

# APPLICATIONS

## Size Exclusion Chromatography Optimization using the European Pharmacopeia Method for Follitropin Oligomers

Zeshan Aqeel, Brian Rivera  
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA



**Brian Rivera**

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



### Introduction

Follitropin is a recombinant form of human follicle-stimulating hormone which is used for ovulation induction with women undergoing in vitro fertilization(1). It is a heterodimeric glycoprotein composed of two subunits. As such, the European Pharmacopeia for Follitropin includes an oligomer assay, which prescribes a 5 µm hydrophilic silica, with the dimensions of 300 x 7.8 mm (2). System suitability requires a resolution of no less than 1.5 between Bovine Albumin and Follitropin monomer, which can be difficult depending on the column used. Further, the demanding resolution specification requires a longer column, as well as the relatively low flow-rate, making for a particularly long analysis. This application shows the method transfer of the Ph. Eur. method from a traditional, 5 µm hydrophilic silica 300 x 7.8 mm column to a 1.8 µm 150 x 4.6 mm column.

### Materials and Methods

Follitropin CRS Standard and Bovine Serum Albumin were obtained from Sigma Chemicals (St. Louis, MO, USA). All solutions and mobile phase were prepared as per the Ph. Eur. 8.0 for Follitropin, concentrated solution.

### HPLC Conditions

HPLC was performed using a 300 x 7.8 mm ID 5 µm hydrophilic silica column as prescribed by the Ph. Eur. and a Yarra<sup>™</sup> 1.8 µm SEC-X150. Both columns were run on a Waters ACQUITY<sup>®</sup> UPLC<sup>®</sup> (Waters Corporation, Milford, MA) with an upper pressure limit of 15,000 psi. Running conditions were as per the method conditions below.

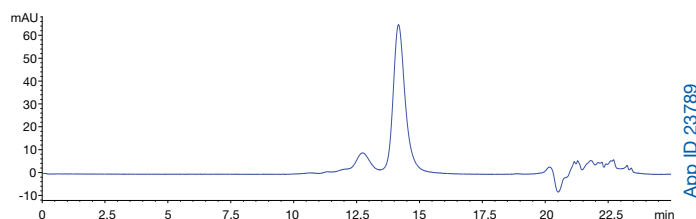
### HPLC Conditions

- Column:** 1) Hydrophilic Silica Column, 5 µm  
2) Yarra 1.8 µm SEC-X150
- Dimensions:** 1) 300 x 7.8 mm  
2) 150 x 4.6 mm
- Mobile Phase:** 100 mM Sodium Phosphate, 200 mM Sodium Sulfate, pH 6.7
- Flow Rate:** 1) 0.5 mL/min  
2) 0.35 mL/min
- Detection:** UV @ 215 nm
- Injection Volume:** 1) 100 µL  
2) 20 µL

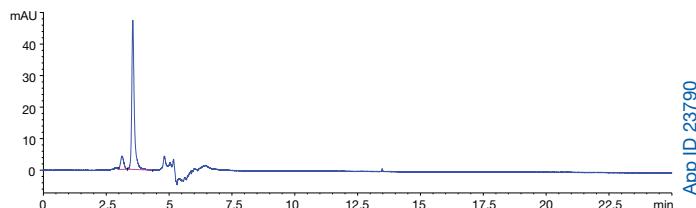
**Table 1.** System Suitability Summary

Column	Analyte	Retention Time (min)	Resolution
5 µm Hydrophilic silica 300 x 7.8 mm	Bovine Albumin	12.736	–
	Follitropin	14.161	1.50
Yarra 1.8 µm SEC-X150, 150 x 4.6 mm	Bovine Albumin	3.129	–
	Follitropin	3.557	2.16

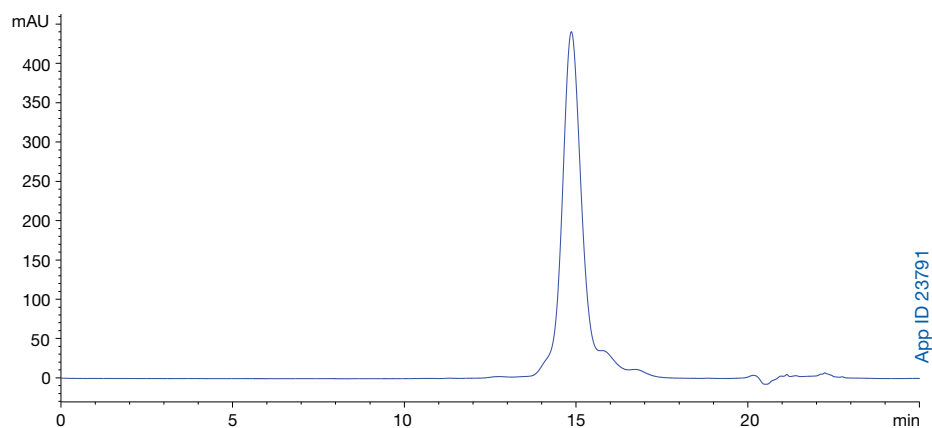
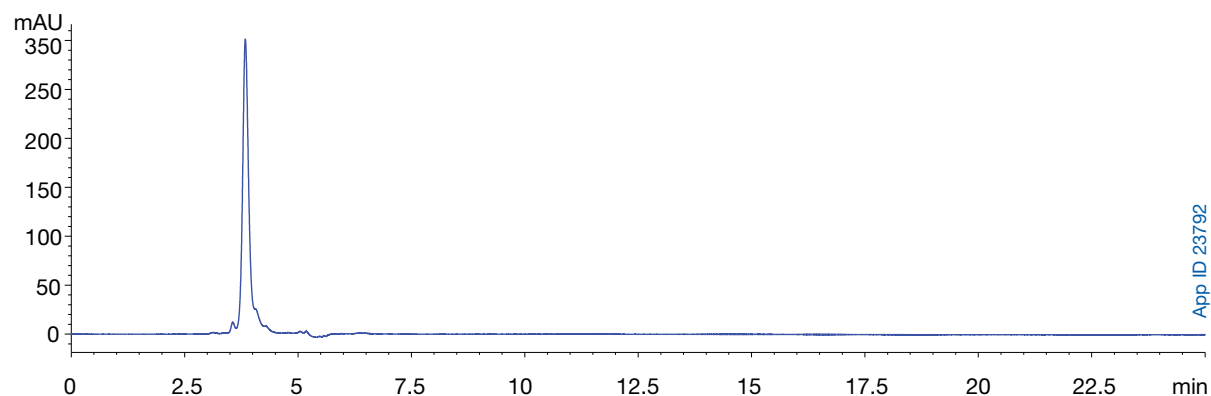
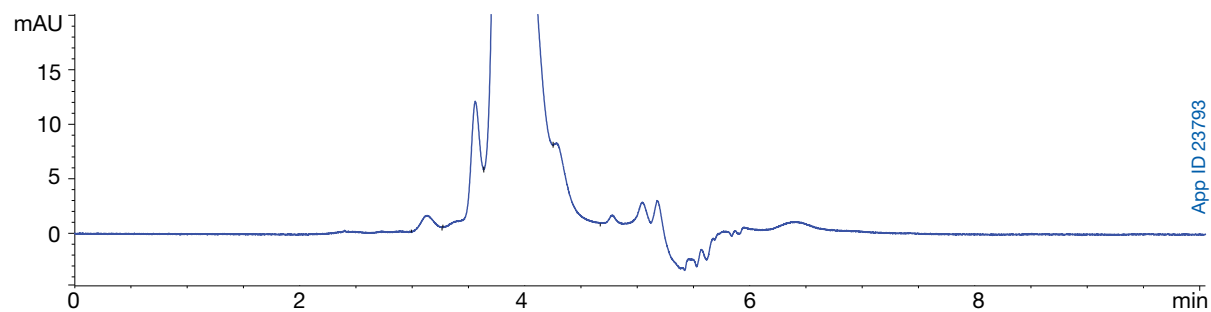
**Figure 1.** System Suitability, 5 µm hydrophilic silica, 300 x 7.8 mm



**Figure 2.** System Suitability, Yarra 1.8 µm SEC-X150, 150 x 4.6 mm



## APPLICATIONS

**Figure 3.** Reference Sample, 5  $\mu$ m Hydrophilic Silica, 300 x 7.8 mm**Figure 4.** Reference Sample, Yarra 1.8  $\mu$ m SEC-X150, 150 x 4.6 mm**Figure 5.** Impurity Profile with Yarra SEC-X150

# APPLICATIONS

## Results and Discussion

**Figures 1 and 2** show the chromatograms for system suitability with Ph.Eur. standards for the 5 $\mu$ m and 1.8 $\mu$ m SEC columns, respectively.

Albumin and Follitropin have a resolution of 1.5 for the 5 $\mu$ m column, which meets system suitability but does not give room for error due to sample preparation variation or column to column variation. However, with the Yarra 1.8 $\mu$ m SEC-X150, Albumin and Follitropin have a resolution of 2.15, which is well within the resolution requirement of the monograph. Data for system suitability, with resolution and retention times, are summarized in **Table 1**.

Run times for Yarra SEC-X150 are significantly shorter than the 5 $\mu$ m method (7.5 minutes compared to the typical 30 minutes). This is both due to the shorter column length (150mm) but also the increase in flow-rate used for the 1.8 $\mu$ m method, which is 0.35mL/min. Note that this is not a direct scaling from 7.8mm ID in the monograph to the smaller 4.6mm ID Yarra SEC-X150 column. **Figures 3 and 4** show the reference sample for Follitropin. In addition to the much shorter run times, the impurity profile shown in **Figure 5** shows expanded detail. Note the high molecular weight pre-peak shoulder and dimer observed in the sample. Partial shouldering might also indicate fragments of the heterodimer protein. This level of characterization cannot be achieved with the 5 $\mu$ m method.

## Conclusion

Traditional GFC/SEC analysis of Follitropin oligomers, as per the Ph.Eur. monograph, may be time prohibitive to a laboratory with many samples for analysis due to the current method's length of time. The demanding method also makes it challenging to simply meet system suitability, leading to failed runs or premature column failure and replacement. Analysis using a high performance column such as the Yarra 1.8 $\mu$ m SEC-X150 can cut run times down significantly simply by running a shorter column dimension. Additionally, with sub-2 $\mu$ m particles, implementing faster flow-rates with no drop in efficiency can lead to even faster run times, giving more flexibility in method development. It is important to note that as per the Ph. Eur. requirements, a 50 % reduction in particle size and a 25 % change in inner diameter for isocratic methods are allowable changes. Since the Ph. Eur. prescribes a 5 $\mu$ m column and 7.5mm ID for Follitropin, this method would require a revalidation.

## References

1. Goa KL, Wagstaff AJ. Follitropin alpha in fertility: a review. *BioDrugs*. 1998 Mar;9(3):235-60.
2. European Pharmacopeia 8.0, Follitropin Concentrated Solution. 2015:2290-2295

## Ordering Information

### Yarra 1.8 $\mu$ m SEC Bio-Inert Columns (mm)

Phases	Analytical 150 x 4.6	Analytical 300 x 4.6 mm
Yarra 1.8 $\mu$ m SEC-X150	00F-4631-E0	00H-4631-E0
Yarra 1.8 $\mu$ m SEC-X300	00F-4743-E0	00H-4743-E0

### Yarra 1.8 $\mu$ m SEC Stainless Steel Columns (mm)

Phases	Analytical 150 x 4.6	Analytical 300 x 4.6 mm	SecurityGuard ULTRA Cartridges***
Yarra 1.8 $\mu$ m SEC-X150	00F-4631-E0-SS	00H-4631-E0-SS	AJ0-9512
Yarra 1.8 $\mu$ m SEC-X300	00F-4743-E0-SS	00H-4743-E0-SS	AJ0-9513

\*\*\*SecurityGuard ULTRA cartridges require holder, Part No.: AJ0-9000

For Stainless Steel  
Only



guarantee

If Yarra analytical columns do not provide you with at least an equivalent separation as any other GFC column of similar porosity, type, and dimensions, return the column with comparative data within 45 days for a FULL REFUND.



# APPLICATIONS

## Australia

t: +61 (0)2-9428-6444  
f: +61 (0)2-9428-6445  
auinfo@phenomenex.com

## Austria

t: +43 (0)1-319-1301  
f: +43 (0)1-319-1300  
anfrage@phenomenex.com

## Belgium

t: +32 (0)2 503 4015 (French)  
t: +32 (0)2 511 8666 (Dutch)  
f: +31 (0)30-2383749  
beinfo@phenomenex.com

## Canada

t: +1 (800) 543-3681  
f: +1 (310) 328-7768  
info@phenomenex.com

## China

t: +86 (0)20 2282-6668  
f: +86 (0)20 2809-8130  
chinainfo@phenomenex.com

## Denmark

t: +45 4824 8048  
f: +45 4810 6265  
nordicinfo@phenomenex.com

## Finland

t: +358 (0)9 4789 0063  
f: +45 4810 6265  
nordicinfo@phenomenex.com

## France

t: +33 (0)1 30 09 21 10  
f: +33 (0)1 30 09 21 11  
franceinfo@phenomenex.com

## Germany

t: +49 (0)6021-58830-0  
f: +49 (0)6021-58830-11  
anfrage@phenomenex.com

## India

t: +91 (0)40-3012 2400  
f: +91 (0)40-3012 2411  
indiainfo@phenomenex.com

## Ireland

t: +353 (0)1 247 5405  
f: +44 1625-501796  
eireinfo@phenomenex.com

## Italy

t: +39 051 6327511  
f: +39 051 6327555  
italiainfo@phenomenex.com

## www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at [international@phenomenex.com](mailto:international@phenomenex.com)

## Luxembourg

t: +31 (0)30-2418700  
f: +31 (0)30-2383749  
nlinfo@phenomenex.com

## Mexico

t: 01-800-844-5226  
f: 001-310-328-7768  
tecnicomx@phenomenex.com

## The Netherlands

t: +31 (0)30-2418700  
f: +31 (0)30-2383749  
nlinfo@phenomenex.com

## New Zealand

t: +64 (0)9-4780951  
f: +64 (0)9-4780952  
nzinfo@phenomenex.com

## Norway

t: +47 810 02 005  
f: +45 4810 6265  
nordicinfo@phenomenex.com

## Puerto Rico

t: +1 (800) 541-HPLC  
f: +1 (310) 328-7768  
info@phenomenex.com

## Spain

t: +34 91-413-8613  
f: +34 91-413-2290  
espinfo@phenomenex.com

## Sweden

t: +46 (0)8 611 6950  
f: +45 4810 6265  
nordicinfo@phenomenex.com

## United Kingdom

t: +44 (0)1625-501367  
f: +44 (0)1625-501796  
ukinfo@phenomenex.com

## USA

t: +1 (310) 212-0555  
f: +1 (310) 328-7768  
info@phenomenex.com

## All other countries Corporate Office USA

t: +1 (310) 212-0555  
f: +1 (310) 328-7768  
info@phenomenex.com



If Yarra analytical columns do not provide you with at least an equivalent separation as any other GFC column of similar porosity, type, and dimensions, return the column with comparative data within 45 days for a FULL REFUND.

### Terms and Conditions

Subject to Phenomenex Standard Terms and Conditions which may be viewed at [www.phenomenex.com/TermsAndConditions](http://www.phenomenex.com/TermsAndConditions).

### Trademarks

Yarra and SecurityGuard are trademarks of Phenomenex. Waters, ACQUITY, and UPLC are registered trademarks of Waters Corporation.

### Disclaimer

Phenomenex is in no way affiliated with Waters Corporation. Chromatographic conditions are the same for all columns unless otherwise noted. Comparative separations may not be representative of all applications.

© 2016 Phenomenex, Inc. All rights reserved.