LC-MS/MS for Simultaneous Determination of Four Major Active Catechins of Tea Polyphenols in Rat Plasma and Its Application to Pharmacokinetics

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Abstract: Objective To develop a liquid chromatography technique coupled with tandem mass spectrometry (LC-MS/MS) for simultaneous determination of four active catechins EGCG, ECG, EGC, and EC of tea polyphenols (TP) in rat plasma in order to further study its multi-component pharmacokinetics. Methods Following a single step liquid-liquid extraction of plasma samples with ethyl acetate, the four catechins were separated on a Hypersil ODS C18 column using an isocratic mobile phase composed of methanol-water (30:70). The detection using a mass spectrometer was performed under negative ESI in the MRM mode. The analytes were identified by reference to both MRM and $t_R$ values and quantified using peak area internal standard method. Results The method was shown to be specific without interference from matrix, metabolites, and impurities present in TP raw material and to be sensitive with LOD and LOQ of 1.5 and 10 ng/mL (EGCG) as well as 0.75 and 5 ng/mL (ECG, EGC, and EC). A good linearity was obtained over a wide range of 10–10 000 ng/mL for EGCG and 5–5000 ng/mL for other three catechins ($r > 0.996$). The method was validated to be reproducible and reliable, as evidenced by intra-batch and inter-batch precision of less than 10% and 11%, accuracy of 97.13%–106.05% and 99.22%–103.14%, respectively. The recovery of extraction ranged from 72.74% to 89.13%, matrix effect from 88.76% to 105.97% for four catechins. The method was successfully used to study the pharmacokinetics of TP iv administered to rats at a dose of 100 mg/kg. Conclusion This method is shown to completely meet requirements for the multi-component pharmacokinetic study of TP in rats.

Key words: EC; ECG; EGC; EGCG; LC-MS/MS; pharmacokinetics; plasma

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Introduction

In recent years, tea polyphenols (TP), a group of polyphenolic compounds extracted from green tea (Camellia sinensis L. O. Ltze, Theaceae), have attracted ever-growing attention just because over the past two decades numerous preclinical and clinical studies have revealed their potent anti-oxidative activity and extensive beneficial roles in human health, such as effects of anti-atherosclerosis, anti-diabetic, anti-obesity, anti-irradiation, anti-aging (Yokozowa and Nakagawa, 2002; Zhao et al., 1992; Sabu, Smitha, and Kutan, 2002; Murase et al., 2002; Zhu et al., 2008), and especially the chemoprevention of cancers and anti-AIDS effects (Katiyar, Lee, and Lu, 2001; Williamson et al., 2006; Lambert et al., 2003; Xie et al., 2003). It is likely that those diseases above and pathological conditions are caused by or associated with oxidative stress or oxidative damage, and thus the anti-oxidative and anti-free radical activities of TP have become a subject of considerable interest.

The aforementioned health-promoting properties of TP have been attributed to catechins present in TP, characterized by di- or tri-hydroxyl group substitution.

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of the B ring and meta-5,7-dihydroxyl substitution of the A ring of flavanols, including \((-\) epigallo-catechin-3-gallate (EGCG), \((-\) epicatechin-3-gallate (ECG), \((-\) epigallocatechin (EGC), and \((-\) epicatechin (EC) (Fig. 1), of which EGCG is the most abundant and active, ECG next, and EC the least (Graham, 1992; Luo, Guo, and Xu, 2005). With the increasing significance of the potential beneficial effects, there is a growing demand for research on their metabolic disposition and pharmacokinetics (PK). However, up to date, the PK study of TP is scarce relative to its considerable pharmacodynamic (PD) studies due to the lack of satisfactory analysis methodology in biological samples. Several methods for determination of catechins have been described in the literature. Most of methods have utilized HPLC coupled with different types of detectors including UV (Fu et al., 2008; Fu et al., 2009), CL (Nakagawa and Miyazawa, 1997), ECD (Yang, Arai, and Kusu, 2000), CEAD (Chu et al., 2004), and LC-MS (Dalluge et al., 1997), among which UV was preferred due to its practicability.

![Chemical structures of four major active catechins present in green tea](image)

Fig. 1 Chemical structures of four major active catechins present in green tea

Recently, we developed RP-HPLC-UV assays with isocratic and gradient mobile phases to determine catechins in biological samples and thereby to investigate PK of TP (Fu et al., 2008; Fu et al., 2009). But it was found that HPLC-UV assay was associated with low sensitivity, difficulty in resolution between multiple components, and long run time for a single analysis, and thus was not an ideal choice for multi-component PK study of TP. In addition, most of studies in currently available reports were directed at mono-component detection and mono-component PK study after administration of single ingredient or TP (Lin et al., 2007; Chow et al., 2001; Lambert and Yang, 2003). A multi-component PK study of TP is essential for deep insight into its PK and PD properties.

With rapid development of bioanalysis, the LC-MS was increasingly applied to PK study due to its ability to provide adequate resolution and high sensitivity. This prompted us to use LC-MS to study multi-component PK of TP. In this paper an LC-MS/MS method for simultaneous determination of four major active catechins of TP in rat plasma was reported. This method takes advantage of liquid-liquid extraction with ethyl acetate to pre-treat plasma samples and a reverse phase C18 column with isocratic mobile phase to separate four catechins. Internal standard and a mass spectrometer under negative ESI in the multiple reaction monitoring (MRM) mode were used to monitor the target ions. To the best of our knowledge, the present method is the only known reported LC-MS/MS method for simultaneous analysis of four active catechins in plasma of rats dosed with TP, a green tea-derived multi-component extract.

Materials and methods

Chemicals and reagents

TP, an extract from green tea (Camellia sinensis L. O. Ltze), was purchased from Wuyuan Tea Plantation (Jiangxi Province, China) with purity > 98%, determined by spectrophotometry with ferrous tartrate as chromogenic agent, and the percent contents of 43.14% EGCG, 16.11% ECG, 5.97% EGC, and 9.21% EC, measured by the HPLC-UV assay in our laboratory (Fu et al., 2009), and was formulated as injection with sterile water for experimental use. EGCG, ECG, and EGC reference substances were purchased from U-sea Biotech Co., Ltd. (Shanghai, China) with purity of 99.0%; EC reference substance was provided by China Institute for Control of Pharmaceutical & Biological Products with purity of 97%; Vanillin as an IS was purchased from Kermel Chemical Reagent Co., Ltd. (Tianjin, China) with purity of 99%; HPLC-grade methanol was from Tedia Company (USA); Ethyl acetate (EtOAc), vitamin C, and all other chemicals used were of analytical purity. The double-distilled water was used for preparing mobile phase and other solutions.

LC-MS/MS conditions

The LC-MS/MS analysis was performed using an
Agilent 1200 model liquid chromatography system equipped with a quaternary pump delivery system, an online degasser, and an API 3200 Triple Quadrupole mass spectrometer (AB, USA) equipped with an electrospray ionization (ESI) source (AB, USA). The system control and data processing were carried out by Analyst 1.4.1 software. The separation was carried out on a Hypersil C_{18} column (150 mm \times 2.1 mm, 5 \mu m, Ilite, Dalian, China) maintained at room temperature (20 \(^\circ\)C) with an isocratic mobile phase composed of methanol-water (30 : 70) running at 0.5 mL/min; Injection volume was 10 \(\mu\)L. After passing through the column, eluant was directly delivered to ESI source operating in negative ionization mode. The tuning parameters were optimized by infusing a solution containing 1000 ng/mL each of EGCG, ECG, EGC, EC, and the IS, delivered via a Harvard syringe pump at a constant flow rate of 10 \(\mu\)L/min. Gas 1 and gas 2 (nitrogen) were set at 379 and 414 kPa. The optimized ionspray voltage and temperature were set at ~4000 V and 590 \(^\circ\)C, respectively. The declustering potentials (DP) were set at ~37, ~40, ~45, ~41, ~30 V and collision energies (CE) were adjusted to optimize the product ion signal as ~27, ~30, ~33, ~41, ~18 eV for EGCG, ECG, EGC, EC, and IS, respectively. The MRM mode was used to monitor the transition of the deprotonated molecule at \(m/z\) 457.0\(\rightarrow\)168.8 (EGCG), 441.0\(\rightarrow\)168.8 (ECG), 305.1\(\rightarrow\)124.8 (EGC), 289.1\(\rightarrow\)108.8 (EC), and 150.7\(\rightarrow\)135.8 (IS) (Fig. 2).

**Preparation of standard solutions**

The primary stock solutions of EGCG (6 mg/mL), ECG (3 mg/mL), EGC (3 mg/mL), and EC (3 mg/mL) standards were individually prepared by dissolving appropriate amount of reference substance in methanol, and then mixed in appropriate ratios to yield mixture stock solutions and then diluted serially in methanol to yield serial mixture standard working solutions containing appropriate concentrations of the above four catechins (0.05, 0.1, 0.25, 1, 5, 25, and 50 \(\mu\)g/mL of EGCG; 0.025, 0.05, 0.125, 0.5, 2.5, 12.5, and 25 \(\mu\)g/mL of ECG; 0.025, 0.05, 0.125, 0.5, 2.5, 12.5, and 25 \(\mu\)g/mL of EGC and 0.025, 0.05, 0.125, 0.5, 2.5, 12.5, and 25 \(\mu\)g/mL of EC). The IS solution was prepared by dissolving vanillin in methanol to produce a concentration of 2.5 \(\mu\)g/mL. All the standard solutions were stored at ~20 \(^\circ\)C until analysis.

**Preparation of plasma calibration standards**

Plasma calibration standards, containing 0.01, 0.02, 0.05, 0.2, 1, 5, and 10 \(\mu\)g/mL of EGCG; 0.005, 0.01, 0.025, 0.1, 0.500, 2.5, and 5 \(\mu\)g/mL of ECG; 0.005, 0.01, 0.025, 0.1, 0.5, 2.5, and 5 \(\mu\)g/mL of EGC; and 0.005, 0.01, 0.025, 0.1, 0.5, 2.5, and 5 \(\mu\)g/mL of EC, respectively, were prepared by addition of 20 \(\mu\)L each of the respective mixture standard working solution, IS.
solution and 20% vitamin solution into 100 µL of blank plasma.

Sample pretreatment

An aliquot of 100 µL plasma sample was transferred into an Eppendorff tube containing 20 µL each of 2.5 µg/mL IS and 20% vitamin C solution and then extracted once with 1 mL of EtOAc by vortex-mixing for 1 min and centrifuged at 10 000 r/min for 10 min at 4 °C. The upper organic phase was transferred into another tube and evaporated to dryness under a gentle N₂ stream at 45 °C. The residue was reconstituted in 100 µL of mobile phase. After centrifuging at 10 000 r/min for 10 min at 4 °C, 10 µL of supernatant was used for LC-MS/MS analysis.

Quantification of the four catechins in plasma samples of rats

The frozen plasma samples of rats were thawed naturally at ambient temperature and then treated as described under section “sample pretreatment”. Then, peak area ratio of analyte/IS was calculated with reference to the recorded chromatogram. Samples were quantified by reference to accompanying calibration extraction curves constructed by plotting the peak area ratios of each analyte/IS vs analyte concentrations in plasma calibration standards.

Experimental animals and PK study

Male SD rats weighing (200–220) g were supplied by Animal Center of Dalian Medical University and housed in standard laboratory conditions and had free access to water and commercial rat chow. They were maintained under a constant 12 h light-dark cycle at an environment temperature of (21–23) °C. All animal experiments were carried out in accordance with institutional guidelines and ethics. Rats were injected via tail vein with TP injection at a dose of 100 mg/kg, equivalent to 43.14, 16.11, 5.97, and 9.21 mg/kg of EGC, ECG, EGC, and EC, respectively. Aliquots of 400 µL blood were collected pre-dose and at different time post-dose from orbital sinus by venipuncture into heparinized tubes from six rats and centrifuged immediately at 10 000 r/min at 4 °C to yield plasma, which were stored at −20 °C until analysis. The concentration-time curves were fitted by compartment model; PK parameters were calculated with 3p97 PK program developed by China Mathematical Pharmacological Society.

Validation of methodology

This LC-MS/MS assay was validated thoroughly in respect to specificity, linearity, precision, accuracy, stability, and extraction recovery according to the Guiding Principles issued by China SFDA (Zheng, 2002). The quality control (QC) samples containing appropriate amount of EGC + ECG + EGC + EC, prepared based on procedures described under section “sample pretreatment”, at high (5 + 2.5 + 2.5 + 2.5) µg/mL, middle (0.2 + 0.1 + 0.1 + 0.1) µg/mL, and low (0.02 + 0.01 + 0.01 + 0.01) µg/mL concentrations for six repeated analyses separately were used for validation.

Results and discussion

Method development

In ESI-MS spectra of four catechins under optimized LC conditions, the most abundant deprotonated molecular ions, [M − H]− m/z 457, 441, 305, 289, and 151 were observed for EGC, ECG, EGC, EC, and IS, respectively. These ions were then used as precursor ions to conduct scan analysis of product ions, the obtained main fragment ions were deprotonated ion of gallic acid [M − H]− (m/z 168.8) for EGC and ECG, and trioxynbenzene deprotonated ion [M − H]− (m/z 124.8) for EGC as well as catechol deprotonated ion [M − H]− (m/z 108.8) for EC, and de-methylated vanillin ion [M − H]− (m/z 135.8), therefore, the ion reactions used for quantitative analysis were selected as m/z 457.0→168.8 (EGCG), 441.0→168.8 (ECG), 305.1→124.8 (EGC), 289.1→108.8 (EC), and 150.7→135.8 (IS) (Fig. 2).

The methanol-water was selected as mobile phase, a 30 : 70 ratio of methanol to water was the best choice for four catechins and IS resolution with short tₑ (< 4 min for a single analysis). This mobile phase was free from organic acid additive, such as acetic acid and trifluoroacetic acid as described by Masukawa, Lin, and Chu et al (Lin et al, 2007; Masukawa et al, 2006; Chu et al, 2004), who deemed that acidic additive provided the most abundant deprotonated molecular ions [M − H]− characteristic for green tea catechins under negative ESI. But the mobile phase employed by us resulted in an increased sensitivity and better resolution as compared with acetic acid or ammonium acetate containing mobile phases, which led to attenuated ion signal of analytes and IS.

An appropriate IS is required for accurate
quantitative bioanalysis. Unfortunately, most of tea catechin PK studies took advantage of external standard methods. The only available report on IS method is the one by Dr. LIN Ei-chwen and PELILLO M, who chose theophyllin and tyrosol as IS (Lin et al., 2007; Pelillo et al., 2002). In our study vanillin was chosen, because it was not only similar to catechins in chemical structure, but also peaked among four catechins (Fig. 2) and was superior to theophyllin and tyrosol.

A simple pretreatment procedure with 6% perchloric acid for deproteinizing plasma was tried, but failed due to the dilution and ion suppression effect and thereby decreased sensitivity and contamination of ion source. Finally, liquid-liquid extraction with EtOAc was chosen, yielding a concentrating effect and elimination of interference from matrix.

**Specificity**

In this method MRM mode in mass spectrometry behavior combined with a differentiated $t_R$ in chromatography behavior was used to identify various analytes, conferring on the method of a high specificity. As seen in Fig. 3, the four catechins and IS were well resolved from each other with $t_R$ being 1.70, 3.53, 1.18, 2.29, and 2.86 min for EGCG, ECG, EGC, EC, and IS, respectively. No

![Fig. 3 Representative MRM chromatograms of EGCG, ECG, EGC, EC, and IS](image)

A: EGCG ($t_R$ = 1.70 min); B: ECG ($t_R$ = 3.53 min); C: EGC ($t_R$ = 1.18 min); D: EC ($t_R$ = 2.29 min); E: IS ($t_R$ = 2.86 min). I: drug-free plasma; II: blank plasma spiked with individual standards or IS; III: plasma sample collected at 8 h after intravenous injection of TP to rats.
interference from matrix and metabolites was observed.

**Calibration curves and linearity**

Calibration curves were constructed by performing weighed linear regression analysis (\( w_i = 1/C_i^2 \)) of peak area ratios (\( R \)) of respective analyte to IS vs the corresponding concentrations (\( C \), ng/mL). The obtained calibration curves showed a good linearity over the range of 0.01–10 \( \mu \)g/mL for EGCG and 0.005–5 \( \mu \)g/mL for ECG, EGC, and EC; The regression equations and their correlation coefficient (\( r \)) were calculated as follows:

- **EGCG**,
  \[ R = 0.000989C + 0.00166 \ (r = 0.9982) \]
- **ECG**,
  \[ R = 0.000745C + 0.00102 \ (r = 0.9968) \]
- **EGC**,
  \[ R = 0.00047C + 0.000986 \ (r = 0.9966) \]
- **EC**,
  \[ R = 0.00031C + 0.000608 \ (r = 0.9973) \]

The LOD (\( S/N = 3 \)) and LOQ (\( S/N = 20 \)) were determined as 1.5 and 10 ng/mL for EGCG, 0.75 and 5 ng/mL for ECG, EGC, and EC, respectively.

**Precision and accuracy**

The precision and accuracy of this method were presented in Table 1. The intra-batch and inter-batch precisions, expressed as relative SD (RSD), were obtained by analyzing QC samples containing the four catechins at low, middle, and high concentrations in six replicates of each concentration for each batch and calculated by SD of measured concentrations from a batch (within one day) and from three batches (on three consecutive days) divided by the mean measured concentration. The RSD was found to be 4.24%–10.27% for intra-batch precision and 2.71%–11.57% for inter-batch precision, indicating that this method had a good reproducibility.

**Table 1  Precision and accuracy (\( x \pm s \))**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Doses / (( \mu )g·mL(^{-1} ))</th>
<th>Intra-batch (( n = 6 ))</th>
<th>Inter-batch (( n = 3 \times 6 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Found / (ng·mL(^{-1} ))</td>
<td>RSD / %</td>
<td>Accuracy / %</td>
</tr>
<tr>
<td>EGCG</td>
<td>0.02</td>
<td>20.23 ± 1.04</td>
<td>4.24</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>207.0 ± 11.3</td>
<td>10.27</td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>4911 ± 203.7</td>
<td>4.62</td>
</tr>
<tr>
<td>ECG</td>
<td>0.01</td>
<td>10.23 ± 0.65</td>
<td>5.95</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>102.6 ± 9.83</td>
<td>6.65</td>
</tr>
<tr>
<td></td>
<td>2.50</td>
<td>2543 ± 103.5</td>
<td>5.00</td>
</tr>
<tr>
<td>EGC</td>
<td>0.01</td>
<td>10.61 ± 0.58</td>
<td>5.72</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>101.6 ± 11.8</td>
<td>7.97</td>
</tr>
<tr>
<td></td>
<td>2.50</td>
<td>2457 ± 114.1</td>
<td>4.49</td>
</tr>
<tr>
<td>EC</td>
<td>0.01</td>
<td>10.33 ± 0.55</td>
<td>5.16</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>101.3 ± 9.43</td>
<td>6.44</td>
</tr>
<tr>
<td></td>
<td>2.50</td>
<td>2428 ± 95.2</td>
<td>5.10</td>
</tr>
</tbody>
</table>

Accuracy, expressed as percentage of mean measured concentration vs nominal concentration, was estimated between 97.13%—106.05% (intra-batch) and 99.22%—103.14% (inter-batch), indicating high accuracy of this method.

**Recovery of extraction and matrix effect**

The recovery of extraction (RE) and matrix effect (ME) were obtained by performing the procedures described by Matuszewski, Constanzer, and Chavez-Eng (2003).

**Table 2  Recovery extraction and matrix effect (\( x \pm s \), \( n = 6 \))**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>RE / %</th>
<th>ME / %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Middle</td>
</tr>
<tr>
<td>EGCG</td>
<td>89.13 ± 5.76</td>
<td>76.85 ± 4.10</td>
</tr>
<tr>
<td>ECG</td>
<td>78.88 ± 7.19</td>
<td>75.87 ± 3.11</td>
</tr>
<tr>
<td>EGC</td>
<td>78.79 ± 5.61</td>
<td>72.74 ± 4.69</td>
</tr>
<tr>
<td>EC</td>
<td>77.73 ± 7.71</td>
<td>73.01 ± 4.25</td>
</tr>
<tr>
<td>IS</td>
<td>52.23 ± 6.99</td>
<td></td>
</tr>
</tbody>
</table>

As shown in Table 2, the mean ME values ranged from 88.76% to 105.97%, indicating that the ME was minor and could be ignored in the quantitative analysis. The RE was found to be over the range of 72.74%—89.13% for four catechins and 52.23% for IS.

**Stability**

The stability of analytes in plasma samples was investigated at different storage conditions using QC samples and estimated by comparing the concentration assessed at different times with initial concentration.
The relative content > 90% was considered to be stable. It has been demonstrated that the four catechins were stable for at least 30 d during plasma sample storage at −20 °C, 12 h at 4 °C, and 8 h at room temperature.

Application of the methodology in PK study

After a single iv dose of TP 100 mg/kg to five rats, the concentration-time curves were constructed for up to 22 h for EGCG and ECG and 8 h for EGC and EC, as shown in Fig. 4, and could be best fitted to three-compartment model and described by tri-exponential equation: \( C = Ae^{-\alpha t} + Be^{-\beta t} + Ge^{-\gamma t} \), compared with the two-compartment model demonstrated in our previous study, where HPLC-UV assay was used (Fu et al., 2009). It was apparent that use of highly sensitive LC-MS/MS assay led to demonstration of the three-compartment model. Also, unlike HPLC-UV assay, as reported in our previous studies (Fu et al, 2008; Fu et al, 2009), only EGCG and ECG, the two catechins abundant in TP, could be detected due to the low sensitivity, LC-MS/MS assay described in the present study allowed the above four catechins to be all detectable and thus enable investigation of multi-component PK profile of TP.

![Fig. 4 C-T curves of EGCG, ECG, EGC, and EC following iv administration of TP (100 mg/kg⁻¹) to rats](image)

From Table 3 it was evident that the ester-type catechins EGCG and ECG differed greatly from non-ester-type catechins EGC and EC in PK behaviors, reflected by far larger \( V_d \), longer \( t_{1/2} \), and smaller CL for the former than for the latter, due to the difference between the two types of catechins in lipophilicity. All the four catechins showed large \( V_d \) values exceeding total body water volume, suggesting that they were widely distributed in body. In the four catechins EC showed the shortest \( t_{1/2} \) (about 1.5 h), indicating its rapid elimination from body; By contrast, EGCG and ECG showed a \( t_{1/2} \) as long as 10 h, indicating that these two catechins eliminated slowly.

Table 3  PK parameters of four catechins in rats after receiving an iv dose of 100 mg·kg⁻¹ of TP  (\( \bar{X} \pm s, n = 5 \))

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>EGCG</th>
<th>ECG</th>
<th>EGC</th>
<th>EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t_{1/2} )</td>
<td>min</td>
<td>15.78 ± 6.85</td>
<td>76.4 ± 26.7</td>
<td>620 ± 151.7</td>
<td>2001 ± 457</td>
</tr>
<tr>
<td>( t_{1/2} )</td>
<td>min</td>
<td>14.8 ± 7.1</td>
<td>77.1 ± 23.6</td>
<td>630.9 ± 29.3</td>
<td>1101 ± 293</td>
</tr>
<tr>
<td>AUC</td>
<td>μg·mL⁻¹·min</td>
<td>2001 ± 457</td>
<td>2001 ± 457</td>
<td>2001 ± 457</td>
<td>2001 ± 457</td>
</tr>
<tr>
<td>CL</td>
<td>L·kg⁻¹·min</td>
<td>0.022 ± 0.005</td>
<td>0.016 ± 0.005</td>
<td>0.038 ± 0.009</td>
<td>0.038 ± 0.012</td>
</tr>
<tr>
<td>( V_d )</td>
<td>L·kg⁻¹</td>
<td>20.0 ± 6.1</td>
<td>14.1 ± 3.7</td>
<td>4.17 ± 1.13</td>
<td>5.24 ± 2.28</td>
</tr>
</tbody>
</table>

Conclusion

In this study, an LC-MS/MS assay with the detection of the specific target ions performing under negative ESI in the MRM mode was developed for simultaneous determination of four catechins in rat plasma. This assay was validated to be highly specific as a result of MRM mode combined with differentiated \( t_R \) for identification, highly sensitive with LOD and LOQ of as low as 0.75—1.5 ng/mL and 5—10 ng/mL and also rapid with a very short run time ( < 4 min) for a single analysis. Application to the PK study in rats demonstrated that the present LC-MS/MS method completely could meet the requirements for multi-component PK study of TP.

References


