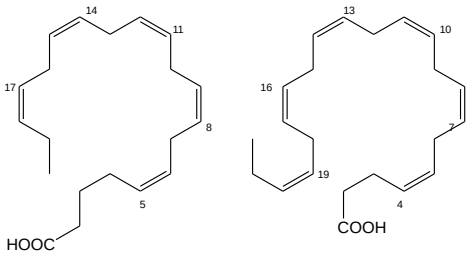
Analysis of Eicosapentaenoic and Docosahexaenoic Acids in Fish Oil Products by HPLC

Eicosapentaenoic acid (EPA) is a 20-carbon-chain unsaturated fatty acid with five *cis* double bonds (positions 5, 8, 11, 14, and 17) (20:5). Similarly, docosahexaenoic acid (DHA) is a 22-carbon-chain unsaturated fatty acid with six *cis* double bonds (positions 4, 7, 10, 13, 16, and 19) (22:6). Both EPA and DHA are classified as ω-3 fatty acids. The oils of blue-backed fish such as sardines and mackerel contain large quantities of these compounds, and because anti-arteriosclerosis, anti-aging, and memory and learning ability improvement effects have been associated with ω-3 fatty acids, many supplements and health foods containing fish oils as a raw material have been put on the market. Although a number of reports describe the use of GC for the analysis of EPA and DHA, introduced here is a method combining fluorescent derivatization with HPLC.



EPA DHA

Figure 1 Structural formula of EPA, DHA

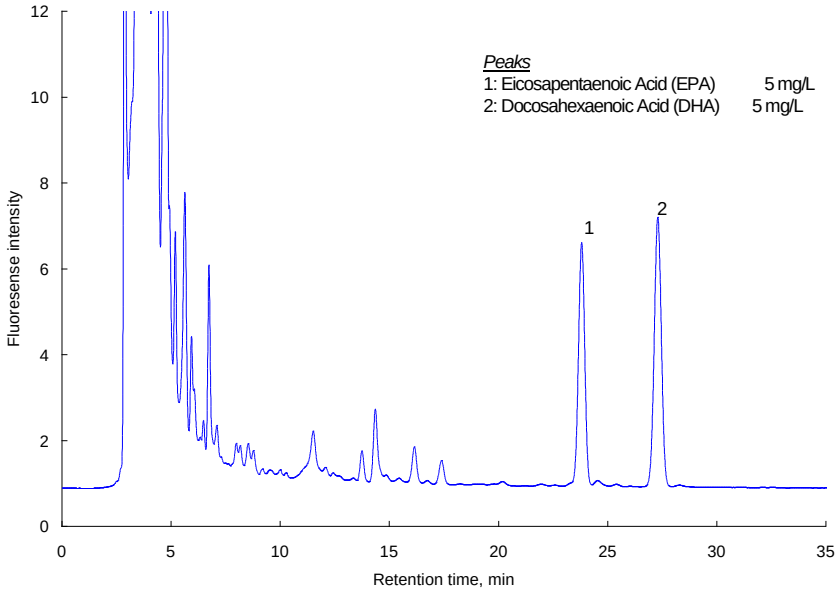


Figure 2 Chromatogram of standard reference

Table 1 Conditions

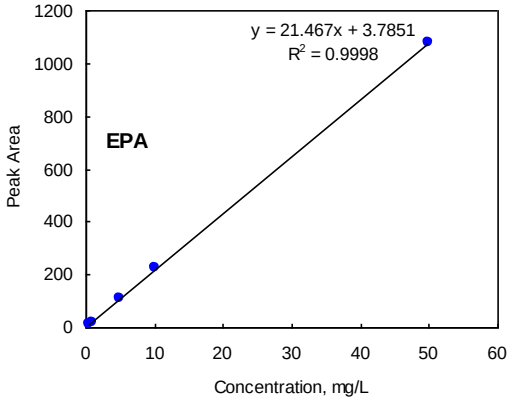
Column: TSKgel ODS-100Z 3μm (4.6 mmI.D. x 25 cm)
Eluent: water/acetonitrile = 5/95
Flow rate: 1.0 mL/min
Injection volume : 10 μL
Column temp.: 40℃
Detection : FLD (Ex; 365 nm, Em; 412 nm)

A TSKgel ODS-100Z 3μm (4.6 mm I.D. × 25 cm) analytical column was used. ADAM (9-anthryldiazomethane) was used as the fluorescent derivatization reagent, and after reacting at 40°C, separation was performed.

Figure 2 shows a chromatogram of a standard sample. Calibration curves having good linearity in the concentration range 0.5 to 50 mg/L were obtained for both EPA and DHA. Under these analytical conditions, the limit of quantitation was 0.02 mg/L. Figure 5 shows a chromatogram from an analysis of EPA and DHA within a fish-oil-containing supplement. The quantity of each analyte was determined without any effects of contaminants, and the recovery was 92 to 95%.

1) 10 mg of ADAM was dissolved in 10 mL of ethyl acetate
↓
2) 10 mL of ethyl acetate was added to 20 mg of sample and dissolved with sonicator
The sample solution was filtered (PTFE, 0.5 μm pore)
The filtrate was diluted 10-fold with ethyl acetate
↓
3) 200 μL of 1) and equal volume of 2) was mixed and reacted at 40°C for 60 min
↓
4) reaction solution was diluted 10-fold with acetonitrile and applied to HPLC

Figure 3 Procedure of pretreatment



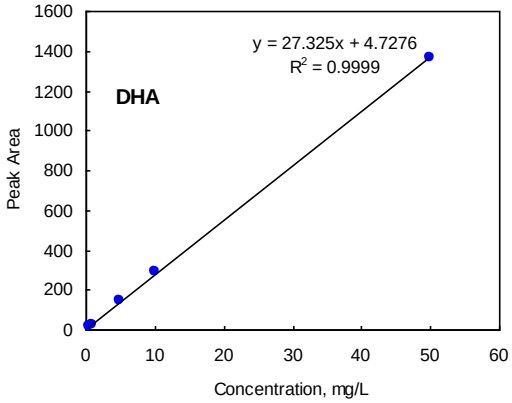


Figure 4 Calibration curves

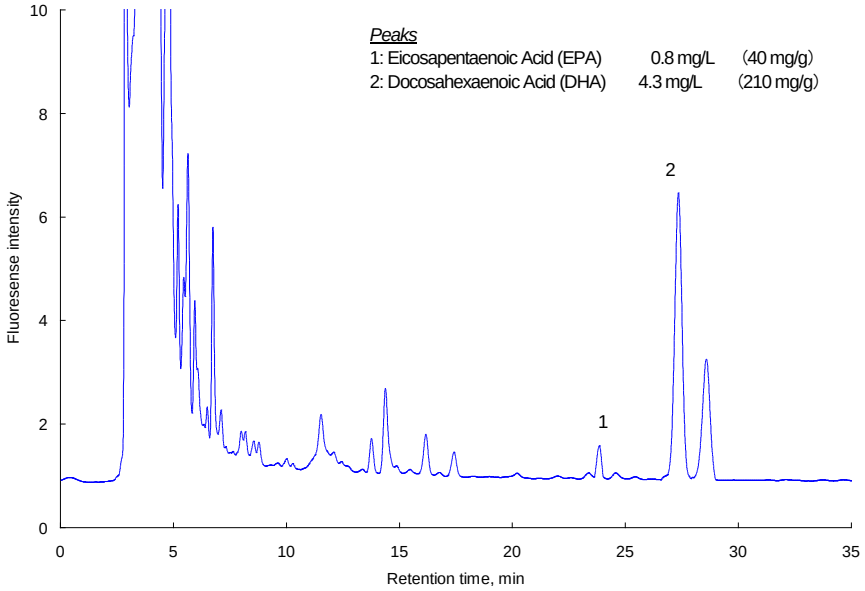


Figure 5 Chromatogram of fish-oil-containing supplement