Application Note

220057H

Analysis of fatty acids by High Performance Liquid Chromatography with Evaporative Light Scattering Detection

Introduction

Due to the poor UV absorbance, fatty acids has been commonly measured by UV detector at short UV wavelength or by RI detector, which takes long time to make baseline stable and the result is easily influenced by the impurities. The method of Pre-column derivatization is another well known approach for fatty acids, but the operation is complicated. Now ELSD is recognized as an effective method for the lipid analysis, by which the high sensitivity and stable baseline can be achieved, while the complex sample pre-preparation is no longer needed. As the sample becomes more volatile when the carbon chain is shorter, ELSD is normally considered inadequate and difficult to measure the fatty acid with relatively shorter carbon chain.

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

Keyword : Saturated, Unsaturated, Fatty acid, C18 Column, ELSD

Experimental

Equipment		Conditions	
Pump:	PU-2089	Column:	Develosil ODS HG-5 (4.6 mmID x 150 mmL, 5 µm)
Autosampler:	AS-2057	Eluent:	A: 0.1% Acetic acid in Acetonitrile, B: 0.1% Acetic acid, C: 0.1% Acetic acid in Acetone
Column oven:	CO-2060		
Detector:	ELS-2041	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.:	40ºC
		ELSD condition:	Nebulizer temp.: 30ºC Evaporator temp.: 18ºC Gas flow rate; 1.4 SLM
		Injection volume:	10 µL
		Standard sample:	8 Fatty acids + Elaidic acid

Result

Fig. 1 shows the chromatogram of standard mixture of 9 fatty acids including the trans fatty acids. 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.

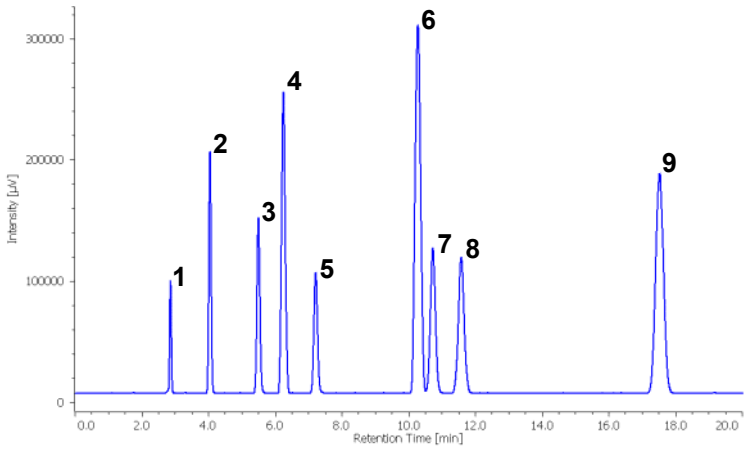

Fig. 1. Chromatogram of standard mixture of 9 fatty acids including trans fatty acids
1: Capric acid (C10) 0.5 mg/mL, 2: Lauric acid (C12) 0.2 mg/mL, 3: Linolenic acid (C18:3) 0.1 mg/mL, 4: Myristic acid (C14) 0.1 mg/mL, 5: Linoleic acid (C18:2) 0.2 mg/mL, 6: Palmitic acid (C16) 0.1 mg/mL, 7: Oleic acid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

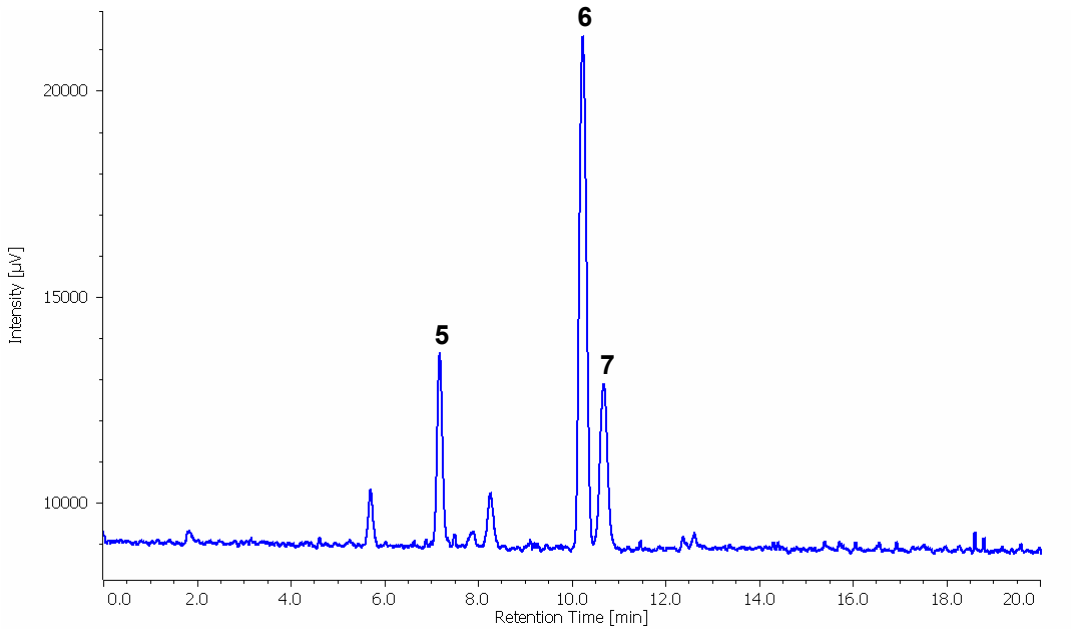

Fig. 2. Chromatogram of rice bran oil
The peak numbers are the same as in Fig. 1. Sample preparation: 1.0 g rice bran oil was dissolved in 10 mL acetone. The solution was then filtrated using membrane filter of 0.45 μm.

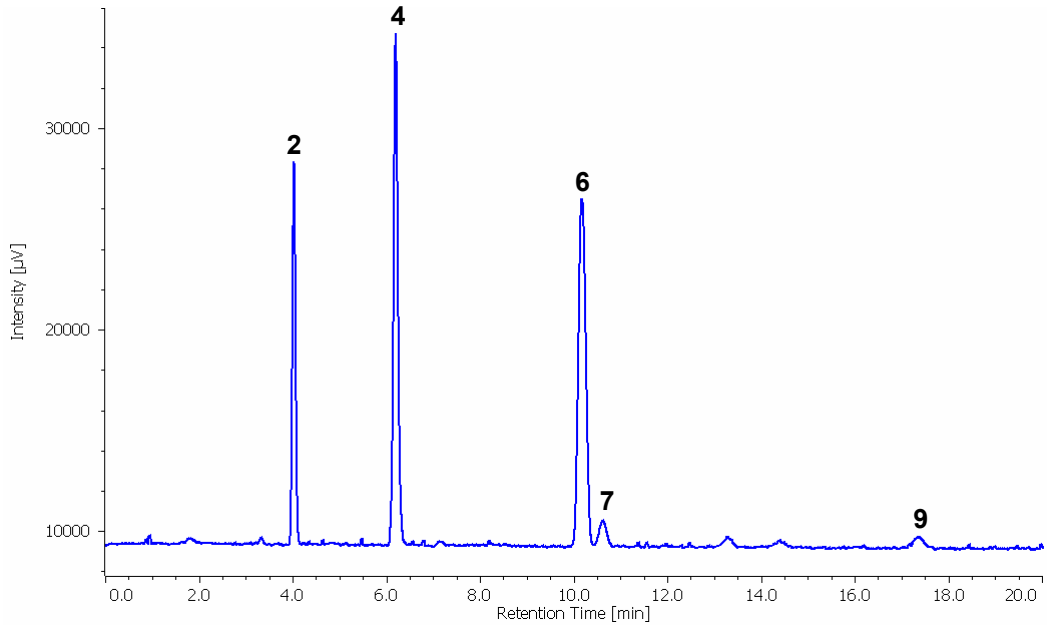

Fig. 3. Chromatogram of coconut oil
The peak numbers are the same as in Fig. 1. Sample preparation: 1.0 g coconut oil was dissolved in 10 mL acetone. The solution was then filtrated using membrane filter of 0.45 μm.

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Trans fatty acid, known as the unsaturated fatty acid with the double bond of trans type, is hardly contained in the natural vegetable oil. It is generated during the manufacture 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 the recent years, more and more countries are regulating the usage of the product which contains the trans fatty acids. The chromatograms of Margarine itself and Margarine added with Elaidic acid are shown in Fig. 4 and Fig. 5 respectively.

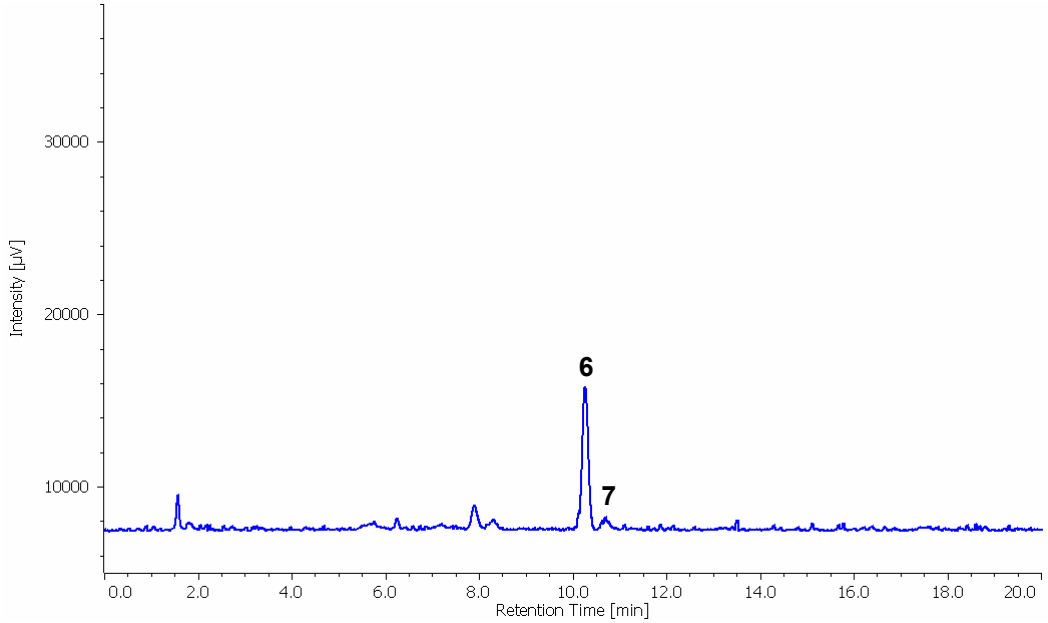

Fig. 4. Chromatogram of Margarine
The peak numbers are the same as in Fig. 1. Sample preparation: 0.5 g Margarine was dissolved in 10 mL acetone. The solution was then filtrated using membrane filter of 0.45 μm.

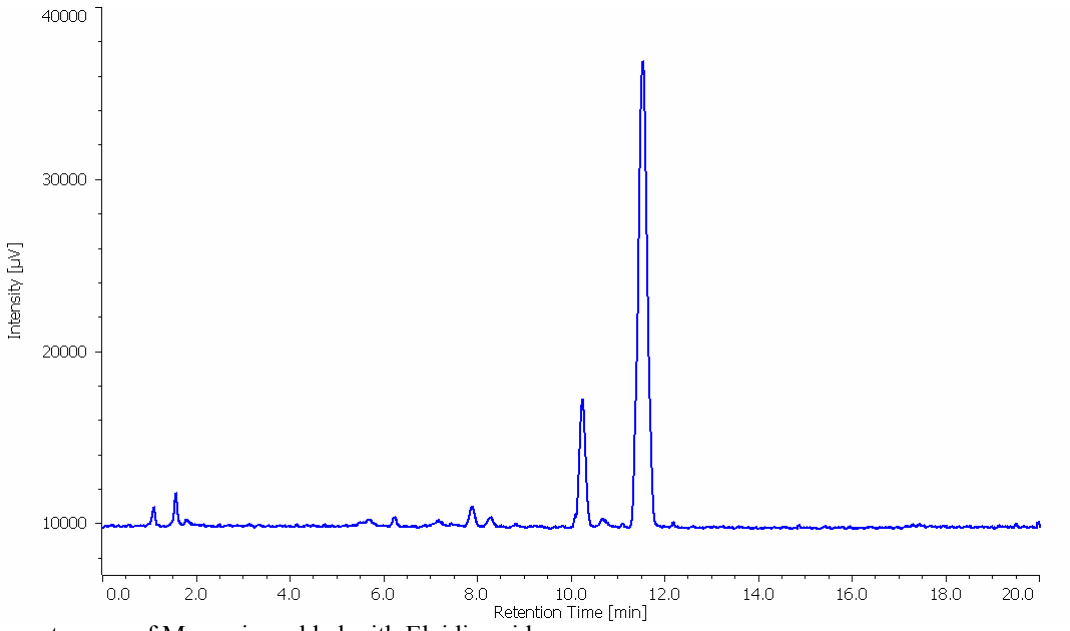

Fig. 5. Chromatogram of Margarine added with Elaidic acid
The peak numbers are the same as in Fig. 1. Sample preparation: Elaidic acid was added to sample of Fig. 4 to adjust the concentration of Elaidic acid to be 0.2 mg/mL

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