

Simultaneous Determination of Cis- and Trans-Palmitoleic acid in Rat Serum by LC-ESI-MS/MS

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Research

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Abstract

Background: *Cis*-palmitoleic acid (*c*POA) and *trans*-palmitoleic acid (*t*POA) are isomers of palmitoleic acid, which are monounsaturated fatty acids, affecting glucose and lipid metabolism, and reducing insulin resistance. *t*POA was better than *c*POA in regulating lipid metabolism in hyperlipidemia mice, but the metabolic transformation and structure-activity relationship haven't been reported.

Method: A precise and accurate liquid chromatography-tandem mass spectroscopy (LC-MS/MS) method was developed to determine *c*POA and *t*POA simultaneously. *c*POA and *t*POA were administered i.g. (intragastric gavage) to rats at 75 mg/kg respectively, serum samples were analyzed by LC-ESI-MS/MS on a reverse-phase BDS C18 column equilibrated and eluted with acetonitrile (A) and water (B) (A:B = 80:20, v/v) at a flow rate of 0.3 mL/min and the injection volume was 1 μ L.

Results: The calibration curves for *c*POA and *t*POA were linear over the range 0.1~12 μ g/mL. Analytes were monitored by selected-reaction monitoring in negative electrospray ionization mode. The T_{max} of *c*POA was 0.94 ± 0.44 h and the C_{max} 8.17 ± 1.97 μ g/L, and the T_{max} of *t*POA was 1.50 ± 0.98 h and the C_{max} 14.77 ± 11.91 μ g/L. AUC_{0-24h} of *c*POA and *t*POA were 59.45 ± 29.83 and 113.88 ± 72.25 mg/L*h.

Conclusions: The method was applied in pharmacokinetic study of *c*POA and *t*POA successfully. Besides, it's found the concentration of *c*POA and *t*POA fluctuated in serum of rats with the consistent trend, which may be reciprocal bio-convert in biological activity in the body.

Highlights

1. An LC-MS/MS method was developed to determine *c*POA and *t*POA simultaneously.
2. The method was applied in pharmacokinetic analysis of *c*POA and *t*POA in serum.
3. It showed that *c*POA and *t*POA might be reciprocal bio-convert in the body.

Background

Palmitoleic acid (C16:1, n-7, POA) is a natural omega-7 monounsaturated fatty acid, abundant in plant and fish oil [1]. *Cis*-POA (*c*POA) and *trans*-POA (*t*POA) are isomers with different space structures. *c*POA is common in natural POA, which has been widely reported. It has been demonstrated that *c*POA may influence favorably glucose and lipid metabolism through multiple mechanisms [2-3]. *c*POA affected the key enzymes of blood glucose metabolism, regulated insulin secretion and reduced insulin resistance [4-5]. Orally administered *c*POA induced satiety, enhanced the release of satiety hormones and decreased food intake in mice [6], in addition, *c*POA reduced body weight gain, ameliorated the development of hyperglycemia and hypertriglyceridemia, and improved insulin sensitivity [7]. However, *trans*-fatty acid is the subject of an ongoing discussion on both suggested positive and negative associations with metabolic and cardiovascular risk factors [8]. While higher *t*POA proportion in plasma phospholipids improved insulin sensitivity or decreased onset of type 2 diabetes disease [9]. *t*POA regulated glycolipid

metabolism, closely related to low density lipoprotein [10–12], which has been used as a biomarker for reducing the risk of type 2 diabetes and coronary heart disease. *c*POA was determined in the subcutaneous fat of human body [13–14], but there was no method was reported to determine *t*POA. Also, the mechanism of the difference in the physiological activity and bio-metabolism of *c*POA and *t*POA is not clear. It is important to develop a method for the quantitative analyses of *c*POA and *t*POA in plasma or serum samples for future pharmacology studies. In the present study, a simple UPLC-MS/MS method for simultaneous determination of *c*POA and *t*POA from rat serum without methyl esterification treatment was developed. This method proved to be selective and sensitive, with a wide range of detection and low limit of detection, used to ascertain the pharmacokinetics of *c*POA and *t*POA.

Materials And Methods

Materials and reagents

*c*POA and *t*POA 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). All other solvents or reagents were commercially available and reagent grade. Blank rat serum was collected from healthy male Sprague-Dawley rats weighing 280 ± 20 g (Laboratory Animal Center of Nanjing University of Chinese Medicine, Nanjing, China).

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 μ m, Thermo Fisher Scientific, USA). The mobile phase was composed of acetonitrile (A) and water (B) (A: B = 80:20, v/v) at a flow rate of 0.3 mL/min and the injection volume was 1 μ L [15].

Mass spectrometric conditions

Identification of *c*POA and *t*POA in serum samples was conducted using an AB 5500 Q-trap LC-MS/MS (ABSCIEX, Framingham, MA, USA) equipped with electro spray ionization (ESI). Quantitative analysis of *c*POA and *t*POA 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, collision gas medium, ion spray voltage at 4500 V, 550 °C, and ion source gases at 50 L/h. AB Analyst 1.6.0 software (ABSCIEX, 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 *c*POA and *t*POA detection optimized using standards. Daughter ions obtained from deprotonated molecular ions of *c*POA and *t*POA

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.

Table 1
Electrospray ionization ESI-MS/MS parameters for *c*POA and *t*POA.

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	Dwell Time (s)	DP(V)	EP(V)	CE(V)	CXP(V)
<i>c</i> POA	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
<i>t</i> POA	253.2	234.7	20	-100	-7.3	-28.0	-8.5
	253.2	127.0	20	-100	-7.3	-26.0	-12.0
	253.2	111.1	20	-100	-7.3	-26.0	-12.0

Stock solutions and working solutions

Individual standard stock solutions of *c*POA and *t*POA (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 *c*POA and *t*POA. All solutions were stored at -20°C and brought to room temperature before use.

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 0.05, 0.1, 0.5, 2.5, 5, 10, 12 µg/mL for *c*POA and *t*POA. QC samples were prepared as compound samples for each concentration at 0.5 µg/mL for *c*POA and *t*POA, 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, upper fluid of that 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 Use [16]. 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 μ L of aqueous solution with formic acid in 5%, 100 μ L methanol, and 1250 μ L MTBE were added and mixed by vortexing for 3 min. After centrifugation at 18,000 \times g for 10 min, the clear supernatant of 1 mL was extracted to a new centrifuge tube, blowed by flowing nitrogen, and redissolved by 200 μ L 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 presented, 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 $(A/B \times 100\%)$ 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 (ICR, 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 administered i.g. (intragastric gavage) of normal saline 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. Rats in the second group were administered i.g. of cPOA with 75 mg/kg body weight. Serial blood samples were collected in tubes via the orbital venous plexus before and at time points of 0, 10, 20, 30, 40, 60 min, 2 h, 3 h, 6 h, 12 h, 24 h, after administration. In the third group, rats were administered i.g. 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 software of PK solution 2TM (Summit Research Service, Montrose, CO, USA): area under curve from zero to the last measurable serum concentration point (AUC_{0-t} , $t = 24\text{h}$), maximum concentration (C_{max}), time-to-maximum concentration (T_{max}).

Results

Determination of cPOA and tPOA by LC-ESI-MS/MS

cPOA and tPOA were determined by liquid chromatography ESI tandem mass spectrometry (LC-ESI-MS/MS). The peak area ratio of the two coupled transitions for each analyte was matched to a standard. 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, B, C). cPOA and tPOA were detected in samples intragastric administration respectively. Both cPOA and tPOA were detected simultaneously in samples after pretreatment. The results showed that the blank serum might contain trace amounts of cPOA and tPOA (Fig. 2D).

Method Validation

Selectivity and Specificity

Selectivity and specificity were assessed by comparing chromatograms of six different batches of blank rat serum samples to corresponding spiked serum samples. No other endogenous substances were observed to interfere with cPOA and tPOA in any samples, but there was certain amount of cPOA and tPOA in blank serum. 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), which showed differences of less than 5%.

Calibration Curve Linearity, Lower Limit of Quantification (LLOQ), and Limit of Detection (LOD)

Standard curves were established by plotting the ratios of chromatogram peak areas of cPOA or tPOA. The curves showed correlation coefficients greater than 0.999 and exhibited good linearity over concentration ranges of 0.1 ~ 12 µg/mL for cPOA and tPOA. Typical calibration equations were $y = 2.39e^{+005x}$ (R = 0.9991) for cPOA, and $y = 9.06e^{+004x}$ (R = 0.9999) for tPOA, where y represented the peak area ratio of an analyte, and x represented an analyte concentration. The lower limits of quantification (LLOQ) for both cPOA and tPOA were defined as 0.1 µg/mL. The limit of detection (LOD) was estimated to be 30 ng/mL, respectively.

Accuracy and precision

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

Table 2
Accuracy and precision for determination of *c*POA and *t*POA in serum samples (n = 6).

Analyte	Concentration ($\mu\text{g/mL}$)	Mean \pm SD ($\mu\text{g/mL}$)	Precision (%)	Accuracy (%)
<i>c</i> POA	0.50	0.56 \pm 0.15	0.98	111.0
	2.50	2.76 \pm 0.86	1.45	110.4
	10.0	8.54 \pm 1.79	2.01	85.4
<i>t</i> POA	0.50	0.54 \pm 0.16	1.48	109.0
	2.50	2.64 \pm 0.65	2.13	105.6
	10.0	8.15 \pm 0.98	4.07	81.5

Recovery and Matrix Effects

The recoveries of *c*POA and *t*POA spiked into rat serum were determined at three QC concentrations. The recoveries of *c*POA were 101.43 \pm 1.37, 102.11 \pm 1.25, and 101.92 \pm 1.84 (n = 6) at concentrations of 0.5, 2.5 and 10 $\mu\text{g/mL}$, and those of *t*POA were 98.28 \pm 1.23, 100.66 \pm 1.82, and 99.75 \pm 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 a standard analyte spiked into blank serum, minus that in the blank serum, were compared with the corresponding peak area obtained by directly injecting the standard analyte in the mobile phase at concentrations of 0.5, 2.5 and 10 $\mu\text{g/mL}$ for *c*POA and *t*POA. In terms of matrix effects, all the ratios defined above were within acceptable limits (89.51% – 94.72%) (Table 3). No significant matrix effect for *c*POA and *t*POA were observed, indicating that ion suppression or enhancement from serum components was negligible for this method.

Table 3
Recovery and matrix effects of *c*POA and *t*POA in serum samples (n = 6).

Analyte	Concentration ($\mu\text{g/mL}$)	Matrix Effects		Recovery	
		Mean \pm SD ($\mu\text{g/mL}$)	RSD (%)	Mean \pm SD ($\mu\text{g/mL}$)	RSD (%)
<i>c</i> POA	0.50	94.12 \pm 1.91	2.96	101.43 \pm 1.37	1.39
	2.50	92.64 \pm 1.67	1.98	102.11 \pm 1.25	1.31
	10.0	91.45 \pm 2.43	2.56	101.92 \pm 1.84	2.09
<i>t</i> POA	0.50	94.72 \pm 1.05	1.27	98.28 \pm 1.23	2.11
	2.50	93.93 \pm 2.47	2.69	100.66 \pm 1.82	1.99
	10.0	89.51 \pm 3.05	3.55	99.75 \pm 3.01	3.58

RSD, relative standard deviation.

Stability

Results from all stability tests indicated that these analytes were stable under routine laboratory conditions (Table 4). The stability of *c*POA and *t*POA was evaluated as described in the experimental section. Therefore, the method was judged to be applicable for routine analyses.

Table 4
Stability of *c*POA and *t*POA (n = 6).

Storage Condition (-80°C)	<i>c</i> POA			<i>t</i> POA		
	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	81.5	2.42
2 freeze-thaw cycle	0.50	105.0	3.02	0.50	99.2	3.02
	2.50	107.6	2.98	2.50	101.4	2.98
	10.0	86.6	2.54	10.0	83.7	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	91.7	2.97
	10.0	82.7	3.87	10.0	85.9	3.26

Pharmacokinetic analyses of *c*POA and *t*POA

The mean concentrations of *c*POA in blank rat serum fluctuated around 5 µg/mL (4 ~ 8 µg/mL) in 24 hours, while, the mean concentrations of *t*POA fluctuated around 2 µg/mL (0 ~ 4 µg/mL) in 2 hours, and we can't quantify *t*POA because of the lower concentration after 2 hours (Fig. 3), it may relate to the concentration of *c*POA in organism. The result showed that *c*POA and *t*POA concentrations fluctuated in the same trend in certain time.

The pharmacokinetic profiles of *c*POA and *t*POA were investigated by the described method following a single i.g. dose at 75 mg/kg body weight of *c*POA to rats (Fig. 4A). The mean serum concentration–time profiles of *c*POA and *t*POA showed that *c*POA was absorbed rapidly by rats such that it increased in serum in 0.3 h after administration and decreased slowly thereafter (Fig. 4A). The T_{max} of *c*POA was 0.94 ± 0.44 h and the C_{max} 8.17 ± 1.97 µg/L (Table 5). The concentration of *c*POA was decreased to the level of blank serum rapidly. Meanwhile, *t*POA was detected simultaneously after *c*POA dosing, mean serum concentration–time profiles of *t*POA fluctuated (4 ~ 7 µg/L) in the same trend with *c*POA. And, the concentration of *t*POA in serum was retain 4 µg/L to 6 µg/L.

The pharmacokinetic profiles of *c*POA and *t*POA were investigated by the described method following a single i.g. dose at 75 mg/kg body weight of *t*POA to rats (Fig. 4B). The mean serum concentration–time profiles of *c*POA and *t*POA showed that *t*POA was absorbed slowly by rats such that it increased in serum in 2 h after administration and decreased rapidly thereafter (Fig. 4B). The T_{max} of *t*POA was 1.50 ± 0.98 h and the C_{max} 14.77 ± 11.91 $\mu\text{g/L}$. *t*POA was decreased to the concentration of original level rapidly. Meanwhile, *c*POA was detected after *t*POA dosing simultaneously, mean serum concentration–time profiles of *c*POA fluctuated ($5 \sim 15$ $\mu\text{g/L}$) in the same trend with *c*POA. And, the concentration of *c*POA in serum was maintain 10 $\mu\text{g/L}$, which was more than original concentration of *c*POA.

The pharmacokinetic analysis has been made on *c*POA and *t*POA, both with pre-existing endogenous levels (Fig. 3). Therefore, the data should be evaluated corrected for pre-existing baseline levels. The endogenous concentrations of *c*POA and *t*POA were variable, therefore, it contributed to the total concentration of *c*POA and *t*POA, and added to the inter- and intra-individual variability. Therefore, a pre-dose adjustment of measured levels was conducted. For each subject and 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 substances [17, 18]. If the measured value was lower than the pre-dose concentration the adjusted concentration was set to zero (Fig. 5).

The pharmacokinetic parameters were determined using non-compartmental analysis from the serum concentrations of *c*POA and *t*POA. The area under the serum concentration-time curve (AUC) was calculated by applying the log-linear trapezoidal model to the measured *c*POA and *t*POA concentrations and the combined sum of *c*POA and *t*POA 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 reported as the AUC from time 0 to 24 h post-dosing (AUC_{0-24h}). AUC_{0-24h} of *c*POA and *t*POA were 59.45 ± 29.83 and 113.88 ± 72.25 $\text{mg/L} \cdot \text{h}$. The pharmacokinetic parameters of *c*POA and *t*POA were calculated and summarized in Table 4.

Table 5
Pharmacokinetic parameters of baseline-corrected *c*POA and *t*POA in rats after i.g. administration of *c*POA and *t*POA respectively at 75 mg/kg body weight.

Parameters	Unit	Mean ± SD	
		<i>c</i> POA	<i>t</i> POA
C_{\max}	mg/L	8.17 ± 1.97	14.77 ± 11.91
T_{\max}	h	0.94 ± 0.44	1.50 ± 0.98
AUC(0-24h)	mg/L*h	59.45 ± 29.83	113.88 ± 72.25

Discussion

*c*POA in organisms, especially in adipose tissue, may be related to the development of cardiovascular disease, so *t*POA has been used as a biomarker for reducing the risk of type 2 diabetes and coronary heart disease [10–12], which may be due to the correlation of *c*POA to *t*POA, playing a decisive role in biological activity in the body.

*t*POA in the body was mainly obtained from food intake and endogenous oxidation reaction. Isooleic acid (18:1, *t*11) in food could be converted into *t*POA (16:1, *t*9) by shortening the chain length, with a conversion rate of 17% [9]. As the concentration of *t*POA in daily diet was relatively low and easy to be oxidized, *t*POA in serum was mainly derived from endogenous transformation of isooleic acid from dietary [19]. By simulating the differentiation of adipocytes in vitro, it was found that the increasing content of *c*POA would increase the content of isooleic acid (18:1, *t*11) and conjugated linoleic acid (18:2, *c*9, *t*11) in adipocytes [21]. Therefore, it could be inferred that exogenous *c*POA (16:1, *c*9) may generate isooleic acid (18:1, *t*11) by lengthening carbon chain and desaturation during metabolism in vivo, and further convert to *t*POA (16:1, *t*9) by shortening chain length (Fig. 5). Huang [21] selected healthy adults to study the effect of oral seabuckthorn fruit oil (main ingredient *c*POA, without *t*POA) on the content changes of *t*POA and *t*POA phosphatide fatty acids in fasting serum, and found that the content of *t*POA in fasting serum increased with high oral dose of *c*POA (1520 mg/d) for 3 consecutive weeks ($P = 0.0668$). In our pharmacokinetic study, it was found that the change of exogenous *c*POA concentration in serum after *c*POA administered i.g. would lead to the fluctuation of (endogenous) *t*POA concentration with the consistent trend, indicated the possibility of *c*POA being metabolized into *t*POA in vivo. Also, we found the change of exogenous *t*POA concentration in serum was correspondent with the fluctuation of (endogenous) *c*POA concentration. So, it's suggested that *c*POA and *t*POA in serum may interconvert of isomeric compound configurations. Previous studies have shown that *t*POA was better than *c*POA in regulating lipid metabolism in hyperlipidemia mice [22]. Therefore, whether *c*POA played a role in regulating lipid metabolism by transforming into *t*POA, and the bioconversion relationship between *c*POA and *t*POA and the conversion rate could be further studied by isotope labeling or fluorescence labeling method.

Conclusions

A rapid, reliable, and sensitive LC-MS/MS method for simultaneous determination of *c*POA and *t*POA, in rat serum was described for the first time. Both compounds were identified in serum after i.g. administration. This method was successfully applied to a pharmacokinetic study of *c*POA and *t*POA in rats. The method may be applied to clinical investigations to elucidate the biological activity and structure-activity relationship (SAR) of *c*POA and *t*POA in humans. Besides, it was suggested that *c*POA and *t*POA in serum may interconvert of isomeric compound configurations.

Abbreviations

POA

Palmitoleic acid, *c*POA: *Cis*-palmitoleic acid, *t*POA: *Trans*-palmitoleic acid, LC-MS/MS: liquid chromatography-tandem mass spectrometry.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable

Availability of data and materials

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

Competing interests

The authors declare no conflict of interest.

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Author contributions

Wenwen Huang, Yiping Zhang and Liping Zhong carried out the experiment and prepared the manuscript. Yiping Zhang, Liping Zhong, Chunlong Sun, Guangyu Yan, Ting Yang, Ruizao Yi, Hao Wu contributed with

valuable discussions and scientific input. Wenwen Huang, Hao Wu, Ting Yang, Rizao Yi (corresponding authors) conducted the experimental design. All authors helped preparing the manuscript and approved the final version.

Conflicts of Interest

The authors declare no conflict of interest.

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Figures

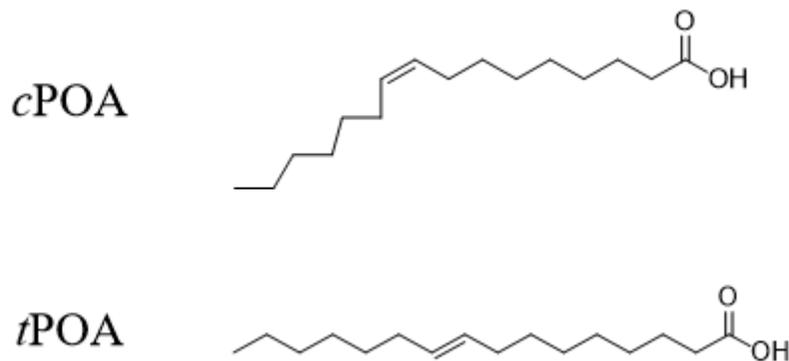


Figure 1

Structures of *c*POA and *t*POA

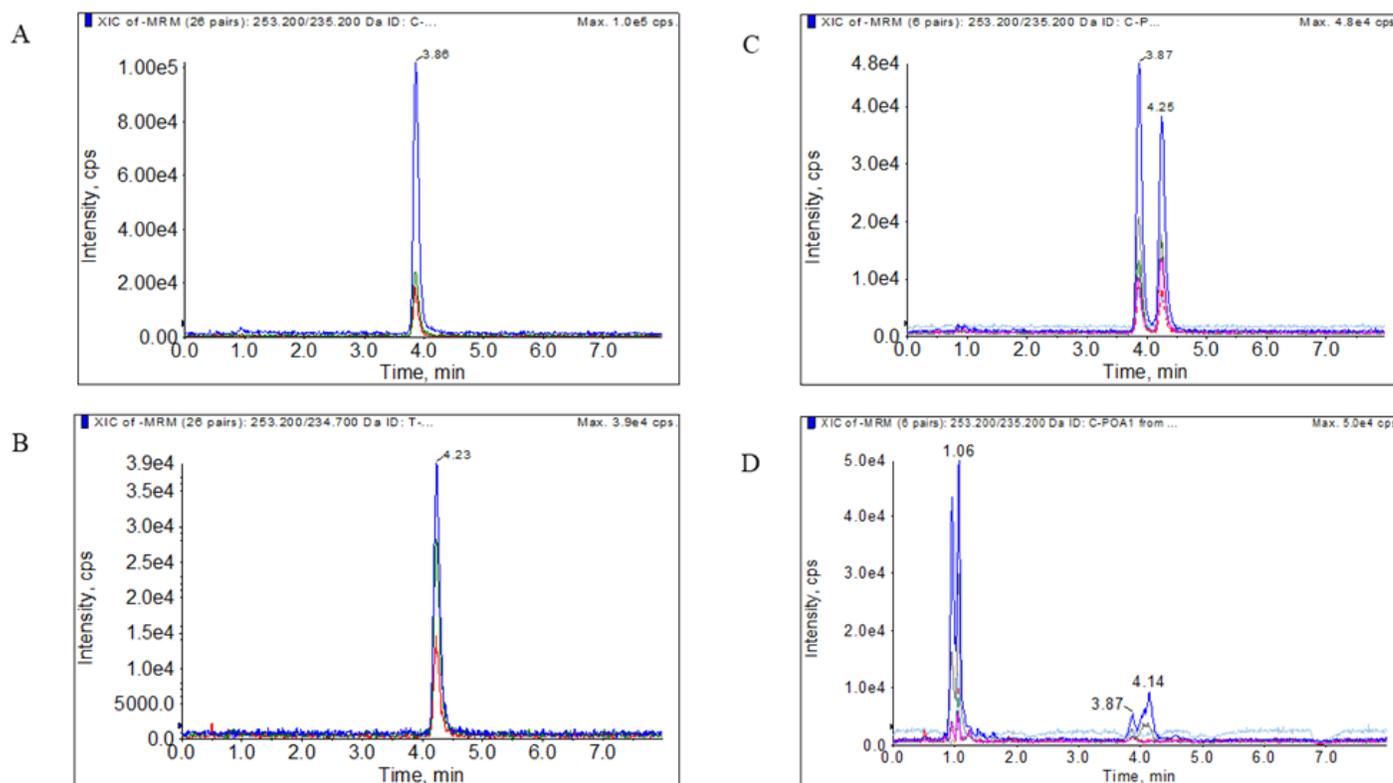


Figure 2

MRM chromatograms of *c*POA and *t*POA (A) total ion chromatogram spiked with *c*POA, (B) total ion chromatogram spiked with *t*POA, (C) total ion chromatogram spiked with *c*POA and *t*POA, (D) blank rat serum sample.

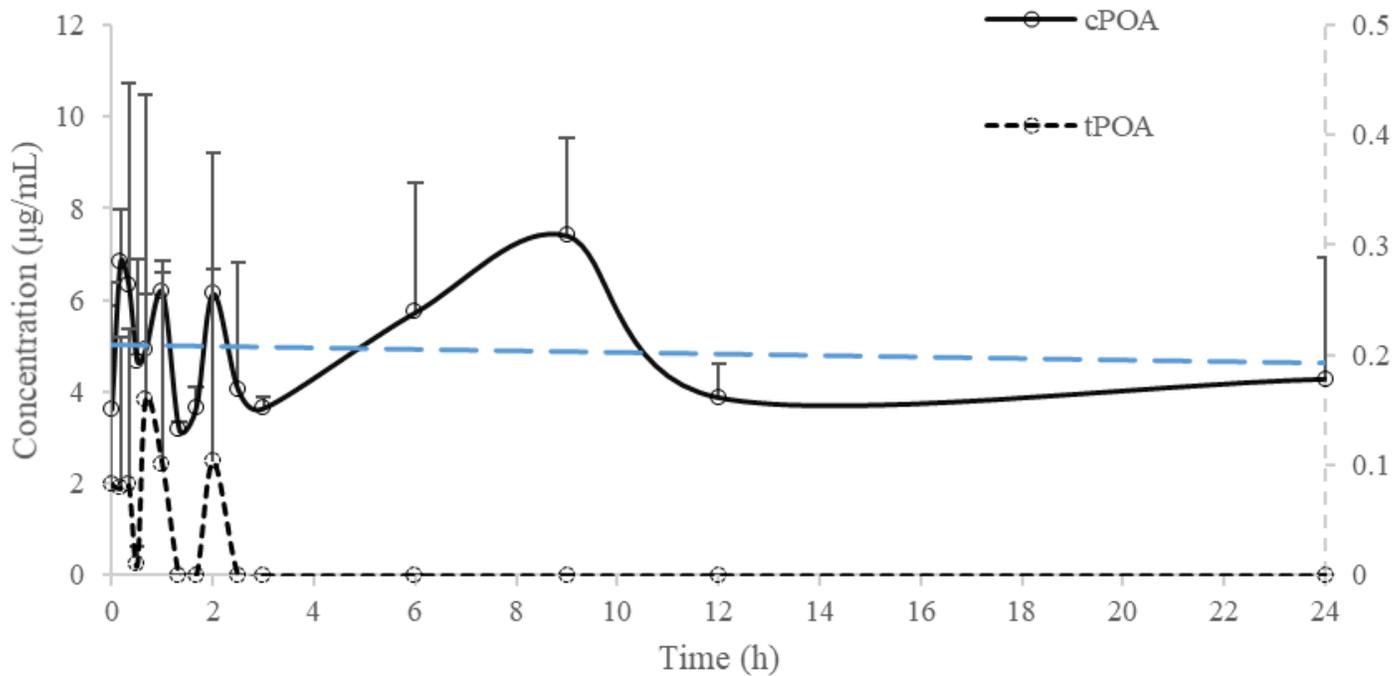


Figure 3

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

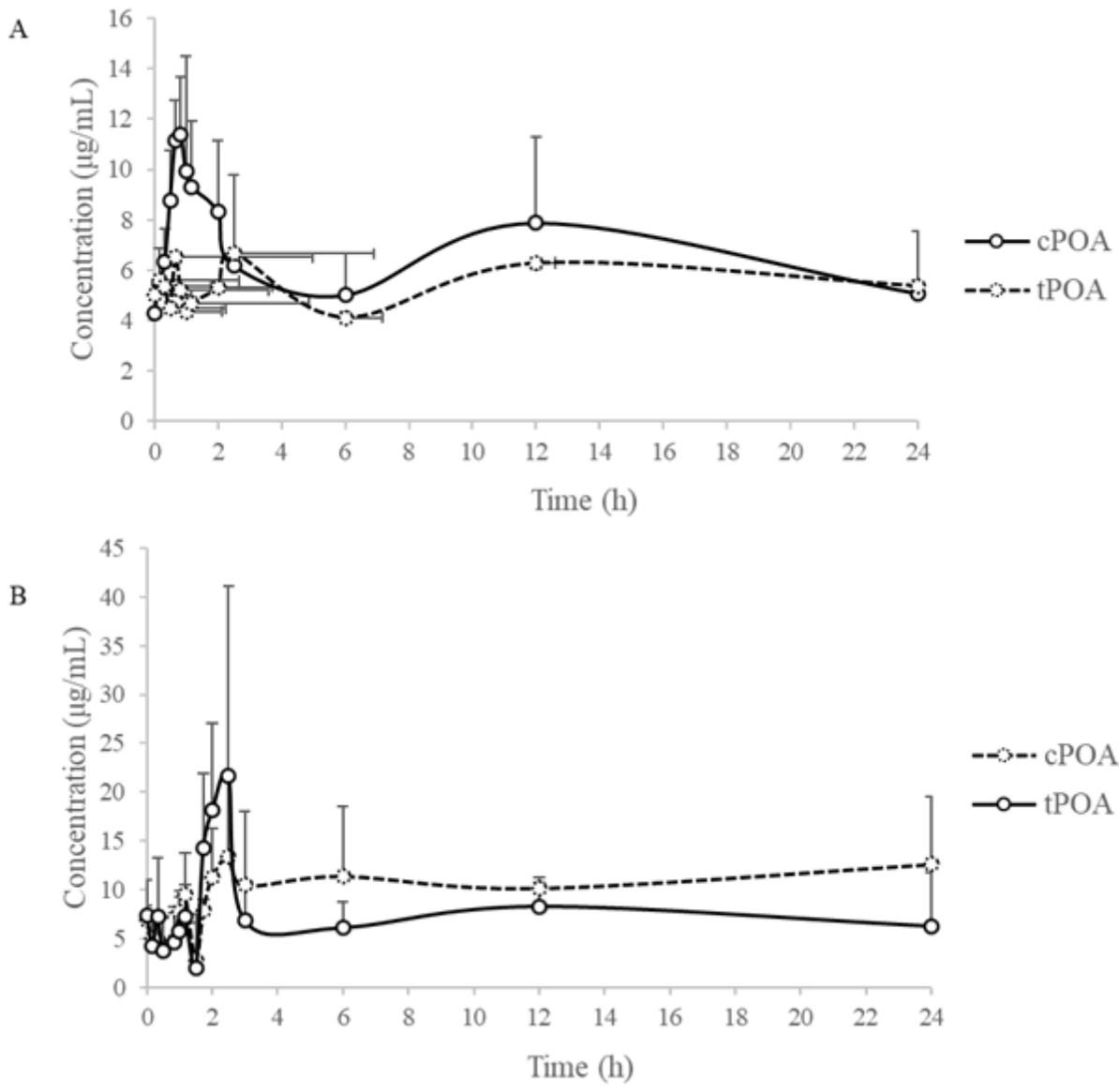


Figure 4

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.

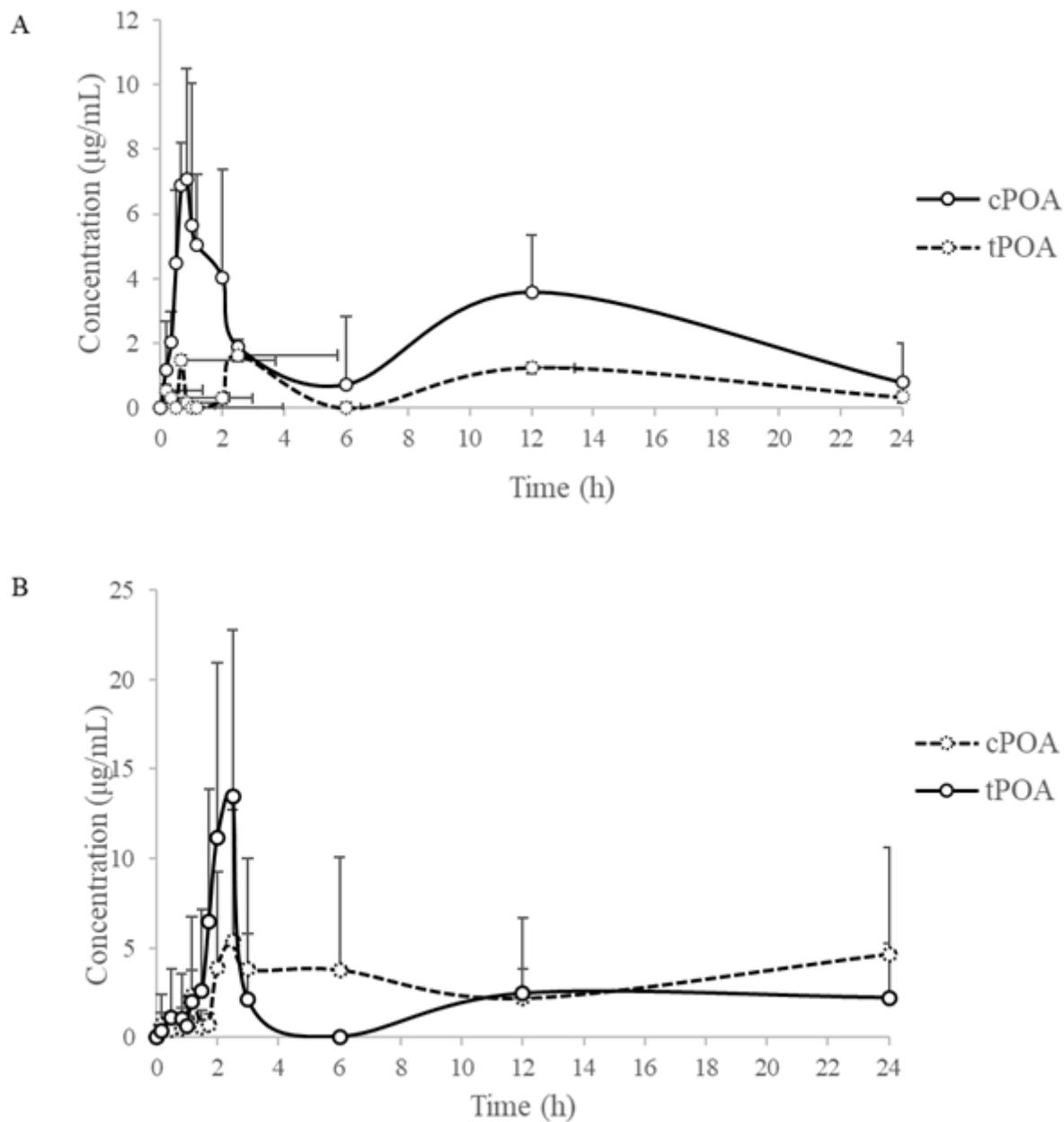


Figure 5

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.

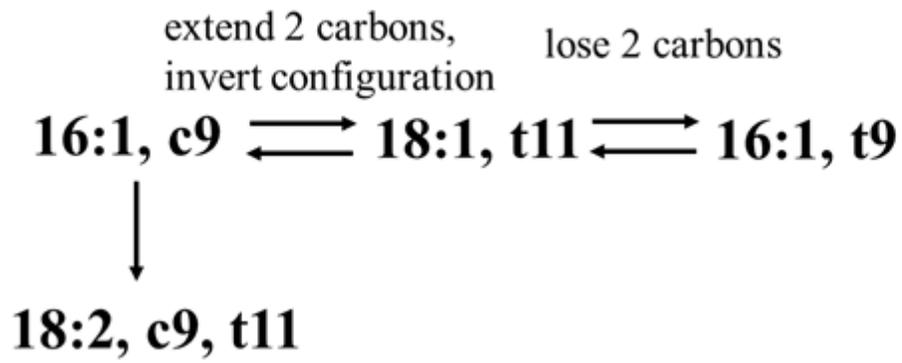


Figure 6

The possible convert mechanism between cPOA and tPOA