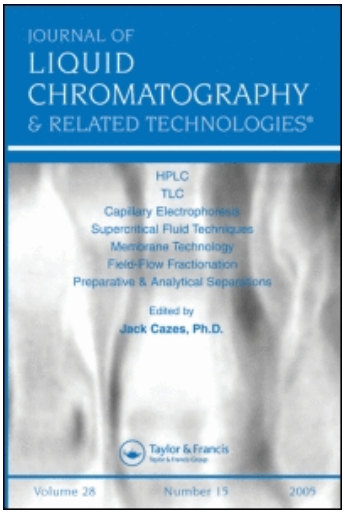

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DETERMINATIONS OF TRILINOLEIN AND 1,2-DILINOLEOYL-3-OLEOYL-GLYCEROL IN VARIOUS PANAX GINSENG BY HPLC

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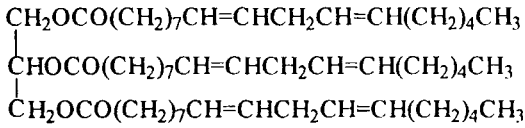
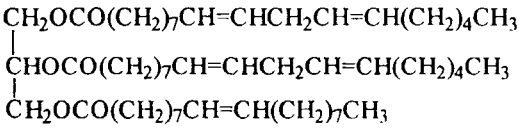
ABSTRACT

A simultaneous analysis of trilinolein and 1,2-dilinoleoyl-3-oleoyl-glycerol (DLO) in *Panax ginseng* C. A. Meyer (ginseng) by a high performance liquid chromatographic method was used in the analysis of various Ginseng Radix. Chromatographic analysis is achieved on an isocratic system consisting of a polymeric reversed phase C_{18} column with a mobile phase of acetonitrile-methanol (50:50, v/v) to elute the trilinolein and DLO. The system was detected at 205 nm. The results indicate that various ginseng extraction from Korean, Japanese and Chinese by n-hexane contained 0.37 ± 0.009, 0.39 ± 0.016, and 0.27 ± 0.009 mg/g, respectively for trilinolein; and 0.41 ± 0.009, 0.45 ± 0.01, and 0.22 ± 0.008 mg/g, respectively for DLO. Quantitative determination of the triacylglycerol content in different parts of ginseng showed that the contents were in the following order: rhizome head > main root > root hair.



Trilinolein



1,2-Dilinoleoyl-3-oleoyl-rac-glycerol

Figure 1. Chemical structures of trilinolein and 1,2-dilinoleoyl-3-oleoyl-*rac*-glycerol (DLO).

INTRODUCTION

Radix Ginseng, is the dry root of *Panax ginseng* C.A. Meyer (Araliaceae), a worldwide well-known traditional Chinese medicine with the popular name "ginseng"[1]. The root of ginseng contains about 4% of ginsenosides, which are generally considered as the pharmacologically active components of ginseng[2]. Except for ginsenosides, the other group of active components, triacylglycerol has been found in Panax pseudo-ginseng[3]. It has been recently reported that trilinolein of pseudo-ginseng inhibits adrenaline-induced human platelet aggregation[4]. This inhibition of trilinolein was accompanied by reduced ATP release and thromboxane B_2 formation[3]. During cardiopulmonary bypass, trilinolein also improves the erythrocyte deformability.[5,6] The typical triacylglycerol, trilinolein and 1,2-dilinoleoyl-3-oleoyl-*rac*-glycerol (DLO) (Figure 1) are triacylglycerol in all three esterified positions of glycerol with linoleic acid or oleic acid, respectively. In our unpublished data it was shown that DLO also possess pharmacological effects in the cardiovascular system. The content of triacylglycerol in ginseng may be one of the major active principles.[7]

In order to further study the actions of ginseng, it is important to investigate triacylglycerol content in original material ginseng. In this work, we used a polymeric reversed phase liquid chromatographic method for the determination of trilinolein and DLO in the content of various ginseng and different parts of ginseng.

MATERIALS AND METHODS

Chemicals and Reagents

Ginseng was purchased from a traditional oriental herbal drug store in Taipei. The origin of material ginseng was identified by the botanist at the National Research Institute of Chinese Medicine. Authentic compounds, trilinolein and DLO, were obtained from Sigma Chem. (St. Louis, MO, USA). Acetonitrile (HPLC far UV grade), n-hexane and methanol (HPLC grade) were obtained from LabScan Chem. (Dublin, Ireland). The stock solutions of trilinolein and DLO were dissolved in n-hexane at a concentration of 1 mg/mL.

Apparatus and Chromatography

The HPLC system consisted of an injector (Rheodyne 7125, Cotati, CA, USA), a variable wavelength UV-VIS detector (Soma, Tokyo, Japan) and a chromatographic pump (ICI model 1110, Australia). Separation was achieved on a HEMA polymeric reversed-phased C_{18} column, 250 x 4 mm, particle size 10 μm (Tessek A/S, The Science Park, Aarhus C, Denmark). The mobile phase was acetonitrile-methanol (50:50, v/v), and the flow rate was 1.0 mL/min. Triacylglycerols were monitored at a wavelength of 205 nm throughout the experiments. The system was operated at room temperature (25°C)..

Extraction

Ginseng powder (0.5 g) was boiled with 50 mL of n-hexane for 10 min. This procedure was repeated twice. The two filtrates were combined and diluted to 100 mL in a volumetric flask.

Precision

To determine the intra-assay variance of trilinolein and DLO, quadruplicate assays were carried out at the same concentrations (1, 5 or 20 μg/mL), at different times during the day.

Inter-assay variance of trilinolein and DLO were determined by assaying in quadruplicate, on days one, two, four and six. Coefficients of variation (C.V.s) were calculated from these values.

Determination of Trilinolein and DLO

Calibration graphs for trilinolein and DLO dissolved in n-hexane were constructed by HPLC of various known amounts of these compounds (0.5, 1, 5, 10 and 20 μg/mL). The contents of trilinolein and DLO in the crude extract of ginseng were determined by the regression equation for the peak area under the curve versus concentrations of these two compounds.

RESULTS AND DISCUSSION

Identification of trilinolein and DLO in the crude extract of ginseng was substantiated by liquid chromatography in the following four modes: (1) on the correct retention times of trilinolein and DLO, compare the retention times of trilinolein and DLO with crude extract of ginseng and authentic standards, respectively; (2) on the correct concentrations of trilinolein and DLO, after spiking with authentic compounds (trilinolein or DLO), in the crude extract of ginseng and measured their peak areas; the peak areas of trilinolein and DLO should be additive; (3) on the modification of mobile phase; by changing the ratio of acetonitrile:methanol from 5:5 to 3:7 (v/v), the retention times of trilinolein and DLO should be delayed owing to the ratio of acetonitrile bring decreased; (4) on the alteration of UV absorbance wavelength; by changing the wavelength from 205 nm to 230 nm, the peaks of trilinolein and DLO should disappear because peaks of trilinolein and DLO both do not show absorbance at 230 nm.

Under the conditions described above, the retention times of trilinolein and DLO were found to be 9.23 and 12.27 min, respectively (Figure 2). The detection limit for trilinolein and DLO, at signal-to-noise ratio of 3, were 0.1 and 0.5 μg/mL, respectively.

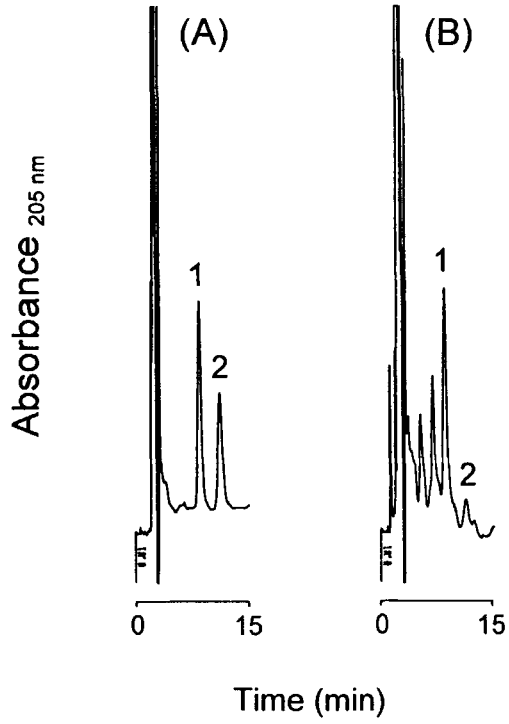


Figure 2. Elution profiles of the injection 20 μL of (A) mixture of trilinolein and 1,2-dilinoleoyl-3-oleoyl-glycerol (DLO), (B) extract of *Panax ginseng* C. A. Meyer was separated by a polymeric reversed phase C_{18} column with 205 nm. 1: trilinolein; 2: DLO.

The contents of trilinolein and DLO in the crude ginseng extract were determined from the linear regression equation of the calibration graphs for these compounds. The calibration curves of trilinolein and DLO were Y = 5.30E-5 X + 0.037 (r^2 = 0.999) and Y = 4.39E-5 X + 0.65 (r^2 = 0.998), respectively; here X is peak-area response and Y is amount of compound. The linearity ranges of trilinolein and DLO were 0.5-20 μg/mL.

The intra-assay C.V.s for the determination of trilinolein and DLO at concentrations of 1, 5, and 20 μg/mL were acceptable with C.V.s of less than 10 %. The inter-assay C.V.s for trilinolein and DLO at the same concentrations were less than 10 %.

Table 1

Trilinolein and 1,2-Dilinoleoyl-3-Oleoyl-Glycerol (DLO) Contents (Mg/G) in Various Ginseng Growth in Different Areas

Origin	Trilinolein	DLO
Korea	0.37 ± 0.009	0.41± 0.009
Japan	0.39 ± 0.016	0.45 ± 0.012
China	0.27 ± 0.009	0.22 ± 0.008

Data are expressed as mean ± SEM (n=6).

Table 2

Trilinolein and 1,2-Dilinoleoyl-3-Oleoyl-Glycerol (DLO) Contents (Mg/G) in Different Parts of China Ginseng

Different parts	Trilinolein	DLO
Rhizome head	0.34 ± 0.005	0.33 ± 0.015
Main root	0.27 ± 0.009	0.22 ± 0.008
Root Hair	0.25 ± 0.006	0.33 ± 0.024

Data are expressed as mean ± SEM (n=6).

Table 1 summarized the contents of trilinolein and DLO in n-hexane extracts of various ginseng. The highest yields of trilinolein and DLO were found in Korean and Japanese ginseng, respectively.

Table 2 shows the contents of different parts of ginseng. The data indicated that the contents were in the following order: rhizome head > main root > root hair for trilinolein; rhizome head = root hair > main root for DLO.

In conclusion, the proposed technique should be useful for the quality control, stability or pharmacokinetics of trilinolein and DLO in the traditional drug of ginseng.

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