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ORIGINAL ARTICLE

UPLC-TQ/MS Determination and Pharmacokinetic Study of Eight Compounds in Normal and Lps-primed Rat Plasma after Oral Administration of Psoralea Fructus ethanol Extract

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ABSTRACT

Background: Psoralea Fructus (PF) is one of the most frequently applied tonic Chinese herbs with various bioactivities, while recently, the idiosyncratic drug-induced liver injury (IDILI) nature of PF was addressed, and liver injury cases were reported in the clinic. Our previous research indicated that many constituents exerted liver injury property, whereas the pharmacokinetic profile of these compounds between normal and immune stress states is still unclear. **Objectives:** This study aims to construct a validated method to simultaneously determine eight analytes (including psoralen, isopsoralen, psoralidin, corylin, psoralenoside, isopsoralenoside, bavachin, and neobavaisoflavone) in PF via UPLC-TQ/MS, and to compare their pharmacokinetic properties in normal and LPS-stimulated rats. **Materials and Methods:** The rats were randomly divided into four groups. The blood samples of different groups were harvested at different time points; the eight analytes were analyzed under UPLC-TQ/MS, and the constructed method was confirmed in terms of specificity, linearity, lower limit of quantitation (LLOQ), precision, accuracy, stability, recovery, and matrix effect. **Results:** The developed UPLC-TQ/MS method showed good linearity (r = 0.9972–0.9994) and specificity; the recovery and matrix effect were acceptable (ranging from 64.75 ± 1.59% to 104.31 ± 3.38%, and from 87.11 ± 1.91% to 115.45 ± 1.63%, respectively), the intra- and inter-day precision of eight analytes were under 13.42%, the intra- and inter-day accuracy was eligible (92.68%–108.85%), and the analytes were stable under storage conditions. All eight analytes in PFE were rapidly absorbed into the circulation, while the relevant pharmacokinetic parameters (including *AUC*, MRT, VRT, λ, V, $t_{1/2}$, C_{max}, $\mathbf{T_{max}}$, and CL) of these analytes were quite different between the normal and model rats. **Conclusion:** A sensitive, accurate, and rapid method was successfully established and validated to determine the plasma characteristics of analytes in normal and LPS-primed rats. The pharmacokinetic profile indicated the body states might appreciably impact the pharmacokinetic profile of the bioactive constituents of PF, further inducing liver injury in specific patients.

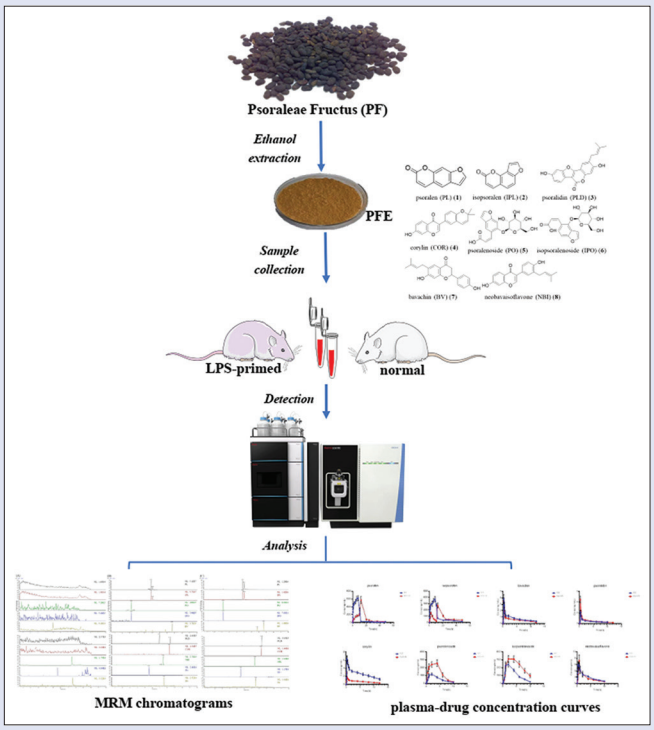
Key words: Idiosyncratic liver injury, LPS-stimulated model, pharmacokinetic, psoraleae fructus, UPLC-TQ/MS

SUMMARY

- A UPLC-TQ/MS method for the determination of eight analytes in Psoraleae Fructus ethanol extract was developed.
- The pharmacokinetics of these compounds in PFE were studied systematically in normal and LPS-stimulated rats for the first time.
- The relevant pharmacokinetic parameters of these analytes in normal and LPS-stimulated rats were compared and analyzed to preliminarily illustrate the liver injury property of PF.

Abbreviations used:

PF: Psoraleae Fructus; **IDILI:** Idiosyncratic liver injury; **PFE:** PF ethanol extract; **LPS:** Lipopolysaccharides; **PL:** Psoralen; **IPL:** Isopsoralen; **PLD:** Psoralidin; **COR:** Corylin; **PO:** Psoralenoside; **IPO:** Isopsoralenoside; **BV:** Bavachin; **NBI:** Neobavaisoflavone; **IS:** Internal standard; **CMC:** Carboxyl methyl cellulose; **MRM:** Multiple reaction monitoring; **LLOQ:** lower limit of quantitation; **S/N:** Signal-to-noise ratio; **QC:** Quality control; AUC: Area under the curve; **MRT:** Mean residence time; **VRT:** The variance of residence time; **λ:** Elimination rate constant; **V:** Apparent volume of distribution; $\mathbf{t_{1/2}}$: half-life; $\mathbf{C_{max}}$**:** Maximum concentration; $\mathbf{T_{max}}$: Maximum peak time; **CL:** Clearance rate; **DMEs:** Drug-metabolizing enzymes;



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INTRODUCTION

Psoraleae Fructus (PF, Buguzhi in Chinese), the dry ripe fruit of *Psoralea corylifolia* L.[1] is one of the most frequently applied tonic Chinese herbs for warming kidneys, activating yang, promoting inspiration, and checking diarrhea.[2] In the view of modern pharmacology, PF exhibits a wide range of bioactivities, including anti-inflammatory, antioxidative, antibacterial, and can be used to treat osteoporosis and vitiligo.[3-5] As the National Adverse Drug Reaction Monitoring Center reported in 2008 and 2016, several types of Chinese patent medicines, such as Zhuang-gu-guan-jie pills and Xian-ling-gu-bao capsules, can induce liver injury, which both contain PF, and increasing clinical reports have indicated the potential hepatotoxicity of patients receiving PF.[6,7] Huang Y *et al.*[8] and Wang X.Y *et al.*[9] also revealed the liver injury effect of PF. Huang Y. *et al.*[8] retrieved the clinical cases in Yinzhou community, and data showed that the gross incidence rate of Xianlinggubao-related liver injury was 0.034%, indicating the rare incidence and the idiosyncratic drug-induced liver injury (IDILI) nature of PF, which means hepatotoxicity associated with PF is not related to the dose, route, or duration of drug administration.[10]

Unlike western medicine, traditional Chinese medicines are a mixture of different bioactive components. Tang *et al.*[11] detected three coumarins (psoralen, isopsoralen, and psoralidin) and six prenylated flavonoids (neobavaisoflavone, isobavachalcone, bavachinin, bavachalcone, isobavachin, and bavachin) from PF by analyzing Xianlinggubao capsules using ultra-performance liquid chromatography-tandem mass spectrometry(UPLC-MS/MS). Ding *et al.*[12] identified 44 components, including 28 flavonoids, 10 coumarins, 2 monoterpenoids, and 4 other compounds from PF using ultra performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS). Our previous research[9] showed that the PF ethanol (PFE) extract and its main constituents (psoralen, isopsoralen, bavachin, isobavachalcone, psoralidin, and bakuchiol) all exerted hepatotoxicity on L02 cells, and the long-term application of PF might cause liver injury as well.[7]

Idiosyncratic drug-induced liver injury (IDILI) only occurs in a minority of the population but different from liver disease, which is independent of dose, route, or duration of drug administration.[13,14] IDILI is a synergistic effect that is caused by body diathesis, environment, and drugs.[13] Considering the IDILI property of PF, its idiosyncratic toxicity might result from different exposures to toxic metabolites.[15] However, the pharmacokinetic profile of these hepatotoxic chemicals in PF on the IDILI-model is still yet unclear. Increasing evidence implies that IDILI cases mostly are immune-mediated,[16,17] and lipopolysaccharides (LPS) were proved to simulate IDILI status.[14]

In this present study, the pharmacokinetic profile of eight analytes (including psoralen, isopsoralen, psoralidin, corylin, psoralenoside, isopsoralenoside, bavachin, and neobavaisoflavone) in PFE were investigated and compared in normal and LPS-mediated rat plasma after oral administration of PFE, to preliminarily illustrate the IDILI property of PF.

MATERIALS AND METHODS

Samples, chemicals, and reagents

Psoraleae Fructus (NO. 20040101) was purchased from Zhengzhou Ruilong Pharmaceutical Co., Ltd. (Zhengzhou, China) and identified by Chen Tianchao, the pharmacist of the First Affiliated Hospital of Henan University of Chinese Medicine. The chemical standards of psoralen (PL) (1), isopsoralen (IPL) (2), psoralidin (PLD) (3), and corylin (COR) (4) were provided by Chengdu Pufei De Biotech CO., Ltd. (Chengdu, China); psoralenoside (PO) (5), isopsoralenoside (IPO) (6), bavachin (BV) (7) and neobavaisoflavone (NBI) (8) were provided by Chengdu Chroma-Biotechnology Co., Ltd. (Chengdu, China), and their purity was above 98% determined by HPLC [Figure 1]. Internal standard (IS) clarithromycin (IS^+) and probenecid (IS^-) were purchased from National Institutes for Food and Drug Control (Beijing, China). Acetonitrile, methanol, and formic acid (HPLC grade) for UPLC analysis were bought from Merck (Darmstadt, Germany) and Beijing Dikema Technology Co., Ltd. (Beijing, China) in order. We have already obtained approval from the ethics committee (NO. YFYDW2020017) on 19th Oct. 2020.

Instrument and analytical conditions

The liquid mass system consisted of a Waters UPLC system (Waters, USA) coupled with an Acquity UPLC® BEH C18 column (2.1 × 100 mm, 1.7 μm), and a Thermo TSQ Altis mass spectrometer (Thermo, the U.S.A.) with electrospray ionization (ESI) ion source. For UPLC separation, the mobile phase consisted of 0.1% formic acid acetonitrile (A) and 0.1% formic acid water (B). The gradient elution program was set as follows: 30%–60% B (1–6 min), 60%–95% B (6–7 min), 95% B (7–9 min), 95%–10% B (9–9.1 min), 95% B (9.1–15 min). The column temperature was set as 30°C; the flow rate of the mobile phase was 0.2 mL·min^{-1}, and the sample injection volume was 1 μL.

The mass spectra were acquired in the multiple reaction monitoring (MRM) of positive or negative modes for the analytes and IS. The optimized mass spectrometry (MS) parameters were fixed as capillary voltage, 3500 V and 2500 V for positive and negative modes in order; sheath gas flow and auxiliary gas, 25 arb and 7 arb in order; collision gas pressure, 1.5 mTorr; capillary temperature, 300°C; ion source temperature, 400°C. The detection parameters of precursor ion, sub-ion ion, collision energy, and lens voltage of eight components of PFE and IS.

Preparation of standard and sample solution

A total of eight standard compounds (including psoralen (PL), isopsoralen (IPL), psoralidin (PLD), corylin (COR), psoralenoside (PO), isopsoralenoside (IPO), bavachin (BV) and neobavaisoflavone (NBI)) were dissolved in methanol, and further diluted into a series of working solutions to obtain each standard curve.

The crude drug of PF was weighed to 1 kg. After immersed in 10-fold volume of 95% ethanol for 0.5 hr, PF was reflux extracted twice, and the filtrate was vacuum concentrated to obtain ethanol extract (PFE) with the concentration of 1.39 g·mL^{-1} (1.37 g·g^{-1}). The contents of eight analytes in PFE were quantitatively evaluated with the constructed method. The contents of PL, IPL, PO, IPO, BV, PLD, COR and NBI were 435.81, 431.24, 627.05, 625.78, 93.75, 110.87, 57.98, 206.21 mg·mL^{-1} in PFE, respectively; and 3.14, 3.10, 4.51, 4.50, 0.67, 0.80, 0.42, 1.48 mg·g^{-1} in crude PF, respectively. The stationary administration dosage of PFE was 3.6 g·kg^{-1} by dissolving the prepared PFE in carboxyl methyl cellulose (CMC).

Preparation of plasma samples

Male Sprague Dawley (SD) rats (SPF degree, 180–190 g) were supplied by the Beijing Vital River Laboratory Animal Technology Co., Ltd. [License No. SCXK (Jing) 2016-0006], and were fed under the proper conditions with freely feeding and watering, and 12-hr light and 12-hr dark cycle. These rats fasted 12 hr with water supplied before experiments. All the animal operations followed the protocols of the Animal Experimentation Committee of the First Affiliated Hospital of Henan University of Chinese Medicine. The experimental animals were evenly divided into two groups: individually applied Psoraleae Fructus, and combined with lipopolysaccharides, named as BGZ group and BGZ-LPS group in order (n = 15).

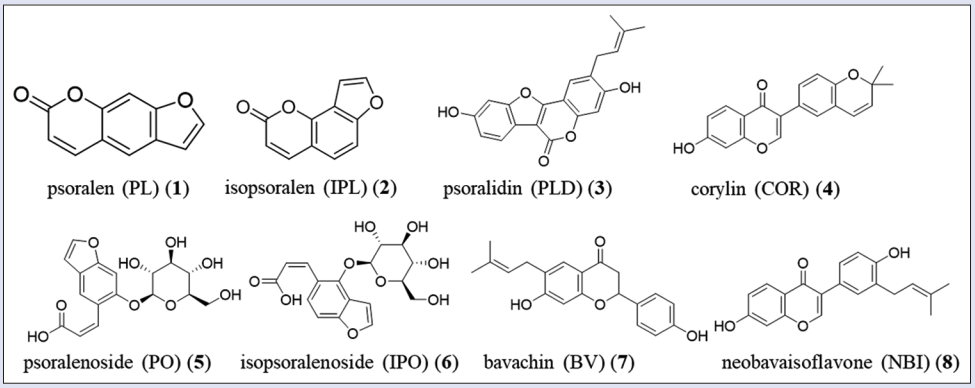

Figure 1: The chemical structures of psoralen (PL) (1), isopsoralen (IPL) (2), psoralidin (PLD). (3), corylin (COR) (4) psoralenoside (PO) (5), isopsoralenoside (IPO) (6), bavachin (BV) (7) and neobavaisoflavone (NBI) (8)

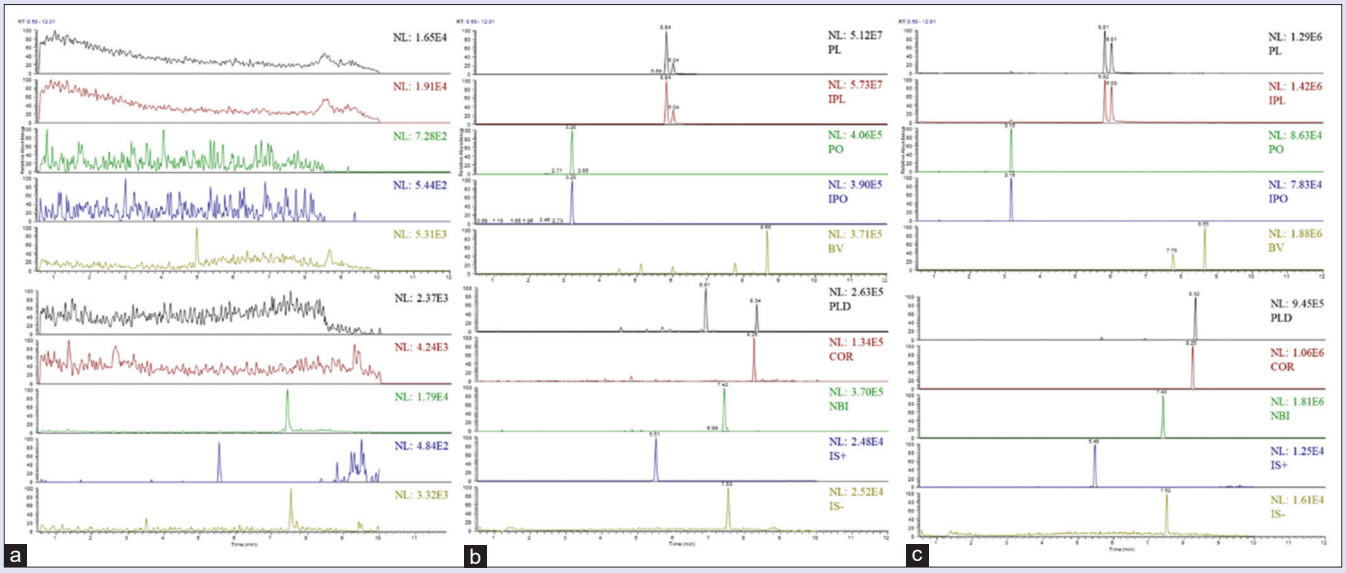

Figure 2: Representative MRM chromatograms of the blank plasma (a), the eight analytes and IS in blank plasma (b), and real plasma after gavage PFE for 15 min (c)

The rats fasted for 12 hr and their tail was intravenously injected with LPS (2.0 mg·kg^{-1}) or its saline vehicle, and 2 hr later, all rats were administrated PFE-CMC (3.6 g·kg^{-1}) by gavage, according to our former research.[18] The blood sample of each rat was collected from orbital venous plexus after 0 hr, 0.083 hr, 0.167 hr, 0.25 hr, 0.5 hr, 0.75 hr, 1 hr, 2 h, 4 hr, 6 hr, 8 hr, 10 hr, 12 hr, 24 hr, 28 hr, 36 hr, and 48 hr of gavage, respectively. Plasma samples were obtained by centrifugation at 3000 rpm for 10 min and stored at −80°C for further analysis.

Plasma sample treatment

Formic acid-diluted water (1:9, *V*:*V*, 20 μL) and internal standard (IS) solution (10 μL) were mixed with each plasma sample (100 μL), and MeOH (300 μL) was added to precipitate protein and other similar impurities, followed by vortex mixing for 5 min and centrifugation at 14,000 rpm for 10 min at 4°C. The supernatant was centrifugally concentrated to dry, and the residuum was dissolved with MeOH (100 μL), vortexed mixing for 3 min, and centrifugated at 14,000 rpm for 10 min, and the supernatant of each sample was collected for further determination.

Method validation

The specificity, linearity, lower limit of quantitation (LLOQ), precision, accuracy, stability, recovery, and matrix effect of this established method was comprehensively investigated.

Specificity, linearity and LLOQ

Blank plasma samples treated with or without standard solutions and IS were compared to the plasma sample of treated rats to expel interferences from endogenous substances or other impurities around the retention time.

A series of working solutions of the mixed standard solution was used to obtain the standard curve of each component in PFE, taking each analyte concentration as *X* and the area ratio of analyte-to-IS as *Y*. Signal-to-noise ratio (S/N) of 10:1 was set as LLOQ.

Recovery and matrix effect

A blank plasma sample was applied to prepare three different concentrations of quality control (QC) solution, after disposed of as seen in the "Plasma sample treatment" section. These QC analytes were

analyzed as in the "Instrument and analytical conditions" section to obtain corresponding peak area A1. The peak area of pretreated blank solution and standard solution redissolved with spiked IS was attributed to A2 and A3, respectively. Six parallel samples at each concentration were used; the ratio of A1-to-A3 was deemed as recovery (%), and the ratio of A2-to-A3 was recognized as matrix effect (%).

Precision and accuracy

Different concentrations of QC solutions and mixed standard solutions in blank plasma with six replicates were applied to investigate the precision and accuracy. The intra- and inter-day precision were evaluated by determining these during a single day and replicating for three continuous days.

Stability

The storage stability (4°C) and long-term stability (−80°C) were evaluated for 24 hr and 30 days in order, using different concentrations of QC solution. The freeze–thaw stability (circular triplicates at −80°C and room temperature) was also investigated.

Pharmacokinetic study

Drug and Statistics Software version 2.0 (DAS 2.0, Mathematical Pharmacology Professional Committee of China, Shanghai, China) was used to calculate the pharmacokinetic parameters including area under the curve (*AUC*), mean residence time (MRT), the variance of residence time (VRT), elimination rate constant (λ), apparent volume of distribution (V), half-life ($t_{1/2}$), maximum concentration (C_{max}), maximum peak time (T_{max}) and clearance rate (CL). The pharmacokinetic behavior of eight components in rat plasma was analyzed, and the relevant results were represented as Mean ± SD.

RESULTS AND DISCUSSION

Optimization of UPLC-MS/MS analysis method

A UPLC-MS/MS method for simultaneously detecting eight analytes in PFE and IS in rat plasma was investigated under both positive and negative modes, aiming to obtain a better separation degree and more symmetrical chromatographic peak shape in a short time. To optimize the mobile phase, acetonitrile-0.1% formic acid in water and 0.1% formic acid in acetonitrile-0.1% formic acid in water were compared. Results indicated that slightly formic acid contributed to the analytes ionization and the signal intensity improvement. In this study, 0.1% formic acid in acetonitrile-0.1% formic acid in water was selected as the mobile phase for separation.

Optimization of plasma sample disposal method

The plasma sample disposal method was optimized in advance to maximize the detection of analytes with low-to-high polarity and minimize the matrix effect and improve the recovery rate. Different organic solvents (methanol and acetonitrile) with three- and five-fold volumes were applied to precipitate proteins in plasma samples, using recovery rate as an index. Considering minuscule difference was detected under these conditions, in this study, a three-fold volume of methanol was used to dispose of plasma samples.

Method validation

A sensitive and reliable analytical method was developed under the optimized UPLC-TQ/MS conditions and validated in terms of specificity, linearity, LLOQ, recovery, matrix effect, precision, accuracy, and stability. The pharmacokinetic profile of eight analytes in PFE was further investigated under the constructed condition.

Specificity

Blank plasma samples were prepared with a protein precipitation procedure ensuring less interference of analytes and IS from plasma.[1] The representative chromatograms for blank rat plasma, standards of the analytes and IS spiked in blank plasma, and rat plasma collected after gavage PFE for 15 min are shown in Figure 2. The results show no significant interference from endogenous substances observed under the current analytical conditions, which indicates the specificity of the elaborated procedures.

Linearity and LLOQ

The standard curve of eight analytes revealed good linearity with correlation coefficients ranging from 0.9972 to 0.9994 (data is shown in Table 1). LLOQ was obtained by analyzing the concentration of mixed standard solution based on the S/N value.

Recovery and matrix effect

The recovery and matrix effect of eight analytes in PFE with three concentration levels were summarized in Table 2. The recovery and matrix effect ranged from 64.75 ± 1.59% to 104.31 ± 3.38%, and from

Table 1: The standard curve, correlation coefficient, linear range, and LLOQ of eight analytes in rat plasma samples

Analyte	Regression Equation	Linearity (*r*)	Linear Range ($ng \cdot mL^{-1}$)	LLOQ ($ng \cdot ml^{-1}$)
PL	Y=0.545X+0.7084	0.9990	2~10000	2
IPL	Y=0.596X+0.6405	0.9982	2~10000	2
PO	$Y=4.97\times10^{-3}X+4.889\times10^{-4}$	0.9990	2~5000	2
IPO	$Y=1.469\times10^{-3}X - 2.934\times10^{-4}$	0.9978	2~5000	2
BV	$Y=0.4769X+6.845\times10^{-2}$	0.9994	0.1~200	0.1
PLD	Y=0.4018X+0.2055	0.9990	0.1~200	0.1
COR	Y=0.5264X+0.206	0.9972	0.1~200	0.1
NBI	Y=0.8012X+0.2296	0.9988	0.1~500	0.1

Table 2: The recovery and matrix effect of eight analytes in rat plasma samples (*n*=6)

Analytes	Concentration ($ng \cdot mL^{-1}$)	Recovery (%)	RSD (%)	Matrix Effect (%)	RSD (%)
PL	7	101.48	2.31	90.67	0.83
	100	96.08	4.63	108.11	1.46
	1000	99.49	0.48	107.10	0.75
IPL	7	104.31	3.38	96.14	4.06
	100	96.54	4.00	111.41	2.67
	1000	96.31	1.77	106.16	1.36
PO	10	79.39	3.55	106.30	2.37
	30	67.35	1.66	92.30	3.05
	500	64.75	1.59	87.11	1.91
IPO	10	84.63	5.12	100.04	7.50
	30	90.61	9.05	101.64	3.41
	500	72.06	4.07	91.39	2.31
BV	1.5	92.51	1.02	94.19	2.32
	45	85.56	9.29	106.21	0.90
	150	93.76	0.44	100.85	1.58
PLD	0.5	96.79	2.56	96.50	1.28
	10	91.28	0.94	108.42	0.69
	100	85.53	2.57	95.34	0.27
COR	0.5	92.64	4.30	104.74	2.99
	10	98.75	2.08	115.45	1.63
	100	90.73	1.24	99.52	0.60
NBI	0.5	99.20	1.73	111.23	6.72
	20	95.78	4.15	101.72	1.50
	200	91.40	1.74	103.03	1.18

87.11 ± 1.91% to 115.45 ± 1.63%, respectively. The RSD of recovery and matrix effect was under 9.29% and 7.50% in order, which indicates the acceptable

Precision and accuracy

Three concentrations of eight analytes in QC samples were used to investigate the intra- and inter-day precision and accuracy (the data was shown in Table 3). The RSD range of intra- and inter-day precision was 2.31%–11.47% and 1.48%–13.42% in order. The RSD range of intra- and inter-day accuracy was 95.71%–105.63% and 92.68%–108.85%, respectively. All of these were eligible.

Stability

The storage stability (4°C), long-term stability (−80°C), and freeze–thaw stability of the eight analytes in PFE with three concentrations were listed in Table 4. The RSD range of eight analytes at 4°C for 24 hr was 2.23%–12.27%, and the range of long-term stability (−80°C) and freeze–thaw stability was 1.34%–13.42% and 0.76%–12.05% in order, indicating good stability within the testing conditions.

Pharmacokinetic profile

The metabolic interactions between PF constituents and human drug-metabolizing enzymes (DMEs) might be important in drug disposition and endogenous metabolism, and further influence the effect and toxicity of PF.[4] Most of the constituents in PF can be metabolized by both phase I and phase II metabolizing enzymes in mammals.[4] Therefore, different body states might induce different reflections of PF in the body. In this study, the pharmacokinetic profile of various bioactive compounds in PF was investigated under normal and immune stress states to uncover the biological essence of the IDILI nature of PF.

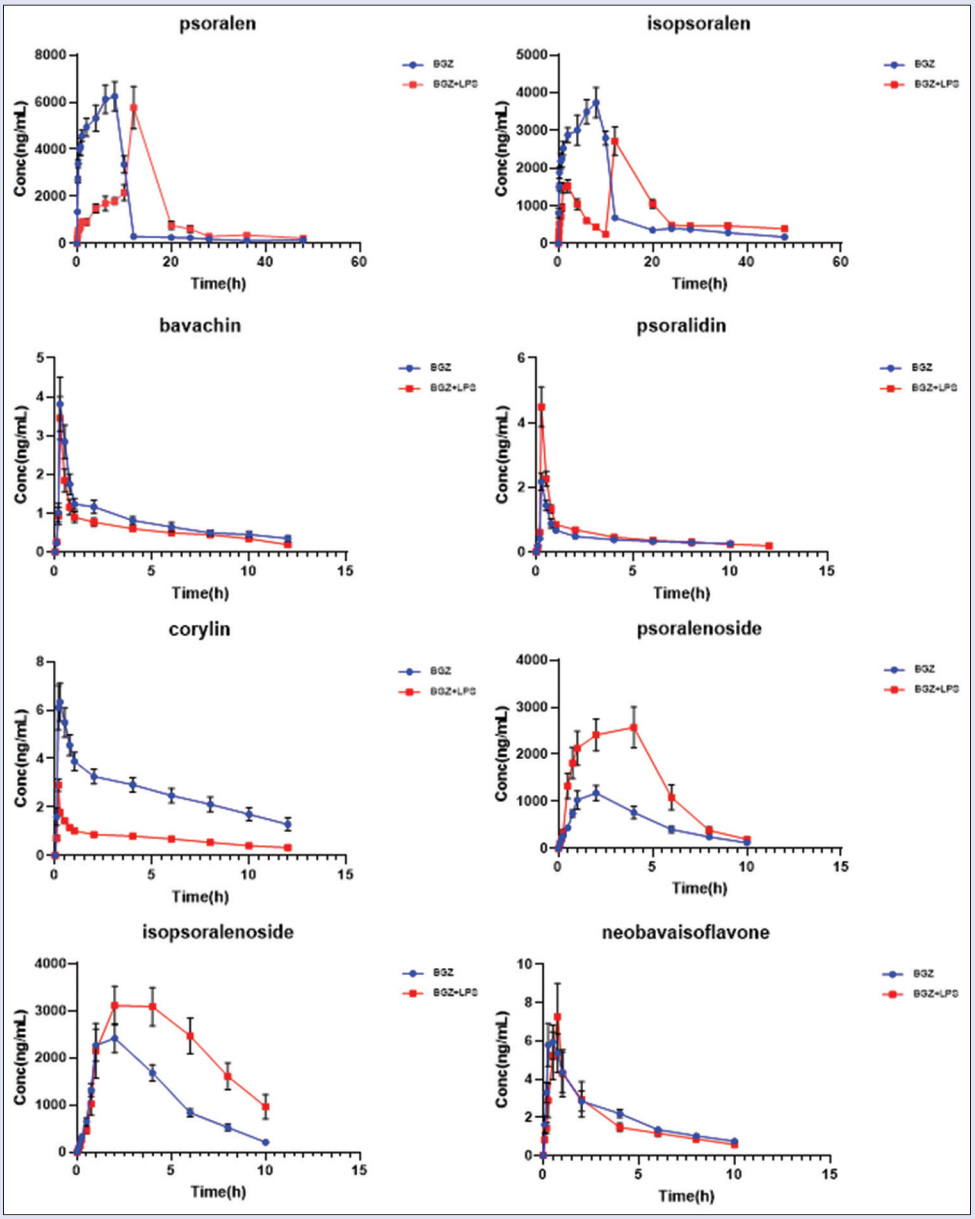

Figure 3: The plasma-drug concentration curves of eight analytes of PFE in plasma samples from normal and LPS-simulated (model) rats ($n = 12$, $\bar{x} \pm sx$)

Table 3: The precision and accuracy of eight analytes in rat plasma samples (n=6, $\bar{x}\pm s$)

Analytes	Concentration (ng·mL^{-1})	Inter-day			Intra-day		
		Content (ng·mL^{-1})	RR%	RSD%	Content (ng·mL^{-1})	RR%	RSD%
PL	7	7.05±0.25	100.75	3.52	7.01±0.47	100.12	6.65
	100	101.17±4.18	101.17	4.13	104.63±4.61	104.63	4.41
	1000	990.75±47.54	99.81	4.80	1009.18±55.11	100.92	5.46
IPL	7	7.37±0.22	105.23	3.01	7.93±0.62	108.63	7.88
	100	100.80±7.03	100.80	6.97	103.46±5.89	103.46	5.70
	1000	1017.76±79.28	105.63	7.79	1024.34±60.34	102.43	5.89
PO	10	9.76±1.12	97.61	11.47	10.61±0.62	106.06	5.85
	30	30.18±2.97	100.59	9.84	30.59±2.10	101.98	6.88
	500	504.04±14.54	101.36	2.88	497.18±21.23	99.44	4.27
IPO	10	10.13±0.83	101.31	8.23	9.84±1.32	98.36	13.42
	30	30.87±2.06	102.90	6.68	31.11±2.34	103.70	7.53
	500	522.82±50.09	103.35	9.58	544.26±28.61	108.85	5.26
BV	1.5	1.14±0.14	95.71	4.96	1.39±0.07	92.68	4.82
	45	43.47±1.26	96.60	2.89	44.17±1.14	98.16	2.58
	150	152.71±10.06	101.81	6.58	151.00±2.24	100.67	1.48
PLD	0.5	0.52±0.17	103.33	3.17	0.49±0.06	98.27	12.27
	10	9.73±0.74	97.28	7.60	9.85±0.33	98.48	3.39
	100	96.91±6.26	98.23	6.46	95.77±2.63	95.77	2.75
COR	0.5	0.51±0.03	101.77	6.86	0.50±0.05	100.47	10.51
	10	9.60±0.91	96.05	9.46	9.89±1.30	98.93	13.13
	100	98.96±2.29	98.96	2.31	100.07±2.28	100.07	2.28
NBI	0.5	0.49±0.05	98.03	9.37	0.49±0.04	98.33	8.60
	20	19.38±0.88	96.91	4.55	20.09±0.80	100.47	3.99
	200	191.64±8.41	95.82	4.39	200.17±15.45	100.08	7.72

Table 4: The stability of 8 analytes in rat plasma samples (n=6, $\bar{x}\pm s$)

Analytes	Concentration (ng·mL^{-1})	Storage (4°C, 24 h)		Freeze–Thaw (triplicates)		Long-term (–80°C, 30 d)	
		Content (ng·mL^{-1})	RSD%	Content (ng·mL^{-1})	RSD%	Content (ng·mL^{-1})	RSD%
PL	7	7.00±0.29	4.12	7.47±0.30	4.01	7.26±0.23	3.10
	100	104.14±4.54	4.35	101.29±3.37	3.33	102.23±2.39	2.34
	1000	1004.85±51.73	5.14	1021.13±24.16	2.37	1015.53±46.43	4.57
IPL	7	7.54±0.44	5.84	7.27±0.30	4.11	7.60±0.17	2.18
	100	103.35±6.19	6.00	103.06±2.63	2.55	102.65±3.57	3.48
	1000	1016.47±61.12	6.01	1032.30±21.92	2.12	982.38±23.21	2.36
PO	10	10.46±0.96	9.17	10.83±0.67	6.19	10.61±0.62	5.85
	30	30.42±2.17	7.14	30.06±0.89	2.96	30.41±1.86	6.13
	500	496.08±17.67	3.56	502.07±20.45	4.07	497.18±21.23	4.27
IPO	10	9.20±0.93	9.82	9.38±0.97	10.33	9.84±1.32	13.42
	30	31.26±2.99	9.58	31.22±1.11	3.56	28.14±3.22	11.43
	500	534.22±28.37	5.33	556.01±19.88	3.58	544.17±28.47	5.23
BV	1.5	1.33±0.12	8.76	1.21±0.03	2.19	1.39±0.07	4.82
	45	43.89±1.14	2.61	44.87±0.64	1.42	44.17±1.14	2.58
	150	151.56±6.80	4.45	147.82±3.14	2.13	150.86±2.03	1.34
PLD	0.5	0.49±0.06	12.27	0.53±0.06	12.05	0.46±0.04	9.58
	10	9.81±0.41	4.14	10.03±0.28	2.78	9.67±0.31	3.25
	100	97.49±3.98	4.08	102.16±9.89	9.68	95.65±4.94	5.17
COR	1	0.50±0.06	12.16	0.49±0.05	9.60	0.54±0.03	4.71
	10	9.87±1.18	11.98	9.00±0.24	2.63	9.08±0.30	3.30
	100	99.66±2.23	2.23	95.73±1.86	1.94	100.07±2.28	2.28
NBI	0.5	0.50±0.04	8.13	0.56±0.03	5.13	0.52±0.02	3.81
	20	19.84±0.79	3.98	20.38±0.16	0.76	19.81±0.69	3.47
	200	193.57±8.85	4.55	189.23±4.20	2.22	196.38±2.72	1.38

The pharmacokinetic profile of PFE in normal and LPS-stimulated rats was analyzed, in which the contents of eight analytes were qualified by the developed method of UPLC-TQ/MS during the period of 48 hr after PFE administration. The blood concentration–time curve of each analyte is shown in Figure 3, and relevant pharmacokinetic parameters (including *AUC*, MRT, VRT, λ, V, $t_{1/2}$, C_{max}, T_{max}, and CL) calculated based on blood concentration are listed in Table 5.

Results indicated that all eight analytes in PFE were rapidly absorbed into the circulation, which was in accordance with previous studies.[19] It was observed that the *AUC* (0–t) of psoralenoside, isopsoralenoside, and psoralidin in the LPS-primed model group was significantly increased compared with the normal group, while the *AUC* (0–t) of corylin in the model group was significantly decreased. Meanwhile, in comparison to the normal group, the CL of psoralenoside and isopsoralenoside in the model group was nearly half decreased, whereas the CL of psoralidin

Table 5: The relevant pharmacokinetic parameters of eight analytes in normal and LPS-simulated (model) rat plasma samples (n=12, $\bar{x}$±s)

Analytes	Group	*AUC* (0-t)	MRT (0-t)	VRT (0-t)	λz	$t_{1/2}z$	T_{max}	Vz	CLz	C_{max}
		ng/ml·h	h	h^2	1/h	h	h	L/kg	L/h/kg	ng·mL^{-1}
PL	Control	61231.02±15017.05	7.18±0.60	41.05±16.66	0.05±0.03	19.86±12.96	5.96±2.62	448590.31±252258.48	17003.71±6069.65	6813.96±1967.81
	Model	59346.95±20280.01	14.24±0.99**	91.50±20.67**	0.05±0.03	27.25±31.78	11.17±1.80**	539352.20±488046.49	16550.84±5269.47	5956.87±2992.54
IPL	Control	46938.54±8408.78	11.44±1.32	117.81±17.53	0.05±0.01	16.19±4.12	7.00±1.35	478372.41±159699.66	20183.67±2970.77	4014.98±1364.44
	Model	40271.62±9180.33	18.39±2.26**	154.25±31.63**	0.03±0.04	48.13±42.14*	9.33±4.83	900719.17±412227.67**	17667.50±6829.46	2921.18±1058.04*
PO	Control	5732.16± 2550.11	3.51±0.41	5.43±1.27	0.31±0.18	5.53±10.49	1.79±0.88	1386026.75±1803068.06	260032.85±141951.27	1356.45±671.06
	Model	14101.75±5663.49**	3.65±0.34	4.54±1.34	0.49±0.26	2.27±2.18	2.50±1.65	456585.72±599252.00	113705.96±52848.79**	3064.08±1291.35**
IPO	Control	11902.75±4289.77	3.62±0.31	5.21±0.77	0.40±0.13	2.01±1.00	1.67±0.89	5.06±2.91	1.80±0.74	2627.03±1133.11
	Model	22362.13±9764.73**	4.76±0.67**	6.51±1.11**	0.29±0.20	4.86±5.10	2.42±1.24	4.84±5.06	0.91±0.56**	3637.22±1655.29
BV	Control	9.42±3.81	4.11±0.56	11.08±1.95	0.19±0.13	7.67±6.90	0.25±0.00	37284.47±16982.12	2507.07±1001.52	2.17±0.91
	Model	7.09±2.59	4.34±0.47	11.46±0.90	0.18±0.11	5.59±3.63	0.25±0.00	27306.98±10402.19	2391.28±1066.08	4.49±2.13**
PLD	Control	4.53±1.12	3.94±0.54	9.73±1.71	0.10±0.09	15.65±17.23	0.19±0.04	4650.21±1910.16	536.85±317.63	7.79±2.92
	Model	6.19±1.06**	3.76±0.43	11.58±1.14**	0.10±0.06	11.16±10.93	0.17±0.00	14189.31±4776.17**	1890.89±433.44**	2.90±0.85**
COR	Control	31.29±11.37	4.70±0.45	11.34±1.33	0.13±0.08	7.98±5.66	0.35±0.23	17936.35±16655.09	1878.82±752.77	3.96±2.35
	Model	8.42±1.56**	4.64±0.39	11.39±0.98	0.15±0.06	5.48±2.34	0.25±0.00	19226.43±13776.30	2495.78±956.23	3.45±1.92
NBI	Control	21.12±6.77	3.40±0.55	7.22±1.52	0.21±0.11	4.77±3.48	0.60±0.28	4918.71±2101.17	843.84±318.32	8.60±4.45
	Model	18.53±12.16	3.48±0.61	7.42±1.53	0.12±0.04*	7.77±6.87	0.67±0.12	9875.94±6577.21*	945.97±438.01	8.48±6.03

Note: Compared with normal group, *P<0.05, **P<0.01

was dramatically increased. Both the MRT (0–t) and VRT (0–t) of psoralen, isopsoralen, and isopsoralenoside in the model group were markedly enhanced. The λz of neobavaisoflavone, C_{max} of isopsoralen, and psoralidin were obviously decreased in the model group. The $t_{1/2}$ of isopsoralen, T_{max} of psoralen, Vz of isopsoralen, psoralidin, and neobavaisoflavone were all significantly increased in LPS-primed rats.

According to the plasma concentration profile and pharmacokinetic parameters of eight PFE analytes, the apparent volume of distribution (V) of psoralenoside was the largest among all the analytes, suggesting a wider distribution of PO in the body, and the distribution of isopsoralen, psoralidin, and neobavaisoflavone were all significantly increased in LPS-primed rats. The T_{max} of PL and IPL were relatively longer than the other six analytes in both normal and model rats, indicating the slower absorbance velocity of both analytes, and the absorbance of PL was significantly reduced in LPS-primed rats. The C_{max} of PL, IPL, PO, and IPO was much higher than the other analytes due to their high content in PFE. The C_{max} of IPL was remarkedly reduced in model rats, whereas the C_{max} of PO and BV became much higher.

The *AUC* (0–t), indicating relative bioavailability, of PO, IPO and PLD had a nearly two-fold increase in model rats, while the *AUC* (0–t) of COR was dramatically reducing. This indicates that both the different analytes contents and the state of the body lead to different systemic exposure. The $t_{1/2}$ of PL, IPL, and PLD was much higher among these eight analytes, indicating the slow elimination of them.

CONCLUSION

A simple, sensitive and reliable UPLC-TQ/MS method was constructed to simultaneously determined eight analytes (including psoralen, isopsoralen, psoralidin, corylin, psoralenoside, isopsoralenoside, bavachin, and neobavaisoflavone) in Psoraleae Fructus ethanol extract. This method was also acceptably validated and applied to examine the pharmacokinetic profile of these analytes after oral administration with the aid of normal and IDILI-simulated rats, and to further explain the related efficacy and IDILI property of PF. By comparison, in the LPS-stimulated rats, the pharmacokinetic parameters of the eight analytes in PFE were dramatically different, which indicated that the immune stress state might appreciably impact the pharmacokinetic profile of the bioactive constituents of PF. Therefore, the clinical administration of PF should fully consider the different states of the patients to avoid the occurrence of clinical heterogeneous liver injury.

Credit authorship contribution statement

Weixia Li: Methodology, Writing - review and editing, Conceptualization; Xiaoyan Wang: Funding acquisition, Investigation; Hui Zhang: Formal analysis, Software, Data curation; Mingliang Zhang: Methodology, Project administration; Yali Wu: Funding acquisition, Writing - original draft, Validation; Lu Niu: Project administration, Visualization; Shuqi Zhang: Project administration, Software; Xiaofei Chen: Investigation, Validation; Jinfa Tang: Supervision, Funding acquisition.

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Conflicts of interest

There are no conflicts of interest.

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