

APPLICATIONS

Quick, Accurate Testing of FAMES in Olive Oil by GC/FID Using a ZebronTM ZB-FAME GC Column

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Tim was raised in Texas where it was completely too hot, then moved to Pennsylvania and Ohio where it was entirely too cold. He finally settled on California where the weather is just right.



Testing of fatty acids in olive oil is commonly performed to ensure authenticity and to prevent adulteration. The long column lengths traditionally required for analysis produce extremely long run times; by using a column with targeted selectivity and a shorter length, fast, accurate results can be achieved for increased sample throughput.

Introduction

Olive oil is one of the most adulterated food products worldwide. Various fatty acids such as those shown in **Figure 1** can be found in olive oil; understanding the profile of these fatty acids helps the marketplace provide an authentic and reliable product to the tables of consumers across the globe. In addition to product integrity, the fatty acid profile of olive oil is useful in determining nutritional quality and other flavor characteristics. For these reasons, fatty acids from olive oil are commonly tested among food laboratories worldwide.

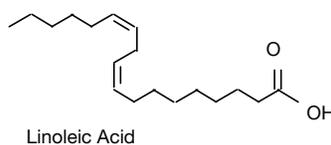
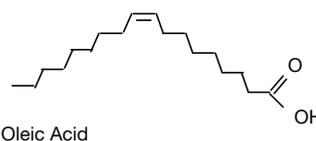
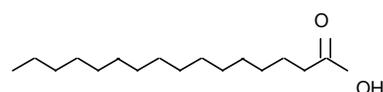
Underivatized free fatty acids are highly polar and tend to form hydrogen bonds, which can lead to tailing and poor peak shapes. Because they are adsorptive and lack volatility (longer chain acids), free fatty acids are commonly derivatized and converted to fatty acid methyl esters (FAMES) to improve accuracy and quantitation. Once derivatized, FAMES analysis by GC typically employs a highly polar GC column to separate cis and trans orientations on long carbon bonded tails; these columns are also generally long in length (100 m), which can result in run times of 60 minutes or more.

In this technical note, we demonstrate the use of a new, shorter column with targeted selectivity that can produce good results as well as increased sample throughput. A Zebron ZB-FAME GC column is used for the testing of olive oil following the International Olive Council (IOC) method COI/T.20/Doc. No 25 (the global method for the detection of extraneous oils in olive oils), with good accuracy and fast run times for the method.

Materials and Methods

Regular and extra virgin olive oil was procured from a local supermarket (Torrance, CA). IOC official method COI/T.20/Doc. No 25 was followed for sample preparation and GC instrument parameters. A Zebron ZB-FAME column of 30 meter x 0.25 mm x 0.20 μ m dimensions was used for the GC analysis. Samples were extracted from each olive oil and then esterified to their respective fatty acid methyl esters.

Figure 1.
Structures of various fatty acid compounds found in olive oil.



Experimental Conditions

Solid Phase Extraction (SPE) Method

Cartridge:	Strata [®] Si-1, 1g/6mL (Part No.: 8B-S012-JCH) (on vacuum or positive pressure manifold)
Wash:	6 mL Hexane
Load:	Oil solution (0.12 g of oil in 0.5 mL of hexane)
Elute:	10 mL of hexane/diethyl ether (87:13)
Dry Down:	Evaporate eluate under nitrogen, steady stream
Dissolve:	Purified oil residue in 1 mL hexane
Add:	0.1 mL 2N potassium hydroxide in methanol
Shake:	Cap tube and shake vigorously for 15 seconds; leave to separate until upper layer becomes clear
Extract:	Upper layer for analysis (the heptane solution is suitable for injection into the GC)



Figure 2.
Extra virgin olive oil FAMES

GC Conditions

Conditions same for both separations:

Column: Zebron™ ZB-FAME

Dimensions: 30 meter x 0.25 mm x 0.20 μm

Part No.: 7HG-G033-10

Injection: Split 50:1 @ 240 °C, 1 μL

Carrier Gas: Helium @ 1.2 mL/min (constant flow)

Oven Program: 100 °C for 2 min to 140 °C @ 10 °C/min to 190 °C
@ 3 °C/min to 260 °C @ 30 °C/min for 2 min

Detector: FID @ 260 °C

Recommended Liner: Zebron PLUS Single Taper with Wool

Liner Part No.: AG2-0A11-05 (for Agilent® system)

Sample: Analytes are diluted 5:1 in heptane

1. C11:0*
 2. C16:0
 3. C16:1 cis 9
 4. C18:0
 5. C18:1 cis 9
 6. C18:2 cis 9,12
 7. C18:3 cis 9,12,15
 8. C20:0
 9. C20:1 cis 11
- * internal standard

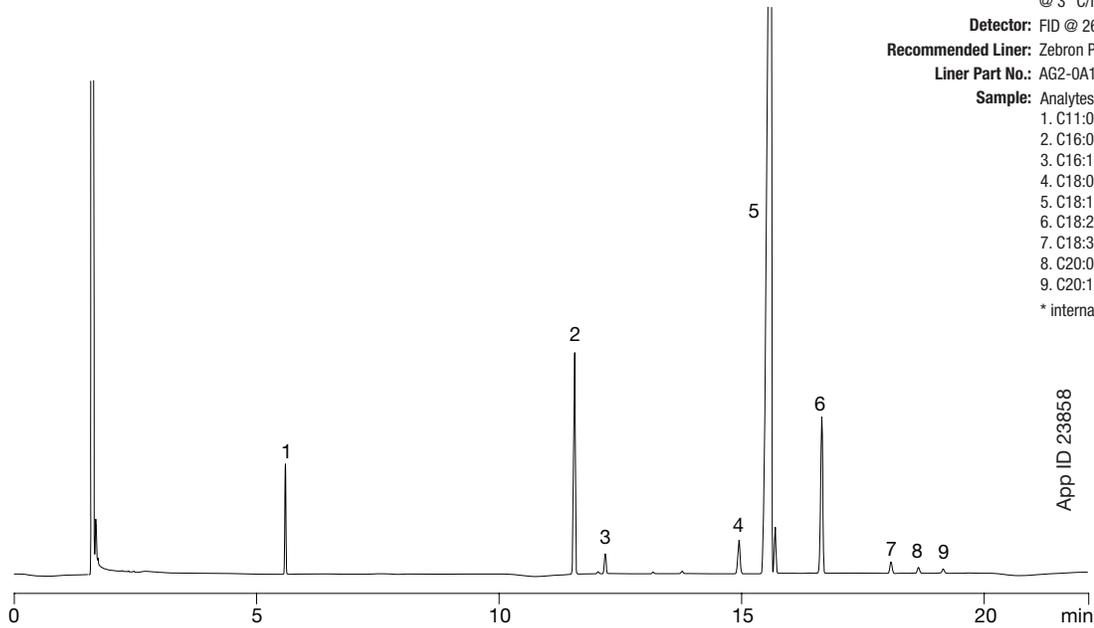
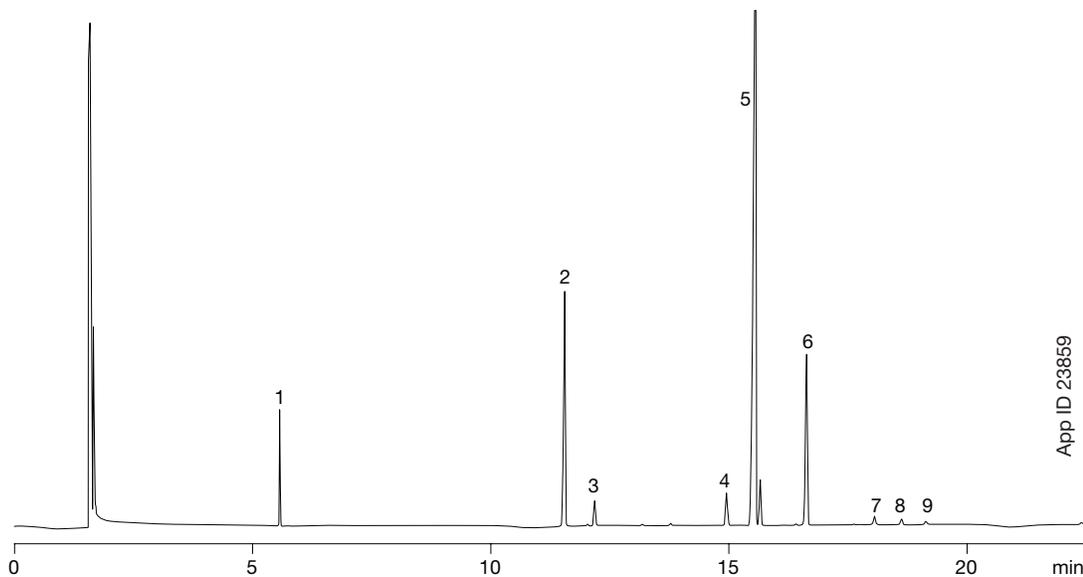


Figure 3.
Regular olive oil FAMES



Results and Discussion

The International Olive Council (IOC) outlines specific methods designed to test for various compounds in olive oil. In this experiment, extracted and esterified samples were analyzed by GC according to the official IOC olive oil method COI/T.20/Doc. No 25 for determination of fatty acid methyl esters.

The phase chemistry of GC columns traditionally used for this analysis is generally high in polarity; FAMEs testing typically requires a chemistry for adequate separation of the various isomers. In addition, long column lengths are commonly used to increase the resolution for both the cis and trans isomers as well as separate the analytes of interest from matrix interferences. The Zebtron™ ZB-FAME column used for this analysis provided a high-cyanopropyl chemistry that allowed for both optimal selectivity and use of short dimensions to decrease the overall run time.

The primary FAMEs of interest in both regular and extra virgin olive oil were fully resolved in under 20 minutes using a short 30 meter ZB-FAME GC column as demonstrated in **Figures 2 and 3**. The large oleic acid methyl ester peak is also clearly resolved from the other methyl esters of interest. Furthermore, there were no significant matrix interferences with the analytes of interest.

Even with this short run time, additional optimization of the method can be performed to further shorten the run or add bakeout steps that increase column lifetime and sample throughput.

Conclusion

Good separation and results were achieved for 9 FAMEs isomers in regular and extra virgin olive oil using a Zebtron ZB-FAME GC column. Due to the optimized phase chemistry, a short 30 meter length could be used to achieve fast 20 minute run times while maintaining separation of the various isomers, which presents significant time savings when compared to the traditional 50 – 60 meter columns used for this analysis.

Laboratories seeking to increase their sample throughput for FAMEs testing can greatly improve productivity by considering optimized columns targeted for this analysis; improved phase chemistry and optimized column dimensions can ultimately provide faster run times and still achieve separation of cis/trans isomers.



APPLICATIONS

Ordering Information

Zebron™ ZB-FAME GC Columns

Length (m)	ID (mm)	Film (µm)	Temp. Limits (°C)	Standard	5 m Guardian™
				Part No.	Part No.
20	0.18	0.15	- 60 to 280	7FD-G033-05	-
30	0.25	0.20	- 60 to 280	7HG-G033-10	7HG-G033-10-GGA
60	0.25	0.20	- 60 to 280	7KG-G033-10	-

Zebron PLUS GC Inlet Liners

Description	Dimensions	Part No.	Unit
For Agilent® and Thermo Scientific® GC Systems			
Single Taper with Wool	4 x 78.5	AG2-0A11-05 AG2-0A11-25	5/pk 25/pk



Strata® SPE Silica (Si-1)

Format	Sorbent Mass	Part Number	Unit
Tube			
	100 mg	8B-S012-EAK	1 mL (100/box)
	200 mg	8B-S012-FBJ	3 mL (50/box)
	500 mg	8B-S012-HBJ**	3 mL (50/box)
	500 mg	8B-S012-HCH**	6 mL (30/box)
	1 g	8B-S012-JCH**	6 mL (30/box)
Giga™ Tube			
	500 mg	8B-S012-HDG	12 mL (20/box)
	1 g	8B-S012-JDG	12 mL (20/box)
	2 g	8B-S012-KDG	12 mL (20/box)
	5 g	8B-S012-LEG	20 mL (20/box)
	10 g	8B-S012-MFF	60 mL (16/box)
	20 g	8B-S012-VFF	60 mL (16/box)
	50 g	8B-S012-YSN	150 mL (8/box)
	70 g	8B-S012-ZSN	150 mL (8/box)

**Tab-less tubes available. Contact Phenomenex for details.



If products mentioned in this technical note do not provide at least an equivalent separation as compared to any other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

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