Analysis of Fatty Acids by Pre-column HPLC

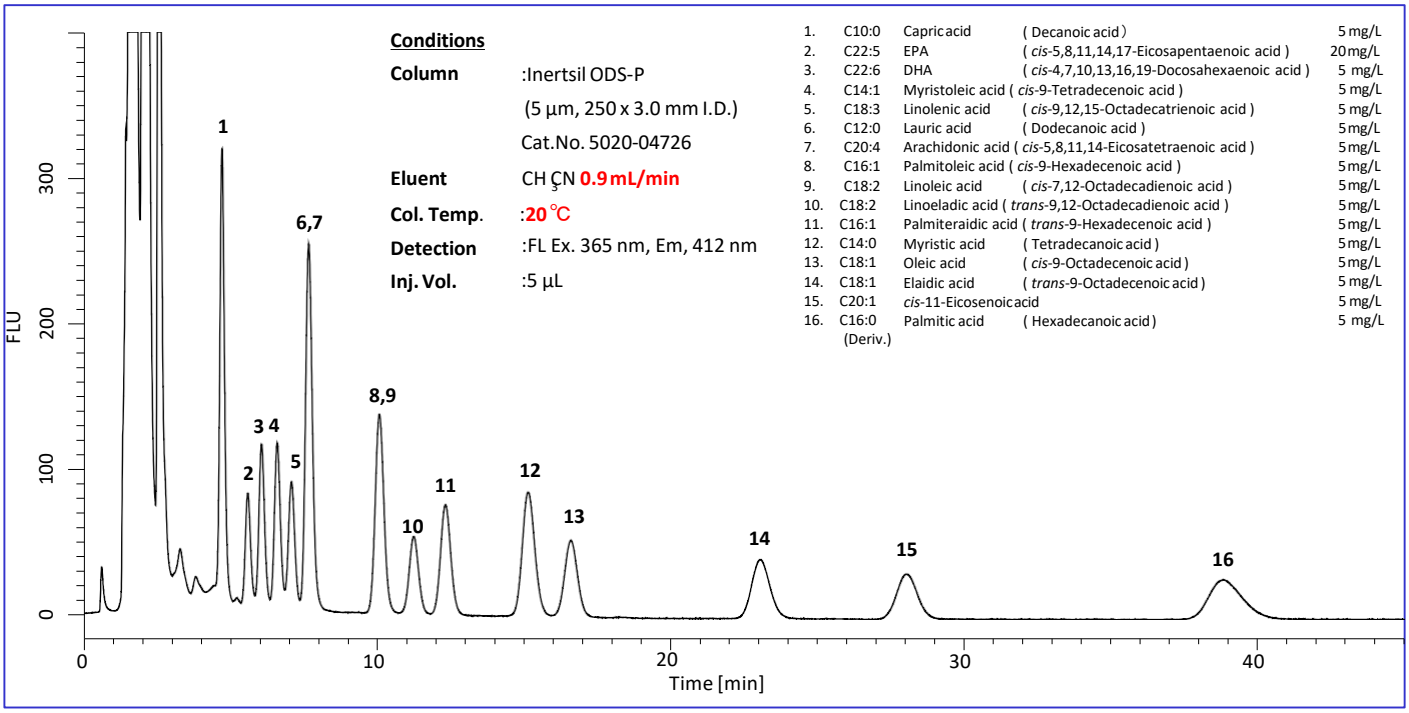
Fatty acids are major lipid components, and their content ratios exhibit some features of lipids. In this note, utilizing ADAM (9-anthryldiazomethane) as a fluorescent derivatization reagent, fatty acids are determined by HPLC-fluorescence detection system.

In example 1, the derivatives of representative fatty acids, such as EPA (*cis*-5,8,11,14,17-eicosapentaenoic acid) and DHA (*cis*-4,7,10,13,16,19-docosahexaenoic acid), are successfully separated. However, run time becomes too long if more hydrophobic fatty acids are analyzed under the condition. Therefore, another condition shown in example 2 was also described in this note.

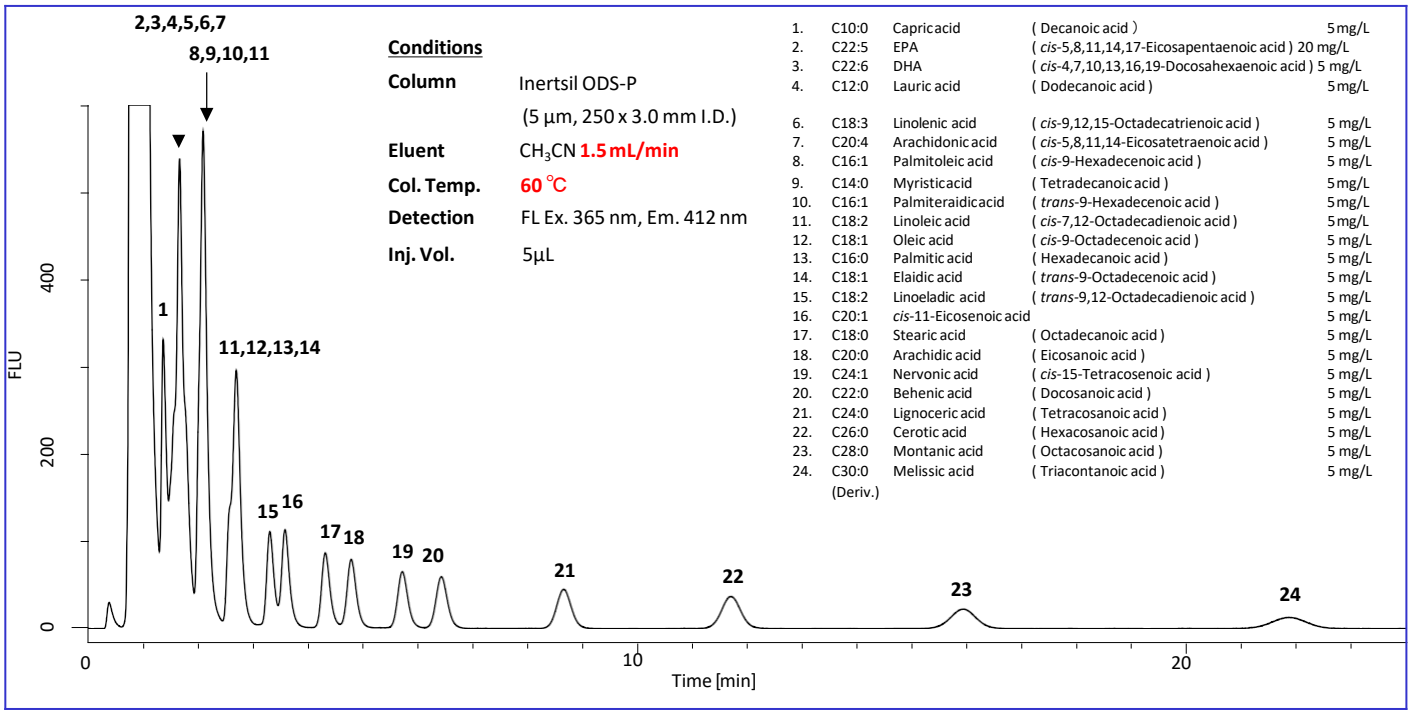
The derivatization reaction was peroformed by adding 100µL of 0.1 % ADAM solution to 500 µL of standard solution of fatty acids. After waiting for 1 hour, the reaction solution was injected into the HPLC system.

(K.Suzuki)

Example 1



Example 2



Conditions

Column	Inertsil ODS-P (5 µm, 250 x 3.0 mm I.D.)
Eluent	CH_3CN **1.5 mL/min**
Col. Temp.	**60** ℃
Detection	FL Ex. 365 nm, Em. 412 nm
Inj. Vol.	5µL

1.	C10:0	Capric acid	(Decanoic acid)	5 mg/L
2.	C22:5	EPA	(*cis*-5,8,11,14,17-Eicosapentaenoic acid)	20 mg/L
3.	C22:6	DHA	(*cis*-4,7,10,13,16,19-Docosahexaenoic acid)	5 mg/L
4.	C12:0	Lauric acid	(Dodecanoic acid)	5 mg/L
6.	C18:3	Linolenic acid	(*cis*-9,12,15-Octadecatrienoic acid)	5 mg/L
7.	C20:4	Arachidonic acid	(*cis*-5,8,11,14-Eicosatetraenoic acid)	5 mg/L
8.	C16:1	Palmitoleic acid	(*cis*-9-Hexadecenoic acid)	5 mg/L
9.	C14:0	Myristic acid	(Tetradecanoic acid)	5 mg/L
10.	C16:1	Palmiteraidic acid	(*trans*-9-Hexadecenoic acid)	5 mg/L
11.	C18:2	Linoleic acid	(*cis*-7,12-Octadecadienoic acid)	5 mg/L
12.	C18:1	Oleic acid	(*cis*-9-Octadecenoic acid)	5 mg/L
13.	C16:0	Palmitic acid	(Hexadecanoic acid)	5 mg/L
14.	C18:1	Elaidic acid	(*trans*-9-Octadecenoic acid)	5 mg/L
15.	C18:2	Linoeladic acid	(*trans*-9,12-Octadecadienoic acid)	5 mg/L
16.	C20:1	*cis*-11-Eicosenoic acid		5 mg/L
17.	C18:0	Stearic acid	(Octadecanoic acid)	5 mg/L
18.	C20:0	Arachidic acid	(Eicosanoic acid)	5 mg/L
19.	C24:1	Nervonic acid	(*cis*-15-Tetracosenoic acid)	5 mg/L
20.	C22:0	Behenic acid	(Docosanoic acid)	5 mg/L
21.	C24:0	Lignoceric acid	(Tetracosanoic acid)	5 mg/L
22.	C26:0	Cerotic acid	(Hexacosanoic acid)	5 mg/L
23.	C28:0	Montanic acid	(Octacosanoic acid)	5 mg/L
24.	C30:0 (Deriv.)	Melissic acid	(Triacontanoic acid)	5 mg/L

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