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Identification and dynamic analysis of the purine alkaloids in rat plasma after oral administration of green tea by liquid chromatography hybrid ion trap time-of-flight mass spectrometry

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ABSTRACT

A liquid chromatography hybrid ion trap time-of-flight mass spectrometric (LC–IT-TOF-MS) method was developed and validated for identification and simultaneous determination of the potential bioactive components from green tea in rat plasma. The plasma samples were extracted by liquid–liquid extraction with ethyl acetate and separated on Shim-pack XR-ODS II column by a gradient elution within a runtime of 8.0 min. The mobile phase consisted of A (0.1% formic acid in acetonitrile) and B (0.1% formic acid in water) at a flow rate of 0.4 ml/min. Two prototype components and one metabolite were successfully identified as caffeine, theobromine and theophylline according to their retention times, accurate molecule weight, and major fragment ions. Then they were determined with the addition of two internal standards, hypoxanthine and paracetamol. The linear range was 10–10,000 ng/ml for caffeine, 2.0–2000 ng/ml for theobromine and 1.0–1000 ng/ml for theophylline, respectively. Intra-day and inter-day precision were within 6.0% and 10.9%, and accuracy was less than 4.8% and 6.5%, respectively. The validated method was successfully applied to investigate the dynamic change rules of caffeine, theobromine and theophylline in rat plasma after oral administration of caffeine, theobromine and green tea extract. The comparative analysis of the pharmacokinetic parameters indicated that there were obvious differences between green tea extract administration and single substances administration.

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1. Introduction

Green tea, the unfermented leaf of *Camellia sinensis* (L.) O. Kuntze, is one of the oldest and most popular beverage in the world [1]. It is usually used for refreshing the brain and regaining consciousness. Those effects are attributed to the purine alkaloids, major including caffeine (CAF), theobromine (TB) and theophylline (TP) [2]. The three compounds show similar bioactivities due to their similar chemical structures (shown in Fig. 1). As adenosine and γ-aminobutyrate (GABA) receptor antagonists, the three compounds can increase the release of various neurotransmitters and inhibit phosphodiesterase [3]. Accordingly, they display multiple pharmacological effects, such as enhancing cognitive function [4], increasing endurance [5], and reliving anxiety [6]. Furthermore, TP also shows the effect on relieving cough and asthma [7].

As a Traditional Chinese Medicine (TCM), the chemical composition of green tea is complex. According to the theory of plasma pharmacochemistry, only the components absorbed into the blood have the chance to show pharmacological bioactivities [8]. Therefore, it is of great significance to identify the absorbed components and their metabolites in plasma after administration of green tea.

Earlier publications have developed methods for the determination of purine alkaloids in plasma using RP-HPLC [9,10]. But most reports seemed to be lack of the information on the identification of the analytes and the dynamic changes among the analytes after oral administration of the single substances and the plant. In addition, almost all reports used a single internal standard to analyze the analytes at different concentrations which could not meet the requirement for accurate quantitative determination. Thus, it is necessary to use two internal standards with different concentrations in the study when the plasma concentrations of the analytes were significantly different.

The LC–IT-TOF-MS, gathering the advantage of both the ion trap in producing multistage tandem (MS1-10) fragmentations and the TOF in high resolution and accurate mass measurement, has been confirmed as a useful tool in the identification of unknown compounds [11–13]. Previous study also demonstrated the instrument with enough sensitivity would meet the requirements for quantitative analysis [14]. Therefore, in this study, based on the theory of plasma pharmacochemistry, an LC–IT-TOF-MS method

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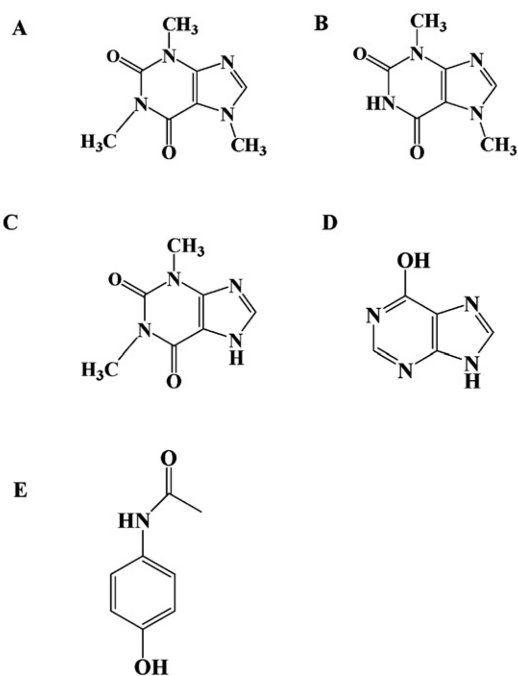

Fig. 1. Chemical structures of CAF (A), TB (B), TP (C), IS-1 (D) and IS-2 (E).

was established for the first time to screen for multiple components in rat plasma after oral administration of green tea and to study their dynamic process *in vivo*. Furthermore, the pharmacokinetic behavior of the components in rat plasma was compared after oral administration of single substances and green tea extract.

2. Experiment

2.1. Chemicals and materials

CAF, TB and TP (purity > 99.0%) as well as internal standards, hypoxanthine and paracetamol (purity > 99.0%), were purchased from the National Institutes for Food and Drug Control (Beijing, China). Acetonitrile (HPLC-grade) was purchased from Merck KGaA (Darmstadt, Germany). Formic acid (HPLC-grade) was purchased from Aladdin Chemistry Co. Ltd. (Shanghai, China). Water was purified using a Milli-Q system (Millipore, Bedford, MA). Green tea samples were collected from *Dragon well* area (Hangzhou, China).

2.2. Animals

Eighteen Sprague-Dawley rats (male, mean weight 200 ± 20 g) were purchased form the Animal Center of Guangdong Province (Guangzhou, China). Animals were acclimatized to food and water *ad libitum* for a 72 h period before use and were group-housed under a 12 h light/dark cycle in an environmentally controlled animal facility. All procedures were carried out under strict compliance with the national guide for the care and use of laboratory animals according to the protocol approved by General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China.

2.3. Apparatus and LC–IT-TOF-MS conditions

LC experiments were conducted on a Shimadzu (Kyoto, Japan) HPLC system consisting of an LC-10AD binary pump, a DGU-14A degasser, SIL-20AC autosampler and a CTO-20AC column oven. Chromatographic separation was achieved on a Shim-pack XR-ODS column (75 mm × 2.0 mm, I.D. 2.2 μm, Shimadzu). The mobile phase (delivered at 0.4 ml/min) consisted of A (0.1% formic acid in acetonitrile) and B (0.1% formic acid in water). A binary gradient elution was applied from 95 to 92% of solvent B from 0 to 5 min, then quickly returned to initial 95% B and maintained till 8 min for column balance. The temperature of column and autosampler were maintained at 40 °C and 4 °C, respectively. The mass spectrometer of LC–IT-TOF-MS (Shimadzu, Japan) was equipped with an ESI source in positive ion mode. The optimized analytical conditions were as follows: detector voltage, 1.70 kV; curved desolvation line (CDL) temperature 250 °C, heat block temperature 200 °C; nebulizing gas (N_2) flow, 1.5 l/min; dry gas (N_2) pressure, 50 kPa; pressure of TOF region, 1.5×10^{-4} Pa; ion trap pressure, 1.7×10^{-2} Pa; ion accumulated time, 30 ms; precursor ion selected width, 3.0 amu. Accurate mass determination was corrected by calibration using the sodium trifluoroacetate clusters as reference. For qualitative analysis: mass spectrometry was conducted in the full scan mode at the range of m/z 100–300.

2.4. Preparation of standard solutions and quality control samples

The stock solutions of CAF (1 mg/ml), TB (200 μg/ml), TP (100 μg/ml) and two internal standards, hypoxanthine (IS-1, 100 μg/ml) and paracetamol (IS-2, 100 μg/ml) were prepared in acetonitrile–water (5:95, v/v), separately. A series of mixture standard working solutions with the concentrations of 10–10,000 ng/ml for CAF, 2.0–2000 ng/ml for TB and 1.0–1000 ng/ml for TP were obtained by diluting the mixture of the stock solutions with acetonitrile–water (5:95, v/v). In addition, the stock solutions of two internal standards were diluted to the concentrations of 3000 ng/ml for hypoxanthine and 400 ng/ml for paracetamol with acetonitrile–water (5:95, v/v). All solutions were kept at 4 °C and were brought to room temperature before use.

Calibration standards of CAF (10, 20, 100, 400, 2000, 5000 and 10,000 ng/ml), TB (2.0, 4.0, 20, 80, 400, 1000 and 2000 ng/ml) and TP (1.0, 2.0, 10, 40, 200, 500 and 1000 ng/ml) were prepared by adding appropriate amount of the mixture standard working solution to the blank rat plasma. Three levels of quality control (QC) samples (20, 400 and 8000 ng/ml for CAF; 4.0, 80 and 1600 ng/ml for TB and 2.0, 40 and 800 ng/ml for TP) in plasma were prepared separately in the same fashion.

2.5. Sample preparation

Plasma samples (200 μl) were spiked with 10 μl mixture solutions of two internal standards and 20 μl acetonitrile, and then extracted with 2 ml ethyl acetate by vortexing for 5 min. After centrifugation at 12,000 rpm for 10 min, the organic phase was transferred to another vial and evaporated to dryness at 35 °C under a slight stream of nitrogen. Then the residue was reconstituted with 100 μl of acetonitrile–water (5:95, v/v), and 20 μl of the solution was used for LC–MS analysis.

2.6. Method validation

The method was fully validated in accordance with US FDA guidelines [15]. Specificity was assessed by comparing chromatograms of six different batches of blank plasma obtained from six rats with those of corresponding standard plasma samples spiked with CAF, TB, TP and two internal standards, and a plasma sample after oral administration of aqueous extract of green tea.

The linearity of the assay was assessed by analyzing the calibration curves (10–10,000 ng/ml for CAF, 2.0–2000 ng/ml for TB and 1.0–1000 ng/ml for TP) in plasma using least-squares linear regression of the peak area ratio of CAF to IS-1, TB and TP to IS-2 *vs.* the nominal concentration of the calibration standard with a weighed

factor ($1/x^2$). The lower limit of quantification (LLOQ) was defined as the lowest concentration on the calibration curve with an acceptable precision within 20% deviation and accuracy between 80% and 120%.

The precision and accuracy were evaluated by assaying six replicates of QC samples at low, medium and high concentrations on the same day and for three consecutive days. Precision was measured by intra- and inter-day relative standard deviation (RSD) and accuracy was described as relative error (RE).

The recoveries were determined at three QC levels with six replicates by comparing the peak areas from extracted samples with those in post-extracted blank plasma samples spiked with the analytes at the same concentration. The recoveries of internal standards were determined in the same way at the concentration of 200 ng/ml. The matrix effect was measured at three QC levels by comparing the peak response of blank plasma extracts spiked with analytes (A) with that of pure standard solution containing equivalent amounts of the compounds (B). The ratio ($A/B \times 100$) % was used to evaluate the matrix effect.

Stability studies in plasma samples were also conducted at three QC levels in several different storage conditions: at room temperature for 8 h, at −20 °C for at least 7 d, after three freeze–thaw cycles, and at 4 °C for 12 h in processed samples.

2.7. Application of the method to pharmacokinetic study

The method was used to determine CAF, TB and TP in rat plasma after oral administration of TB (4 mg/kg), CAF (73 mg/kg) and aqueous extract of green tea (400 mg/kg at a dosing containing TB 4 mg/kg and CAF 73 mg/kg) by using stomach tube, respectively. Animals were randomly divided into three groups, with six rats each. Blood samples were collected from the suborbital vein into heparinized tubes before administration and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48 h after dosing, then immediately centrifuged at 12,000 rpm for 5 min. Plasma was separated from all blood samples and transferred into 1 ml micro centrifuge tubes and were stored at −20 °C until analysis.

The pharmacokinetic parameters of CAF, TB and TP were calculated by the non-compartmental analysis of plasma concentration *vs.* time data using the DAS 2.1 software package (Chinese Pharmacological Society). The comparison of pharmacokinetic parameters between administration of the single substance and aqueous extract of green tea was possessed by SPSS 19.0 (Statistical Package for the Social Science). $AUC_{(0-t)}$, $AUC_{(0-\infty)}$ and C_{max} were compared *via* an independent-samples t-test after logarithmic transformation; $T_{1/2}$ and T_{max} were measured using a non-parametric statistical test (Mann–Whitney test) [16–19]. $P < 0.05$ was considered statistically significant for all the tests.

3. Results and discussion

3.1. Investigation of the components absorbed into the blood

Using LC–IT-TOF-MS, we acquired chromatographic profiles of blank plasma (Fig. 2A), green tea extract (Fig. 2B) and rat plasma after administration of green tea extract (Fig. 2D). First of all, as shown in Fig. 2A, no peak appeared in the blank plasma profile, which showed that there is no endogenous interference. While Fig. 2D shows that three peaks were detected in the rat plasma after administration of green tea extract, which might indicate that the three peaks were all the signals of components absorbed into the blood. They might be seen as the potential bioactive components from green tea. Furthermore, as shown in Fig. 2B, peak 1 and peak 2 were detected in the profile of green tea extract, so they could be defined as prototype components. However, peak 3 did not appear in green tea extract, which could be considered as a metabolite derived from green tea.

In order to explain the dynamic process of two prototype components, similar studies after single administration of them were further performed. The components observed in the plasma are shown in Table 1. The results indicated that just like green tea extract, all three peaks were also observed in the rat plasma after oral administration of CAF, which confirmed that peak 2 and peak 3 were both the metabolites of CAF.

3.2. Structure speculation and identification of bioactive components

Two prototype components and one metabolite had been detected in plasma, and their chemical structures were identified according to their retention time, accurate molecule weight and the m/z of their fragment ions (shown in Table 1). First of all, the structures of the fragment ions in rat plasma were speculated according to the corresponding m/z. Secondly, according to the fragment ions and the accurate molecule weight of the components, the chemical structures of the three components (peaks 1–3) were speculated. Finally, the structures of the three components were identified with the standards.

3.3. Optimization of chromatographic and mass conditions

Under the optimized electrospray ionization condition, all the analytes and two internal standards (shown in Fig. 1) exhibited higher sensitivity in the positive mode. The full-scan positive ion spectrum of the analytes after direct injection in MS proved that the most abundant ions were all $[M+H]^+$, and the responses were very stable and showed good linearity. Whereas, there were hardly any responses to these analytes under negative ion mode, such as $[M-H]^-$, $[M+HCOO]^-$ or $[M+CF_3COO]^-$. So the quantitative analysis was carried out as follows: $[M+H]^+$ m/z 195.08 for CAF, $[M+H]^+$ m/z 181.07 for TB and TP, $[M+H]^+$ m/z 137.04 for IS-1 and $[M+H]^+$ m/z 152.07 for IS-2.

The separation and ionization of CAF, TB, TP and two internal standards were affected by the composition of the mobile phase. Therefore, the composition of the mobile phase is important for improving peak shape, detection sensitivity and shortening run time. With addition of formic acid to the mobile phase, the peak symmetry of all the analytes was improved greatly. So the concentration of formic acid in mobile phase was optimized from 0.05% to 0.2%, the result showed that the addition of 0.1% formic acid was better for all analytes.

3.4. Selection of internal standards

Concentration of the internal standard plays an important role for the quantitative determination of the analytes. In the study, the differences of the plasma concentrations of the analytes were so significant that a single internal standard could not meet the needs of simultaneously quantitative determination of the analytes accurately. Therefore, two internal standards with different concentrations were chosen. Hypoxanthine and paracetamol were used as the internal standards due to their stable extraction recovery, proper chromatographic retention time and similar ionization response in ESI positive mode with the analytes. Hypoxanthine with the concentration of 3000 ng/ml was selected as the IS for CAF, for its suitable response on LC–IT-TOF-MS. Similarly, paracetamol with the concentration of 400 ng/ml was selected as the IS for TB and TP.

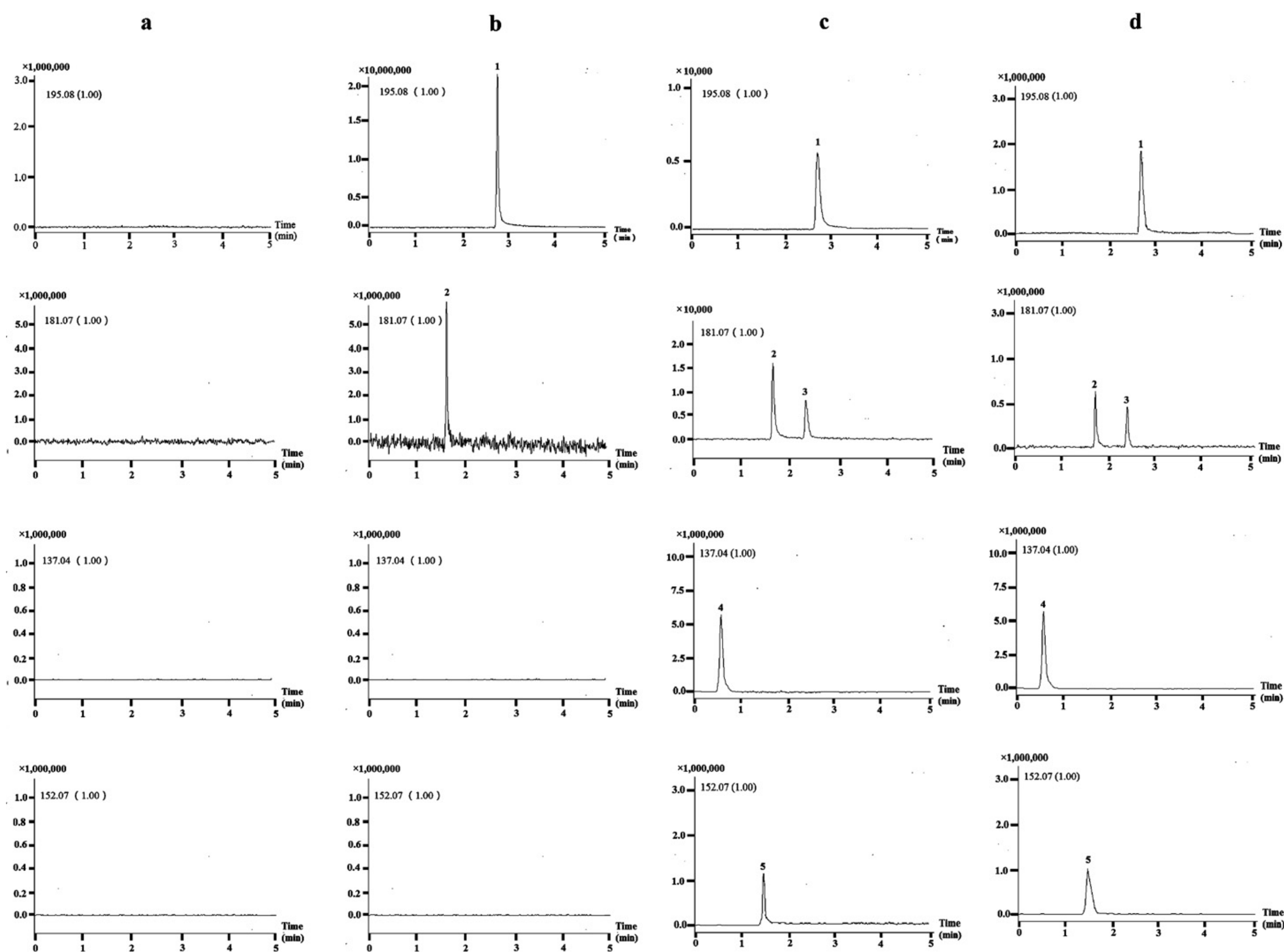

Fig. 2. Represent chromatograms obtained from blank rat plasma (A) green tea extract (B) blank plasma spiked with CAF (10 ng/ml, LLOQ), TB (2.0 ng/ml, LLOQ) TP (1.0 ng/ml, LLOQ) and IS (C) and rat plasma 2 h after oral administration of green tea (D) 1, CAF; 2, TB; 3, TP; 4, IS-1; 5, IS-2.

3.5. Sample preparation

During the development of the extraction method, protein supernatant and liquid–liquid extraction were explored. Liquid–liquid extraction is more effective in producing a clean sample. Thus liquid–liquid extraction was used for the sample preparation in this method. Various organic solvents, such as ether and ethyl acetate were evaluated. Finally, ethyl acetate was found to be optimal for its higher extraction recovery and less matrix interference.

3.6. Method validation

3.6.1. Specificity

The retention times of CAF, TB, TP and two internal standards were 2.87, 1.50, 2.46, 0.74 (IS-1) and 1.80 min (IS-2), respectively.

Table 1
Identified components in green tea and rat plasma.

Peak no.	t_R (min)	$[M+H]^+$ (m/z)	ESI-MS^n (m/z)[a] (100% base peak)	Identification	Green tea extract	Rat plasma[b] Green tea extract	Rat plasma CAF	Rat plasma TB
1	2.87	195.08	MS^2[195.08]: 110.07, 138.07 MS^3[195.08–138.07]: 69.04, 110.07	CAF	+[c]	+	+	–[d]
2	1.50	181.07	MS^2[181.07]: 82.05, 138.07 MS^3[181.07–138.07]: 69.04, 110.07	TB	+	+	+	+
3	2.46	181.07	MS^2[181.07]: 124.05 MS^3[181.07–124.05]: 69.04, 96.06	TP	–	+	+	–

[a] The fragment ions are shown in Fig. 3.
[b] Rat plasma after oral administration of different substances.
[c] The component was detected in green tea extract or in rat plasma.
[d] The component was not detected in green tea extract or in rat plasma.

No significant interference and ion suppression from endogenous substances were observed at the retention time of the analytes and internal standards. Fig. 2 shows the representative LC–MS chromatograms. With the help of suitable system and appropriate mobile phase, a total analysis time of 8 min was achieved, which could make high-throughput analysis possible.

3.6.2. Linearity and sensitivity

The linearity was evaluated on three separate occasions with two sets of calibration curves per occasion. Calibration curves were linear over the concentration ranges of 10–10,000 ng/ml for CAF, 2.0–2000 ng/ml for TB and 1.0–1000 ng/ml for TP, with the corresponding linear regression equation of $y = 6.408 \times 10^{-2}x + 8.92 \times 10^{-3}$ ($r = 0.9976$), $y = 2.610 \times 10^{-3}x + 1.407 \times 10^{-2}$ ($r = 0.9936$), and $y = 3.799 \times 10^{-2}x + 1.156 \times 10^{-2}$ ($r = 0.9989$), respectively. Where y was the peak area ratio of the analyte to the corresponding IS and x was the concentration of the analyte.

The LLOQ of the assay was 10 ng/ml for CAF, 2.0 ng/ml for TB and 1.0 ng/ml for TP, respectively. The data above demonstrated that this method was sensitive enough for the pharmacokinetic study of CAF, TB and TP *in vivo*. The chromatograms of the sample at LLOQ are shown in Fig. 2C.

3.6.3. Precision and accuracy

The precision and accuracy shown in Table 2 were evaluated at three levels of QC samples, respectively. All results for the tested samples were within the acceptable criteria of ±15%, which indicated that the present method had a good precision and accuracy.

3.6.4. Extraction recovery and matrix effect

The extraction efficiencies ranged from 90.6 to 92.2% for CAF, from 71.22 to 72.85% for TB and from 86.1 to 89.3% for TP (Table 2). The mean recoveries of IS-1 and IS-2 were 77.41% and 83.6%, separately. The results indicated that recoveries were consistent and precise at different concentrations.

The results of the matrix effect are shown in Table 2 and are within the range. In addition, the matrix effects for internal standards were 91.5% for hypoxanthine and 92.1% for paracetamol. These results indicated that the endogenous substances showed no significant effect on the ionization for both the analytes and the internal standards.

3.6.5. Stability

The stability of CAF, TB and TP in rat plasma under different storage conditions is summarized in Table 3. The results indicated that all the analytes were stable in plasma at room temperature for 8 h, at −20 °C for at least 7 d, after three freeze–thaw cycles. In addition, the prepared samples in mobile phase with the IS in the autosampler were also stable at 4 °C for at least 12 h.

3.7. Pharmacokinetic application

The developed method was successfully applied to a pharmacokinetic study of CAF, TB and TP in rat plasma after oral administration of TB (4 mg/kg), CAF (73 mg/kg), and an aqueous extract of green tea (at a dose containing TB 4 mg/kg and CAF 73 mg/kg). The concentration–time curves (mean ± SD) for CAF, TB and TP are presented in Fig. 3 and the corresponding pharmacokinetic parameters are listed in Table 4.

As shown in Table 4, significant differences existed in the pharmacokinetic results of CAF between rats receiving the single substance and aqueous extract of green tea. Compared with the values after oral administration of the aqueous extract of green tea, the $AUC_{(0-t)}$, $AUC_{(0-\infty)}$ and $C_{\max}$ of CAF were remarkably increased ($P < 0.05$ for AUC and $P < 0.01$ for $C_{\max}$) after oral administration of

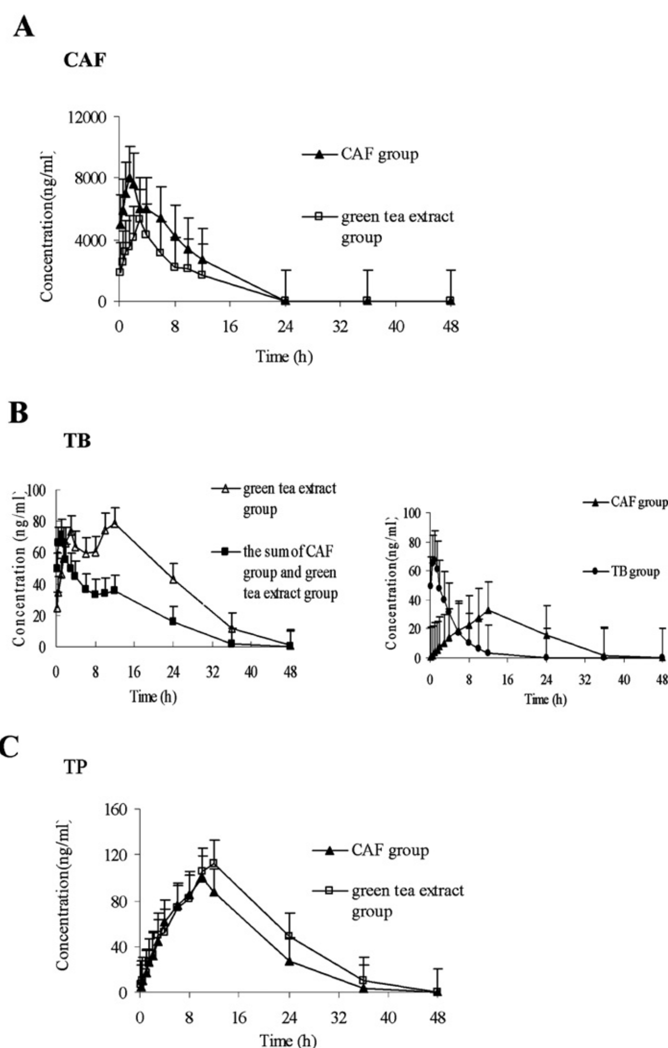

Fig. 3. Concentration–time curves for CAF (A), TB (B) and TP (C) in rat plasma after oral administration of CAF (73 mg/kg), TB (4 mg/kg), and green tea extract (400 mg/kg).

CAF, which indicated that the absorption of CAF was inhibited after oral administration of an aqueous extract of green tea. In contrast, no difference was observed in the pharmacokinetic parameters of TP, which suggested that the biotransformation from CAF to TP was not influenced by other ingredients in green tea.

The pharmacokinetic results of TB were more complex. First of all, as discussed in Section 3.1, TB (peak 2) was a metabolite of CAF (peak 1), which was in accordance with the conclusion of the related studies [20]. Furthermore, TB was also a prototype component in the green tea, which might indicate that TB detected in the rat plasma after oral administration of the aqueous extract of green tea was the sum of the prototype component and the metabolite. As shown in Fig. 3B-1, after oral administration of an aqueous extract of green tea, the mean plasma concentration–time curve exhibited double-peaks at 3 h and 12 h, while Fig. 3B-2 shows that after oral administration of TB, only one peak appeared at 1 h. The difference indicated that the reason for the appearance of double-peaks might be the influence of other components in green tea rather than the resorption of TB itself. Fig. 3B-2 also displays the concentration–time curve of TB after oral administration of CAF, only one peak appeared at 12 h.

In order to study the influence of green tea on the absorption of TB and the biotransformation from CAF to TB in rat plasma, a mean plasma concentration–time curve of the sum of the plasma

Table 2
Precision, accuracy, matrix effect, and recovery for analysis of CAF, TB and TP in rat plasma ($n = 6$).

	Concentration spiked (ng/ml)	Intra-day RSD (%)	Inter-day RSD (%)	Accuracy (RE, %)	Matrix effect (%, mean ± SD)	Recovery (%, mean ± SD)
CAF	20	0.6	2.1	−0.1	93.3 ± 0.6	91.5 ± 5.8
	400	0.5	4.7	−5.7	92.1 ± 3.2	90.6 ± 2.1
	8000	0.7	5.6	−6.6	97.9 ± 2.4	92.2 ± 4.7
TB	4.0	1.1	10.2	8.9	92.1 ± 0.7	71.22 ± 4.62
	80	1.1	3.5	1.2	93.4 ± 4.9	72.85 ± 5.65
	1600	0.7	0.8	−4.7	95.5 ± 2.3	71.36 ± 4.23
TP	2.0	3.7	3.6	−4.6	92.3 ± 3.7	89.3 ± 3.4
	40	6.8	4.0	−3.6	94.2 ± 4.9	87.2 ± 4.2
	800	1.7	6.8	−5.5	95.6 ± 4.1	86.1 ± 6.5

Table 3
Stability of CAF, TB and TP in rat plasma ($n = 3$).

Conditions	CAF			TB			TP		
	Spiked (ng/ml)	RE (%)	RSD (%)	Spiked (ng/ml)	RE (%)	RSD (%)	Spiked (ng/ml)	RE (%)	RSD (%)
Room temperature for 8 h	20	−5.7	2.5	4.0	14.2	2.7	2.0	−12.2	2.0
	400	6.0	7.2	80	−3.2	4.8	40	9.1	9.9
	8000	−8.3	3.6	1600	5.5	3.7	800	5.9	3.8
Frozen for 7 days	20	−10.9	5.2	4.0	12.7	3.8	2.0	−14.8	7.7
	400	−5.7	4.8	80	2.9	3.7	40	−5.4	5.7
	8000	−7.6	6.4	1600	−4.1	6.0	800	6.3	6.5
Three freeze and thaw cycles	20	0.3	7.3	4.0	13.9	5.6	2.0	−13.6	11.5
	400	5.4	5.1	80	−7.4	6.2	40	6.0	3.2
	8000	6.9	4.3	1600	9.8	4.1	800	4.0	4.1
4 °C in autosampler for 12 h in processed samples	20	13.5	6.9	4.0	10.4	6.3	2.0	9.1	5.8
	400	−5.0	4.6	80	−6.5	5.6	40	13.8	1.0
	8000	1.3	7.5	1600	8.0	5.8	800	14.3	9.6

Table 4
Main pharmacokinetic parameters for CAF, TB and TP after oral administration of TB, CAF and green tea extract (mean ± SD; $n = 6$).

Parameters	CAF		TB			TP	
	CAF alone	Green tea extract	TB alone	CAF alone	Green tea extract	CAF alone	Green tea extract
$AUC_{(0-t)}$ (ng h/ml)	$6.374 \times 10^4 \pm 1.049 \times 10^4$*	$3.968 \times 10^4 \pm 1.133 \times 10^4$	293.2 ± 24.0***	554.6 ± 174.8***	1827 ± 426	1680 ± 423	2177 ± 1065
$AUC_{(0-\infty)}$ (ng h/ml)	$9.90 \times 10^4 \pm 2.14 \times 10^4$*	$5.277 \times 10^4 \pm 2.482 \times 10^4$	305.7 ± 24.2***	1474 ± 1021***	2752 ± 984	1695 ± 426	2668 ± 1125
C_{max} (ng/ml)	$9.18 \times 10^3 \pm 1.39 \times 10^3$**	5500 ± 1655	74.25 ± 8.57	33.86 ± 11.23***	82.1 ± 10.4	108.7 ± 18.0	121.2 ± 64.6
T_{max} (h)	2.1 ± 1.0	3.0 ± 0.6	1.0 ± 0.3	11.3 ± 1.0	10.2 ± 3.6	10.3 ± 1.5	11.3 ± 1.0
$T_{1/2}$ (h)	9.0 ± 6.9	5.4 ± 3.4	2.4 ± 0.6**	11.3 ± 6.3	13.0 ± 8.0	4.8 ± 0.6	10.2 ± 7.2

* $P < 0.05$ compared with green tea extract.
** $P < 0.01$ compared with green tea extract.
*** $P < 0.001$ compared with green tea extract.

concentrations after oral administration of TB and CAF was obtained and then compared with the curve of the plasma concentrations after oral administration of green tea point-to-point. As shown in Fig. 3B-1, both the two curves exhibited double peaks. The T_{max} of the peaks were 1 h and 12 h for the former, 3 h and 12 h for the latter. The results might indicate that green tea could postpone the absorption of TB. Furthermore, the plasma concentrations of the latter were significantly higher than the former at each point after 1.5 h, which might indicate that green tea could promote the biotransformation from CAF to TB. Another explanation was unknown components existed in green tea might be metabolized into TB and made the plasma concentration significantly increase. After 12 h, the plasma concentrations of the latter decreased more significantly than the former, which indicated that green tea would accelerate the elimination of TB.

4. Conclusions

A sensitive and efficient LC–IT-TOF-MS method was established for screening the components absorbed into the blood and analyzing their dynamic process in rat plasma. As a result, three components were successfully identified as CAF, TB and TP, respectively. Furthermore, a comparative pharmacokinetic study of the analytes after oral administration of the single substances and an aqueous extract of green tea was performed. The method of the quantitative analysis showed high sensitivity, with the LLOQ of 10 ng/ml for CAF, 2.0 ng/ml for TB and 1.0 ng/ml for TP. The results indicated that green tea could inhibit the absorption of CAF and promote the biotransformation from CAF to TB. Meanwhile, green tea could also postpone the absorption and accelerate the elimination of TB. The method developed could be useful in screening bioactive components in rat plasma and in further pharmacokinetic studies on CAF and green tea.

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