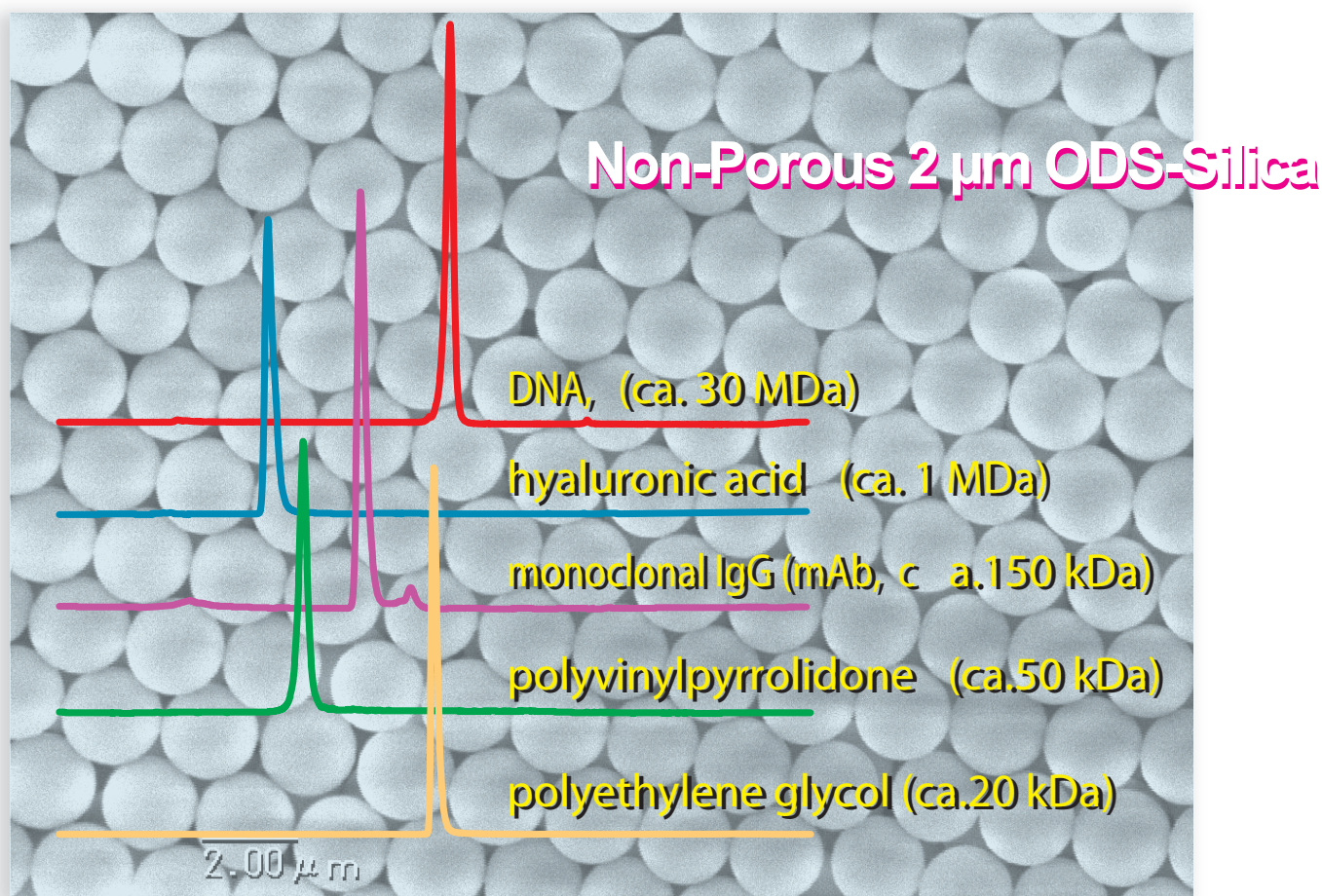


High Resolution 2 μm Non-Porous ODS Column

Reversed-phase separation for bio- and synthetic-polymers up to 30 MDa
 Amazing number of peaks for peptides and proteins
 Different selectivity from porous ODS columns
 250mm length high-resolution column with 2 μm particle
 High-efficiency with low flow rate compatible with conventional HPLC systems

Non-Porous Spherical Silica / 2 μm / ODS

Changing polymer separation history



Imtakt has developed a novel 2 μm non-porous high resolution ODS column.

There are several shortcomings for porous ODS columns for polymer separation:

- Poor peak shape of solutes due to wide range in pore size distribution
- Poor recovery of solutes due to micro-pores and meso-pores
- Reduced column efficiency due to high mass transfer resistance

Presto FF-C18 can overcome these shortcomings for polymer separation. This high resolution, non-porous ODS column is quite different from conventional ODS columns, and will create new opportunities for 21st century separation science.

Peptides

Presto FF- C18 performs exceptionally well with amino acid residue recognition for peptides and protein separations. The number of peaks for Presto FF- C18 is twice that of porous ODS - and shows improved recovery for peptides and proteins due to lack of micro- pores. The 250mm length Presto FF- C18 (2um particle) column will be an important tool for high resolution peptide and protein separations.

● Peptide Mapping

A: water /TFA = 100 /0.1
 B: acetonitrile /TFA = 100 /0.1
 50deg.C, 220nm
 Tryptic digest of alpha-casein
 5uL

250 x 4.6 mm

268 peaks

Presto FF-C18 (2um)
 1-35%B (0-150min)
 0.4mL/min (25MPa)

131 peaks

Porous ODS (3um)
 Cadenza CD-C18
 5-45%B (0-150min)
 1mL/min (14MPa)

0 50 100 150 min

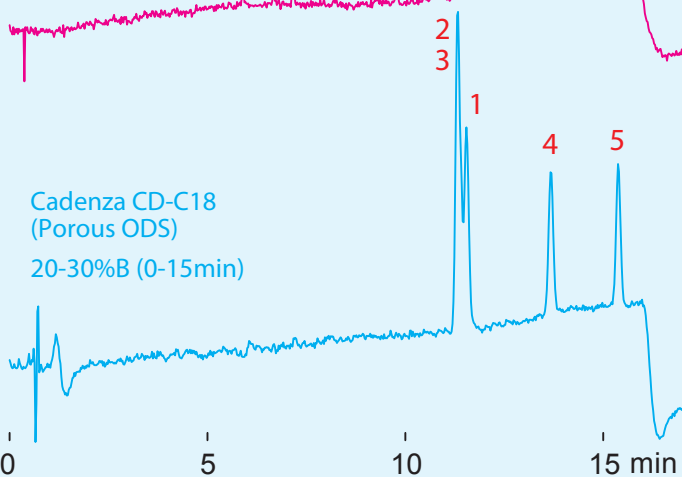
Presto FF-C18 enables unbelievable separation power for peptide mapping. Accurate trace analysis for peptides is very important for proteome analysis, but can be compromised with porous ODS column - due to adsorption in micro-pores.

Presto FF-C18 offers twice the number of peaks and improved recovery over porous ODS columns, and will change the proteomics world dramatically.

● Different Selectivity for Peptide Separation

50 x 4.6 mm
 A: water /TFA = 100 /0.1
 B: acetonitrile /TFA = 100 /0.1
 1mL/min, 37deg.C, 220nm

Presto FF-C18
 (Non-porous ODS)
 15-30%B (0-15min)



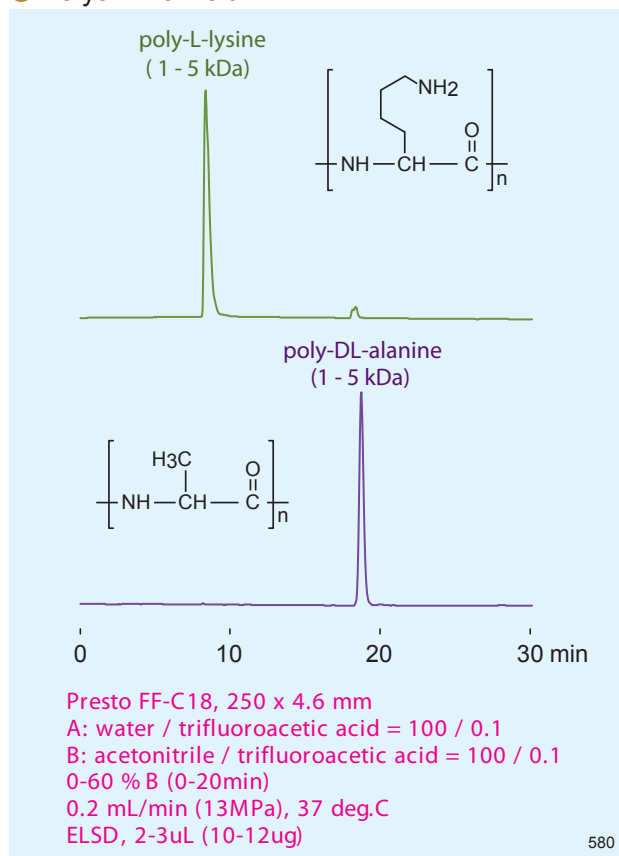
- 1 Angiotensin IV (Human)
Val-Tyr-Ile-His-Pro-Phe
- 2 Angiotensin III (Human)
Arg-Val-Tyr-Ile-His-Pro-Phe
- 3 Angiotensin II (Human)
Asp-Arg-Val-Tyr-Ile-His-Pro-Phe
- 4 [Val⁵]-Angiotensin I (Bovine)
Asp-Arg-Val-Tyr-Val-His-Pro-Phe-His-Ile
- 5 Angiotensin I (Human)
Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Ile

The surface area for non-porous ODS is extremely low compared to porous ODS. In order to obtain similar retention as porous ODS, the organic composition should be decreased when using non-porous ODS. This can be advantageous as the difference in eluent composition can contribute to different elution profile (as can be seen with the separation of angiotensin I, II and III).

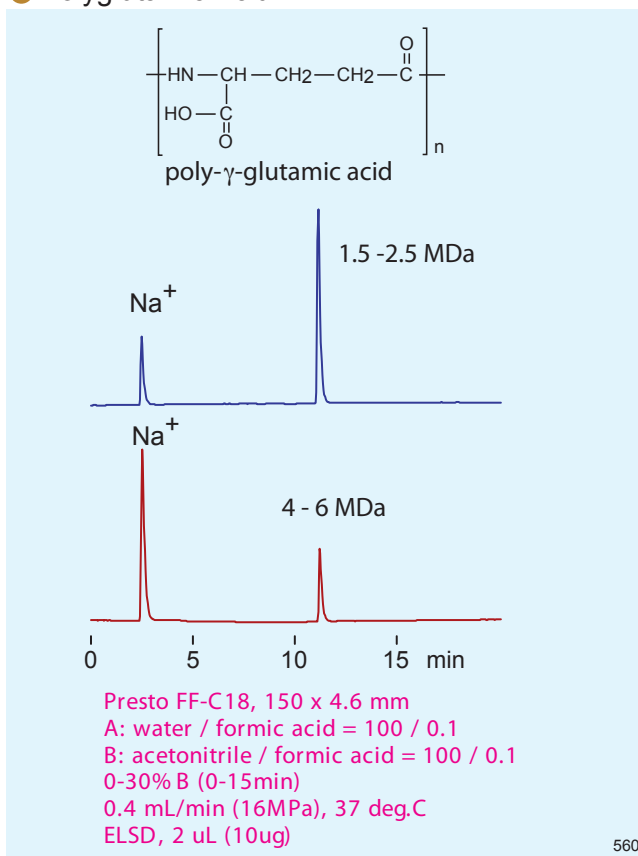
Polypeptides and Proteins

Presto FF- C18 excels at (different) peptide bond structure recognition. It is useful for a wide range of molecular weight separations - from small peptides to large proteins.

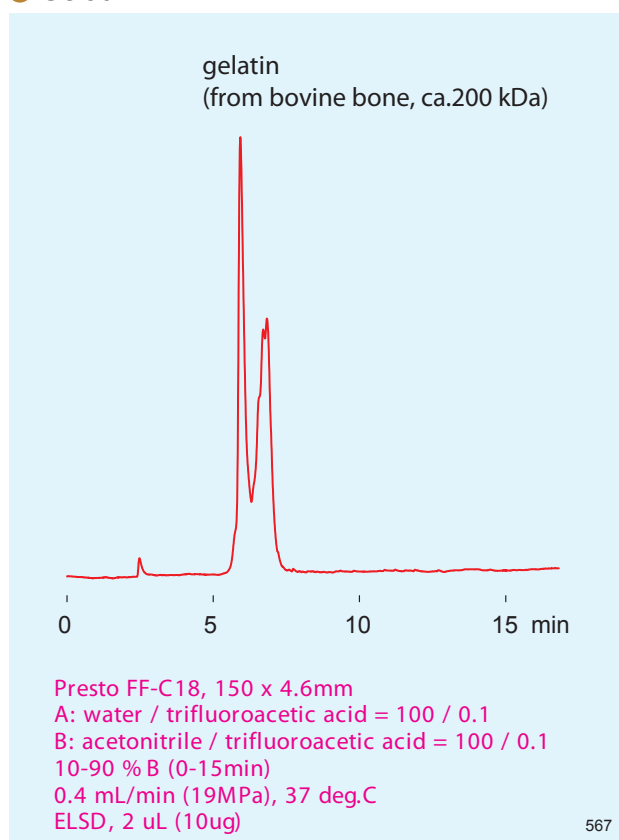
● Polyamino Acid



● Polyglutamic Acid



● Gelatin



Polyamino acids are polymers made up of repeating units of amino acids (shown are homo polyamino acids for L-lysine and DL-alanine). The two homopolymers provide very different retention times. Although the polyamino acids have a molecular weight distribution, the data shows only one peak for each polymer.

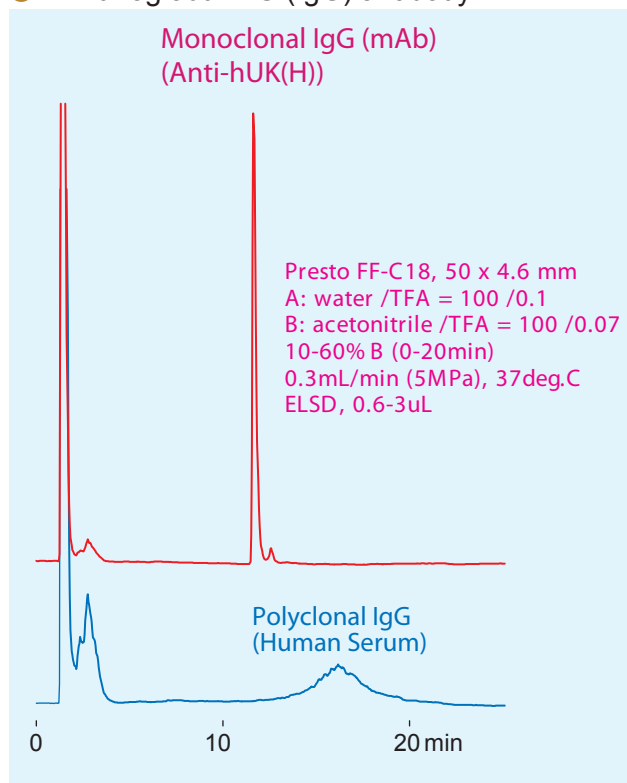
Polyglutamic acid (MW equals several MDa) has a wide range molecular weight distribution. However, polymers of similar molecular weight co-elute to form one peak. The reason for the poor recognition of chain length is as follows: the size of the polypeptide has reached a critical mass where the contact area to stationary phase is effectively the same for all polymers. The molecular interaction between solute and stationary phase is very similar, regardless of molecular weight of polymers - making it difficult to differentiate between the structures.

In contrast, proteins consist of a multitude of different amino acid residues. As a result, they are well differentiated on Presto FF-C18 (regardless of the molecular weight). Gelatin consists of molecular weight distribution - and can be separated by Presto FF-C18.

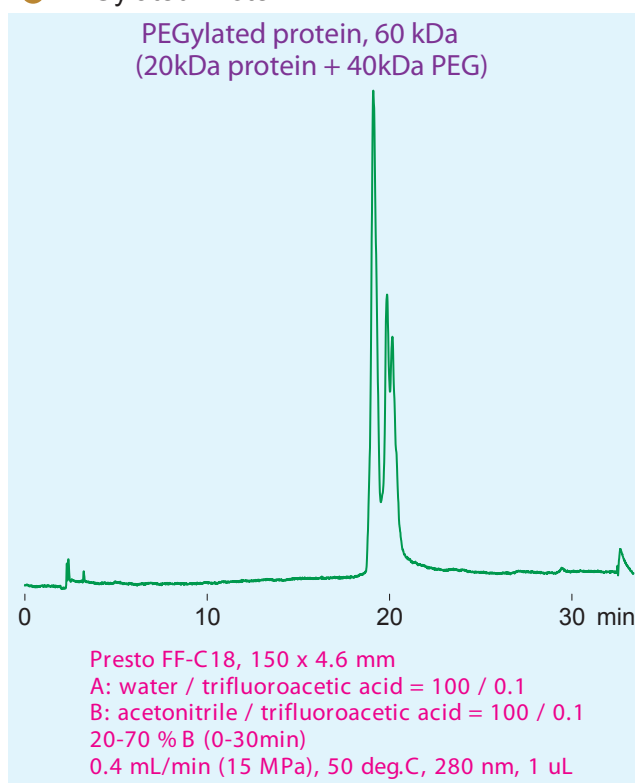
Medical Related Proteins

Presto FF- C18 also offers excellent results for medical related proteins. The more traditional modes of separation for proteins, IEX or SEC, can be replaced with reversed- phase using non- porous ODS.

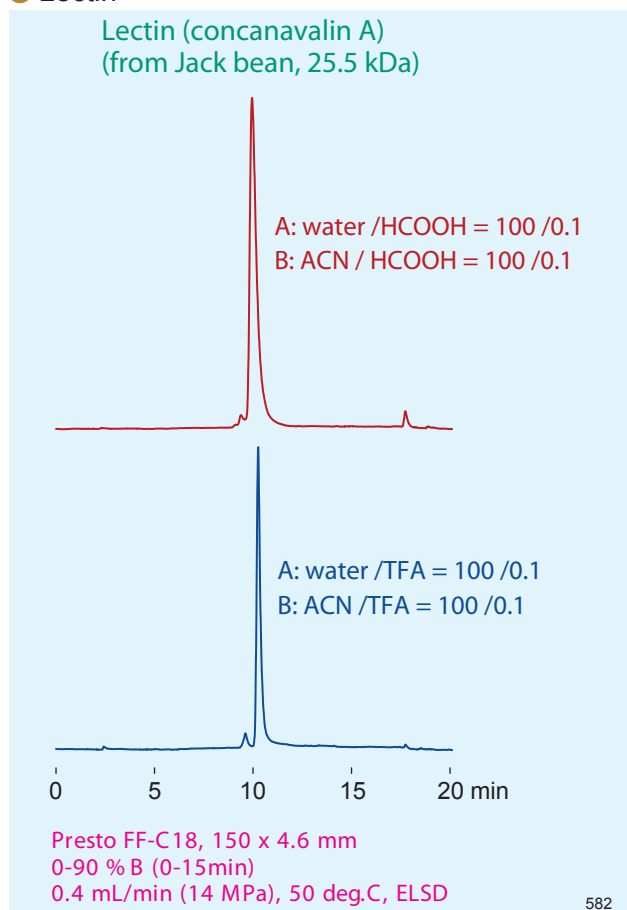
● Immunoglobulin G (IgG) antibody



● PEGylated Protein



● Lectin



582

The Immunoglobulin G (IgG,) antibody, which consists of a large quaternary structure (MW ca. 150kDa), can exhibit poor peak shape when injected onto porous RP columns (due to pore size distribution and mass transfer resistance). Presto FF-C18 can provide improved peak shape and recognition between mAb (same amino acid sequence) and polyclonal antibody (different amino acid sequence).

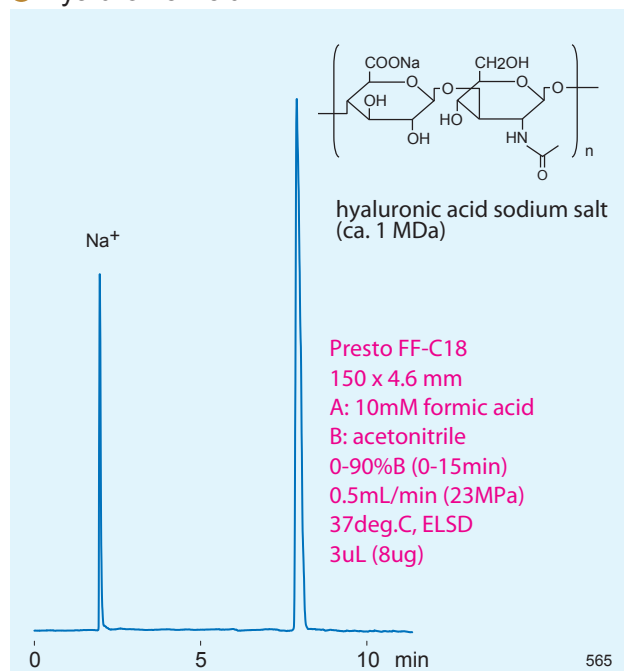
The PEGylation of proteins offers many advantages for drug and protein therapeutics. The above data shows several peaks due to various PEG oligomers and / or different yields of PEGylation. Presto FF-C18 will be useful for purity check, structural analysis, and QC production regarding almost any protein.

Lectin, a protein which binds to specific sugars, is important for medical purposes. The Concanavalin A protein elutes easily using formic acid / acetonitrile gradient.

Polysaccharides (Ionic)

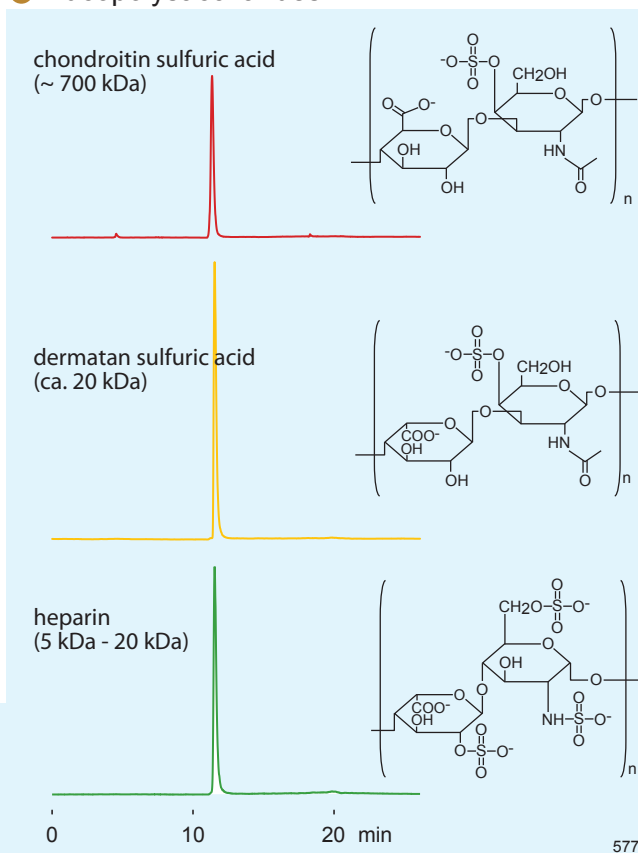
Presto FF-C18 (non-porous ODS) can be useful for polysaccharide separations. In comparison to SEC columns, Presto FF-C18 can achieve sharper peaks using gradient elution, making RP the preferred mode for quantitative analysis.

● Hyaluronic Acid

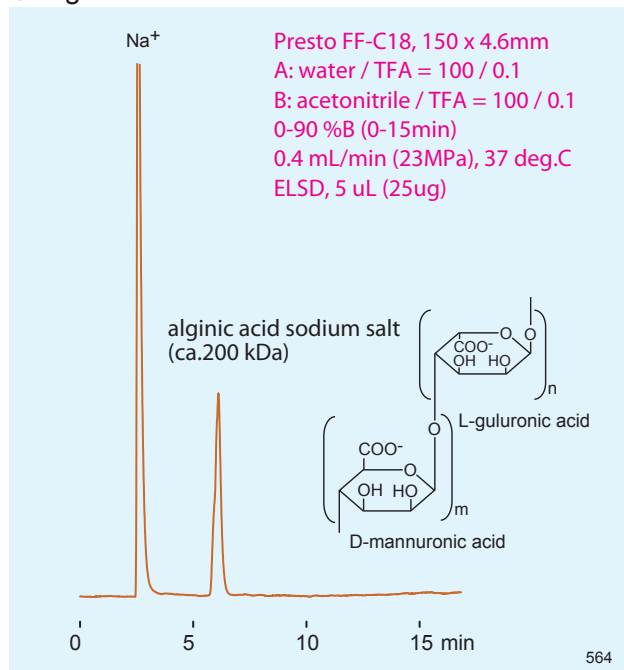


Presto FF-C18, 250 x 4.6 mm
 A: water /triethylamine /acetic acid = 100 /1.1 /0.5
 B: acetonitrile
 0-40% B (0-15min)
 0.35 mL/min (24 MPa), 37 deg.C, ELSD, 2 uL (10 ug)

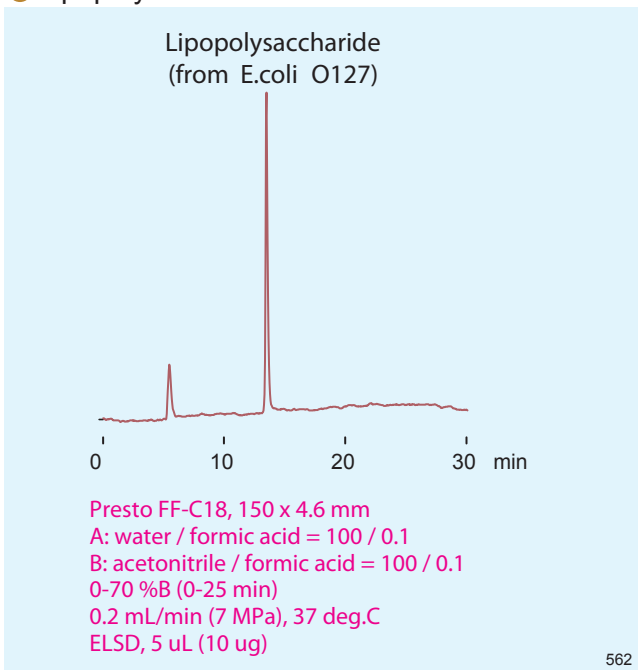
● Mucopolysaccharides



● Alginic Acid



● Lipopolysaccharide

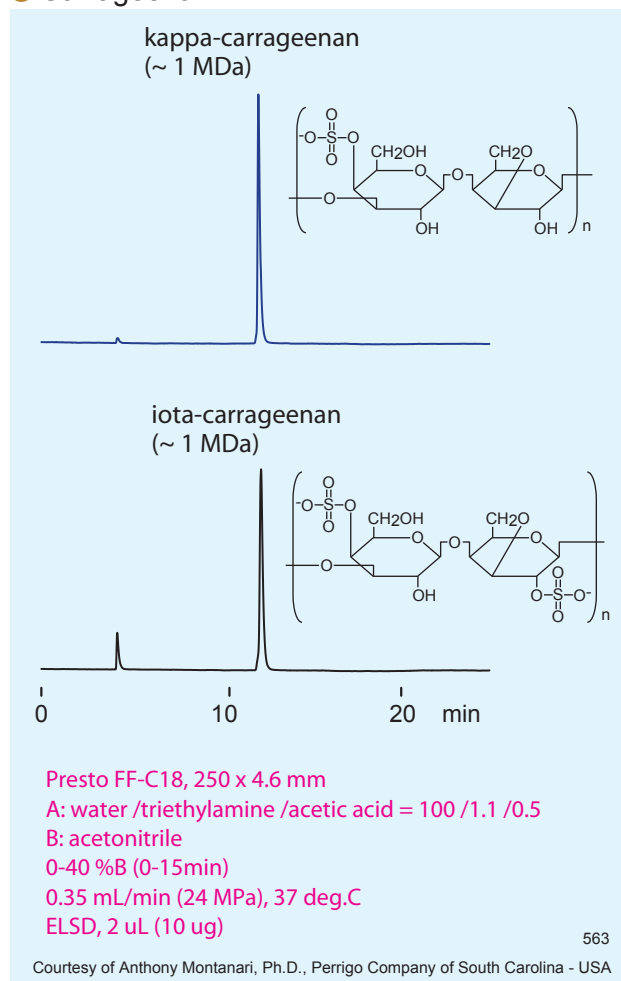


pH modifiers are required when ionic polysaccharides are injected on to Presto FF-C18. Hyaluronic acid, a mucopolysaccharide, elutes with formic acid (even though it contains a carboxyl group). In contrast, chondroitin, dermatan, and heparin contain sulfur group(s) and require triethylamine acetate (ion-pairing modifier) under neutral pH conditions. Retention behavior for these three polymers is similar due to similar structure and molecular weight. Trifluoroacetic acid (TFA), which was used for alginic acid, can also be useful for ionic polysaccharide separations.

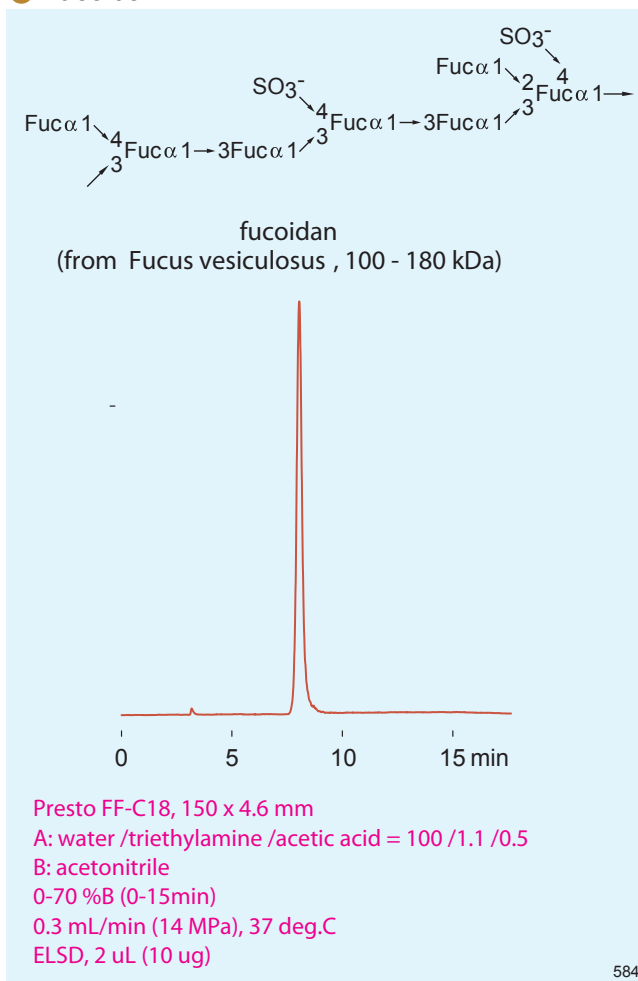
Polysaccharides (Ionic)

Presto FF- C18 is useful for polysaccharide analysis.

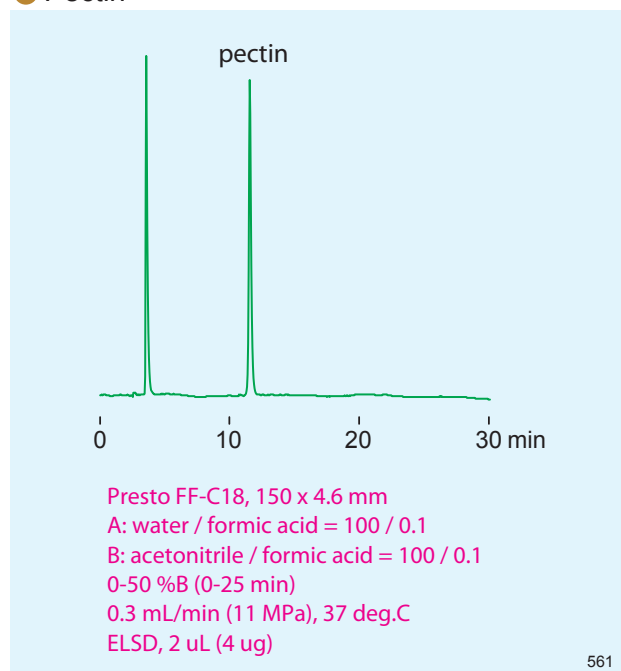
● Carrageenan



● Fucoidan



● Pectin



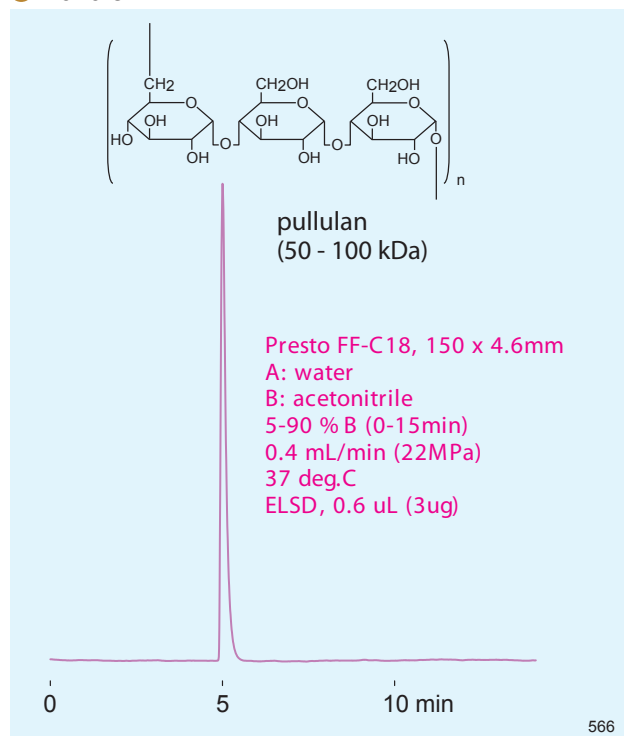
A pH modifier is required to analyze ionic polysaccharides. Triethylamine acetate in particular is effective for polysaccharides that contain sulfur groups. The two carrageenan (κ -, ι -) shown here have a different number of sulfur groups. However, retention (and peak shape) is similar for both polysaccharides due to their similar structures and molecular weights. Fucoidan contains sulfur groups and results in excellent peak shape when triethylamine acetate pH modifier is used.

Pectin is comprised of repeating units of galacturonic acid. Although a majority of these units are esterified - a percentage of the units are not. Formic acid was found to be a useful pH modifier for this application.

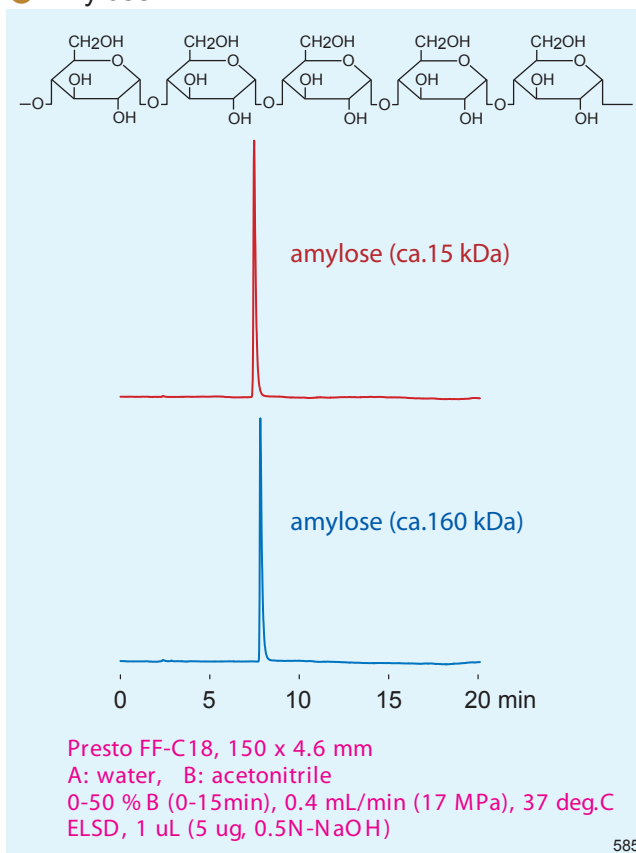
Non-Ionic Polysaccharides

Presto FF- C18 separates polysaccharides and does so with sharper peaks and lower cost than SEC columns.

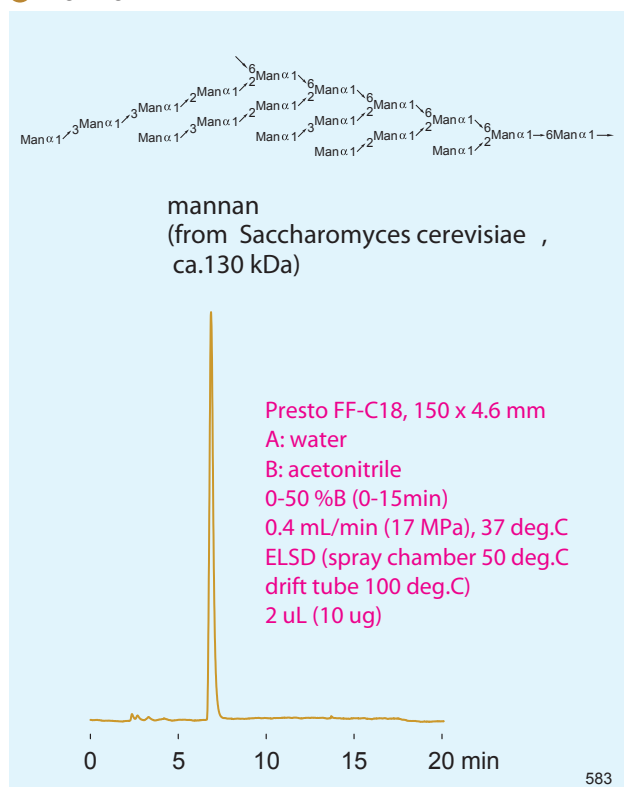
● Pullulan



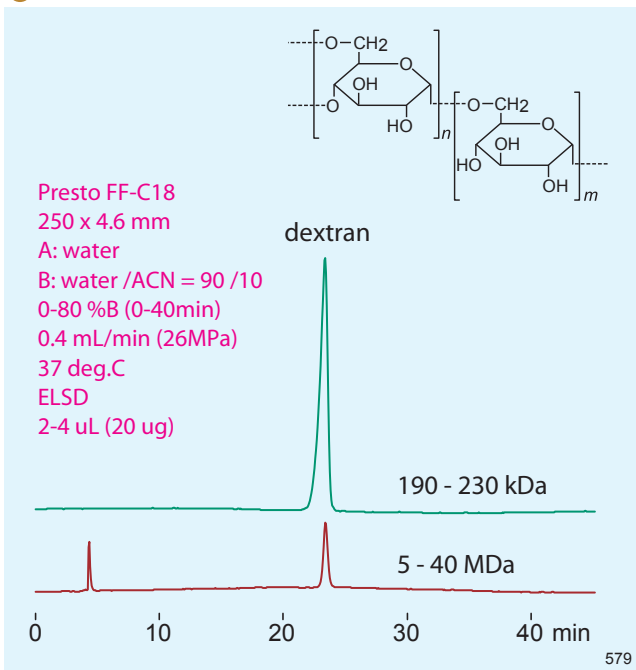
● Amylose



● Mannan



● Dextran

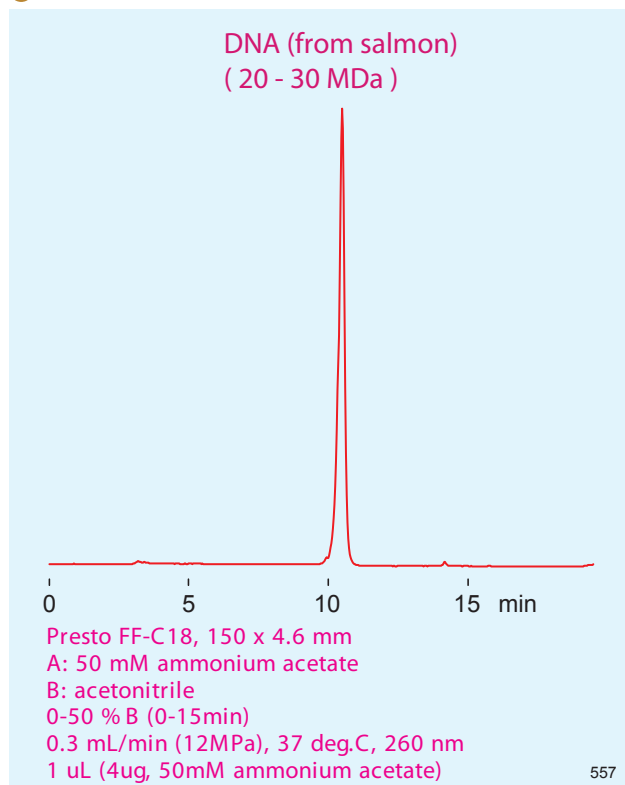


Reversed-phase separation for non-ionic polysaccharides on Presto FF-C18 does not require any pH modifier. Excellent peak shape was observed for several non-ionic polysaccharides using water / acetonitrile gradients. Polysaccharides contain multiple OH groups, and different concentrations of acetonitrile may be required for proper elution. The data shows that retention will be similar for homo polysaccharides larger than 10kDa. Peak shape is dependent upon molecular size distribution.

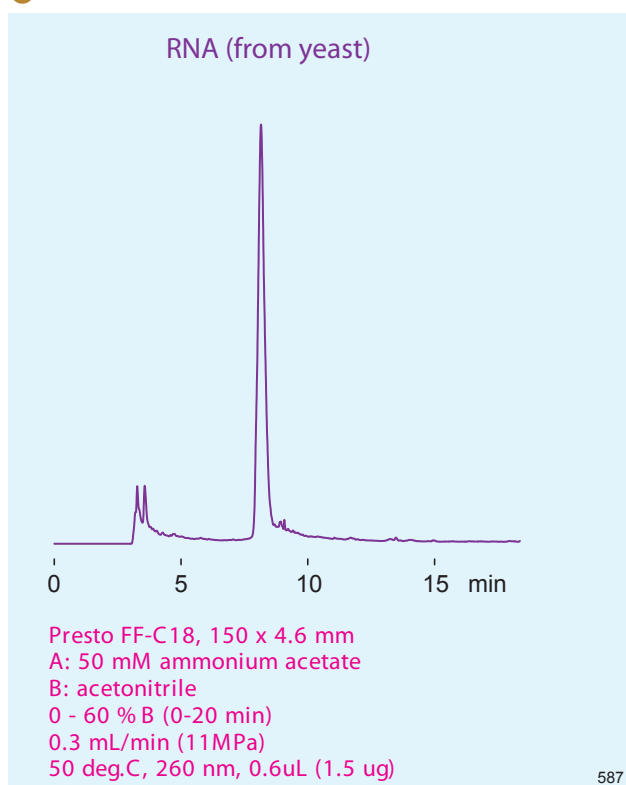
Nucleic Acids

Presto FF- C18 can analyze nucleic acids. Historically, nucleic acids have been difficult to analyze by HPLC, due to the extremely large size of these biopolymers. The non-porous ODS, Presto FF- C18, makes this possible.

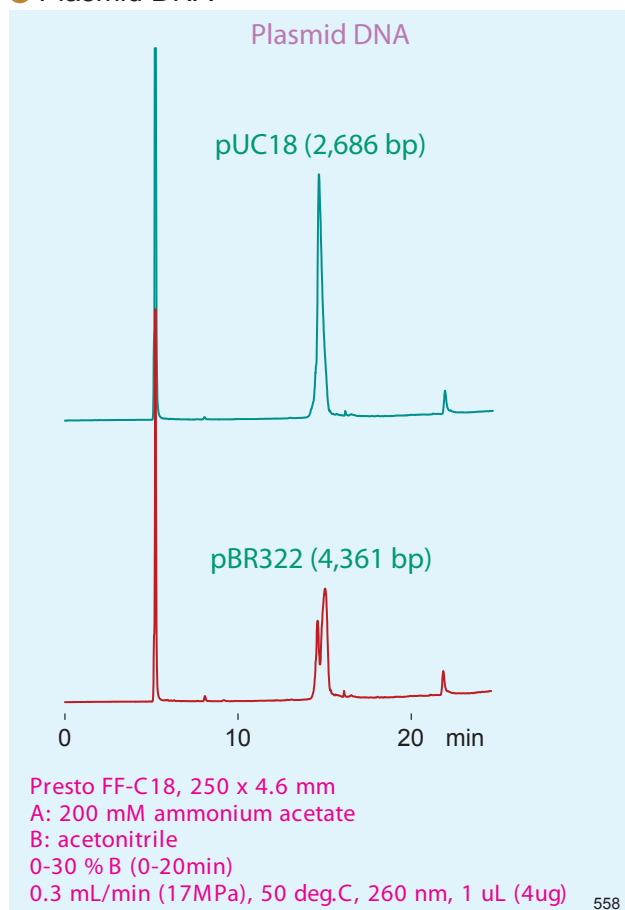
DNA



RNA



Plasmid DNA



Double stranded DNA is a tremendously large molecule that is normally hydrolyzed into fragments (via nuclease) prior to analysis. Presto FF-C18 can analyze intact DNA as well as DNA fragments.

Increasing column temperature to 50 deg.C can help to improve peak shape for both DNA and RNA. In addition, 50mM ammonium acetate, used as a neutral pH modifier, can also help to improve peak shape.

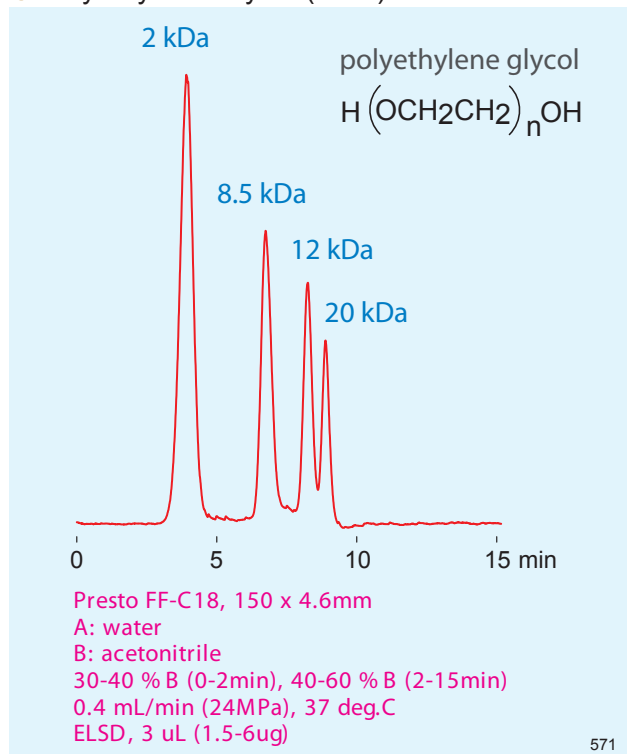
Plasmid DNA consists of covalently closed circular form and has a different structure from nuclear DNA. In this experiment, a high concentration of ammonium acetate (200mM) was required for elution.

Porous RP columns traditionally have struggled with DNA and RNA analysis. Presto FF-C18 can improve both analysis and isolation of these large biopolymers.

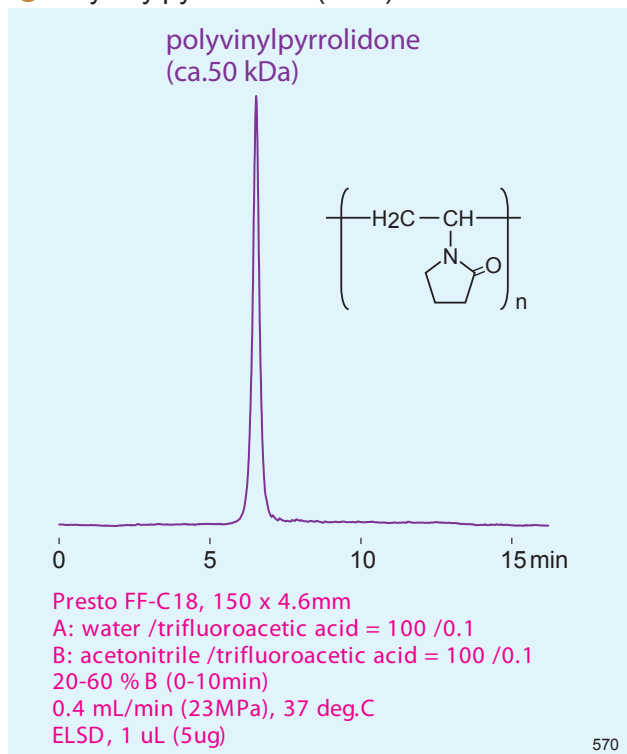
Synthetic Polymers (Hydrophilic)

Presto FF- C18 can achieve better peak shape for synthetic polymers, which have traditionally been analyzed on SEC mode. In addition, peak width under RP mode is dependent upon molecular size distribution.

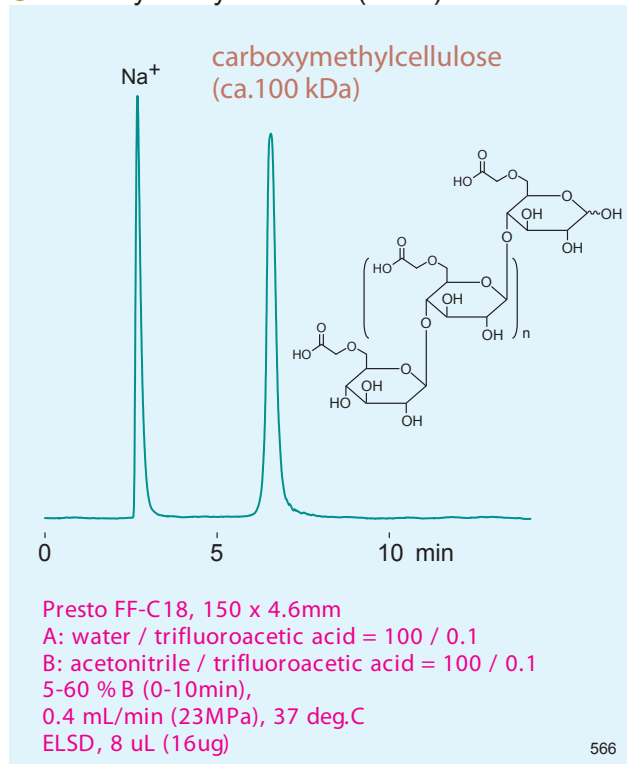
● Polyethylene Glycol (PEG)



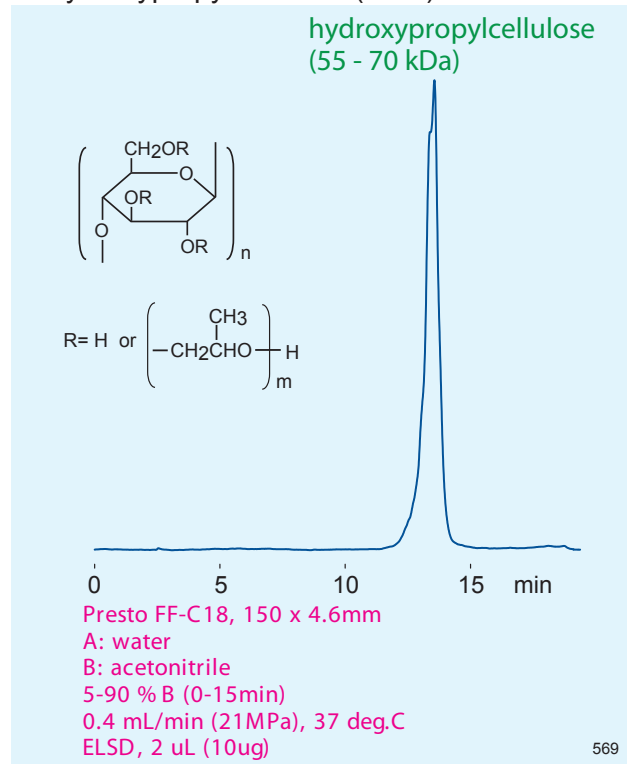
● Polyvinylpyrrolidone (PVP)



● Carboxymethylcellulose (CMC)



● Hydroxypropylcellulose (HPC)



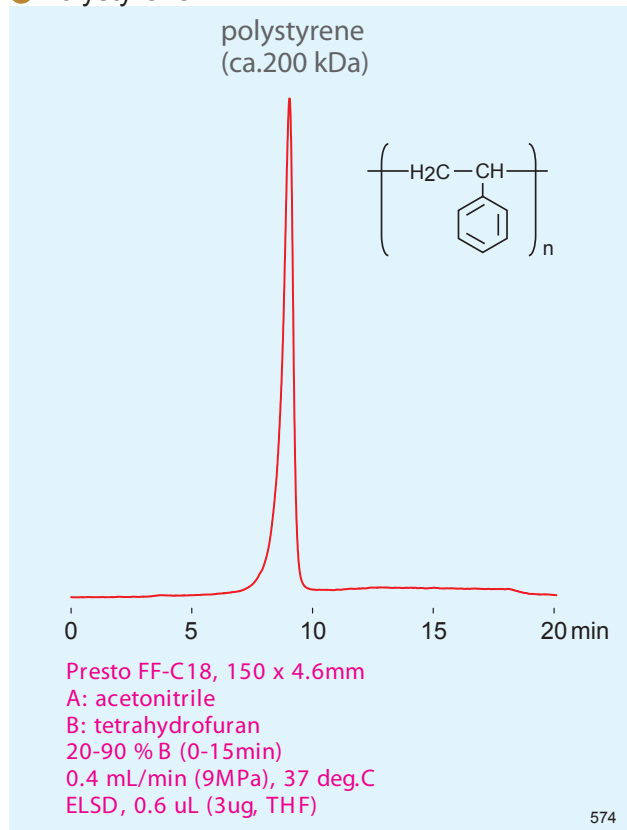
Hydrophilic polymers, which have been traditionally analyzed on aqueous SEC (GFC) mode, may now also be analyzed on Presto FF-C18 with RP mode and gradient elution.

The PEG data shows that relatively small polymers (up to 100kDa) can be separated from each other. Ionic polymers, such as CMC, require the addition of pH modifier to the eluent.

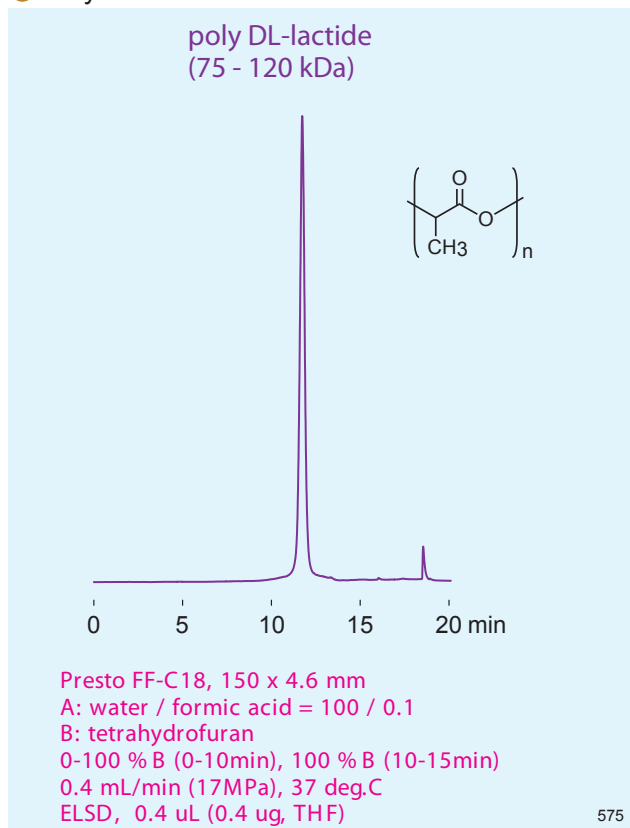
Synthetic Polymers (Hydrophobic)

Presto FF-C18 can be applied to hydrophobic polymer analysis. The solubility and elution properties of the solute need to be taken into account when preparing the mobile phase. There is an opportunity to reduce costs and convert the current GPC method to an RP method with Presto FF-C18.

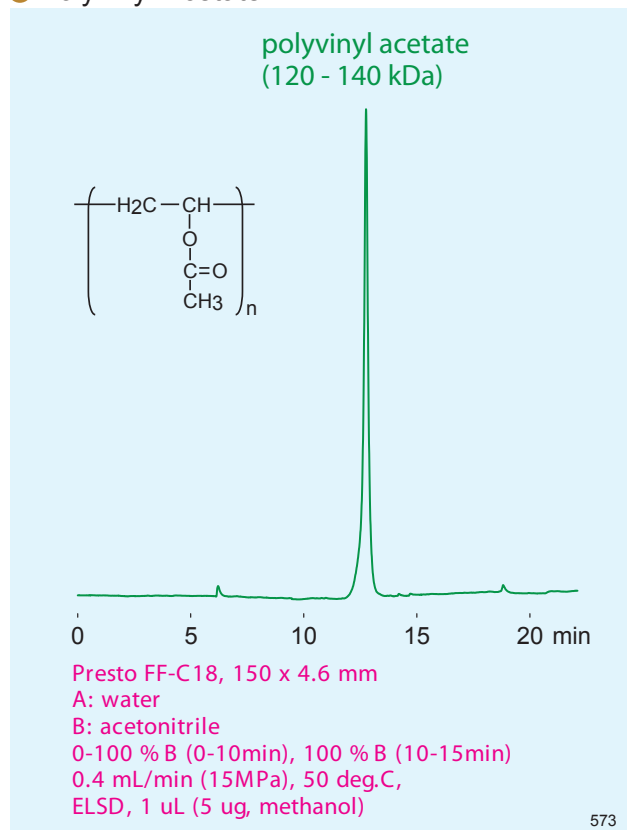
● Polystyrene



● Polylactide



● Polyvinyl Acetate



Polystyrene, a large molecule, is very hydrophobic and difficult to analyze on porous RP columns. Usually it is analyzed using GPC. Presto FF-C18 can be used with non-aqueous elution. Peak width will be affected by molecular size distribution.

Polylactide is hydrophobic polymer, but does have some hydrophilic properties due to its large abundance of oxygen. As a result, gradient elution from water to THF was required.

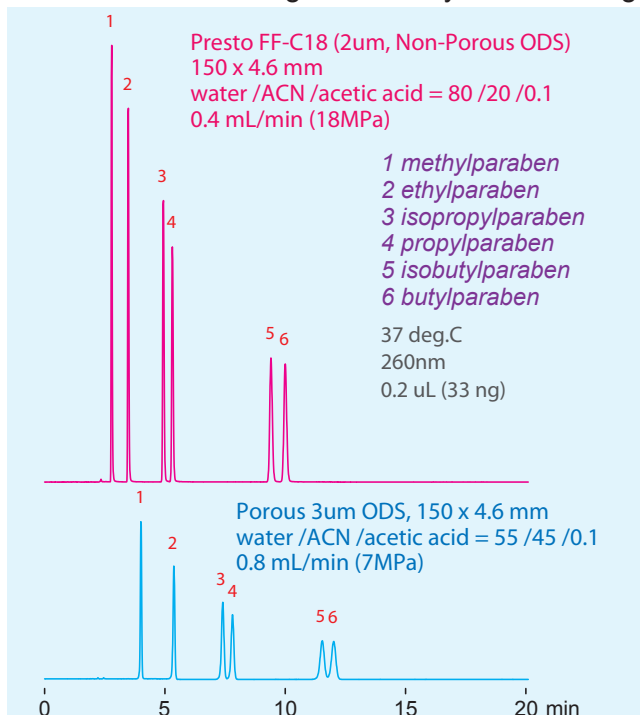
Similarly, polyvinyl acetate is a hydrophobic polymer with some hydrophilic properties. It required gradient elution from water to acetonitrile.

Presto FF-C18 can create new possibilities for hydrophobic polymer separations under RP mode.

Small Compounds

Due to its extremely low surface area (non-porous), Presto FF-C18 is usually not recommended for small molecule analysis. However, depending on the solute structure, non-porous ODS can be advantageous for small molecule separations.

● Low Flow Rate - High Sensitivity and Amazing Separation

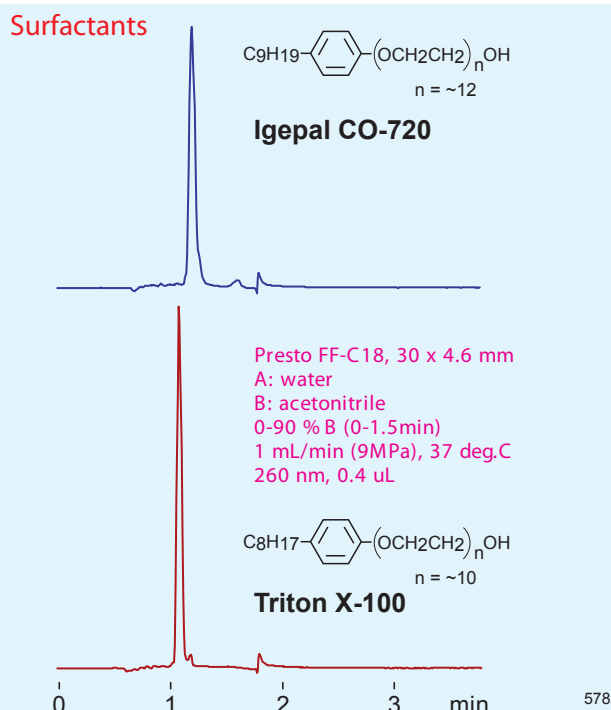


When mobile phase composition and flow rate is optimized, Presto FF-C18 can at times achieve amazing peak shape for small molecules.

Porous ODS has a high mass transfer resistance (resulting in band broadening). In contrast, non-porous ODS does not have this issue. As a result, for the alkylparabens, Presto FF-C18 provided better peak shape (less band broadening) than porous ODS. In addition, lower flow rate (ex: half the flow rate of porous ODS column) provides improved sensitivity and resolution.

Although Presto FF-C18 consists of 2µm particles, there are many advantages to operating at low flow rate: higher resolution, improved sensitivity, and lower pressures for conventional HPLC systems.

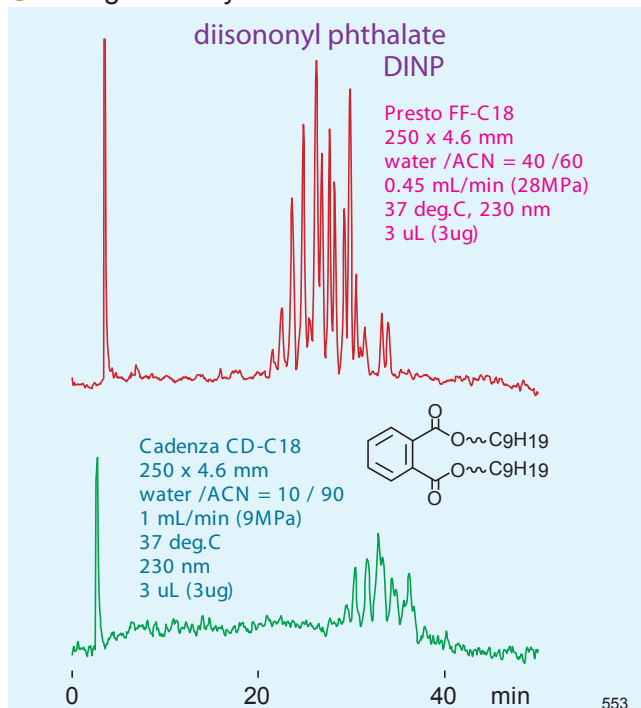
● Peaks Co-Elute to Make One Peak



With small compounds, Presto FF-C18 sometimes shows worse separation than porous ODS columns. Igepal and Triton are mixtures of different oxyethylene chain lengths. Porous ODS column can easily separate the oligomers, while Presto FF-C18 provides poor selectivity due to low oxygen recognition. However, having the oligomers co-elute on Presto FF-C18 can be favorable since it produces one peak in a short time analysis.

Presto FF-C18 can expand separation possibilities when coupled with porous ODS columns.

● Recognize Alkyl Branched-Chain



On conventional porous ODS, Phthalic di-ester has numerous branched-chains with the same MW that co-elute. In contrast, Presto FF-C18, at a low flow rate, recognizes the alkyl chain isomers and is more sensitive.

Recommendations for Presto FF-C18

Presto FF-C18 is a non-porous ODS column consisting of 2µm non-porous silica particles. The specific surface area is much lower than conventional porous ODS column. As a result, eluent composition should be optimized in order to obtain retention equivalent to a porous ODS column. Presto FF-C18 works great at low flow rates and can be used on conventional HPLC systems. Higher flow rates (i.e. same flow rate as porous ODS column) will require the use of a UHPLC system.

● Mobile Phase Composition

Retention is greatly reduced on non-porous ODS due to its extremely low surface area. In order to get similar retention to porous ODS, organic solvent ratio should be reduced by 1/2 - 1/3. But this leads to another benefit as it decreases solvent consumption.

● Flow Rate

Presto FF-C18 consists of 2µm particles and can be used at operating pressures up to 50MPa for 6mm or less I.D. columns. In addition, because Presto FF-C18 is non-porous (and thus no diffusion in pores), excellent performance is also achieved at lower flow rates. This has the added effect of being able to use 2µm particle on conventional HPLC systems. Additionally, lower flow rates, such as 1/2 - 1/3 of porous ODS columns, improves resolution and sensitivity.

● Sample Solvent and Injection Volume

The surface area of Presto FF-C18 is extremely low and can be affected by sample injection volume. In addition, peak shape may be poor when polar compounds are dissolved in organic solvent and operated under highly aqueous eluent conditions. Under this scenario, the organic solvent becomes the eluent - causing some solutes to elute more quickly than others (resulting in poor peak shape). To avoid this potential problem, sample solvent should be highly aqueous (which also allows the use of larger injection volumes).

● Retention of Polar Compounds

Presto FF-C18 can struggle to retain polar compounds. But, 100% aqueous eluent can be used with this column and analysis of polar compounds can be optimized by using 0.1-1% organic.

● Elution Mode

Presto FF-C18 has a disadvantage for isocratic elution due to low surface area. It is therefore recommended to use gradient elution as often as possible. If isocratic elution is required, peak shape and retention reproducibility can be improved by increasing the ionic strength of the buffer.

● Recommendations for HPLC Operation

It is recommended that low dispersion systems and high pressure binary pumps are used with Presto FF-C18. Low pressure gradient pumps have too much dead volume which can have a negative effect on the separation. It is highly recommended to use gradient elution with Presto FF-C18. If isocratic elution is required, it is strongly recommended to pre-mix the mobile phase prior to use. Due to the non-porous silica, the kinetics within Presto FF-C18 are extremely fast. Therefore, a mixer is not required with this column (t-union is sufficient for HT analysis).

Ordering Information

Presto FF-C18 2µm Non-Porous Silica, Octadecyl Ligand, End-Capped

| Length (mm) | Product Code | | | | | |
|-------------|--------------|-----------|-----------|-------------|-----------|------------|
| | 1 mm I.D. | 2 mm I.D. | 3 mm I.D. | 4.6 mm I.D. | 6 mm I.D. | 10 mm I.D. |
| 10 | - | FF020 | FF030 | FF000 | - | - |
| 20 | - | FF029 | FF039 | FF009 | - | - |
| 30 | FF011 | FF021 | FF031 | FF001 | FF061 | FF0P1 |
| 50 | FF012 | FF022 | FF032 | FF002 | FF062 | FF0P2 |
| 75 | FF013 | FF023 | FF033 | FF003 | FF063 | FF0P3 |
| 100 | FF014 | FF024 | FF034 | FF004 | FF064 | FF0P4 |
| 150 | FF015 | FF025 | FF035 | FF005 | FF065 | FF0P5 |
| 250 | FF016 | FF026 | FF036 | FF006 | FF066 | FF0P6 |

Micro / Nano columns are also available.