Simultaneous determination of cis- and trans-palmitoleic acid in rat serum by UPLC–MS/MS

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Palmitoleic acid, a monounsaturated fatty acid which could affect glucose and lipid metabolism and reduce insulin resistance has two isomers, i.e. cis-palmitoleic acid (cPOA) and trans-palmitoleic acid (tPOA). However, the pharmacokinetic, metabolic transformation and structure–activity relationship of the two isomers have not been reported. A precise and accurate ultra performance liquid chromatography–tandem mass spectroscopy (UPLC–MS/MS) method was developed to determine cPOA and tPOA simultaneously. Both the cPOA and tPOA were administered i.g. (intragastric gavage) to rats at 75 mg/kg. Serum samples were collected and analyzed for the two isomers by UPLC–MS/MS on a reverse-phase BDS C18 column equilibrated and eluted with water (A) and acetonitrile (B) at a flow rate of 0.3 mL/min. The calibration curves for cPOA and tPOA were linear over the range 0.1–12 μg/mL. Analytes were monitored by selected-reaction monitoring in negative electrospray ionization mode. The Tmax of cPOA was 0.94 ± 0.44 h and the Cmax 8.17 ± 1.97 μg/L, and the Tmax and Cmax of tPOA were 1.50 ± 0.98 h and 14.77 ± 11.91 μg/L, respectively. AUC 0–24 h of cPOA and tPOA were 59.45 ± 29.83 and 113.88 ± 72.25 mg/L·h. The method was applied in pharmacokinetic study of cPOA and tPOA in rat serum successfully. Besides, the concentrations of cPOA and tPOA in rat serums were observed fluctuating with a consistent trend, which may be due to reciprocal bio-convert in the body.

Abbreviations
POA  Palmitoleic acid
cPOA  Cis-Palmitoleic acid
tPOA  Trans-Palmitoleic acid
UPLC–MS/MS  Ultra performance liquid chromatography–tandem mass spectrometry

Palmitoleic acid (POA, C16:1, n-7) is an omega-7 monounsaturated fatty acid in plant and fish oil1. It has two isomers, i.e. cis-POA (cPOA) and trans-POA (tPOA), which are with different space structures (Fig. 1). cPOA is common in natural POA, which has been widely reported affecting favorably glucose and lipid metabolism through multiple mechanisms2–4. cPOA was observed affecting the key enzymes of blood glucose metabolism, regulating insulin secretion and reducing insulin resistance5. Orally administered cPOA induced satiety, enhanced the release of satiety hormones and decreased food intake in mice6, as well as reduced body weight, ameliorated the development of hypertriglyceridemia and hyperglycemia, and improved insulin sensitivity7. On the other side, trans-fatty acid is a subject of ongoing discussions on both suggested positive and negative associations with cardiovascular and metabolic risk factors8. Higher tPOA proportion in plasma phospholipids improved insulin sensitivity or decreased onset of type 2 diabetes disease9. tPOA regulated glycolipid metabolism, closely related to low density lipoprotein10–12. It has been used as a biomarker for reducing the risk of coronary heart disease and type 2 diabetes. Most of the studies analysed fatty acids using GC-FID or GC-Ms13. The fatty acids samples used in the process requires separation and methylation before the measurement. This may destroy the original molecular structure of fatty acids in the biological matrix, and require big biological sample size or amount than other methods14,15. In previous studies, cPOA was determined in the subcutaneous fat of human

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body, and tPOA was determined in the 200 µL plasma of human by GC thorough, aminopropyl SPE columns for the separation (Isolute, Biotage) and transmethylated with methanolic-HCl into fatty acid methyl esters (FAME)16–18, but no method was available to determine cPOA and tPOA at the same time. So, it's important to develop a method for the simultaneous determination of cPOA and tPOA in biological samples to explore the biological activity, drug delivery systems or regenerative medicine. In the present paper, a quantification method using UPLC–MS/MS to determine cPOA and tPOA simultaneously in rat serum was developed. It was proved to be selective and sensitive, with a wide range of detection and low limit of detection, which could be used to ascertain the pharmacokinetics of cPOA and tPOA.

**Materials and methods**

**Materials and animals.** cPOA and tPOA were purchased from NU-CHEK (> 99%, Elysian, USA). HPLC-grade formic acid was obtained from Roe Scientific Inc. (Powell, OH, USA). HPLC-grade acetonitrile, methanol and MTBE (methyl tert-butyl ether) were obtained from Merck KGaA (Darmstadt, Germany). Ultrapure water was from a Millipore Milli-Q system (Millipore Corp., Billerica, MA, USA). The other solvents or reagents were commercially available and reagent grade.

The use of rat approved by the Experimental Animal Ethics Committee of Nanjing University of Traditional Chinese Medicine in accordance with NIH guidelines. Ethical approval number: 201811A027. The authors complied with the ARRIVE guidelines. The 18 male Sprague-Dawley rats weighing 280 ± 20 g were maintained with 12 h:12 h light/dark cycles in a temperature-controlled room throughout the study. Standard rat chow and tap water were supplied ad libitum. Rat serum was collected from vein of rat after narcotized by intraperitoneal injection of pentobarbital sodium (Produlab Pharma B.V., 40 mg/kg).

**Chromatographic and mass spectrometric conditions.** Liquid chromatography conditions. Analyte separations were performed on an Agilent UPLC-1290 system (Agilent Corp., Milford, MA, USA) using a BDS C18 column (2.1 × 100 mm, 2.1 µm, Thermo Fisher Scientific, USA). The mobile phase was included water (A) and acetonitrile (B) (A:B = 20:80, v/v) at a flow rate of 0.3 mL/min, and the injection volume was 1 µL.

Mass spectrometric conditions. Identification of cPOA and tPOA in serum samples was conducted using an AB 5500 Q-trap UPLC–MS/MS (AB Sciex, Framingham, MA, USA) equipped with electro spray ionization (ESI). Quantitative analysis of cPOA and tPOA were also performed by UPLC–MS/MS. Detection was performed in negative ion mode under the following conditions: curtain gas at 35.0 L/h, and ion source gases at 50 L/h. AB Analyst 1.6.0 software (AB Sciex, Framingham, MA, USA) was used for system control and data acquisition. ESI-MS/MS parameters are shown in Table 1. Detection was performed in the negative ion mode and conditions for cPOA and tPOA detection optimized using standards. Product ions obtained from deprotonated molecular ions of cPOA and tPOA included three main ions from each compound at m/z 235.2, 126.9, 111.0 and at m/z 234.7, 127, and 111.1, respectively.

**Stock solutions and working solutions.** Individual standard stock solutions of cPOA and tPOA (2.50 mg/mL, 2.24 mg/mL respectively) were prepared in acetonitrile. These stock solutions were serially diluted with acetonitrile to provide standard working solutions in the concentration range of 0.175–42.0 μg/mL for cPOA and tPOA. All solutions were stored at −20 °C and brought to room temperature before use.

| Analyte | Precursor ion (m/z) | Daughter ion (m/z) | Dwell time (s) | DP (V) | EP (V) | CE (V) | CXP (V) |
|---------|---------------------|-------------------|----------------|--------|--------|--------|--------|
| cPOA   | 253.2               | 235.2             | 20             | −100   | −8.2   | −25.8  | −11.0  |
|         | 253.2               | 126.9             | 20             | −100   | −8.2   | −31.0  | −11.0  |
|         | 253.2               | 111.0             | 20             | −100   | −8.2   | −29.0  | −11.0  |
| tPOA   | 253.2               | 234.7             | 20             | −100   | −7.3   | −28.0  | −8.50  |
|         | 253.2               | 127.0             | 20             | −100   | −7.3   | −26.0  | −12.0  |
|         | 253.2               | 111.1             | 20             | −100   | −7.3   | −26.0  | −12.0  |
Calibration standard curves and QC samples. Calibration standard (CS) curves were prepared by spiking 20 μL of the appropriate analyst working solution into 50 μL of blank rat serum. The effective concentrations were 0.1, 0.5, 2.5, 5, 10, 12 μg/mL for cPOA and tPOA. QC samples were prepared as compound samples for each concentration at 0.5 μg/mL for cPOA and tPOA, and stored at –20 °C until use. Rat serum samples, serving as QCs, were processed the following sample procedure as for unknown samples.

Serum sample preparation. 50 μL of serum sample (blank, or pharmacokinetics serum sample) in a 2.0 mL centrifuge tube, 60 μL of aqueous solution with formic acid in 5%, 100 μL methanol, and 1250 μL MTBE were added and mixed by vortexing for 3 min. After centrifugation at 18,000×g for 10 min, the clear supernatant of 1 mL was extracted to a new centrifuge tube, blown by flowing nitrogen, and redissolved by 200 μL acetonitrile solution. Centrifugation at 18,000×g for 10 min again, supernatant was injected into the UPLC–MS/MS system.

Method validation. Assay validation performed was based on the currently accepted FDA prescription and per guidelines of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use18. Each blank serum sample was processed through the extraction procedure and tested to ensure no rat serum interference with the analyte. While the serum sample preparation was 60 μL of aqueous solution with formic acid in 5%, 100 μL methanol, and 1250 μL MTBE were added and mixed by vortexing for 3 min. After centrifugation at 18,000×g for 10 min, the clear supernatant of 1 mL was extracted to a new centrifuge tube, blown by flowing nitrogen, and redissolved by 200 μL acetonitrile solution.

The determination of the extraction recoveries of cPOA and tPOA was at three QC concentrations. And the calculation of the recoveries was by comparing analyte peak area ratios for each analyte in serum samples with those of analytes in the serum matrices by extracting analyte-free serum samples which were prior to chromatography. In extracted rat serum, matrix effects from endogenous substances were present, which might have caused ion signal suppression or enhancement. Matrix effects at three QC concentrations (0.5, 2.5, and 10 ng/mL) were measured by comparing peak responses of samples post-extraction (A) with that of pure standard solution which contained equivalent amounts of the two compounds (B). The ratio (A/B × 100%) was used to evaluate the matrix effect and the extraction recovery and matrix effect of cPOA and tPOA were evaluated simultaneously by the same method.

During sample storage and processing procedures, the stability of cPOA and tPOA in rat serum was assessed by analyzing replicates (n = 6) of three QC concentrations. The freeze–thaw stability was determined through three freeze–thaw cycles. All stability testing of QC samples were determined according to calibration curves of freshly prepared standards.

Pharmacokinetic studies of cPOA and tPOA. Male rats (Sprague-Dawley, 280 ± 20 g) were obtained from the Laboratory Animal Center of Nanjing University of Chinese Medicine (Nanjing, China). Animal handling procedures followed standard operating procedure approved by the institutional animal care and use committee. All rats were dosed following overnight fasting except for water ad libitum. For pharmacokinetic studies, 18 male rats were randomly divided into three groups. In the first group, rats were given the same volume of solvent saline intragastrically. Blood samples were collected at the time points of 0, 10, 20, 30, 40, 60 min, and 2 h, 3 h, 6 h, 12 h, 24 h. Rats in the second group were administered i.g. of cPOA with 75 mg/kg body weight. Blood samples were collected at the time points of 0, 10, 20, 30, 40, 60 min, and 2 h, 3 h, 6 h, 12 h, 24 h after administration. In the third group, rats were administered intragastric gavage of tPOA with 75 mg/kg body weight. Blood samples were collected at the time points of 0, 10, 20, 30, 40, 60 min, and 2 h, 3 h, 6 h, 12 h, 24 h. Serum was separated and stored frozen at –80 °C until analysis. The following main pharmacokinetic parameters were analyzed using the non-compartmental pharmacokinetics data analysis soft-ware of PK solution 2 TM (Summit Research Service, Montrose, CO, USA): area under curve following main pharmacokinetic parameters were analyzed using the non-compartmental pharmacokinetics data analysis soft-ware of PK solution 2 TM (Summit Research Service, Montrose, CO, USA): area under curve time-to-maximum concentration (AUC 0–t, t = 24 h), maximum concentration (Cmax), time-to-maximum concentration (Tmax).

Results

Determination of cPOA and tPOA by UPLC–MS/MS. cPOA and tPOA were determined by ultra performance liquid chromatography ESI tandem mass spectrometry (UPLC–MS/MS). The total chromatography time was 8.0 min and retention time cPOA and tPOA were 3.86 min and 4.23 min, respectively (Fig. 2a–c). cPOA and tPOA were detected simultaneously in serum samples by intragastric administration after pretreatment. The results showed that the blank serum might contain trace amounts of cPOA and tPOA (Fig. 2d,e).

Method validation. Selectivity and specificity. Selectivity and specificity were investigated by comparing chromatograms of 6 different blank rat serum samples to those of corresponding spiked serum samples. No other endogenous substances were observed to interfere with cPOA and tPOA in any samples, but trace amounts of cPOA and tPOA were observed in blanks. Specificity was verified by comparing retention times of cPOA and tPOA (3.86 and 4.23 min, respectively) in quality control (QC) samples (n = 6). The relative standard deviations of selectivity and specificity were less than 5%.

Calibration curve linearity, limit of detection (LOD), and Lower limit of quantification (LLOQ). Standard curves of cPOA or tPOA were obtained by plotting the ratios of peak areas of associated Standard Solutions. The curves were observed with correlation coefficients greater than 0.999 exhibiting good linearity over concentration.
ranges of 0.1–12 μg/mL for cPOA and tPOA. Typical calibration equations were $y = 2.39e^{0.05x} (R = 0.9991)$ for cPOA, and $y = 9.06e^{0.05x} (R = 0.9999)$ for tPOA, respectively, where $y$ represented the peak area ratio of an ana-

Figure 2. MRM chromatograms of cPOA and tPOA. (a) total ion chromatogram spiked with cPOA standard sample, (b) total ion chromatogram spiked with tPOA standard sample, (c) total ion chromatogram spiked with cPOA and tPOA standard sample, (d) blank rat serum sample, (e) rat serum sample after cPOA intragastric gavage.
lyte, and x represented an analyte concentration. The limit of detection (LOD) was estimated to be 30 ng/mL. The lower limits of quantification (LLOQ) for both cPOA and tPOA were defined as 0.1 μg/mL.

**Accuracy and precision.** Accuracy and precision of the present method were summarized in Table 2. Accuracy was required to be within ±15% (20% for LLOQ) and precision not to exceed ±15% (20% for LLOQ), according to the criteria for biological sample analysis suggested by U.S. Food and Drug Administration (FDA) guidelines. Our results showed that the method was accurate and precise for simultaneous analysis of cPOA and tPOA in rat serum samples.

**Recovery and matrix effects.** The recoveries of cPOA and tPOA spiked into rat serum were determined at three QC levels. The recoveries of cPOA were 101.43 ± 1.37, 102.11 ± 1.25, and 101.92 ± 1.84 (n = 6) at concentrations of 0.5, 2.5 and 10 μg/mL, and those of tPOA were 98.28 ± 1.23, 100.66 ± 1.82, and 99.75 ± 3.01 (n = 6), respectively (Table 3). Matrix effects in the present study were investigated by a post-extraction spike method. The peak area of the standard analyte spiked into blank serum was compared with the corresponding peak area obtained were compared with that of injecting the standard analyte in the mobile phase at concentrations of 0.5, 2.5 and 10 μg/mL, all performed in triplicate. All the ratios of matrix effects defined above were within acceptable limits (89.51–94.72%) (Table 3). No significant matrix effect for cPOA and tPOA were observed, showing that ion suppression or enhancement from serum components was negligible for this method.

**Stability.** Results of stability tests indicated that the analytes were stable in laboratory conditions (Table 4). The stability of target compounds was evaluated as described in the experimental section.

**Pharmacokinetic analyses of cPOA and tPOA.** The mean concentrations of cPOA in blank group fluctuated around 5 μg/mL (4–8 μg/mL) in 24 h, while, those of tPOA fluctuated around 2 μg/mL (0–4 μg/mL) in the beginning 2 h, and remained low after 2 h (Fig. 3), suggesting original cPOA may exist in organism. The above observations also showed that the concentrations of cPOA and tPOA fluctuated in a similar trend in certain time.

The pharmacokinetic profiles of cPOA and tPOA were investigated by the treatment at 75 mg/kg i.g. dose of cPOA to rats (Fig. 4a). The mean concentration–time profiles of cPOA and tPOA in serum showed that cPOA was absorbed rapidly by rats and increased in serum in 0.3 h after administration and decreased slowly thereafter (Fig. 4a). The Tmax of cPOA was 0.94 ± 0.44 h and the Cmax 8.17 ± 1.97 μg/L (Table 5). The concentration of cPOA decreased to the blank level rapidly. Meanwhile, tPOA was detected simultaneously after cPOA dosing whose concentration–time profile fluctuated (4–7 μg/L) showed the same trend with cPOA. And, the concentration of tPOA in serum was retain 4–6 μg/L. The pharmacokinetic profiles of cPOA and tPOA were investigated following a single i.g. dose at 75 mg/kg body weight of tPOA to rats (Fig. 4b). The mean serum concentration–time profiles of cPOA and tPOA

| Analyte | Concentration (μg/mL) | Mean ± SD (μg/mL) | Accuracy (%) | Precision (%) |
|---------|-----------------------|-------------------|--------------|---------------|
| cPOA    | 0.50                  | 0.56 ± 0.15       | 111.0        | 0.98          |
|         | 2.50                  | 2.76 ± 0.86       | 110.4        | 1.45          |
|         | 10.0                  | 8.54 ± 1.79       | 85.40        | 2.01          |
| tPOA    | 0.50                  | 0.54 ± 0.16       | 109.0        | 1.48          |
|         | 2.50                  | 2.64 ± 0.65       | 105.6        | 2.13          |
|         | 10.0                  | 8.15 ± 0.98       | 81.50        | 4.07          |

Table 2. Accuracy and precision for determination of cPOA and tPOA in serum samples (n = 6).

| Analyte concentration (μg/mL) | Matrix effects | Recovery |
|------------------------------|----------------|---------|
|                              | Mean ± SD (μg/mL) | RSD (%) | Mean ± SD (μg/mL) | RSD (%) |
| cPOA                         |                 |         |                 |         |
| 0.50                         | 94.12 ± 1.91    | 2.96    | 101.43 ± 1.37   | 1.39    |
| 2.50                         | 92.64 ± 1.67    | 1.98    | 102.11 ± 1.25   | 1.31    |
| 10.0                         | 91.45 ± 2.43    | 2.56    | 101.92 ± 1.84   | 2.09    |
| tPOA                         |                 |         |                 |         |
| 0.50                         | 94.72 ± 1.05    | 1.27    | 98.28 ± 1.23    | 2.11    |
| 2.50                         | 93.93 ± 2.47    | 2.69    | 100.66 ± 1.82   | 1.99    |
| 10.0                         | 89.51 ± 3.05    | 3.55    | 99.75 ± 3.01    | 3.58    |

Table 3. Recovery and matrix effects of cPOA and tPOA in serum samples (n = 6). RSD relative standard deviation.
Table 4. Stability of cPOA and tPOA (n = 6).

| Storage condition (−80 °C) | cPOA | tPOA |
|---------------------------|------|------|
|                           | Concentration (μg/mL) | Accuracy (%) | RSD (%) | Concentration (μg/mL) | Accuracy (%) | RSD (%) |
| 1 Freeze–thaw cycle       | 0.50  | 111.0 | 1.27    | 0.50    | 109.0   | 1.27    |
|                           | 2.50  | 110.4 | 1.53    | 2.50    | 105.6   | 1.53    |
|                           | 10.0  | 85.4  | 2.42    | 10.0    | 81.50   | 2.42    |
| 2 Freeze–thaw cycle       | 0.50  | 105.0 | 3.02    | 0.50    | 99.20   | 3.02    |
|                           | 2.50  | 107.6 | 2.98    | 2.50    | 101.4   | 2.98    |
|                           | 10.0  | 86.6  | 2.54    | 10.0    | 83.70   | 2.54    |
| 3 Freeze–thaw cycle       | 0.50  | 96.4  | 2.84    | 0.50    | 103.2   | 2.12    |
|                           | 2.50  | 99.2  | 3.07    | 2.50    | 91.70   | 2.97    |
|                           | 10.0  | 82.7  | 3.87    | 10.0    | 85.90   | 3.26    |

Figure 3. Mean serum concentration–time profiles of cPOA and tPOA in blank rat serum in 24 h (n = 6, mean ± SD). — shows mean serum concentration–time profiles of cPOA, ---- shows mean serum concentration–time profiles of tPOA.

Figure 4. Mean serum concentration–time profiles of cPOA and tPOA (n = 6). (a) Showed cPOA and tPOA concentrations after i.g. administration of cPOA in rats at 75 mg/kg body weight, (b) showed cPOA and tPOA concentrations after i.g. administration of tPOA in rats at 75 mg/kg body weight.
showed that tPOA was absorbed slowly by rats such that it increased in serum in 2 h after administration and decreased rapidly thereafter (Fig. 4b). The Tmax of tPOA was 1.50 ± 0.98 h and the Cmax 14.77  ± 11.91 μg/L. tPOA was decreased to the concentration of original level rapidly. Meanwhile, cPOA was detected after tPOA dosing simultaneously, mean serum concentration–time profiles of cPOA fluctuated (5–15 μg/L) in the same trend with tPOA. And, the concentration of cPOA in serum was maintain 10 μg/L, which was more than original concentration of cPOA.

As mentioned above, pharmacokinetic analysis was made on cPOA and tPOA, both of which were with pre-existing endogenous levels (Fig. 3). Therefore, a correction based on the pre-existing baseline were necessary. Since the endogenous concentrations of cPOA and tPOA were variable, when discussing the total concentration of the two isomers, the contents of the inter- and intra-individual compounds were added some variabilities. So, a pre-dose adjustment of measured levels was conducted. For each rat at any test period, the pre-dose concentrations were subtracted from the measured serum concentrations, and the pharmacokinetic parameters was calculated on the baseline-adjusted concentrations according to regulatory guidelines for bioequivalence testing of endogenous substances21,22. If the measured value was lower than the pre-dose concentration, the adjusted one was set to zero (Fig. 5a,b).

Pharmacokinetic parameters including Cmax, Tmax and AUC 0–24 h were determined using non-compartmental analysis according to the concentrations of cPOA and tPOA in the serum (Table 5). The area under the serum concentration–time curve (AUC) was calculated by applying the log-linear trapezoidal model to the measured concentrations of cPOA, tPOA and the combined sum of them at the actual sampling time points. Because of the fluctuated pre-existing endogenous levels, it was not possible to define a terminal elimination phase. The elimination rate constant was therefore not calculated, and exposure was described as the AUC from time 0 to 24 h post-dosing (AUC0–24h). AUC0–24h of cPOA and tPOA were 59.45 ± 29.83 and 113.88 ± 72.25 mg/L·h.

Table 5. Pharmacokinetic parameters of baseline-corrected cPOA and tPOA in rats after i.g. administration of cPOA and tPOA at 75 mg/kg body weight.

| Parameters | Unit | Mean ± SD  |
|------------|------|------------|
| Cmax | mg/L | 8.17 ± 1.97 | 14.77 ± 11.91 |
| Tmax | h | 0.94 ± 0.44 | 1.50 ± 0.98 |
| AUC (0–24 h) | mg/L·h | 59.45 ± 29.83 | 113.88 ± 72.25 |

Figure 5. Baseline-corrected serum concentration–time profiles of cPOA and tPOA (n = 6). (a) Showed cPOA and tPOA concentrations after i.g. administration of cPOA in rats at 75 mg/kg body weight, (b) showed cPOA and tPOA concentrations after i.g. administration of tPOA in rats at 75 mg/kg body weight.
Discussion

cPOA in organisms, especially in adipose tissue, may be related to the development of cardiovascular disease and tPOA has been used as a biomarker for reducing the risks of type 2 diabetes and coronary heart disease\textsuperscript{10–12}. The correlation between cPOA to tPOA may play a decisive role in related biological activities in the body.

We established a UPLC–MS/MS method with highly precision to detect trace amounts of cPOA and tPOA in animal serum, which did not require sample esterification pretreatment. Meanwhile, it was successfully used in the pharmacokinetic research, finding that the change of exogenous cPOA in animal serum, which did not require sample esterification pretreatment. Meanwhile, it was successfully used in the pharmacokinetic research, finding that the change of exogenous cPOA concentration in serum was also correspondent with the fluctuation of exogenous tPOA concentration.

Huang et al. studied the effect of oral seabuckthorn fruit oil (main ingredient cPOA, without tPOA) on the content changes of tPOA and iPOA phosphatidate fatty acids in fasting serum of healthy adults, and found that the content of iPOA increased with high oral dose of cPOA (1520 mg/day) for 3 consecutive weeks\textsuperscript{22}. By simulating the differentiation of adipocytes in vitro, it was found that the increasing content of iPOA would increase the contents of VA (18:1, t11) and conjugated linoleic acid (CLA, 18:2, c9, t11) in adipocytes\textsuperscript{25}. tPOA in the body was mainly from food intake and endogenous oxidation reaction. Isoleic acid (VA, 18:1, t11) in food could be converted into iPOA (16:1, t9) by shortening the chain length, with a conversion rate of 17%\textsuperscript{26}. As tPOA in daily diet was with relatively low amount and easy to be oxidized, that in serum was mainly derived from endogenous transformation of VA from dietary\textsuperscript{25}. Therefore, we speculated that cPOA and tPOA in organism may interconvert each other. Of course, this inference based on a comparative observation needed to be confirmed by further studies.

The pretreatment process of GC method was cumbersome and had a long total chromatography time and retention time. Establishing rapid, accurate and simultaneous detection of cPOA and iPOA in biological samples would benefit laboratorial and clinical research and real-time detecting cPOA and tPOA in organisms. Our method could be applied to clinical investigations to elucidate the biological activity and structure–activity relationship (SAP) of cPOA and tPOA in humans and favor the development of biological activity, drug delivery systems or regenerative medicine to define the metabolism and transformation between fatty acids. Besides, previous studies have shown that tPOA was better than cPOA in regulating lipid metabolism in hyperlipidemia mice\textsuperscript{26}. Therefore, whether cPOA played a role in regulating lipid metabolism by transforming into tPOA, and the bioconversion relationship between cPOA and tPOA and the conversion rate could be further studied by isotope labeling or fluorescence labeling method.

Conclusions

A sensitive and rapid UPLC–MS/MS method for determination of cPOA and tPOA simultaneously was established and used to identify them in rat serum after i.g. administration. Trace of isomers cPOA and tPOA could be quickly isolated and quantified in 5 min using this method. And it was further used in a pharmacokinetic study of cPOA and tPOA in rats. The results suggested that the cPOA concentration in serum was correspondent with the fluctuation of iPOA concentration indicating cPOA and tPOA in organism may interconvert each other.

Data availability

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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**Author contributions**
W.H., Y.Z. and L.Z. carried out the experiment and prepared the manuscript. Y.Z., L.Z., C.S. wrote the main manuscript text. Z.Z. edited the English language. All authors reviewed the manuscript.

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**Competing interests**
The authors declare no competing interests.

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