Separation of Fatty Acid Methyl Esters Using a High-Polarity, Phase-Optimized GC Column and a GC/FID Detection Technique

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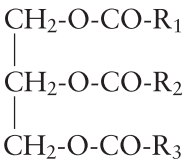
Key Words
TRACE TR-FAME GC column, fatty acid methyl esters (FAME), *cis* and *trans* fatty acids

Abstract
This application note demonstrates the analysis of five fatty acid methyl esters (FAME) separated using a column containing a highly polar stationary phase, the Thermo Scientific™ TRACE™ TR-FAME GC column. It also demonstrates resolution of *cis* and *trans* compounds.

Introduction

Fats consist of glycerol esters and long chain aliphatic acids (fatty acids). The general glycerol structure is shown below.



Fatty acid methyl esters (FAME) analysis is an important tool both for characterizing fats and oils and for determining the total fat content in foods. Fats can be extracted from a matrix using a non-polar solvent and saponified to produce salts of the free fatty acids. After derivatizing the free acids to form methyl esters, the mixture can be analyzed by gas chromatography (GC), due to the volatility and thermal stability of the FAME. Gas chromatography has become an important technique in fats and oils analysis because accurate results can be obtained for complex sample matrices.

FAME analyses were among the first applications for gas chromatography, so many of the GC methods originally written for the analysis of fats and oils utilized packed column technology. Capillary columns offer significant advantages over packed columns, including producing more efficient separations. When analyzing fats and oils with complex fatty acid profiles, such as the *cis* and *trans* forms of polyunsaturated fatty acids, higher efficiencies are needed to resolve the individual components.

In this application, a mixture of five FAME reference standards containing *cis* and *trans* stereoisomers and other components including methyl stearate were separated on a Thermo Scientific TRACE TR-FAME 100 m × 0.25 mm x 0.2 µm GC column

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Experimental Details

Consumables		Part Number
Column:	TRACE TR-FAME, 100 m x 0.25 mm x 0.20 µm	260M238P
Septum:	BTO, 17 mm	31303211
Liner:	Thermo Scientific FOCUS™ Split liner, 3 x 8 x 105 mm	45350031
Column ferrules:	100% graphite ferrules for TRACE injector 0.1–0.25 mm ID	29053488
Injection syringe:	10 µL fixed needle syringe for a Thermo Scientific TriPlus™ Autosampler	36500525
Sample handling vials and closure:	Thermo Scientific Chromacol™ 9 mm screw 0.3 mL fixed insert amber Micro+ vials Thermo Scientific Chromacol 9 mm screw caps with silicone/PTFE septa	03-FISV (A) 9-SC(B)-ST101
Fisher Scientific™ HPLC grade hexane		H/0403/15

Preparation of solutions
Methyl stearate (10 mg/mL in n-heptane)
Methyl *trans*-9-octadecenoic (10 mg/mL in n-heptane)
Methyl *cis*-9-octadecenoate (10 mg/mL in n-heptane)
trans-9,12-octadecadienoic acid methyl ester (10 mg/mL in n-heptane)
cis-9,12-octadecadienoic acid methyl ester (10 mg/mL in n-heptane)

Sample Preparation
A solution of all of the standards at concentrations of 1 µg/mL was prepared in n-heptane.

Separation Conditions	
Instrumentation:	Thermo Scientific TRACE GC Ultra gas chromatograph
Carrier gas:	Helium
Split flow:	10 mL/min
Split ratio:	10:1
Column flow:	1.0 mL/min, constant flow
Oven temperature:	120 °C (0.0 min), 20 °C/min, 210 °C (10 minutes), 40 °C/min, 250 °C (1 minute)
Injector type:	Split/Splitless
Injector mode:	Split, constant flow
Injector temperature:	240 °C
Detector type:	Flame ionization detector (FID)
Detector temperature:	240 °C
Detector air flow:	350 mL/min
Detector hydrogen flow:	35 mL/min
Detector nitrogen flow:	30 mL/min

Injection Conditions	
Instrumentation:	Thermo Scientific TriPlus Autosampler
Injection volume:	2.0 µL

Results

The analysis was performed on a TRACE TR-FAME GC column with excellent separation and resolution of *cis* and *trans* isomers. The trans isomer elutes before the *cis* isomer with proper resolution of all five components (Figure 1). Table 1 shows the retention times for five FAME standards with their levels of detection of 1 µg/mL.

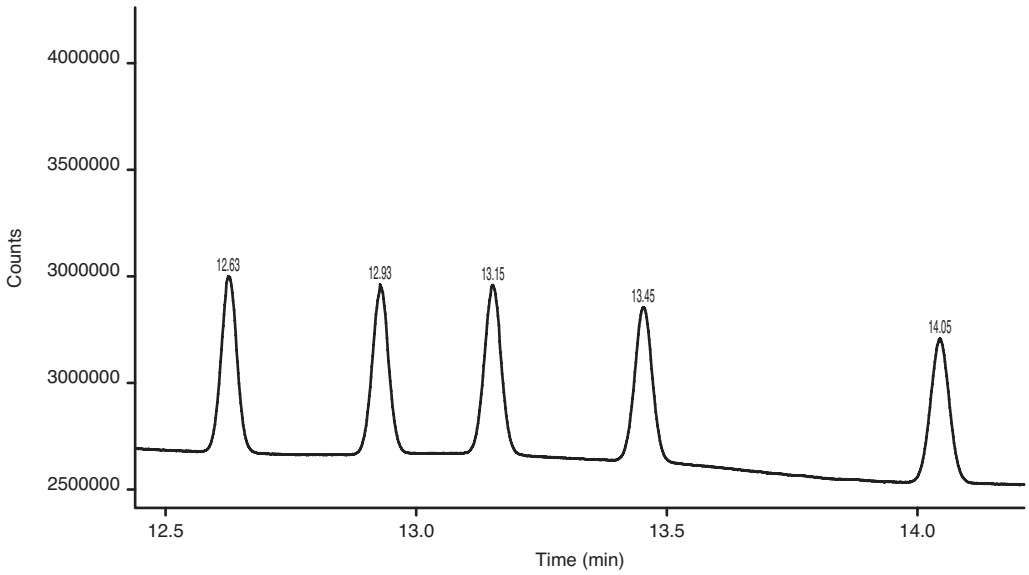

Figure 1: Expanded chromatogram of five components of FAME mixture on a TRACE TR-FAME 100 m × 0.25 mm × 0.20 µm GC column

Elution order	Compound	Concentration 1 ppm	t_R min
1	Methyl stearate	0.01	12.63
2	Methyl *trans*-9-octadecenoic	0.01	12.93
3	Methyl *cis*-9-octadecenoate	0.01	13.15
4	*trans*-9,12-octadecadienoic acid methyl ester	0.01	13.45
5	*cis*-9,12-octadecadienoic acid methyl ester	0.01	14.05

Table 1: FAME peaks observed and their retention times (t_R)

Conclusion

This application note demonstrates the capabilities of TRACE TR-FAME GC columns to separate five different FAME standards, resolving *cis* and *trans* isomers in which the trans isomer eluted earlier than the cis analog. The column provides excellent peak shapes and well-separated peak profiles of five different FAME standards in a reasonably short run time. The run time can be optimized based on the presence of other FAME standards using the same column with optimization of temperature and flow rate.

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