



Harvest and clarification

Endotoxin removal by Emphaze AEX hybrid purifier

Rituparna Sengupta, Ph.D and Rebecca Hochstein, Ph.D.
Thermo Fisher Scientific, Inc

Emphaze AEX hybrid purifier laboratory capsules for endotoxin removal in pre-clinical and early stage clinical recombinant proteins

The Thermo Scientific™ Emphaze™ AEX Hybrid Purifier is a synthetic, single-use anion exchange product that utilizes quaternary amine (Q) functionalized non-woven hydrogel media for clarification and purification of process fluids in biopharmaceutical purification. Emphaze AEX hybrid purifier redefines and simplifies biologics manufacturing processes by utilizing chromatography for particle control and reduction of DNA, host cell proteins, endotoxin, and bioburden. The anion exchange chromatography of the functional nonwoven in Emphaze AEX hybrid purifier provides substantial reduction of soluble impurities and of insoluble particles below 0.1 microns for superior turbidity reduction.

The introduction of two small laboratory capsule sizes (BV1R and BV0.3R) for the Emphaze AEX hybrid purifier enables the use of this device for endotoxin removal in laboratory applications using peristaltic pumps, as well as using syringe filtration. The laboratory capsules allow for fast and easy processing of small volumes of early stage drug candidates during



pre-clinical and early stage clinical studies in animal-based systems, where endotoxin removal is a requirement in order to avoid eliciting an unwanted immune response while still being able to observe drug efficacy.

Application data for endotoxin removal from protein solutions using Emphaze AEX hybrid purifier

Protein solutions in phosphate-buffered saline (PBS) buffer were used to represent early stage drug candidates in pre-clinical trials. Bovine serum albumin (BSA, pI = 4.5) and a biosimilar IgG1 (pI = 8.5) were chosen as model proteins to demonstrate endotoxin removal by Emphaze AEX hybrid purifier over a practical range of isoelectric points that models the diversity of biopharmaceutical drug candidates. Proteins were spiked with 10^2 and 10^3 EU/mL of stock endotoxin from a lysed and clarified *E. coli* culture.

Emphaze AEX hybrid purifier successfully reduced endotoxin to below WFI (water for injection) levels (<0.25 EU/mL)¹ in protein solutions at challenge loads of >1 kg/L. Flow rates of 1, 5, and 10 BV/min all reduced endotoxin levels to below WFI, demonstrating that the performance was unaffected by flow rate (Fig. 1). Syringe filtration (~ 23 BV/min) using Emphaze AEX hybrid purifier also removed endotoxin to below WFI levels (Fig. 2).

Robust endotoxin removal to below WFI with 90 – 100% protein recovery across isoelectric points and flow rates by Emphaze AEX hybrid purifier

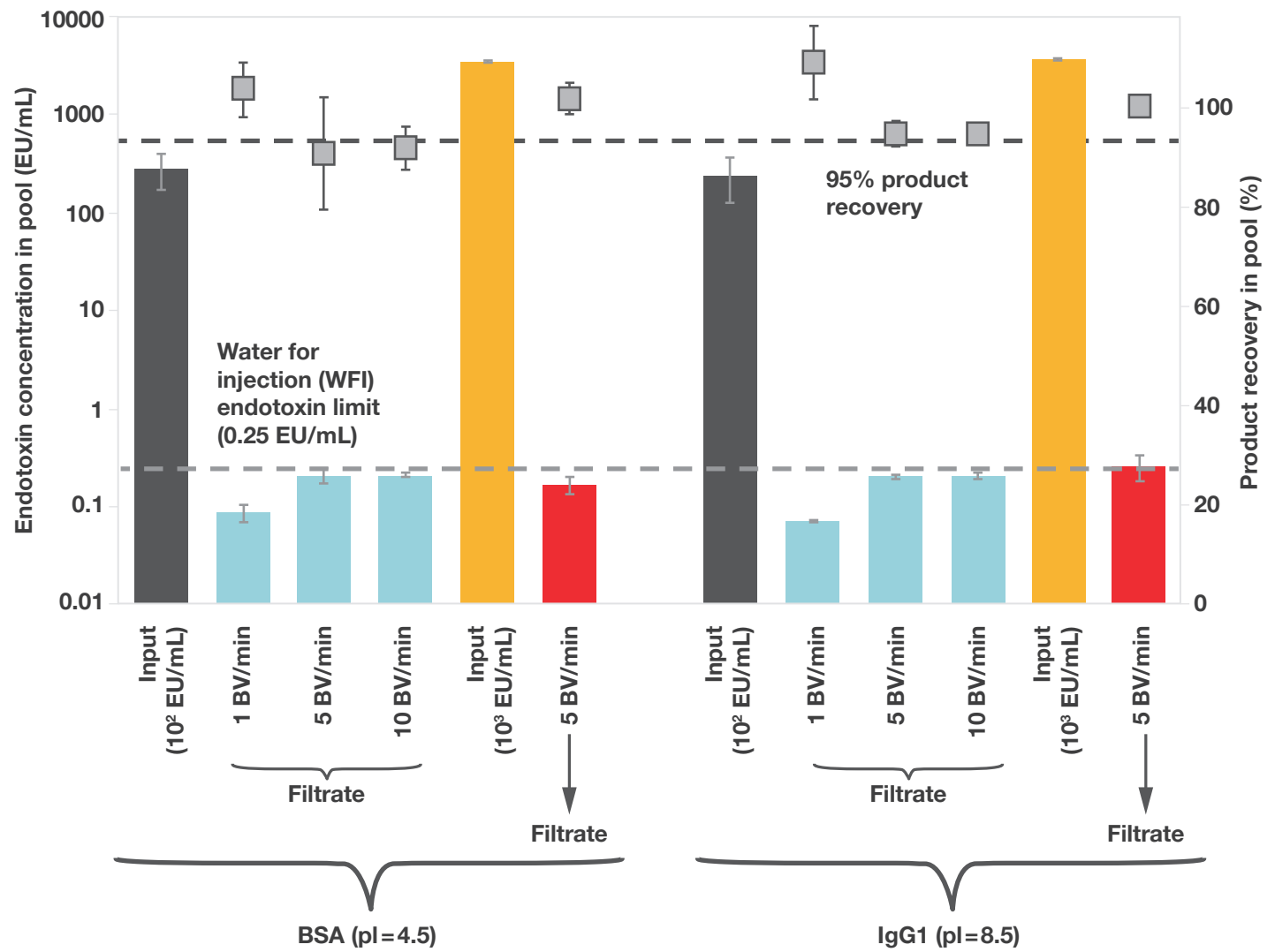


Figure 1. Endotoxin removal by Thermo Scientific™ Emphaze™ AEX Hybrid Purifier for two different proteins (BSA and IgG1) at different flow rates (1 BV/min, 5 BV/min and 10 BV/min) using two different input concentrations for endotoxin (10² and 10³ EU/mL). Protein isoelectric points (pIs) were experimentally determined by isoelectric focusing. In all instances, endotoxin was reduced to below WFI (<0.25 EU/mL) levels and protein recovery was greater than 95%.

Endotoxin removal by Emphaze AEX hybrid purifier using syringe filtration

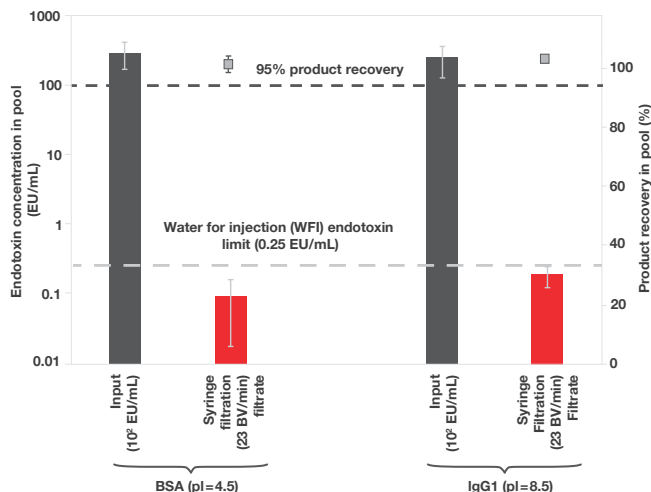


Figure 2. Syringe filtration using Thermo Scientific™ Emphaze™ AEX Hybrid Purifier demonstrates endotoxin reduction to below WFI levels for an endotoxin input of 10² EU/mL at protein challenge loads of greater than 1 kg/L.

Comparison of Emphaze AEX hybrid purifier with a standard AEX pre-packed resin column with Q-chemistry

Endotoxin removal was performed using a standard quaternary amine pre-packed AEX resin column as a comparison to Emphaze AEX hybrid purifier. The column showed decreased endotoxin reduction at flow rates higher than 1 BV/min (Fig. 3) and levels above WFI.

Emphaze AEX hybrid purifier is ideal for endotoxin removal applications in a laboratory setting, especially for high throughput processing of protein samples for screening during pre-clinical and early-stage clinical testing. The insensitivity to flow rate, unlike

a diffusion-limited resin column, allows for the use of less complex pumping systems, such as a peristaltic pump or syringe filtration, enabling multiple samples to be processed simultaneously or in quick succession. The high capacity of Emphaze AEX hybrid purifier allows for high protein loads, and scalable devices allow for easy transition to pilot or production scale processes. Emphaze AEX hybrid purifier is a robust and simple solution to the challenging problem of endotoxin removal to help accelerate the preclinical development of biopharmaceutical drug candidates.

Endotoxin removal performance is reduced at high flow rates when using a standard AEX pre-packed resin column with Q-chemistry

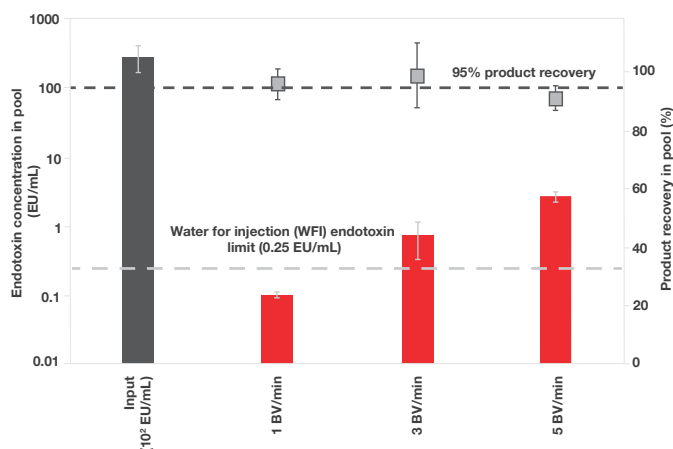


Figure 3. Endotoxin removal by a standard AEX pre-packed resin column with Q-chemistry, using BSA and an input endotoxin concentration of 10² EU/mL, shows endotoxin reduction to below WFI only when the column is operated at a flow rate of 1 BV/min. At higher flow rates, endotoxin levels were above WFI levels.

References

- 1 United States Pharmacopeia and National Formulary.
USP 29-NF 24 chapter 1231. Water for Pharmaceutical Purposes.



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