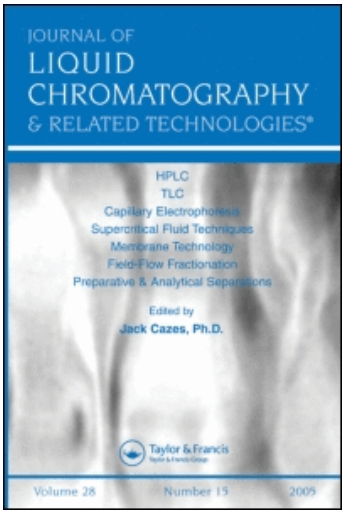

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DETERMINATION OF TRIACYLGLYCEROLS IN *PANAX PSEUDO-GINSENG* BY HPLC POLYMERIC COLUMN

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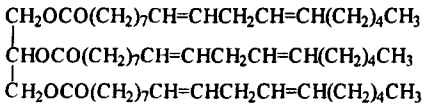
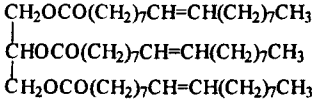
ABSTRACT

An improved high performance liquid chromatographic method was developed to determinate the active triacylglycerol principles of *Panax pseudo-ginseng* Wall. var. notoginseng Hoo & Tseng extracts. Chromatographic analysis is achieved on an isocratic system consisting of a polymeric reverse phase C_{18} column with a mobile phase of acetonitrile-methanol (50:50, v/v) to elute the trilinolein and triolein. The eluted triacylglycerol are detected at 205 nm. The linear calibration range was 0.5-20 and 20-500 μg/mL for trilinolein and triolein, respectively. The detection limit was 0.1 μg/mL for trilinolein and 10 μg/mL for triolein. It was found that n-hexane extracts of *Panax pseudo-ginseng* Wall. var. notoginseng Hoo & Tseng contained 0.21±0.021 mg/g of trilinolein. However, triolein was not detectable in the same herbal extract.



Trilinolein



Triolein

Figure 1. Chemical structures of trilinolein and triolein.

INTRODUCTION

Trilinolein and triolein (Fig. 1) are triacylglycerol in all three esterified positions of glycerol with linoleic acid and oleic acid, respectively. Trilinolein is also the active principle of the root of *Panax pseudo-ginseng* Wall. var. notoginseng (Burk.) Hoo & Tseng (Chinese name: Sanchi).[1] Sanchi is a herbal drug widely used in traditional Chinese medicine for the treatment of cardiovascular diseases.[2]

It was recently reported that, both saponins[3] and trilinolein[4] of Sanchi inhibit adrenaline-induced human platelet aggregation. This inhibition of trilinolein was accompanied by reduced ATP release and thromboxane B_2 formation[1]. During the cardiopulmonary bypass, trilinolein also improves the erythrocyte deformability.[5,6]

To determine fatty acids or triacylglycerols, a straight phase with silica column,[7] cyanopropyl column,[8] reverse phase[9] HPLC with UV detection, pre-column[10] and post-column[11,12] with fluorescent detection and HPLC-mass[13] have been reported. However, most of the methods for lipids measurement are not generally specific for trilinolein or triolein. Although the deuterium-labeled of [$^2H_{12}$]trilinolein and [$^2H_{18}$]triolein have been synthesized,[1] the contamination of radioisotope-labeled concerns prohibit their applications. In this work, we used a polymeric reverse phase method for the determination of trilinolein and triolein in the content of *Panax pseudo-ginseng* Wall. var. notoginseng (Burk.) Hoo & Tseng.

MATERIALS AND METHODS

Chemicals and Reagents

Panax pseudo-ginseng Wall. var. notoginseng (Burk.) Hoo & Tseng was purchased from a traditional Chinese herbal drug store in Taipei. Authentic compounds, trilinolein and triolein were obtained from Sigma Chem. (St. Louis, MO, USA). Acetonitrile (HPLC far UV grade), n-hexane, ethanol and methanol (HPLC grade) were obtained from LabScan Chem. (Dublin, Ireland). The stock solutions of trilinolein and triolein were dissolved in n-hexane at 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 (Waters model 510). Separation was achieved on a HEMA reverse phased C_{18} polymer column, 250 x 4 mm, particle size 10 μm (P.J. Cobert Asso. St. Louis, MO, USA). 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

Panax pseudo-ginseng Wall. var. notoginseng Hoo & Tseng powder (0.5 g) was boiled with 50 mL of extraction solvent [n-hexane, methanol, ethanol (99.5 %), ethanol (50 %) or water] 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, quadruplicate assays were carried out on the same concentrations (1, 5 or 20 μg/mL) at different times during the day. Inter-assay variance was determined by assaying in quadruplicate, on days one, two, four and six. Coefficients of variation (C.V.s) were calculated from these values.

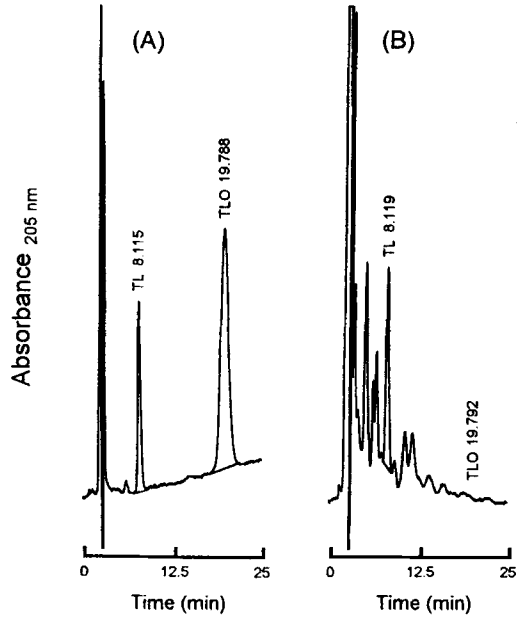


Figure 2. Elution profile of the injection 20 μL of (A) mixture of TL (trilinolein; 10 μg/mL) and TLO (triolein; 250 μg/mL), (B) extract of *Panax pseudo-ginseng* Wall. var. notoginseng Hoo & Tseng (20 mL/g) was separated by a polymeric reverse phase C_{18} column with 205 nm.

Determination of Trilinolein and Triolein

Calibration graphs for trilinolein and triolein 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 triolein in the crude extract of *Panax pseudo-ginseng* Wall. var. notoginseng Hoo & Tseng were determined by the regression equation for the area under the curve versus concentration of these two compounds.

RESULTS AND DISCUSSION

Ultraviolet detector was a popular HPLC detector for lipids analysis[15]. However, the only functional groups detectable in the UV region (190-210 nm) are the carbonyl (C=O) and double bonds in the fatty acid chains (C=C)[16]. Thus, monitoring the elution of triacyl glycerols UV detection at 205 nm is

Table 1

Contents of Trilinolein and Triolein in Different Extracts of 1 Gm. of *Panax Pseudo-Ginseng* Wall. Var. Notoginseng Hoo & Tseng.

Extraction Solvents	Trilinolein	Triolein
n-Hexane	0.21 ± 0.021	n.d.
Methanol	n.d	n.d
Ethanol (99.5 %)	n.d.	n.d.
Ethanol (50 %)	n.d.	n.d.
Water	n.d.	n.d.

Data are expressed as mean ± SEM (mg/g, n=6).
n.d.: not detectable.

often used. But the solvents with significant absorption above 200 nm cannot be used in the mobile phase.

Under the conditions described above, the retention times of trilinolein and triolein were found to be 8.12 and 19.79 min, respectively (Fig. 2). The triolein content in the herbal extracts was under the detection limit (Fig. 2), thus, its validation was omitted. The detection limit for trilinolein and triolein, at signal-to-noise ratio of 4, were 0.1 and 10 μg/mL, respectively.

The content of trilinolein in the crude herbal extract was determined from the linear regression equation of the calibration graph for the compound. The equation for trilinolein was $Y = 5.301E\text{-}5\ X + 0.037$ ($r^2 = 0.999$), here, X is peak-area response and Y is amount of compound. The linearity range of trilinolein was 0.5-20 μg/mL.

The recovery tests were carried out by adding trilinolein (20 μg/mL) and triolein (100 μg/mL) to crude extract of herbal drug. The recoveries were 99.5 % and 99.3 % for trilinolein and triolein, respectively.

The intra-assay variation for the determination of trilinolein at concentrations of 1, 5, and 20 μg/mL were acceptable with C.V.s of less than 8 %. The inter-assay C.V.s for trilinolein at the same concentration were less than 10 %.

Table 1 summarized the contents of trilinolein (triolein is not detectable) in extracts of Panax pseudo-ginseng Wall. var. notoginseng Hoo & Tseng obtained with different solvents. Highest yield of trilinolein was founded from n-hexane extraction.

In conclusion, the proposed technique should be useful for the quality control of triacylglycerol in the herbal drug of *Panax pseudo-ginseng* Wall. var. notoginseng Hoo & Tseng for stability and for pharmacokinetic study of trilinolein.

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