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Reliable Ion Exchange LC: Consistent Performance from MonoBeads® Support

B. Edlén, Amersham Pharmacia Biotech

Mono Q and Mono S columns are highly efficient, pH-stable columns designed for high performance ion exchange separations of proteins, peptides, and polynucleotides, in applications including peptide mapping and monoclonal antibody purification. The unique properties of these columns are based on MonoBeads support – a beaded hydrophilic material with the narrowest particle size distribution of any chromatographic support: 10±0.3µm. This monodispersity permits high flow rates at relatively low backpressures. Stringent product testing ensures highly consistent performance from Mono Q and Mono S columns, prepared with these particles.

For any chromatographic medium, physical and chemical characteristics should be highly consistent over time, so that when a column is replaced, the user can obtain equivalent results with the replacement column. A quality product is one for which a column bought today has the same selectivity as one purchased a week, month, year, or years ago. Pharmacia® chromatographic media based on MonoBeads® support provide outstanding reproducibility that chromatographers can rely on.

For the user, quality means no worries about product consistency. In order to ensure consistency the manufacturer must begin with the raw materials, whose critical variables must be known and controlled. Amersham Pharmacia Biotech chemists test physical and chemical variables for the raw materials, the bulk gel, and the final column, to ensure that every column will behave as expected by the user (Table 1). The electron micrograph in Figure A and the particle size distribution plot in Figure B show the monodispersity of MonoBeads support (1). The extremely narrow particle size distribution permits high flow rates at relatively low backpressures. Protein separation tests are run on every batch of bulk gel, to ensure that retention and selectivity are within specified limits. Although chromatographers very rarely use the full capacity of an ion exchange column, capacity is not allowed to fall outside specifications developed more than a decade ago, in order to maintain consistent performance from Mono Q® columns (strong anion exchanger; quaternary amino functional groups remain equally charged over the column's entire useful pH range) and Mono S® columns (strong cation exchanger; sulfonic acid functional groups). Figure C shows a typical batch test chromatogram for Mono Q packing material. Figure D shows the negligible variations in protein retention times and ion exchange capacity for Mono Q material, from lots produced in 1982 to lots produced in 1993. Lots of Mono S packing material are tested under conditions similar to those used for the Mono Q material.

Since their introduction, MonoBeads support-based columns have proven their reliability and reproducibility. Mono Q and Mono S columns are manufactured to close, carefully maintained

Table 1. Column Characteristics: Mono Q and Mono S Columns

Dimensions:	50 x 5mm
Bed Volume:	1mL
Flow Rate:	0.5-2mL/min
Max. Backpressure:	750psi (5MPa)
Temperature:	4°-40°C
pH:	2-12
Max. Loading Capacity:	25mg
Protein Binding Capacity:	65mg human serum albumin (Mono Q) 75mg immunoglobulin G (Mono S)
Typical Separation Times:	5-20 min

Figure A. MonoBeads Particles Are Highly Uniform in Size

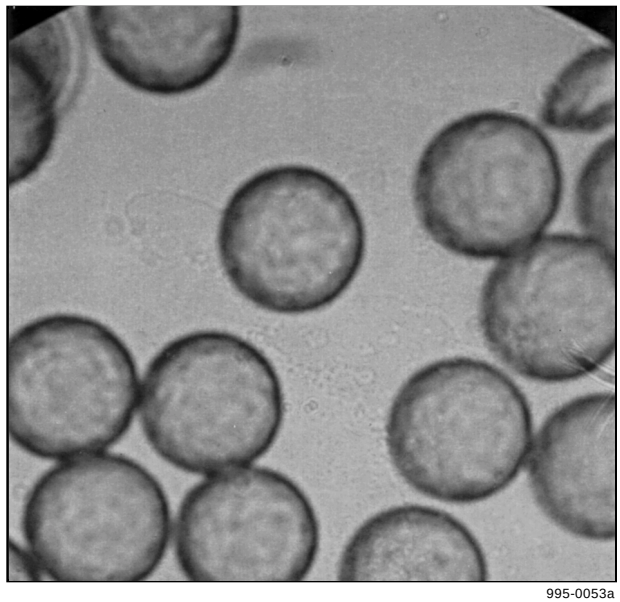

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Figure B. Narrow Particle Size Distribution for MonoBeads Particles (Coulter Counter technique)

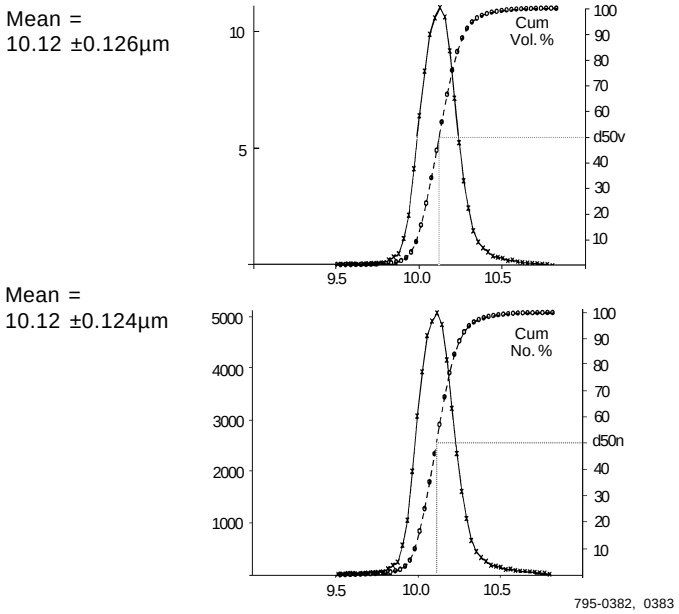
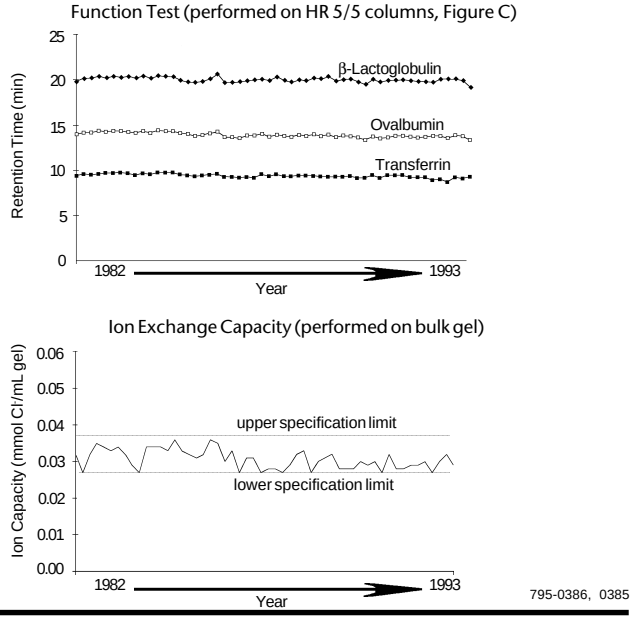


Figure D. Mono Q Columns Are Highly Consistent

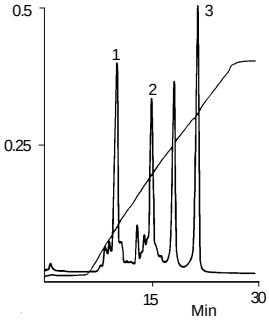


specifications, to make their routine use in preparative and analytical work highly reliable. Column stability, another important factor, also has been documented for Mono Q and Mono S columns (2). In the words of the Amersham Pharmacia Biotech chemists: "Quality assurance is an important part of the manufacturing process. Every step on the way to the final product is well documented. The exact result of those steps in the production that are subject to variation should be verified by tests. This will guarantee that a customer can always get media or columns with performance equal to those previously bought (3)."

If you are performing ion exchange separations of proteins, peptides, or polynucleotides, and wish to be sure of highly consistent performance from column to column, we highly recommend Mono Q and Mono S columns to you.

Figure C. Typical Results: Function Test for Mono Q Columns

Column: **Mono Q HR 5/5, 5cm x 5mm ID, 10µm particles**
Cat. No.: **54807**
Mobile Phase: A = 0.02M piperazine, pH 6.0
B = 0.02M piperazine/0.3M NaCl, pH 6.0
0.5% B to 100% B in 20 min
Flow Rate: 1mL/min
Det.: UV, 280nm



1. Transferrin, 1mg/mL
2. Ovalbumin, 2mg/mL
3. β-Lactoglobulin, 2mg/mL

Ordering Information:

Description	Cat. No.
Mono Q HR 5/5 Column 5cm x 5mm, 10µm particles	**54807**
Mono S HR 5/5 Column 5cm x 5mm, 10µm particles	**54808**

References
1. Göransson, B., *Part. Part. Sys. Charact.* **7**: 6 (1990).
2. Johansson, B.-L. and N. Strafström, *J. Chromatogr.* **314**: 396 (1984).
3. Edlén, B., *Amer. Biotechnol. Lab.*, Nov. 1994 (pp 40-41).

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