Simultaneous Determination of Cis- and Trans-palmitoleic Acid in Rat Serum by UPLC-MS/MS

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Research Article

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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 pharmacokinetic, metabolic transformation and structure-activity relationship have not been reported.

Method: A precise and accurate ultra performance liquid chromatography-tandem mass spectroscopy (UPLC–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, serum samples were analyzed 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.

Results: The calibration curves for *c*POA and *t*POA 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 *c*POA was 0.94±0.44 h and the Cmax 8.17±1.97 μg/L, and the Tmax of *t*POA was 1.50±0.98 h and the Cmax 14.77±11.91 μg/L. $AUC_{0\text{-}24h}$ 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 in rat serum 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 the body.

1. Introduction

Palmitoleic acid (POA, C16:1, n-7) is an omega-7 monounsaturated fatty acid in plant and fish oil [1]. C*is*-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 influent 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, ameliorated the development of hypertriglyceridemia and hyperglycemia, and improved insulin sensitivity [7]. However, *trans*-fatty acid is the subject of an ongoing discussion on both suggested positive and negative associations with cardiovascular and metabolic 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 coronary heart disease and type 2 diabetes. Most of the reports which analysed fatty acids or fatty acid profiles used GC-FID or GC-Ms, while, the detection pretreatment of the biological matrix requires separated and methylation of fatty acids before measured. Additionally, biological samples contain kinds of fatty acids, and the pretreat method may destroy the original molecular structure of fatty acids in the biological matrix and the result incomprehensive, besides, requring more biological sample size or

content [13–14]. *c*POA was determined in the subcutaneous fat of human body, and *t*POA 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) [15–17], but there was no method reported to determine *c*POA and *t*POA in simultaneously. So, it's important to develop a method for the qrauantitative analyses of *c*POA and *t*POA in biological samples for the studies of biological activity, drug delivery systems or regenerative medicine. In the present paper, a UPLC-MS/MS method for quantification of simultaneous determination direct of *c*POA and *t*POA in rat serum was developed. This method proved to be selective and sensitive, with a wide range of detectionand low limit of detection, used to ascertain the phar-macokinetics of *c*POA and *t*POA.

2. Materials And Methods

2.1 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 (Darm-stadt, 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).

2.2 Chromatographic and mass spectrometric conditions

2.2.1 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.

2.2.2 Mass spectrometric conditions

Identification of *c*POA and *t*POA in serum samples was conducted using an AB 5500 Q-trap UPLC-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, 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. Product 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.

2.3 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℃ and brought to room temperature before use.

2.4 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 *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℃ until use. Rat serum samples, serving as QCs, were processed the following sample procedure as for unknown samples.

2.5 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, blowed 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.

2.6 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 [18]. 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, blowed by flowing nitrogen, and redissolved by 200 μL acetonitrile solution.

The determination of the extraction recoveries of *c*POA and *t*POA 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 × 100%) was used to evaluate the matrix effect and the extraction recovery and matrix effect of *c*POA and *t*POA were evaluated simultaneously by the same method.

During sample storage and processing procedures，the stability of *c*POA and *t*POA 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.

2.7 Pharmacokinetic studies of *c*POA and *t*POA

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 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 *c*POA 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 intragastric gavage of *t*POA 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℃ until analysis. The following main pharmacokinetic parameters were analyzed using the non-compartmental pharmacokinetics data analysis soft-ware of PK solution 2TM (Summit ResearchService, Montrose, CO, USA): area under curve from zero to the last measurable serum concentration point (AUC_{0-t}, t=24h), maximum concentration (Cmax), time-to-maximum concen-tration (Tmax).

3. Results

3.1 Determination of *c*POA and *t*POA by UPLC-MS/MS

*c*POA and *t*POA were determined by ultra performance liquid chromatography ESI tandem mass pectrometry (UPLC-MS/MS). The total chromatography time was 8.0 min and retention time *c*POA and *t*POA were 3.86 min and 4.23 min, respectively **(Fig.2 A, B, C)**. *c*POA and *t*POA were detected in serum samples intragastric administration. Both *c*POA and *t*POA were detected simultaneously in samples after pretreatment. The results showed that the blank serum might contain trace amounts of *c*POA and *t*POA **(Fig.2 D, E)**.

3.2 Method Validation

3.2.1 Selectivity and Specificity

Selectivity and specificity were investigated by comparing chromatograms of 6 different blank rat serum samples to corresponding spiked serum samples. No other endogenous substances were observed to interfere with *c*POA and *t*POA in any samples, but there was certain amount of *c*POA and *t*POA in blank serum. Specificity was verified by comparing retention times of *c*POA and *t*POA (3.86 and 4.23 min, respectively) in quality control (QC) samples (n=6), which indicated differences of less than 5%.

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

Standard curves were obtained by plotting the ratios of peak areas of *c*POA or *t*POA. The curves showed correlation coefficients greater than 0.999 and exhibited good linearity over concentration ranges of 0.1~12 μg/mL for *c*POA and *t*POA. Typical calibration equations were $y = 2.39e^{+005x}$ (R = 0.9991) for *c*POA, and $y = 9.06e^{+004x}$ (R = 0.9999) for *t*POA, where y represented the peak area ratio of an analyte, 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 *c*POA and *t*POA were defined as 0.1 μg/mL.

3.2.3 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[19]. The present results suggested that the method was accurate and precise for simultaneous analysis of *c*POA and *t*POA in rat serum samples.

3.2.4 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±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 *t*POA were 98.28±1.23, 100.66±1.82, and 99.75±3.01 (n=6), respectively (**Table 3**). The peak area of the standard analyte spiked into blank serum, minus that in the blank serum, were compared with that of injecting the standard analyte of 0.5, 2.5 and 10 μg/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, showed that ion suppression or enhancement from serum components was negligible for this method.

3.2.5 Stability

Results of stability tests indicated that the analytes were stable in the laboratory conditions (**Table 4**). The stability of *c*POA and *t*POA was evaluated as described in the experimental section.

3.3 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

lower concentration after 2 hours (**Fig. 3**), it may relate to the concentration of original *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 method at 75 mg/kg i.g. dose of *c*POA to rats (**Fig. 4A**). The mean concentration–time profiles of *c*POA and *t*POA in serum 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 Tmax of *c*POA was 0.94±0.44 h and the Cmax 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 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 Tmax of *t*POA was 1.50±0.98 h and the Cmax 14.77±11.91 μg/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 ~ 15 μg/L) in the same trend with *c*POA. And, the concentration of *c*POA in serum was maintain 10 μg/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 rat 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 [20, 21]. 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 ($AUC_{0\text{-}24h}$). $AUC_{0\text{-}24h}$ of *c*POA and *t*POA were 59.45±29.83 and 113.88±72.25 mg/L*h. The pharmacokinetic parameters of *c*POA and *t*POA were calculated and summarized in **Table 4**.

4. Discussion

*c*POA in organisms, especially in adipose tissue, may be related to the development of cardiovascular disease, *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 (VA, 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 VA from dietary [22]. By simulating the differentiation of adipocytes in vitro, it was found that the increasing content of *c*POA would increase the content of VA and conjugated linoleic acid (CLA, 18:2, *c*9, *t*11) in adipocytes [23]. Therefore, it could be inferred that exogenous *c*POA (16:1, *c*9) may generate VA (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. 6). Huang [24–25] 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 leaded 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 (endogenous) *c*POA concentration in serum was correspondent with the fluctuation of exogenous *t*POA concentration. So, it's suggested that *c*POA and *t*POA in serum may interconvert of isomeric compound configurations. The pretreatment process of GC method was cumbersome and has a long total chromatography time and retention time. Establishing rapid, accurate and simultaneous detection of *c*POA and *t*POA in biological samples has important research value for laboratory, clinical research and real-time detection *c*POA and *t*POA of organism. The method may be applied to clinical investigations to elucidate the biological activity and structure-activity relationship (SAP) of *c*POA and *t*POA in humans. And favoring 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 *t*POA was better than *c*POA in regulating lipid metabolism in hyperlipidemia mice [26]. It was suggested that *c*POA and *t*POA in serum may interconvert of isomeric compound configurations. 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.

5. Conclusions

A sensitive and rapid UPLC-MS/MS method for determination of *c*POA and *t*POA simultaneously in rat serum was established, which were identified in serum after i.g. administration. And the method was used to a pharmacokinetic study of *c*POA and *t*POA in rats. The result suggested that the endogenous *c*POA

concentration in serum was correspondent with the fluctuation of exogenous *t*POA concentration, and *c*POA and *t*POA in organism may interconvert of isomeric compound configurations, which of the metabolic transformation mechanism needs further investigation.

Abbreviations

POA: Palmitoleic acid, *c*POA: *Cis*-palmitoleic acid, *t*POA: *Trans*-palmitoleic acid, UPLC-MS/MS: Ultra Performance liquid chromatography-tandem mass pectrometry.

Declarations

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

All 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 contributed with valuable discussions and scientific input. Wenwen Huang conducted the experimental design. All authors participated in preparing the manuscript and approved the final version.

Conflicts of Interest

All authors declare no conflict of interest.

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Tables

Table 1

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

Analyte	Precursor Ion (m/z)	Daughter Ion (m/z)	Dwell Time (s)	DP(V)	EP(V)	CE(V)	CXP(V)
*c*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
*t*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

Table 2

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

Analyte	Concentration (μg/mL)	Mean±SD (μg/mL)	Accuracy (%)	Precision (%)
*c*POA	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	85.4	2.01
*t*POA	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	81.5	4.07

Table 3

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

Analyte	Concentration (μg/mL)	Matrix Effects		Recovery	
		Mean±SD (μg/mL)	RSD (%)	Mean±SD (μg/mL)	RSD (%)
*c*POA	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
*t*POA	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

RSD, relative standard deviation.

Table 4

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

Storage Condition (-80°C)	*c*POA			*t*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

Table 5

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

Parameters	Unit	Mean±SD	
		*c*POA	*t*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

Figures

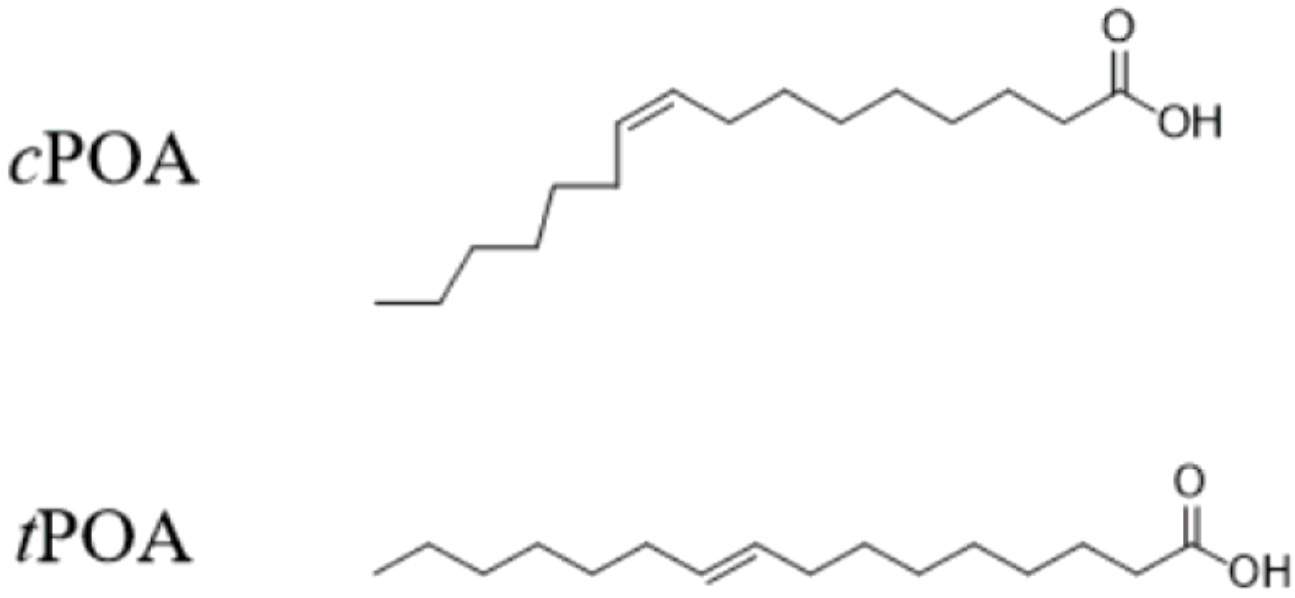

Figure 1

Structures of *c*POA and *t*POA

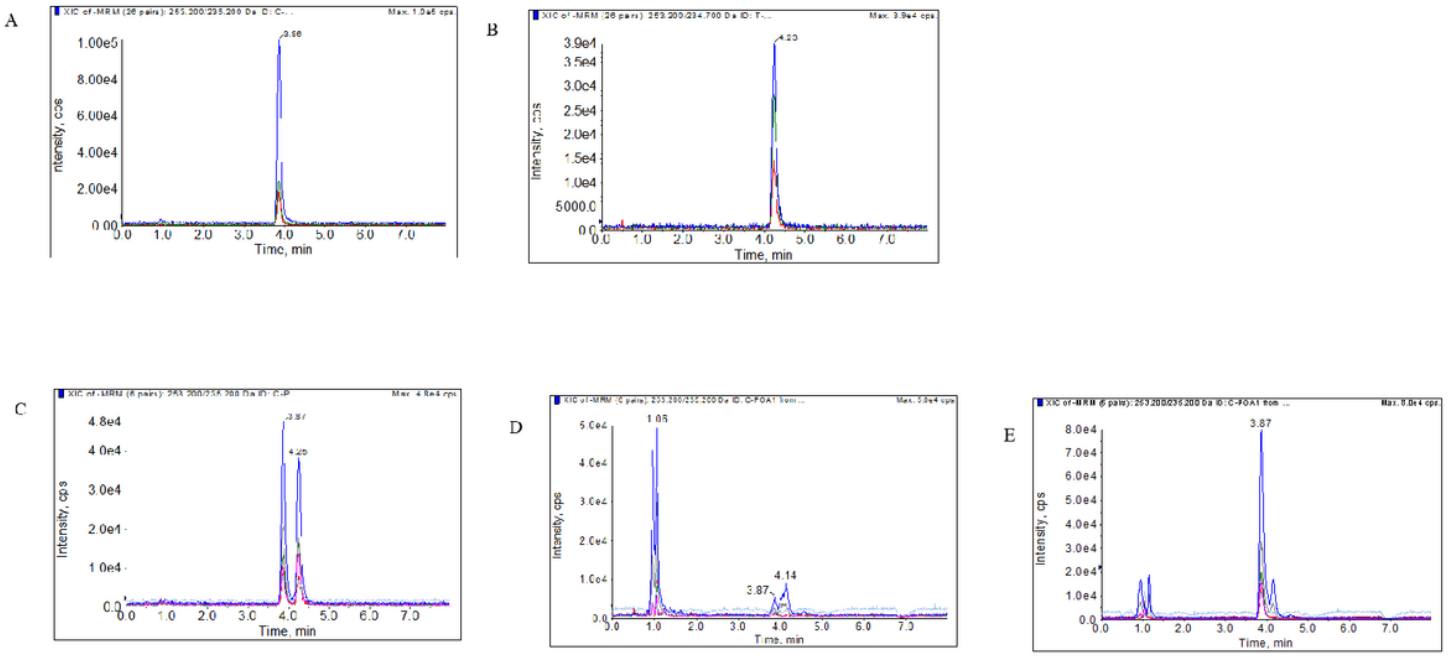

Figure 2

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, (E) rat serum sample.

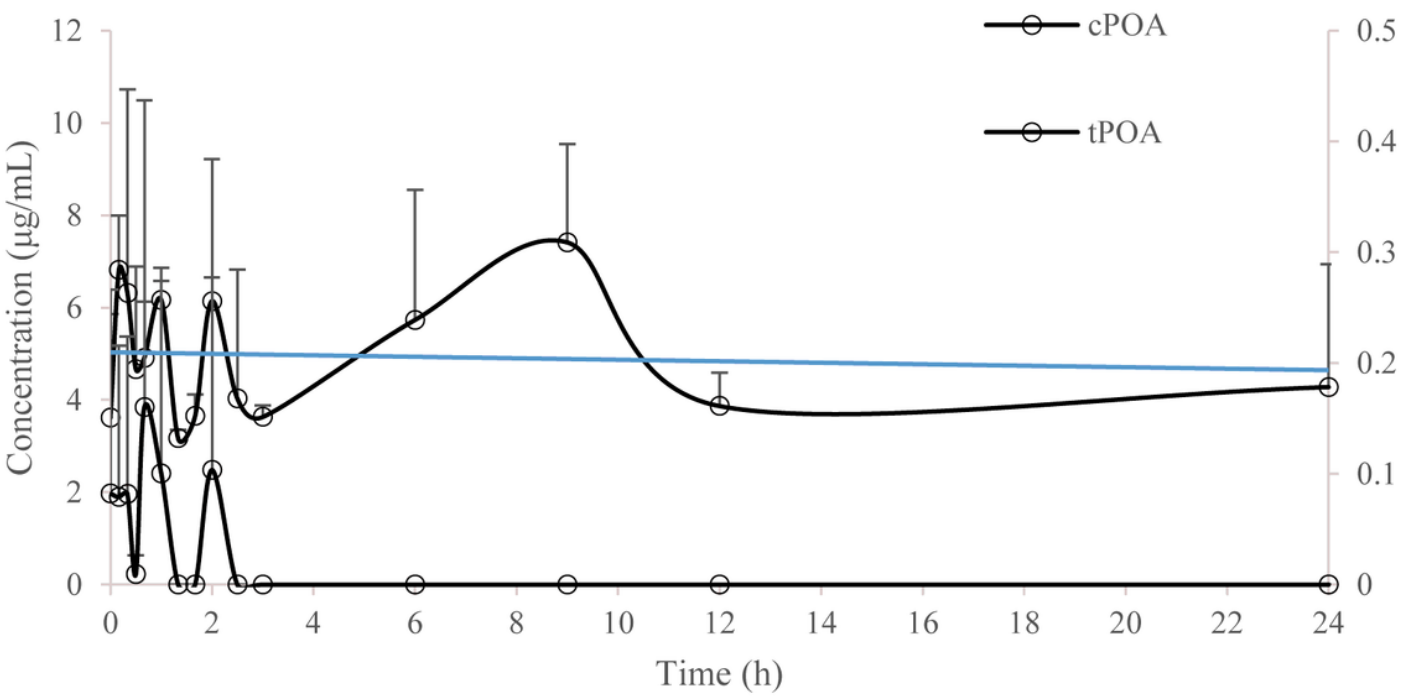

Fig. 3 Mean serum concentration-time profiles of cPOA and tPOA in blank rat serum in 24h (n=6, mean ± SD)

Figure 3

Mean serum concentration-time profiles of *c*POA and *t*POA in blank rat serum in 24h (n=6, mean±SD)

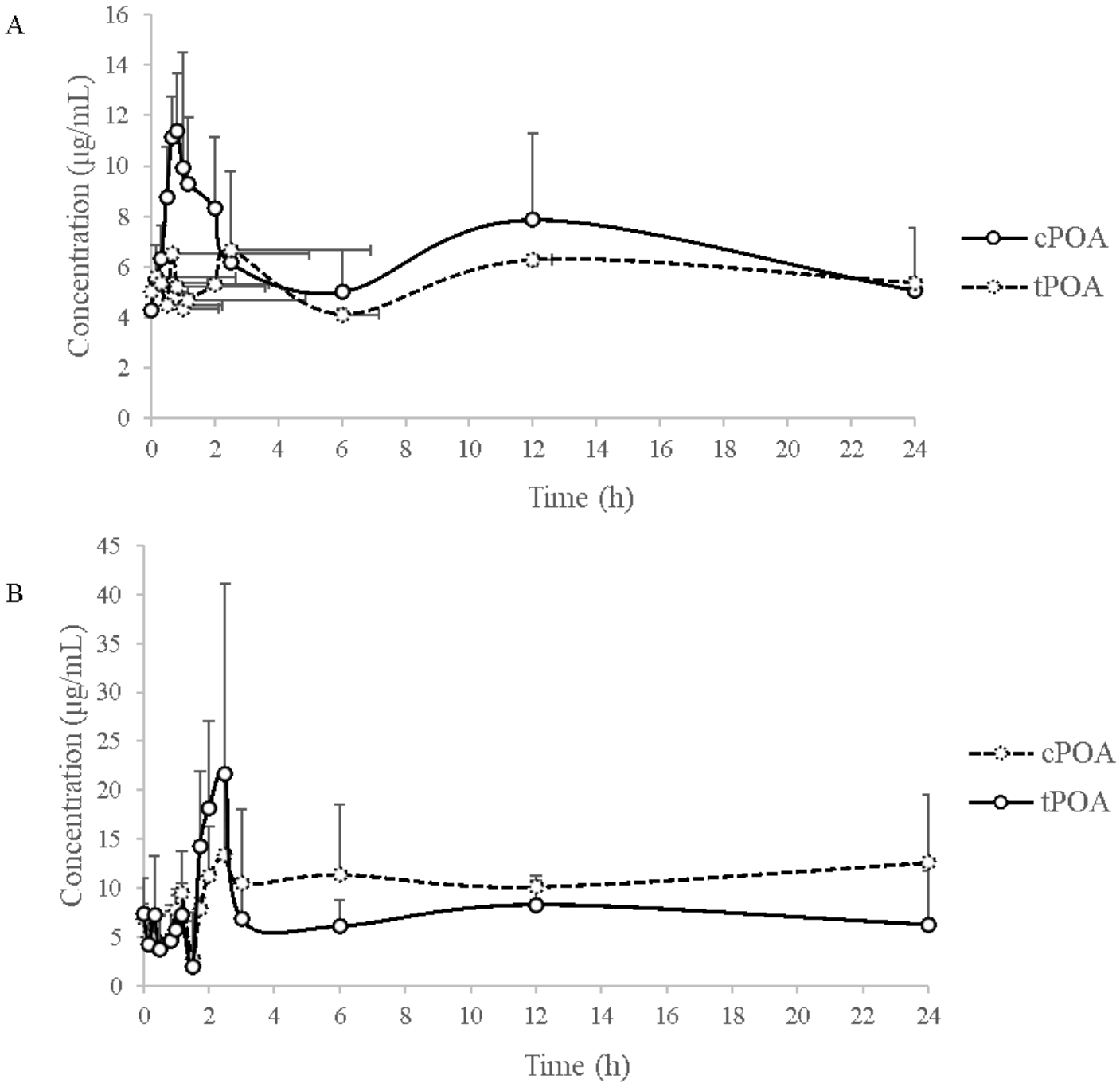

Figure 4

Mean serum concentration-time profiles of *c*POA and *t*POA (n=6). A showed *c*POA and *t*POA concentrations after i.g. administration of *c*POA in rats at 75 mg/kg body weight, B showed *c*POA and *t*POA concentrations after i.g. administration of *t*POA in rats at 75 mg/kg body weight.

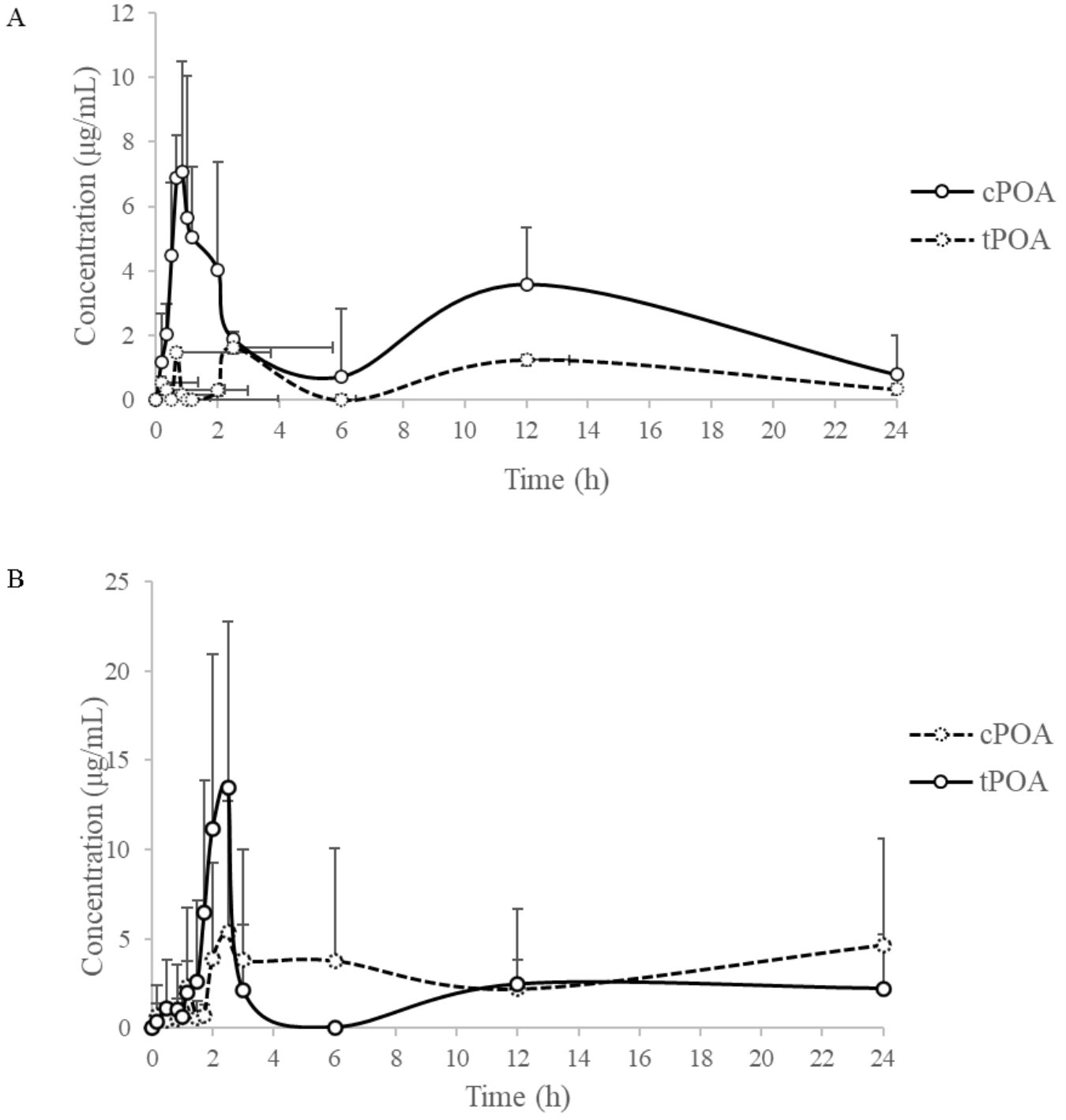

Figure 5

Baseline-corrected serum concentration-time profiles of *c*POA and *t*POA (n=6). A showed *c*POA and *t*POA concentrations after i.g. administration of *c*POA in rats at 75 mg/kg body weight, B showed *c*POA and *t*POA concentrations after i.g. administration of *t*POA in rats at 75 mg/kg body weight.

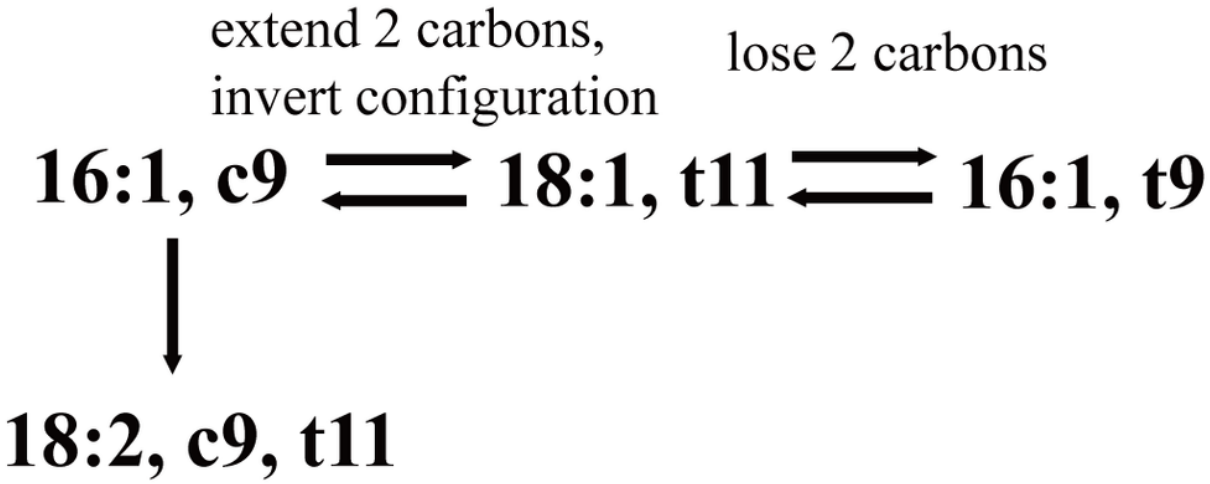

Figure 6

The possible convert mechanism between *c*POA and *t*POA. *c*POA（16:1, *c*9） is converted to VA（18:1, *t*11） through extends 2 carbons and inverts configuration by desaturation. Then, VA（18:1, *t*11）is converted to *t*POA （16:1, *t*9）through shortening the chain length by losing 2 carbons. Besides, *c*POA（16:1, *c*9） converted to conjugated linoleic acid（CLA（18:2, *c*9, *t*11）.