound	Concentration (ng/mL)		Precision (RSD (%))		Accuracy (RE (%))
	Spiked	Measured	Intra-day	Inter-day	
Psoralen	1	1.0 ± 0.0	2.7	3.1	3.2
	2	2.0 ± 0.1	4.6	2.1	−1.7
	50	49.7 ± 2.3	4.8	2.6	−0.5
	200	204.1 ± 9.6	4.6	5.2	2.1
Isopsoralen	1	1.0 ± 0.0	3.8	4.2	2.0
	2	2.0 ± 0.1	4.1	4.7	−0.4
	50	49.5 ± 2.6	5.5	2.1	−1.0
	200	203.5 ± 10.9	5.6	3.4	1.8

Table 2
Stability of psoralen and isopsoralen in rat plasma ($n = 3$).

Storage conditions	Concentration (ng/mL)			RE (%)	
	Spiked	Measured (Mean ± SD)		Psoralen	Isopsoralen
		Psoralen	Isopsoralen		
Short-term stability (at room temperature for 6 h)	2	2.1 ± 0.2	2.1 ± 0.1	5.4	5.6
	50	52.2 ± 0.6	49.7 ± 1.4	4.3	−0.6
	200	197.4 ± 9.9	188.7 ± 2.2	−1.3	−5.7
Three freeze–thaw cycle	2	2.0 ± 0.1	2.1 ± 0.0	1.0	6.0
	50	49.8 ± 3.7	51.0 ± 2.3	−0.5	1.9
	200	204.7 ± 11.3	209.3 ± 10.9	2.4	4.7
At 10 °C in the autosampler for 12 h	2	1.9 ± 0.1	1.9 ± 0.0	−3.5	−3.3
	50	50.2 ± 3.9	48.0 ± 1.3	0.4	−4.1
	200	205.6 ± 12.9	204.8 ± 18.8	2.8	2.4
Long-term stability (at −20 °C for 30 days)	2	2.0 ± 0.1	2.1 ± 0.0	1.5	3.5
	50	54.0 ± 0.4	52.1 ± 1.2	8.1	4.2
	200	217.1 ± 7.9	217.8 ± 1.1	8.5	8.9

3.5.5. Recovery

Under the given set of operating conditions, the extraction recoveries of psoralen and isopsoralen were 78.5 ± 6.7% and 81.9 ± 8.0% for three QC levels, respectively. The recoveries were consistent over its calibration range, indicating that the extraction efficiency of the current assay is independent of the concentrations in the ranges studied. The recovery of the IS was 64.1 ± 1.3% at the concentration of 500 ng/mL and was steady throughout.

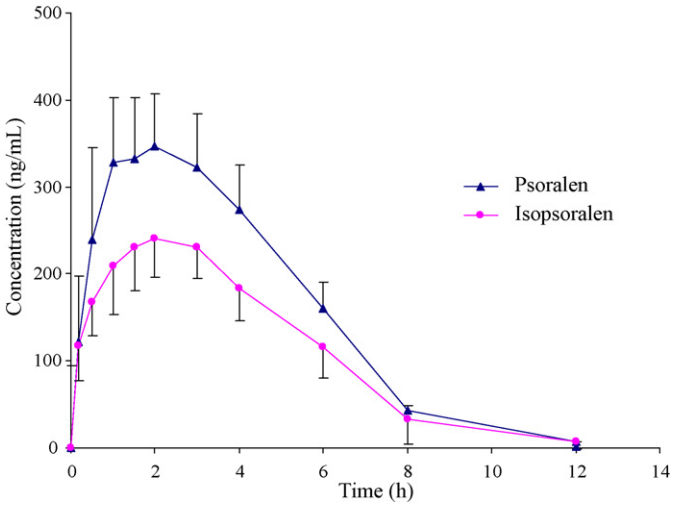

Fig. 3. Mean plasma concentration–time profile of psoralen and isopsoralen after oral administration of Haigou Pill (1.6 g/kg) to rats. Each point represents mean ± SD ($n = 6$).

3.5.6. Stability

Results of the stability tests are summarized in Table 2. Psoralen and isopsoralen were stable in rat plasma after being placed at ambient temperature for 6 h, after being stored at −20 °C for 30 days or through three freeze–thaw cycles. On the other hand, processed samples were found to be stable at 10 °C in the autosampler for a period of 12 h, indicating that a large number of samples could be processed in each analytical run.

3.6. Pharmacokinetic study

We successfully applied the present method to the pharmacokinetic study of psoralen and isopsoralen in six Wistar rats after oral administration of Haigou Pill of 1.6 g/kg. Plasma concentrations of psoralen and isopsoralen were determined up to 12 h and remained within the calibration curve range throughout the study. The mean plasma concentration versus time curves is presented in Fig. 3.

Table 3
Main pharmacokinetic parameters of psoralen and isopsoralen after oral administration of Haigou Pill (1.6 g/kg) to rats ($n = 6$, mean ± SD).

Parameters	Psoralen	Isopsoralen
	Mean ± SD	Mean ± SD
$t_{1/2}$ (h)	1.31 ± 0.16	1.33 ± 0.31
K_e (1/h)	0.54 ± 0.06	0.54 ± 0.11
V1/F (L/kg)	3.15 ± 0.55	3.48 ± 0.57
CL/F (L/h/kg)	1.69 ± 0.36	1.87 ± 0.39
AUC_{0-t} (ng h/mL)	1713.82 ± 415.17	1167.47 ± 296.52
$AUC_{0-\infty}$ (ng h/mL)	1742.33 ± 433.33	1196.51 ± 322.79
MRT_{0-t} (h)	3.50 ± 0.26	3.53 ± 0.43
$MRT_{0-\infty}$ (h)	3.62 ± 0.33	3.68 ± 0.55
T_{max} (h)	1.58 ± 0.49	2.08 ± 0.49
C_{max} (ng/mL)	383.19 ± 78.72	261.49 ± 30.61

The major pharmacokinetic parameters of psoralen and isopsoralen were calculated by one-compartment model and demonstrated in Table 3. The result of pharmacokinetic data analysis showed that both isomers have similar pharmacokinetic characteristics.

4. Conclusions

A rapid, sensitive and specific UPLC/MS/MS method was developed and validated for the separation and determination of psoralen and isopsoralen in rat plasma, which allowed sufficient sample throughput. The method was successfully applied to characterize the pharmacokinetics of psoralen and isopsoralen in rats after oral administration of Haigou Pill. The results showed that the developed bioanalytical method was a valuable analytic technique for determination of the isomers in biological matrix and met the requirements for the pharmacokinetics of psoralen and isopsoralen.

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