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Analysis of daidzein in nanoparticles after oral co-administration with sodium caprate to rats by ultra-performance liquid chromatography–quadrupole-time-of-flight mass spectrometry

Yiran Ma[a], Li Zhang[b], Xinyi Zhao[a], Qi Shen[a,*]

[a] School of Pharmacy, Shanghai Jiao Tong University, Dongchuan Road 800, Shanghai 200240, China
[b] Instrumental Analysis Center, Shanghai Jiao Tong University, Dongchuan Road 800, Shanghai 200240, China

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ABSTRACT

An ultra-performance-liquid-chromatography–quadrupole-time-of-flight mass spectrometry (UPLC/Q-TOF-MS) method was developed and validated for the quantitation of daidzein (DZ) in rat plasma. Ethylparaben was chosen as internal standards (IS). DZ was linear over the range of 0.001–5 μg/mL. The lower limit of quantification (LLOQ) was 0.001 μg/mL and the limit of detection (LOD) was 0.0005 μg/mL. The intra-day and inter-day relative standard deviations (RSDs) were ranged from 3.59% to 6.43% and 5.35% to 7.25%, respectively. This UPLC/Q-TOF-MS method provided good specifity, highly sensitivity, accurate and high-speed detection (6 min), applicable to the pharmacokinetics study in rats *in vivo* after oral administration of free daidzein solution, daidzein-loaded poly (lactide-*co*-glycolide) (PLGA) nanoparticles (D-NPs) suspension and D-NPs co-administered with sodium caprate (C_{10}) which as the oral absorption promoter. It was shown that the pharmacokinetics behavior was significantly improved after the oral administration of D-NPs suspension co-administered with absorption promoter C_{10} by the fact that the relative bioavailability were enhanced about 4.24-fold, compared to that of DZ suspension.

1. Introduction

Daidzein [(4,7-dihydroxylisoflavone), CAS 486-66-8, Fig. 1A] (DZ) is one of the metabolism of soy isoflavones, mainly exists in leguminous plants, especially in soybeans, soy foods and traditional Chinese medicines such as *Puerariae radix*, and human fluids [1,2]. As one kind of isoflavone, DZ has become of immense interest to clinical nutritionists because of its potential to modulate and prevent numerous hormonal and non-hormonal-dependent diseases [3,4]. It has also been reported that it possessed antigiardial activity [5], antioxidant action, and potential antidiabetic properties [6,7]. Several methods have been covered to analyze DZ in urine, feces, and blood plasma after the intake of soy products. However, its poor oral absorption and bioavailability limit its clinical samples detection, which could result from its poor solubility in water; in particular, the strong metabolism that occurs in the intestine and liver [8,9]. Therefore a sensitive and accurate analytical method for the determination of DZ in biological samples is of great importance. A variety of analytical techniques have been applied to the quantitation of DZ in biological fluids, including high-performance liquid chromatography (HPLC) [10,11], liquid chromatography coupled to electrospray mass spectrometry (LC/ES-MS) [12], gas chromatography–mass spectrometry (GC–MS) [13] and other analytical methodologies. However, most of these are limited in some way. As to the HPLC method, it requires fewer steps for sample preparation and analysis, we have been successfully developed HPLC method for the quantitation of DZ in rat plasma after DZ was encapsulated into PLGA nanoparticles, but this method shows less sensitivity (LOD was 0.05 μg/mL) for tentatively identify the pharmacokinetic behavior of D-NPs with or without C_{10} after oral administration to the SD rats and require relatively large volumes of blood samples. A tedious derivatization process is often required for GC-MS detection. Compared with HPLC, LC/ES-MS method also shows much more sensitivity and specificity, but the biological matrix must be investigated which also makes the analysis process becomes very tedious. In clinical samples detection, one kind of successful assay method must simultaneously meet the following conditions: simple sample pre-treatment, high accuracy, high efficiency with appropriate sensitivity, reproduced experimental data, and so on. Unfortunately, the above three methods do not meet the clinical requirement. This study firstly developing ultra-performance-liquid-chromatography–quadrupole-time-of-flight mass spectrometry (UPLC/Q-TOF-MS) method for tentatively identifies the pharmacokinetic behavior of D-NPs with or without C_{10} after oral administration to the SD rats.

* Corresponding author. Tel.: +86 21 34204049; fax: +86 21 34204049.
E-mail address: qshen@sjtu.edu.cn (Q. Shen).

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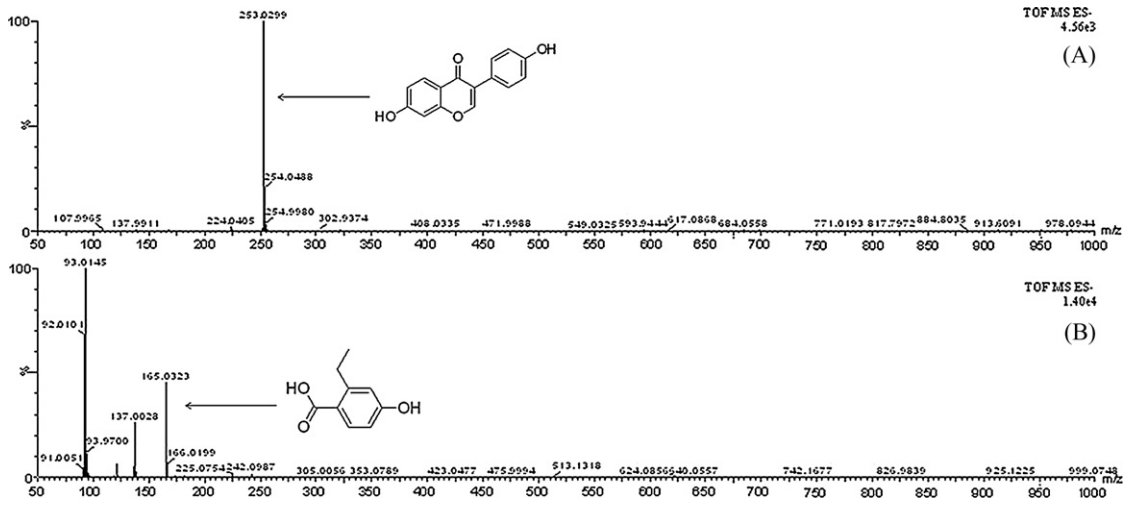

Fig. 1. The product-ion mass spectra of $[M-H]^-$ ions of daidzein (DZ, A) and ethylparaben (IS, B).

In the present work, firstly, an UPLC-Q-TOF-MS-based analytical method was developed for the determination of DZ in rat plasma within the addition of the internal standard ethylparaben (IS, Fig. 1B) as a tool for quality control. The validation results showed a higher sensitivity (an LLOQ as low as 1 ng/mL), a relatively shorter analytical time (6 min per sample). Secondly, the developed method was successfully applied to the pharmacokinetic behavior study of DZ formulations (DZ suspension, D-NPs, and D-NPs co-administered with C_{10}) in rats *in vivo*. Some studies have employed bile salts, such as deoxycholic acid, for improving oral delivery with existing biodegradable systems and demonstrated that deoxycholic acid can offer protection in the stomach as well as improved bioavailability [14]. Furthermore, using absorption promoter on oral drug delivery platforms did not involve new chemical entities and could be fine-tuned to apply to a range of poorly permeable drugs [15]. In our study it was found that the oral bioavailability of D-NPs co-administered with C_{10} in rats was significantly improved, compared to that of DZ suspension or D-NPs suspension without the addition of absorption promoter. To our best knowledge, few assay methods cover the pharmacokinetic behavior in rats after the oral co-administration of D-NPs suspension and C_{10} (CH_3–$(CH_2)_8$–COONa, which widely exists in foodstuffs and dairy products) as an absorption promoter. It would be helpful in understanding of health-promoting properties of DZ.

2. Experimental

2.1. Chemicals and reagents

Daidzein (DZ, purity > 98%, Fig. 1A) and ethylparaben (99.8%, IS, Fig. 1B) were purchased from Qingze Co. Ltd. (Nanjing, China). Sodium caprate was purchased from Sigma (98.0%, St. Louis, MO, USA). Hydroxypropyl-beta-cyclodextrin (HP-β-CD), sodium carboxymethyl cellulose (CMC-Na), acetonitrile, methylene chloride, chloroform and dimethyl sulfoxide (DMSO) were purchased from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China). Polyvinyl alcohol (PVA, viscosity 11–14 cp) was purchased from Kayon biological technology Co. Ltd. (Shanghai, China). Distilled water was produced by a Milli-Q purification system (Millipore, Billerica, MA, USA). The rest chemicals and reagents were of analytical grade.

2.2. Animal handling

Healthy male Sprague–Dawley (SD) rats (220 ± 20 g) were obtained from the Laboratory Animal Center, Shanghai Jiao Tong University, China. The animal experimental protocols were performed according to the guideline of the Experimental Animal Ethics Committee of Shanghai Jiao Tong University. Rats were kept in a room under a controlled temperature of 21 ± 1 °C and a relative humidity of 50 ± 10%, and housed in groups of three in stainless-steel wiremesh cages.

2.3. UPLC–MS instruments and analytical conditions

UPLC was performed on an ACQUITY UPLC™ system (Waters Corp., Milford, MA, USA), equipped with a binary solvent delivery system, and a sample manger coupled to Micromass Q-TOF Premier™ mass spectrometer (Waters Corporation, Milford, MA, USA) equipped with an electrospray interface. Chromatographic separation was performed on an ACQUITY UPLC Hypersil Gold C_{18} column (1.9 μm; 100 × 2.1 mm; Thermo, scientific, Co.) at 25 °C and using a mobile phase that consisted of water (A) and acetonitrile (B) with the following gradient program: 85% A:15% B (v/v) at 0 → 6.0 min; 40% A:60% B (v/v) at 6.0 → 6.3 min; 5% A:95% B (v/v) at 6.3 → 7.0 min; 85% A:15% B (v/v) at 7.0 → 9.0 min. The auto-sampler temperature was conditioned at 4 °C. The injection volume was 7 μL using the partial loop mode for sample injection. The chromatographic run time per sample was 6 min. The separated compounds were detected by a diode array detector. The mass spectrometer was operated with electrospray ionization (ESI) interface in negative ionization mode for DZ and IS. The ionization source conditions were: capillary voltage 3.0 kV, cone voltage 55 V, source temperature 100 °C and desolvation temperature 250 °C. The data was collected between 50 and 1000 *m/z* with the optimized collision energy at 6.0 V for DZ and IS. The cone and desolvation gas flow rates were 50 and 600 L/h, respectively. Under these conditions, For ESI^- the target ions were *m/z* 253.03 for DZ and *m/z* 165.03 for IS, respectively. The raw data files were then processed by MassLynx 4.1. From the acquired full scan data, the drug-specific ions were extracted for construction of the chromatograms and evaluation of the results.

2.4. Preparation of samples

2.4.1. Preparation of standards and quality control samples

The stock solutions of DZ and IS were prepared at 100 μg/mL in methanol, respectively, and stored at −20 °C; standard solutions were prepared by diluting the stock solutions above with methanol. The concentration of working solution for internal standard was 0.5 μg/mL. Calibration standards of DZ at concentrations of 0.001,

0.005, 0.05, 0.5, 1 and 5 μg/mL were prepared by spiking appropriate amounts of the standard solutions in blank plasma. The quality control (QC) samples were prepared at concentrations of 0.005, 0.1 and 4 μg/mL in a similar way to the calibration standards.

2.4.2. Preparation of plasma samples

100 μL blank plasma was obtained by centrifugation of orbital blood and then spiked with 10 μL of DZ working solutions and IS (0.5 μg/mL) respectively; 900 μL acetonitrile was added and the mixture was vortexed for approximate 1 min, then centrifuged at 15,000 rpm for 15 min to remove protein and extracted the drugs, the supernatant acetonitrile was withdrawn and dried by nitrogen gas. Finally the residue was reconstituted in 100 μL methanol and vortexed for 1 min, and then 7 μL of the solution was injected for analyses.

2.5. Method validation

The method was validated for specificity, matrix effect (ME), linearity, LLOQ, accuracy, precision, extraction recovery and stability. The different validation parameters and the values for acceptance of the range of validation parameters were in accordance with international guidelines and Food and Drug Administration guidelines.

2.5.1. Specificity

The specificity of the method was investigated by comparing the UPLC/Q-TOF-MS base peak intensity (BPI) chromatograms of blank plasma, plasma sample spiked with DZ and IS, and plasma sample at 2 h after oral administration of D-NPs spiked with IS. The blank plasma samples were obtained from six rats. There should be no interference from endogenous or exogenous materials observed at the retention time of DZ and the IS.

2.5.2. Calibration curves, linearity, LLOQ and LOD

Calibration curves were prepared using six standard plasma samples over the range 0.001–5 μg/mL. The values of A_{DZ}/A_{IS} (y) and the analyte concentrations of DZ (x) were used to plot the calibration curve ($y = ax + b$), where a is an intercept and b is a slope concentration. The lower limits of quantitation (LLOQ) was defined as the lowest concentration of analytes that can be determined with an acceptable accuracy and precision by a particular method at which the signal-to-noise ratios (S/N) were greater than 10. In our method the LLOQ values were established at the lowest concentration of the linear calibration range, while the limit of detection (LOD) was estimated as the amount of DZ which caused a signal three times to noise.

2.5.3. Precision

Precision was defined as the relative standard deviation (RSD%). The intra- and inter-run precision of the UPLC/Q-TOF-MS method was assessed from the results of QC samples at three concentration levels (0.005, 0.1 and 4 μg/mL). The mean values and RSD for QCs at three concentration levels were calculated over three validation runs. Six replicates of each QC level were determined in each run. These data were then used to calculate the intra- and inter-run precision (RSD) by a one-way analysis of variance.

2.5.4. Extraction recovery

The matrix effect was evaluated by analyzing six different blank plasma samples, spiked at the concentration of LLOQ. Extraction recovery was determined using the extraction and analysis of three QC samples (0.005, 0.1 and 4 μg/mL). The extraction recovery of IS was determined in the same way at a concentration of 0.5 μg/mL. Each control level was prepared with six replicates.

2.5.5. Stability

Sample stability was determined by analyzing QC samples after three freeze–thaw cycles, at room temperature for 4 h, −20 °C for 7 days (at the concentration of 0.005, 0.1, and 4 μg/mL) ($n = 6$). The DZ remaining (%) was determined by comparing the means of the calculated concentrations of DZ samples at the above conditions with that of freshly prepared QC samples.

2.5.6. Application of the assay to pharmacokinetic studies

The health SD rats (220 ± 20 g) were randomly divided into three groups, with six rats in each and fasted overnight with free access to water before the experiments. They were administered orally with D-NPs, D-NPs with the addition of C_{10} (100 mg/kg) and DZ suspension (DZ dispersed in 0.5% sodium carboxymethyl cellulose solution) at a dose of 10 mg/kg, respectively. After administration, blood samples (about 0.25 mL) were collected from the retro-orbital plexus before dosing and at the predetermined time points of 0.5, 1, 1.5, 2, 4, 8, 12, 24, 36, and 48 h. Then the blood samples were immediately transferred to heparinized tubes (100 IU/mL blood) and centrifuged at 5000 rpm for 5 min. The plasma samples obtained were stored at −80 °C until analysis.

The UPLC/Q-TOF-MS data of DZ and IS were analyzed by the MarkerLynx software (version 4.1; Waters, Manchester, UK). All the plasma concentration time profiles for each individual and mean concentration for each dose group were analyzed using the micro-extravascular model with two-compartmental method and expressed as the mean ± SD of at least six experiments. The area under the concentration–time curve ($AUC_{0-48\,h}$) from zero to the last time point (48 h), the peak plasma concentration (C_{max}), the time taken to reach peak concentrations (T_{max}), the time taken for the plasma concentration to decrease by half and thereby reflecting the rate of elimination ($T_{1/2}$) and the mean residence time (MRT) provide an indication of absorption characteristics were all obtained. Statistical significance was assessed by Student's t-test or Dunnett's test for multiple comparisons with $p < 0.05$ as the minimal level of significance.

3. Results

3.1. Method development

3.1.1. Sensitivity

Under the optimum UPLC/Q-TOF-MS conditions mentioned above, DZ and IS all showed higher responses in the negative ion mode than in the positive ion mode, the spectra showed the most abundant ions at m/z 253.03 for DZ and 165.03 for IS, in addition, it also showed high abundance fragment ions at m/z 93 and 137 for IS. The product-ion spectrum of DZ and IS are shown in Fig. 1. The quantitative analysis was also carried out at m/z 253.03 for DZ and 165.03 for IS, respectively.

3.1.2. Specificity

Representative UPLC/Q-TOF-MS base peak intensity (BPI) chromatogram of DZ and IS in rat plasma samples are shown in Fig. 2. From the results, DZ and IS were separated on baseline with retention times of 2.78 and 3.49 min, respectively; there were also no typical ion peaks (m/z 253.03 for DZ and 165.03 for IS) can be found in blank plasma samples. So these observations indicated that the assay had adequate specificity.

3.1.3. Linearity, LLOQ and LOD

The standard curves were linear in the concentration range of 0.001–5 μg/mL. The calibration equation was $y = (7.69 \pm 0.31)x + (8.67 \pm 0.27)$ ($r^2 = 0.9988$, $n = 6$). The LLOQ of DZ in plasma was 0.001 μg/mL (lowest standard level, signal-to-noise > 10:1) which is appropriate for quantitative detection

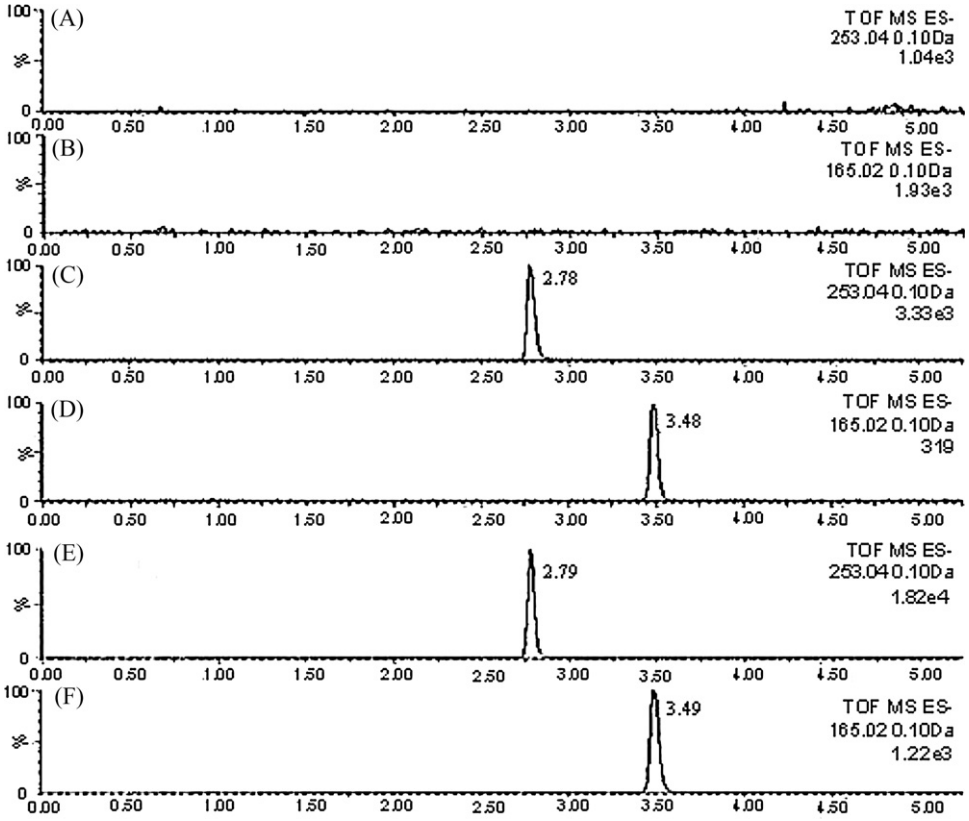

Fig. 2. UPLC/Q-TOF-MS base peak intensity (BPI) chromatograms of daidzein and ethylparaben (IS) in rat plasmas: (A and B) blank rat plasma samples; (C and D) a blank rat plasma sample spiked with daidzein (0.5 μg/mL) and IS (0.5 μg/mL); (E and F) a SD rat plasma sample at 2 h after oral administration of D-NPs spiked with IS (0.5 μg/mL).

of analytes in the pharmacokinetic studies. The LOD of DZ was 0.0005 μg/mL which produced a signal-to-noise of 3:1. The data above showed that the assay was sensitive enough for pharmacokinetics study of DZ *in vivo*.

3.1.4. Precision

The intra- and inter-day precisions of this method were evaluated using the above three QC samples which were prepared with DZ working solutions and at the concentration of 0.005, 0.1 or 4 μg/mL ($n = 6$). The intra- and inter-day precisions (RSD%) were ranged from 3.59% to 6.43% and 5.21% to 7.25%, respectively. The data of the precision in the assay are within the recommendations. These results indicate that this method has good precision.

3.1.5. Extraction recovery and stability

The mean extraction recoveries of 6 repeated DZ QC samples at concentrations of 0.005, 0.1, and 4 μg/mL ($n = 6$) were found to be 73.52 ± 5.23%, 76.93 ± 3.81% and 79.70 ± 7.59%, respectively. The mean extraction recovery of the IS was 77.21 ± 1.63% (at the concentration of 0.5 μg/mL). The results are listed in Table 1. There is no significant difference in the recoveries of DZ among low, medium, and high concentrations. The stability of DZ in rat plasma were also evaluated by analyzing six replicates of quality control samples mentioned above after three freeze–thaw cycles, at room temperature for 4 h, −20 °C for 7 days. All the samples displayed after various stability tests. The results are listed in Table 2.

Table 1
Extraction recoveries of DZ from rat plasma ($n = 6$).

Concentration (μg/mL)	Recoveries ± SD (%)	RSD (%)
0.005	73.52 ± 5.23	10.30
0.10	76.93 ± 3.81	7.38
4.00	79.70 ± 7.59	8.39
IS (0.5)	77.21 ± 1.63	10.35

3.2. Application to a pharmacokinetic study in rats

The presented method was successfully applied to the determination of DZ in plasma obtained from the SD rats following oral administrations of D-NPs co-administrated with C_{10} suspension, D-NPs and DZ suspension at a dose of 10 mg/kg, respectively. The acetonitrile protein precipitation method was used in the plasma treatment. All pharmacokinetic analyses of the rats dosed with DZ solution, D-NPs suspension and D-NPs co-administrated with C_{10} suspension were calculated using ten time points (0.5, 1, 1.5, 2, 4, 8, 12, 24, 36 and 48 h). The mean plasma concentrations *versus* time profiles are shown in Fig. 3. A remarkable increase in the plasma concentrations of DZ was observed when D-NPs suspension and D-NPs co-administrated with C_{10} suspension were studied than that of pure DZ. The curves also clearly showed that there was a

Table 2
Stability study of DZ in rat plasma ($n = 6$).

Measured concentration (μg/mL)	Spiked concentration (μg/mL)		
	0.005	0.1	4
Stability at room temperature for 4 h			
Mean ± SD	0.0051 ± 0.0006	0.12 ± 0.01	4.36 ± 0.33
RSD (%)	11.80	10.30	7.60
Three freeze and thaw stability			
Mean ± SD	0.0049 ± 0.0007	0.13 ± 0.02	4.15± 0.38
RSD (%)	14.30	12.40	9.20
Stability for 7 days at −20 °C			
Mean ± SD	0.0052 ± 0.0005	0.12 ± 0.01	4.31 ± 0.24
RSD (%)	9.60	8.40	5.60

Table 3
Pharmacokinetic parameters of daidzein after oral administration of daidzein suspension, D-NPs suspension, and D-NPs co-administered with C_{10} (100 mg/kg). Each was administered at a dose of 10 mg/kg. Values were calculated as mean ± SD (n = 6).

Parameter	Unit	DZ suspension	D-NPs	D-NPs + C_{10}
T_{max}	h	1.21 ± 0.16	1.58 ± 0.28	2.13 ± 0.49[a]
C_{max}	mg/mL	1.18 ± 0.39	2.39 ± 0.53[a]	4.02 ± 0.98[b]
$T_{1/2}$	h	1.48 ± 0.01	4.30 ± 1.59[b]	7.68 ± 1.54[b]
MRT	h	19.21 ± 1.38	33.74 ± 1.74[a]	50.94 ± 1.96[b]
$AUC_{0-48\,h}$	μg h mL^{-1}	11.47 ± 0.24	34.64 ± 0.38[b]	48.59 ± 0.16[b]
F_{rel} (%)		–	302	424

F_{rel}, relative bioavailability.
[a] $P < 0.05$ compared with DZ suspension group.
[b] $P < 0.01$ compared with DZ suspension group.

great difference in bioavailability between the D-NPs group and D-NPs co-administrated with C_{10} group. In the case, the profile of the D-NPs curve can be divided in two parts: in the first part, D-NPs had a high sharp absorption peak at 1.5 h; in the second part D-NPs had a low absorption peak at 8 h. Surprisingly, D-NPs co-administrated with C_{10} group had only one higher sharp absorption peak at 2 h. Through the developed method, it can be identified that the plasma concentrations of DZ in D-NPs co-administrated with C_{10} group were much higher compared to DZ suspension. The pharmacokinetic parameters of DZ in rat plasma were determined and presented in Table 3. Compared to the control group, both of the nanoparticles treated rats groups have higher concentrations of DZ in plasma during 1.5–24 h, and the area under the plasma concentration–time curve ($AUC_{0-48\,h}$) is increased by 3.02 and 4.24 times, respectively. The mean residence time (MRT), the half-life ($T_{1/2}$) and the maximum plasma concentrations (C_{max}) of D-NPs were also higher than the DZ control group.

4. Discussion

For the quantitative and confirmatory analysis of DZ in the blood samples, a new method based on UPLC/Q-TOF-MS was developed and applied. In this study, a series of preliminary experiments was carried on different mobile phases including acetonitrile/water, methanol/water for complete chromatographic resolution of DZ and IS (data not shown). From the test we knew that the use of methanol in the mobile phase to perform fast separation has some limitations when compared to acetonitrile, since it has a higher viscosity resulting in higher pressure, which can reduce maximum solvent flow rate allowed within the chromatographic system maximum pressure and increase separation time required for the best separation of DZ and IS. Otherwise, the use of the columns with small 1.9 μm particles in UPLC leads to enhanced chromatographic separation efficiencies that translate in narrow peaks. With the 100 mm × 2.1 mm Hypersil Gold C_{18} column used, the chromatographic separation of DZ and IS could be achieved in less than 6 min (see Fig. 2). In this study, a sensitive and efficient UPLC/Q-TOF-MS method was developed and validated for the determination of DZ in rat plasma for the first time using gradient elution with a mobile phase composed of solvent A (water) and solvent B (acetonitrile). The BPI (based peak intensity) chromatogram obtained from the analysis of DZ and IS, respectively. The LOD and LLOQ of this method were 0.5 ng/mL and 1 ng/mL respectively and the runtime was within 6 min which produced significant improvements in method sensitivity, speed, and resolution when compared to conventional HPLC–MS method [12]. So this method was successfully applied to the comparative pharmacokinetic study of DZ after oral administration of D-NPs and D-NPs co-administered with C_{10} compared with DZ suspension as control.

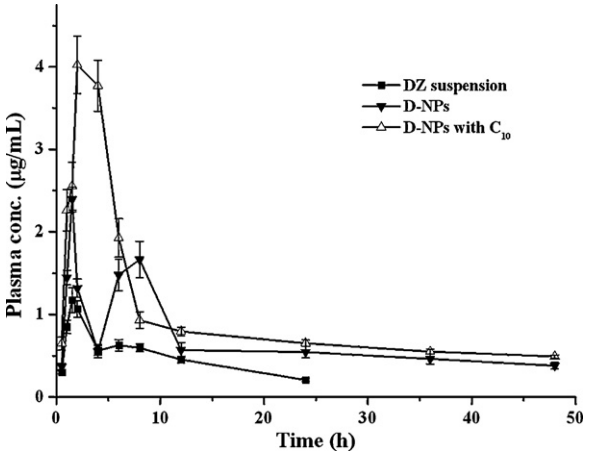

Fig. 3. Mean plasma concentration–time profiles of daidzein in SD rats (mean ± SD, n = 6) after orally of daidzein suspension, D-NPs, and D-NPs with C_{10} (100 mg/kg) suspension at the dose of 10 mg/kg, respectively.

The pharmacokinetic behavior of DZ was investigated in healthy SD rats after oral administration. The drug concentration–time curves of DZ are shown in Fig. 3. After administration, plasma concentrations of DZ suspension by oral route as control decreased rapidly after 1.5 h, it was showed very low drug concentration and bioavailability which was accordant with Qiu's report [16]. On the contrary, D-NPs groups showed a prolonged circulation time of DZ in blood which could still be measured in plasma at 48 h and obtained with higher C_{max}, larger AUC and longer $T_{1/2}$. Interestingly, a double-peak profile were found from the D-NPs group while the second lower absorption peak at 8 h was disappeared from the D-NPs added with C_{10} group. Importantly, C_{max} of the concentration–time curves was significantly higher ($p < 0.05$) in D-NPs with C_{10} (1.68-fold) compared with D-NPs-dosed rats and also observed increased bioavailability since the D-NPs with C_{10} yielded an $AUC_{0-48\,h}$ of 48.59 μg h mL^{-1} compared to 34.64 μg h mL^{-1} without C_{10}. This increased absorption could be resulted from several factors. On the one hand, PLGA is often chosen due to its biocompatibility and versatility in encapsulating a variety of drugs and biologics, as well as the ability to tune the dynamics of drug release by varying monomer ratios and polymer molecular weight [17–19]. Oral delivery of PLGA particles and uptake by intestinal cells has also been well studied [20,21]. Therefore, D-NPs delivery system for oral administration could maintain DZ levels in the effective range for improve the bioavailability of DZ *in vivo*. On the other hand, many studies have confirmed that C_{10} could increases the flux of many different types of poorly permeable agents across intestinal epithelia *in vitro* and has no impact on human health which is approved by the FDA [22–28]. D-NPs containing C_{10} (at the dose of 100 mg/kg) was employed to assess the utility of C_{10} on D-NPs delivery and efficacy [29]. In present study, it is clearly showed that C_{10} could improve oral delivery of D-NPs, it had a higher concentration of absorption for a shorter time (2–4 h after dosing) when employed D-NPs with C_{10}. This phenomenon can be explained that C_{10} could open the tight junction of intestinal epithelium rapidly and reversibly first, and then the most

of D-NPs could be rapidly absorpted into the intestinal epithelium, the rest of them could be absorpted slowly. This maybe the reason why the first absorption peak at 2 h is much higher than the C_{10}-free formulation and the second lower absorption peak at 8 h is disappeared.

5. Conclusion

A sensitive, selective and fast UPLC/Q-TOF-MS method for the determination of DZ in rat plasma was developed and validated. The acetonitrile protein precipitation method was used in the plasma treatment, which provided clean sample and consistent high extraction recovery. The analytes were separated by UPLC Hypersil Gold C_{18} column (1.9 μm; 2.1 × 100 mm), and detected by Q-TOF-MS equipped with an electrospray ionization (ESI) source. The developed method allows satisfactory separation, highly sensitivity, accurate and high-speed detection for tentatively identify the pharmacokinetic behavior of D-NPs with or without C_{10} after oral administration to the SD rats.

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