Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 852 (2007) 85–91
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Simultaneous determination of 1,5-dicaffeoylquinic acid and its active metabolites in human plasma by liquid chromatography–tandem mass spectrometry for pharmacokinetic studies

Ruolan Gu [a], Guifang Dou [a], Jing Wang [a], Junxing Dong [b], Zhiyun Meng [a,*]

[a] *Laboratory of Drug Metabolism and Pharmacokinetics, Beijing Institute of Transfusion Medicine, 27 Taiping Road, Beijing 100850, China*
[b] *Beijing Institute of Radiation Medicine, 27 Taiping Road, Beijing 100850, China*

Received 25 October 2006; accepted 31 December 2006
Available online 13 January 2007

Abstract

1,5-Dicaffeoylquinic acid (1,5-DCQA), a potent HIV-1 integrase inhibitor, is undergoing an evaluation as a promising novel HIV therapeutic agent. Here, we report a simple, rapid and robust LC–MS/MS method for simultaneous determination of 1,5-DCQA and its two active metabolites, 1-caffeoyl-5-feruoylquinic acid (1,5-CFQA) and 1,5-*O*-diferuoylquinic acid (1,5-DFQA) in human plasma. The quantitation of the target compounds was determined by selected reaction monitoring (SRM) mode using electrospray ionization (ESI). Good linearity was obtained in the 3–500 ng/ml range for each analyte and the analytical method was validated in terms of specificity, precision, accuracy, recovery, stability and matrix effect. These assays gave R.S.D.% values for precision always lower than 13.8% and R.E.% values for accuracy between −8.9 and 0.9%. In addition, the specificity, extraction recovery, stability and matrix effect were satisfactory too. Using the measured plasma concentrations of 1,5-DCQA and its active metabolites in five healthy volunteers, pharmacokinetic profiles of 1,5-DCQA and its active metabolites were evaluated, which supported the clinical pharmacokinetic studies successfully. Due to its high sensitivity, specificity and simplicity, the method could be used for pharmacokinetic studies of both 1,5-DCQA and its active metabolite, and for routine monitoring of their levels in human plasma.

Keywords: LC–MS–MS; 1,5-Dicaffeoylquinic acid; 1-Caffeoyl-5-feruoylquinic acid; 1,5-*O*-Diferuoylquinic acid; Pharmacokinetics

1. Introduction

Dicaffeoylquinic acids (DCQAs) are a class of natural polyphenolic compounds widely distributed in plants [1–4] such as coffee beans, sweet potato leaves, fennels and mugwort flowering tops and possess a broad spectrum of pharmacological activities [4–10]. Recently, DCQAs have received increasing recognition as a potent HIV-1 integrase inhibitor for drug development of a novel HIV therapeutic agent. Treatment of HIV-1 infection/AIDS currently consists of the use of combinations of HIV-1 inhibitors directed against reverse transcriptase and protease. However, their numerous side effects and the rapid emergence of drug-resistant variants require urgently the appearance of new antiviral drugs, as well as the development of newer strategies and viral targets [11–13]. Like all retroviruses, HIV-1 requires the integration of the proviral double-stranded DNA, arising from the reverse transcription step, into the host chromosome for its efficient replication, maintenance of a stably infected state and productive infection. DNA integration is carried out by integrase, so HIV-1 integrase represents a key area in developing new antiretroviral therapy [14]. And as no cellular homologue of HIV-1 integrase has been described, this kind of potential inhibitors for HIV-1 integrase could be relatively non-toxic [11,15–19]. DCQAs have been confirmed to be an effective HIV-1 integrase inhibitor [20] by selectively inhibiting HIV-1 integrase irreversibly toward its conserved amino acid residues in the central core domain of catalysis and preventing HIV-1 replication. Robinson [21] reported that the combinations of it with already existing inhibitors for HIV reverse transcriptase and protease have been suggested to be strongly synergistic.

1,5-Dicaffeoylquinic acid (1,5-DCQA) is a potent and non-toxic HIV-1 integrase inhibitor that has demonstrated

* Corresponding author. Tel.: +86 10 63796513; fax: +86 10 63751728.
E-mail address: mengzhiyun@vip.163.com (Z. Meng).

1570-0232/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.12.055

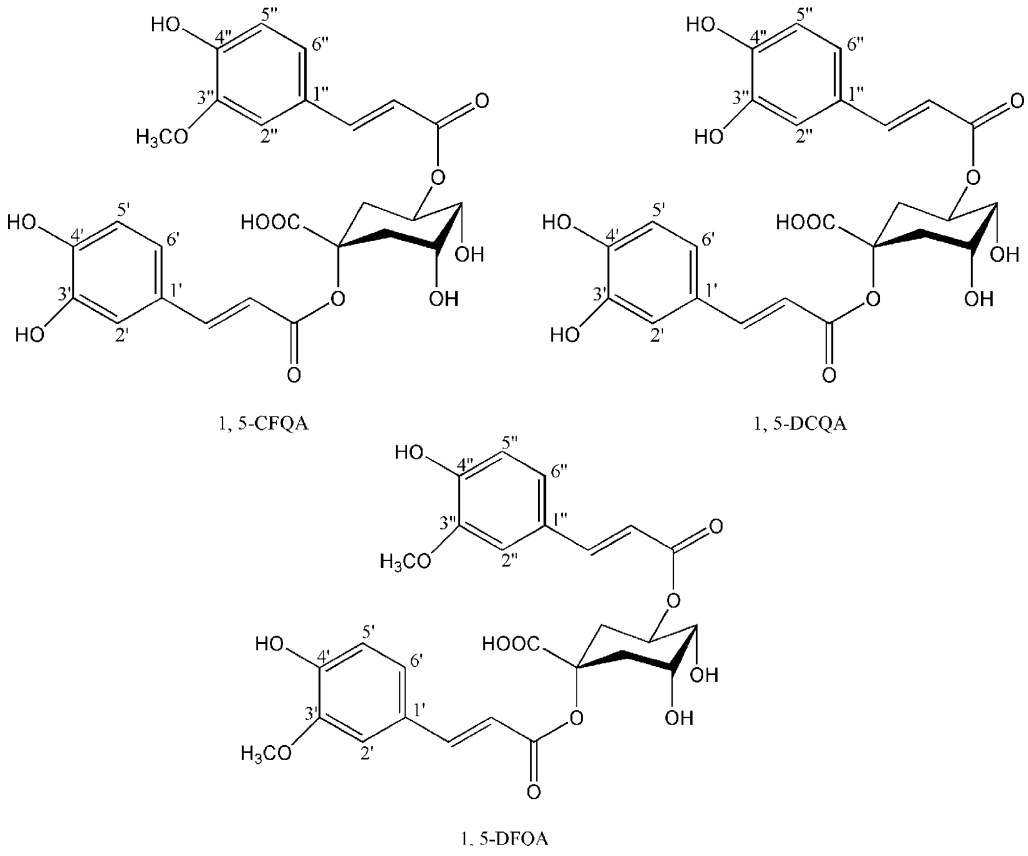

Fig. 1. The chemical structures of 1,5-DCQA, 1,5-CFQA and 1,5-DFQA.

anti-HIV activity in vitro and in vivo against a variety of animal experimental models. Its monomethylated metabolite 1-caffeoyl-5-feruoylquinic acid (1,5-CFQA) and di-methylated metabolite 1,5-*O*-diferuoylquinic acid (1,5-DFQA), according to the preclinical study [22], are the two active metabolites whose anti-HIV activity is comparable to or slightly weaker than that of 1,5-DCQA, respectively (Fig. 1). These encouraging preclinical results have prompted extensive clinical evaluation of 1,5-DCQA. The evaluation of the pharmacokinetics of 1,5-DCQA and, ideally, its known active metabolites is an essential component of many of these clinical studies. But, to our knowledge, little is known regarding the quantitation method of either 1,5-DCQA or its active metabolites in biological matrixes, except for one paper [23] that had developed an HPLC assay with UV detection to determine the 1,5-DCQA levels in rat plasma following oral administration, which had a complicated, time consuming sample pretreatment procedure and less than ideal sensitivity, and did not quantitate either 1,5-DCQA or its metabolites. In order to satisfy the urgent request of clinical study, in this research, we develop a LC/MS/MS method that employed a simple acetonitrile precipitation procedure for analyte recovery and that allowed sensitive and rapid quantitation of 1,5-DCQA and its two active metabolites, 1,5-CFQA and 1,5-DFQA in human plasma simultaneously. This method is currently being applied in a clinical pharmacokinetic study of 1,5-DCQA following the oral administration successfully.

2. Experimental

2.1. Reagents and materials

1,5-DCQA, 1,5-CFQA and 1,5-DFQA were graciously provided by Beijing Institute of Radiation Medicine (Beijing, China). Acetonitrile was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Analytical grade ammonium acetate and glacial acetic acid were purchased from Beijing Chemical Reagents Company (Beijing, China). Distilled water was purified using a SZ-93A auto-double distillation apparatus (YaRong Corp., Shanghai, China). Argon (≥99.999%) and liquid nitrogen (≥99.999%) were purchased from the Beijing Capital Oxygen Manufactory (Beijing, China). Blank human plasma was obtained from the Beijing Red Cross Blood Center (Beijing, China).

2.2. Chromatography

The LC system consisted of a Finnigan autosampler, a Finnigan LC pump (Thermo Electron Corporation, USA), an Inertsil ODS-3 column (5 μm, 2.1 mm × 150 mm, GL Sciences Inc., Japan) and a mobile phase where mobile phase solvent A was acetonitrile and mobile phase solvent B was 5 mM ammonium acetate (pH 5.0). The initial mobile phase composition of 5% solvent A and 95% solvent B was maintained for 2 min. Between 2 and 6 min, the percentage of solvent A was changed to 50%.

Between 6 and 7 min, the percentage of solvent A was then returned to 5%. The flow rate was 0.2 ml/min. The overall run time was 7 min.

2.3. Mass spectrometer

Mass detection was carried out using a Finnigan TSQ Quantum Ultra equipped with a heated-electrospray ion source and operated by XCalibur software Version 2.0 (Thermo Electron Corporation, USA), with negative-ion, selected reaction monitoring (SRM) mode. The settings of the mass spectrometer were as follows: spray voltage −3.0 kV; capillary offset −35 V; and capillary temperature 300 °C. The sheath gas and auxiliary gas pressures were 45 and 18 psi, respectively. The collision pressure was 1.4 psi. The ionspray interface and mass spectrometric parameters were optimized to obtain maximum sensitivity at unit resolution. The SRM *m/z* transitions monitored for each analyte, the tube lens offset and the optimized collision energy for each analyte are shown in Table 1.

2.4. Preparation of stock and working solutions

Stock solutions containing 3 mg/ml 1,5-DCQA, 1,5-CFQA or 1,5-DFQA were prepared by dissolving each respective compound in mobile phase (50:50, v/v). These solutions were stored at 4 °C until further dilutions were made for working solutions. Stock solutions were combined in order to obtain a single solution that had a final concentration of 1 mg/ml for each analyte, and then a serial dilution was made to achieve the desired serial concentrations of working solutions.

2.5. Preparation of calibration standards and quality control (QC) samples

Known amounts of working solutions were added to the control human plasma to achieve calibration standards of 3–500 ng/ml for 1,5-DCQA, 1,5-CFQA and 1,5-DFQA, respectively, in a total volume of 200 μl. Three quality control samples at 5 ng/ml (low), 100 ng/ml (medium) and 500 ng/ml (high) were prepared independent of those used for the calibration curves. These QC samples were prepared on the day of analysis in the same way as calibration standards.

2.6. Sample preparation

To 200 μl calibration standards, QC samples, or clinical plasma samples, 6 μl glacial acetic acid was added to keep the analytes stable and 400 μl of acetonitrile was added to precipitate protein. Then the samples were vortexed for 2 min. After centrifugation at 4 °C, 15,000 × *g* for 15 min, 10 μl of the supernatant was injected directly into the analytical column for immediate analysis.

Table 1
Ion transition, tube lens offset and collision energy for 1,5-DCQA, 1,5-CFQA and 1,5-DFQA

Compound	Transition	Tube lens offset (V)	Collision energy (eV)
1,5-DCQA	*m/z* 515.0 → 191.0	−145	37
1,5-CFQA	*m/z* 529.0 → 367.0	−148	16
1,5-DFQA	*m/z* 543.0 → 193.0	−151	21

2.7. Method validation

Specificity was tested by an analysis of four different blank human plasma samples to ensure the absence of endogenous compounds with the same retention times as 1,5-DCQA, 1,5-CFQA and 1,5-DFQA.

The linearity was evaluated by processing calibration curves ranging from 3 to 500 ng/ml including a zero level spiked into blank human plasma samples on three different assays, using an external standard method. The peak area was plotted against the nominal concentration of 1,5-DCQA, 1,5-CFQA and 1,5-DFQA, respectively. Then a linear least-squares regression analysis was conducted to demonstrate linearity of the method.

The accuracy and precision of the proposed assay were determined by an analysis of the QC samples. Three sequential assays were performed with six replicate analyses of QC samples (5, 100 and 500 ng/ml) on each single assay. Precision was expressed as %relative standard deviation (R.S.D.) and accuracy was expressed as %relative error (R.E.) between the measured and true value. The results were calculated using one-way analysis of variance (ANOVA).

The lower limit of detection (LLOD) was defined as the lowest detectable concentration, taking into consideration a signal-to-noise ratio of 3. Lower limit of quantification (LLOQ) was determined at the lowest concentration at which the precision (R.S.D.) and the accuracy (R.E.) were less than 20%.

The stability of each analyte spiked into blank human plasma was evaluated in triplicate using concentrations of 5 and 500 ng/ml, respectively. The long-term stability was investigated by analyzing the samples stored at −20 °C for 6 weeks. The freeze–thaw stability was evaluated by analyzing the samples subjected to three daily freeze–thaw cycles at −20 °C. To test the short-term, post-preparative stability of each analyte, the prepared samples were placed into the autosampler at room temperature for 24 h. Stock solution stability was also examined after storage at −20 °C for 8 weeks.

The recovery of each analyte was determined in triplicate using concentrations of 5, 100 and 500 ng/ml, respectively, by comparing the peak areas obtained from QC samples with the standard working solutions containing the same amount of each analyte.

Matrix effect was assessed in a similar fashion. Analyte for each of the three compounds were added to the extract of precipitated blank plasma to achieve concentrations of 5, 100 and 500 ng/ml. These peak areas were compared with those obtained by adding the same concentration of analytes in mobile phase.

2.8. Analysis of clinical samples

Blood samples were obtained from five healthy volunteers before and at 0.25, 0.5, 0.75, 1.0, 1.5, 2, 4, 6, 8, 10 and 12 h after the ingestion of a 600 mg dose of 1,5-DCQA. Blood samples

were collected in heparinized containers to undergo an anticoagulation procedure and centrifuged for 5 min at 12000 × *g* and 4 °C. The resulting plasma was transferred to polypropylene tubes and stored at −20 °C until analysis. The volunteers who provided samples were enrolled in a protocol and gave written informed consent before they participated in the study.

3. Results

3.1. Method development

To establish sample pretreatment conditions, a liquid–liquid extraction method using ethyl acetate, described in previous reports [23], was evaluated and found that it was time consuming and the greatest disadvantage of it was that the extraction recovery of 1,5-DFQA was very poor. And finally we adopt precipitation of protein with acetonitrile as the sample pretreatment method, which proved to be quite simple and have perfect recovery of all analytes.

The analytes were easily cleaved to generate negative product ions, so the negative-ion mode was applied for analysis. When optimizing the setting of mass spectrometry conditions, each analyte was continuously infused into the mass spectrometer in order to obtain a Q1 scan of the parent compound. Specifically, a 5 μg/ml solution of each compound in mobile phase (50:50, v/v) was infused at a rate of 10 μl/min into the HPLC flow, which was an isocratic mobile phase of acetonitrile:ammonium acetate (50:50, v/v) pumped at 0.2 ml/min. The tuning parameters of the mass spectrometer were adjusted to maximize the intensity of the parent ion of each analyte. The resulting spectrum of each analyte mostly consisted of $[M–H]^-$ ions. And the mass spectrometric parameters were adjusted to maximize the intensity of the $[M–H]^-$ ion, which was selected as the precursor ion for SRM detection. Under these optimized conditions, unit mass resolution was achieved with each analyte. The collision energy was adjusted to maximize the intensity of the product ions for each analyte.

At the time of choosing the transitions for SRM analysis of 1,5-DCQA, 1,5-CFQA and 1,5-DFQA, we did acquire two transitions for each analyte at the early stage of the method development, which was *m/z* 515/191, 353 for 1,5-DCQA, *m/z* 529/367, 353 for 1,5-CFQA and *m/z* 543/193, 135 for 1,5-DFQA and found that probably due to the structural similarity of 1,5-DCQA and its two methylated metabolites, the product ions generated from them were mostly the same, and simultaneously acquiring two transitions for each analyte would probably cause the "cross talk" phenomenon, which had impaired the accuracy and linearity of simultaneous quantitation of the three analytes. We also found that it was the first transition of each analyte chosen that showed the good specificity, and the intensities of them were the strongest, while each of the second product ions chosen showed non-specificity, much lower intensities and were not likely to improve the sensitivity when used in SRM mode. So, in order to achieve a timesaving and exact analysis, as shown in Table 1, we finally decided to choose only one transition of *m/z* 515/191 for 1,5-DCQA, *m/z* 529/367 for 1,5-CFQA and *m/z* 543/193 for 1,5-DFQA, which showed the best specificity, accuracy and sensitivity in simultaneous quantitation of the three analytes.

The standard solutions were added to the blank human plasma for specific evaluation. At the initial 2 min of analysis, a mobile phase composition of 95% ammonium acetate and 5% acetonitrile was used to quickly elute lots of undesirable compounds existing in human blank plasma, then the mobile phase was changed to the optimized ratio of 1:1 to elute each analyte without any endogenous interference. Under this optimized conditions, good Specificity was achieved in the assay. As shown in Fig. 2B, the retention times of 1,5-DCQA, 1,5-CFQA and 1,5-DFQA were approximately 5.3, 5.5 and 5.8 min, respectively. And Fig. 2A showed that there was no endogenous compound with the same retention times as the three analytes.

3.2. Linearity, LLOD and LLOQ

The linear regression analysis was constructed by plotting the peak area of analytes versus analyte concentration (ng/ml) in plasma samples. The assay proved to be linear and acceptable. The typical regression equation of these curves and their corre-

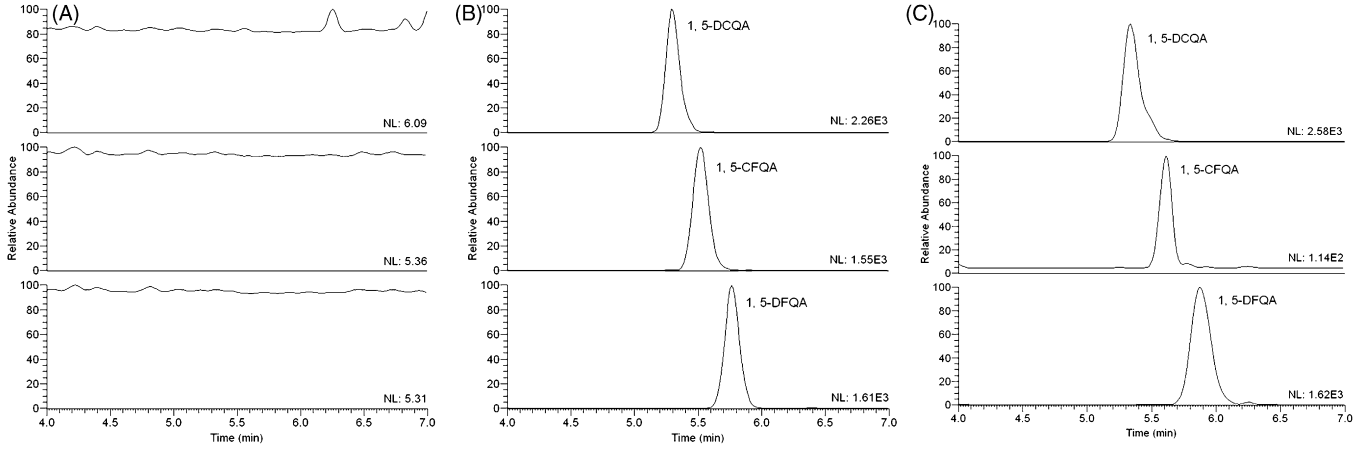

Fig. 2. SRM chromatograms from (A) blank plasma, (B) blank plasma spicked with 60 ng/ml 1,5-DCQA, 1,5-CFQA and 1,5-DFQA, respectively, (C) plasma sample of a healthy volunteer containing 83.3, 5.3 and 62.9 ng/ml of 1,5-DCQA, 1,5-CFQA and 1,5-DFQA, respectively, 2 h after a single oral administration of 600 mg 1,5-DCQA.

lation coefficients (r^2) were calculated as follows: 1,5-DCQA, $y = (-662.232 \pm 15.374) + (-262.330 \pm 14.361)x$, $r^2 = 0.9956 \pm 0.0041$; 1,5-CFQA, $y = (-565.382 \pm 8.593) + (-217.042 \pm 13.854)x$, $r^2 = 0.9964 \pm 0.0050$; 1,5-DFQA, $y = (-877.575 \pm 52.680) + (-298.701 \pm 18.305)x$, $r^2 = 0.9987 \pm 0.0012$.

The error for back-calculated concentration of each calibration point was within 11.8% except for the lowest concentration with an error of below 18.9% and the LLOD and LLOQ were 1 and 3 ng/ml for each analyte, respectively.

3.3. Accuracy and precision

The inter-assay and intra-assay back-calculated concentration with standard deviation, R.S.D. for precision and R.E. for accuracy are displayed in Table 2. The R.E. for both inter- and intra-assay accuracy were between −8.9 and 0.9%. The R.S.D. for intra-assay variability was less than 7.4% at each concentration studied, while R.S.D. for inter-assay variability was a little higher with which the maximal value reached 13.8%, probably because of the slight variety of system and environmental conditions such as humidity and temperature between different assays, all of the values were acceptable.

3.4. Recovery and matrix effect

As shown in Table 3, the recovery for each analyte was higher than 94% and the R.S.D. was lower than 9.9% for each concentration. And no matrix effect was observed; the relative recovery for each analyte was higher than 93%; and the R.S.D. was lower than 9.4%.

3.5. Stability

The stability of all the plasma samples investigated in both low and high concentrations for each analyte was satisfactory with value always higher than 90% and R.S.D. less than 13.8%. And the stability of stock solution was also excellent with values higher than 95% and R.S.D. less than 1.0%. The detailed information are shown in Table 4.

3.6. Application to clinical sample analysis

When applied to clinical samples, the assay proved suitable for quantitating the concentrations of 1,5-DCQA and its active metabolites in the plasma of five healthy volunteers treated with an oral dose of 1,5-DCQA (600 mg). By using the method described above, none of these samples posed any problem for the quantitation of the analytes, and no interference from the matrix was found in any of them. Fig. 2C showed the plasma sample of a healthy volunteer containing 83.3, 5.3 and 62.9 ng/ml of 1,5-DCQA, 1,5-CFQA and 1,5-DFQA, respectively, 2 h after a single oral administration of 600 mg 1,5-DCQA. And Fig. 3 presents the mean plasma concentration–time profiles of 1,5-DCQA, 1,5-CFQA

Table 2
Intra- and inter-assay accuracy and precision for 1,5-DCQA, 1,5-CFQA and 1,5-DFQA ($n = 18$)

Compound	Added[a]	Mean[b]	Intra-S.D.	Inter-S.D.	Intra-R.S.D.%	Inter-R.S.D.%	R.E.%
1,5-DCQA	5	5.0	0.2	0.2	4.0	4.0	−0.3
	100	93.0	2.9	7.1	3.1	7.6	−7.0
	500	504.5	10.8	5.9	2.1	1.2	0.9
1,5-CFQA	5	4.7	0.3	0.6	7.4	13.8	−6.2
	100	94.5	2.5	2.1	2.6	2.2	−5.5
	500	512.2	5.9	24.3	1.2	4.7	2.4
1,5-DFQA	5	4.9	0.2	0.5	5.1	10.9	−2.2
	100	94.7	3.0	10.0	3.1	10.6	−5.3
	500	459.1	9.6	29.6	2.1	6.4	−8.9

[a] Added concentration (ng/ml).
[b] Mean concentration calculated (ng/ml).

Table 3
Matrix effect and absolute recovery of 1,5-DCQA, 1,5-CFQA and 1,5-DFQA ($n = 3$)

Compound	Concentration (ng/ml)	Matrix effect		Absolute recovery (%)	
		Mean ± S.D.	R.S.D.	Mean ± S.D.	R.S.D.
1,5-DCQA	5	105.6 ± 8.9	8.4	100.2 ± 2.8	2.8
	100	94.4 ± 2.8	2.9	97.1 ± 2.0	2.0
	500	94.5 ± 1.5	1.6	97.3 ± 8.4	8.6
1,5-CFQA	5	93.0 ± 1.3	1.4	99.9 ± 0.8	0.8
	100	97.0 ± 4.7	4.9	98.5 ± 2.3	2.3
	500	100.5 ± 0.3	0.3	94.2 ± 9.3	9.9
1,5-DFQA	5	98.8 ± 9.3	9.4	96.2 ± 3.4	3.5
	100	99.6 ± 2.1	2.1	95.4 ± 6.1	6.4
	500	99.0 ± 0.7	0.7	95.4 ± 4.7	4.9

Table 4
Stability of stock solution and plasma sample of 1,5-DCQA, 1,5-CFQA and 1,5-DFQA ($n = 3$)

Compound	Concentration (ng/ml)	Stock solution %recovery		Sample A %recovery		Sample B %recovery		Sample C %recovery	
		Mean ± S.D.	R.S.D.	Mean ± S.D.	R.S.D.	Mean ± S.D.	R.S.D.	Mean ± S.D.	R.S.D.
1,5-DCQA	5	–	–	97.5 ± 1.4	1.5	97.0 ± 2.2	2.3	96.1 ± 2.2	1.5
	500	97.6 ± 0.5	0.6	107.1 ± 0.7	0.7	98.5 ± 2.2	2.3	106.6 ± 2.5	2.4
1,5-CFQA	5	–	–	102.9 ± 5.1	4.9	93.8 ± 8.1	8.6	89.5 ± 2.3	2.6
	500	94.5 ± 0.9	1.0	100.5 ± 1.9	1.9	102.0 ± 1.7	1.7	98.1 ± 2.9	2.9
1,5-DFQA	5	–	–	97.3 ± 6.4	6.6	93.1 ± 5.1	5.5	108.0 ± 14.9	13.8
	500	96.9 ± 0.4	0.5	97.8 ± 2.6	2.7	96.3 ± 0.4	0.4	95.5 ± 2.5	2.6

Sample A refers to the plasma samples unprepared at −20 °C for 6 weeks; Sample B refers to the plasma samples prepared for 24 h at room temperature; Sample C refers to the plasma samples subjected to 3 days freeze–thaw cycles.

and 1,5 DFQA. According to the measured plasma concentrations, the main pharmacokinetic parameters were obtained as follows: for 1,5-DCQA and 1,5-DFQA, respectively, maximum concentration (C_{max}), 73.1 ± 17.2 and 35.6 ± 19.5 ng/ml; time to reach maximum concentration (T_{max}), 1.9 ± 0.2 and 2.8 ± 1.1 h; area under the curve from time 0 to 24 h ($AUC_{0–24}$), 379.8 ± 83.8 and 271.6 ± 129.8 μg h/l; elimination half-life ($T_{1/2}$), 2.7 ± 1.3 and 3.8 ± 0.9 h. Each of these values represented the first description of such information in reported papers, and it is evident that the data may help to establish appropriate dose and frequency of clinical study largely. In this application, at 12 h after ingestion of 1,5-DCQA, plasma concentrations of 1,5-DCQA and 1,5-DFQA were still in excess of the LLOQ for both of them, which proved the applicability of the method in pharmacokinetic studies. And there was no 1,5-CFQA found in these human plasma samples except for one volunteer whose plasma contained low level of 1,5-CFQA with C_{max} of 5.3 ng/ml and T_{max} of 2 h, which suggested that the main metabolic type of 1,5-DCQA metabolized to active metabolite in human plasma was dimethylation.

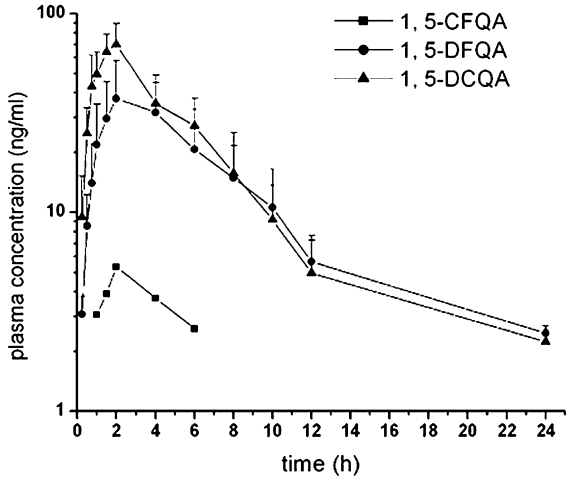

Fig. 3. Mean plasma concentration–time profiles of 1,5-DCQA, 1,5-CFQA and 1,5-DFQA in healthy volunteers. The above values are mean plasma concentrations obtained from five healthy volunteers who were administered a single oral dose of 600 mg 1,5-DCQA, except for 1,5-CFQA which obtained from only one of the five volunteers. The vertical bars represent standard deviation.

4. Conclusion

The new drug class of HIV-1 integrase inhibitor is in development and may soon be available in the clinic [24]. Development of HIV-1 integrase inhibitors could have favorable implication for combination therapy, including synergy with currently available inhibitors, as well as prevention of the chronic carrier state and the emergence of resistant mutants [19].

1,5-DCQA is a promising HIV-1 integrase inhibitor according to the findings reported already. The extensive existence in nature and the striking anti-HIV activities make it an attractive target for new drug development as a novel HIV therapeutic agent. In order to satisfy the urgent demands for quantitating method of 1,5-DCQA in clinical study, we have established a simple, rapid and robust LC–MS/MS assay for simultaneous determination of 1,5-DCQA and its two active metabolites in human plasma. Only 200 μl of plasma is required for the sample pretreatment procedures, and this method is currently being applied in a clinical pharmacokinetic study of 1,5-DCQA following the oral administration successfully. The ability of the assay to simultaneously quantitate 1,5-DCQA and its active metabolites provide information regarding the concentration versus time profile of both parent compound and its active metabolites, which can be used to formulate the most appropriate dose, route and frequency of administration for 1,5-DCQA. An external standard method was used for quantitative analysis successfully with high precision and accuracy because of the simple and timesaving sample pretreatment procedure. Due to its simplicity, selectivity and sensitivity, the assay is suitable for both clinical pharmacokinetic studies and routine monitoring of plasma levels of 1,5-DCQA and its active metabolites. In addition, we find that, the assay is also suitable for human urine samples after oral administration that good linearity was obtained in the 5–500 ng/ml range for each analyte, and that 1,5-CFQA and 1,5-DFQA were found together in all the five volunteers, which indicates the differences of metabolites metabolized from 1,5-DCQA between human plasma and urine.

Acknowledgements

This work was supported by Grant 30371669 from the National Natural Sciences Foundation of China, Grant 2003AA2Z347B from the National High Technology Research

and Development Program of China (863 Program) and by Grant 2005DFA30080 from the International Technology Cooperation Item.

References

[1] R.F. Moreira, L.C. Trugo, C.A. de Maria, A.G. Matos, S.M. Santos, J.M. Leite, Arch. Latinoam. Nutr. 51 (2001) 95.
[2] D. Fraisse, A. Carnat, A.P. Carnat, D. Guedon, J.L. Lamaison, Ann. Pharm. Fr. 61 (2003) 265.
[3] I. Parejo, F. Viladomat, J. Bastida, C. Codina, Anal. Chim. Acta 512 (2004) 271.
[4] M. Yoshimoto, S. Yahara, S. Okuno, M.S. Islam, K. Ishiguro, O. Yamakawa, Biosci. Biotechnol. Biochem. 66 (2002) 2336.
[5] Y. Li, P.P. But, V.E. Ooi, Antiviral Res. 68 (2005) 1.
[6] H.J. Kim, Y.S. Lee, Planta Med. 71 (2005) 871.
[7] S. Mishima, C. Yoshida, S. Akino, T. Sakamoto, Biol. Pharm. Bull. 28 (2005) 1909.
[8] S. Mishima, Y. Inoh, Y. Narita, S. Ohta, T. Sakamoto, Y. Araki, K.M. Suzuki, Y. Akao, Y. Nozawa, Bioorg. Med. Chem. 13 (2005) 5814.
[9] J. Choi, J.K. Park, K.T. Lee, K.K. Park, W.B. Kim, J.H. Lee, H.J. Jung, H.J. Park, J. Med. Food. 8 (2005) 348.
[10] X. Zhu, H. Zhang, R. Lo, J. Agric. Food Chem. 52 (2004) 7272.
[11] K. Sayasith, G. Sauve, J. Yelle, Expert Opin. Ther. Targets 5 (2001) 443.
[12] M. Witvrouw, B. Van Maele, J. Vercammen, A. Hantson, Y. Engelborghs, E. De Clercq, C. Pannecouque, Z. Debyser, Curr. Drug Metab. 5 (2004) 291.
[13] M.T. Makhija, Curr. Med. Chem. 13 (2006) 2429.
[14] L. Tarrago-Livak, M.L. Andreola, M. Fournier, G.A. Nevinsky, V. Parissi, V.R. de Soultrait, S. Litvak, Curr. Pharm. Des. 8 (2002) 595.
[15] B. McDougall, P.J. King, B.W. Wu, Z. Hostomsky, M.G. Reinecke, W.E. Robinson Jr., Antimicrob. Agents Chemother. 42 (1998) 140.
[16] W.E. Robinson Jr., M. Cordeiro, S. Abdel-Malek, Q. Jia, S.A. Chow, M.G. Reinecke, W.M. Mitchell, Mol. Pharmacol. 50 (1996) 846.
[17] W.E. Robinson Jr., M.G. Reinecke, S. Abdel-Malek, Q. Jia, S.A. Chow, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 6326.
[18] P.J. King, G. Ma, W. Miao, Q. Jia, B.R. McDougall, M.G. Reinecke, C. Cornell, J. Kuan, T.R. Kim, W.E. Robinson Jr., J. Med. Chem. 42 (1999) 497.
[19] A. Pani, M.E. Marongiu, Curr. Pharm. Des. 6 (2000) 569.
[20] K. Zhu, M.L. Cordeiro, J. Atienza, W.E. Robinson Jr., S.A. Chow, J. Virol. 73 (1999) 3309.
[21] W.E. Robinson Jr., Antiviral Res. 39 (1998) 101.
[22] B. Yang, Z. Meng, J. Dong, L. Yan, L. Zou, Z. Tang, G. Dou, Drug Metab. Dispos. 33 (2005) 930.
[23] B. Yang, Z.Y. Meng, L.P. Yan, J.X. Dong, L.B. Zou, Z.M. Tang, G.F. Dou, J. Pharm. Biomed. Anal. 40 (2006) 417.
[24] M. Lataillade, M.J. Kozal, AIDS Patient Care STDS 20 (2006) 489.