# Bead size and column format influence the resolution. A comparison using HiScreen and HiTrap prepacked columns.

**Intellectual Property Notice:** The Biopharma business of GE Healthcare was acquired by Danaher on 31 March 2020 and now operates under the Cytiva™ brand. Certain collateral materials (such as application notes, scientific posters, and white papers) were created prior to the Danaher acquisition and contain various GE owned trademarks and font designs. In order to maintain the familiarity of those materials for long-serving customers and to preserve the integrity of those scientific documents, those GE owned trademarks and font designs remain in place, it being specifically acknowledged by Danaher and the Cytiva business that GE owns such GE trademarks and font designs.

**cytiva.com**



CY15124-11Jul20-PT

# Bead size and column format influence the resolution. A comparison using HiScreen™ and HiTrap™ prepacked columns.

*Anna Heijbel and Karin Torstenson*

*GE Healthcare Bio-Sciences AB, Björkgatan 30, SE-751 84 Uppsala, Sweden*

## Introduction

The use of prepacked columns offers time saving, convenience and reproducible results. In addition, different column formats meet the need for different separations and purifications. The chromatography medium volume defines the capacity of the column, while in combination with the column format the purification time and resolution are defined. A short and wide column can be run at higher flow rate to reduce time and a long and narrower column will increase the resolution. Besides beads size and column format the slope of the gradient also affects the resolution. In this study we have chosen to do a comparison of how different column formats and different bead sizes affects the resolution.

The HiTrap and HiScreen column families are prepacked with different chromatography media for different purification techniques such as ion exchange (IEX), hydrophobic interaction (HIC), affinity (AC) and desalting (DS). These two column families are ideal for small scale protein purification, method optimization, screening and robustness testing. They are easily used on chromatography systems such as ÄKTA™ design, and HiTrap columns can also be used manually with a syringe. The column formats are different for these two column families and will influence the purification results, especially in combination with the bead size of the chromatography medium. If necessary, two columns can easily be connected in series to give a bed height of 20 cm (HiScreen) and 5 cm (HiTrap).


HiScreen columns


HiTrap columns

## Column characteristics

| | HiScreen | HiTrap, 5 ml | HiTrap, 1 ml |
|---|---|---|---|
| Column length | 10 cm | 2.5 cm | 2.5 cm |
| Column i.d. | 0.77 cm | 1.6 cm | 0.7 cm |
| Column volume | 4.7 ml | 5 ml | 1 ml |
| Column tube thickness | 2.15 mm | 1.65 mm | 1.65 mm |
| Fittings | 1/16" (valco) | 1/16" (valco) | 1/16" (valco) |

## HiScreen AIEX versus HiTrap AIEX

A study of three different anion exchange chromatography media prepacked in HiScreen and HiTrap (5 ml) columns was performed (Fig. 1). The sample was a mixture of three standard proteins and the same linear flow rate (cm/hour) was used.

HiScreen columns gave higher resolution compared to HiTrap columns. Q Sepharose™ High Performance gave better resolution due to the smaller bead size (34 µm beads) as compared to the Q Sepharose Fast Flow (90 µm beads) and Capto™ Q media (90 µm beads).

### *Running conditions*

| | |
|---|---|
| *Columns:* | HiScreen Q HP, 4.7 ml and HiTrap Q HP, 5 ml<br>HiScreen Q FF, 4.7 ml and HiTrap Q FF, 5 ml<br>HiScreen Capto Q, 4.7 ml and HiTrap Capto Q, 5 ml |
| *Sample:* | 5 ml mixture of apo-transferrin (0.3 mg/ml),<br>α-lactoalbumin (0.4 mg/ml) and<br>soya trypsin inhibitor (0.6 mg/ml) in start buffer |
| *Start buffer:* | 50 mM Tris-HCl, pH 7.3 |
| *Elution buffer:* | 50 mM Tris-HCl, 0.5 M NaCl, pH 7.3 |
| *Flow rates:* | HiScreen Q HP and HiTrap Q HP:<br>150 cm/h (1.2 ml/min and 5 ml/min)<br>HiScreen Q FF and HiTrap Q FF:<br>300 cm/h (2.3 ml/min and 10 ml/min)<br>HiScreen Capto Q and HiTrap Capto Q:<br>400 cm/h (3.1 ml/min and 13 ml/min) |
| *Linear gradient:* | 0% to 100% elution buffer in 20 column volumes |

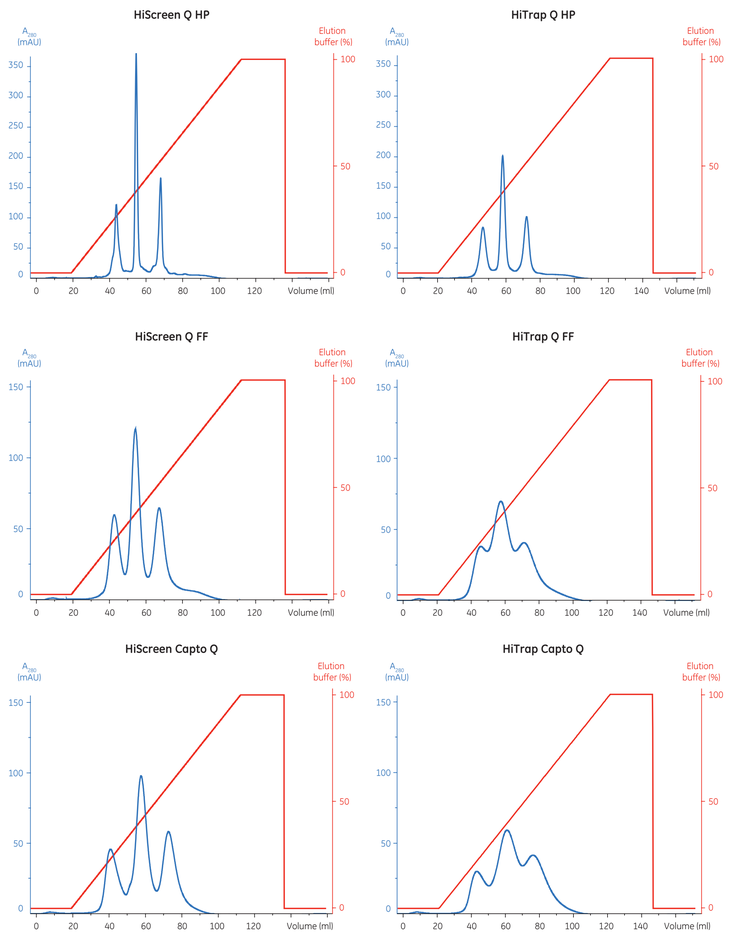


**Fig 1.** Comparision of separations of a standard protein mixture on three different anion exchangers, Q Sepharose High Performance, Q Sepharose Fast Flow and Capto Q prepacked in HiScreen and HiTrap (5 ml) column formats.

## HiScreen HIC versus HiTrap HIC

HiScreen Phenyl HP and HiTrap Phenyl HP (5 ml) are prepacked with Phenyl Sepharose High Performance (34 µm bead size). HiScreen Phenyl FF (high sub) and HiTrap Phenyl FF (high sub) (5 ml) are prepacked with Phenyl Sepharose 6 Fast Flow (high sub) (90 µm bead size). These four columns were compared for resolution when a separation of a standard protein mixture was run with the same linear flow rate (cm/hour) (Fig. 2). The highest resolution was obtained with HiScreen Phenyl HP.

### *Running conditions*

| | |
|---|---|
| *Columns:* | HiScreen Phenyl HP, 4.7 ml<br>HiTrap Phenyl HP, 5 ml<br>HiScreen Phenyl FF (high sub), 4.7 ml<br>HiTrap Phenyl FF (high sub), 5 ml |
| *Sample:* | 1 ml mixture of cytochrome C,<br>ribonuclease A, lysosyme and chymotrypsinogen (1:3:1:1)<br>dissolved in start buffer |
| *Start/wash buffer:* | 100 mM $Na_2HPO_4$, 1.7 M $(NH_4)_2SO_4$, pH 7.0 |
| *Elution buffer:* | 100 mM $Na_2HPO_4$, pH 7.0 |
| *Flow rates:* | HiScreen Phenyl HP and HiTrap Phenyl HP:<br>150 cm/h (1.2 ml/min and 5 ml/min)<br>HiScreen Phenyl FF (high sub) and<br>HiTrap Phenyl FF (high sub):<br>300 cm/h (2.3 ml/min and 10 ml/min) |
| *Linear gradient:* | 0% to 100% elution buffer in 10 column volumes |

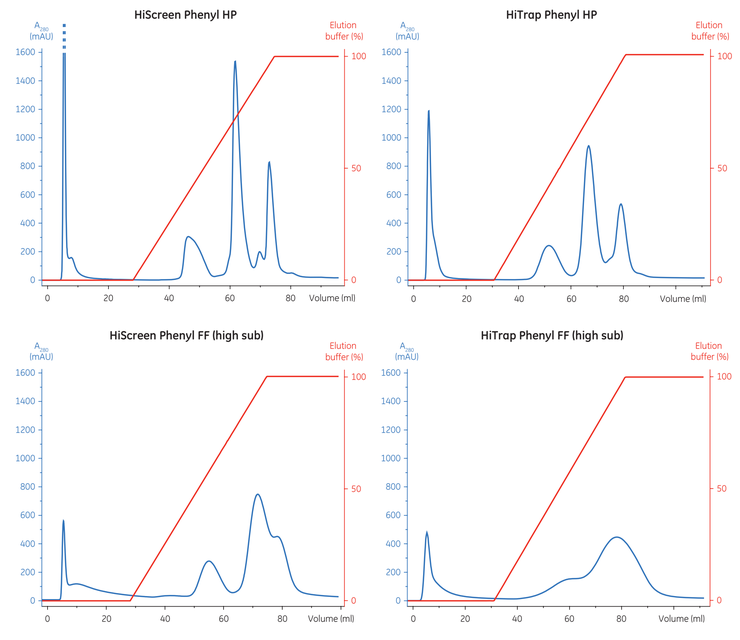


**Fig 2.** Comparision of separations of a standard protein mixture on two different hydrophobic interaction chromatography media prepacked in HiScreen and HiTrap (5 ml) column formats.

## Discussion and conclusions

This study clearly shows the importance of selecting appropriate column format and bead size of the chromatography medium.

Larger bead size and columns with larger diameter allow higher flow rates and are preferable for scaling up studies.

When striving for high resolution, longer and narrower columns with small bead size is preferable.

imagination at work



GE Healthcare Bio-Sciences AB, Björkgatan 30, 751 84 Uppsala, Sweden.

28-9609-28 AA 09/2009