



HPLC columns

ProPac WAX-10/SAX-10 columns

Product manual

Safety and special notices

Make sure you follow the precautionary statements presented in this guide.
The safety and other special notices appear in boxes.

Safety and special notices include the following



Safety

Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury



Warning

Indicates a potentially hazardous situation which, if not avoided, could result in damage to equipment



Caution

Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. Also used to identify a situation or practice that may seriously damage the instrument, but will not cause injury.



Note

Indicates information of general interest



Important

Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system



Tip

Highlights helpful information that can make a task easier

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Introduction

The Thermo Scientific™ ProPac™ protein columns are specifically designed to provide high resolution and high efficiency separations of proteins and glycoproteins with pI = 3-10 MW: >10,000 units.

The packing material is composed of a 10 µm, solvent compatible, non-porous ethylvinylbenzene cross-linked with 55% divinylbenzene polymer substrate. This resin is covered with a highly hydrophilic, neutral polymer, to minimize non-specific interactions between the surface and the biopolymer. On the hydrophilic layer a controlled polymer chain is grafted to introduce the anion exchange functionality.

For the weak anion exchange column (Thermo Scientific™ ProPac™ WAX-10), the surface is grafted with a polymer chain bearing tertiary amine groups.

For the strong anion exchanger (Thermo Scientific™ ProPac™ SAX-10), the surface is grafted with a polymer chain bearing quaternary ammonium groups. Figure 1 below illustrates this surface structure.

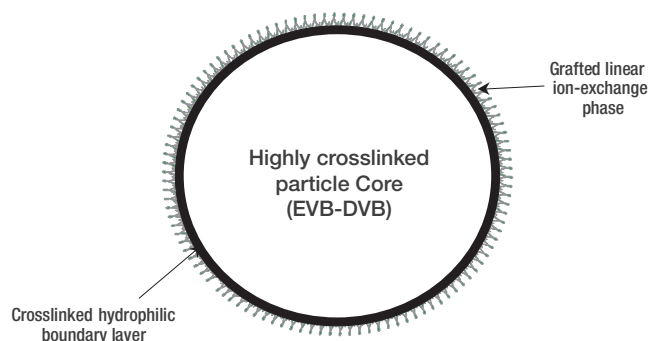


Figure 1. Schematic diagram of the ProPac phase for protein separations

Installation

The ProPac columns were designed to be used with a standard bore HPLC system having a gradient pump module, injection valve and a UV-Vis detector.

A metal-free pump system is recommended for halide-salt eluents which may cause corrosion of metallic components leading to decreased column performance from metal contamination. A metal-free pump is recommended to avoid denaturation of the protein samples. Use of stainless steel tubing, ferrule and bolt assemblies is not recommended because they may damage the threads of the PEEK end fittings.

System void volume

Tubing between the injection valve and detector should be <0.010" I.D. PEEK tubing. Minimize the length of all liquid lines, but especially the tubing between the column and the detector. The use of larger diameter and/or longer tubing may decrease peak efficiency and peak resolution for small I.D. columns.

Operational parameters

Description	Details
pH range	pH = 2-12
Temperature limit	60 °C
Pressure limit	3,000 psi
Organic solvent limit	80% acetonitrile or acetone if required for cleaning
Detergent compatibility	Nonionic, cationic or zwitterionic detergents. Do not use anionic detergents.
Typical eluents	Sodium, potassium salts of phosphate, chloride, or acetate
Flow rate (recommended)	2 × 250 mm: 0.25 mL 4 × 250 mm: 1.0 mL/min 4 × 150 mm: 1.0 mL/min 4 × 100 mm: 1.0 mL/min 9 × 250 mm: 5 mL/min 22 × 250 mm: Up to 30 mL/min
Dynamic capacity (suggested loading amount)	2 × 250 mm: 25 µg 4 × 50 mm: 20 µg 4 × 100 mm: 40 µg 4 × 150 mm: 60 µg 4 × 250 mm: 100 µg 9 × 250 mm: 500 µg 22 × 250 mm: 3,000 µg

Physical characteristics

Parameter	Recommendations
Substrate particle size	10 µm
Substrate pore size	Non-porous
Substrate monomers	Ethylvinylbenzene-divinylbenzene
Substrate cross-linking	55%
Mode of interaction	Anion exchange
Functional group	WAX-10 – Tertiary amine SAX-10 – Quaternary ammonium

Eluent limitations

The ProPac anion exchange columns are compatible with typical eluents such as sodium or potassium chloride or sulfate salts in Tris, phosphate or acetate buffers, up to their limit of solubility. Use of organic solvents in the eluent is usually unnecessary. If you decide to use one, test the solubility limit of eluents in the presence of the chosen organic solvents. Some combinations of eluent salts and organic solvents are not miscible.



Note

Anionic detergents will irreversibly bind to the column and their use should be avoided.

Installation (continued)

Chemical purity requirements

Obtaining reliable, consistent and accurate results requires eluents that are free of impurities. Chemicals, solvents and deionized water used to prepare eluents must be the highest purity available. Low trace impurities and low particle levels in eluents will extend the life of your ion exchange columns and system components. Thermo Fisher Scientific cannot guarantee proper column performance when the quality of the chemicals, solvents and water used to prepare eluents has been compromised.

Inorganic, organic chemicals

Reagent grade or better inorganic chemicals should always be used to prepare eluents. Whenever possible, inorganic chemicals that meet or surpass the latest American Chemical Society standard for purity should be used. These chemicals will detail the purity by having an actual lot analysis on each label.

When using solvents, HPLC Grade products or equivalent should be used to prepare eluents.

Deionized water

The deionized water (D.I.) used to prepare eluents should be Type I Reagent Grade Water with specific resistance of 18.2 megohm-cm. The deionized water should be free of ionized impurities, organics, microorganisms and particulate matter larger than 0.2 μm .

Eluent preparation

Adjusting the pH of the eluent

The eluent solution should contain all the electrolytes before adjusting the pH. To make sure that the pH reading is correct, the pH meter needs to be calibrated at least once a day. Stirring and temperature correction should be employed. Care should be taken to ensure the accuracy of the pH electrode for Tris buffers. Some electrodes will give erroneous results with Tris.

Filtering the eluent

To extend the lifetime of your column as well as your HPLC pump, all eluent buffers should be filtered using a 0.2 μm membrane filter to remove insoluble contaminants from the eluents.

Degassing the eluent

Before use, the eluents must be degassed. The degassing can be done using a vacuum pump. Vacuum degas the solvent by placing the eluent reservoir in a sonicator and drawing vacuum on the filled reservoir with a vacuum pump for 5-10 minutes while sonicating.

Operation

Sample preparation

The protein samples are best dissolved in the initial run buffer or in pure D.I. water. The concentration should be determined so the column is not overloaded by the injected sample. The loading capacity of the column is about 10-100 μg protein/column. The sample loop typically used for the 4 \times 250 mm column size is 10-100 μL . If the protein sample contains particulate contamination, the sample should be filtered through a 0.2 μm syringe filter.

Column equilibration

1. The WAX-10 is shipped in 20 mM Tris pH 8.0/0.1% sodium azide.
2. The SAX-10 is shipped in 10 mM Tris pH 8.5/0.1% sodium azide.

Before performing a run, equilibrate the column with the starting run buffer using approximately 10 times the column volume (i.e. 15 mL in the case of a 4 \times 250 mm column). After cleaning the column or when switching to a different buffer type, a longer equilibration time is recommended. Use an eluent volume of 10 times the column volume to ensure the column is well equilibrated.

Test chromatograms

Production test chromatogram – SAX-10 columns

Each column is individually tested to ensure the quality of the product. A tight set of tolerances surround the final test chromatogram to ensure low column to column variability for the protein applications the columns will undertake.

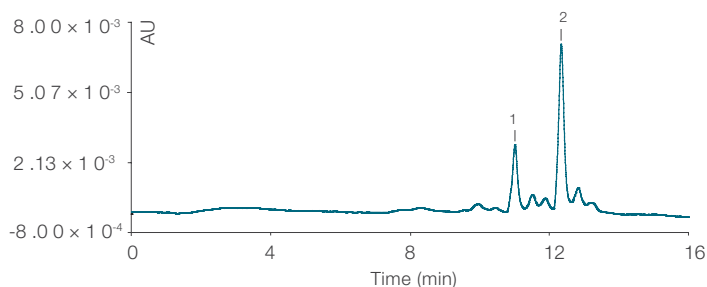


Figure 2. ProPac SAX-10 column (4 x 250 mm) test chromatogram

ProPac SAX-10 column, 4 x 250 mm		
Cat. no.	054997	
Mobile phase	A: 10 mM Tris pH 8.50 B: 10 mM Tris + 0.5 M NaCl pH 8.50	
Flow rate	1.0 mL/min	
Inj. volume	10 µL	
Detection	UV at 280 nm	
Storage	A: + 0.1% sodium azide	
Analytes	1. Ovalbumin 1 2. Ovalbumin 2	
Gradient	A:	B:
0.0	100	0
0.4	100	0
0.5	100	0
15.0	50	50
16.0	0	100
17.0	100	0
25.0	100	0

Production test chromatogram – ProPac WAX-10 column

Each column is individually tested to ensure the quality of the product. A tight set of tolerances surround the final test chromatogram to ensure low column to column variability for the protein applications the columns will undertake.

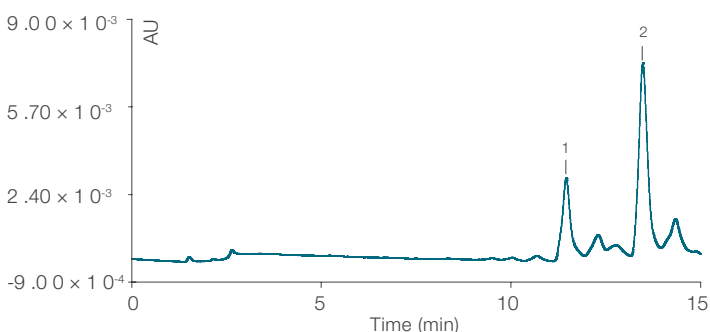


Figure 3. ProPac WAX-10 column (4 x 250 mm) test chromatogram

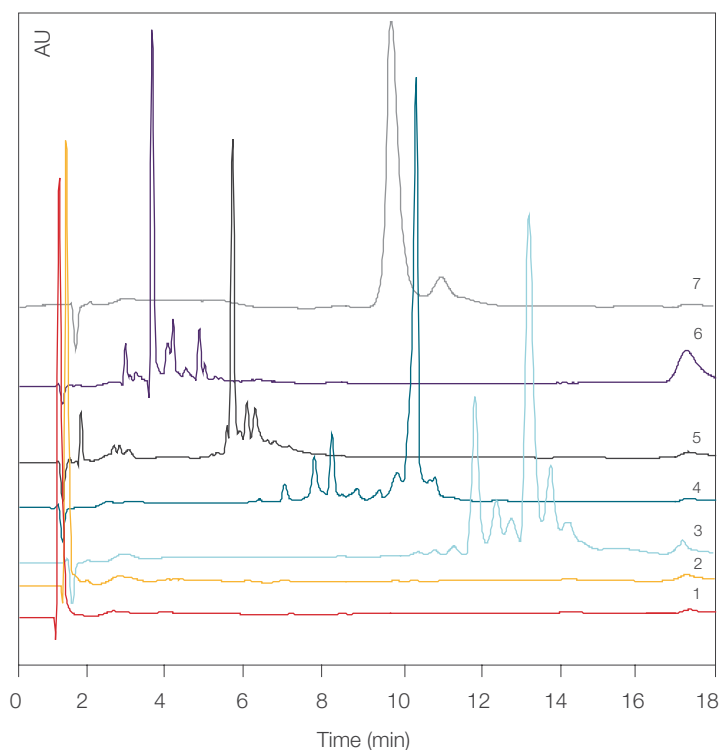
ProPac WAX-10 column, (4 x 250 mm)		
Cat. no.	054999	
Mobile phase	A: 20 mM Tris pH 8.00 20 mM Tris + 0.5 M NaCl pH 8.00	
Flow rate	1.0 mL/min	
Inj. volume	10 µL	
Detection	UV at 280 nm	
Storage	A: + 0.1% sodium azide	
Analytes	1. Ovalbumin 1 2. Ovalbumin 2	
Gradient	A:	B:
0.0	100	0
0.4	100	0
0.5	100	0
15.0	50	50
16.0	0	100
17.0	100	0
25.0	100	0

Example applications

Elution profiles on a ProPac SAX-10 anion exchange column

A series of proteins were chromatographed to give a general impression of the capability of the ProPac anion exchange column. Elution profiles for a couple of basic proteins, lysozyme and cytochrome c, are shown to demonstrate that the surface of the column possesses only an anion exchange characteristic and that residual cation exchange sites are absent, as evidenced by the lack of retention for basic proteins. Trypsin inhibitor is also shown as it has been reported that it is not always possible to resolve all three inhibitors in anion exchange.

Ovalbumin has been noted to have two possible phosphorylation sites could result in a series of closely related variants. In the literature it has been shown that creatine kinase has four closely related forms which have pI values which differ by about 0.1 pH units. Elution profiles for transferrin are shown to demonstrate the selectivity the column demonstrates towards variations in protein sialylation. BSA is also known to exist in solution with a small percentage in the dimerized form.



ProPac SAX-10 column, 4 x 250 mm	
Cat. no.	054997
Mobile phase	A: Water B: Water C: 0.2 M Tris/HCl, pH 8.5
Flow rate	1.0 mL/min
Inj. volume	50 µL (1 mg/mL)
Detection	214 nm
Analytes	1. Lysozyme (B) 2. Cytochrome c, bovine (B) 3. Ovalbumin (B) 4. Trypsin inhibitor, soy (A) 5. Creatine kinase, rabbit (B) 6. Carbonic anhydrase (A) 7. BSA (A)
Gradient	A: 0-0.5 M NaCl in 15 min B: 0-0.25 M NaCl in 15 min 20 mM Tris/HCl throughout

Figure 4. Elution profiles on a ProPac SAX-10 column

Example applications (continued)

Effect of acetonitrile and temperature on the elution profiles of ovalbumin

In this evaluation it was demonstrated that the column exhibited minimal kinetic resistances and that no appreciable secondary hydrophobic interactions were observed for the separation of ovalbumin.

By increasing the temperature at which the chromatography is conducted the rates associated with diffusion and the kinetics of binding are increased. As no significant change is observed in

the elution profiles as function of temperature it can be inferred that such effects do not significantly affect the performance of the column at room temperature. Likewise, for hydrophobic interactions the similarity of the elution profiles of the proteins with and without acetonitrile, which will reduce any hydrophobic interaction between the protein and the stationary phase, implies that hydrophobic interactions are essentially absent.

ProPac SAX-10 column, 4 x 250 mm	
Cat. no.	054997
Mobile phase	A: Water B: Water, 20% v/v ACN C: 2.0 M NaCl D: 0.2 M Tris/HCl (pH 8.5)
Flow rate	1.0 mL/min
Inj. volume	50 µL (1 mg/mL)
Detection	214 nm
Sample	Ovalbumin
Gradient	0-0.25 M NaCl in 15 min

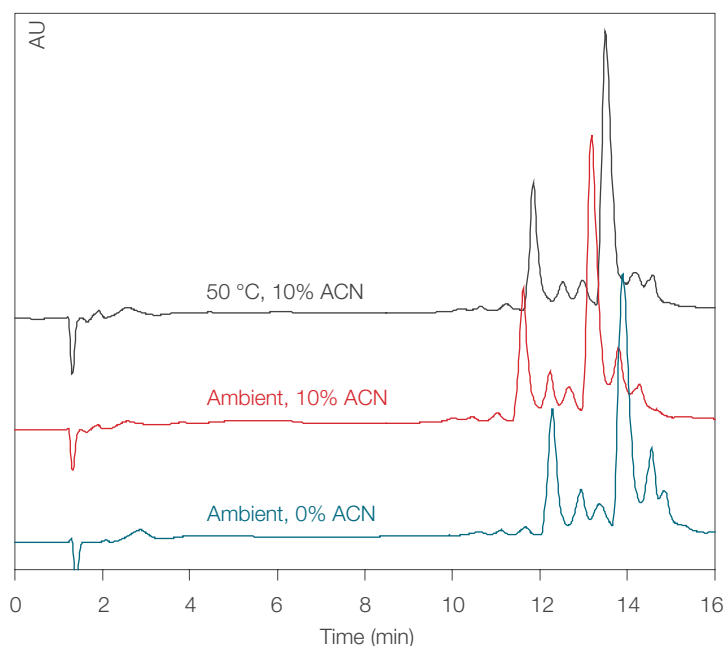


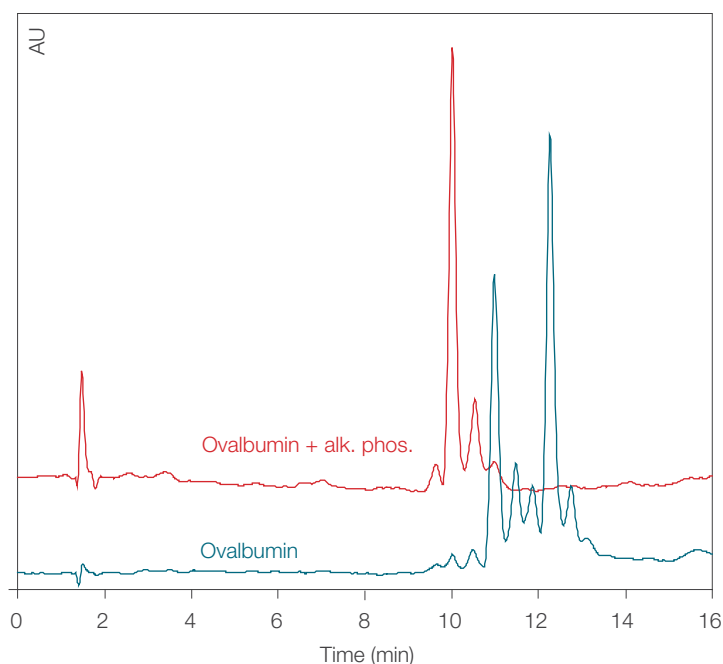
Figure 5. Effect of acetonitrile and temperature on the elution profiles of ovalbumin

Example applications (continued)

Effect of alkaline phosphatase on ovalbumin elution profiles on an anion exchange analytical column

Resolution of phosphorylation variants is important in the characterization of bio-macromolecules, bio-macromolecules.⁴ We resolved several phosphorylation isoforms of ovalbumin using a simple linear gradient on the ProPac strong anion exchange column. It is seen that eight peaks are visible in the ovalbumin chromatogram profile. Upon alkaline phosphatase digestion of

ovalbumin to remove phosphate from the protein, the ovalbumin profile simplifies from eight peaks to one major and three minor peaks. The modification(s) responsible for the three minor peaks has not been identified.



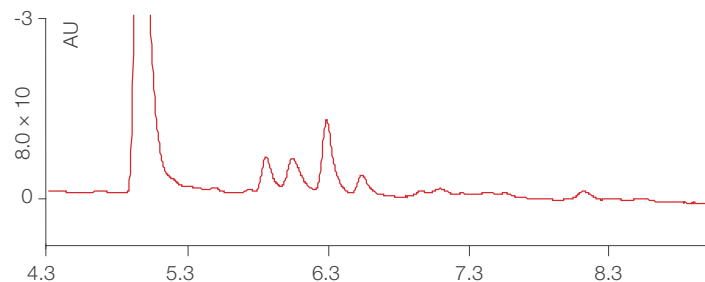
ProPac SAX-10 column, 4 × 250 mm	
Cat. no.	054997
Mobile phase	A: Water B: Water C: 2.0 M NaCl D: 0.2 M Tris/HCl (pH 8.5)
Flow rate	1.0 mL/min
Inj. volume	30 µg (1 mg/mL)
Detection	214 nm
Samples	Ovalbumin before and after treatment with alkaline phosphatase treatment 20 mM Tris/HCl; 0-25 min 0.0-0.25 M NaCl; 0-15 min
Gradient	0.5 M NaCl; 17-19 min 0.0 M NaCl; 17-25 min

Figure 6. Effect of alkaline phosphatase on ovalbumin elution profiles on a strong anion exchange analytical column

Example applications (continued)

Selectivity comparison of anion exchange columns

ProPac SAX-10 and ProPac WAX-10 columns have high selectivity for proteins. These columns can even separate the proteins with minor components, one charge difference and minor structure variations. One example shown here is the separation of carbonic anhydrase from the minor components.



ProPac SAX-10 column, 4 x 250 mm

Cat. no. [054997](#)

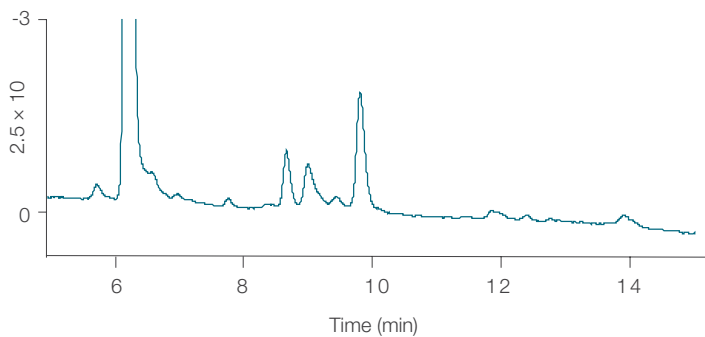
Mobile phase 10 mM Tris (pH 8.5)
0.0-0.15 M NaCl; 0-15 min

Flow rate 1.0 mL/min

Inj. volume 10 μ L

Detection 214 nm

Samples Carbonic anhydrase



ProPac WAX-10 column, 4 x 250 mm

Cat. no. [054999](#)

Mobile phase 10 mM Tris (pH 8.0)
0.0-0.1 M NaCl; 0-30 min

Flow rate 1.0 mL/min

Inj. volume 10 μ L

Detection 214 nm

Samples Carbonic anhydrase

Figure 7. High selectivity of anion exchange column

Example applications (continued)

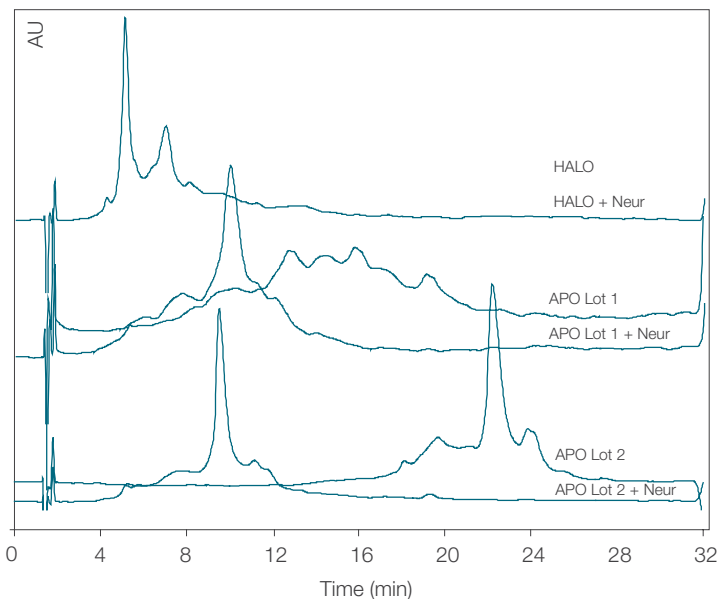
Effect of sialylation on transferrin chromatography

Transferrins are a group of metal-binding glycoproteins, which function in the transport of iron in cells. Human transferrin has two iron binding sites and has a molecular mass of ~ 75,000 daltons. It has two N-linked glycosylation sites (Asn413 and Asn611) which are occupied by bi-, tri- or tetra-antennary N-acetyllactosamine oligosaccharides.¹

Recent data suggests that different isoform profiles of transferrin are diagnostic of different clinical conditions and may be clinically significant. For example it is known that pregnant women in their last trimester have transferrin with increased oligosaccharide

branching and increased sialylation. Alternatively, alcoholics exhibit decreased sialylation of transferrin, an alteration in their isoform profile, which is reversible with abstinence.²

In this application we demonstrate that elution profiles of different transferrins result from differences in the sialylation of the protein.³ Three transferrin samples, one iron rich (Holo) and two from different iron poor (Apo) manufacturer lots, exhibited unique isoform profiles by anion exchange on the ProPac column. When the different transferrin samples are digested with neuraminidase to remove sialic acid, the profiles collapse into a similar pattern.



ProPac SAX-10 column, 4 × 250 mm	
Cat. no.	054997
Mobile phase	A: Water B: Water C: 2.0 M NaCl D: 0.2 M Tris/HCl (pH 9)
Flow rate	1.0 mL/min
Inj. volume	50 µg (1 mg/mL)
Detection	214 nm
Samples	HALO (iron rich) and APO (iron poor) human transferrin samples before and after Neuraminidase treatment. Digestions were made overnight at 37 °C in sodium acetate buffer at pH 5
Gradient	20 mM Tris/HCl; 0-30 min 0.008-0.14 M NaCl; 0-30 min 0.5 M NaCl; 17-19 min 0.0 M NaCl; 17-25 min

Figure 8. Effect of sialylation on transferrin chromatography

Example applications (continued)

Profiling dairy milk caseins

Cow's milk consists of 3-3½% proteins, 80% of which are caseins. Caseins are acidic proteins that are insoluble at their iso-electric point, pH 4.6, and exist in nature in solution as micelles. The other 20% of cow's milk proteins largely consists of serum proteins; that include β -lactoglobulin A and B, α -lactalbumin, serum albumin and the immunoglobulins.⁵

In the dairy industry, cow's milk protein profiling is used to assess adulteration and the effects of processing. It is known that cows milk protein profiling is dependent on the species of animal as

well as on the stage of lactation and the nutritional status of the animal.⁶ Hence, high resolution chromatographic separations of milk proteins is useful in the regulatory monitoring of milk based products.

In this application a high resolution separation is shown for a sample of bovine caseins, including α , β and κ caseins. The disruption of the micelles was achieved by dissolving the milk proteins, and running the chromatography with solvents containing urea and 2-mercaptoethanol.

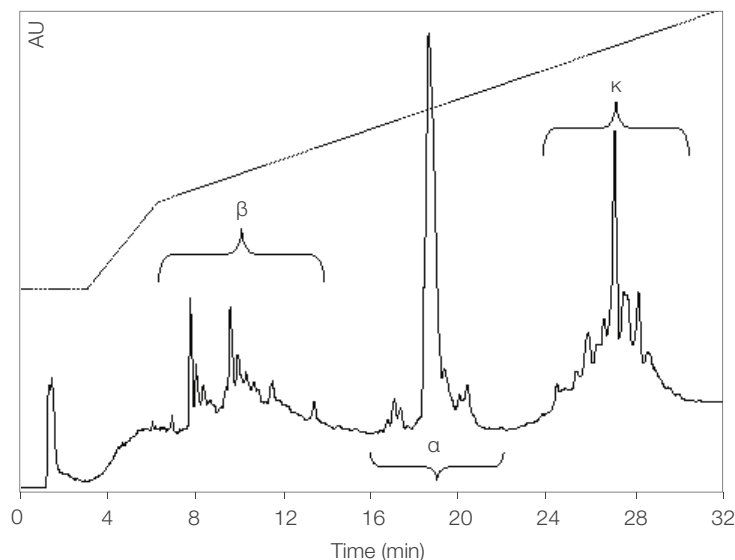


Figure 9. Profiling dairy milk caseins

ProPac SAX-10 column, 4 x 250 mm	
Cat. no.	054997
Mobile phase	A: 4 M urea, 0.01 M 2-mercaptoethanol, 0.01 M HEPES, pH 7.3 B: 1.0 M NaCl, 4 M urea, 0.01 M 2-mercaptoethanol, 0.01 M HEPES, pH 7.3
Flow rate	1.0 mL/min
Inj. volume	50 μ g (1 mg/mL)
Detection	280 nm
Gradient	3 min %B = 10 30 min %B = 35

Troubleshooting guide

Finding the source of high system back pressure

1. A significant increase in the system back pressure may be caused by a plugged inlet frit (bed support) or from the instrument.
2. Check for pinched tubing or obstructed fittings from the pump outlet, throughout the eluent flow path to the detector cell outlet. To do this, disconnect the eluent line at the pump outlet and observe the back pressure at the usual flow rate. It should not exceed 50 psi (0.3 MPa). Continue adding components (injection valve, column, detector) one by one while monitoring the system back pressure. The 4 × 250 mm ProPac WAX-10 and SAX-10 columns should add no more than 1,500 psi back pressure at 1 mL/min. The 4 × 50 mm ProPac WAX-10 and SAX-10 columns should add no more than 400 psi (2.6 MPa) back pressure at 1 mL/min. No other component should add more than 100 psi (0.7 mPa) to the system back pressure.
3. If the high back pressure is due to the column, try cleaning (washing) the column. If the high back pressure persists, replace the column bed support at the inlet of the column.

Column performance is deteriorated

Peak efficiency and resolution is decreasing, loss of efficiency

1. If changes to the system plumbing have been made, check for excess lengths of tubing, tubing diameters larger than 0.010 in I.D.
2. Check the flow rate and the gradient profile to make sure your gradient pump is working correctly.
3. The column may be fouled. Clean the column using the recommended cleaning conditions in the “Column care” section.
4. If there seems to be a permanent loss of efficiency check to see if headspace has developed in the column. This is usually due to improper use of the column such as submitting it to high backpressure. If the resin doesn't fill the column body all the way to the top, the resin bed has collapsed, creating a headspace. The column must be replaced.
5. If the peak shape looks good, but the efficiency number is low, check and optimize the integration parameters. If necessary, correct the integration manually, so the start-, maximum- and end of the peak are correctly identified.

Unidentified peaks appear as well as the expected analyte peaks

1. The sample may be degrading. Proteins tend to degrade faster in solutions; therefore, store your protein samples appropriately, and prepare only a small amount of solution/mixture for analysis.
2. The eluent may be contaminated. Prepare fresh, filtered eluent. The presence of unidentified peaks on a chromatographic column can result from a myriad of causes. However, in the case of the anion exchange columns a unique source of these peak has been identified. As Tris-type buffer solutions age it has been observed that extra, spurious peak can be seen on the chromatogram, mainly during the low ionic strength portion of the gradient. It is easily possible to minimize the deleterious effects of this by making up the buffer solution regularly, by equilibrating the column and by starting the gradient at 15-20 mM of the eluting salt e.g. NaCl. This small amount of NaCl is enough to prevent the accumulation of the buffer “degradation byproduct” on the column and to permit a clear blank chromatogram to be observed.
3. Run a blank gradient to determine if the column is contaminated. If ghost peaks appear, clean the column.

Column care

New column equilibration

The columns are shipped in 10 mM Tris pH = 8.0 buffer containing 0.1% sodium azide. Before use, wash the column with approximately 20 mL of the starting eluent (20 min at 1 mL/min).

Column clean-up



Note

When cleaning an analytical and guard column in series, move the guard column after the analytical column in the eluent flow path. Otherwise contaminants that have accumulated on the guard column will be eluted onto the analytical column.

Cleanup solution

150 potassium nitrate in 80% acetonitrile, pH 2.0 (adjust pH with HCl).

Column cleanup procedure

1. Rinse the column for 15 minutes with 10 mM Tris pH 8.0 before pumping the clean-up solution over the column
2. Prepare 500 mL cleanup solution.
3. Set the pump flow rate to 1 mL/min for the 4 mm I.D. columns, 0.25 mL/min for the 2 mm I.D. columns, or 5.0 mL/min for the 9 mm I.D. columns.
4. Pump the cleanup solution through the column for 60 minutes.
5. Equilibrate the column(s) with starting eluent for at least 30 minutes before resuming normal operation.
6. Place the guard column back in-line before the analytical column if the system was originally configured with a guard column.

Column storage

Short term storage

For short term storage, use the low salt concentration eluent (pH 3-10) as the column storage solution.

Long term storage

1. For long term storage, use 20 mM Tris pH = 8.0 eluent with 0.1% sodium azide added to avoid bacteria growth on the column.
2. Alternatively, use 20 mM Tris pH = 8.0 buffer in 80:20 water/acetonitrile.
3. Flush the column with at least 10 mL of the storage eluent. Cap both ends, securely, using the plugs supplied with the column.

Replacing column bed support assemblies



Note

Replace the inlet bed support only if the column is determined to be the cause of high system back pressure, and cleaning of the column does not solve the problem.

1. Carefully unscrew the inlet (top) column fitting. Use two open end wrenches.
2. Remove the bed support. Tap the end fitting against a hard, flat surface to remove the bed support and seal assembly. Do not scratch the wall or threads of the end fitting. Discard the old bed support assembly.
3. Removal of the bed support may permit a small amount of resin to extrude from the column. Carefully remove this with a flat surface such as a razor blade. Make sure the end of the column is clean and free of any particulate matter. Any resin on the end of the column tube will prevent a proper seal. Insert a new bed support assembly into the end fitting and carefully thread the end fitting and bed support assembly onto the supported column.
4. Tighten the end fitting fingertight, then an additional ¼ turn (25 in x lb.). Tighten further only if leaks are observed.



Caution

If the end of the column tube is not clean when inserted into the end fitting, particulate matter may prevent a proper seal between the end of the column tube and the bed support assembly. If this is the case, additional tightening may not seal the column but instead damage the column tube or break the end fitting.

Reference

1. Coddeville, B. et al. *Glycoconjugate Journal*, (1998), 15, 265-273.
2. Stibler, H., S. Borg and C. Allgulander. *Acta Med Scand*, (1979), 206, 275-281.
3. Rohrer, J. S., and N. Avdalovic. *Protein Expression and Purification*, (1996), 7,39-44.
4. Frenz, J., C. P.Quan, J. Cacia. C. Democko, R. Bridenbaugh and T. McNerney. *Anal.Chem.*, (1994), 66, 335-340.
5. Nollet, L. "Food Analysis by HPLC," Marcel Dekker, 1992.
6. Davies, D. T., and A. J. R. Law. *Journal of Dairy Research*, (1980), 47, 83-90.

Ordering information

ProPac WAX-10 and ProPac SAX-10 columns

Description	Particle size	Dimensions	Cat. no
ProPac WAX-10 columns			
ProPac WAX-10 analytical columns	10 µm	22 × 250 mm	088771
	10 µm	9 × 250 mm	063707
	10 µm	4 × 250 mm	054999
	10 µm	2 × 250 mm	063464
ProPac WAX-10G guard columns	10 µm	4 × 50 mm	055150
	10 µm	2 × 50 mm	063470
ProPac SAX-10 columns			
ProPac SAX-10 analytical column	10 µm	22 × 250 mm	088770
	10 µm	9 × 250 mm	063703
	10 µm	4 × 250 mm	054997
	10 µm	4 × 50 mm	078990
	10 µm	2 × 250 mm	063448
ProPac SAX-10 analytical column, 3 lots × 1 column	10 µm	4 × 250 mm	088775
ProPac SAX-10G guard columns	10 µm	4 × 50 mm	054998
	10 µm	2 × 50 mm	063454

Learn more at [thermofisher.com/biolc](https://www.thermofisher.com/biolc)