Analysis of Fatty Oil in Semen Ziziphi Spinosae by Capillary Gas Chromatography

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Abstract

A simple and fast capillary gas chromatographic (CGC) method with flame ionization detection is developed for the analysis of fatty oil in Semen Ziziphi Spinosae. After methyl-esterification, eight components are identified by gas chromatography–mass spectrometry. The derivatization condition is investigated in order to validate this method. Palmitic acid and stearic acid are quantitated simultaneously. The limits of detection are 5.024 µg/mL for palmitic acid and 6.957 µg/mL for stearic acid, respectively. The limits of quantitation are 16.76 µg/mL for palmitic acid and 23.19 µg/mL for stearic acid, respectively. The percent recoveries of palmitic and stearic acid are 97.4% and 96.6%. CGC is shown to be a quick and informative tool for the analysis of fatty oil in Semen Ziziphi Spinosae.

Introduction

Semen Ziziphi Spinosae [the seed of the *Ziziphus jujuba* Mill. Var. spinosa (Bunge) Hu ex H. F. Chou] is included in the Chinese Pharmacopoeia (1) and has been used since antiquity as a famous traditional Chinese medicine for the treatment of insomnia. Chemical studies have shown that there is a large amount of fatty oil and other components in it (2). Most of the fatty oils are long-chain unsaturated fatty acids (3). Fatty acids are very important compounds in biological systems because they are structural elements of many valuable compounds (such as linoein), and they are important sources of energy. In recent years, more and more research has been carried out on fatty oil and the results show that the oil possesses significant sedative and hypnotic effects (4). It also has effects on platelet aggregation and lipids (5).

The separation and analysis of long-chain fatty acid mixture has been applied extensively to obtain information on a number of biological systems. However, the inherent low volatility of fatty oil has led to some difficulties in the analytical work. Consequently, they are first converted into more volatile derivatives (such as fatty acid methyl esters) and then are analyzed by CGC. Accurate determination of the components has not been reported so far. Therefore it is important and necessary to develop a new method for the analysis of the fatty oil because it is the main constituent of Semen Ziziphi Spinosae and it is involved in very important pharmacological activities.

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Experimental

Materials

Sodium hydroxide, methanol, petroleum ether (60–90°C, boiling point), boron trifluoride in ether (47.0–47.7%, v/v), and *n*-hexane were of analytical grade and were purchased from Yu-wang Chemical Factory (Shandong, China). Palmitic and stearic acids were supplied by Shanghai Reagent Company (Shanghai, China).

Semen Ziziphi Spinosae was purchased from the Shandong province of China (Jinan, Shandong, China) and was identified by Senior Engineer Xu Chun-Quan (Shenyang Pharmaceutical University). Fatty oil was extracted from Semen Ziziphi Spinosae by petroleum ether (60–90°C).

Apparatus

The apparatus consisted of a Finnigan Trace 2000 gas chromatography (GC)–mass spectrometry (MS) system (Finnigan, San Jose, CA). GC analysis was performed on a 30-m × 0.25-mm i.d. HP-5 capillary column (Hewlett-Packard, Boston, MA).

Operating conditions

For the separation procedure, the instrumental parameters were as follows: carrier gas, helium; injector temperature, 250°C; flame ioinization detection temperature, 250°C; and injected volume, 1 µL. The oven temperature program was 40°C for 2 min, then programmed to 160°C at 2°C/min, then to 250°C at 6°C/min

and held for 17 min. The conditions of MS detection were as follows: interface temprature, 280°C; ionization mode, EI+; electron energy, 70 ev; full scan acquisition mode; and mass range, 20–700 amu.

Sample pretreatment

Extraction fatty oil

Semen Ziziphi Spinosae was refluxed for 24 h in a Soxhlet's extractor by petroleum ether (60–90°C). The petroleum ether was evaporated to dryness. Then the fatty oil was prepared for derivatization.

Derivatization of fatty oil and palmitic and stearic acids

A sample of approximately 40 mg of fatty oil was weighed accurately, and the sample was put into a little flask with a ground-glass stopper for derivatization. After adding 5 mL 0.5 mol/L sodium hydroxide in methanol, the mixture was allowed to react for 30 min at 90°C in a water bath. One milliliter of boron trifluoride in ether was added to the solution and kept for 2 min. When cooled down to the room temperature, 3 mL of saturated sodium chloride solution and 4 mL of *n*-hexane were accurately added to the mixture. The flask was covered and shaken for 5 min. The supernatant was transferred into a test tube with a ground-glass stopper.

Ten milligrams of palmitic acid and 7.8 mg of stearic acid were accurately weighed out. The mixture was derivatized (with the same method as for fatty oil) and then diluted with *n*-hexane to form stock solutions of palmitic acid (1.250 mg/mL) and stearic acid (0.975 mg/mL). Quantities of 0.1, 0.2, 0.4, 0.8, 1.2, and 1.6 mL of the above stock solutions were diluted to 2 mL for the preparation of the calibration curve.

Results and Discussion

Extraction recovery of fatty oil

Two grams of Semen Ziziphi Spinosae was refluxed in Soxhlet's extractor for 12, 16, 20, 24, 28, and 32 h, respectively, and the extraction recovery was evaluated by the loss of the weight of raw herb powder. Twenty-four hours was long enough to get the fixed recovery of fatty oil. The extraction procedure was performed in triplicate and the mean recovery was 30.4% with a coefficient of variation of 2.1%. The extraction recovery was close to the previously reported research (2). The result is shown in Figure 1.

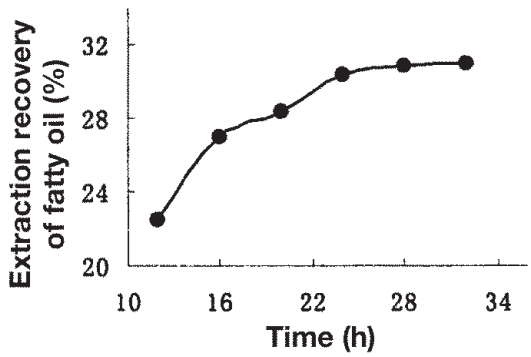

Figure 1. Effect of extraction duration on extraction recovery of fatty oil.

Optimization of derivatization conditions

In this study, derivatization temperature, duration, and the consumption of sodium hydroxide in methanol all had an effect on the yield of derivatization. In order to obtain the optimal conditions, the above factors were investigated. These three factors were arranged in an orthogonal table ($L_9 3^4$) (Table I). Factor A, B, and C referred to derivatization temperature, duration, and the consumption of sodium hydroxide in methanol, respectively. Every factor had three levels: factor A (1, 90°C; 2, 75°C; and 3, 60°C); factor B (1, 30 min; 2, 20 min; and 3, 10 min); and factor C (1, 1 mL; 2, 3 mL; and 3, 5 mL). Nine experiments were performed following Table I. The contents of palmitic and stearic acid were selected as the assessment indexes of derivatization conditions. From the range and analysis-of-variance analysis, a conclusion was made that derivatization temperature and consumption of sodium hydroxide in methanol played a more important role than derivatization duration did in the reaction procedure. Finally, 90°C, 30 min, and 3 mL of sodium hydroxide in methanol were selected as the optimal derivatization conditions.

Identification of eight fatty acids

One microliter of the sample was injected manually into the GC–MS system and analyzed under full-scan acquisition mode. Palmitic and stearic acids were identified by the comparison of their retention times and mass spectra with those obtained from

Table I. Results of the Determination of Palmitic and Stearic Acids in the Orthoganol Test

Test no.	A	B	C	Palmitic acid (μg/mg)	Stearic acid (μg/mg)
1	1	1	1	59.00	46.18
2	1	2	2	100.50	75.50
3	1	3	3	64.22	50.82
4	2	1	2	34.85	24.77
5	2	2	3	10.76	8.769
6	2	3	1	24.50	19.26
7	3	1	3	79.80	63.54
8	3	2	1	33.24	25.39
9	3	3	2	42.63	29.84

Table II. Compounds Identified in the Test Sample

Peak no.	Retention time (min)	Molecular weight	Compound
1	50.7	270	Palmitic acid
2	53.3	294	Linoleic acid
3	53.7	296	Oleic acid
4	54.1	298	Stearic acid
5	56.7	324	Gadoleic acid
6	57.1	326	Arachidic acid
7	58.2	354	Behenic acid
8	60.7	382	Carnaubic acid

the injected standards. The identification of the rest of the six fatty acids was accomplished by the aid of an MS library. The eight characterized components with their respective retention times and molecular weights are listed in Table II.

Quantitation of palmitic and stearic acids

One microliter of the sample solution and of the standard solutions was injected with a split injector (1:30, v/v) into the GC. All the data were acquired and processed with a Finnigan workstation. The chromatograms are shown in Figure 2. Palmitic acid (No. 1) and stearic acid (No. 4) were quantitated from the corresponding peak areas using linear equations. For this purpose, standard solutions of pure sample of palmitic and stearic acids were run three times. The average peak areas were calculated and plotted, and linear regression analysis was performed. The linear relationships between the concentration and the corresponding peak area were found in the concentration range of 0.06250–1.000 mg/mL for palmitic acid and 0.04875–0.7800 mg/mL for stearic acid, respectively. The regression equations were as follows:

$Y = 161.0X + 15.91$(palmitic acid) Eq. 1
$R = 0.9994$

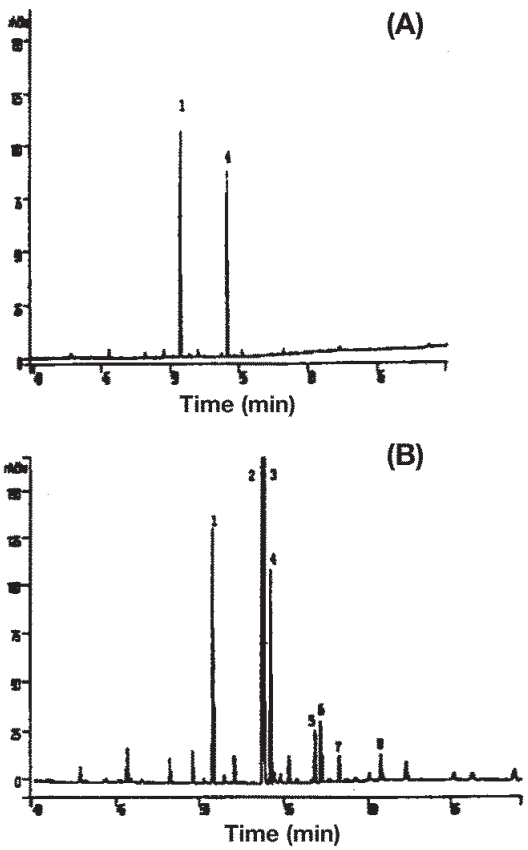

Figure 2. CGC chromatograms of standard (A) and test (B) solutions. The peak are palmitic acid (1), linoleic acid (2), oleic acid (3), stearic acid (4), gadoleic acid (5), arachidic acid (6), behenic acid (7), and carnaubic acid (8), respectively.

$Y = 189.1X + 14.42$(stearic acid) Eq. 2
$R = 0.9989$

where Y is the peak area (mvolts × min) and X is the concentration (mg/mL) for each compound.

The contents of palmitic and stearic acids were 6.3% (w/w) and 4.2% (w/w). The limit of detection (LOD) and limit of quantitation (LOQ) were calculated by measuring ten blanks using the maximal sensitivity allowed by the system and calculating the standard deviation (SD) of this response. LOD was estimated by multiplying the SD by a factor of three. The LOQ was defined as ten times the SD. In this study, LODs were 5.024 µg/mL for palmitic acid and 6.975 µg/mL for stearic acid. LOQs of palmitic and stearic acids were 16.756 and 23.19 µg/mL, respectively. The repeatability of the total procedure was tested using the sample of fatty oil, and the relative standard deviations (RSD) (n = 6) were 2.6% for palmitic acid and 2.8% for stearic acid. The average recoveries of palmitic and stearic acids are 97.4% (RSD = 2.7%) and 96.6% (RSD = 3.0%), respectively. The results showed that the method had good recovery and repeatability.

Because of a lack of standard acids, six other fatty acids were not quantitated simultaneously in this paper. However, their contents (w/w) could be calculated. As a result, the contents of linoleic acid (18:2, $\varnothing^{9,12}$), oleic acid (18:1, $\varnothing^{9}$), gadoleic acid (20:1, $\varnothing^{11}$), arachidic acid (20:0, w/w), behenic acid (22:0, w/w), and carnaubic acid (24:0, w/w) were 25%, 50%, 1.4%, 1.8%, 0.25%, and 0.32%, respectively. Much of the linoleic and oleic acid might have played an important role in its pharmacological activities.

Conclusion

For the first time, a CGC method is reported that could be used to analyze the fatty oil in Semen Ziziphi Spinosae. It gives excellent recoveries and reproducibilities. It could be utilized as a quality control method for medicinal herbs and preparations containing fatty oil from Semen Ziziphi Spinosae.

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