Fast, Selective Triglyceride Analysis

Triglycerides are naturally occurring esters of fatty acids and glycerols. They are widely analyzed in the food industry for natural oil and fat characterization as well as fat adulteration. The triglycerides usually are converted to their methyl esters (FAMEs) to determine the fatty acid composition and percent saturated vs. unsaturated fat. Triglyceride analyses have become very important in recent years since health conscious consumers are concerned with minimizing their dietary intake of saturated fats to reduce the risk of heart disease.

Fatty acid groups in the triglyceride molecule can be classified as saturated [myristic acid (C14:0), palmitic acid (C16:0), or stearic acid (C18:0)], monounsaturated [oleic acid (C18:1)], or polyunsaturated [linoleic acid (C18:2) or linolenic acid (C18:3)]. Typically, triglycerides are characterized by degree of unsaturation. For example, a triglyceride molecule containing the groups stearic acid, oleic acid, and linoleic acid (denoted SOL) would have a greater degree of unsaturation than that of tripalmitin (denoted PPP). Table I shows the nomenclature of fatty acids and triglycerides.[1]

Capillary GC columns are the preferred analytical tool for triglyceride analysis because they yield shorter analysis times, higher efficiency, and better quantitation than packed column GC, HPLC, or SFC. The Rtx®-65TG column is truly an improvement over classical packed columns because triglycerides with the same carbon number but different degrees of unsaturation can be well separated. Also, minimal sample preparation is required for capillary GC analysis. The sample is liquefied by warming and diluting to 50pg/µL with dichloromethane or diisopropyl ether[2,3]. Additional sample clean up is required if significant amounts of monoglycerides, diglycerides, and fatty acids are known to be present in the sample[2].

Column Selection for Triglyceride Analysis

The high molecular weights of triglycerides require capillary columns with high thermal stability. Low bleed columns also are extremely important for accurate triglyceride quantitation. Triglyceride polarity increases with the degree of unsaturation in the fatty acid (i.e., the number of double bonds present). The triglyceride with the most double bonds has the highest polarity and the longest retention time. Therefore, a high temperature is required to elute the higher polarity triglycerides and maintain a short analysis time. Lower response of high molecular weight triglycerides has been observed and originally was attributed to thermal decomposition of triglycerides in the injection port. However, the decreased response also can be caused by both high molecular weight discrimination in the

Table I

Fatty acid and triglyceride nomenclature (after Geeraert and Sandra)

Fatty Acids

Abbreviation	Common Name	IUPAC Name	Short Form
La	lauric acid	dodecanoic acid	C 12:0
M	myristic acid	tetradecanoic acid	C 14:0
P	palmitic acid	hexadecanoic acid	C 16:0
S	stearic acid	octadecanoic acid	C 18:0
A	arachidic acid	eicosanoic acid	C 20:0
Be	behenic acid	dodosanoic acid	C 22:0
Lg	lignoceric acid	tetracosanoic acid	C 24:0
O	oleic acid	*cis*-9-octadecenoic acid	C 18:1
L	linoleic acid	*cis, cis*-9,12-octadecadienoic acid	C 18:2
Ln	linolenic acid	*cis, cis, cis*-9,12,15-octadecatrienoic acid	C 18:3
Ga	gadoleic acid	*cis*-11-eicosenoic acid	C 20:1

Glycerides

Abbreviation	Common Name	Carbon Number	# of Unsaturated Fatty Acids
PPP	tripalmitin	48	0
PLO	palmito-linoleo-olein	52	2

injection port and increased solute band broadening in the stationary phase.

Triglycerides are separated according to carbon number or molecular weight on non-polar, methyl silicone columns (AOAC and AOCS Methods use this type of column). However, no resolution is achieved for differences in unsaturation within the unsaturated fatty acids. Therefore, triglycerides such as POP, SOS, and POS would all appear as a single peak. Triglyceride separation by degree of unsaturation, as well as carbon number requires a highly polar stationary phase. While polar stationary phases such as 50% phenyl/50% methyl offer the necessary selectivity, they traditionally have suffered from relatively low thermal stability. Phenyl/methyl polysiloxanes generally exhibit lower maximum operating temperatures when compared with methyl silicones.

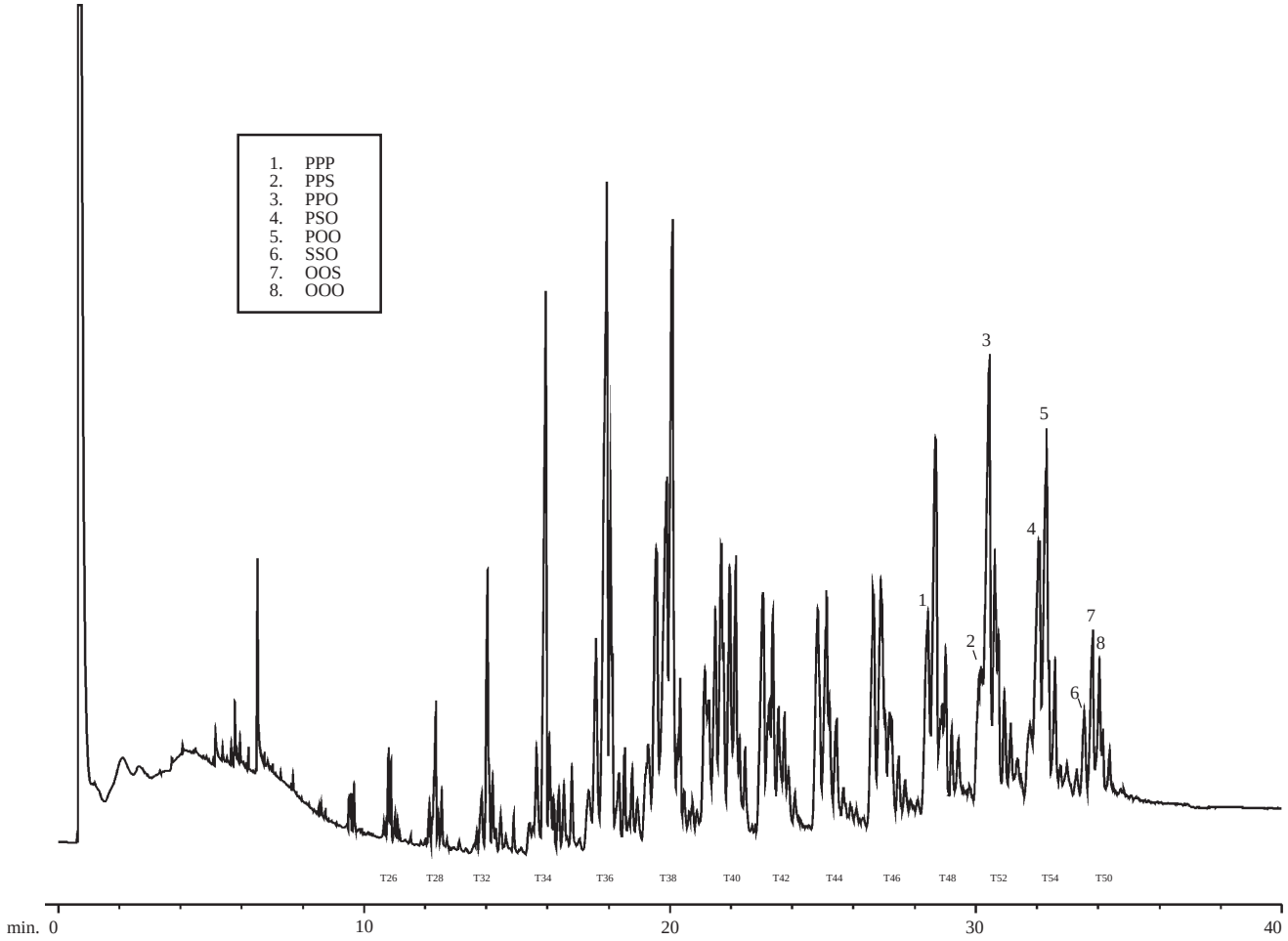
Separate Triglycerides by Carbon Number and Degree of Unsaturation Using an Rtx®-65TG Column

The chemists at Restek have combined innovative polymer synthesis with advanced deactivation techniques to create a highly polar, uniquely selective stationary phase with the extended thermal stability required for triglyceride analysis. This produces columns that last longer and exhibit less bleed at temperatures as high as 370°C. The Rtx®-65TG column (65% phenyl/35% methyl polysiloxane) is selective for resolving triglycerides according to degree of unsaturation as well as carbon number.

Figure 1 shows butter fat triglycerides run on a 30m, 0.25mm, 0.10µm Rtx®-65TG column using a septum-equipped programmable injector (SPI). The SPI injector reduces molecular weight

Figure 1

The Rtx®-65TG column exhibits excellent resolution of butter fat triglycerides in under 35 minutes at 365°C.



30m, 0.25mm ID, 0.10µm Rtx®-65TG (cat.# 17008)
0.2µL direct injection of 1% butter fat in isooctane.

Oven temp.:	80°C (hold 1 min.) to 240°C @ 30°C/min. to 365°C @ 4°C/min. (hold 5 min.)
Det. temp.:	380°C
Carrier gas:	hydrogen
Linear velocity:	70cm/sec.
FID sensitivity:	16 x 10^{-11} AFS
SPI injector:	high-performance capillary insert 60°C, 300°C/min. to 400°C (hold 5 min.)

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discrimination and thermal degradation, which is common with normal split/splitless injectors. The column shows excellent separation and peak symmetry of the triglycerides by both degree of unsaturation and carbon number. Fine structure resolution of T50 (PPS/PPO), T52 (PSS/PSO/POO), and T54 (SSS/SSO/SOO/OOO) triglycerides is obtained with minimal column bleed at 365°C. The optimized analysis time is less than 35 minutes. Figure 2 shows the analysis of canola, corn, and olive oils on a 15m, 0.25mm ID, 0.10µm Rtx®-65TG column. The unsaturates are all well separated in less than 8 minutes with minimal column bleed at 365°C.

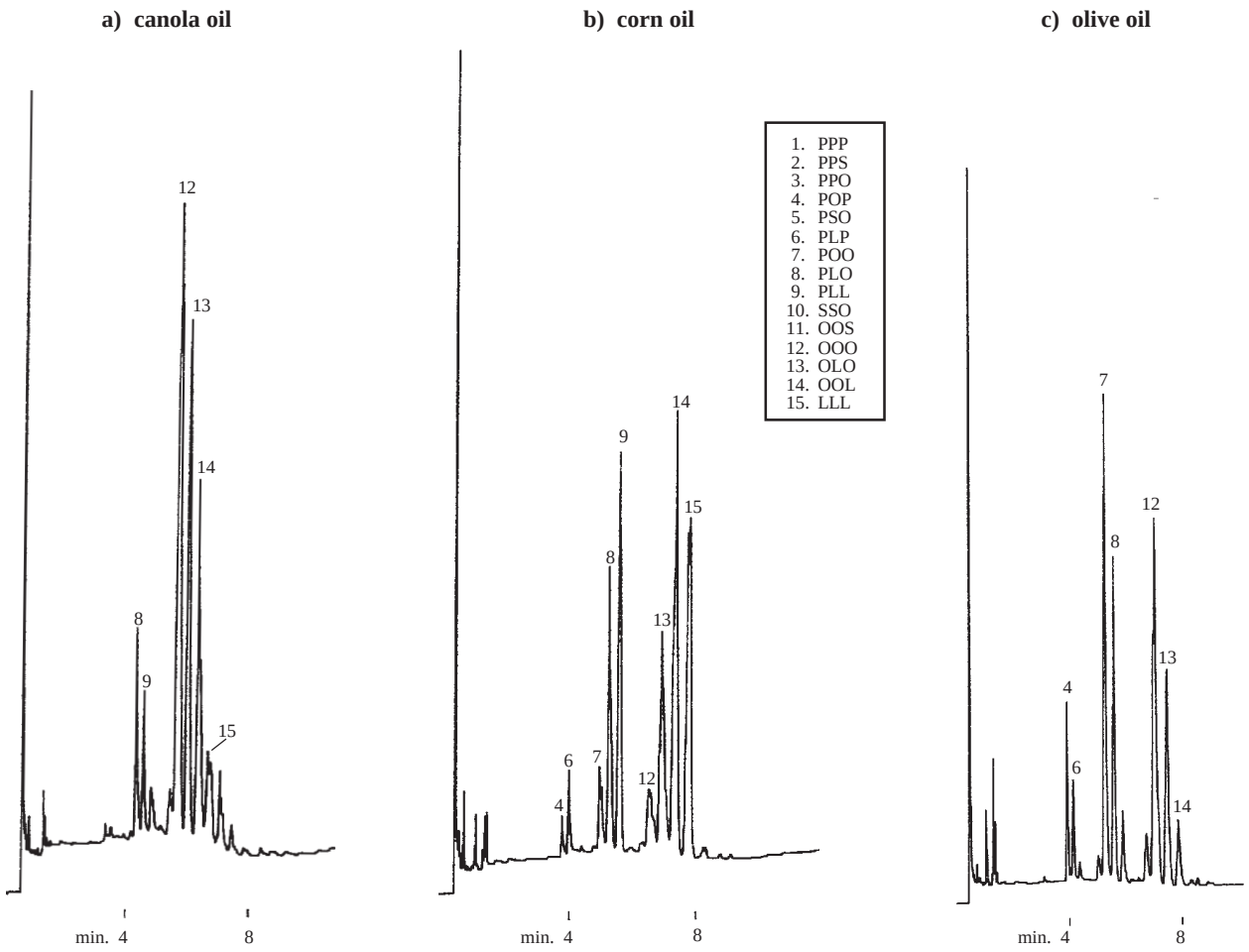
The Rtx®-65TG column is ideal for triglyceride analysis. Separation by degree of unsaturation as well as carbon number is obtained in under 35 minutes with minimal column bleed at 365°C. Because Rtx®-65 columns are individually tested with a temperature-programmed triglyceride test mixture, low bleed and high efficiency are guaranteed. Rtx®-65TG columns are available in both 15- and 30-meter lengths in 0.25, 0.32, and 0.53mm IDs with a 0.10µm film thickness.

References:

1. Geeraert and Sandra, "Capillary GC of Triglycerides in Fats and Oils Using a High Temperature Phenyl Methyl Silicone Stationary Phase," *Journal of HRC & CC, Vol. 8, Aug 1985, pp. 415-419.*
2. Association of Analytical Chemists, Official Methods of Analysis of the AOAC, 17th ed., Method 986.19.
3. American Oil Chemists Society, Official Methods and Recommended Practices (1994), Method Ce 5-86.

Figure 2

Obtain fine structure resolution by degree of unsaturation with common oils on a 15-meter Rtx®-65TG column.



15m, 0.25mm ID, 0.10µm Rtx®-65TG (cat.# 17005)
0.8µL split injection of triglycerides.

Oven temp.:
a) 355°C (hold 1 min.) to 365°C @ 1°C/min.
b) 350°C (hold 0.5 min.) to 365°C @ 1°C/min.
c) 350°C (hold 1 min.) to 365°C @ 0.5°C/min.

Inj. & det. temp.: 365°C
Carrier gas: hydrogen
Linear velocity: 50cm/sec.
FID sensitivity: 8 x 10^{-11} AFS
Split ratio: 40:1

Product Listing

Rtx®-65TG Columns

ID	df (µm)	Temp. Limits	15m	30m
0.25mm	0.10	40 to 370°C	17005	17008
0.32mm	0.10	40 to 370°C	17006	17009
0.53mm	0.10	40 to 360/370°C	17007	17010

MXT®-65TG Columns

ID	df (µm)	Temp. Limits	15m	30m
0.25mm	0.10	20 to 370°C	77005	77008
0.53mm	0.10	20 to 370°C	77007	77010

Inlet Liners

PTV Liners for Agilent GCs	ID/OD & Length (mm)	ea.	cat.# 5-pk.	10-pk.
Straight Glass Inlet Liner	2.0 ID 3.0 OD x 71	—	—	21157
Baffled Glass Inlet Liner	1.5 ID 3.0 OD x 71	—	—	21704
Glass Inlet Liner with Wool*	2.0 ID 3.0 OD x 71	—	—	21156

SPI Liners for Varian GCs	ID/OD & length (mm)	ea.	cat.# 5-pk.	25-pk.
0.5mm SPI	0.53 ID 4.6 OD x 54	20775	20776	20777
0.8mm SPI	0.80 ID 4.6 OD x 54	20778	20779	20780
SPI with Buffer	2.4 ID 4.6 OD x 54	20850	20851	20852

**Liner is packed with fused silica wool. To order glass wool instead, add the suffix "-202" to the liner's catalog number.*

17A PTV Liners for Shimadzu GCs	ID/OD & Length (mm)	ea.	cat.# 5-pk.	25-pk.
17A PTV Sleeve*	1.6 ID 4.0 OD x 95	21705	21706	21707

PTV Liners for Perkin-Elmer GCs	ID/OD & Length (mm)	ea.	cat.# 5-pk.	25-pk.
PTV Press-Tight®	1.0 ID 2.0 OD x 88	20733	20734	20735
PTV Injector	1.0 ID 2.0 OD x 88	20742	20743	20744

Siltek™ Inlet Liners

For Siltek™-deactivated inlet liners, add the corresponding suffix number to your liner catalog number.

qty.	Siltek™	Siltek™ with Siltek™ wool	Siltek™ with CarboFrit™
each	-214.1	-213.1	-216.1
5-pk.	-214.5	-213.5	-216.5
25-pk.	-214.25	-213.25	-216.25