Application Note

220056X

Ultra High-Speed Analysis of Free Fatty Acids in Vegetable Oil using ADAM Derivatization by Ultra High-performance Liquid Chromatography

Introduction

In fatty acid analysis, UV detection at a short wavelength, refractive index detection and evaporative light scattering detection (ELSD) are generally. However, in trace analysis, a derivatization method is preferred because it offers much higher sensitivity. There are several derivatization methods for the detection of free fatty acids that require either UV or fluorescence detection. Among them, 9-Anthryldiazomethane (ADAM) is most commonly used because it reacts with fatty acids easily at room temperature and enables highly sensitive and selective analysis

Here, the separation and fluorescence detection of free fatty acids in vegetable oil are demonstrated using ADAM derivatization by Ultra High-performance Liquid Chromatography (UHPLC).

Keyword: UHPLC, Lipid, Free fatty acids, 2.0 µm, C18 column, ADAM, pre-column derivatization, fluorescence detector

Experimental

Equipment		Conditions	
Pump:	X-LC 3185PU x 2	Column:	X-PressPak V-C18-W (3.0 mmID x 50 mmL, 2.0 µm)
Degasser:	X-LC 3080DG	Eluent A:	Water/Methanol (5/95)
Mixer:	X-LC 3180MX	Eluent B:	Acetonitrile/THF (90/10)
Column oven:	X-LC 3067CO	Gradient condition:	(A/B), 0 min (100/0) →3 min (100/0) → 4.5 min (0/100) →7.5 min (0/100) →7.55 min (100/0) 1cycle: 10 min
Autosampler:	X-LC 3159AS		
Detector:	X-LC 3120FP	Flow rate:	1.0 mL/min
		Column temp.:	40ºC
		Wavelength:	Ex. 365 nm, Em. 412 nm, Gain x100
		Injection volume:	1 µL
		Standard sample:	ADAM derivatized fatty acids (C8 - C22) 0.05 mg/mL each in Ethyl acetate

Result

Fig. 1 shows the procedure for ADAM derivatization and Fig. 2. shows a reaction mechanism of ADAM reagent.

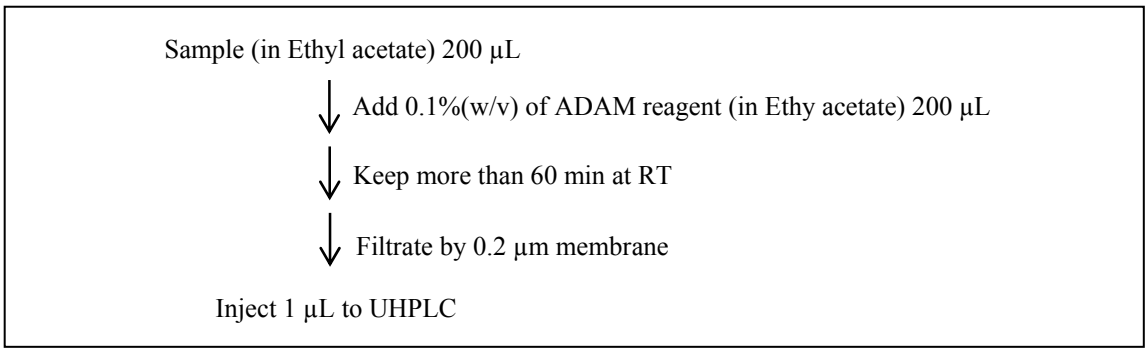

Fig. 1. Procedure for ADAM derivatization.

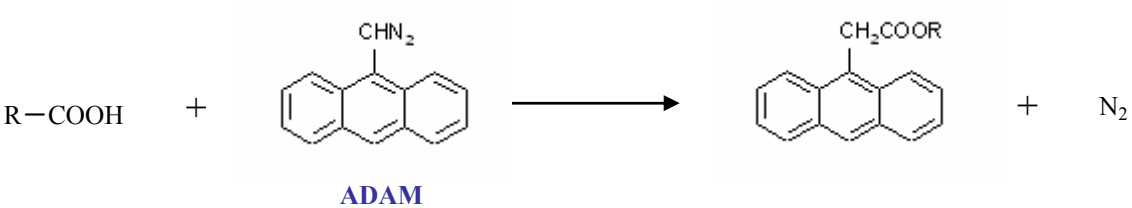

Fig. 2. Reaction mechanism of ADAM reagent with fatty acid

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Fig. 3 shows the chromatogram of the standard mixture of fatty acids derivatized with ADAM. Eleven different fatty acids were separated within 7 min.

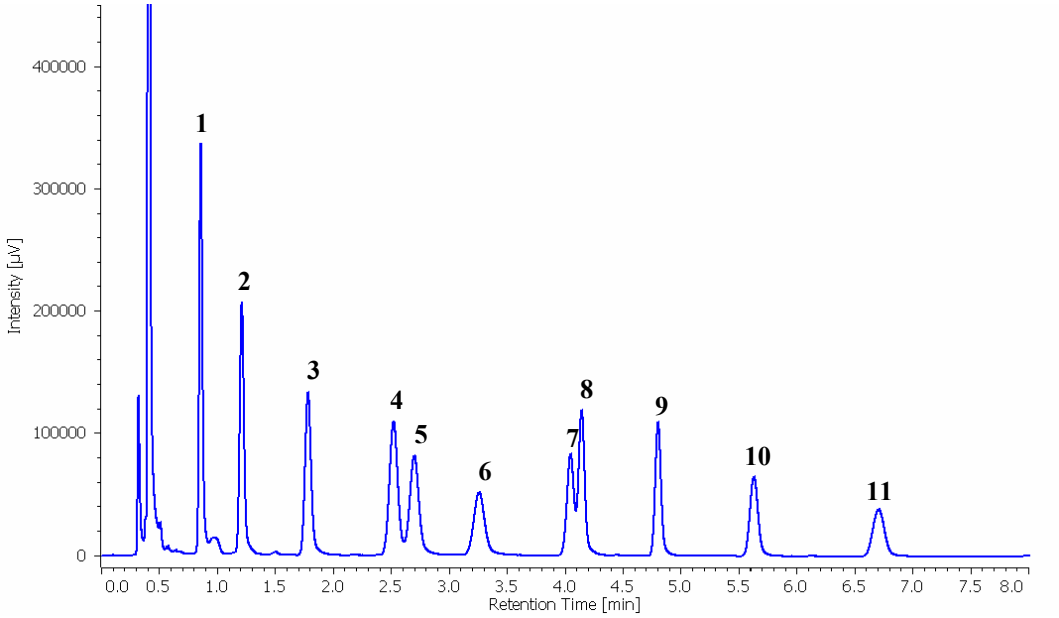

Fig. 3. Chromatogram of standard mixture of fatty acid derivatized by ADAM. 1: Caprylic acid (C8), 2: Capric acid (C10), 3: Lauric acid (C12), 4: Linolenic acid (C18:3), 5: Myristic acid (C14), 6: Linoleic acid (C18:2), 7: Palmitic acid (C16), 8: Oleic acid (C18:1), 9: Stearic acid (C18), 10: Arachidic acid (C20), 11: Behenic acid (C22) (0.05 mg/mL each in ethyl acetate)

Figs. 4 and 5 show the chromatograms of fatty acids in rice oil and coconut oil derivatized with ADAM, respectively.

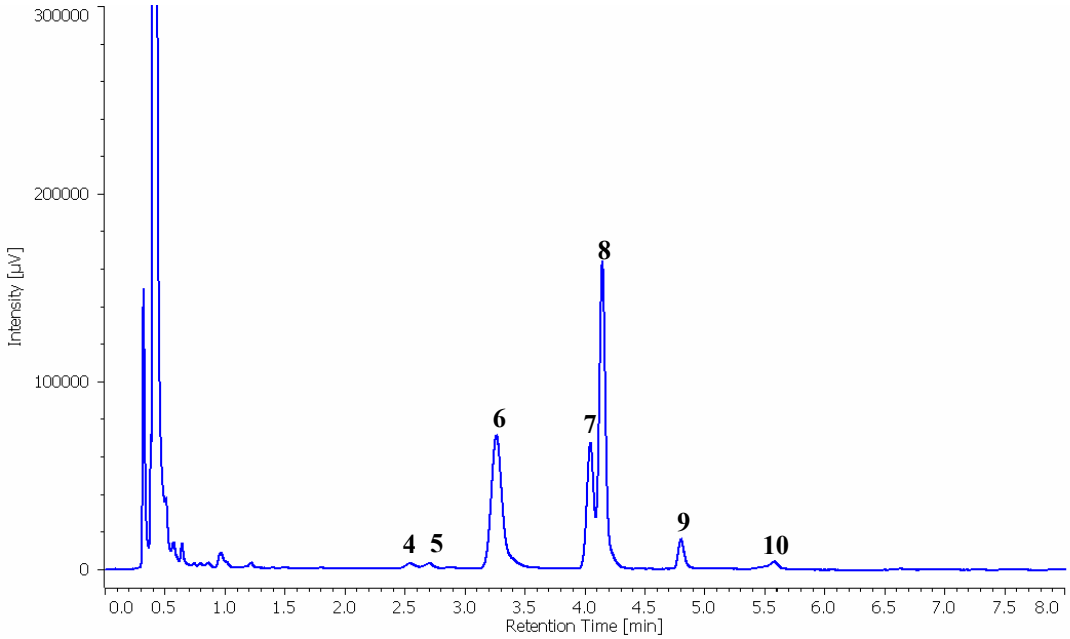

Fig. 4. Chromatogram of ADAM derivative of rice oil. The peak numbers and corresponding compounds are the same as in Fig. 3.

Preparation. Dissolve 1.0 g of rice oil in 10 mL of ethyl acetate and then follow the procedure for ADAM derivatization as in Fig. 1.

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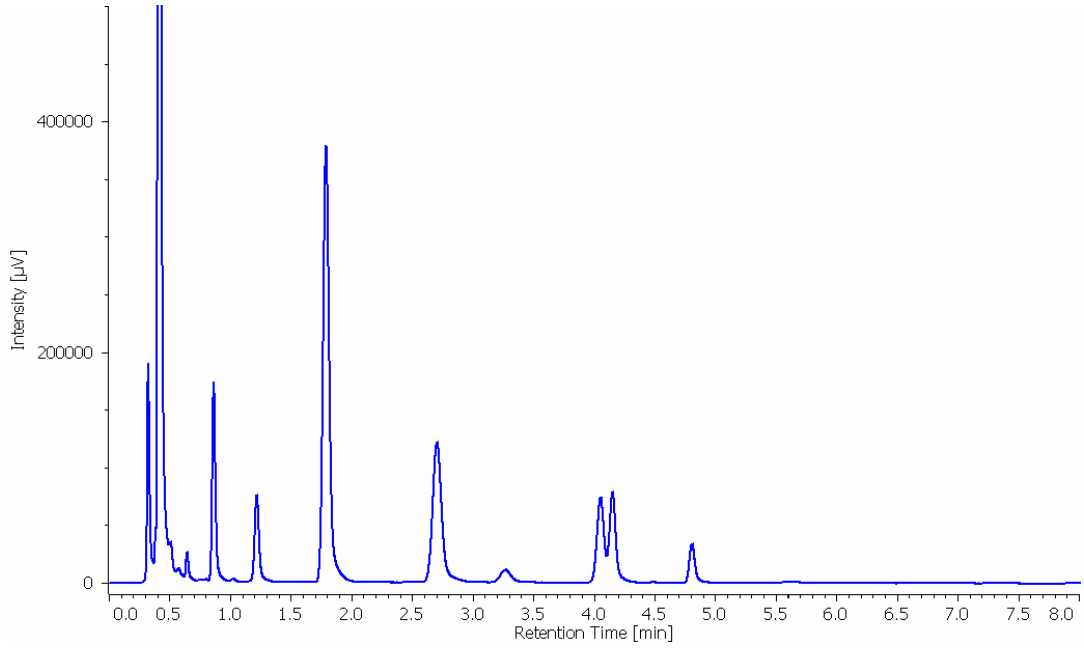

Fig. 5. Chromatogram of ADAM derivative of coconut oil. The peak numbers are the same as in Fig. 3.
Preparation : Dissolve 1.0g of coconut oil in ethyl acetate, to make the volume up to 10 mL and then follow the procedure of ADAM derivatization as in Fig. 1.

Table 1 shows quantitative value of rice oil and coconut oil.

Table 1. Quantitative value (mg/g) of rice oil and coconut oil

Fatty acids	Rice oil	Coconut oil
C8	-	0.017
C10	-	0.012
C12	-	0.85
C14	0.010	0.45
C16	0.23	0.26
C18	0.044	0.089
C18:1	0.41	0.20
C18:2	0.38	0.055
C18:3	0.007	-
C20	0.020	-
C22	-	-

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