

## Analysis of Free Trans Fatty Acids in Oil and Fat by HPLC

Trans fatty acid is a collective name that refers to geometric isomers (cis-trans) of unsaturated fatty acids. Trans fatty acids are contained in processed fats such as margarine and shortening and in processed foods containing these ingredients. They also occur naturally in trace amounts in beef and beef fat. Trans fatty acids are believed to be produced during the processing of vegetable fats and oils (hydrogenation), when oils are heated to high temperatures, and in the stomachs of ruminant animals. As in vivo effects, ingestion of trans fatty acids through food reportedly elevates LDL cholesterol and increases the risk of heart disease such as arteriosclerosis.

GC analysis following methyl esterification is the officially prescribed method for analyzing trans fatty acids. Shown here are examples of analyses of trans fatty acids by HPLC after fluorescent derivatization using an ADAM reagent. Elaidic acid (9t-18:1, trans isomer of oleic acid), a major trans fatty acid, was targeted for analysis. The quantitation limit (S/N=10) for elaidic acid converted to fatty oil and fat was 0.03mg/g.

**Table 1. Analytical conditions**

Column:	<b>TSKgel ODS-120A, 4.6mm ID x 15cm</b>
Mobile phase:	A: acetonitrile/water = 95/5 B: acetonitrile
Gradient:	0min (0%B)→ 15min (100%B)→30min (100%B)
Flow rate:	1.2mL/min
Detection:	FL (Ex: 365nm, Em: 412nm)
Temperature:	25°C
Injection vol.:	5µL

**Figure 1. Derivatization procedure for free fatty acids in fatty oil and fat**

1. Prepare ADAM reagent solution (0.1% methanol solution)  
Prepare 1% acetone solution, and dilute 10 fold with methanol
  2. Prepare sample solution (4g/L acetone solution)  
Dissolve 20mg of fatty oil and fat in 5mL of acetone
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3. Mix 200µL of ADAM reagent solution and 200µL of sample solution, and let stand at room temperature for 60 minutes

**Figure 2. Chromatograms of fatty acid reference standard, used cooking oil, and used palm oil**

