Millipore®
Filtration, Separation
& Preparation

ChromaboIt® Prepacked Chromatography Columns Performance Guide

A family of pre-validated columns prepacked with a selection of our chromatography resins and designed for early clinical stage manufacturing

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How to use the guide

The Chromabolt® Performance Guide is a reference document to provide you with assistance in evaluating Chromabolt® prepacked columns for your chromatography process step. This guide includes general guidelines on various performance aspects of prepacked columns and the physical resin characteristics that may be considered and evaluated by potential users of Chromabolt® columns. This Performance Guide also includes summaries of application tests performed at Merck. These studies have been designed or selected for inclusion in order to provide you with an insightful understanding of Chromabolt® column performance.

Results of these studies are intended as general examples and are not to be construed as product claims or specifications. While results included in this guide summarize outcomes and observations obtained in the specific application studies with the particular experimental conditions, they may or may not be representative of results obtained by other feedstreams or different test conditions and should be confirmed by the end user using feedstream and process conditions representative of the specific application.

Contents

Design Summary

Introduction

Chromabolt® columns are pre-validated and prepacked with a selection of our comprehensive chromatography resin portfolio. These columns provide an economic advantage by freeing up end users valuable time and resources by eliminating manual packing and cleaning. Chromabolt® prepacked columns are manufactured using automated equipment in a clean room environment. This ensures consistent cleanliness and robust, reproducible packed beds. Chromabolt® column design was optimized for end users ease of use and the columns can be effortlessly run by one operator.

Ergonomics: Column Design

Chromabolt® columns were designed with the goal of maintianing the performance characteristics of the packed resin in day-to-day operations. Utilizing a broad range of end user feedback on prepacked columns many key features were designed into Chromabolt®. These include liquid shedding surfaces to enhance the wipe-down cleaning process, a handle and wheels to eliminate strenuous lifting and potential risk of damage of the prepacked bed when transporting the column within the facility and an elongated functional tubing to provide easy access to the bottom of the column.

Scaling

Chromabolt® columns come in three sizes of inner diameter (id) and empty column volume: 10 cm (1.6L), 20 cm (6.5L), and 32 cm (16.1L). All sizes are compatible with all currently available chromatography systems as well as connections and are scalable in terms of their performance. The below graph demonstrates the scalability of Chromabolt® columns in all three sizes for 1 resin Fractogel® EMD TMAE HiCap. Pressure-Flow curves of Fractogel® EMD TMAE HiCap resin measured in 160mM NaCl solution at diverse differential pressures between the inlet and outlet of the Chromabolt® columns are linear and closely overlaying at all three column sizes as seen in **Figure 1**.

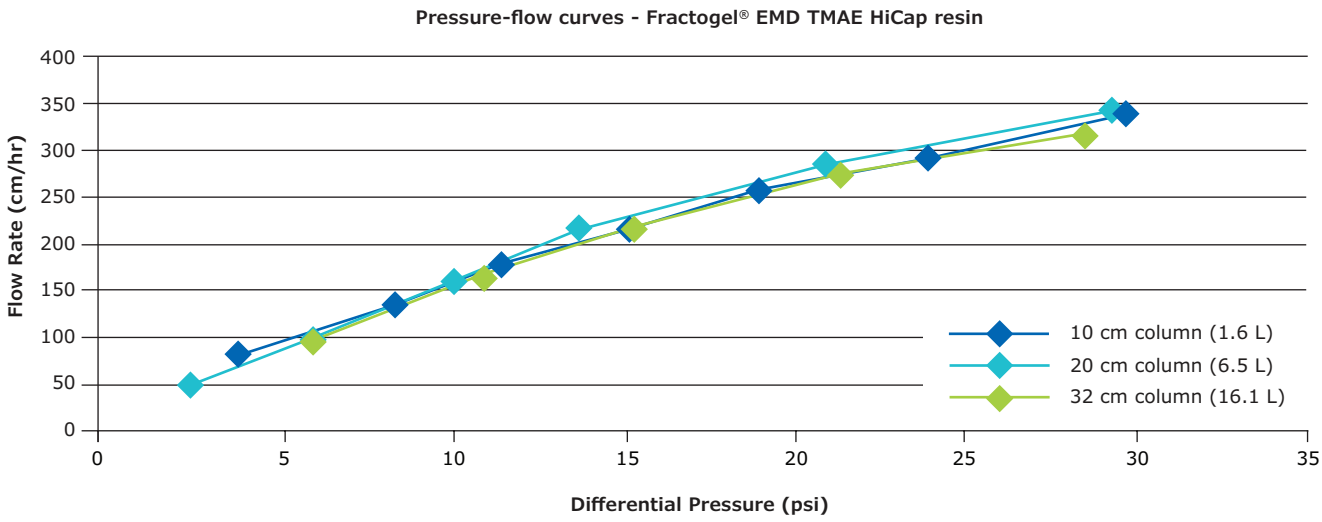


Figure 1. Pressure-flow curves were generated in 160mM NaCl and measured at a differential pressure between the inlet and outlet of the column.

Sanitization

Goal:

To assess the effectiveness of the sanitization process by evaluating the bioburden levels.

Material and Methods:

A 10 cm id column packed with Fractogel® EMD TMAE Hicap resin was sanitized using 0.5 N NaOH by a standard sanitization protocol. To assess the effectiveness of the sanitization process, a worst-case scenario was created, whereby the column was first spiked with bacteria. At the end of the sanitization process, the column was equilibrated into 150 mM NaCl solution, and the flow through samples were collected in each of the steps. The steps are detailed in **Table 1**.

Table 1. Steps followed to show effectiveness of sanitization process.

Flow Steps	Volume	Comments
Sterile 150 mM NaCl solution	4-5CV	Collect flow through sample
Microbial spike	3CV	Collect flow through sample
Sterilization in NaOH	3CV	3hr hold time
Sterile DI water	6CV	Collect flow through sample after neutralization
Sterile 150 mM NaCl solution	6CV	Collect flow through sample
Sterile 150 mM NaCl solution after overnight incubation	1CV	Collect flow through sample

Results and Discussion:

While the varying loose resins have been proven to be sanitizable using 0.5 N NaOH, the goal of this study was to show that this sanitant is also effective on resin packed into a Chromabolt® column.

As shown in **Table 2**, the bioburden levels dropped below 1 cfu/mL even after bacterial spike of circa. 10^8 cfu/mL through the column proving the effectiveness of the sanitization process.

Table 2. Bioburden levels during different steps of sanitization process

Flow Steps	TSA Bioburden (CFU/mL)	SDA Bioburden (CFU/mL)
	Spec ≤100cfu	Spec ≤100cfu
Initial Bioburden in Column	0.004	0.020
Bacterial spike	4.20×10^8	3.50×10^8
Bacteria in flow-through	$< 1 \times 10^3$	$< 1 \times 10^3$
NaOH sanitization	0.009	0.002
Bioburden post-sanitization (same day)	0	0
Bioburden post-sanitization (next day)	0.002	0

Table 3. Probability of failure of bioburden or endotoxin failure for Chromabolt® columns

	% probability of failure	
Resin	Bioburden	Endotoxin
Eshmuno®	< 0.1	< 6
Fractogel® EMD	< 0.1	< 0.1

The sanitization process was applied to the column without bacterial spike, and statistical probability of bioburden and endotoxin failure was calculated.

Figure 1. Eshmuno® Resins' Endotoxin Results – all outcomes well below the spec of 1 EU/mL

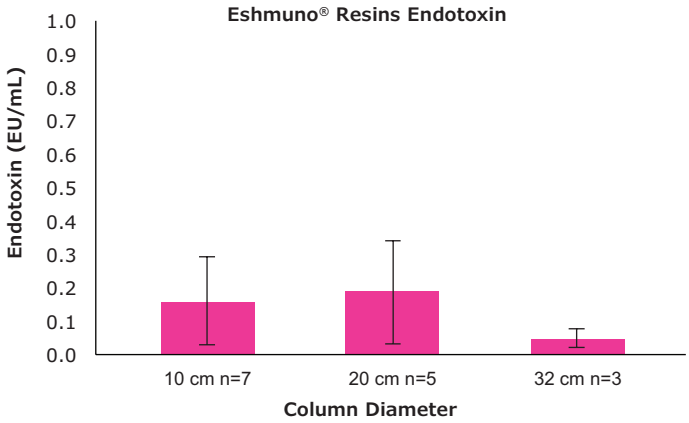


Figure 2. Fractogel® Resins' Endotoxin Results – all outcomes well below the spec of 1 EU/mL

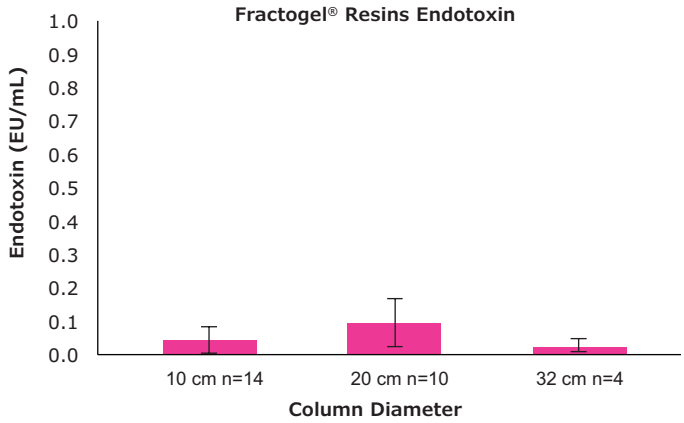


Figure 3. Eshmuno® Resins' Bioburden Results – all outcomes well below the spec of 100 CFU/mL

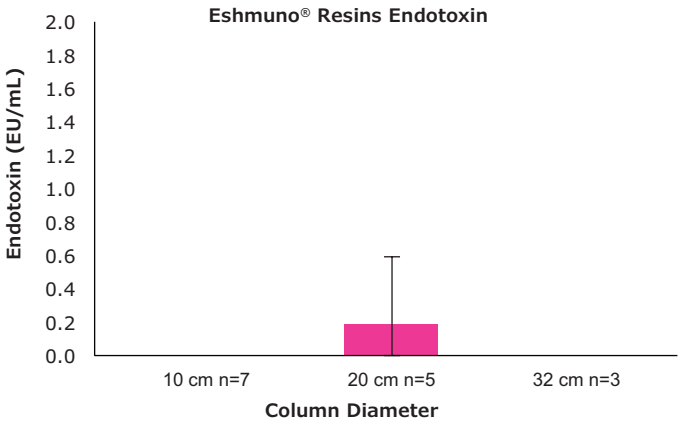
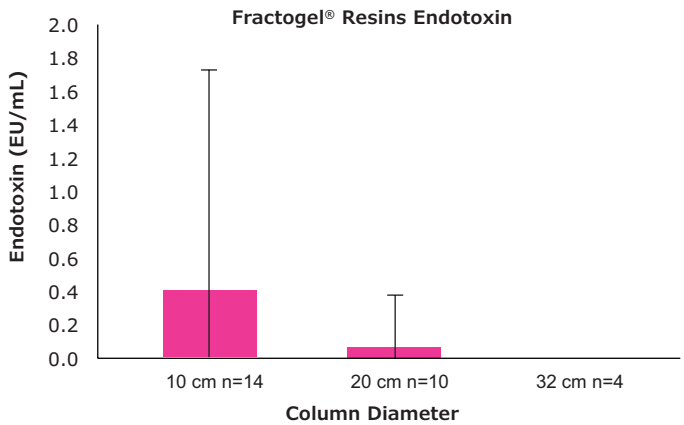


Figure 4. Fractogel® Resins' Bioburden Results – all outcomes well below the spec of 100 CFU/mL



The sanitization process is applied to all the Chromabolt® column generated (>50) and the success rate was calculated in terms of process capability. **Table 3** shows the process capability for bioburden and endotoxins for Eshmuno® and Fractogel® EMD resins represented in terms of probabilities of failures to pass the specification. It should be noted that none of the columns failed the specifications.

As a final step each column is tested for bioburden and endotoxin and only released from manufacturing when it passes the specification.

Conclusion:

Based on the results of bacterial spike experiment and the probability of failure estimations, it was validated that the sanitization process adapted for Chromabolt® columns meets and exceeds the specification.

Extractables Analysis

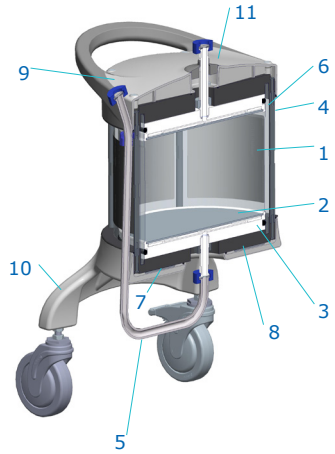
Goal:

To analyze the worst case scenario levels of extractable compounds derived from the USP Class VI Chromabolt® column parts in extreme pH solutions.

Material and Methods:

All components which are in the flow path of the column were analyzed in this experiment. The parts tested include the header, column tube, sanitary adapter, frit, and the O-ring which creates the seal around the header. Testing for extractables was done with respective material samples instead of a complete component to standardize the testing. Component base material is identical between the different column sizes, only the dimensions are altered.

Component samples were immersed in DI water, 1 N HCl and 1 N NaOH for 7, 38, and 76 days respectively at 40 °C. This accelerated testing at increased temperatures corresponds with time points of 0.5, 3, and 6 months at room temperature. This information is displayed in **Table 1**, which lists out the conditions for each sample. At each time point, the TOC levels in the immersion solution were measured. Each sample type was done in triplicate and, correspondingly, the recorded values are averages of these three samples. TOC results displayed in this Performance Guide are shown in ppm levels with respect to one column volume of liquid which could be in the column. Thus for each different column size, a different column volume and component surface area was used in the TOC concentration calculation.



No.	Part	Material of construction
1	Tube	Acrylic
2	Frit	Polyethylene
3	Header/TC	Polyproplene
4	Hardware	Stainless steel
5	Flex Tube	Tygon® tubing
6	O-rings	EPDM
7	O-rings not in fluid path	Silicone
8	Endcaps	PVC
9	Hats and Handles	PBT
10	Shoe	Glass Filled Polyproplene
11	Flashing	Silicone

Table 1. Sample Conditions

Sample Number	Solution	Temperature (°C)	Accelerated Testing Time (Days)	Real Time Equivalent (Months)
1	DI Water	40	7	0.5
2	DI Water	40	38	3
3	DI Water	40	7	0.5
4	1 N HCl	40	7	0.5
5	1 N HCl	40	38	3
6	1 N HCl	40	7	0.5
7	1 N NaOH	40	7	0.5
8	1 N NaOH	40	38	3
9	1 N NaOH	40	7	0.5

Results and Discussion:

Tables 2-4 display TOC data in ppm for the major fluid path components of the Chromabolt® columns. It should be noted that for the header and frit components the area scales directly with column diameter and, therefore, analysis of all sizes are identical. Column tube refers to the tube within which the resin is packed and contacts the resin circumferentially around the perimeter. The analysis of the extractables due to the headers and frits takes into account that there are two of each in the column; the numbers displayed in **Tables 2-4** reflect the total surface area of both components. Data is not displayed for the sanitary adapters or the O-rings as their contribution of extractables is negligible. This is due to the very minimal surface area that these parts contribute to the fluid flow path when compared with the other column components.

Table 2. Column Tube TOC counts

Immersion Solution	Real Time Equivalent (Months)	10 cm column TOC (ppm)	20 cm column TOC (ppm)	32 cm column TOC (ppm)
DI Water	0.5	0.21	0.11	0.07
	3	0.57	0.29	0.18
	6	0.89	0.43	0.27
1 N HCl	0.5	0.12	0.06	0.04
	3	0.27	0.14	0.09
	6	0.70	0.35	0.22
1 N NaOH	0.5	0.48	0.24	0.15
	3	0.75	0.37	0.23
	6	0.73	0.36	0.23

Table 3. Header TOC

Immersion Solution	Real Time Equivalent (Months)	TOC (ppm)
DI Water	0.5	0.02
	3	0.57
	6	0.89
1 N HCl	0.5	0.02
	3	0.02
	6	0.04
1 N NaOH	0.5	0.07
	3	0.08
	6	0.07

Table 4. Frit TOC counts

Immersion Solution	Real Time Equivalent (Months)	TOC (ppm)
DI Water	0.5	0.04
	3	0.05
	6	0.05
1 N HCl	0.5	0.35
	3	0.16
	6	0.11
1 N NaOH	0.5	0.04
	3	0.04
	6	0.00

For all components, the TOC levels were below 1 ppm under all conditions. This suggests very low quantities of compounds are being extracted from Chromabolt® columns. Harsh pH conditions proved to have limited impact on the quantity of extractables. Specific chemical identification of these extractables was not feasible due to lack of a sensitive and direct analytical method for chemical identification at such low concentrations.

Conclusion:

All components within the fluid path of the Chromabolt® family columns were analyzed for extractables. The results indicate low levels of organic compounds can be removed from the components even under varied harsh pH conditions. This is due to careful material selection and production, which results in a safe and trustworthy device for purification.

Resin Compression

Goal:

To determine the nominal amount of resin packed in a column and the nominal compression factor of the resin bed in the column.

Material and Methods:

Tables 1-3 display data for several different resins. This data was collected by allowing desired packing volumes of resin to settle by gravity within a graduated cylinder. The settled bed volume was compared to the final column volume in order to determine the percent compression that the resin will be subjected to. This was done for volumes related to the 10 cm column. The volumes of resin required to pack 20 and 32 cm diameter columns were then calculated through multiplication by the column volume ratios. The bed compression remains consistent regardless of column size. The volume associated with each resin type and column size is displayed in **Table 4**. Overall this data shows a high degree of reproducibility in our manufacturing procedure which allows for automated packing close to a specific resin compression target for each resin.

Results and Discussion:

The compression characteristic is a key packing parameter for Chromabolt® columns which have been packed so that they can be shipped worldwide. Current compression has been developed so as to maintain bed stability under all shipping destinations and conditions. As part of the Chromabolt® prepacked column design and validation, compression factors for all resins are tightly controlled and regulated in our automated manufacturing process. The compression factors for Fractogel® EMD and Eshmuno® resins packed in Chromabolt® columns were optimized to ensure that the columns maintain the target performance characteristics.

Table 1. Gravity Settled Bed Volume and Bed Compression for Fractogel® EMD TMAE HiCap (M) Resin

Sr. No.	Settled bed volume (mL)	Volume of 10 cm column (ml)	% Bed Compression
1	2225	1600	28.09
2	2115	1600	24.35
3	2125	1600	24.71
4	2140	1600	25.23
5	2050	1600	21.95
Average	**2131**		**24.87**
Standard Deviation	**62.8**		**2.19**

Table 2. Gravity Settled Bed Volume and Bed Compression for Fractogel® EMD SO_3 (M) Resin

Sr. No.	Settled bed volume (ml)	Volume of 10 cm column (ml)	% Bed Compression
1	2285	1600	29.98
2	2230	1600	28.25
3	2275	1600	29.67
4	2230	1600	28.25
5	2275	1600	29.67
Average	**2259**		**29.16**
Standard Deviation	**26.8**		**0.84**

Table 3. Gravity Settled Bed Volume and Bed Compression for Eshmuno® S Resin

Sr. No.	Settled bed volume (mL)	Volume of 10 cm column (mL)	% Bed Compression
1	1800	1600	11.11
2	1775	1600	9.86
3	1790	1600	10.61
4	1725	1600	7.25
Average	**1772.5**		**9.71**
Standard Deviation	**33.3**		**1.8**

Table 4. Volume of Gravity Settled Resin in 20 & 32 cm:

Column Size	Column Volumes (L)	Ratio of Column Volumes	Estimated Volume of Resin (mL)		
			Fractogel® EMD TMAE HiCap	Fractogel® EMD SO_3	Eshmuno® S
10	1.6	1	2131	2259	1773
20	6.48	4.05	8631	9149	7179
32	16.14	10.1	21496	22788	17880

Summary of resin compression data

Resin	Manufacturer suggested Compression Range for Non-prepacked Columns	Chromabolt® Column Resin Compression
Fractogel® EMD TMAE	25% - 30%	30%
Fractogel® EMD TMAE HiCap	25% - 30%	25%
Fractogel® EMD SO_3	25% - 30%	30%
Fractogel® EMD SE HiCap	25% - 30%	25%
Fractogel® EMD COO	25% - 30%	30%
Fractogel® EMD DEAE	25% - 30%	28%
Eshmuno® S	8% - 10%	10%
Eshmuno® Q	8% - 10%	12%
Eshmuno® A	8% - 12%	13%

Conclusion:

The data demonstrates that we are successfully able to pack different types of resin (base bead size and chemistry) into Chromabolt® columns using our automated equipment robustly and reliably. Scale up from the 10 cm to 32 cm: showed no deviation in performance or compression of the resin.

Shipping

Goal:

To measure and analyze the effect of transportation forces on Chromabolt® columns by measuring bed performance both before and after shipping tests.

Material and Methods:

Both standardized and real world shipping tests were conducted with Chromabolt® columns in their final packaging.

Table 1. Overview of ISTA-1E test method

Sequence #	Test Category	Test Type	Test Level
1	Vibration	Random	Overall Grms level of 1.15
2	Shock	Incline Impact	69 in (1.7 m) per second impact velocity
3	Shock	Rotational Edge Drop	8 in (200 mm)

Standardized shipping tests were done following protocols outlined in the ISTA-1E non-simulated integrity performance test procedure. **Table 1** displays the general testing requirements. The container is first subjected to random vibration at a root means squared acceleration (Grms) value of 1.15 for at least an hour. The container is then drawn down an incline at 1.7 meters per second before being stopped with an instantaneous impact. This is done for all vertical faces of the container. The final test involves a rotational edge drop of 200 mm for the container's bottom edges. This protocol exceeds expected impacts and vibration experienced by a container during shipping and proves the robustness of both the column and the packaging.

Testing done to show resin bed performance includes parameters of HETP, asymmetry, and flow rate through the column at 15 PSI differential pressure. All testing was performed after columns were allowed to adjust to ambient room temperature.

Real world shipping tests were sent to international testing locations in both Asia and Europe to give maximum travel distance from the manufacturing site in the United States. The column's bed performance was tested before shipping and then once again at the testing sites using identical procedures. The column temperatures were tracked throughout their journey to show an example of effective temperature range.

Results and Discussion:

Figure 1 – 6 display bed performance behavior before and after columns have undergone the designated ISTA-1E shipping protocol. Columns are grouped into categories by base bead matrix; this includes Fractogel® EMD and Eshmuno® base bead resin types. Blue lines have been added to indicate what is the performance specification.

Figure 1. Fractogel® EMD Resins Asymmetry – data remains consistent after shipping ISTA-1E

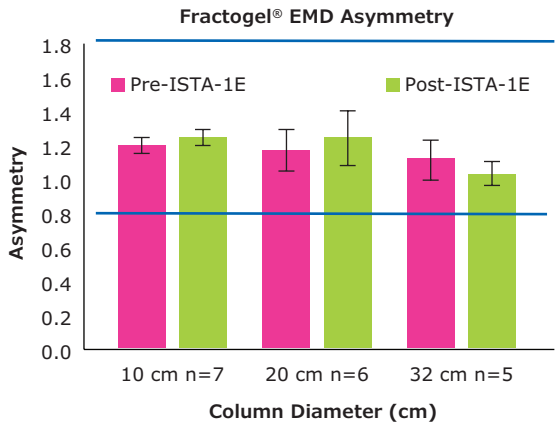


Figure 2. Eshmuno® Resins Asymmetry – data remains consistent after shipping ISTA-1E

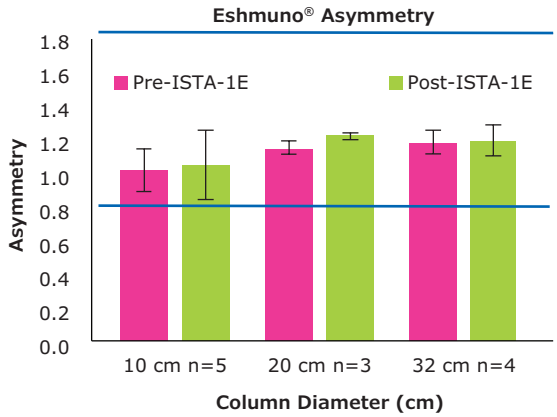


Figure 3. Fractogel® EMD Resins HETP – data remains consistent after shipping ISTA-1E

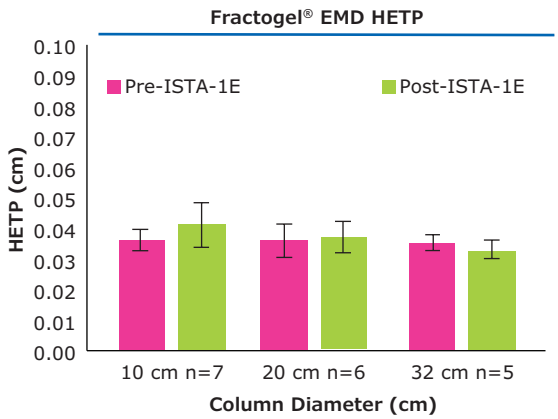


Figure 4. Eshmuno® Resins HETP – data remains consistent after shipping ISTA-1E

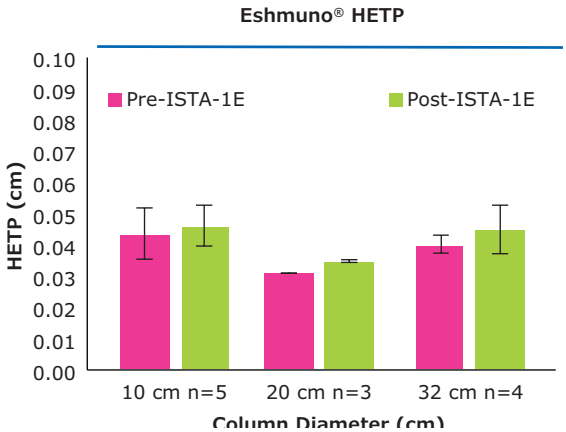


Figure 5. Fractogel® EMD Resins Flow rates at 15 PSI differential pressure – data remains consistent after shipping ISTA-1E

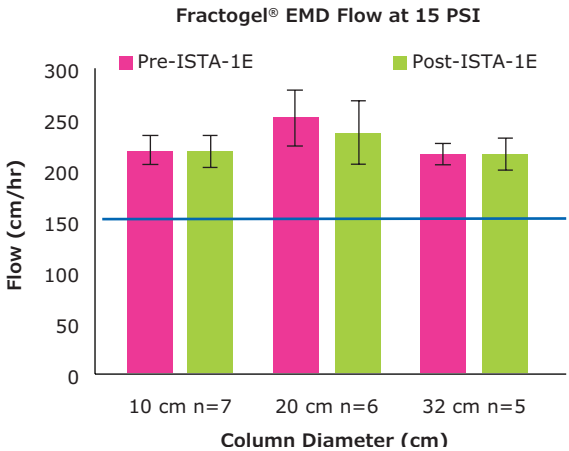
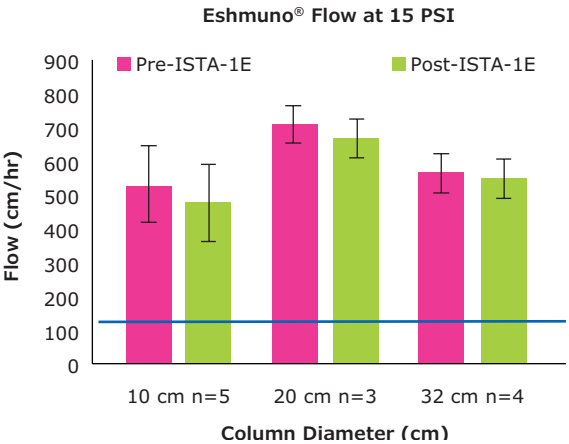


Figure 6. Eshmuno® Resins flow rates at 15 PSI differential pressure – data remains consistent after shipping ISTA-1E



Temperature was recorded for one column shipped to Europe and one column shipped to Asia. The temperature data is displayed below in **Figures 7** and **8**. The temperature recordings begin when the column left the manufacturing facility and end when they were opened at the final destination. The data for bed performance for these temperature tracked columns is shown in **Table 2**.

Figure 7. Temperature tracking of shipment from USA to site 1

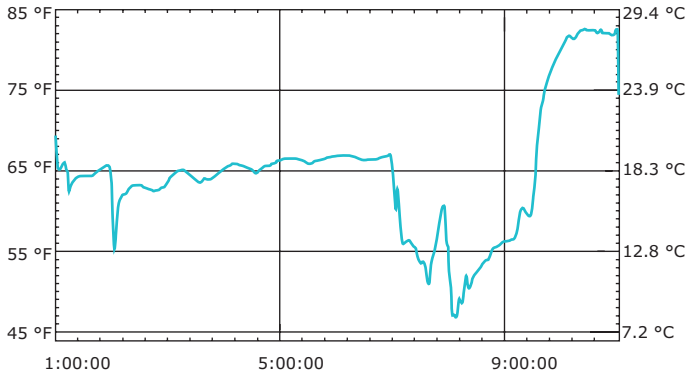


Figure 8. Temperature tracking of shipment from USA to site 2

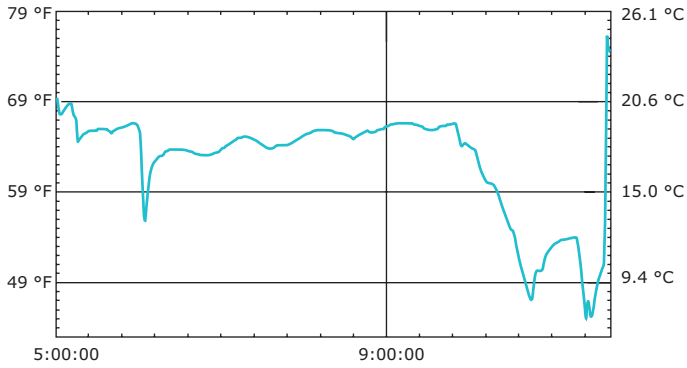


Table 2. Resin bed performance after shipping

	Site 1: Eshmuno® S 10 cm column	Site 2: Fractogel® EMD TMAE HiCap 10 cm column
HETP (cm)	0.0184	0.0400
ASYM	1.20	1.28
Flow at 15 PSI (cm/hr)	450	163

Conclusion:

The Chromabolt® column family is able to maintain a stable and functional resin bed throughout adverse shipping conditions. This is due to the advanced column design, validated packing procedures, and custom crating. The data displayed shows the performance characteristics of all column sizes and all ion exchange resins available in the Chromabolt® platform.

Economic Case Study

Introduction:

Prepacked columns offer significant savings in time because they eliminate the need for the manual packing process.

Economic Advantages of Prepacked Columns

- Time savings of the manual packing process
- Cleaning and validation of column hardware between campaigns
- Reduction in buffers used for packing
- Capital equipment purchases (prepacked columns are considered an expense)
- Spare parts and service contracts for hardware
- Footprint of hardware

Time savings: Column Economics

By using Chromabolt® columns, the user eliminates laborious packing and cleaning of hardware and effectively enjoys a quick setup time. The advantages of this fast turnaround time for Chromabolt® columns are illustrated in **Figure 1** where the hours required for setup and cleaning of traditional hardware is compared to a prepacked column.

Data:

Customer monetary savings are difficult to calculate when looked at holistically due to the influence of many different factors as well as local labor rates etc. However, it is possible to calculate the time savings when implementing columns. The data below is based off industry standards and customer feedback, and assumes that manual packing is 100% successful every time.

Figure 1. Prepacked time savings of 10 cm, 20 cm, and 32 cm columns

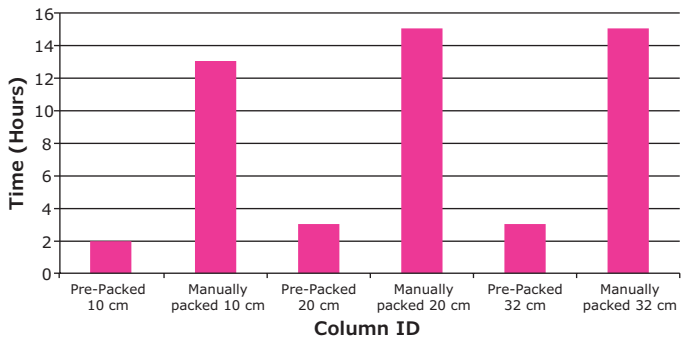


Table 1. Prepacked time savings of 10 cm, 20 cm, and 32 cm columns

	Prepacked 10 cm	Manually packed 10 cm	Prepacked 20 cm	Manually packed 20 cm	Prepacked 32 cm	Manually packed 32 cm
Buffer preparation (hrs)	1	1	2	2	2	2
Column packing (including buffer exchange) (hrs)	0	8	0	8	0	8
Pre-use qualification (hrs)	0.5	0.5	0.5	0.5	0.5	0.5
Pre-use sanitization(hrs)	0.5	0.5	0.5	0.5	0.5	0.5
Column unpacking (hrs)	0	1	0	2	0	2
Equipment cleaning (hrs)	0	2	0	2	0	2
Totals (hrs)	**2**	**13**	**3**	**15**	**3**	**15**

Conclusion:

The above data demonstrates the advantages of fast turnaround time for Chromabolt® prepacked chromatography columns when compared to the hours required for setup and cleaning of traditional hardware columns. Prepacked columns allow manufacturers and pilot facilities to utilize scarce resources more efficiently and to improve plant flexibility. The ability to provide a consistent packed column using an automated process for all three sizes of Chromabolt® reduces variability and provides a powerful new tool for end users.

To place an order or receive technical assistance

In Europe, please call Customer Service:
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Germany: 069 86798021
Italy: 848 845 645
Spain: 901 516 645 Option 1
Switzerland: 0848 645 645
United Kingdom: 0870 900 4645
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