Application Note 20733

A GC-FID Method for the Comparison of Acid- and Base-Catalyzed Derivatization of Fatty Acids to FAMEs in Three Edible Oils

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Key Words

TR-FAME, fatty acid methyl esters (FAMEs), BF_3-methanol, derivatization, *cis*- and *trans*-fatty acid

Abstract

This application note demonstrates the analysis of 37 fatty acid methyl esters (FAMEs) separated by a highly polar phased Thermo Scientific™ TRACE™ TR-FAME GC column. Results from two derivatization methods (acid and base esterification) were compared for their efficiency in converting fatty acids to their methyl esters on three different fat matrices prior to GC analysis.

Introduction

Gas chromatography is the preferred analytical method for the determination of fatty acid methyl esters (FAMEs). The fatty acid content of food was analyzed after derivatization to their methyl ester products. This conversion involved either an acid or base esterification process.

In this application, separation of a mixture of 37 FAMEs in a reference standard was achieved on a TRACE TR-FAME 100 m × 0.25 mm × 0.2 µm GC column. The reference standard contained a wide range of carbon chain lengths (C4–C24), with concentrations between 2–6% wt/wt. The high polarity phase GC column is optimized for separating complex mixtures of *cis*- and *trans*-fatty acids.

The base esterification method [1] was used to derivatize the fatty acid content in three fat matrices (palm oil, margarine, butter) and this was compared with acid catalyzed esterification [2] under equivalent conditions. The FAME components in the three fat matrices were then identified using the retention times established using a 37 component reference standard.

BF_3-methanol is one of the fastest and most convenient ways to convert fatty acids to their methyl ester derivatives. The reagent is supplied in an easy-to-use, septum-sealed Hypo vial and offers convenient syringe removal of the reagent without exposing it to air. Use of the BF_3-methanol reagent results in improved detection of fatty acids in a fatty food matrix while maintaining good chromatographic peak shape.

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Experimental Details

Consumables		Part Number
Column:	TRACE TR-FAME GC column, 100 m × 0.25 mm × 0.20 µm	260M238P
Septum:	Thermo Scientific BTO, 17 mm	31303211
Liner:	Thermo Scientific™ Split FocusLiner™, 3 × 8 × 105 mm	45350031
Column ferrules:	100% graphite ferrules for Thermo Scientific™ TRACE™ injector 0.1–0.25 mm i.d.	29053488
Column ferrules:	Graphite/Vespel® for transfer line 0.1–0.25 mm i.d.	29033496
Injection syringe:	10 µL fixed needle syringe for Thermo Scientific™ TriPlus™ Autosampler	36500525
Vials and closures:	Thermo Scientific™ Chromacol™ 9 mm screw 0.3 mL fixed insert amber Micro+ vials	03-FISV (A)
	Chromacol 9 mm screw caps with Silicone/PTFE septa	9-SC(B)-ST101
Syringes:	Thermo Scientific™ National™ 30 mm GMF Syringe filter membrane, 3.1 µm pore size	F2500-20
	Thermo Scientific™ National™ Target™ 3 mL plastic disposable syringes	S7510-3

Sample Handling Equipment	Part Number
Thermo Scientific™ Reacti-Therm™ III Heating/Stirring Module	TS-18823
Thermo Scientific™ Reacti-Vap™ III Evaporator	TS-18826
Thermo Scientific Reacti-Vap Block	TS-18814
Thermo Scientific™ Reacti-Vial™ Reaction Vials 10 mL	TS-13225

Chemicals and Reagents	Part Number
Fisher Scientific™ HPLC grade hexane	H/0403/15
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade methanol	M/4056/17
Fisher Scientific HPLC grade potassium hydroxide	S/9220/PB08
Thermo Scientific 14% BF_3-methanol esterification reagent	TS-49370

Sample Preparation

Butter, margarine, and palm oil were treated by two derivatization methods. In the first method, potassium hydroxide / methanol was used to esterify the fat samples [1]. This was then compared to an acidic derivatization method involving BF_3-methanol [2].

Base esterification:	A 50 mg liquid fat sample was weighed into Reacti-Vial containing a magnetic stirrer and 1 mL of hexane and 2 mL of 4 mol/L potassium hydroxide / methanol were added. The Reacti-Vial was capped and placed in the Reacti-Therm module for 30 minutes at 50 °C. The mixture was cooled to room temperature and 1 mL of water was then added. After phase separation, an aliquot of the organic layer was transferred to a fixed insert GC vial.
Acid esterification:	A 50 mg liquid fat sample was weighed into a Reacti-Vial containing a magnetic stirrer and 1 mL of hexane and 0.5 mL of Thermo Scientific 14% BF_3-methanol was added. The Reacti-Vial was capped and placed in the Reacti-Therm module for 30 minutes at 50 °C. The mixture was cooled to room temperature and 1 mL of water was then added. After phase separation, an aliquot of the organic layer was transferred to a fixed insert GC vial.

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:	100 °C (0.2 min), 2 °C/min, 240 °C (15 min)
Injector type:	Split/Splitless
Injector mode:	Split, constant septum purge
Injector temperature:	240 °C
Detector type:	Flame ionization detector (FID)
Detector temperature:	250 °C
Detector air flow:	350 mL/min
Detector hydrogen flow:	35 mL/min
Detector nitrogen flow:	30 mL/min

Injection Conditions	
Instrumentation:	TriPlus Autosampler
Injection volume:	1 µL

Results

The analysis of a 37 component FAME reference standard was successfully carried out using a TR-FAME GC column (Figure 1). The high polarity phase on the TRACE TR-FAME GC column provided baseline resolution of the majority of FAME components, apart from C20:3 [*cis*-8, 11, 14], C22:1 [*cis*-13], and C20:3 [*cis*-8, 14, 17], which were partially separated. These compounds, two of which are isomeric, are known to be difficult to separate by GC due to their structural similarities, which results in poor resolution. All FAME components exhibited excellent chromatographic peak shape.

A qualitative analysis was performed by comparing the FAME peaks in the fat matrices using the two derivatization methods. The components were identified using the retention times in the FAME reference standard in Figure 1. The results from the two methods are compared in Figures 2 and 3, under equivalent conditions (see Table 2 for comparison).

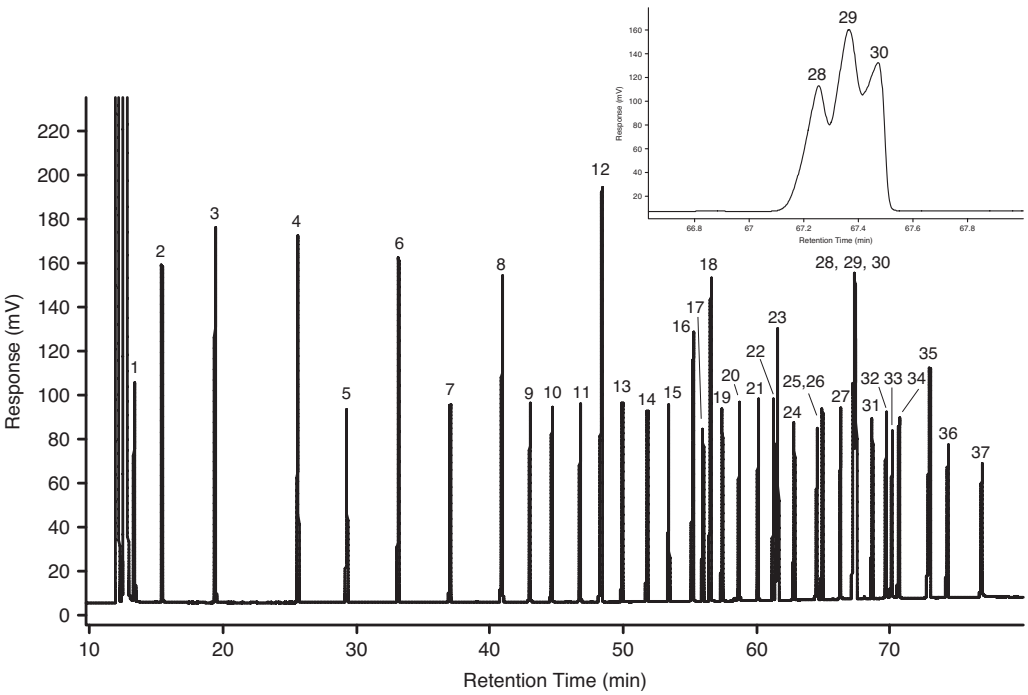

Figure 1: Chromatogram of 37 components FAME mixture (reference standard) separated on a TR-FAME 100 m × 0.25 mm × 0.20 µm GC column

Elution order	Compound	Concentration %wt/wt	t_R/min
1	Methyl butyrate (C4:0)	4	13.40
2	Methyl caproate (C6:0)	4	15.53
3	Methyl capylate (C8:0)	4	19.60
4	Methyl decanoate (C10:0)	4	25.90
5	Methyl undecanoate (C11:0)	2	29.56
6	Methyl dodecanoate (C12:0)	4	33.50
7	Methyl tridecanoate (C13:0)	2	37.36
8	Methyl myristate (C14:0)	4	41.30
9	Methyl myristoleate (C14:1 [*cis*-9])	2	43.36
10	Methyl pentadecanoate (C15:0)	2	45.01
11	Methyl pentadenoate (C15:1 [*cis*-10])	2	47.12
12	Methyl palmitate (C16:0)	6	48.79
13	Methyl palmitoleate (C16:1 [*cis*-9])	2	50.30
14	Methyl heptadecanoate (C17:0)	2	52.18
15	Methyl heptadenoate (C17:1 [*cis*-10])	2	53.78
16	Methyl stearate (C18:0)	4	55.67
17	Methyl octadecenoate (C18:1 [*trans*-9])	2	56.38
18	Methyl oleate (C18:1 [*cis*-9])	4	56.96
19	Methyl linoleaidate(C18:2 [*trans*-9,12])	2	57.79
20	Methyl linoleate (C18:2 [*cis*-9,12])	2	59.06
21	Methyl arachidate (C20:0)	4	60.48
22	Methyl linolenate (C18:3 [*cis*-6,9,12])	2	61.61
23	Methyl (C20:1 [*cis*-11])	2	62.01
24	Methyl linolenate (C18:3 [*cis*-9,12,15])	2	63.20
25	Methyl heneicosanoate(C21:0)	2	64.96
26	Methyl eicosadienoate (C20:2 [*cis*-11,14])	2	65.31
27	Methyl behenate (C22:0 FAME)	4	66.66
28	Methyl eicosatrienoate (C20:3 [*cis*-8,11,14)	2	67.60
29	Methyl erucate (C22:1 [*cis*-13])	2	67.72
30	Methyl eicosatrienoate (C20:3 [*cis*-11,14,17])	2	67.87
31	Methyl arachidonate (C20:4 [*cis*-5,8,77,14])	2	69.06
32	Methyl tricosanoate (C23:0)	2	70.06
33	Methyl docosadienoate (C22:2 [*cis*-13,16])	2	70.57
34	Methyl lignocerate (C24:0)	4	71.06
35	Methyl *cis*-5,8,11,14,17-eicosapentaenoate	2	73.41
36	Methyl nervonate (C24:1 [*cis*-15])	2	74.74
37	Methyl *cis*-4,7,10,13,16-docosahexenoate	2	77.23

Table 1: FAMEs according to the elution order and retention times for the reference standard

The base esterification process resulted in fewer FAME peaks observed in the fat samples compared to the acid esterification process (see Table 2 for comparison). Some emulsification occurred during phase separation and the esterified solution required filtering with a syringe filter prior to GC analysis.

In contrast, acidic esterification produced more FAME peaks than base esterification. The strong Lewis acid BF_3-methanol can more efficiently esterify fatty acids compared with the base esterification method, with no white emulsion appearing when reaction is worked up with water.

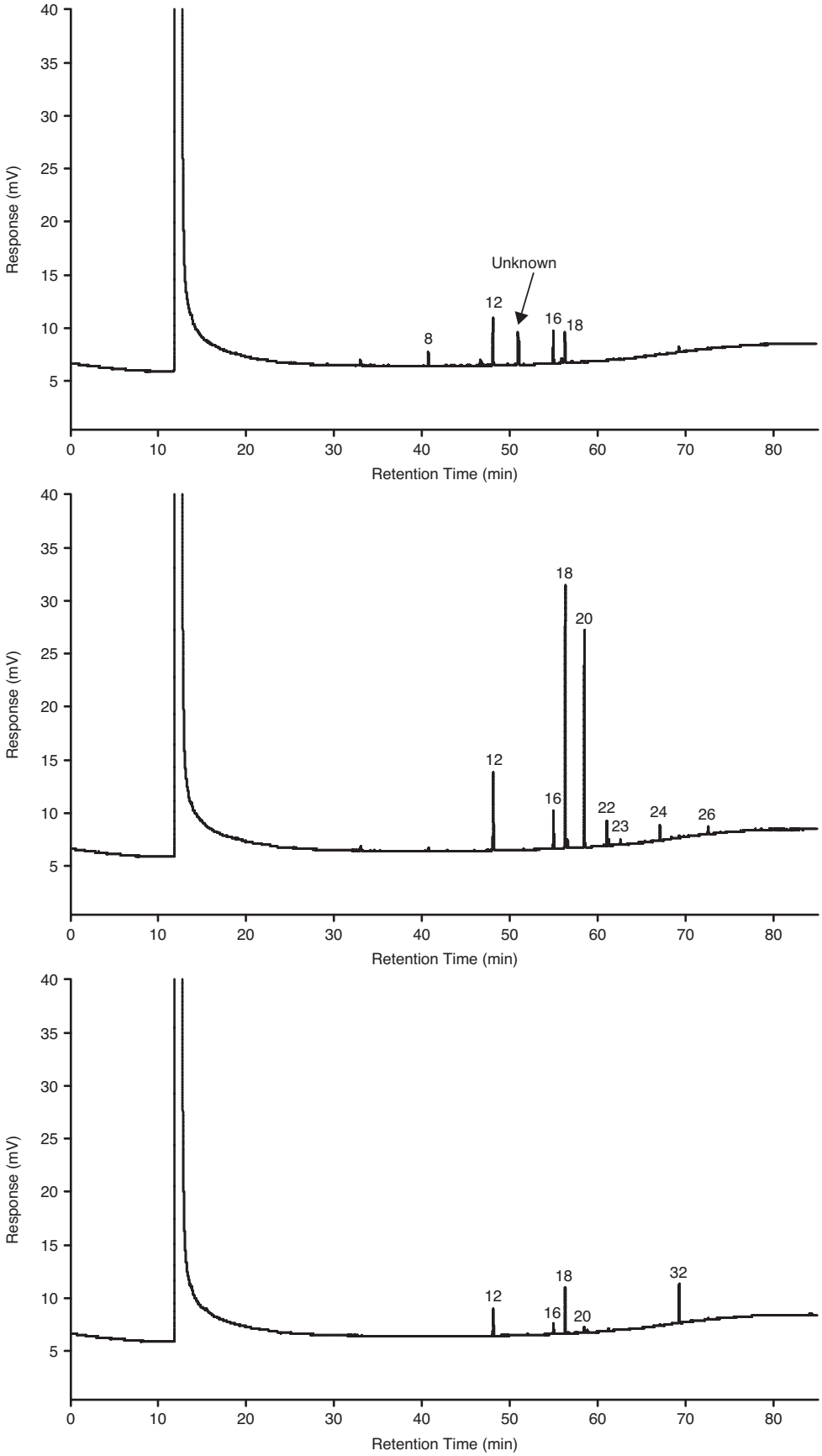

Figure 2: Chromatograms of (top) butter, (middle) margarine, and (bottom) palm oil sample derivatized by potassium hydroxide / methanol

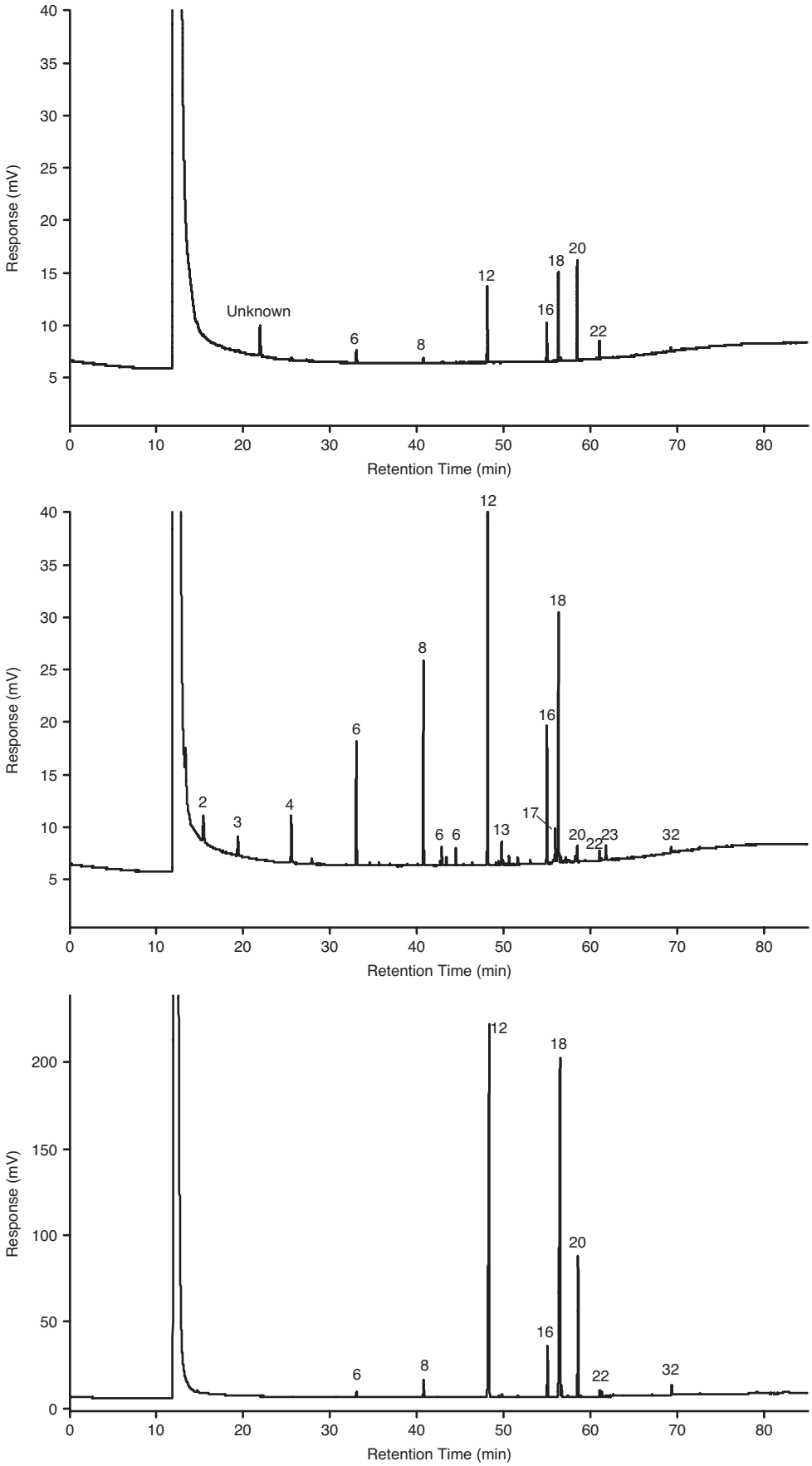

Figure 3: Chromatograms of (top) butter, (middle) margarine, and (bottom) palm oil sample derivatized by Thermo Scientific Reagent BF_3-methanol

Matrix	Base esterified-FAMEs using KOH / methanol (Figure 2)	Acid esterified- FAMEs using BF_3-methanol (Figure 3)
Butter	-	(6) C12:0
	(8) C14:0	(8) C14:0
	(12) C16:0	(12) C16:0
	(16) C18:0	(16) C18:0
	(18) C18:1 [*cis*-9]	(18) C18:1 [*cis*-9]
	-	(20) C18:2 [*cis*-9,12]
	-	(22) C18:3 [*cis*-6,9,12]
Margarine	-	(2) C6:0
	-	(3) C8:0
	-	(4) C10:0
	-	(6) C12:0
	-	(8) C14:0
	-	(9) C14:1 [*cis*-9]
	-	(10) C15:0
	(12) C16:0	(12) C16:0
	-	(13) C16:1 [*cis*-9]
	(16) C18:0	(16) C18:0
	-	(17) C18:1 [*trans*-9]
	(18) C18:1 [*cis*-9]	(18) C18:1 [*cis*-9]
	(20) C18:2 [*cis*-9,12]	(20) C18:2 [*cis*-9,12]
	(22) C18:3 [*cis*-6,9,12]	(22) C18:3 [*cis*-6,9,12]
	(23) C20:1 [*cis*-11]	(23) C20:1 [*cis*-11]
	(24) C18:3 [*cis*-9,12,15]	-
	(32) C23:0	(32) C23:0
Palm oil	-	(6) C12:0
	-	(8) C14:0
	(12) C16:0	(12) C16:0
	(16) C18:0	(16) C18:0
	(18) C18:1 [*cis*-9]	(18) C18:1 [*cis*-9]
	(20) C18:2 [*cis*-9,12]	(20) C18:2 [*cis*-9,12]
	-	(22) C18:3 [*cis*-6,9,12]
	(32) C23:0	(32) C23:0

Table 2: FAMEs peaks observed for two derivatization methods in 30 minutes reaction time

Conclusion

The Thermo Scientific reagent BF_3-methanol provides a fast and efficient way of converting fatty acids to their methyl esters in fat samples. The TRACE TR-FAME GC column can separate a complex mixture of 37 FAMEs with excellent peak shapes.

References

[1] Chinese Official method SN/T 1945-2007. Determination of fatty acids in food-Capillary gas chromatography

[2] Thermo Scientific Reagents, Solvents and Accessories Brochure. Ref BR20535_E 06/12S

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