APPLICATIONS

Size Exclusion Chromatography Method Optimization using the European Pharmacopoeia for Insulin Fibrils (Ph. Eur. Monograph 838)

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

Introduction

Insulin is a peptide hormone which plays a central role in metabolism. In its monomer form, insulin can partially misfold, causing a cascade of non-native cross assembly of beta-pleated sheets or amyloid-like fibrils[1]. For the insulin analog manufacturer, this is especially problematic, especially since insulin analogs can have a higher propensity for aggregation than native insulin[2].

As such, when a new formulation or delivery device is used, insulin fibrillation, commonly known as high molecular weight proteins (HMWP), should be assessed. A common method for analyzing insulin HMWP is size exclusion chromatography (SEC). The European Pharmacopoeia (Ph. Eur.) prescribes a 300 x 7.5 mm hydrophilic silica column[3]. However, the method requires a lengthy 35 minute run time which is time prohibitive for any laboratory with many samples to run and data points to generate.

This application shows the method transfer of the Ph. Eur. method from a traditional, 10 µm hydrophilic silica 300 x 7.8 mm column to a high performance, 1.8 µm hydrophilic silica 150 x 4.6 mm column.

Materials and Methods

Human insulin resolution solution was prepared as per the Ph. Eur. Sample was diluted in 0.01 M hydrochloric acid to a concentration of approximately 4 mg/mL. Insulin analog was prepared by stressing at 37 °C and diluted 0.01 M hydrochloric acid to ~4 mg/mL prior to injection.

HPLC Conditions

HPLC was performed using a 10 µm hydrophilic silica 300 x 7.8 mm column as prescribed by the Ph. Eur. and a Yarra™ 1.8 µm SEC-X150 150 x 4.6 mm column. Both columns were run on a Waters® ACQUITY® UPLC® (Waters Corporation, Milford, MA). Running conditions were as per the method conditions on page 2.

Results and Discussion

Figures 1 and **2** shows the chromatograms for system suitability with Ph. Eur. insulin standards. Monomer and dimer for the 10 µm column have a resolution of 2.15, which meets the system suitability requirement of ≥ 2.0. However, with the Yarra 1.8 µm SEC-X150 column, monomer and dimer have a resolution of 2.86, which far exceeds the resolution requirement of the monograph. Peak areas for dimer and HMWP for both columns are within the < 1 % system suitability requirement. Note total analysis time for system suitability is approximately 12 minutes for Yarra 1.8 µm SEC-X150 column and 35 minutes for the traditional 10 µm hydrophilic silica column.

Figure 1.
System Suitability
10 µm 300 x 7.8 mm Column

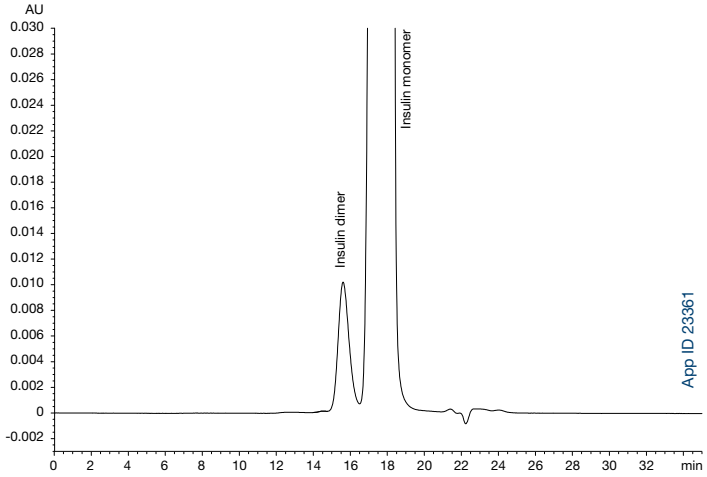
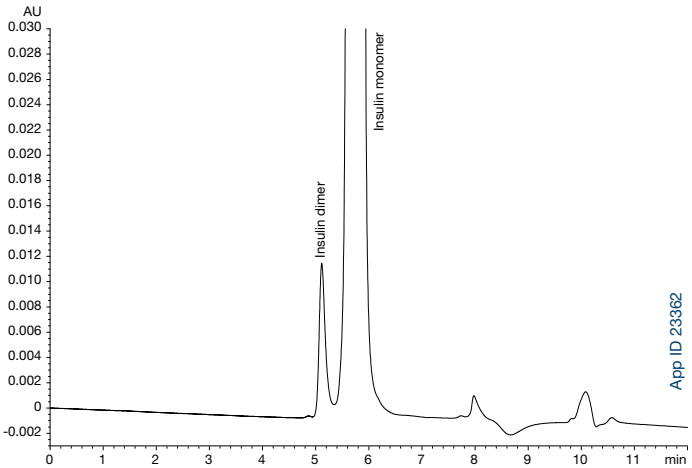


Figure 2.
System Suitability
Yarra 1.8 µm SEC-X150 150 x 4.6 mm Column



Data for system suitability is summarized in **Table 1**. Note the higher efficiency (Plate Count, Ph. Eur.) for all peaks using the Yarra™ 1.8 µm SEC-X150 column. This higher efficiency not only gives better resolution for monomer and dimer, but also allows for the resolution calculation for HMWP and dimer.

Figures 3 and **4** show the degraded insulin analog sample. At 1.96, resolution is acceptable between monomer and dimer for the 10 µm column. This is in comparison to 2.06 for Yarra 1.8 µm SEC-X150 column. Sum of areas of HMWP and analog exceeds 1 % for both columns, indicating the sample would not pass the test limits of <1 % for insulin HMWP. Again, note the difference in total run time between the two methods.

Data is summarized in **Table 2**. Improvements are seen in efficiency for monomer and dimer peaks in the Yarra 1.8 µm SEC-X150 column method, again lending to the increased resolution seen with the updated method.

Conclusions

Traditional SEC analysis of the insulin fibrils, as per the Ph. Eur. monograph 838, may be time prohibitive to a laboratory requiring several data points for analysis. Analysis using a sub-2 µm column such as the Yarra 1.8 µm SEC-X150 column can cut down on run times by more than 15 minutes. System suitability for peak areas and resolution are easily met, and there are improvements when compared to the traditional 10 µm column method. Improvements from the 10 µm method are also seen with the degraded insulin analog sample, indicating that the Yarra 1.8 µm SEC-X150 column is suitable for determining method limits of 1 % HMWP.

It is important to note that as per the Ph. Eur. requirements, a 50 % reduction in particle size and a 25 % change in the column inner diameter for isocratic methods are the maximum allowable adjustments. Since the Ph. Eur. prescribes a 10 µm particle size and 7.5 mm ID for many insulin methods, this method moving to the 1.8 µm particle size and 4.6 mm ID would require a revalidation.

Figure 3.
Degraded Insulin Analog
10 µm 300 x 7.8 mm Column

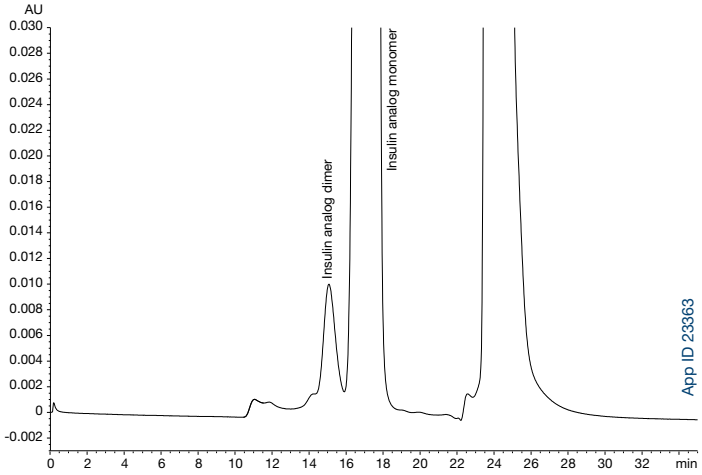
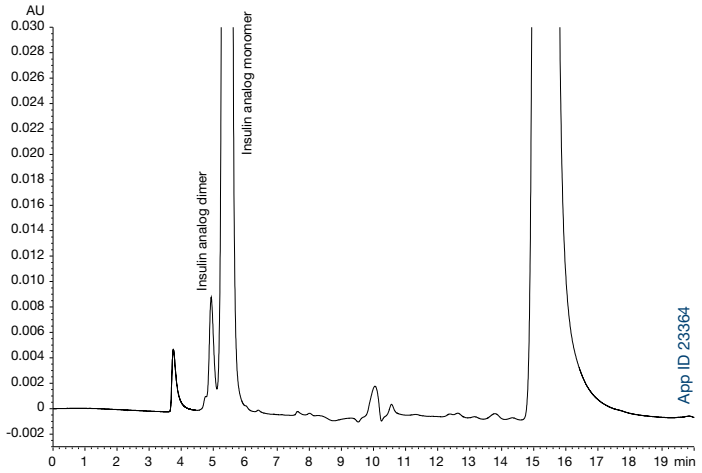


Figure 4.
Degraded Insulin Analog
Yarra 1.8 µm SEC-X150 150 x 4.6 mm Column



HPLC Conditions

Column:	1. 10 µm Hydrophilic Silica Column 2. Yarra 1.8 µm SEC-X150
Dimensions	1. 300 x 7.8 mm 2. 150 x 4.6 mm
Mobile Phase:	L-arginine (1.0 g/L)/acetic acid (99 %)/acetonitrile;65/15/20 (v/v/v)
Flow Rate:	1. 0.5 mL/min 2. 0.2 mL/min
Detection:	UV @ 276 nm
Injection Volume:	1. 100 µL 2. 10 µL

Table 1.
System Suitability Criteria

Column	Analyte	Retention Time (min)	Area	% Area	Resolution	Plate Count (Ph. Eur.)
10 µm 300 x 7.8 mm	HMWP	14.519	3036	0.01	-	-
	Insulin dimer	15.609	425713	0.94	-	3262
	Monomer	18.031	44938179	99.05	2.15	3854
Yarra™ 1.8 µm SEC-X150 150 x 4.6 mm	HMWP	4.87	757	0.01	-	12374
	Insulin dimer	5.11	103223	0.92	1.29	9766
	Monomer	5.83	11116010	99.07	2.86	6205

Table 2.
Insulin Analog

Column	Analyte	Retention Time (min)	Area	% Area	Resolution	Plate Count (Ph. Eur.)
10 µm 300 x 7.8 mm	HMWP Peak 1	11.055	57429	0.15	-	-
	HMWP Peak 2	11.838	65836	0.17	-	-
	HMWP Peak 3	14.243	67374	0.17	-	-
	Insulin analog dimer	15.074	497875	1.26	-	2215
	Insulin analog monomer	17.531	38823177	98.26	1.94	3110
Yarra 1.8 µm SEC-X150 150 x 4.6 mm	HMWP Peak 1	3.756	54228	0.67	-	
	HMWP Peak 2	4.767	5740	0.07	-	
	Insulin analog dimer	4.936	88801	1.09	-	5692
	Insulin analog monomer	5.505	7975341	98.17	2.06	5654

Acknowledgements

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References

1. Yang, Y., A. Petkova, K. Huang, B. Xu, Q.-X. Hua, I-J. Ye, Y.-C. Chu, S.-Q. Hu, N. B. Phillips, J. Whittaker, F. Ismail-Beigi, R. B. Mackin, P. G. Katsoyannis, R. Tycko, and M. A. Weiss. "An Achilles' Heel in an Amyloidogenic Protein and Its Repair: INSULIN FIBRILLATION AND THERAPEUTIC DESIGN." *Journal of Biological Chemistry* **2010:** 10806-0821. Print.
2. Librizzi, Fabio, and Christian Rischel. "The Kinetic Behavior of Insulin Fibrillation Is Determined by Heterogeneous Nucleation Pathways." *Protein Science : A Publication of the Protein Society* 14.12 (2005): 3129–3134. PMC. Web. **5 Oct. 2015**.
3. European Pharmacopeia 8.6, Human Insulin Monograph, 2015:5299-5301

Ordering Information

Yarra 1.8 µm SEC Bio-Inert Columns (mm)

	Analytical	Analytical
Phases	**150 x 4.6**	**300 x 4.6 mm**
Yarra 1.8 µm SEC-X150	00F-4631-E0	00H-4631-E0
Yarra 1.8 µm SEC-X300	00F-4743-E0	00H-4743-E0

Yarra 1.8 µm SEC Stainless Steel Columns (mm)

	Analytical	Analytical	SecurityGuard ULTRA Cartridges***
Phases	**150 x 4.6**	**300 x 4.6 mm**	**3/pk**
Yarra 1.8 µm SEC-X150	00F-4631-E0-SS	00H-4631-E0-SS	AJ0-9512
Yarra 1.8 µm 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

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