Agilent Zorbax Bio Series GF-450 Column

Datasheet

General Description

Zorbax Bio-Series GF-450 is a surface-stabilized, hydrophilic gel filtration column useful for the size separation of biological macromolecules. This column has been specifically designed to provide superior efficiency, pH stability, and operational lifetime when using typical aqueous buffer solutions (pH 3.0-8.5) as the mobile phase.

The GF-450 column separates compounds by gel filtration (exclusion). Separations depend on the size of the sample molecules and the effective pore diameter of the packing material. Typically, the elution order or retention time obtained for a given molecule is inversely proportional to the logarithm of its molecular size. Molecular size is related to the molecular weight. Very large molecules are excluded from the pores, have the shortest path through the column, and elute first. Medium size molecules partially diffuse into the pores, and elute later. The smallest molecules, which can totally permeate the pore volume of the packing, elute last.

These mechanisms determine the three areas of separation within a gel filtration column, i.e., the exclusion range, the linear separation range, and the total permeation volume of the column. For a more detailed treatment of gel filtration see References 1-15.

The GF-450 and GF-250 columns are complimentary. The GF-450 column provides high performance gel filtration separation of high molecular weight biomolecules which are excluded from the linear separation range of the smaller pore sized GF-250 column.[13] The optimal separation ranges for the columns are 900,000 to 25,000 Daltons (D) for the GF-450 column and 250,000 to 10,000 D for the GF-250, assuming spherical macro-molecules.

Product Description

The GF-450 column is the result of a major research effort in DuPont laboratories concerning the separation of biological macromolecules. This research examined the critical parameters which would produce a more stable and more efficient protein gel filtration column than was generally available for this application. These parameters include particle characteristics, surface chemistry, and column configuration. Each parameter has been carefully engineered to produce a highly efficient column which provides a wide pH range of operation (pH 3.0-8.5) and has a long projected operational lifetime.

The GF-450 column packing material is a wide pore (300Å) zirconia-stabilized silica support. The material has been further modified with a hydrophilic organosilane to produce a homogeneous, monomeric, hydrophilic stationary phase. This combination of the Zr-stabilization process[14] and the monomeric bonded surface gives the GF-450 column its superior performance characteristics. This packing material is suitable for the gel filtration chromatography of proteins, enzymes, peptides, and other biological macromolecules, and is highly resistant to base catalyzed hydrolysis.[15]

The ability to manufacture a surface-stabilized silica eliminates the requirement for an extended polymeric coating to protect the silica from hydrolytic attack. The monomeric hydrophilic surface coating employed in the GF-450 column allows the use of a smaller pore size support to obtain separations of molecules in a given molecular weight range, since a monomolecular layer does not reduce the pore diameter of the packing as does a polymeric coating technique. Smaller pore size packings are preferred since they are more mechanically stable than thinner-walled, wider pore packings.

Column Characteristics

Column Packing

- Particle - Spherical Silica
- Surface Modification - Zirconium-stabilized
- Bonded Phase - Hydrophilic molecular monolayer (diol type)
- Pore Diameter - 300Å
- Surface Area - 54 m^2/gram
- Particle Diameter - Nominal 6.0 µm

Column Configuration

- Diameter - 9.4 mm ID or 21.2 mm ID
- Length - 250 mm

Separations

The chromatogram in Figure 1 is that of the protein test mixture used to evaluate Zorbax GF-450 columns. Figure 2 shows the separation range achieved using a companion GF-250 column, using the same protein test mixture as in Figure 1. Note the difference in separation range produced by the GF-450 column (Figure 1) and the GF-250 column.

Column Performance

Column Quality Control

Each packing lot is thoroughly tested prior to use for column manufacture. This test includes a separation of a standard protein test mixture. When calibrating your column, use a freshly prepared protein mixture of commercially available lyophilized proteins. Measure column efficiency using a small-molecule, permeation peak, e.g., sodium azide. Prepare samples by dissolving protein and/or sodium azide in mobile phase collected from the detector waste effluent. Using this liquid eliminates refractive index disturbances in the chromatogram.

Efficiency

The GF-450 packing material, a small particle (nominally 6.0 micron) spherical silica, produces a highly efficient gel filtration column. High efficiency means better resolution. Therefore, one GF-450 column may provide the needed resolution where previously two "High Performance Gel Filtration" columns were required.

Theoretical Plates

The GF-450 columns typically exhibit over 10,000 plates per column for a small-molecule sample (e.g., sodium azide). The actual performance of your column is provided on the Column Performance Report which accompanies the column.

Stability

The hydrolytic stability of this product was determined by continuously pumping 0.1M $(NH_4)_2SO_4$, 0.05M Tris, 0.005% sodium azide, pH 8.25 ± .05, for three weeks, while evaluating the column's separation performance. This test corresponds to 4-6 months of heavy use. At high pH (above 8.5) column life will likely be reduced.

Speed

At normal flow rates (1.0 mL/min), a 9.4 x 250mm Zorbax GF-450 column can effect separations in less than 20 minutes, compared with the several hours to several days required for conventional organic gel-based gel filtration media.

Recovery

Protein recoveries from the GF-450 column are typically 85-100% of the total protein applied to the column. This recovery level is dependent on the protein in use and the mobile phase selected. Typically, biological activity of macromolecules is fully retained, assuming mobile phase compatibility with the biological substance.

Safety Considerations

Some important points to keep in mind for safe operation with LC components:

- All points of connection in liquid chromatographic systems are potential sources of leaks. Users of liquid chromatographic equipment should be aware of the potential hazards from such leaks due to the toxicity or flammability of the chosen mobile phase.
- Because of its small particle size, dry Zorbax packing is respirable. Columns should only be opened in a well ventilated area.
- These columns have not been approved for use in processing products for human use.
- Sodium azide is toxic and potentially a powerful and sensitive explosive.
- In the presence of azide, moisture, and carbon dioxide, heavy metals (e.g., copper) are capable of forming a number of basic azides which are explosive.
- In the presence of acids, azide can produce hydrazoic acid which is also explosive.
- Sodium azide should be neutralized, e.g., by decomposition. Once decomposed, it may be disposed of in accordance with standard safety procedures for acid solutions.
- Material Safety Data Sheets will be provided upon request.

Operational Guidelines

Installation

- Remove the protective screw plugs from the column.
- Attach the column inlet to the appropriate port on your injector system. There is an arrow on the column to indicate the direction of flow of the mobile phase.
- All components should be coupled as closely as possible. Dead volume, excess mixing volumes, and extra lengths of tubing should be minimized. The use of low dead volume connectors is advised.
- DO NOT overtighten fittings since this can damage the fittings and the 1/16" tubing.
- The column is shipped in aqueous phosphate buffer including sodium azide. Flush the column with at least 10 column volumes (120mL) of new buffer before use.
- Check the mobile phase for sample solubility and biocompatibility prior to use. All solvents should also be filtered and degassed prior to use.
- When the column has been completely purged with new mobile phase, connect it to the detector.
- Recommended flow rates are from 0.5-2.0 mL/min, typically, 1.0 mL/min. Resolution is flow dependent[2], therefore, the effect of flow rate on sample component resolution should be examined before finalizing method conditions.
- Zorbax GF-450 columns may be operated at temperatures up to 40°C and at any lower temperature which does not induce the formation of solids in the mobile phase. The system back pressure will increase markedly near the freezing point of the mobile phase, as a result of increasing viscosity.
- Rapid changes in column temperature are best avoided, particularly when mobile phase is not being pumped through the column.

Mobile Phase Selection

For biological separations, the choice of mobile phase is critical and must consider both column performance and the maintenance of biological function. Typical buffers used for classical protein gel filtration

are acceptable for the GF-450 column. These include denaturing buffers and those containing detergents.

All silica-based gel filtration columns possess a slight negative charge. This charge is likely due to unreacted silanol groups on the silica surface. The GF-450 column has a slightly higher negative charge density than some other silica-based columns, primarily due to the zirconia stabilization process. This slight negative charge does not affect the separation of biomacromolecules when using typical buffers of 0.1-0.5 molarity, and pH levels of 5.0-8.5. At low pH (4.0) and low ionic strength (less than 0.05M), an ion exchange effect may be noticed. This effect can be eliminated by either raising the pH or the ionic strength of the buffer. *An example of a routinely used buffer is 0.2M sodium phosphate, pH 7.0.* Using buffers with pH values above 8.5 does cause a slow base-catalyzed dissolution of the silica packing, but can be tolerated for short periods at the expense of somewhat reduced column life. Buffers with pH values below 5.0 should be avoided, not because of any deleterious effect they have on the packing, but because of possible alteration of the samples of interest. These sample alterations may adversely affect the expected separations. The column itself is stable to pH 3.0. The use of an antimicrobial agent in the mobile phase is also recommended.

The GF-450 column is compatible with most organic solvents. It may be used in series with other HPLC gel filtration columns, such as the GF-250 column (PN 884973-901). This combination extends the separation range obtained with a single column. However, the separation obtained is not linear. When used in combination, you should connect these columns in range order of decreasing pore diameter, i.e., first the GF-450, then the GF-250.

Sample Introduction

- Samples should be free of any particulates. Filter if necessary.
- Samples should be injected using a continuous flow injection device. Standard loop injectors on most HPLC systems are suitable devices.
- When using a single GF-450 column, injection volumes of 1-100 µL may be used without any significant alterations of resolution. Injection volumes of 300 µL or greater may be used; however, a major decrease in resolution may be observed.
- Concentrated proteins may be injected directly on the column as long as major differences in viscosity be-

Mobile Phase: 0.2 Na_2HPO_4, pH 7.5
Flow Rate: 1 cm^3 / min
Temperature: Ambient
Detector: UV (250 nm)

Peak Identity:	Molecular Weight (Daltons)
1. Purified IgM (mouse)	900,000
2. Thyroglobin	669,000
3. β-Galactosidase	570,000
4. IgG (goat)	150,000
5. BSA	67,000
6. Ovalbumin	45,000
7. Myoglobin	17,500
8. Sodium Azide	65

FIGURE 1
Separation of Protein Mixture
Bio Series GF-450 Column

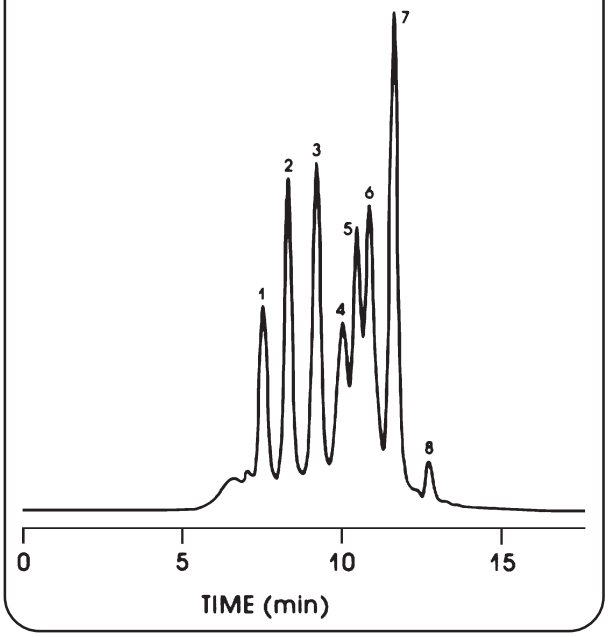
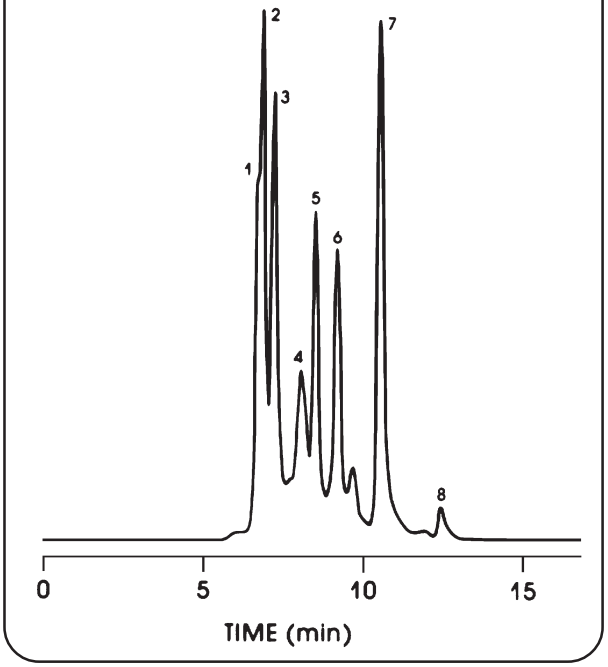


FIGURE 2
Separation of Protein Mixture
Bio Series GF-250 Column



tween the sample and the mobile phase are avoided.

- When injecting reaction mixtures (such as enzyme digests, etc.), always check compatibility of the sample and mobile phase and solubility of the mixture prior to use.

Applications

Typical uses of the GF-450 include:

- Purification of Large Biomolecules
- Monitoring Specific Proteins in a Mixture
- Evaluating Reaction Mixtures
- Screening Commercial Products
- Binding Studies
- Quantitation Studies
- Buffer Changes
- Protein-identification Assay for Other Purification Steps

Column Care

To protect the GF-450 column and increase its life, we recommend the following:

- A guard column, inserted between the injector and the column, to protect against particulates in the sample. The Reliance Cartridge guard column is recommended. This requires a fittings kit (PN 820777-901) and one cartridge (4 Pack: PN 820950-911).
- In-line filters.

Halide salts, such as sodium chloride, are corrosive to steel. If it is necessary to use such salts, thoroughly flush the HPLC system after use. Store the column in a mobile phase which does not contain halides.
If the column becomes plugged, try to clear the blockage by backflushing the column.
If this procedure is not successful, replace the column inlet frit using the following procedure:

- Remove the column from the HPLC system and carefully open the inlet fitting.
- Inspect the frit and top of the packed bed for discoloration.
- Replace the inlet frit (PN 280959-001) and reassemble.

If the packing is significantly discolored, back-flush the column (after reassembly) with 30% isopropanol; 30% isopropanol with 1mM EDTA (pH 4.5); or 50% acetonitrile, containing 0.05% TFA. This last cleaning procedure is quite stringent and may restore column performance.. If not, you can assume that the column has been compromised beyond repair.

Storage Recommendations

- When the column is in frequent use, it is not necessary to flush out the mobile phase daily, although care should be taken to avoid potential bacterial growth. If the column will not be used for several days, it is advised that the mobile phase be flushed and replaced with one containing an antimicrobial agent (e.g., sodium azide).
- Do not store the column in a mobile phase which contains halides.
- Storage in 100% methanol or ethanol is not necessary or recommended. A recommended long-term storage liquid is 0.1M sodium phosphate, pH 7.0, containing an antimicrobial agent (0.005% sodium azide).
- Remove the column from the HPLC instrument and seal the ends with the protective screw fittings used during shipping.
- Columns should be stored at room temperature.

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