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# Research Article
# Pharmacokinetics and Tissue Distribution of Oleic and Linoleic Acids Following Oral and Rectal Administration of *Brucea javanica* Oil in Rats

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## Abstract

**Background:** *Brucea javanica* oil (BJO) is an important traditional Chinese medicine used for treatment of cancer, amoebic dysentery and malaria. To provide a rational basis for the use of this herb in clinical practice, the study investigated the *in vivo* distribution and pharmacokinetics of the marker agents oleic and linoleic acids following oral administration of BJO emulsion (BJOE) or rectal administration of BJO suppository (BJOS). **Materials and Methods:** Male Sprague-Dawley rats were given BJOE orally and BJOS via rectal administration. Samples from plasma and internal organs (the heart, liver, spleen, lungs, kidneys, brain, rectum and prostate) were collected. The concentrations of oleic and linoleic acids were determined using a Gas Chromatograph (GC) coupled with a flame ionization detector. Pharmacokinetic parameters were estimated using non-compartmental methods. **Results:** The GC procedure showed good precision and stability and was suitable for determining oleic and linoleic acids in the biological samples. Following administration via the two routes, oleic and linoleic acids were detected in all examined tissues with the highest levels found in the prostate. However, in comparison with BJOE, local BJOS application improved the maximum concentration ($C_{max}$) of oleic and linoleic acids in plasma and shortened the time to reach $C_{max}$ ($T_{max}$). Furthermore, BJOS showed a higher relative prostate-to-tissues $AUC_{0-t}$ ratio than BJOE. **Conclusion:** The BJO accumulated in the prostate after administration of either emulsion or suppository, providing a meaningful basis for clinical trials of prostate cancer treatment with this herb. The BJO was rapidly absorbed into the plasma after rectal administration, which may lead to a rapid pharmacological effect. Local application to the rectum might be a promising delivery route for BJO.

Key words: *Brucea javanica* oil, emulsion, suppository, pharmacokinetics, tissue distribution

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

*Brucea javanica* oil (BJO), a traditional herbal medicine is extracted from dried mature fruit of *Brucea javanica* (L.) Merr. (Simaroubaceae), which is widely distributed in areas from Southeast Asia to Northern Australia[1]. The BJO exhibits potent pharmacological activities, including anticancer, antimalarial and amoebic dysentery suppressive activities[2-4]. Oleic and linoleic acids are the two major components of BJO[4]. Both of these acids have been reported to induce apoptotic death of breast cancer, lung cancer, prostate cancer and lymphoma cells and also have cytotoxic effects on AML cell lines HL-60 and U937[4-8]. Oleic and linoleic acids have also been considered as the indicative components for BJO determination in pharmacokinetic and bioavailability studies on BJO[9-11]. The chemical structures of oleic and linoleic acids are shown in Fig. 1.

The BJO formulations are indicated for clinical treatment of malignant tumors including lung cancer, gastrointestinal cancer, hepatic cancer, esophageal cancer and bladder cancer[12,13]. The emulsion formulation (BJOE) is the most commonly used in clinical applications and is administered mainly through the oral route. However, BJOE may demonstrate flocculation, delamination and demulsification resulting from the increasing interfacial area following emulsification during storage and transportation[14,15]. In addition, oral administration of the emulsion may lead to stimulus of the intestinal tract and vomiting owing to the cold nature and the bitter, unpleasant taste of BJO. Therefore, the exploration of novel formulations and delivery routes for BJO holds particular commercial, clinical and scientific interest[16]. To alleviate the adverse reactions induced by BJO, rectal delivery could serve as an alternative to the oral route. The generous blood flow from the hemorrhoidal veins in the distal part of the rectum into the vena cava allows much of the absorbed drug to enter the systemic circulation directly, thus bypassing the portal vein and the liver[17-19]. Additionally, the rectal suppository is a traditional, favorable dosage form for children and non-cooperating patients.

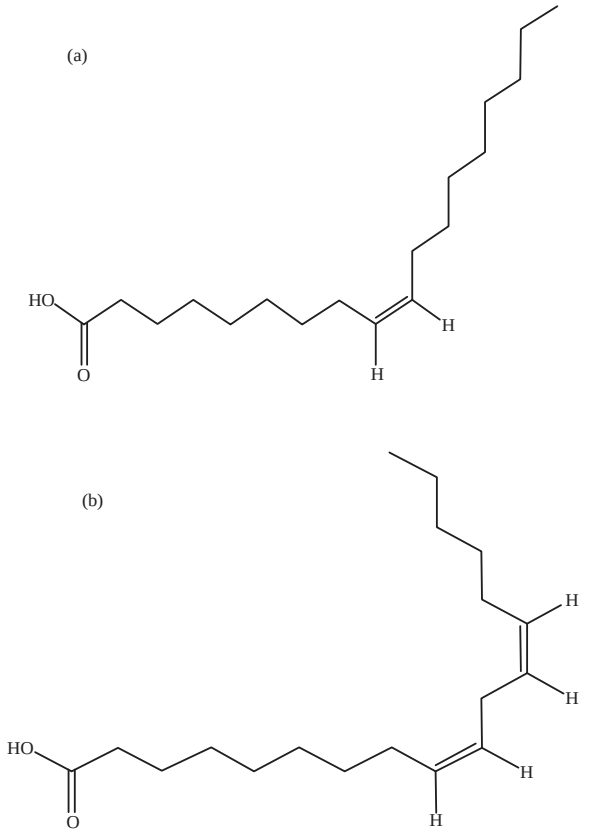


Fig. 1(a-b): Chemical structures of (a) Oleic acid and (b) Linoleic acid

Some pharmacokinetic studies on BJO formulations have been reported presently. The BJO incorporated in microemulsion is released slowly into rat plasma after oral administration[9]. Pharmacokinetic analysis of BJO liposomes using high-performance liquid chromatography has proven that this formulation shows a greater circulating time in rat plasma in comparison with intravenously administered BJOE[20]. *In vivo* studies have indicated that BJO has synergistic effects when combined with certain anticancer drugs in intravenous microemulsion[10,21]. However, to our knowledge, there are no reports on the pharmacokinetic profiles and tissue distribution of BJOE administered through the oral route.

In this study, the aim was to establish a simple and feasible method to analyze the BJO marker agents (oleic and linoleic acids) in biological samples, to render it amenable to further experiments. Experiments to assess pharmacokinetics and tissue distribution of BJO after oral administration of BJOE and rectal suppository (BJOS) administration were conducted to increase our understanding of its *in vivo* actions and efficacy. Finally, the pharmacokinetic parameters and prostate-to-tissue $AUC_{0-t}$ ratios were evaluated to compare the emulsion and suppository formulation.

## MATERIALS AND METHODS

**Chemicals and reagents:** The BJO containing 31.74% oleic acid and 42.31% linoleic acid was purchased from Jishuizhongnan Natural Refinery (Jiangxi, China). Oleic and linoleic acids standards were obtained from Sigma-Aldrich (Shanghai, China). Internal Standard (IS) phenyl benzoate was purchased from Alfa Aesar (Beijing, China). Cremophor RH-40 (polyoxyl 40-hydrogenated castor oil) was kindly

gifted by BASF (Ludwigshafen, Germany). The PEG6000 (polyethyleneglycol 6000) was purchased from Damao Chemical Reagent Factory (Tianjin, China). All other chemicals and reagents were of analytical grade.

**Animals:** Male Sprague-Dawley rats (180-220 g) were obtained from the Laboratory Animal Center of Guangzhou University of Chinese Medicine (Guangzhou, China). Animals were fasted for 12 h and allowed free access to water prior to the experiments. Animal experimental protocols were approved by the Animal Ethical Committee (Guangdong Pharmaceutical University, Guangzhou, China) and all animal studies were carried out according to the Guide for Care and Use of Laboratory Animals.

**Measurement of oleic and linoleic acid components by gas chromatography:** The effective components of oleic and linoleic acids in plasma and tissues were determined using Gas Chromatography (GC). Due to the high boiling points of oleic and linoleic acids, esterification was performed to achieve high sensitivity in GC[22]. The oleic and linoleic acids in plasma samples (100 μL) and tissue homogenates (0.5 g) were extracted by adding a mixture of dimethyl carbinol, n-hexane and c-glacial acetic acid (40:10:1, v/v/v). Then, 1 mL n-hexane and 1 mL distilled water were added and the mixture was vortexed for 30 sec. The supernatant was transferred to a centrifuge tube and evaporated to dryness under nitrogen. The residue was reconstituted with 2 mL of 0.5 mol $L^{-1}$ KOH solution in methanol and placed in a water bath at 60°C for 25 min for saponification until all oil droplets were dissolved. After cooling down to room temperature, 2 mL of 15% boron trifluoride ether solution was added and the mixture was placed in a water bath at 60°C for 2 min for esterification. Then the test solution was vortex-mixed with 2 mL n-hexane prior to mixing with 1 mL saturated NaCl solution. The upper n-hexane solution was removed and vortexed with the same amount of a certain concentration of IS. The mixture was filtered through a membrane filter (0.22 μm) and 2 μL was injected into an Agilent GC 6820 gas chromatograph (Agilent, USA) equipped with a DPFF-AP column (30 m×0.25 mm×0.25 μm) (Agilent, USA). The injector was maintained at 250°C. High-purity nitrogen was used as the carrier gas. The column oven was maintained at 205°C and the detector was a hydrogen flame ionization detector at 250°C.

**Preparation of calibration standards and Quality Control (QC) samples:** A stock solution containing more than two compounds with 15.62 mg $mL^{-1}$ oleic acid and 16.48 mg $mL^{-1}$ linoleic acid was prepared in n-hexane. The stock solution was serially diluted with n-hexane to provide working standard solutions of desired concentrations. An IS stock solution of 8.53 mg $mL^{-1}$ was also prepared in n-hexane. Calibration standards of plasma-derived working solutions with oleic and linoleic acids at final concentrations of 15.62-1562 and 16.48-1648 μg $mL^{-1}$, respectively were prepared. The final standard concentrations of tissue samples were 12.50-248.05 μg $mL^{-1}$ for oleic acid and 13.18-241.00 μg $mL^{-1}$ for linoleic acid. The IS working solution was prepared at a final concentration of 170.67 μg $mL^{-1}$ in n-hexane. For method validation, QC plasma samples of oleic and linoleic acids were prepared separately at three concentrations of oleic acid 30.70, 122.80 and 491.20 μg $mL^{-1}$ and three concentration of linoleic acid 33.81, 135.20 and 541.00 μg $mL^{-1}$, respectively. Tissue homogenate QC samples were prepared at three concentrations of 62.02, 93.03 and 155.05 μg $mL^{-1}$ of oleic acid and of 60.25, 90.38 and 150.63 μg $mL^{-1}$ of linoleic acid.

**Biosample preparation:** Blood samples (0.50 mL) were obtained by retro-orbital puncture at various time intervals (before administration and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 18 and 24 h after administration) and collected in heparinized tubes. The samples were immediately centrifuged at 900×g for 10 min to separate the plasma fraction and stored at -20°C until analysis. The rats were sacrificed by decapitation at various time intervals (before administration and at 2, 4, 6, 8, 12, 24 h after administration) and tissue samples (heart, liver, spleen, lung, kidney, brain, rectum and prostate) were collected. The samples were washed with normal saline to remove the blood and dried with filter paper. The tissues were weighed and homogenized in normal saline (1:4, w/v). The homogenates were stored at -20°C until analysis. The blood and homogenate samples were analyzed using the GC method. Quantitative analysis of the oleic and linoleic acids was performed using the IS method.

**Validation procedures:** The specificity of the method was investigated by comparing the chromatograms of blank plasma and tissue homogenates with samples spiked with standard compounds and IS, samples obtained after an oral dose of emulsion and samples obtained after rectal administration. The lower limit of quantification (LLOQ) was determined as the amount that could be detected with a signal-to-noise ratio of 5. The linearity of each calibration curve was determined by plotting the peak area ratio (y) of the analytes to the IS versus the nominal concentration (x) of analytes with weighted ($1/x^2$) least-squares linear regression. Accuracy, intra and inter-day precision were estimated by

analyzing three QC samples (five replicates for each) at low, middle and high concentrations on the same day and on three consecutive validation days, respectively. Extraction recovery was assessed by comparing the peak responses of three QC samples (five replicates for each) with the responses of analytes from standard solutions spiked in post-extracted black plasma at equivalent concentrations. Relative recovery was measured via comparison of the peak responses obtained from three QC samples (five replicates for each) to those obtained from neat standard solutions at equivalent concentrations. The stability of oleic and linoleic acids was evaluated by analyzing the plasma and tissue QC samples at high, medium and low concentrations. Three replicates were stored at room (25°C) and refrigerated (4°C) temperatures to evaluate short-term stability, while long-term stability was investigated using samples stored at -20°C. Stability was assessed by comparing the mean concentrations of the stored samples with those of freshly prepared samples.

**Preparation of BJOE and BJOS:** The BJOE was prepared according to the Chinese traditional patent formulation issued by the Ministry of Health[23]. A solution of 15 g soybean lecithin in water was rapidly added to 10 mL preheated BJO followed by high-speed shear mixing (ULTRA-TURRAX T 18 basic homogenizer; IKA, Staufen, Germany) at 8000 rpm for 9 min and continuous stirring for 9 min. The coarse emulsion was made up to 100 mL with water for injection and subjected to high-pressure homogenization (ATS AH-2010, Canadian) at 900 bar for 5 cycles to form the final emulsion. The drug content in the emulsion was 9% (w/v). The BJO suppository (BJOS) was prepared using the hot-melt method. The PEG6000 was melted and blended with a mixture of RH-40 and BJO under continuous agitation. The mixture was thoroughly stirred prior to pouring into a mold lubricated with liquid paraffin and cooled down to 25°C. The final forms were removed from the mold, packed and kept at 4°C in a refrigerator until further investigation. The drug content in the suppositories was 11% (w/w).

**Pharmacokinetic and tissue distribution study:** Based on the oral BJOE dose applied in clinical conditions, the recommended human Single Dose (SD) is 20 mL. The human-equivalent SD for rats was calculated according to a dosage conversion formula based on the body surface area[24]. The converted dosage of BJOE administered orally for rats is 2 mL $kg^{-1}$ b.wt., that of BJOS administered rectally is 1.6 g $kg^{-1}$ b.wt. (Supplementary material). For the pharmacokinetic study, rats were divided into two groups (n = 6 per group). After overnight fasting, BJOE was administered by oral gavage and BJOS was rectally administered to each animal. After dosing, blood samples were collected in heparinized tubes at various time points as mentioned above. For the tissue distribution study, thirteen groups of rats (n = 3 per group) were orally administered BJOE and rectally administered BJOS as described for the pharmacokinetic study. The rats were sacrificed at various time points and tissue samples were collected.

**Data analysis:** Because oleic and linoleic acids are endogenous compounds, the concentrations in rat plasma and tissues were corrected by subtracting the concentration of a blank sample from each animal to obtain the authentic drug distribution profiles. Pharmacokinetic parameters were calculated by non-compartmental analysis using the DAS software (Mathematical Pharmacology Professional Committee of China, Shanghai, version 3.1.1). Selected pharmacokinetic parameters included the area under the concentration-time curve from time zero up to the last measurable time point ($AUC_{0-t}$), peak plasma concentration ($C_{max}$) and median time to attain $C_{max}$ ($T_{max}$). All results are expressed as the mean±standard deviation. Differences in pharmacokinetic parameters among groups were tested by one-way analysis of variance (ANOVA) using SPSS 16.0 (SPSS IBM, Armonk, NY, USA). The differences were considered to be significant when $p<0.05$ or $p<0.01$. A specific calibration curve was separately prepared for each tissue sample obtained from drug-free rats. The concentrations of oleic and linoleic acids in each sample were expressed in terms of milligram per gram tissue and calculated by the equation[25]:

$$Ct = \frac{CsVs}{P}$$

where, $C_t$ is the tissue concentration (mg $g^{-1}$), $C_s$ is the supernatant concentration, Vs is the supernatant volume and P is the weight of the sample.

## RESULTS

**Method validation:** Typical chromatograms of blank and spiked plasma or tissues with analytes and IS are shown in the Supplementary Fig. S1-S31. Because oleic and linoleic acids are endogenous unsaturated fatty acids circulating in rat plasma and tissues, the response values corresponding to oleic and linoleic acids could be detected in both blank plasma and tissues. All calibration curves for oleic and linoleic acids displayed good linearity (all correlation coefficients [$r^2$] >0.99). The calibration plot equations of the analytes, their

$r^2$ and linear ranges were calculated and are listed in Table S1. On the basis of the signal-to-noise ratio, the LLOQ of oleic and linoleic acids was 15.62 and 16.48 μg $mL^{-1}$ for plasma samples, while it was 12.50 μg $mL^{-1}$ of oleic acid and 13.18 μg $mL^{-1}$ of linoleic acid for the heart, liver, spleen, lung, kidney, brain, rectal and prostate tissue homogenates, respectively. The Relative Standard Deviation (RSD%) for both intra and inter-day analysis was below 10% as shown in the Supplementary Table S2. The recovery rates of oleic and linoleic acids in rat plasma and tissues are presented in the Supplementary Table S3. The RSDs of the recovery rates were less than 9%. Both the oleic and linoleic acids were stable in rat plasma and tissues for at least 1 day at room temperature, 3 days at refrigeration and 7 days at freezing temperature since no obvious degradation occurred in the samples under the storage conditions tested.

**Pharmacokinetic analysis:** The plasma drug concentration-time profiles after single-dose oral administration of BJOE and rectal administration of BJOS in rats are presented in Fig. 2 and the pharmacokinetic parameters are shown in Table 1. Figure 2 showed different pharmacokinetic profile of oleic and linoleic acids between two formulations. It can be seen that the oleic and linoleic acids of BJOS achieved higher plasma levels than BJOE during absorption phase in which the $T_{max}$ are shorter for BJOS. Table 1 shows that the $AUC_{0-t}$ of the oleic and linoleic acids showed no significant differences between the two formulations ($p>0.05$). However, the $C_{max}$ values for oleic and linoleic acids in the plasma after application of BJOS were 1.81-fold and 2.27 higher, respectively, than after oral application of BJOE. In addition, the $T_{max}$ for BJOS was shorter than that for BJOE for both oleic and linoleic acids.

**Tissue distribution analysis:** The concentration-time data for oleic and linoleic acids in the tissues after administration of the BJOE and BJOS are shown in Fig. 3. In Fig. 3, both oleic and linoleic acids concentrations in the heart and liver were slightly higher for orally administered BJOE than for rectally administered BJOS. As for the spleen and kidney, there were no consistent significant differences between the two formulations. Furthermore, the levels of oleic and linoleic acids in the prostate were markedly higher than those in other tissues. The overall trend in tissue distribution of the bioactive components was $AUC_{Prostate}>AUC_{Lung}>AUC_{Liver}>AUC_{Kidney}>AUC_{Heart}>AUC_{Rectum}>AUC_{Spleen}>AUC_{Brain}$, as shown in Fig. 4. To better compare the two formulations, the prostate-to-tissue (P/T) $AUC_{0-t}$ ratios of oleic and linoleic acids were calculated using the following formula:

$$P/T = (AUC_{Prostate})/(AUC_{Heart}+AUC_{Liver}+AUC_{Spleen}+AUC_{Lung}+AUC_{Kidney}+AUC_{Brain}+AUC_{Rectum})$$

Table 1: Comparison of pharmacokinetic parameters of the oleic and linoleic acids in plasma following oral administration of BJOE and rectal administration of BJOS in rats (n = 6)

| | Oleic acid | | Linoleic acid | |
|---|---|---|---|---|
| Pharmacokinetic parameters | BJOS | BJOE | BJOS | BJOE |
| $AUC_{(0-t)}$ (μg h $mL^{-1}$) | 1959.87±233.05 | 2153.99±489.64 | 1790.55±514.45 | 2599.47±778.26 |
| $C_{max}$ (μg $mL^{-1}$) | 245.60±65.49* | 135.81±34.360 | 418.33±46.78** | 184.14±89.79 |
| $T_{max}$ (h) | 4.00±0.0 | 15.6 0±6.40 | 4.00±0.00 | 14.00±3.7 |

*p<0.05 versus BJOE and **p<0.01 versus BJOE

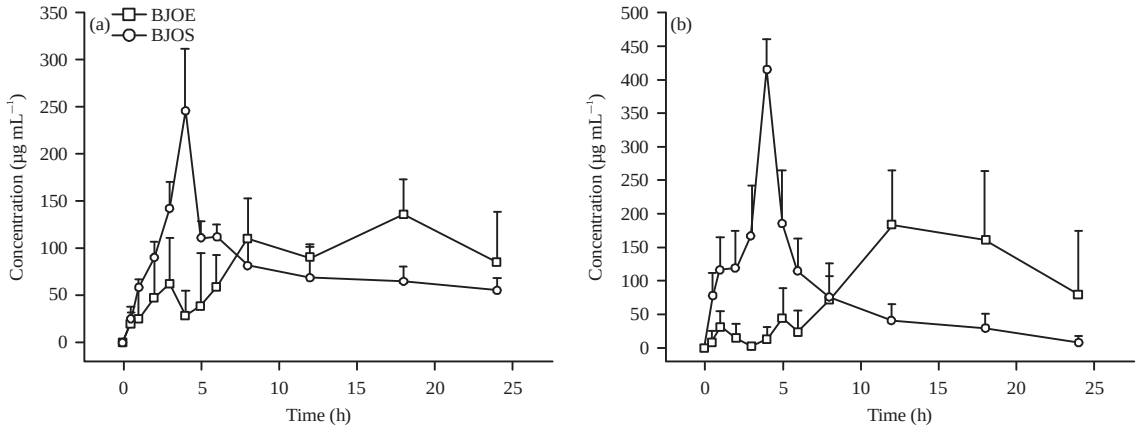


Fig. 2(a-b): Mean plasma drug concentration-time profiles of (a) Oleic acid and (b) Linoleic acid after oral administration of BJOE and rectal administration of BJOS in rats (n = 6)

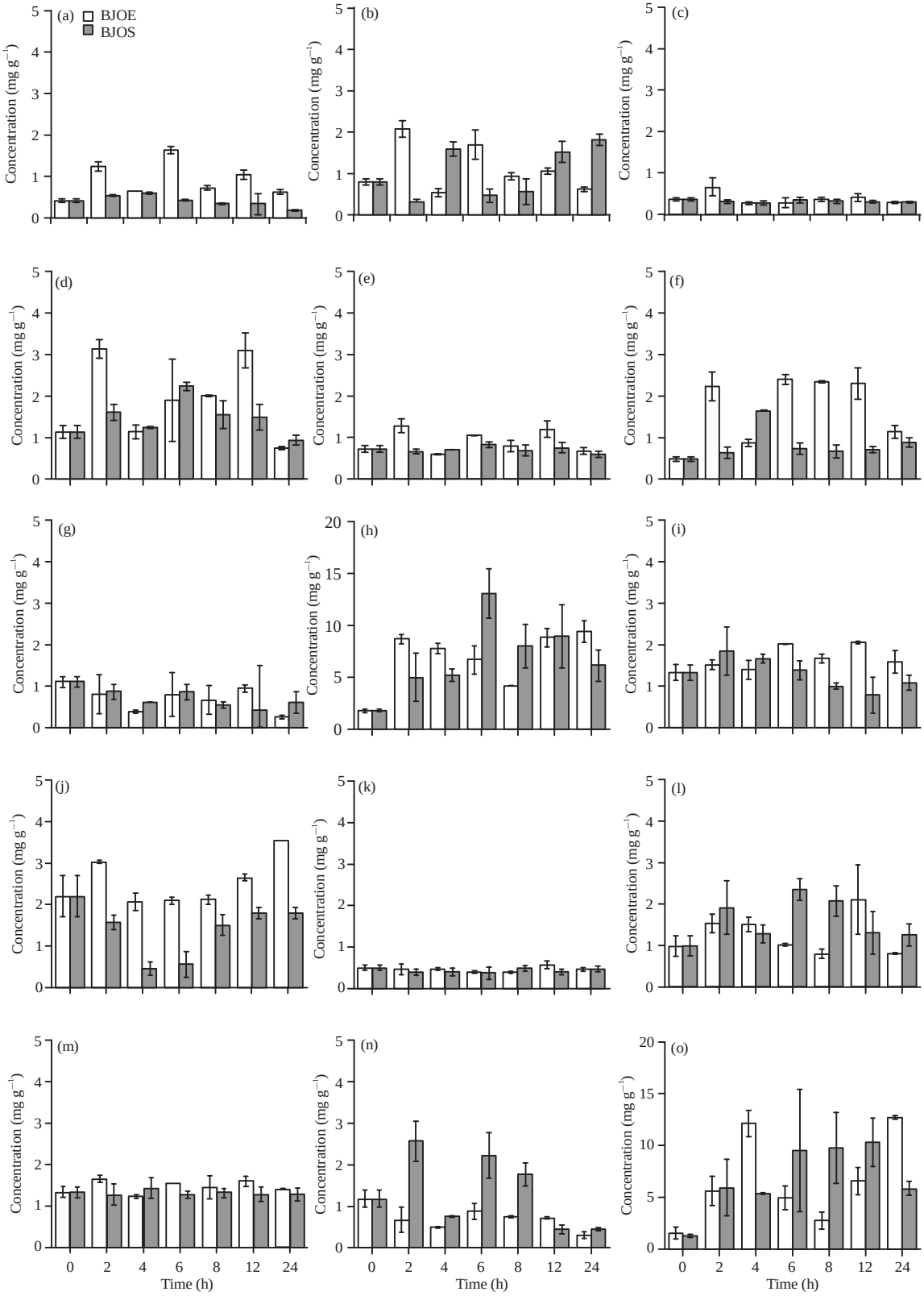


Fig. 3(a-o): Concentration versus time data of oleic acid in the (a) Heart, (b) Liver, (c) Spleen, (d) Lungs, (e) Kidneys, (f) Brain, (g) Rectum, (h) Prostate and of linoleic acid in the (i) Heart, (j) Liver, (k) Spleen, (l) Lungs, (m) Kidneys, (n) Rectum and (o) Prostate following oral administration of BJOE and rectal administration of BJOS (n = 3)

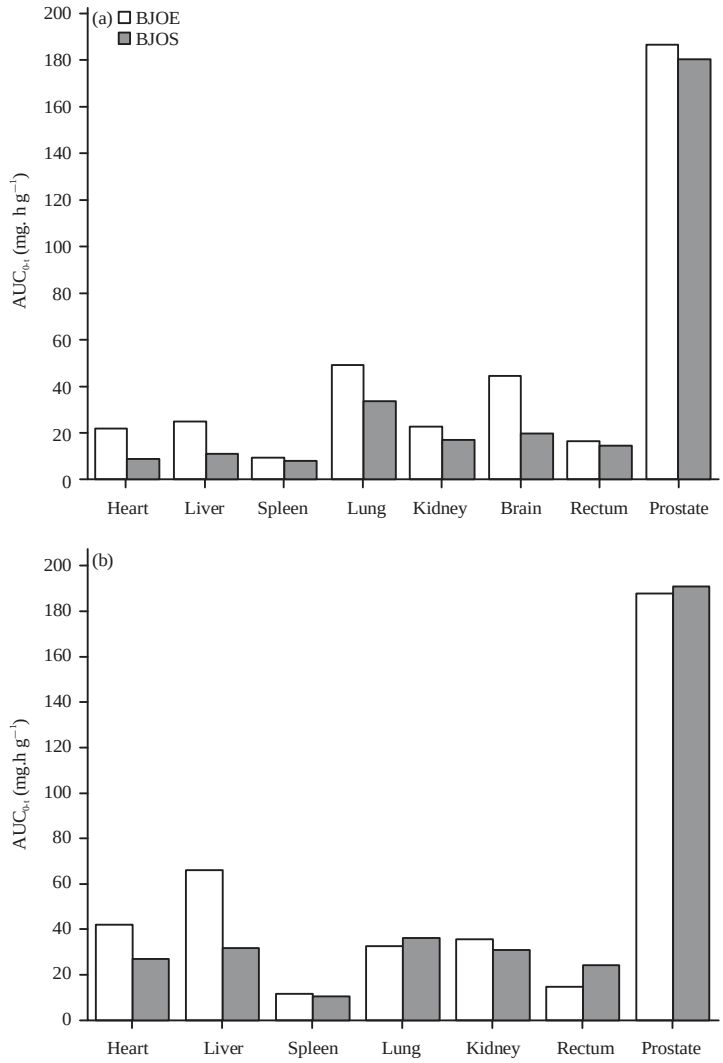


Fig. 4(a-b): Distribution of (a) Oleic acid and (b) Linoleic acid in different organs calculated as the $AUC_{0-t}$ following oral administration of BJOE and rectal administration of BJOS

The BJOS yielded a higher prostate-to-tissue $AUC_{0-t}$ ratio as 1.63 of oleic acid and 1.19 of linoleic acid than BJOE as 0.99 of oleic acid and 0.92 of linoleic acid, which may due to lower exposure in other tissues.

## DISCUSSION

After a single dose of drugs into rats, a single peak is expected in the plasma concentration-time curve; however, for the BJOE, a second peak appeared. This might be due to a variety of causes including delayed gastric emptying[26], variable absorption in different regions of the enteral canal[27], enterohepatic recirculation[28] and reabsorption after tissue distribution as BJO is highly lipophilic and associates tightly with tissues after oral administration. Following a rectal dose of BJO in rats, the drug loaded in the suppository was effectively transported into the circulation in a short time. Generous blood outflow from the distal part of the rectum directly into the vena cava might cause the absorbed drug to enter systemic circulation directly after rectal administration of the suppository[29,30], thus yielding higher $C_{max}$ and shorter $T_{max}$. Compared to the previous study of other BJO formulations[9,20], the BJOS showed an improved pharmacokinetic profile, i.e., higher $C_{max}$ and shorter $T_{max}$, which is preferable for effective treatment. Rectal administration of BJOS can be reserved for situations in which oral administration is difficult.

Tissue distribution studies demonstrated that both the oleic and linoleic acids were distributed to all examined tissues, except for the brain, where no linoleic acid was

detected. The movement of a compound across the blood-brain barrier not only depends on the lipophilicity and molecular size of the compound but also is regulated by a specific carrier-mediated transport system that can export it from endothelial cells into the blood stream[31]. Further detailed studies will be required to elucidate the mechanism underlying our observation. Oleic and linoleic acids were distributed in organs with abundant blood supply such as the lungs, kidneys and liver, which implied that the distribution of the compounds depends on the blood flow or perfusion rates of the organs. The relatively high concentrations in the liver and lungs confirmed the findings of previous reports that BJOE shows good curative effect in liver and lung cancer treatments[32-35].

Within 24 h of administration, maximum exposure of the analytes was observed in the prostate. In general, drug penetration into the prostate gland is thought to be governed by principles determining drug passage across biological, lipid-containing membranes; thus, the lipid solubility of the molecule determines the rate of diffusion of drugs across the prostatic epithelium[36]. This may explain why the BJO showed high accumulation in the prostate after administration through both the oral and rectal routes. Additionally, the blood in the common prostato-rectal arteries arising from the internal pudendal artery may be shunted into prostatic artery and middle rectal artery[37], allowing the drug to accumulate in the prostate after BJOS administration. To ascertain the mechanism of this phenomenon in tissue distribution, more detailed studies are needed.

In present clinical application, BJO formulations are used to treat different cancers such as lung cancer, liver cancer, lung cancer with brain metastasis and digestive-tract cancer. The results suggest that the drug concentrates in the prostate after oral and rectal administration and possibly shows efficacy; therefore, BJO may be also effective for the treatment of prostate cancer. Further study is needed to confirm the exact effect.

## CONCLUSION

To the best of our knowledge, this is the first report of a pharmacokinetic study of BJOE and BJOS in rat biological samples after oral and rectal administration, respectively. A sensitive and reliable GC method was established and successfully applied for comparison of oleic and linoleic acids in rat plasma and tissues following different administration routes. Based on the detectable oleic and linoleic acids in various tissues, the highest concentrations were found in the prostate, indicating that the drug accumulates in this gland. The BJOS not only yielded the highest plasma concentration of oleic and linoleic acids but also a short time to peak accumulation, which demonstrated that the main components of BJO in the suppository formulation were rapidly absorbed, which may lead to rapid-onset pharmacological effect. The results of the current study suggest that application of BJOS via the rectum offers a potential alternative to oral application and provide a significant basis for further development of the suppository formulation. Finally, our findings may lead to a novel therapeutic strategy for prostate cancer treatment.

## ACKNOWLEDGMENTS

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## SUPPLEMENTARY MATERIAL

**Dose conversion:** The dose conversion between "milligram per milliliter" and "milligram per kilogram" was conducted as follows: Referring to the oral BJOE applied in clinic, the recommended human dose at a single time is 20 mL. The converted dose of BJOE administered in rats was calculated according to the dosage conversion formula based on body surface area:

$$\text{BJOE}: \text{Dose (mL kg}^{-1}) = \frac{\text{Clinical dose (mL)}}{\text{Human weight (kg)}} \times \frac{\text{Body surface area ration (rat)}}{\text{Body surface area ration (human)}} = \frac{20\text{ mL}}{60\text{ kg}} \times \frac{0.47}{0.08} = 2\text{ mL kg}^{-1}$$

$$\text{BJO}: \text{Dose (g kg}^{-1}) = \text{Dose of BJOE (mL kg}^{-1} \times \text{content (g / 100 mL)} = 2\text{ mL kg}^{-1} \times 9\text{ g / 100 mL} = 1.8\text{ g kg}^{-1}$$

$$\text{BJOS}: \text{Dose (g kg}^{-1}) = \frac{\text{Dose of BJO (g kg}^{-1})}{\text{Content (g / 100) g}} = \frac{1.8\text{ g kg}^{-1}}{11\text{ g / 100 g}} = 1.6\text{ g kg}^{-1}$$

In this study, the converted dosage of BJOE administered orally is 2 mL kg$^{-1}$. As the drug loading of BJOE was 9% (w/v), the equal dose of BJO was calculated to be 1.8 g kg$^{-1}$. Because the drug loading in BJOS was 11% (w/w), the human-equivalent dose was 1.6 g kg$^{-1}$.

**Specificity:** The concentrations of analyte standards were 100 μg $mL^{-1}$ for oleic acid, 100 μg $mL^{-1}$ for linoleic acid and 170.67 μg $mL^{-1}$ for internal standard. All samples were obtained at 2 h after rectal administration of 1.6 mg $kg^{-1}$ BJOS. Peak identification, 1: Oleic acid, 2: Linoleic acid and 3: Phenyl benzoate (IS).

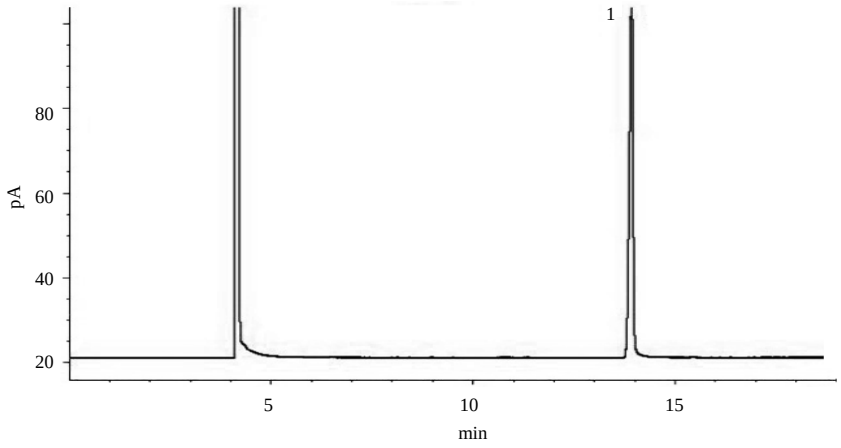


Fig. S1: Oleic acid standard

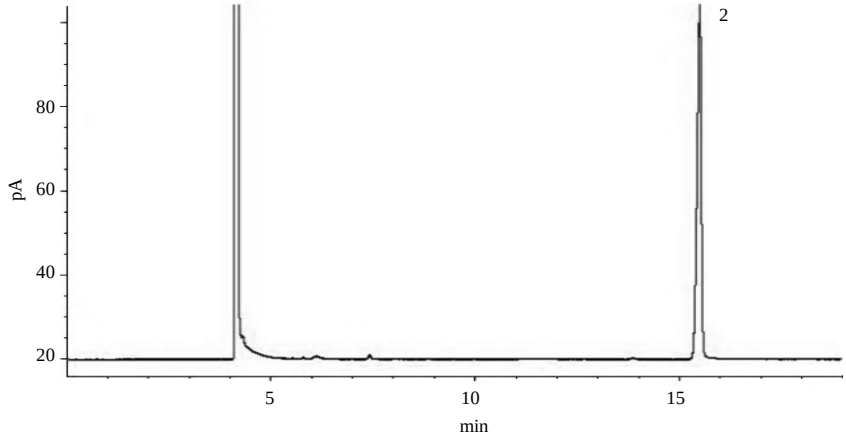


Fig. S2: Linoleic acid standard

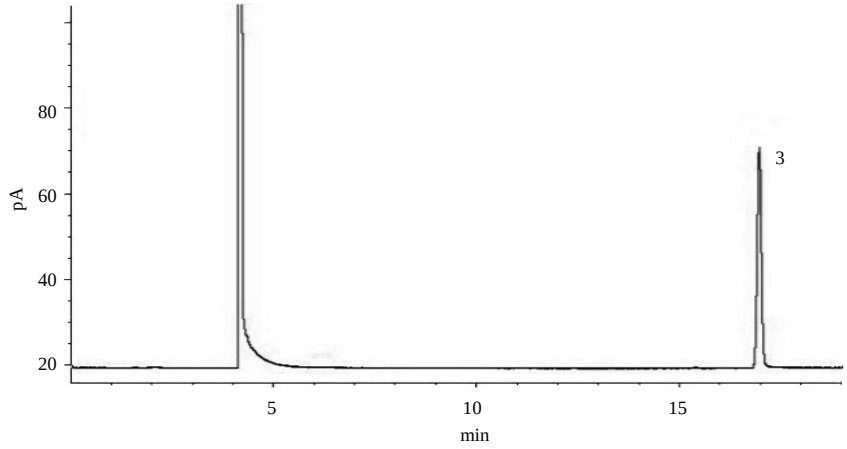


Fig. S3: Internal standard (phenyl benzoate)

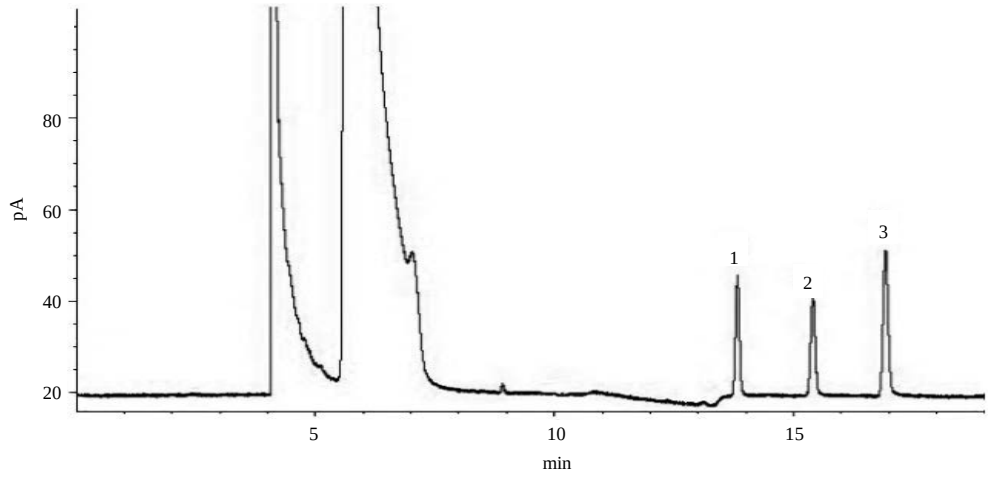


Fig. S4: Mixture standard of active components

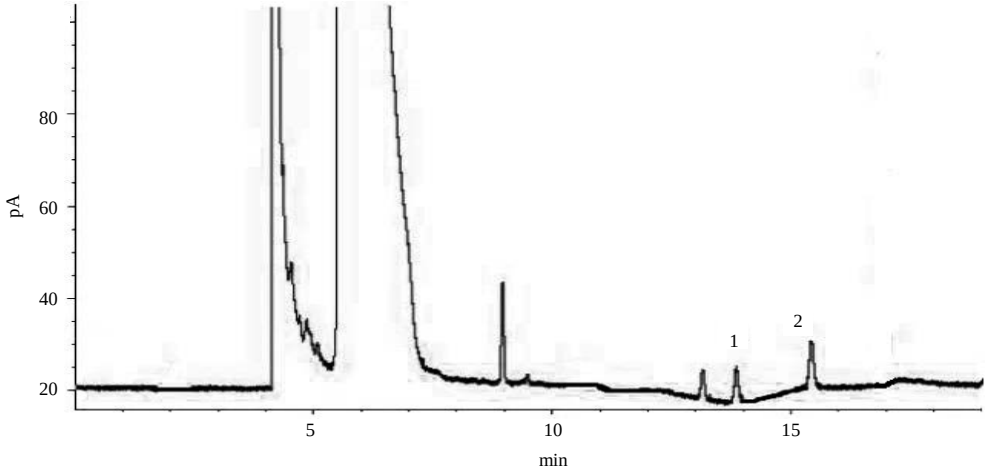


Fig. S5: Blank plasma sample

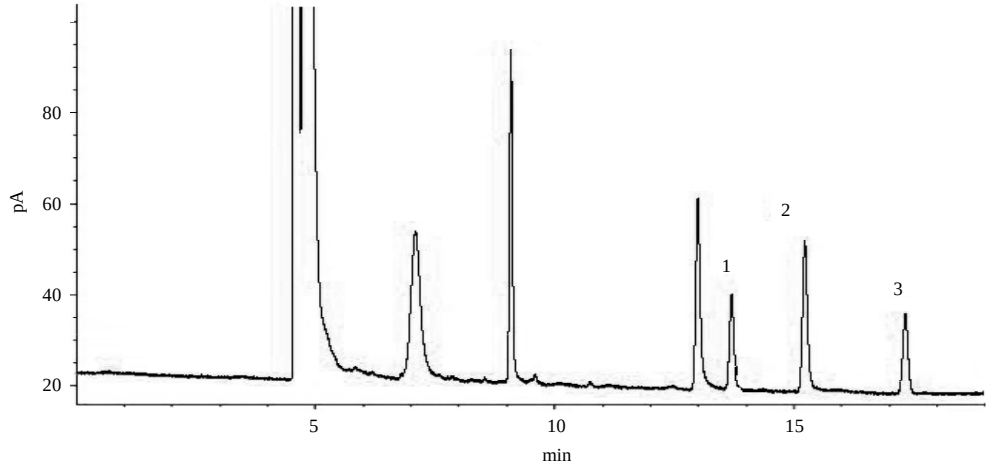


Fig. S6: Blank plasma sample spiked with internal standard

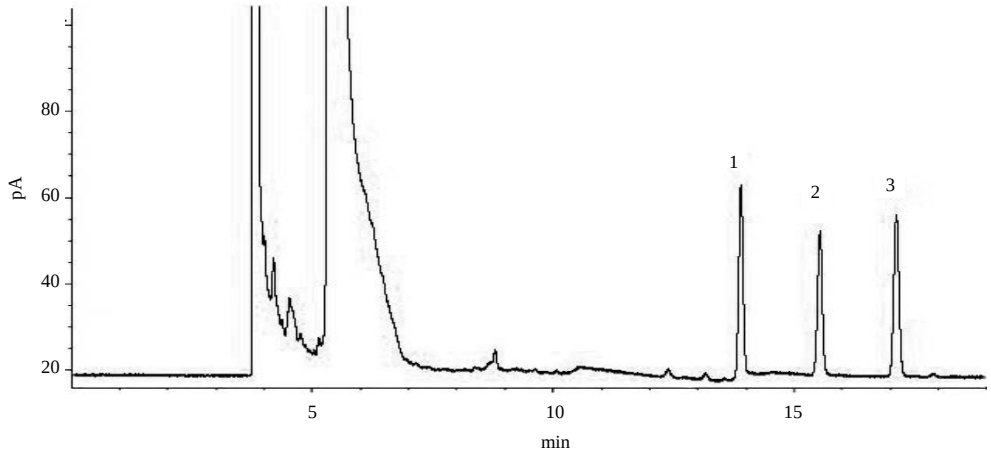


Fig. S7: Plasma sample obtained after rectal administration of BJOS

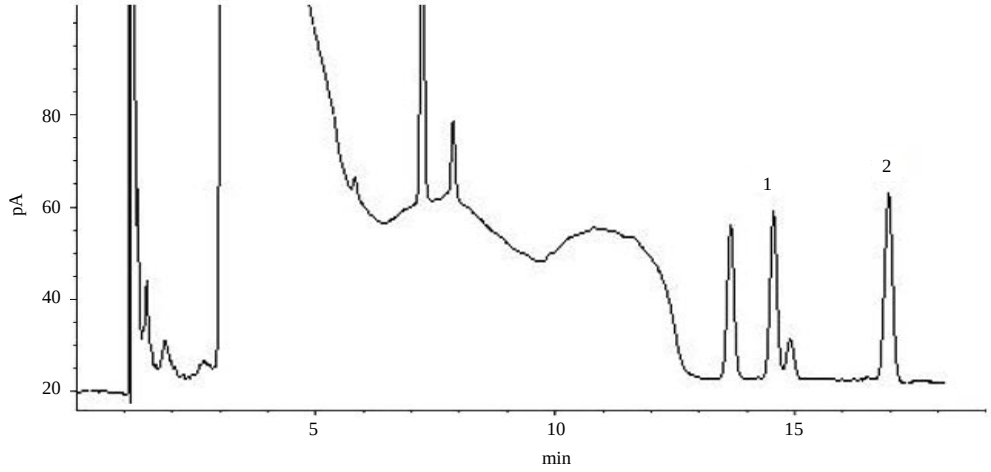


Fig. S8: Blank heart sample

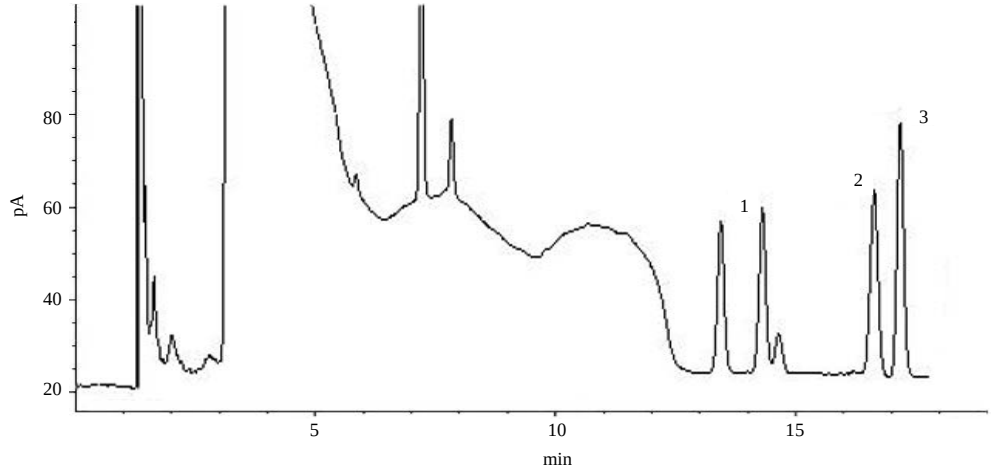


Fig. S9: Blank heart sample spiked with internal standard

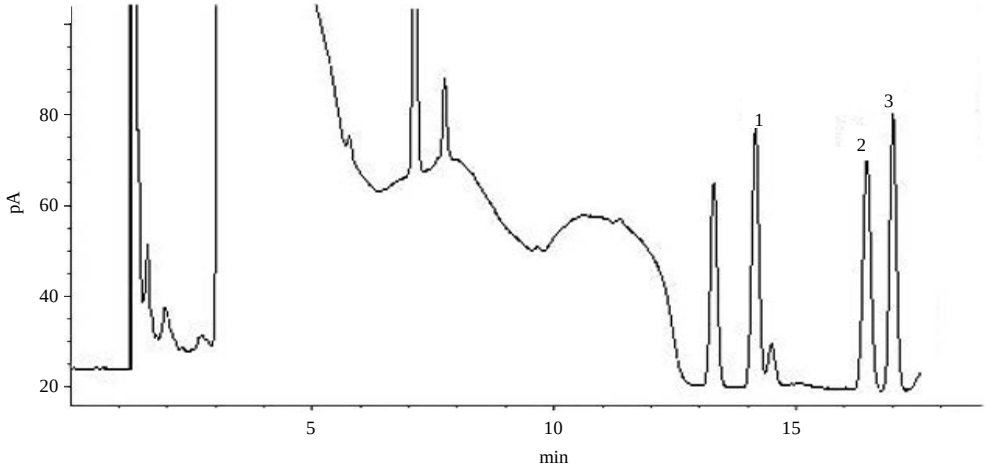


Fig. S10: Heart sample obtained after rectal administration of BJOS

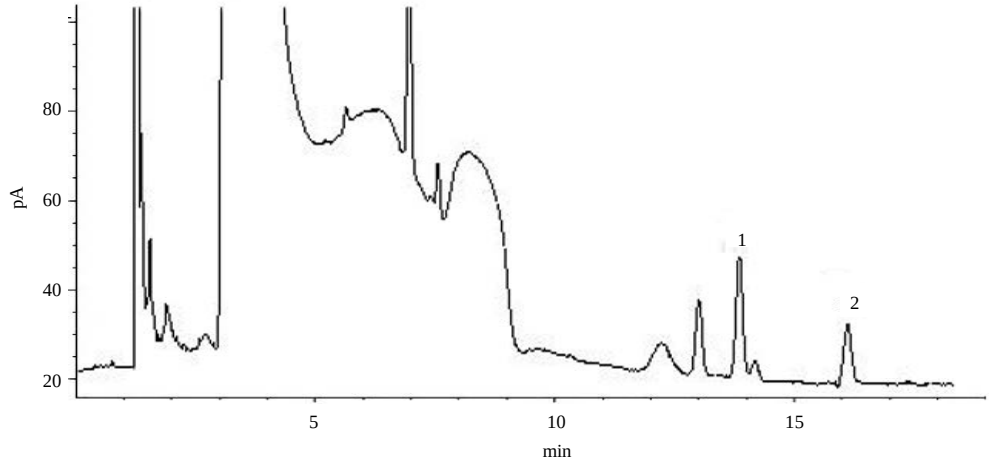


Fig. S11: Blank liver sample

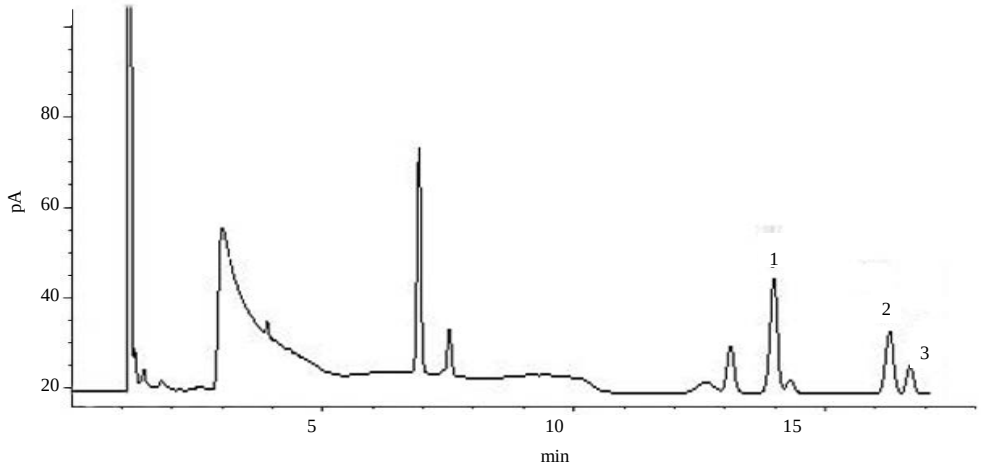


Fig. S12: Blank liver sample spiked with internal standard

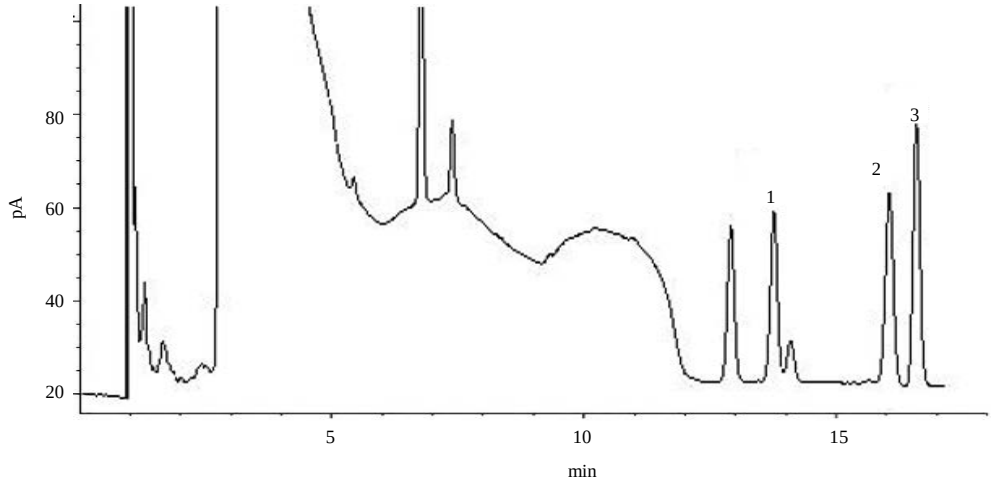


Fig. S13: Liver sample obtained after rectal administration of BJOS

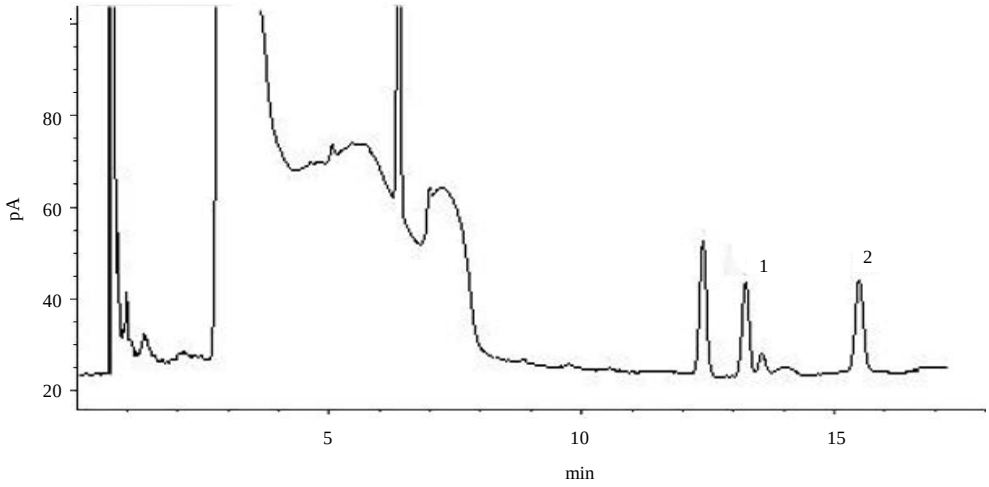


Fig. S14: Blank spleen sample

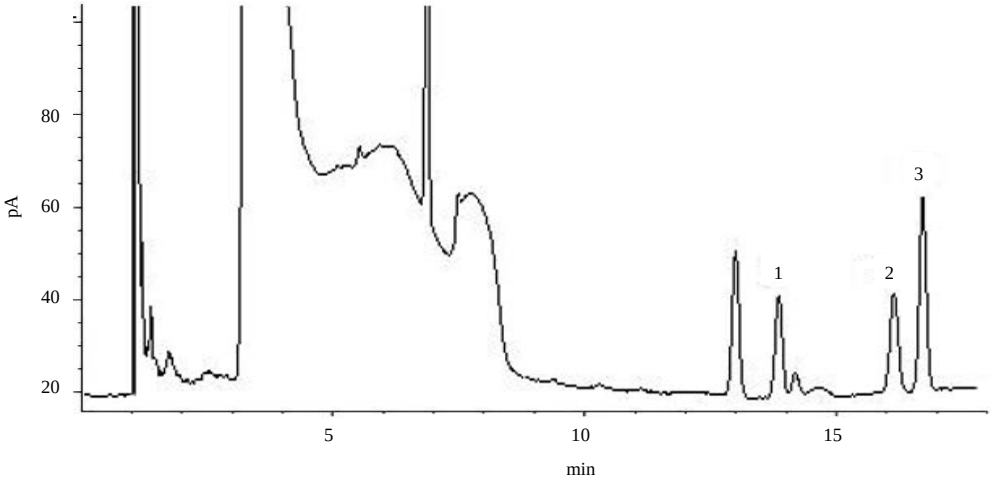


Fig. S15: Blank spleen sample spiked with internal standard

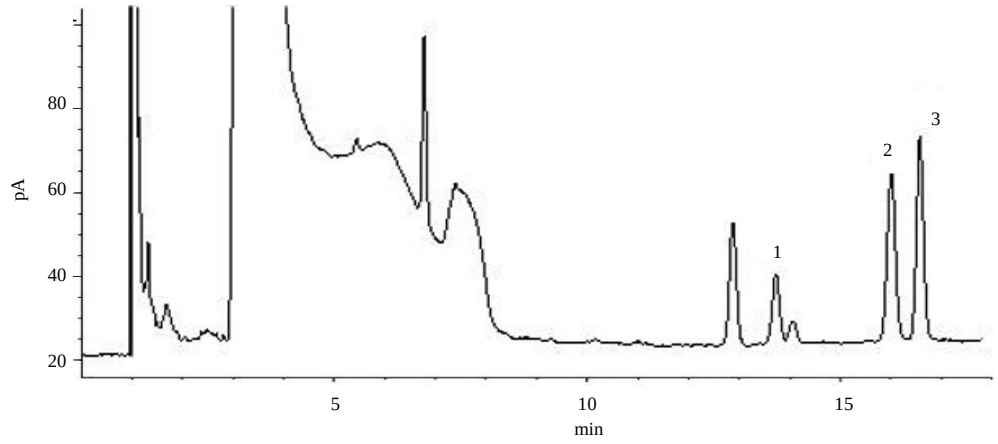


Fig. S16: Spleen sample obtained after rectal administration of BJOS

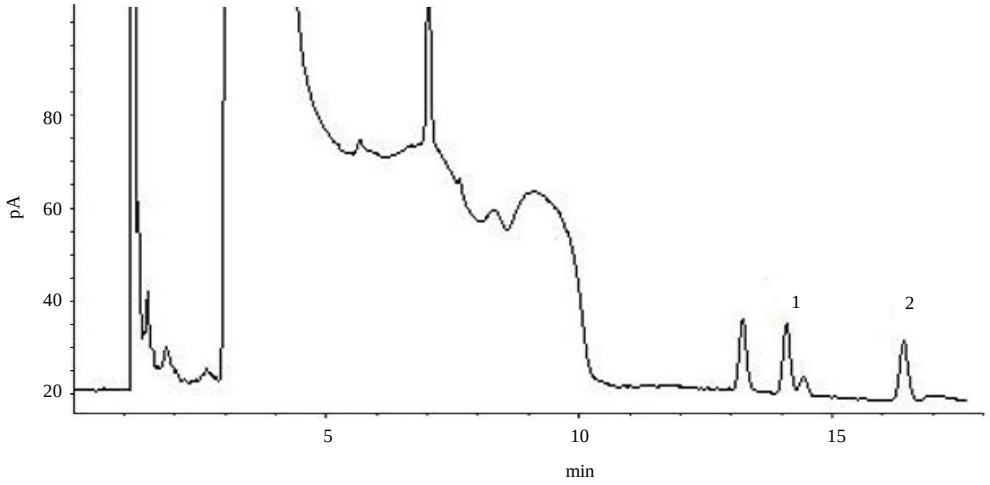


Fig. S17: Blank lung sample

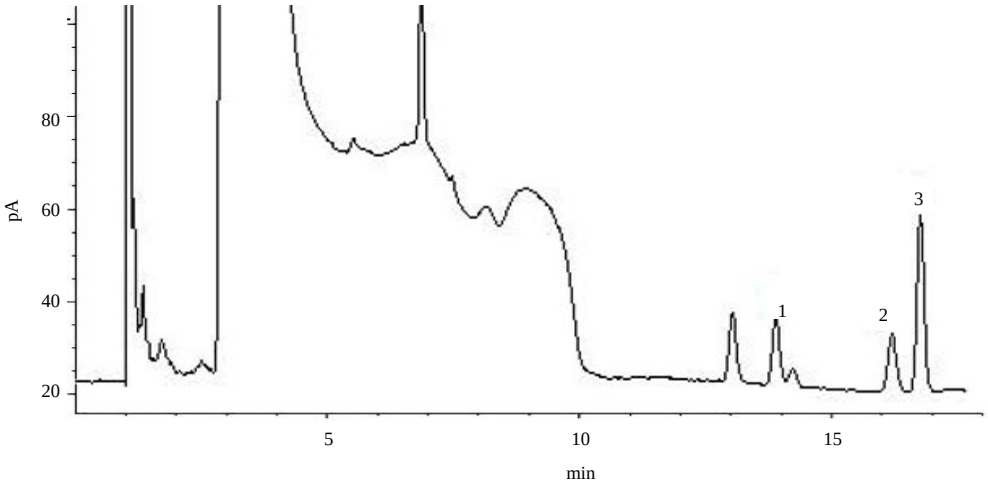


Fig. S18: Blank lung sample spiked with internal standard

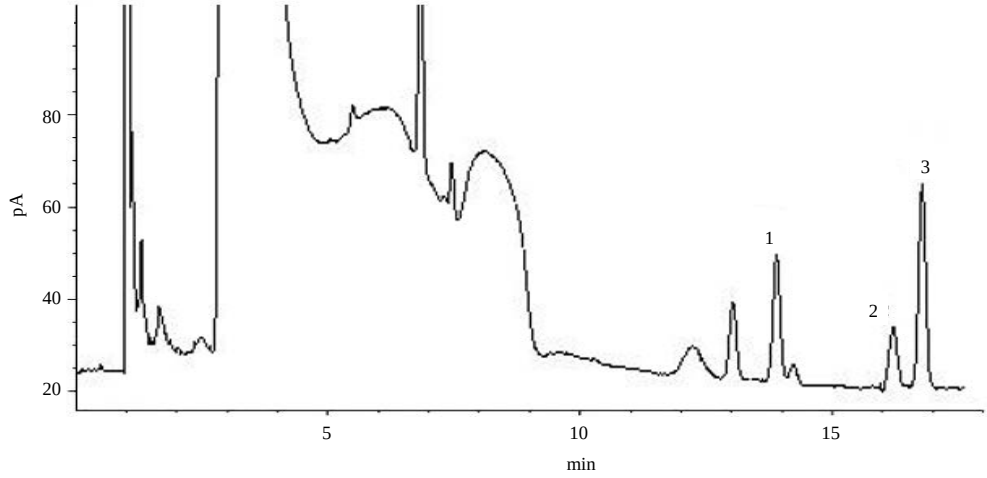


Fig. S19: Lung sample obtained after rectal administration of BJOS

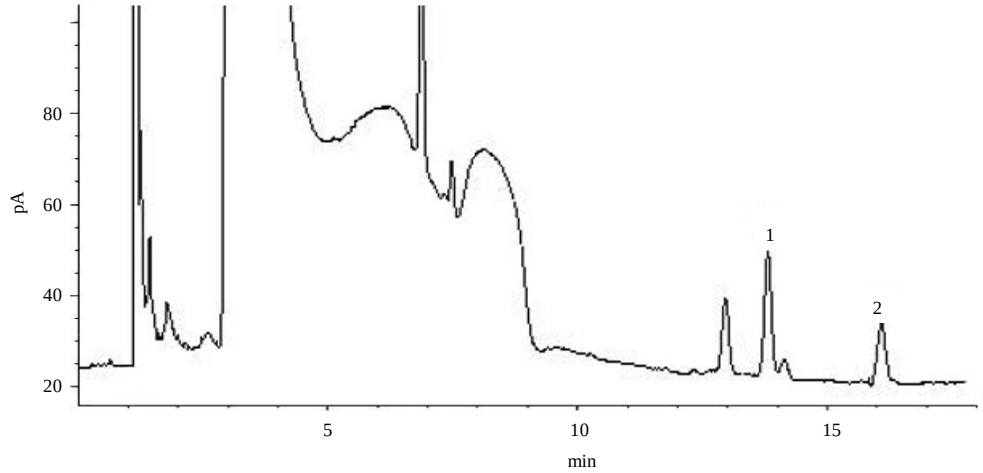


Fig. S20: Blank kidney sample

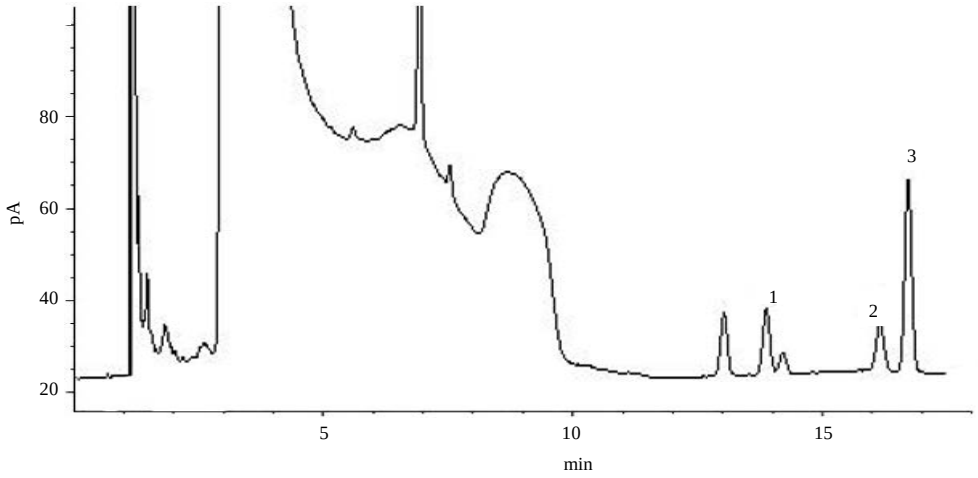


Fig. S21: Blank kidney sample spiked with internal standard

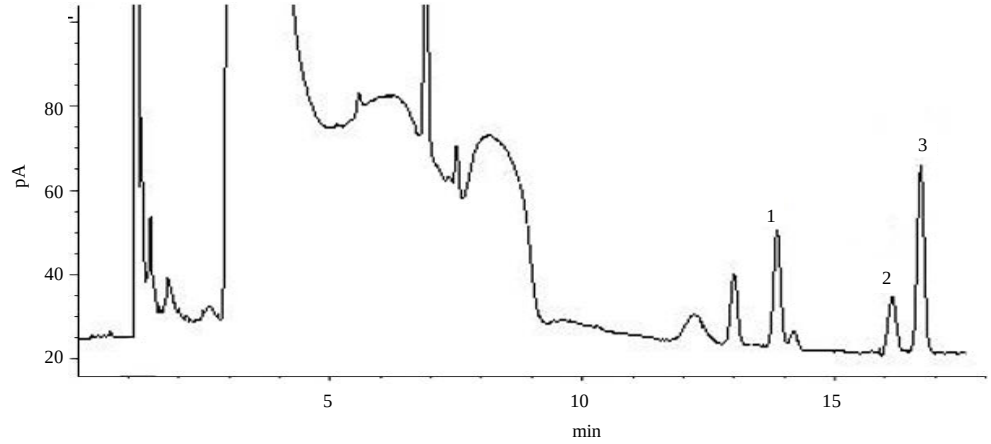


Fig. S22: Kidney sample obtained after rectal administration of BJOS

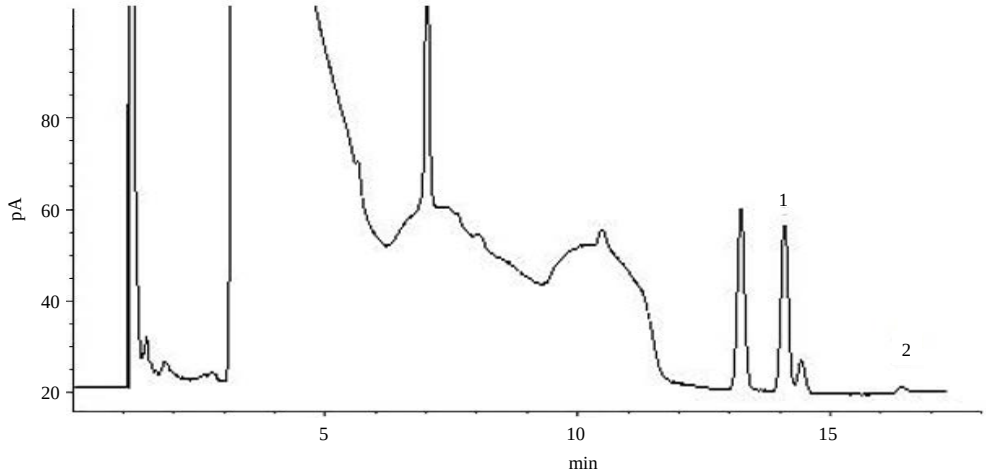


Fig. S23: Blank brain sample

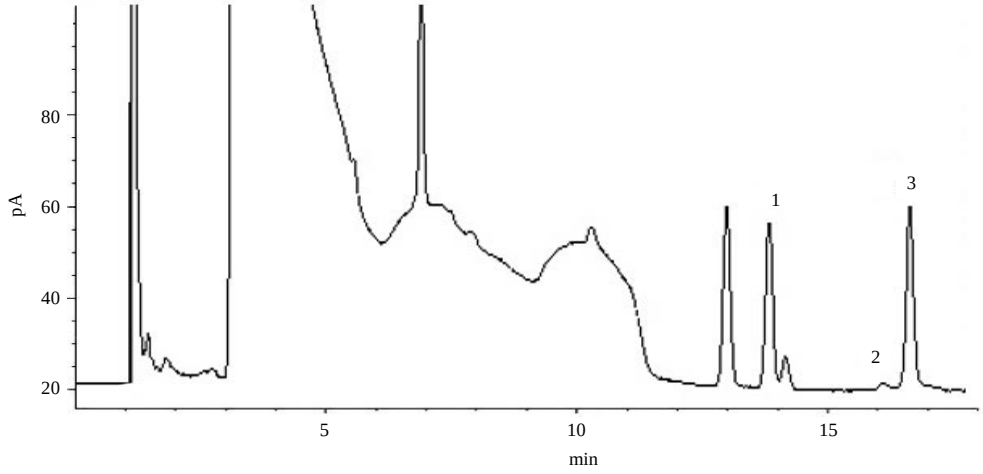


Fig. S24: Blank brain sample spiked with internal standard

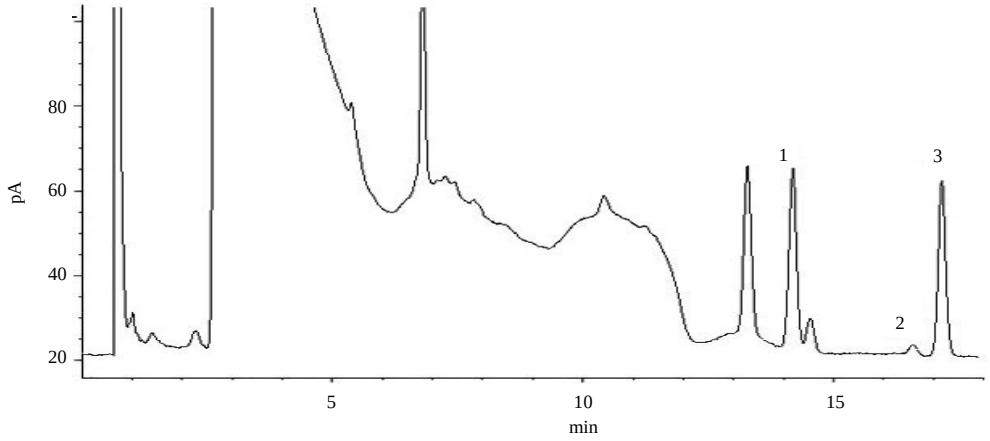


Fig. S25: Brain sample obtained after rectal administration of BJOS

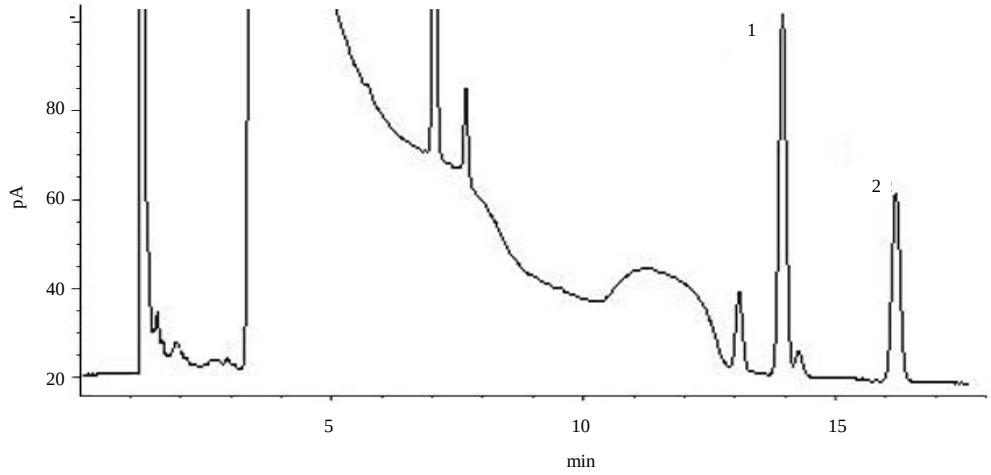


Fig. S26: Blank prostate sample

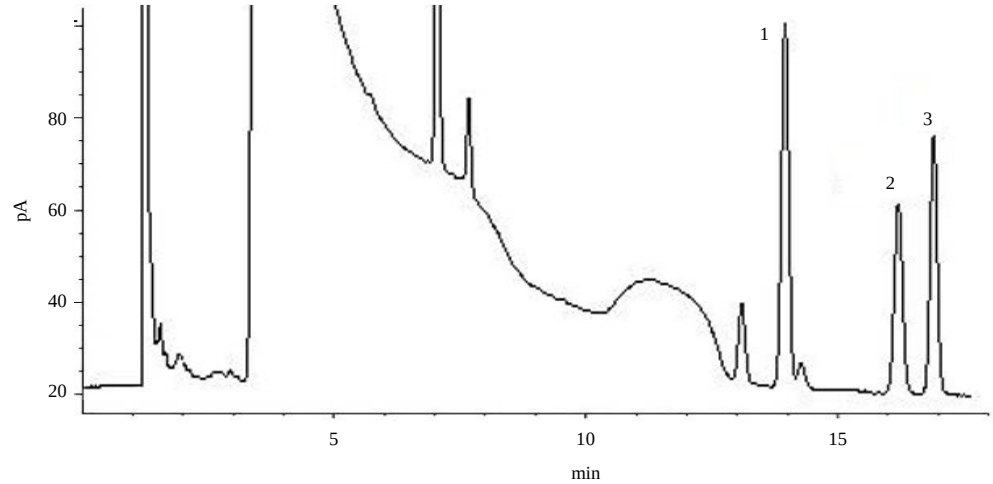


Fig. S27: Blank prostate sample spiked with internal standard

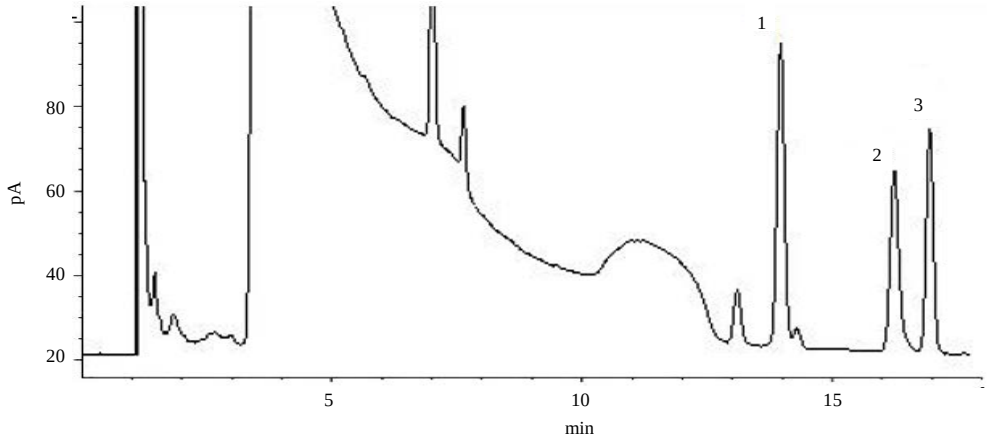


Fig. S28: Prostate sample obtained after rectal administration of BJOS

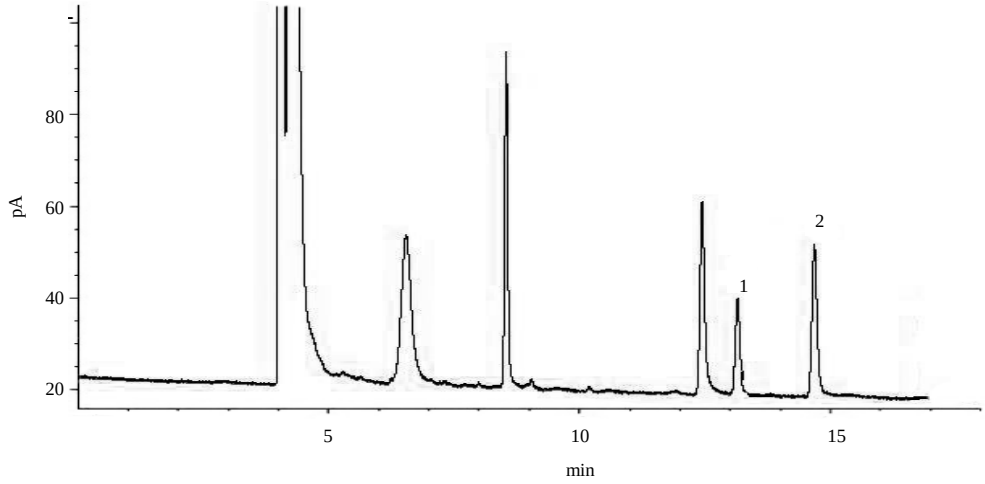


Fig. S29: Blank rectum sample

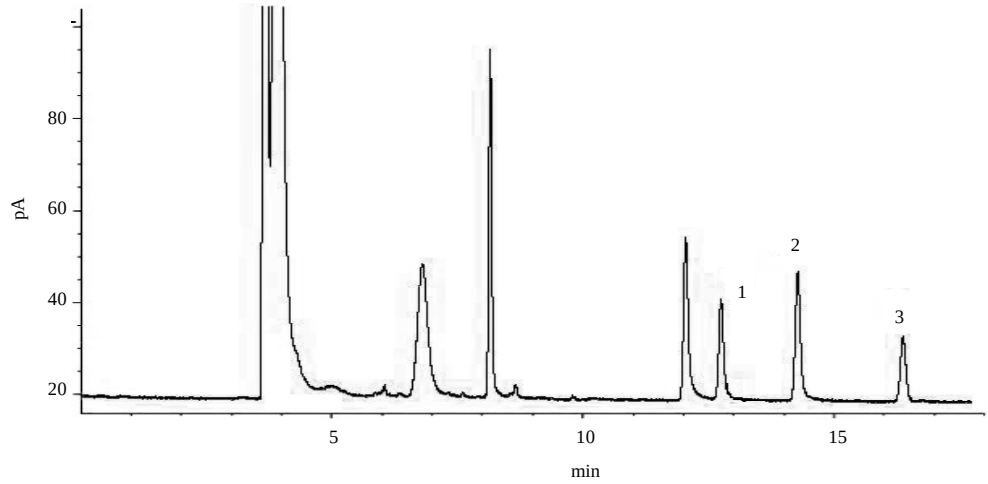


Fig. S30: Blank rectum sample spiked with internal standard

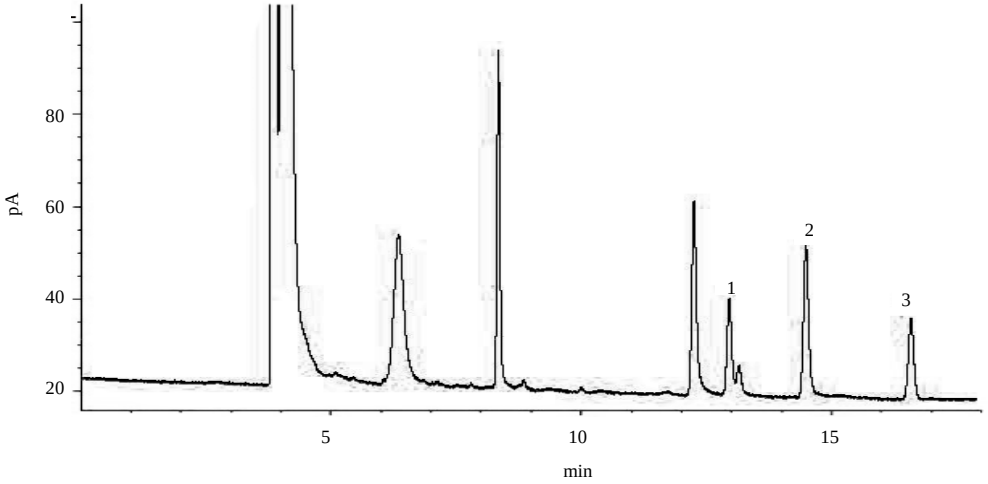


Fig. S31: Rectum sample obtained after rectal administration of BJOS

**Linearity and LLOQ**

Table S1: Calibration curves and linear ranges of oleic and linoleic acids in plasma and tissues in rats

| Tissues | Analytes | Regression equation | Linear range (μg mL$^{-1}$) | Correlation coefficient | LLOQ (μg mL$^{-1}$) |
|---|---|---|---|---|---|
| Plasma | Oleic acid | Y = 0.0041X+0.0428 | 15.62-1562 | 0.9984 | 15.62 |
| | Linoleic acid | Y = 0.0037X+0.0028 | 16.48-1648 | 0.9992 | 16.48 |
| Heart | Oleic acid | Y = 0.0142X+0.038 | 12.50-248.05 | 0.9997 | 12.50 |
| | Linoleic acid | Y = 0.0135X+0.0313 | 13.18-241.00 | 0.9998 | 13.18 |
| Liver | Oleic acid | Y = 0.0138X+0.047 | 12.50-248.05 | 0.9990 | 12.50 |
| | Linoleic acid | Y = 0.0132X+0.0412 | 13.18-241.00 | 0.9978 | 13.18 |
| Spleen | Oleic acid | Y = 0.0164X+0.068 | 12.50-248.05 | 0.9997 | 12.50 |
| | Linoleic acid | Y = 0.0128X+0.0268 | 13.18-241.00 | 0.9997 | 13.18 |
| Lung | Oleic acid | Y = 0.0145X+0.104 | 12.50-248.05 | 0.9991 | 12.50 |
| | Linoleic acid | Y = 0.0142X+0.0123 | 13.18-241.00 | 0.9993 | 13.18 |
| Kidney | Oleic acid | Y = 0.0165X+0.052 | 12.50-248.05 | 0.9993 | 12.50 |
| | Linoleic acid | Y = 0.0135X+0.0152 | 13.18-241.00 | 0.9995 | 13.18 |
| Brain | Oleic acid | Y = 0.0182X+0.038 | 12.50-248.05 | 0.9996 | 12.50 |
| | Linoleic acid | Y = 0.0152X+0.0183 | 13.18-241.00 | 0.9993 | 13.18 |
| Rectum | Oleic acid | Y = 0.0132X+0.034 | 12.50-248.05 | 0.9995 | 12.50 |
| | Linoleic acid | Y = 0.0134X+0.0236 | 13.18-241.00 | 0.9995 | 13.18 |
| Prostate | Oleic acid | Y = 0.0187X+0.075 | 12.50-248.05 | 0.9993 | 12.50 |
| | Linoleic acid | Y = 0.0137X+0.0155 | 13.18-241.00 | 0.9968 | 13.18 |

**Precision**

Table S2: Precision of the method for the analysis of oleic and linoleic acids in plasma and tissues in rats (3 days with 3 repetitions per day)

| | | RSD (%) | | | RSD (%) | |
|---|---|---|---|---|---|---|
| Tissues | Oleic acid (μg mL$^{-1}$) | Intra-day | Inter-day | Linoleic acid (μg mL$^{-1}$) | Intra-day | Inter-day |
| Plasma | 30.70 | 4.30 | 6.00 | 33.81 | 3.60 | 2.00 |
| | 122.80 | 6.30 | 3.00 | 135.20 | 5.70 | 3.90 |
| | 491.20 | 1.70 | 2.10 | 541.00 | 1.30 | 1.00 |
| Heart | 62.02 | 1.35 | 3.36 | 60.25 | 1.58 | 2.54 |
| | 93.03 | 1.33 | 3.24 | 90.38 | 1.26 | 2.86 |
| | 155.05 | 1.42 | 2.55 | 150.63 | 1.57 | 2.49 |
| Liver | 62.02 | 1.22 | 4.21 | 60.25 | 1.43 | 4.29 |
| | 93.03 | 1.34 | 4.35 | 90.38 | 1.58 | 4.36 |
| | 155.05 | 1.02 | 4.69 | 150.63 | 1.64 | 4.81 |

Table S2: Continue

| | | RSD (%) | | | RSD (%) | |
|---|---|---|---|---|---|---|
| Tissues | Oleic acid (μg mL$^{-1}$) | Intra-day | Inter-day | Linoleic acid (μg mL$^{-1}$) | Intra-day | Inter-day |
| Spleen | 62.02 | 2.12 | 3.82 | 60.25 | 1.84 | 2.34 |
| | 93.03 | 2.09 | 3.45 | 90.38 | 1.67 | 2.16 |
| | 155.05 | 1.87 | 3.68 | 150.63 | 1.56 | 2.48 |
| Lung | 62.02 | 1.56 | 5.64 | 60.25 | 1.65 | 3.46 |
| | 93.03 | 1.63 | 5.26 | 90.38 | 1.89 | 3.59 |
| | 155.05 | 1.75 | 6.17 | 150.63 | 1.54 | 3.75 |
| Kidney | 62.02 | 1.35 | 3.38 | 60.25 | 1.38 | 2.67 |
| | 93.03 | 1.54 | 3.16 | 90.38 | 1.61 | 3.14 |
| | 155.05 | 1.46 | 2.78 | 150.63 | 1.35 | 3.52 |
| Brain | 62.02 | 1.26 | 4.15 | 60.25 | 1.26 | 4.15 |
| | 93.03 | 1.21 | 4.34 | 90.38 | 1.34 | 3.85 |
| | 155.05 | 1.18 | 4.03 | 150.63 | 1.25 | 3.49 |
| Rectum | 62.02 | 1.61 | 3.14 | 60.25 | 1.31 | 3.23 |
| | 93.03 | 1.38 | 4.42 | 90.38 | 2.13 | 3.46 |
| | 155.05 | 2.53 | 3.56 | 150.63 | 2.07 | 3.62 |
| Prostate | 62.02 | 2.61 | 4.14 | 60.25 | 2.31 | 3.20 |
| | 93.03 | 2.38 | 4.52 | 90.38 | 2.15 | 3.35 |
| | 155.05 | 2.55 | 3.56 | 150.63 | 2.07 | 3.92 |

**Extraction recovery**

Table S3: Extraction and relative recoveries of oleic and linoleic acids in rat plasma and tissues (n = 5)

| | | | Extraction recovery | | Relative recovery | |
|---|---|---|---|---|---|---|
| Tissues | Analytes | Concentration (μg mL$^{-1}$) | Mean | RSD (%) | Mean | RSD (%) |
| Plasma | Oleic acid | 30.70 | 103.90 | 2.6 | 99.16 | 4.3 |
| | | 122.80 | 95.69 | 5.4 | 92.80 | 6.3 |
| | | 491.20 | 105.10 | 1.7 | 102.40 | 1.7 |
| | Linoleic acid | 33.81 | 93.06 | 3.2 | 98.36 | 3.6 |
| | | 135.20 | 92.53 | 4.4 | 99.47 | 5.7 |
| | | 541.00 | 95.28 | 1.3 | 102.90 | 1.3 |
| Heart | Oleic acid | 62.02 | 88.80 | 1.3 | 96.60 | 2.5 |
| | | 93.03 | 83.30 | 1.5 | 91.00 | 2.4 |
| | | 155.05 | 91.60 | 1.6 | 93.30 | 2.7 |
| | Linoleic acid | 60.25 | 92.20 | 1.8 | 102.90 | 2.5 |
| | | 90.38 | 85.90 | 2.1 | 98.40 | 2.6 |
| | | 150.63 | 93.30 | 2.5 | 98.30 | 2.5 |
| Liver | Oleic acid | 62.02 | 91.90 | 3.2 | 97.40 | 5.4 |
| | | 93.03 | 95.80 | 3.5 | 105.70 | 6.1 |
| | | 155.05 | 84.60 | 4.7 | 92.00 | 4.3 |
| | Linoleic acid | 60.25 | 86.60 | 2.9 | 97.60 | 4.5 |
| | | 90.38 | 82.20 | 2.4 | 93.70 | 5.1 |
| | | 150.63 | 94.90 | 2.7 | 92.70 | 4.6 |
| Spleen | Oleic acid | 62.02 | 108.70 | 3.9 | 93.40 | 5.1 |
| | | 93.03 | 86.40 | 3.4 | 89.00 | 4.3 |
| | | 155.05 | 89.70 | 2.7 | 95.20 | 2.7 |
| | Linoleic acid | 60.25 | 111.10 | 3.5 | 98.10 | 5.0 |
| | | 90.38 | 118.40 | 3.1 | 96.20 | 4.3 |
| | | 150.63 | 85.00 | 2.7 | 96.00 | 3.3 |
| Lung | Oleic acid | 62.02 | 87.90 | 5.4 | 106.00 | 4.5 |
| | | 93.03 | 111.90 | 5.3 | 91.50 | 4.5 |
| | | 155.05 | 115.60 | 6.5 | 95.10 | 6.3 |
| | Linoleic acid | 60.25 | 93.40 | 4.6 | 98.90 | 5.6 |
| | | 90.38 | 116.50 | 4.1 | 96.50 | 5.1 |
| | | 150.63 | 117.70 | 4.8 | 95.90 | 6.8 |

Table S3: Continue

| Tissues | Analytes | Concentration (μg mL$^{-1}$) | Extraction recovery | | Relative recovery | |
|---|---|---|---|---|---|---|
| | | | Mean | RSD (%) | Mean | RSD (%) |
| Kidney | Oleic acid | 62.02 | 80.20 | 2.4 | 92.60 | 3.4 |
| | | 93.03 | 104.70 | 2.6 | 95.50 | 2.1 |
| | | 155.05 | 81.10 | 3.1 | 91.30 | 3.3 |
| | Linoleic acid | 60.25 | 84.80 | 2.1 | 93.10 | 3.7 |
| | | 90.38 | 110.10 | 2.3 | 96.30 | 2.6 |
| | | 150.63 | 86.90 | 1.9 | 92.30 | 3.4 |
| Brain | Oleic acid | 62.02 | 85.50 | 3.4 | 91.80 | 2.4 |
| | | 93.03 | 86.40 | 2.1 | 94.00 | 2.7 |
| | | 155.05 | 83.60 | 1.8 | 90.80 | 3.1 |
| | Linoleic acid | 60.25 | - | - | - | - |
| | | 90.38 | - | - | - | - |
| | | 150.63 | - | - | - | - |
| Rectum | Oleic acid | 62.02 | 86.80 | 5.7 | 96.80 | 6.2 |
| | | 93.03 | 86.60 | 7.6 | 97.60 | 5.4 |
| | | 155.05 | 84.40 | 6.3 | 95.10 | 6.3 |
| | Linoleic acid | 60.25 | 91.10 | 5.1 | 91.10 | 6.3 |
| | | 90.38 | 89.70 | 6.3 | 91.70 | 4.4 |
| | | 150.63 | 90.80 | 5.4 | 90.50 | 7.4 |
| Prostate | Oleic acid | 62.02 | 106.80 | 6.7 | 97.20 | 7.3 |
| | | 93.03 | 96.60 | 6.4 | 105.10 | 7.5 |
| | | 155.05 | 94.00 | 7.3 | 104.20 | 8.2 |
| | Linoleic acid | 60.25 | 117.10 | 6.1 | 102.70 | 7.9 |
| | | 90.38 | 119.70 | 6.4 | 104.70 | 7.3 |
| | | 150.63 | 90.40 | 6.4 | 108.40 | 8.1 |

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