

# APPLICATIONS

## Oligomer Composition of Over-The-Counter Omega-3 Fish Oil Capsules by Gel Permeation Chromatography (Ph. Eur. Monograph 1912)

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In addition to chromatography, Brian also has a passion for ice cream-making, and enjoys experimenting with bold, new flavors.

Among the variety of over-the-counter (OTC) vitamin and nutraceutical products sold to consumers, fish oils containing omega-3 fatty acids are some of the most widely endorsed by healthcare professionals. Specifically, several fatty acids such as DHA and EPA have shown numerous health benefits. Three different OTC fish oil supplements were analyzed by Gel Permeation Chromatography to determine oligomer composition.

### Introduction

A number of studies have shown that plasma concentration of highly unsaturated, n-3 (omega) fatty acids are indicative of overall health, with a balanced ratio of omega-3 and omega-6 fatty acids being ideal<sup>1,2,3</sup>. However, the typical Western diet is heavy in red meat, eggs, and other foods which are high in omega-6 fatty acids. Dietary supplementation with marine food-derived omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which, helps to balance this ratio.

Typically taken in the form of over-the-counter (OTC) "fish oil" capsules, supplementation of omega-3 fatty acids helps lower triglycerides<sup>4</sup>, may reduce the risk of birth complications and premature birth<sup>5</sup>, and even reduces depression with similar effects to pharmaceutical drugs such as fluoxetine<sup>6</sup>.

One critical test for fish oil supplements is oligomer analysis. In this study, we assessed the omega-3 fatty acid content of 3 different fish oil supplements using gel permeation chromatography (GPC), and using a reference standard, compared relative retention times and peak areas to determine the oligomer composition of each OTC fish oil supplement.

### Materials and Methods

#### Reagents and Chemicals

Standards were purchased from Sigma Chemical (St. Louis, MO). Reference standards were prepared as per the European Pharmacopeia 8.0; 50 mg of monodocosahexaenoin R, 30 mg of didocosahexaenoin R, and 20 mg of tridocosahexaenoin R was dissolved in 100 mL of THF. OTC Fish Oil samples were obtained from local nutraceutical shops.

### Experimental Conditions

#### HPLC Conditions

GPC separations were performed on an Agilent<sup>®</sup> 1260 HPLC system with autosampler, RI detector, and ChemStation<sup>™</sup> software (Palo Alto, CA, USA). The method run was based upon the European Pharmacopeia 8.0 method for analyzing triglycerides in fish oils.

Three Phenogel<sup>™</sup> fixed pore GPC columns were connected in series as follows: Injector – Phenogel 500 Å, Phenogel 100 Å, Phenogel 50 Å – Detector.

<b>Columns:</b>	Phenogel 5 µm 500 Å	300 x 7.8 mm	(P/N 00H-0443-K0)
	Phenogel 5 µm 100 Å	300 x 7.8 mm	(P/N 00H-0442-K0)
	Phenogel 5 µm 50 Å	300 x 7.8 mm	(P/N 00H-0441-K0)

**Mobile Phase:** THF  
**Flow Rate:** 0.8 mL/min  
**Detection:** RI (35° C)  
**Injection:** 40 µL  
**Column Temp:** 30° C



**Results and Discussion**

The acceptance criteria for the Ph. Eur. are no more than 1.5% oligomer of fatty acid composition. An oligomer is identified as any peaks eluting before the triglyceride reference standard, and relative peak areas are used to determine oligomer composition in the sample.

**Figure 1** shows the Ph. Eur. reference standard test mixture. Elution order is tridocosahexaenoin R, didocosahexaenoin R, and monodocosahexaenoin R. Any peaks eluting before the triglyceride peak at 25.61 min would indicate oligomer.

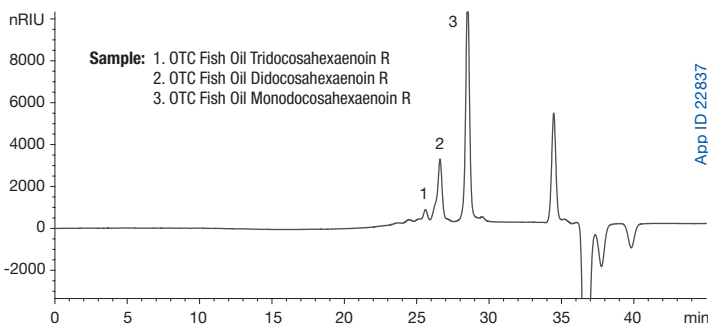
Sample OTC-1 (**Figure 2**), shows an oligomer 1.8% of the total peak area, which would not meet the Ph. Eur. acceptance criteria. Samples OTC-2 (**Figure 3**) and OTC-3 (**Figure 4**) do not show any oligomer peaks. All retention times and relative peak areas can be found in **Table 1**.

Further identification of samples, including characterization of the oligomer peak, would require GC-FID analysis, which was outside the scope of this study.

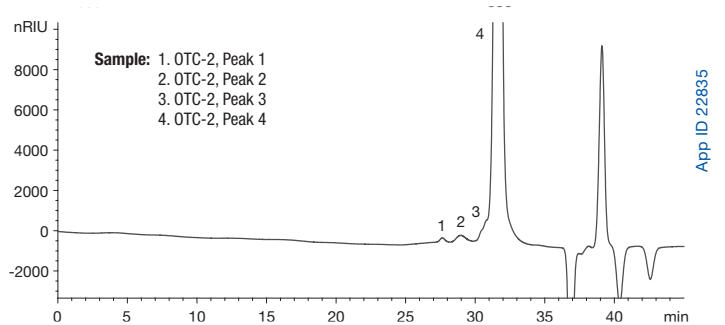
**Table 1.**  
Retention Times and Relative Peak Areas

Analyte	Retention Time (min)	Area (%)
Ph. Eur. Reference Standard		
Tridocosahexaenoin R	25.61	
Didocosahexaenoin R	26.61	
Monodocosahexaenoin R	28.52	
OTC-1		
Oligomer	23.90	1.80
OTC-1, Peak 1	25.83	93.99
OTC-1, Peak 2	26.87	4.21
OTC-2		
OTC-2, Peak 1	27.64	0.47
OTC-2, Peak 2	28.96	0.56
OTC-2, Peak 3	30.83	1.60
OTC-2, Peak 4	31.77	97.37
OTC-3		
OTC-3, Peak 1	30.57	100

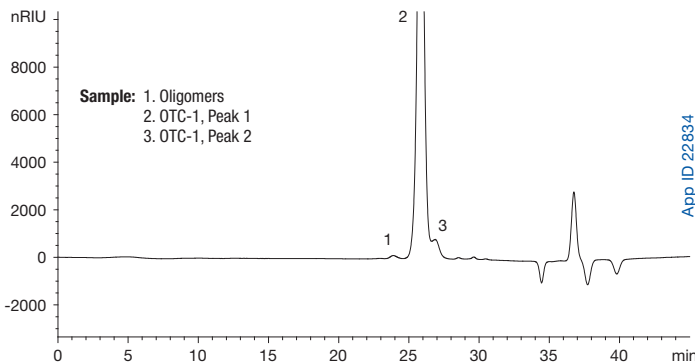
**Figure 1.**  
Ph. Eur. Standards Reference Standard Chromatogram



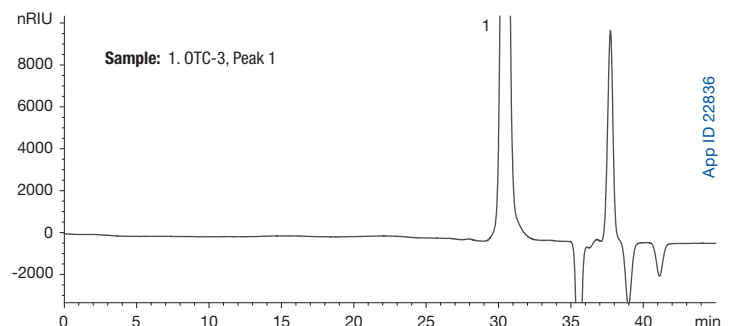
**Figure 3.**  
Sample OTC-2 Chromatogram



**Figure 2.**  
Sample OTC-1 Chromatogram



**Figure 4.**  
Sample OTC-3 Chromatogram



## Conclusion

In this study, we analyzed 3 different over-the-counter fish oil supplements using Phenogel fixed pore GPC columns run in series as per the European Pharmacopoeia 8.0<sup>7</sup>. One of the three samples analyzed had an oligomer content that would not have met acceptance criteria of the Ph. Eur. monograph. Further analysis would need to be performed to characterize the exact composition of omega-3 fatty acids in sample OTC-1.

This data suggests that Phenogel fixed pore columns are suitable to determine the oligomer composition in OTC fish oil supplements as per the Ph. Eur. monograph.

## References

- [1] Kaur G, et al. "Docosapentaenoic acid (22:5n-3): a review of its biological effects." *Prog Lipid Res.* (2011)
- [2] Kuriki K, et al. "Plasma concentrations of (n-3) highly unsaturated fatty acids are good biomarkers of relative dietary fatty acid intakes: a cross-sectional study." *J Nutr.* (2003)
- [3] Stark KD, et al. "Comparison of bloodstream fatty acid composition from African-American women at gestation, delivery, and postpartum." *J Lipid Res.* (2005)
- [4] Cazzola R, et al. "Age- and dose-dependent effects of an eicosapentaenoic acid-rich oil on cardiovascular risk factors in healthy male subjects." *Atherosclerosis.* (2007)
- [5] Olsen SF, et al. "Randomised controlled trial of effect of fish-oil supplementation on pregnancy duration." *Lancet.* (1992)
- [6] Laino CH, et al. "Potentiation of omega-3 fatty acid antidepressant-like effects with low non-antidepressant doses of fluoxetine and mirtazapine." *Eur J Pharmacol.* (2010)
- [7] Council of Europe. *European Pharmacopoeia*. 8<sup>th</sup> Ed. Strasbourg: Council of Europe; 2007. Fish Oil, Rich in Omega-3 Acids. 2236-2238

## Ordering Information

### Phenogel™ SEC/GPC Columns

5 μm Analytical Columns (mm)			SecurityGuard™ Cartridges (mm)
300 x 7.8			4 x 3.0*
Pore Size	MW Range		
50 Å	100-3 K	00H-0441-K0	AJO-9292
100 Å	500-6 K	00H-0442-K0	AJO-9292
500 Å	1 K-15 K	00H-0443-K0	AJO-9292
10 <sup>3</sup> Å	1 K-75 K	00H-0444-K0	AJO-9292
10 <sup>4</sup> Å	5 K-500 K	00H-0445-K0	AJO-9292
10 <sup>5</sup> Å	10 K-1,000 K	00H-0446-K0	AJO-9292
10 <sup>6</sup> Å	60 K-10,000 K	00H-0447-K0	AJO-9292
300 x 7.8			4 x 3.0*
Mixed Beds			
Linear(2)	100-10,000 K	00H-3259-K0	AJO-9292

for 3.2-8.0 mm ID

5 μm Narrow Bore (NB) Columns (mm)			SecurityGuard™ Cartridges (mm)
300 x 4.6			4 x 3.0*
Pore Size	MW Range		
50 Å	100-3 K	00H-0441-E0	AJO-9292
100 Å	500-6 K	00H-0442-E0	AJO-9292
500 Å	1 K-15 K	00H-0443-E0	AJO-9292
10 <sup>3</sup> Å	1 K-75 K	00H-0444-E0	AJO-9292
10 <sup>4</sup> Å	5 K-500 K	00H-0445-E0	AJO-9292
10 <sup>5</sup> Å	10 K-1,000 K	00H-0446-E0	AJO-9292
10 <sup>6</sup> Å	60 K-10,000 K	00H-0447-E0	AJO-9292
300 x 4.6			4 x 3.0*
Mixed Beds			
Linear(2)	100-10,000 K	00H-3259-E0	AJO-9292

for 3.2-8.0 mm ID

## Ordering Information con't.

10 μm Analytical Columns (mm)			SecurityGuard™ Cartridges (mm)
300 x 7.8			4 x 3.0*
Pore Size	MW Range		
50 Å	100-3 K	00H-0641-K0	AJO-9292
100 Å	500-6 K	00H-0642-K0	AJO-9292
500 Å	1 K-15 K	00H-0643-K0	AJO-9292
10 <sup>3</sup> Å	1 K-75 K	00H-0644-K0	AJO-9292
10 <sup>4</sup> Å	5 K-500 K	00H-0645-K0	AJO-9292
10 <sup>5</sup> Å	10 K-1,000 K	00H-0646-K0	AJO-9292
10 <sup>6</sup> Å	60 K-10,000 K	00H-0647-K0	AJO-9292
300 x 7.8			4 x 3.0*
Mixed Beds			
Linear(2)	100-10,000 K	00H-3260-K0	AJO-9292

for 3.2-8.0 mm ID

5 μm Preparative Columns (mm)			Guards
300 x 21.2			50 x 21.2
Pore Size	MW Range		
100 Å	500-6 K	00H-0442-P0	03B-0642-P0

10 μm Preparative Columns (mm)			Guards
300 x 21.2			50 x 21.2
Pore Size	MW Range		
100 Å	500-6 K	00H-0642-P0	03B-0642-P0

\* SecurityGuard Analytical Cartridges require holder, Part No.: KJO-4282

### Guard Cartridge Holder

Part No.	Description
KJO-4282	Reusable Holder (SecurityGuard Kit)

Note: SecurityGuard cartridges for Non-Aqueous Polymer GPC columns are not compatible with HFIP solvent.

### Column Union

Part No.	Description
AQO-8507	Zero Dead Volume Union, SS, with 10-32 fittings

Note: Additional union (AQO-8507) may be necessary for SecurityGuard to fit in column oven with less than 30 cm length capacity.

Phenogel columns are routinely shipped in THF. Please contact your Phenomenex representative for information about other shipping solvents.



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SecurityGuard is patented by Phenomenex. U.S. Patent No. 6,162, 362

Caution: this patent applies to the analytical-sized guard cartridge holder, and does not apply to SemiPrep, PREP, or ULTRA holders, or to any cartridges.

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