Application Note

Analysis of fatty acids in rice bran oil, coconut oil and margarine by HPLC-ELSD

Introduction

Due to the poor UV absorbance, fatty acids have been commonly measured at a short UV wavelength or by RI detector, which takes a long time to establish a stable baseline. The method of pre-column derivatization is another well known approach for fatty acids, but the operation is complicated. ELSD is recognized as an effective method for the lipid analysis providing high sensitivity and a stable baseline while also eliminating the complex sample pre-preparation. As the sample becomes more volatile with a shorter carbon chain, ELSD is normally considered inadequate and difficult to measure the fatty acid.

This report describes the analysis using the ELS-2041, which is equipped with the cooling capability in the evaporator, of the saturated and unsaturated fatty acids of C10 ~ C18.

Keyword : HPLC, Saturated, Unsaturated, Fatty acid, C18 Column, ELSD, Capric acid, Lauric acid, Linolenic acid, Myristic acid, Linoleic acid, Palmitic acid, Oleic acid, Elaidic acid, Stearic acid, rice bran oil, coconut oil, margarine

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Experimental Equipment:

Pump:	PU-2089
Autosampler:	AS-2057
Column oven:	CO-2060
Detector:	ELS-2041

Conditions:

Column:	Develosil ODS HG-5 (4.6 mmID x 150 mmL, 5 µm)
Eluent:	A: 0.1% Acetic acid in Acetonitrile, B: 0.1% Acetic acid, C: 0.1% Acetic acid in Acetone
Gradient condition:	(A/B/C), 0 min (85/15/0) , 20 min (85/15/0), 20.05 min (5/0/95), 30 min (5/0/95),30.05 min (85/15/0) 1cycle: 45 min
Flow rate:	1.0 mL/min
Column temp.:	40°C
ELSD condition:	Nebulizer temp.: 30°C Evaporator temp.: 18°C Gas flow rate; 1.4 SLM
Injection volume:	10 µL
Standard sample:	8 Fatty acids + Elaidic acid

Results and Discussion

Figure 1 shows the chromatogram of a standard mixture of 9 fatty acids including a trans fatty acid. A good separation within 20 minutes was achieved for both saturated and unsaturated fatty acids of C10 ~ C18, including the Elaidic acid of trans fatty acid.

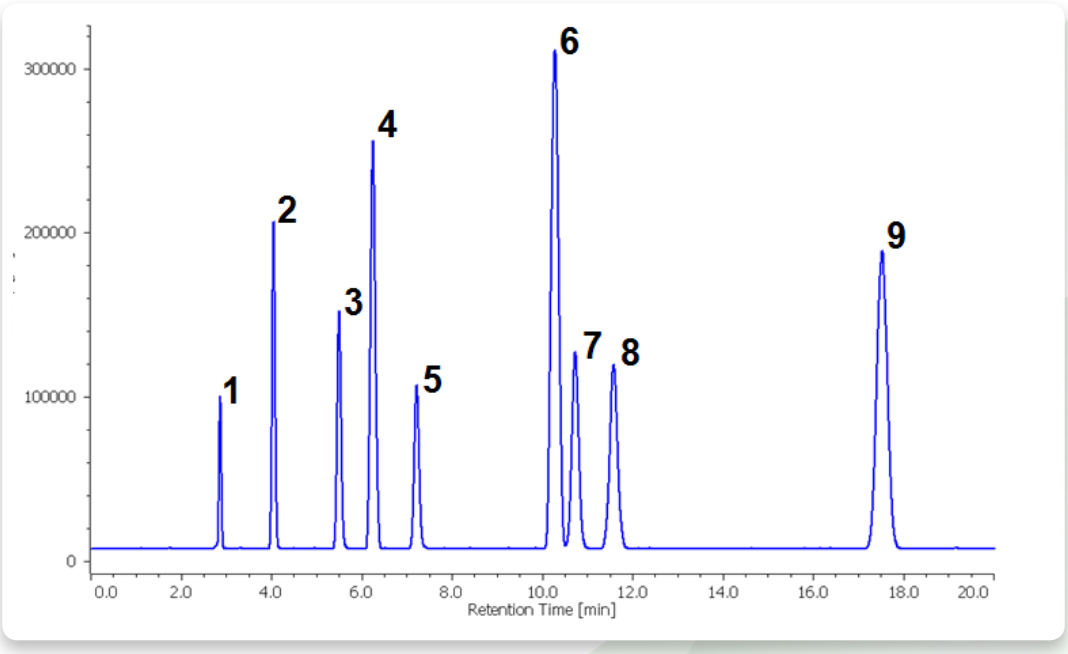

Figure 1. Chromatogram of the standard mixture of 9 fatty acids including a trans fatty acid. 1: Capric acid (C10) 0.5 mg/mL, 2: Lauric acid (C12) 0.2 mg/mL, 3: Linolenic acid (C18:3) 0.1 mg/mL, 4: Myristic acid (C14) 0.1 mg/mL, 5: Linoleic acid (C18:2) 0.2 mg/mL, 6: Palmitic acid (C16) 0.1 mg/mL, 7: Oleic acid (C18:1) 0.2 mg/mL, 8: Elaidic acid 0.2 mg/mL, 9: Stearic acid (C18) 0.1 mg/mL

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The chromatograms of rice bran oil and coconut oil are shown in figure 2 and 3, respectively.

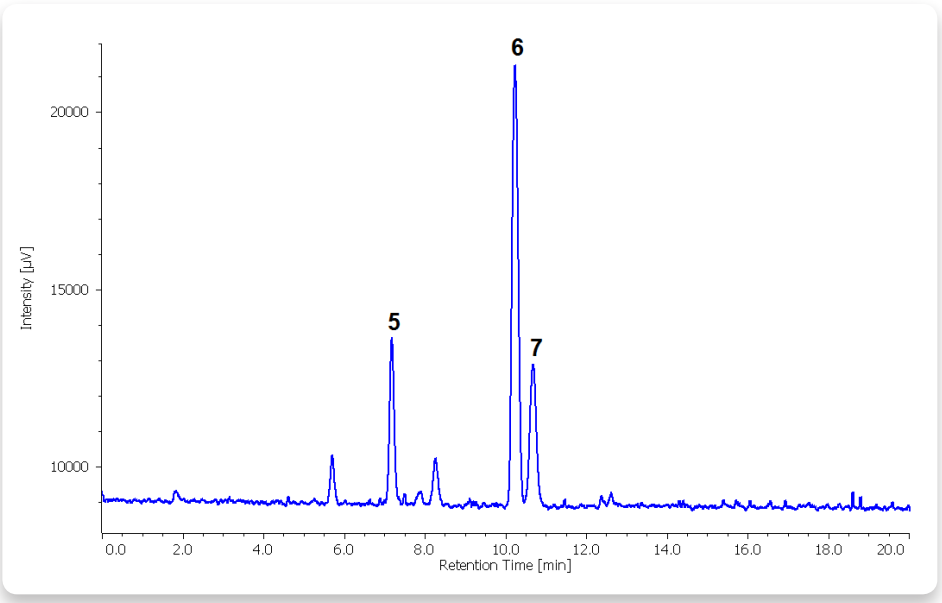

Figure 2. Chromatogram of rice bran oil.
The peak names are the same as in figure 1. Sample preparation: 1.0 g rice bran oil was dissolved in 10 mL of acetone. The solution was then filtered through a 0.45 µm filter.

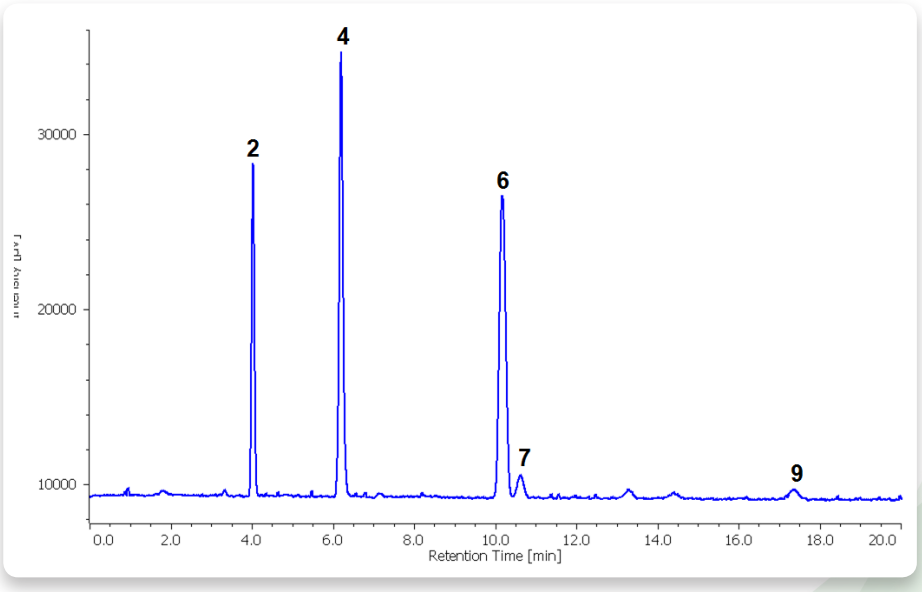

Figure 3. Chromatogram of coconut oil
The peak names are the same as in figure 1. Sample preparation: 1.0 g coconut oil was dissolved in 10 mL of acetone. The solution was then filtered through a 0.45 µm filter.

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A trans fatty acid, known as the unsaturated fatty acid with the double bond of trans type, is not contained in natural vegetable oil. It is generated during the manufacturing process of hydrogenated oil, such as margarine or shortening , when hydrogen is added. Since the possibility of causing the health impairment, such as heart disease, has been noticed during recent years, more countries are regulating the usage of products which contain trans fatty acids. The chromatograms of margarine itself and margarine added with Elaidic acid are shown in figure 4 and 5, respectively.

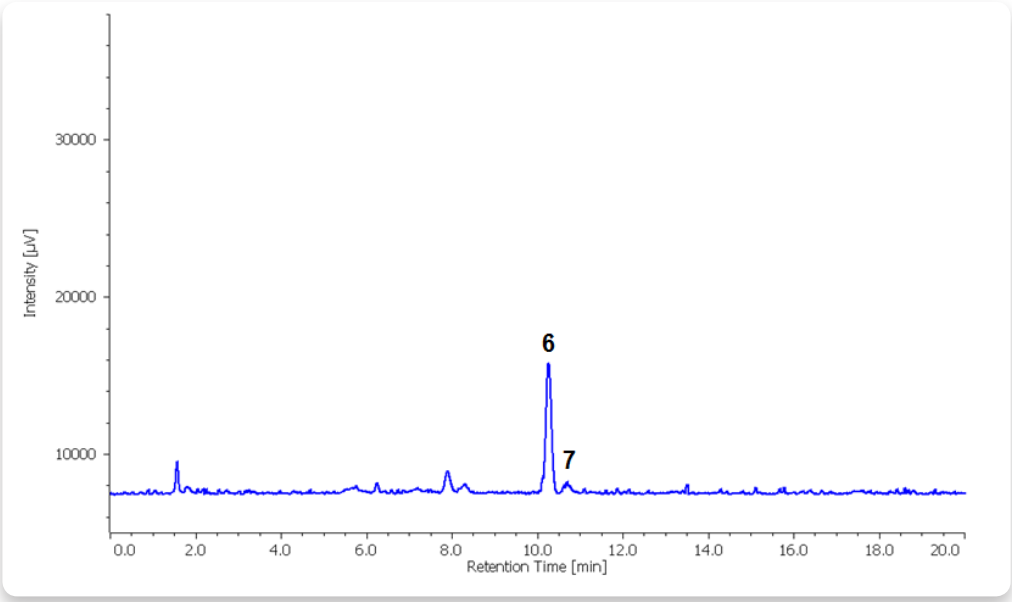

Figure 4. Chromatogram of Margarine.
The peak names are the same as in figure 1. Sample preparation: 0.5 g margarine was dissolved in 10 mL of acetone.

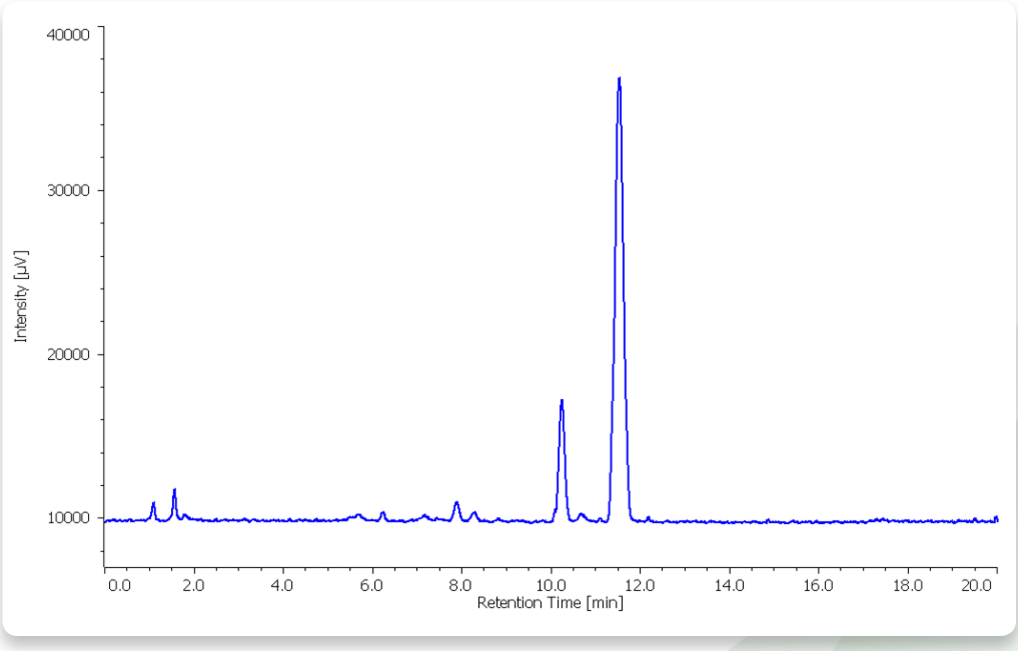

Figure 5. Chromatogram of Margarine added with Elaidic acid.
The peak names are the same as in figure 1. Sample preparation: Elaidic acid was added to sample of figure 4 to adjust the concentration of Elaidic acid to be 0.2 mg/mL.

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