Mass Chromatographic Quantification of Ricinoleic Acid in Common Vegetable Oils and Oil Seeds : A Rapid Communication

J Rehabil Health Sci 2003; 1: 39-41

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Mass Chromatographic Quantification of Ricinoleic Acid in Common Vegetable Oils and Oil Seeds (A Rapid Communication)

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Recieved October 29, 2003; accepted December 3, 2003

Key words: ricinoleic acid (12-hydroxy-*cis*-9-octadecenoic acid); common vegetable oils; common oil seeds; gas chromatography/mass spectrometry; mass chromatography

Introduction

Ricinoleic acid (12-hydroxy-*cis*-9-octadecenoic acid) is known as the characteristic acyl component of castor oil and comprises about 90% of the total acyl components of castor bean triacylglycerols.[1-3] By using the analytical method described in this rapid communication, our research group has determined that ricinoleic acid is also found in common vegetable oils and common oil seeds. To our knowledge, no other papers have reported this.

Materials and Methods

Common vegetable oils, common oil seeds and beef tallow were purchased from local markets. Standard castor oil, methyl ricinoleate (purity 90%) and 3-hydroxyhexadecanoic acid (purity 98%) were obtained from Wako Pure Chemical Industries, Osaka, Japan. Other chemicals were of the highest reagent grade available, and all solvents were distilled before use.

To 200 mg of each sample (from nine kinds of vegetable oils and from the lipids of four kinds of oil seeds extracted by the method of Folch *et al.*[4]), an internal standard (IS) of 3-hydroxyhexadecanoic acid (100 μg) was added. The sample mixture was methanolyzed with 0.5 mol/L KOH/methanol and successively methylated with 14% BF_3/methanol.[5] After adding a saturated NaCl solution, the resultant fatty acid methyl esters were extracted twice with hexane. The combined hexane layers were dehydrated and evaporated to dryness. The residue containing fatty acid methyl esters was placed on a Presep-C silica gel column (Wako Pure Chemical Industries, Osaka, Japan) with hexane (5 mL, ×2) and developed with a solution of 3% ether/hexane (30 mL) to remove esters having straight carbon chains. The remaining esters having hydroxyl groups on their carbon chains were recovered from the column by passing ether (20 mL). The recovered fraction was evaporated to dryness, dissolved in dehydrated pyridine (0.3 mL) and then trimethylsilylated with 0.2 mL of a reagent mixture (trimethylchlorosilane/*N*-trimethylsilylimidazole/*N*,*O*-bis-(trimethylsilyl)trifluoroacetamide, 1:1:1, by vol.) under the usual conditions. After being allowed to stand for 10 min at room temperature, the *O*-trimethylsilylated (-OTMS) methyl ester derivatives thus obtained were analyzed on a DB-5ms capillary column (30 m × 0.25 mm i.d., 0.25 μm film, chemically bonded type, Agilent Technologies, Baloalt, CA) coupled to a Shimadzu QP-5050A mass spectrometer with a computer on-line system. The operating conditions were as follows. Column temperature: 180℃ for 2 min isothermally, to 260℃ at a rate of 5℃/min, then to 310℃ at a rate of 40℃/min, and 310℃ for 10 min isothermally; injector temperature: 250℃; interface temperature: 280℃; split ratio: 1/10; carrier gas: helium (linear gas velocity of 38.4 cm/sec); ionizing energy: 70 eV; and scanning range: 50-450 *m/z* (0.5 sec/cycle).

Results and Discussion

To determine a calibration curve for estimating the amount of ricinoleic acid in food samples, six kinds of standard mixtures containing known amounts of 3-hydroxyhexadecanoic acid as the IS (100 μg) and authentic methyl ricinoleate (0, 10, 50, 100, 200 and 400 μg) were prepared. The standard mixtures were methylated (3-hydroxyhexadecanoic acid was converted to the methyl ester form but methyl ricinoleate remained unchanged), trimethylsilylated and finally analyzed by gas chromatography/mass spectrometry (GC/MS).

Both of these derivatives eluted at 10.5 and 13.5 min as shown in Fig. 1A. In the electron impact mass spectrum of methyl 3-hydroxyhexadecanoate-OTMS (Fig. 1B), the fragment ions at *m/z* 175 and 285 were derived from the α-cleavage at the silyl ether group, and the ion at *m/z* 343 was due to the

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loss of the methyl group (one of three methyl groups of the silyl group) from the molecular ion at *m/z* 358 (M^+-15). The major ion at *m/z* 73, $(CH_3)_3Si^+$, was the typical ion originating from the trimethylsilylated compounds. In the mass spectrum of methyl ricinoleate-OTMS (Fig. 1C), the α-cleavage at the silyl ether group gave the fragment ions at *m/z* 187 and 299. The migration ion (TMS rearrangement ion) at *m/z* 270, CH_2-CH=CH-$(CH_2)_7$-C^+(=OTMS)-OCH_3, was observed, indicating the positions of the hydroxyl group at the C-12 carbon and of the double bond at the C-9 and C-10 carbons. These mass spectral assignments coincided to those in previous papers.[6,7] Based on the mass spectral elucidation, the six kinds of standard mixtures for the calibration curve were mass chromatographed to trace two characteristic fragment ions at *m/z* 175 (for methyl 3-hydroxyhexadecanoate-OTMS) and at *m/z* 187 (for methyl ricinoleate-OTMS).

The value *V* indicating the peak area ratio of the two fragment ions was calculated with the following formula: *V* = peak area of *m/z* 187 / peak area of *m/z* 175, where ions at *m/z* 187 and 175 represent methyl ricinoleate-OTMS and methyl 3-hydroxyhexadecanoate-OTMS, respectively. Plotting the value *V* for the six kinds of standard mixtures against the amounts of methyl ricinoleate gave the calibration curve shown in Fig. 2. Value *V* increased in direct proportion to the increased amounts of methyl ricinoleate in the standard mixtures (0-400 μg). From these results we concluded that our newly-developed GC/MS method is suitable for both detecting and quantifying ricinoleic acid in food samples.

As a model experiment, 200 μg of standard castor oil was added to 200 mg of beef tallow which had been checked as having no ricinoleic acid. The repeated analysis (independently for five times) of this castor oil-spiked beef tallow showed an average 98% of recovery of ricinoleic acid (2.5% CV).

Then, according to the method given in the

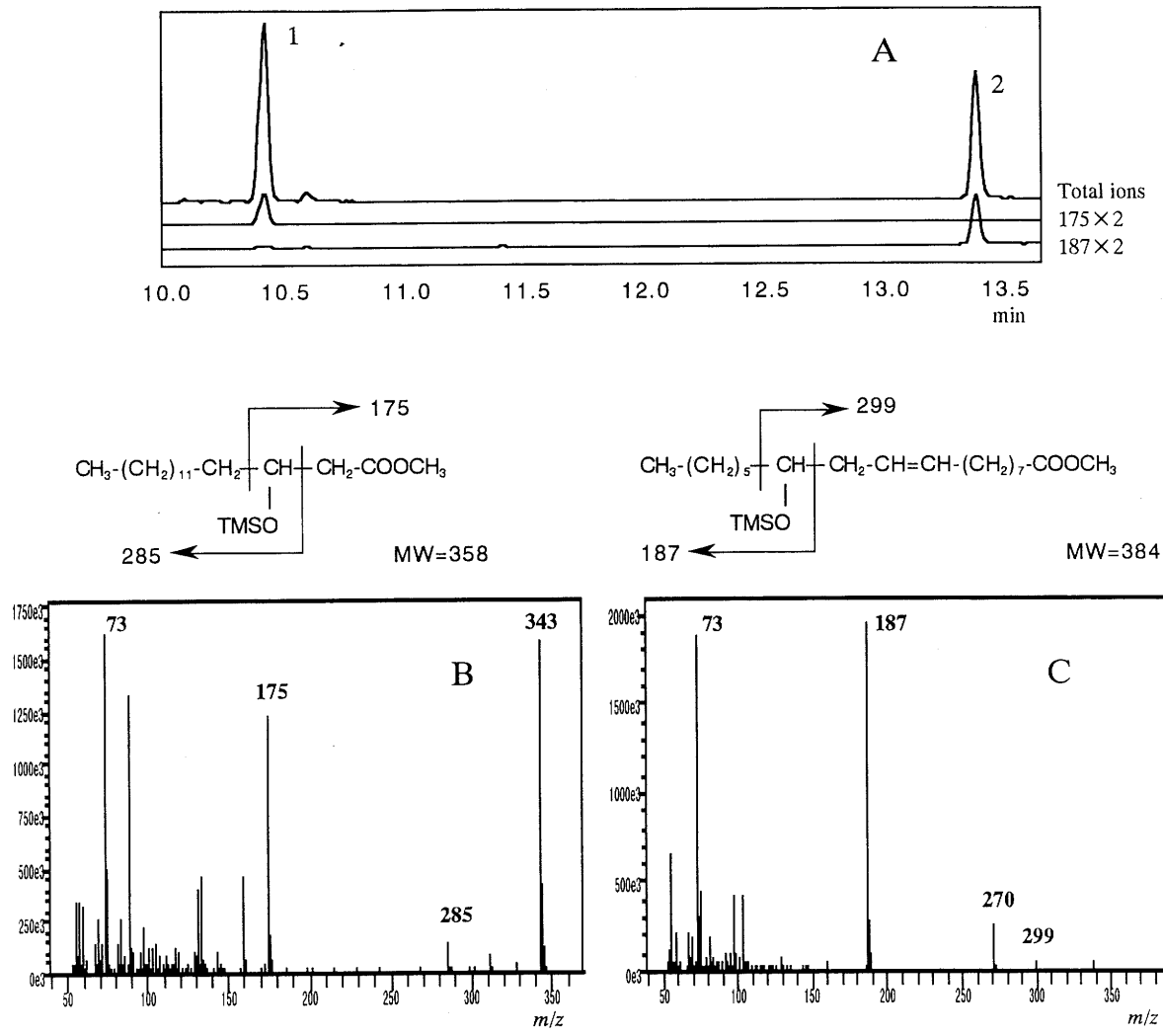

Fig. 1 Mass chromatogram (A) of a mixture of methyl 3-hydroxyhexadecanoate and methyl ricinoleate after trimethylsilylation, and mass spectra of trimethylsilylated derivatives of methyl 3-hydroxyhexadecanoate (B) and methyl ricinoleate (C). In A, peak 1, trimethylsilylated derivative of methyl 3-hydroxyhexadecanoate; peak 2, trimethylsilylated derivative of methyl ricinoleate. Operating conditions are detailed in the text.

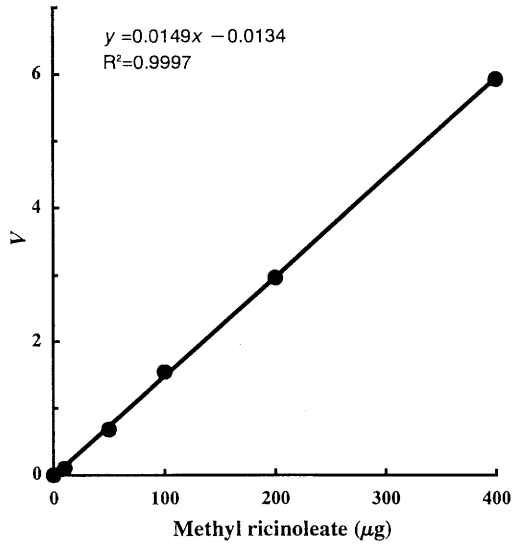

Fig. 2 Calibration curve for determining the amounts of ricinoleic acid.
The amounts of methyl ricinoleate in standard mixtures are plotted on the *x*-axis, and the value *V* on the *y*-axis. The value *V* indicating the peak area ratio of the two fragment ions is calculated with the following formula: *V* = peak area of *m/z* at 187 / peak area of *m/z* 175, where ions at *m/z* 187 and 175 represent the trimethylsilylated derivatives of methyl ricinoleate and methyl 3-hydroxyhexadecanoate, respectively. Each plot is an average of two determinations.

Materials and Methods section, we analyzed common vegetable oils and oil seeds obtained from local markets. On the basis of mass spectral evidence and mass chromatographic tracing, the amounts of ricinoleic acid in each sample were determined as shown in Table 1. All the commercial oils contained ricinoleic acid, though the amounts varied (a minimum of 20 ppm in safflower oil and a maximum of over 2000 ppm in cottonseed oil). We considered the possibility of contamination with castor oil or a similar oil during the refining process for these commercial oils. To rule out this possibility, we extracted the oils from the four different oil seeds according to the usual manner used in lipid laboratories. In these cases, as well, ricinoleic acid was always quantified as shown in Table 1.

From this evidence, we can conclude that ricinoleic acid is present in common vegetable oils and also in common oil seeds. The localization of ricinoleic acid in lipid classes is a triacylglycerol fraction, because commercial oils are composed mostly of triacylglycerols.

In plant lipid biochemistry, the mechanism of the biosynthetic pathway for ricinoleic acid in the castor bean has been well investigated and fully discussed.[3,8,9] The castor bean enzyme system catalyzes the production of ricinoleate by direct hydroxylation of oleate (*cis*-9-octadecenoate) which binds to phosphatidylcholine (the immediate substrate: oleoyl-phosphatidylcholine). It is unknown if this same enzyme system, responsible for biosynthesizing ricinoleic acid in castor bean, is also active in the common oil seeds we tested.

Table 1 Amounts of ricinoleic acid in common vegetable oils and oil seeds (ppm)

Vegetable oil		Oil seed
Olive	50	
Corn	105	
Sesame	167	117
Rice bran	216	
Soybean	437	278
Rapeseed	125	206
Sunflower	145	
Safflower	50	
Cottonseed	2040	2441

Each value is an average of two determinations.

A part of this study was presented at the annual meeting of the Food Hygienics Society of Japan held in Morioka in October, 2003.

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