Application Note

EtoxiClear®

Product Code: 3250

Removal of Endotoxins – from bench to process scale

Endotoxins or lipopolysaccharides (LPS) are highly toxic components of the cell wall of Gram-negative bacteria, which are often present in significant amounts in bacterial cell expression systems such as E.coli.

A number of methods have been adopted for the removal of endotoxin based on adsorption, in particular ion exchange chromatography. Although downstream processing can significantly reduce endotoxin levels in the product, efficient and cost-effective removal of residual endotoxin from biopharmaceutical preparations remains a challenge.

Astrea Bioseparations have developed an affinity chromatography adsorbent, EtoxiClear®, that is highly stable, robust and non-toxic, with a high affinity for bacterial endotoxin and low protein binding. EtoxiClear® is a cost-effective and scalable technology designed for use in endotoxin removal applications including process development, sample/buffer preparation and product polishing steps used during cGMP manufacture of biological molecules. This application note describes the use of EtoxiClear® to effectively remove endotoxin from a purified immunoglobulin protein solution at bench scale using the Evolve® R column, and at process scale with the Evolve® D column.

Materials & Methods

Purified immunoglobulin solutions (~10 mg/mL), containing different levels of endotoxin were used to evaluate the EtoxiClear® adsorbent. The tests involved running the protein solutions through the adsorbent using three pre-packed column sizes: 5 mL, 50 mL (Evolve® R columns), and 385 mL (using a 7 cm Evolve® D column) (Table 1). The runs aimed to demonstrate the performance of the adsorbent at different scales, and at different levels of endotoxin burden. Prior to the runs, the columns were de-pyrogenated for 16 hours using 1 M NaOH, followed by 8 – 10 CV of equilibration buffer. A 2 CV wash with water was used for the 385 mL column before switching to buffer. The immunoglobulin solutions (2 CV)were applied to the columns, and the non-bound samples were collected for analysis. The runs were performed using either an ÄKTA ™ Avant automated workstation, or a low-pressure system.

Table 1

Chromatography conditions for the removal of endotoxin (low and high concentration) from a purified immunoglobulin solution using EtoxiClear® pre-packed into 5 and 50 mL Evolve® R columns and a 385 mL Evolve® D column.

Condition	Description	
Column parameters:	5 mL	10 cm bed height, 0.8 cm diameter
	50 mL	10 cm bed height, 2.5 cm diameter
	385 mL	10 cm bed height, 7 cm diameter
Operational flow rate:	120 cm/h (5 minute residence time)	
Depyrogenation:	1 M NaOH (16 hours)	
Equilibration buffer:	100 mM sodium citrate, 100 mM sodium chloride, pH 6.2	
Feedstock:	2 CV of purified immunoglobulin solution (~ 10 g/L) containing endotoxin (low and high concentration)	
Post load wash buffer:	100 mM sodium citrate, 100 mM sodium chloride, pH 6.2	
Clean-in-Place:	1 M NaOH	

The concentration of the endotoxin present in the immunoglobulin feedstock was measured using a kinetic chromogenic LAL assay with Glucashield® buffer. The concentration of the IgG solution was determined using spectrophotometry at 280 nm (molar extinction coefficient of 1.35 for a 1 mg/mL solution).

Results and Discussion

Six runs were carried out in total, two runs per column size with low and high titre endotoxin-burdened purified immunoglobulin solutions. Figure 1 shows a typical chromatogram of the removal of endotoxin from the immunoglobulin solution using the 50 mL Evolve® R EtoxiClear™ pre-packed column. The profile demonstrates a classic negative step, with the immunoglobulin passing through the column (no interaction). The recovery of IgG was determined spectrophotometrically (Table 2), and the removal of endotoxin is shown in Tables 3 and 4 for the low and high titre endotoxin loading, respectively.

Figure 1

Chromatogram demonstrating the loading the low titre endotoxin burdened immunoglobulin solution (100 mL) onto the 50 mL Evolve® R EtoxiClear® pre-packed column.

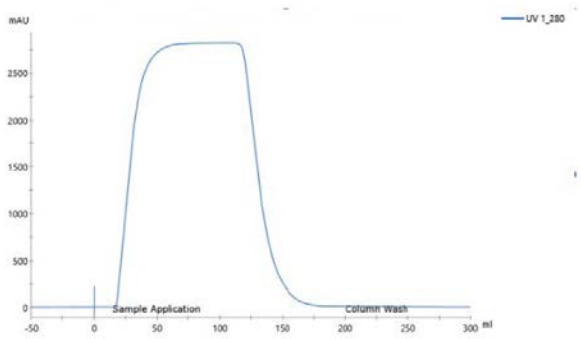

Table 2

Performance data for the recovery of immunoglobulins from the endotoxin clearance runs using EtoxiClear® pre-packed into 5 and 50 mL Evolve® R columns and a 7 cm Evolve® D column.

Column	Sample	Total Immunoglobulin (mg)
5 mL Evolve® R	Load	112.1
	Non-bound	96.5
Recovery: 86.1%		
50 mL Evolve® R	Load	1260.7
	Non-bound	1241.6
Recovery: 98.5%		
7 cm Evolve® D	Load	7939.6
	Non-bound	7865.3
Recovery: 99.1%		

Table 3

Performance data for the removal of a low concentration of endotoxin from the Immunoglobulin solution using EtoxiClear® pre-packed into 5 and 50 mL Evolve® columns and a 7 cm Evolve® D column.

Column	Sample	Endotoxin Concentration (EU/mL)	Endotoxin Concentration (EU/mg)
5 mL Evolve® R	Load	23	2.18
	Non-bound	0.02	0.003
50 mL Evolve® R	Load	36	2.88
	Non-bound	0.06	0.01
7 cm Evolve® D	Load	67	6.39
	Non-bound	0.07	0.01

Table 4

Performance data for the removal of a high concentration of endotoxin from the Immunoglobulin solution using EtoxiClear® pre-packed into 5 and 50 mL Evolve® R columns and a 7 cm Evolve® D column.

Column	Sample	Endotoxin Concentration (EU/mL)	Endotoxin Concentration (EU/mg)
5 mL Evolve® R	Load	1599	143
	Non-bound	0.02	0.002
50 mL Evolve® R	Load	2712	247
	Non-bound	0.05	0.01
7 cm Evolve® D	Load	2185	206
	Non-bound	0.05	0.01

Conclusions

The EtoxiClear® resin provided up to 99% recovery of immunoglobulin product when using pre-packed columns at lab scale, using the Evolve® R columns, as well as at larger scale, in the 7 cm Evolve® D column. The pre-packed EtoxiClear® columns demonstrated superior endotoxin removal at low- or high-titre. With both endotoxin titre levels the pre-packed EtoxiClear® columns cleared down to < 0.07 EU/mL which equated to < 0.01 EU/mg of protein. From the high-titre endotoxin feedstock, there is a > 4.5 log removal of endotoxin. EtoxiClear® shows high performance and scalability for endotoxin clearance with low protein binding at both bench and process scale. Additionally, the Evolve® R and Evolve® D pre-packed column range proved to be well-suited for EtoxiClear® endotoxin removal applications. The columns provide a ready-to-use solution, which requires no packing or packing qualification. Evolve® D and Evolve® R columns are disposable, providing ease of use, significant cost savings and eliminating the need for cleaning validation.

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