Simultaneous Determination of Berberine and Evodiamine in Dog Plasma by LC–ESI–MS Method and Its Application to Pharmacokinetics

Yan Fei* and **Yan Rui**

State Grid Electric Power Research Institute, Wuhan 430074, PR China

Abstract

A simple, specific, and sensitive method is developed for quantitation of berberine and evodiamine in dog plasma using HPLC–MS–MS. The plasma samples were prepared by a single-step protein precipitation with methanol–acetonitrile (2:1,v/v). This method was validated on a Shim-pack ODS (4.6 µm, 150 mm × 2.0 mm i.d., Shimadzu) column with isocratic mobile consisting of water (containing 0.1% formic acid)–methanol (15:85,v/v), at a flow rate of 0.45 mL/min. The ion transitions recorded in selective reaction monitoring mode were *m/z* 336→320 for berberine, *m/z* 304→134 for evodiamine, and *m/z* 172→128 for metronidazole (IS), respectively. The RSD of the assay intra- and inter-day precision was less than 12.4% and the accuracy exceeded 89%. A linear dynamic range of 0.2–20 ng/mL was established with limit of quantitation of 0.2 ng/mL. Stability studies were carried out at different storage conditions proving analytes to be stable. The assay was successfully applied to determine the pharmacokinetic profiles after an oral administration of *rhizoma coptidis–evodia rutaecarpa* herb couple.

Introduction

In traditional Chinese medicine (TCM), herbs are often used in combination to achieve the joint actions of the individual herbs (1). In this case, appropriate exploration on herb couples might be helpful to understand the principles of TCM. Among the common herbs, *rhizoma coptidis* and *evodia rutaecarpa* were wide used in a wealth of TCM prescriptions for a long history. In particular, they were typically used together to achieve synergistic effect (2). The *rhizoma coptidis–evodia rutaecarpa* couple (6:1, g/g) was widely used in many TCM with typically pharmacological activity. Usually, alkaloids such as berberine, palmatine, jateorrhizine, coptisine in *rhizoma coptidis* and evodiamine, rutaecarpine in *evodia rutaecarpa* are thought to be the pharmacologically active constituents. Several investigators have examined the plasma concentration to study the pharmacokinetics of the constituents. Earlier publications have described methods for simultaneous analysis of berberine and palmatine (3), berberine and their main metabolites (4), three alkloids (5), five alkloids (6), plamatine (7), evodiamine (8–10), and rutaecarpine (11) in biological samples. Due to low plasma concentrations, electrospray mass spectrometry (MS) was more and more often employed for its low limit of detection. To this end, however, simultaneous determination of both of the herbs after coupling in dogs has not been reported. Because both of the two herbs are indispensable to the activity, it is incomplete to assay the constituents of one herb without considering the role of the other. The chemical structures of berberine and evodiamine are shown in Figure 1.

In this paper, a sensitive and selective method of liquid chromatography (LC)-electrospray ionization-MS is presented for the simultaneous determination of berberine and evodiamine in dog plasma, the most important pharmacologically active constituents of *rhizoma coptidis* and *evodia rutaecarpa*, respectively. This assay has been successfully applied to a pharmacokinetic study of the two alkaloids after oral administration of *rhizoma coptidis–evodia rutaecarpa* herb couple in beagles.

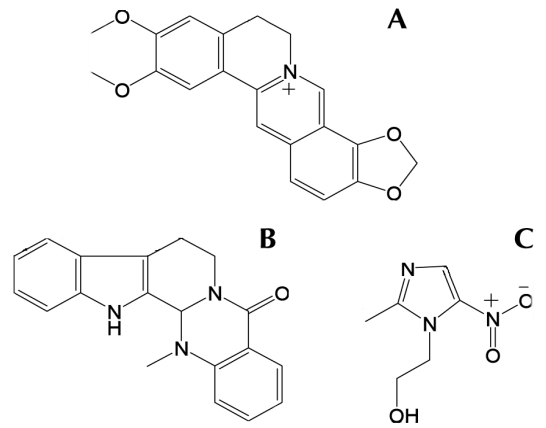

Figure 1. Chemical structures of berberine (A), evodiamine (B), and metronidazole (IS, C).

*Author to whom correspondence should be addressed: email bjjt09@gmail.com.

Experimental

Materials

The reference standards of berberine (purity 98.5%), evodiamine (purity 99.0%), and metronidazole (purity 99.0%) (IS) were all obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). *Rhizoma coptidis* and *evodia rutaecarpa* were purchased from a local drug store in Shenyang. Methanol, acetonitrile, and formic acid were of chromatographic grade from the Yuwang Chemical Factory (Shandong, China). Deionized water was used and all other reagents were of analytical grade.

Content of berberine and evodiamine in the couple

To calculate the administration dosage, the contents of berberine and evodiamine in herb extract were quantitatively determined according to the Chinese Pharmacopeia. The contents of berberine and evodiamine were 38.6 mg/g *rhizoma coptidis* and 1.07 mg/g *evodia rutaecarpa*, respectively.

Instrument and LC–MS–MS conditions

The HPLC system consists of a LC-10ADvp Pump (Shimadzu, Kyoto, Japan) and a SIL-HTA Autosampler (Shimadzu, Kyoto, Japan). Chromatographic separation was carried out on a Shimpack ODS (4.6 µm, 150 mm × 2.0 mm i.d., Shimadzu) column with an EasyGuard C18 Security guard column (8 × 4.0 mm i.d., Dikma, Beijing, China) kept at 25°C. The mobile phase consists of water (containing 0.1% formic acid)–methanol (15:85, v/v), at a flow rate of 0.45 mL/min.

MS detection was performed on a Thermo Finnigan TSQ Quantum triple quadrupole mass spectrometer (San Jose, CA) equipped with an ESI source in the positive ionization mode. The MS operating conditions were optimized as follows: the spray voltage: 4200 V; the heated capillary temperature: 320°C; the sheath gas (nitrogen): 30 psi; the auxiliary gas (nitrogen): 5 psi; the collision gas (argon) pressure: 1.2 mtorr (1 torr = 133.3 Pa); the collision energy: 30 eV for all. Data acquisition was performed by Xcalibur 2.0 software (Thermo Finnigan). Peak integration and calibration were performed using LC Quan software (Thermo Finnigan). Quantitation was obtained by using SRM mode of the transitions at *m/z* 336→320 for berberine, at *m/z* 304→134 for evodiamine, at *m/z* 172→128 for metronidazole (IS) respectively, with a scan time of 0.3 s per transition. The typical full-scan ESI mass spectrum of berberine, evodiamine, and IS is described in Figure 2A–2C.

Preparation of standard solutions

The stock solution of berberine and evodiamine at the concentration of 40 µg/mL for each was prepared in methanol and stored at 4°C until use. Working solution of the analytes was prepared by spiking the blank dog plasma (100 µL) with 10 µL of the appropriate working solutions to yield the following concentrations: 0.2, 0.6, 1.2, 2.5, 5, 10, and 20 ng/mL. Quality control (QC) samples were prepared from blank plasma at concentrations of 0.5, 3, and 18 ng/mL.

Sample preparation

Dog plasma 400 µL was mixed with 2 mL acetonitrile–methanol (1:2, v/v), 10 µL internal standard solution (100 ng/mL). After vortex-mixing 30 s, the mixture was centrifuged at 4000 rpm for 10 min. The supernatant was separated out and blown to dryness with nitrogen at 40°C. Then the residue was reconstituted in 100 µL mobile phase and a 20 µL aliquot of the final testing samples was injected onto the LC–MS–MS system for analysis. The same procedure was used to determine the recovery and precision in plasma.

Method validation

Calibration standards of seven analytes concentration levels were extracted and assayed. The analytes calibration curve was generated by plotting the peak-area ratios of analytes to the IS versus the concentrations of analytes, using weighed least squares linear regression (the weighing factor was $1/C^2$). The lower limit of quantitation (LLOQ) for each analyte in plasma, defined as the lowest concentration at which both precision and accuracy were less than or equal to 20%, is 0.2 ng/mL.

Accuracy and precision were investigated by determining QC samples at three concentration levels of 0.5, 3, and 18 ng/mL (six samples for each concentration level) on 3 different validation days.

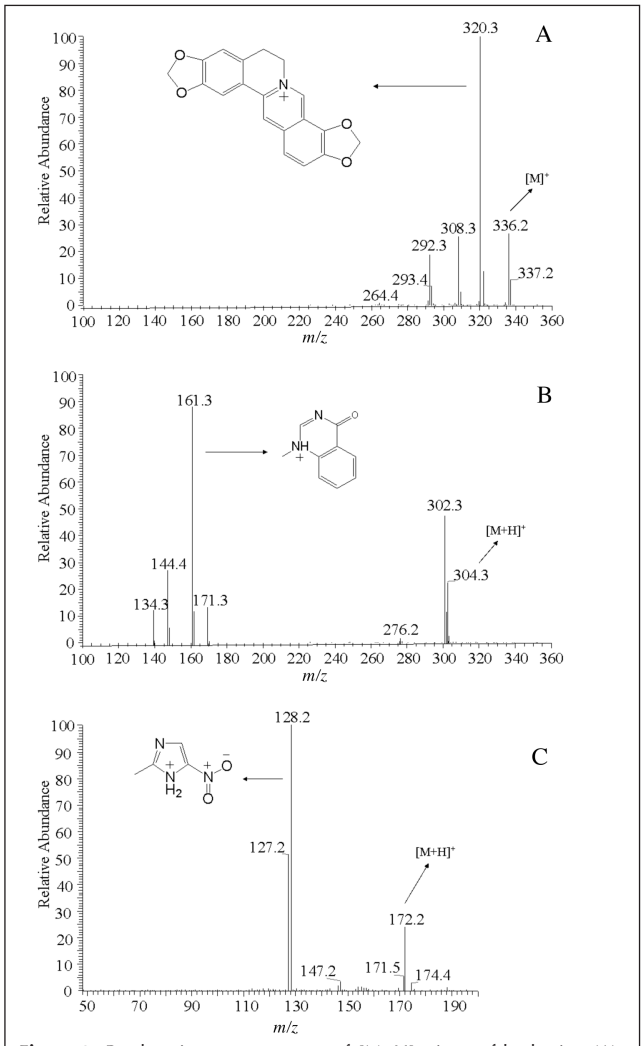

Figure 2. Product ion mass spectra of [M+H]+ ions of berberine (A), evodiamine (B), and IS (C).

The extraction recoveries of berberine and evodiamine were determined at low, medium, and high concentrations. Recoveries were calculated by comparing the analyte/IS peak area ratios obtained from extracted plasma samples with those from blank plasma extracts spiked with standard solution at the same concentration.

The short- and long-term storage stability of analytes was analyzed at three concentration levels (0.5, 3, and 18 ng/mL for each analyte) stored at room temperature for 24 h and –20°C for 7 days, respectively. The freeze-thaw stabilities were determined after three freeze and thaw cycles. The stabilities of analytes were determined by comparing the mean concentration with the initial concentration of freshly prepared samples before storage.

Pharmacokinetic study

Healthy beagle dogs (9.3 ± 1.0 kg), purchased from the experimental Animal Center of Shenyang Pharmaceutical University, were kept in an environment controlled breeding room for 3 days before starting the experiment. Animal study was carried out in accordance with the Guidelines for Animal Experimental of Shenyang Pharmaceutical University and the protocol was approved by Animal Ethics Committee of the institution. *Rhizoma coptidis–evodia rutaecarpa* (6:1, g/g) powders filled in capsules were administered to 5 beagles (0.16 g/kg body weight) per os. Blood samples (1 mL) were obtained from foreleg vein before dosing and subsequently at 0.25, 0.75, 1.25, 1.75, 2.75, 3.75, 4.75, 5.75, 7.75, 9.75, 12, and 24 h following administration, transferred to a heparinized eppendorf tube and centrifuged at 4000 rpm for 10 min. The plasma obtained was frozen at –20°C until analysis.

Results and Discussion

Selectivity

The selectivity was investigated by preparing and analyzing six individual dog blank plasma samples. The typical chromatograms of blank plasma, LLOQ for berberine and evodiamine in plasma sample (0.2 ng/mL), and a plasma sample 1.75 h after oral administration were presented in Figure 3. The selectivity of the assay was evaluated by analyzing six different batches of blank human plasma. All samples were found to be of no interference at the retention times of the analytes or the IS.

As internal standard, metronidazole does not exist in herb extract and functions through an ion channel different from analytes. After separating by HPLC, the retention time of metronidazole was in the middle of analytes. In addition, its recovery rate was 91.0%, which was generally consistent with analytes and thus ensured the ideal result.

Method validation

All calibration curves showed excellent linearity over the range 0.2~20 ng/mL in dog plasma. The regression

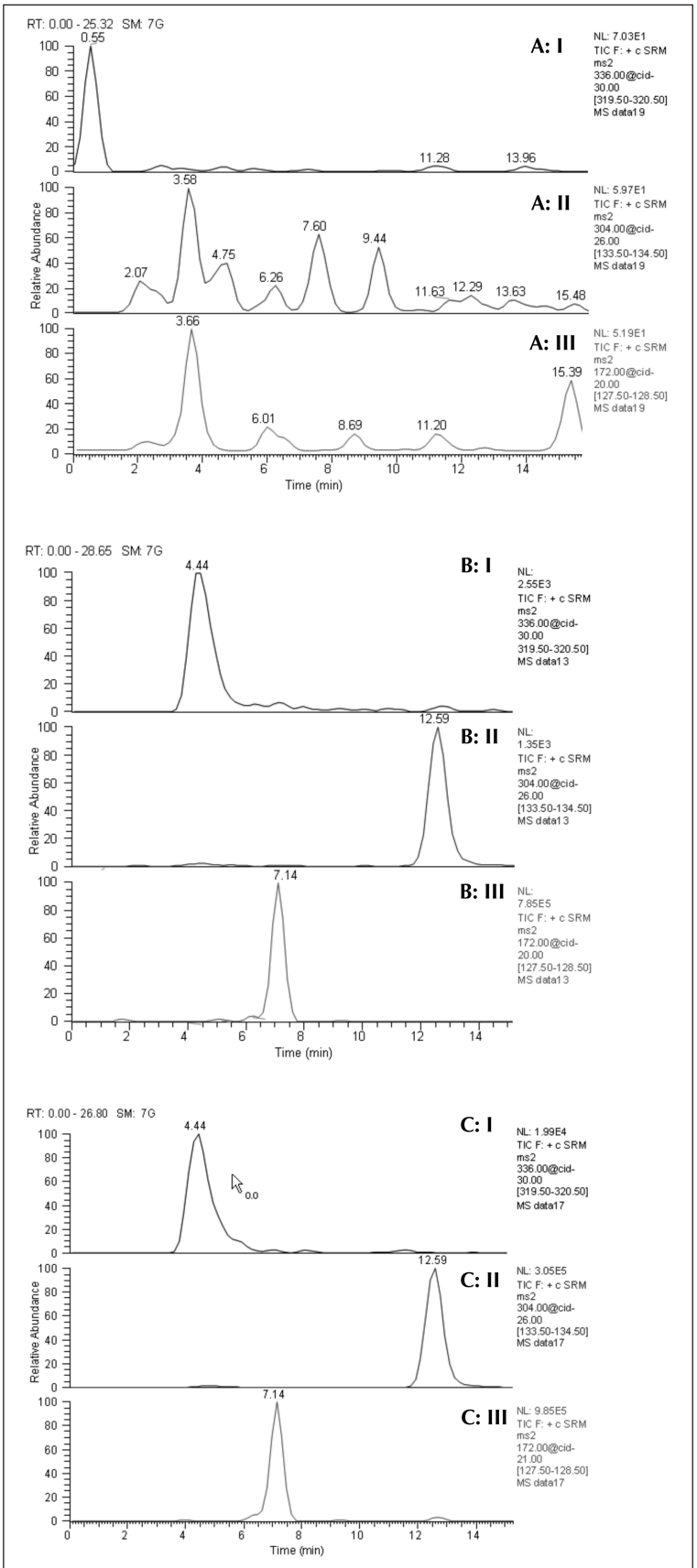

Figure 3. Representative SRM chromatograms for berberine and evodiamine and IS in dog plasma: a blank plasma sample (A); a blank plasma sample spiked with berberine and evodiamine at the LLOQ of 0.2 ng/mL IS at 10 ng/mL (B); plasma sample obtained 105 min after oral administration (C): berberine (I), evodiamine (II), and IS (III).

Table I. Intra- and Inter-day Precision and Accuracy Data for Berberine and Evodiamine in Dog Plasma*

Samples	Spiked conc. (ng/mL)	Measured conc. (mean ± SD)	RSD (%)	RE (%)
Berberine				
Intra-day	0.2	0.23 ± 0.029	12.6	14.9
	0.5	0.491 ± 0.051	14.2	–1.8
	3.0	2.976 ± 0.094	11.3	–0.8
	18.0	18.504 ± 0.558	6.8	2.8
Inter-day	0.5	0.51 ± 0.049	2.5	2.0
	3.0	3.024 ± 0.131	2.9	0.8
	18.0	17.352 ± 1.044	6.0	–3.6
Evodiamine				
Intra-day	0.2	0.223 ± 0.04	17.9	11.5
	0.5	0.51 ± 0.022	12.8	2.0
	3.0	2.868 ± 0.061	14.1	–4.4
	18.0	17.604 ± 0.594	6.2	–2.2
Inter-day	0.5	0.513 ± 0.02	1.8	2.6
	3.0	2.909 ± 0.056	4.1	–3.03
	18.0	17.766 ± 0.918	2.3	–1.3

*3 days, six replicates per day.

Table II. Extraction Recovery of Berberine and Evodiamine in Dog Plasma (n = 5)

Nominal plasma conc. (ng/mL)	Recovery (mean ± SD, %)		RSD (%)	
	Berberine	Evodiamine	Berberine	Evodiamine
0.5	102 ± 2.05	90.0 ± 1.11	8.1	2.9
3.0	93.0 ± 1.19	84.5 ± 0.98	10.8	11.9
18.0	95.4 ± 1.39	86.3 ± 1.12	3.3	0.7

Table III. Stability Data of Berberine and Evodiamine in Dog Plasma Under Various Storage Conditions (n = 3)

Storage condition	Spiked conc. (ng/mL)	Calculated conc. (mean ± SD) (ng/mL)		RE(%)	
		Berberine	Evodiamine	Berberine	Evodiamine
Short-term stability	0.5	0.442 ± 0.01	0.492 ± 0.04	–11.6	–1.625
	3.0	2.729 ± 0.03	2.861 ± 0.199	–9.033	–4.625
	18.0	18.367 ± 0.298	19.148 ± 1.102	2.039	6.375
Long-term stability	0.5	0.485 ± 0.012	0.518 ± 0.023	–3	3.625
	3.0	2.726 ± 0.056	3.158 ± 0.094	–9.133	5.25
	18.0	17.799 ± 0.228	18.338 ± 0.72	–1.117	1.875
Freeze/thaw stability	0.5	0.537 ± 0.01	0.487 ± 0.029	7.4	–2.625
	3.0	2.858 ± 0.042	3.176 ± 0.165	–4.733	5.875
	18.0	18.171 ± 0.259	18.63 ± 1.148	0.95	3.5

Table IV. Pharmocokinetic Parameters of Berberine and Evodiamine After an Oral Administration of Rhizoma Coptidis–Evodia Rutaecarpa powder*

Component	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-t} (ng h/mL)	AUC_{0-inf} (ng h/mL)
Berberine	6.9 ± 2.8	2.8 ± 0.3	6.411 ± 1.6	57.66 ± 11.82	65.59 ± 20.38
Evodiamine	5.3 ± 2.3	5.8 ± 0.8	10.951 ± 2.7	68.61 ± 14.16	73.30 ± 22.78

* (6:1, g/g) 0.16 g/kg body weight

equations were y = 0.0809x + 0.0329 (r = 0.9984, n = 5) for berberine and y = 0.0976x – 0.0016 (r = 0.9994, n = 5) for evodiamine. The LLOQ was established at 0.2 ng/mL, lower than earlier publications (3–6,8,10,11).

Data for intra- and inter-day precision and accuracy of the method for determination of analytes are presented in Table I. All values of accuracy and precision were within recommended limits. The intra- and inter-day precisions were less than 14.2% for each analyte. The bias, determined from QC samples, was within ± 4.4%. Recoveries were more than 80% at different concentrations for two analytes with acceptable variance. Data are shown in Table II.

Table III summarizes the stability data of QC samples. All the results showed that all the samples were stable during these tests and there were no stability related problems during the routine analysis of samples for pharmacokinetic study.

Application to pharmacokinetic studies in dogs

The method described previously was successfully applied to the pharmacokinetic study in which plasma concentrations of berberine and evodiamine were determined for 24 h after oral administration of *rhizoma coptidis–evodia rutaecarpa* herb couple (0.16 g powder/kg body weight). The mean plasma concentration–time profiles (n = 5) are represented in Figure 4. The appearance of multiple blood concentration peaks in Figure 4 is likely due to the fact that the rate of absorption of analytes was limited by the rate of dissolution of constituents from herb powders. It was noted that Deng et al. also reported that berberine, palmatine, and jateorrhizine exhibited three blood concentration peaks in dog body after oral administration of *rhizoma coptidis–evodia rutaecarpa* herb powders (5).

Pharmacokinetic parameters, estimated using a noncompartmental analysis using Drug and Statistics 2.0 (DAS 2.0) (Mathematical Pharmacology Professional Committee of China, Shanghai, China) are listed in Table IV. Although the amount of *evodia rutaecarpa* was administered less than *rhizoma coptidis*, C_{max} and AUC were higher than those of berberine. This demonstrated that the absorption of evodiamine was better than berberine, which probably originates from the fact that the liposolubility of evodiamine is better than that of berberine. There were no reports on pharmacokinetic profiles of berberine or evodiamine in dogs. Huang et al. studied the pharmacokinetic behavior of palmatine in beagle dogs after oral administration of palmatine tablet (7). The absorption appeared slow with a t_{max} of 5 h. After T_{max}, the plasma concentration decreased slowly. Elimination half-lives($t_{1/2}$) after intramuscular and oral dosing were 49 and 56 h, respectively. The relative bioavailability of oral administration to intramuscular injection was only 1.31%. Tsai et al. proved that the significant first-pass extraction severely limited oral bioavailability of berberine and other alkaloids after administration of solo compound (12). The results indicated that some components of *evodia rutaecarpa* raised the oral bioavailability and changed the pharmacokinetic behavior of berberine.

The interaction among multitudinous compounds in different formulas resulted in vastly different profiles of

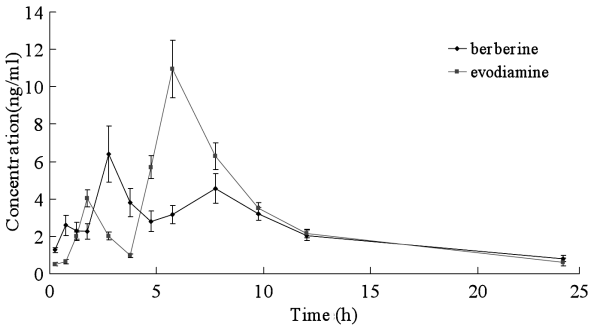

Figure 4. Mean plasma concentration–time curves of berberine and evodiamine after oral administration of rhizoma coptidis–evodia rutaecarpa herb couple 0.16 g/kg body weight.

the same constituent in vivo. At this point, however, there are no systematic studies reported on this issue. Lu et al. demonstrated that $t_{1/2}$ of berberine and palmatine were 8.0 ± 2.9 and 10.5 ± 2.8 h, respectively after *rhizoma coptidis* was administered together with other herb extract (3). Yu et al. presented pharmacokinetic parameters of berberine, palmatine, coptisine, epiberberine, and jatrorrhizine in rats after a dose of *rhizoma coptidis* extract as follows: t_{max} was 3.40 ± 1.47, 0.90 ± 0.95, 2.25 ± 1.82, 1.30 ± 1.56, and 1.69 ± 1.72 h; and MRT was 9.31 ± 0.81, 10.12 ± 1.16, 6.69 ± 2.07, 5.09 ± 2.44 h, and NA, respectively (6). Jeng et al. confirmed that $t_{1/2}$ of evodiamine was 44.56 ± 5.80 min after intravenous administration of evodiamine to a rat(8). It must be other components which delayed its elimination and caused double peak (or peak to peak). In a nut shell, different formulas and different administration routes might impact the pharmacokinetic behavior of analytes.

Conclusions

This paper described and validated a simple and sensitive LC–MS–MS method for the simultaneous determination of berberine and evodiamine in beagle dog plasma. The LC–MS–MS assay was applied to the detailed pharmacokinetic studies of berberine and evodiamine in plasma after oral administration of *rhizoma coptidis–evodia rutaecarpa* herb couple. After necessary modifications, proposed method would play an essential role in some important potential application including the determination of other alkaloids of TCM in plasma.

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