Thermo Scientific

Acclaim SEC Columns

Product Manual

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Thermo SCIENTIFIC

Product Manual

for

Acclaim SEC-1000 Columns

7µm, Analytical, 4.6 x 300 mm (P/N 079724)
7µm, Analytical, 7.8 x 300 mm (P/N 079721)
7µm, Analytical, 7.8 x 150 mm (P/N 079722)
7µm, Guard, 4.6 x 33 mm (P/N 082739)

Acclaim SEC-300 Columns

5µm, Analytical, 4.6 x 300 mm (P/N 079723)
5µm, Analytical, 7.8 x 300 mm (P/N 079725)
5µm, Analytical, 7.8 x 150 mm (P/N 079726)
5µm, Guard, 4.6 x 33 mm (P/N 082740)

Revision History:

Revision 01, December, 2012, Original Publication.

For Research Use Only. Not for use in diagnostic procedures.

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.***

Contents

1. Introduction

Thermo Scientific Acclaim™ SEC-300 and SEC-1000 are high-efficiency and high-resolution size exclusion chromatography (SEC) columns specifically designed for separation of water-soluble polymers and oligomers, e.g. polyethylene glycols, polyvinyl alcohols, polyvinyl pyrrolidones, dextrans, polyacrylic acids, etc.

Acclaim SEC columns are available in two pore sizes, 300 Å and 1000 Å, and cover a broad molecular weight range from 100 to 1,000,000 Dalton. The Acclaim SEC-1000 uses with a nominal pore size of 1000 Å, for separating polymers and oligomers in the MW range of 1,000 to 1,000,000 Dalton; the Acclaim SEC-300 has a nominal pore size of 300 Å, for separating in the MW range of 100 to 50,000 Dalton (calibration curve shown below, Figure 1).

1.1 Main Features of the Acclaim SEC columns include:

- Proprietary mono-dispersed multi-pore hydrophilic resin: no inflection points in calibration curve
- Two columns with different pore sizes cover wide linear range (100 to 1,000,000 Dalton)
- Availability of small particle sizes packed in 4.6 x 300-mm dimensions allows for high resolution analysis at reduced solvent consumption
- Stable surface bonding with low column bleed and compatibility with UV, RI, MS, ELSD and Corona® CAD detection

Table 1 Physical Data

	Acclaim SEC-300	Acclaim SEC-1000
Substrate	Hydrophilic polymethacrylate resin	Hydrophilic polymethacrylate resin
Particle shape	Spherical	Spherical
Particle size	5-µm	7-µm
Pore size	300-Å (multi-pore)	1000-Å (multi-pore)
Separation range for PEO*	100 – 50,000 Daltons	1,000 – 1,000,000 Daltons
Exclusion limit for PEO*	50,000 – 150,000 Daltons	3,000,000 – 7,500,000 Daltons

*PEO = polyethylene oxides

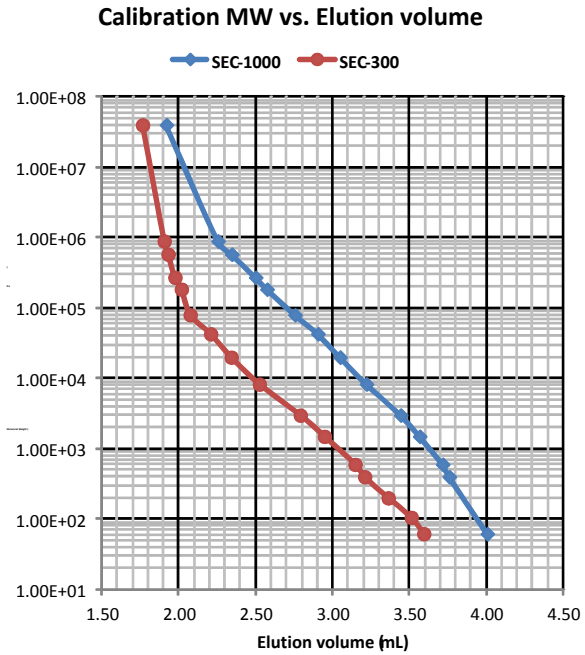
Table 2 Specifications and Recommended Operational Parameters

Column	Dimension (mm)	Flow Rate (mL/min)	Pressure Limit (psi)	Temperature (ºC)	pH Range	Sample Loading (µL)
SEC-300, 5µm	4.6x300	≤ 0.35	< 1200	< 60	2 – 12	< 100
SEC-300, 5µm	7.8x300	≤ 1.00	< 1200	< 60	2 – 12	< 300
SEC-300, 5µm	7.8x150	≤1.00	< 700	< 60	2 – 12	< 150
SEC-1000, 7µm	4.6x300	≤ 0.35	< 600	< 60	2 – 12	< 100
SEC-1000, 7µm	7.8x300	≤ 1.00	< 600	< 60	2 – 12	< 300
SEC-1000, 7µm	7.8x150	≤ 1.00	< 350	< 60	2 – 12	< 150

1.2 Operational Guidelines

- Operate the column according to "Operational Parameters" described above.
- Follow the direction of flow that is marked on the column. Reverse flow should be avoided except for removal of inlet blockage.
- Avoid sudden pressure surge on to the column.
- Avoid air on to the column.
- Use guard columns to protect the analytical column to prolong column lifetime.
- The column is compatible with up to 20% organic solvents. When changing solvents, make gradual changes in solvent composition to protect the bed from compression.
- Salt concentration in mobile phase should be lower than 0.5 M.
- Column cleaning: choose a cleaning solution based on sample type.
- To remove cationic contaminants, use high salt concentration solution (0.5 – 1.0 M).
- To remove hydrophobic contaminants, use a buffer with acetonitrile or methanol.
- Also can use pH2-3 or pH9-12 buffer, or buffers containing urea or SDS.
- Column storage: 0.05 – 0.1% NaN_3 or 20% ethanol for longer term (>24 hours); or mobile phase for short term (< 24 hours).

Figure 1 Calibration Curves of Acclaim SEC-300 and Acclaim SEC-1000



Columns:	Acclaim SEC -300 Acclaim SEC -1000
Dimension :	4.6 x 300 mm
Mobile Phase:	10 mM sodium perchlorate
Flow Rate:	0.35 mL/min
Temperature:	25 °C
Injection Volume:	50 µL
Detection:	RI
Samples:	(0.03% - 0.1% in mobile phase) Dextran (MW 5,000,000 - 40,000,000), PEO (MW 895,000, 580,000, 272,000, 185,000, 80,000, 43,000, and 20,000), PEG (MW 8,300, 3,000, 1,500, 600, 400 and 200), Diethylene glycol (MW 106) and Ethylene glycol (MW 62)

2. Step-by-Step User Guide

It is recommended to run the column performance test upon receiving your new Acclaim SEC column. The purpose of this test is to ensure no damage has occurred during shipping. Steps 1 - 5 below outline the necessary steps to perform this validation test. Test the column using the conditions described on the Quality Assurance (QA) Report enclosed in the column box. Repeat the test periodically to track the column performance over time.

Step 1 – Visually inspect the column

Report any damage to Thermo Fisher Scientific. Depending upon the nature of the damage, we may request that you ship the damaged column back to us for a replacement.

Step 2 – Mobile phase preparation

The Acclaim SEC column can be used with a variety of mobile phases, including D.I. water, ammonium acetate buffer, sodium perchlorate, sodium sulfate, etc. It is highly recommended that the mobile phase is filtered with a 0.2 - 0.5 µm pore size membrane filter or/and a line filter containing membrane is installed on the HPLC system.

2.1.1 De-ionized water

The de-ionized water used to prepare the mobile phase should be Type 1 Reagent Grade water, or HPLC Grade Water. The deionized water must be free of ionized impurities, organics, microorganisms and particulate matters. Many commercial water purifiers are designed for HPLC applications and are suitable for these applications.

2.1.2 Solvents

The solvent must be free from particulate, ionic or UV-absorbing impurities. Use of ultrahigh purity solvents, HPLC grade ensures that your chromatography is not affected by impurities in the solvent.

2.1.3 Mobile phase

Depending on the nature of the polymer of interest, the detection method can be UV, RI, ELSD, CAD, or MS. When using ELSD, CAD or MS detector, a volatile mobile phases, ammonium acetate, ammonium formate, acetic acid or formic acid, must be used. The quality of these buffer salts and acids are critical for good detection and only high-purity (99.9% or better) reagents should be used. For RI detection method, a perchlorate salt is often used to obtain better baseline.

Step 3 – Set up the LC system

Use a standard LC system equipped with a LC pump, a column oven, a suitable detector (e.g. UV, RI, ELSD, CAD, etc) and an injector (or an Autosampler). When using the semi-micro column dimension (4.6x300-mm), the LC system must be optimized for low dead volume; use small id tubing (0.005” I.D. tubing, especially between the injector and detector) and a small volume detector cell (e.g. micro flow cell). The system should be thoroughly primed before use.

Step 4 – Condition the column

When a new column is used for the first time, it should be washed thoroughly with D.I. water for three column volumes at a low flow rate (e.g. 0.2 mL/min for 4.6x300-mm column format).

When switching to a new mobile phase, make sure that the new mobile phase is compatible with the previous mobile phase in the column to avoid column clogging due to precipitation. The column should be thoroughly conditioned before any injection is made (e.g. ~3 column volumes).

When switching from a nonvolatile mobile phase (e.g. phosphate buffer) to a volatile mobile phase (e.g. ammonium acetate buffer) for applications using MS or CAD detection method, the column must be washed thoroughly off-line with 100 mM ammonium acetate, pH5 for three column volumes and then with acetonitrile/100 mM ammonium acetate (10:90, v/v) for three column volumes before equilibrated with the desired mobile phase for three column volumes.

Step 5 – Reproduce the chromatogram in the Quality Assurance Report

Perform the column performance test using the conditions described in the Quality Assurance Report, and compare the result with the one in the report. After the column is fully equilibrated, multiple injections of a standard containing formamide or another void marker should be made until the reproducible retention is obtained.

It is recommended that you run the column performance test upon receiving your new Acclaim SEC column. Repeat the test periodically to track the column performance over time.

Step 6 – Real sample analysis

Once the column performance is satisfactorily confirmed in Step 5, the column is ready for real sample analysis.

NOTE

It is recommended that the column performance test be performed periodically to monitor the condition of the column.

3. Considerations in Method Development

3.1 Column Selection

Refer to Table 1 for selecting a suitable column for specific applications. In general, Acclaim SEC-1000 is a good starting point for polymers with unknown or larger than 20,000 Dalton molecular weights because of its wide separation range. For polymers whose molecular weights are lower than 20,000 Dalton, the Acclaim SEC-300 may provide better result.

3.2 Mobile Phase

Proper mobile phase is critical to maximize molecular sieving mechanism and to minimize secondary effects such as ionic and hydrophobic interaction between the analyte and the stationary phase. Depending on the sample, buffer type, pH and concentration should be considered for optimal resolution and recovery.

The stationary phases (polymethacrylate resin) packed in Acclaim SEC columns contain small quantity of residual charged anionic groups. For anionic polymers, a salt such as sodium nitrate or sodium perchlorate can be added to D.I. water as the mobile phase. When using a mobile phase with a low ionic strength, anionic polymers are excluded by ionic repulsion, resulting in an earlier elution time than expected based on their molecular size. Generally, a concentration of less than 0.1 M is sufficient to overcome undesirable ionic interactions. On the other hand, cationic polymers are retained by electrostatic attractions between them and the column, resulting in elution times later than theoretically predicted. To minimize this undesired interaction the mobile phase should contain up to 0.5 M of salt and/or be kept at an acidic condition to keep the surface of the stationary phase surface neutral. Nonionic water soluble polymers such as polyethylene glycols can simply be analyzed with D.I. water as the mobile phase.

If hydrophobic interaction occurs between the analyte and the stationary phase, up to 20% water miscible organic solvent can be added to the mobile phase to mask such secondary interaction. It also helps prevent columns from fouling by removing hydrophobic impurities in the sample. The commonly used solvents include acetonitrile, acetone, ethanol or methanol. Note that care should be taken to prevent in the process of changing solvent-containing mobile phase, such as low flow rate (20% of regular operating flow rate) and gradual change in solvent content.

3.3 Sample Loading

Sample loading on a SEC column is limited due to the absence of a stationary phase that participates in the retention process. High sample loads distort peak shapes and cause an overall decrease in efficiency due to column overload. Optimal sample load highly depends on the sample properties (sample matrix) and the separation task. For analytical columns, sample concentrations of 1-20 mg/mL are recommended. Proteins can be loaded at higher concentrations and higher total loads than synthetic macromolecules.

4. Column Care

4.1 Column storage

Column storage: 0.05 – 0.1% NaN_3 in D.I. water or up to 20% ethanol for longer term (>24 hours); or mobile phase for short term (< 24 hours).

4.2 Operating pH range : pH 2 to 12

Acclaim SEC columns must be operated within pH 2 to 12. Using a narrow pH range, such as pH 3 to 11, will help achieve better column usage time.

4.3 Operating temperature limit : 10 - 60 ºC

Acclaim SEC columns can be used at an elevated temperature up to 60 ºC. When a sub room temperature is needed, the flow rate should be adjusted (lowered) to prevent it from exceeding pressure limit.

4.4 Pressure limit

It is extremely important not to impose a sudden pressure surge to the column. The pressure limits can be found in Table 2.

4.5 Flow rate

See Table 2 for details. When switching the mobile phase, special care should be taken to avoid formation of precipitation in the column and pressure surge during the transition. As a cautionary measure, a lower flow (~ 50% of typical flow rate) should be applied to maintain the integrity of the column.

4.6 Guard cartridge

When analyzing real-life samples, a guard cartridge must be used with the analytical column, and replaced periodically depending on the nature of the sample. Failing to do so will result in rapid column deterioration and premature column failure.

4.7 Column cleaning procedure

Particulates in the sample or the mobile phase larger than 0.5 µm will plug the column inlet frit. It is important the samples and mobile phases be free of particulates. If solvent flow appears to be restricted (high column back-pressure), check first to see that solvent flow is unobstructed up to the column inlet. If the column has the restriction, there may be particulate matter on the inlet frit. An attempt should be made to remove any inlet debris by back-flushing 25 to 30 mL of mobile phase through the column. If this fails to return the column to near its original operating pressure, consider replacing the column.

All samples should be pre-treated and filtered before being injected on the column. In the event that column washing/cleaning is needed, the following procedure can be used as a guideline:

1. Flush the column with D.I. water for at least 3 column volumes. Note: flow rate is 0.15 mL/min for 4.6-mm i.d. column and 0.45 mL/min for 7.8-mm i.d. column.
2. Flush the column with a 0.5 M salt solution (a higher concentration up to 1.0 M can be used depending on the nature of the contaminants) for at least 5 column volumes. For poly-cationic species, the solution should be acidic (~pH3). For anionic or neutral contaminants, the solution pH should be in the range of 5 – 8.
3. Flush the column with a 0.01 – 0.05 M salt or buffer solution containing 20% solvent (e.g. methanol or acetonitrile) for at least 5 column volumes. For hydrophobic cationic polymers, the solution should be acidic (~pH3). For anionic or neutral contaminants, the solution pH should be in the range of 5 – 8.
4. Flush the column with D.I. water for at least 3 column volumes.
5. Flush the column with the mobile phase.

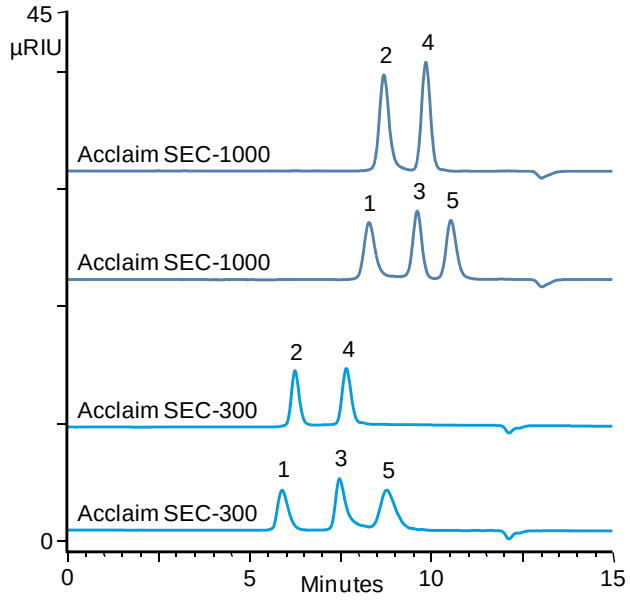
5. Applications

The Acclaim SEC columns offer good resolution for a variety of molecular weight polymers or oligomers (Figure 2).

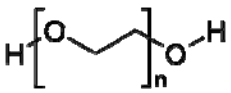
5.1 Polyethylene Glycols

Polyethylene glycol (PEG), Polyethylene oxide (PEO), and Polyoxyethylene (POE) all refer to oligomers/polymers of ethylene oxide. PEG usually means < 20,000 MW; PEO usually means > 20,000 MW Depending on their molecular weights PEGs can be liquids or low-melting solids. While PEG and PEO with different molecular weights find use in different applications, and have different physical properties due to chain length effects, their chemical properties are nearly identical. PEGs have many applications including medical, biological, chemical, consumer products and other industrial areas. Figure 2 shows the profiles of five PEGs of different molecular weights obtained and Acclaim SEC-300 and Acclaim SEC-1000.

Figure 2 Polyethylene Glycols: Acclaim SEC-300 vs. Acclaim SEC-1000



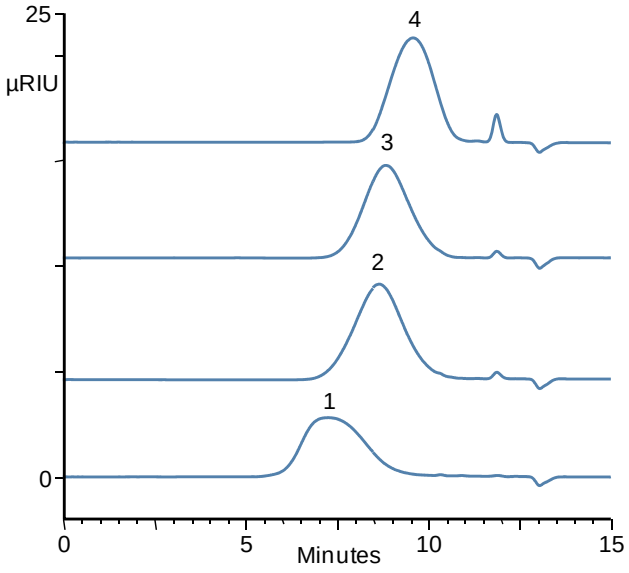
Columns:	Acclaim SEC-300
	Acclaim SEC-1000
Dimension:	4.6 x 300 mm
Mobile Phase:	10 mM sodium perchlorate
Flow Rate:	0.35 mL/min
Temperature:	30 °C
Injection Volume:	5 µL
Detection:	RI
Samples:	5 mg/mL in mobile phase
	1. PEG MW 35,000
	2. PEG MW 12,000
	3. PEG MW 3,400
	4. PEG MW 2,000
	5. PEG MW 300



5.2 Polyvinylpyrrolidones (PVP)

Polyvinylpyrrolidone (PVP), a water-soluble polymer made from the monomer N-vinylpyrrolidone, is extensively used in pharmaceutical, medical, cosmetics, foods, and industrial applications. While the Acclaim SEC-1000 is suited to determination of PVP samples with a wide molecular weight range (Figure 3), the Acclaim SEC-300 provides more detailed information on molecular weight distribution of smaller PVP (Figure 4).

Figure 3 Polyvinylpyrrolidones (PVP) on Acclaim SEC-1000



Columns:	Acclaim SEC-1000
Dimension:	4.6 x 300 mm
Mobile Phase:	10 mM sodium perchlorate
Flow Rate:	0.35 mL/min
Temperature:	30 °C
Injection Volume:	5 µL
Detection:	RI
Samples:	5 mg/mL in mobile phase
	1. PVP, MW 360,000
	2. PVP, MW 55,000
	3. PVP, MW 24,000
	4. PVP, MW 10,000

PVP = Polyvinylpyrrolidone

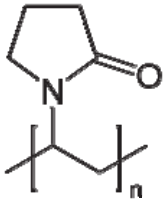
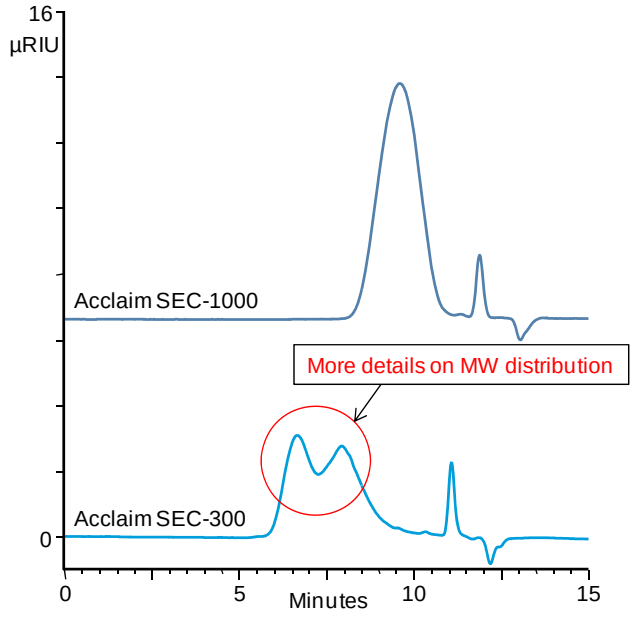


Figure 4 Polyvinylpyrrolidone: Acclaim SEC-300 vs. Acclaim SEC-1000

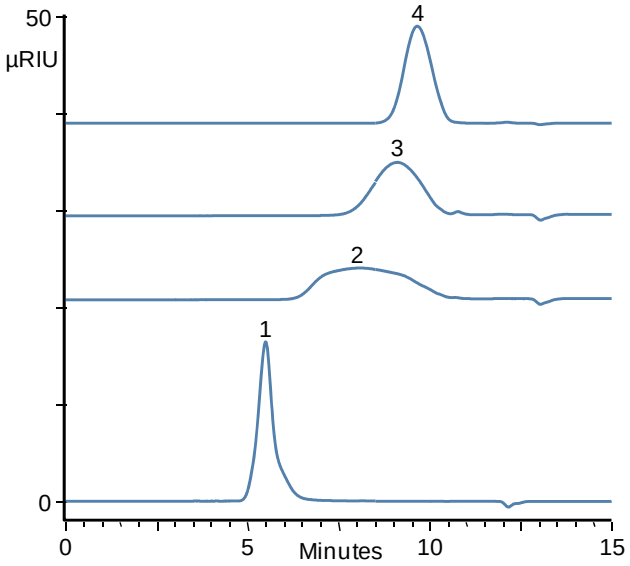


Columns:	Acclaim SEC-300 Acclaim SEC-1000
Dimension:	4.6 x 300 mm
Mobile Phase:	10 mM sodium perchlorate
Flow Rate:	0.35 mL/min
Temperature:	30 °C
Injection Volume:	5 µL
Detection:	RI
Sample:	Polyvinylpyrrolidone (PVP), MW 10,000 (5 mg/mL in mobile phase)

5.3 Dextrans

Dextran is a class of complex, branched polymers called glucan that is composed of chains of various lengths (from 3 to 2000 kilodaltons). It has broad medical and industrial applications. Figure 5 shows the overlay of chromatogram traces of dextrans with different sizes obtained on Acclaim SEC-1000. For smaller dextran (MW 10,000), the Acclaim SEC-300 elutes the analytes farther away from the void, resulting in better data quality (Figure 6).

Figure 5 Dextrans on Acclaim SEC-1000



Columns:	Acclaim SEC-1000
Dimension:	4.6 x 300 mm
Mobile Phase:	10 mM sodium perchlorate
Flow Rate:	0.35 mL/min
Temperature:	30 °C
Injection Volume:	5 µL
Detection:	RI
Samples:	5 mg/mL in mobile phase
	1. Dextran, MW 580,000
	2. Dextran, MW 200,000 – 300,000
	3. Dextran, MW 35,000 – 50,000
	4. Dextran, MW 10,000

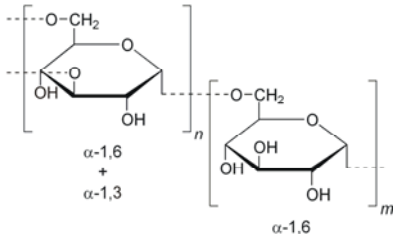
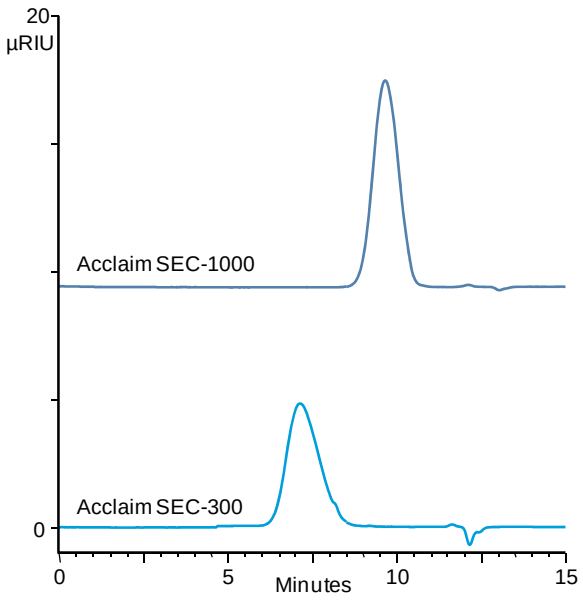
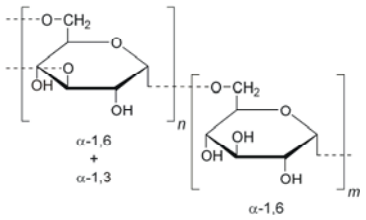


Figure 6 Dextran: Acclaim SEC-300 vs. Acclaim SEC-1000



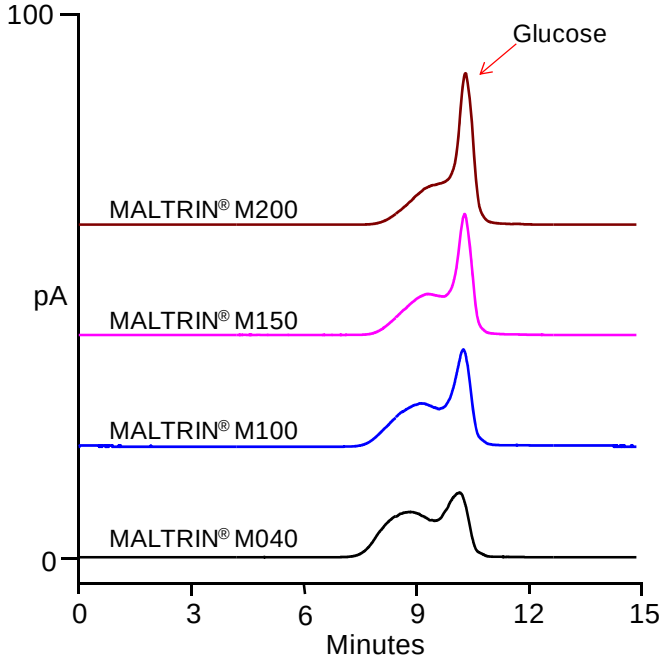
Columns:	Acclaim SEC-300
	Acclaim SEC-1000
Dimension:	4.6 x 300 mm
Mobile Phase:	10 mM sodium perchlorate
Flow Rate:	0.35 mL/min
Temperature:	30 °C
Injection Volume:	5 µL
Detection:	RI
Sample:	Dextran, MW 10,000 (5 mg/mL in mobile phase)



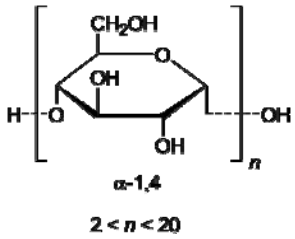
5.4 Maltodextrins

Maltodextrin is a polysaccharide that is used as a food additive. It is produced from starch by partial hydrolysis. Maltodextrin is easily digestible, being absorbed as rapidly as glucose and is commonly used as an ingredient in a variety of food production, such as sodas and candy. As shown in Figure 7, maltodextrin samples with different degree of hydrolysis are clearly illustrated using the Acclaim SEC-1000 column.

Figure 7 Maltodextrins



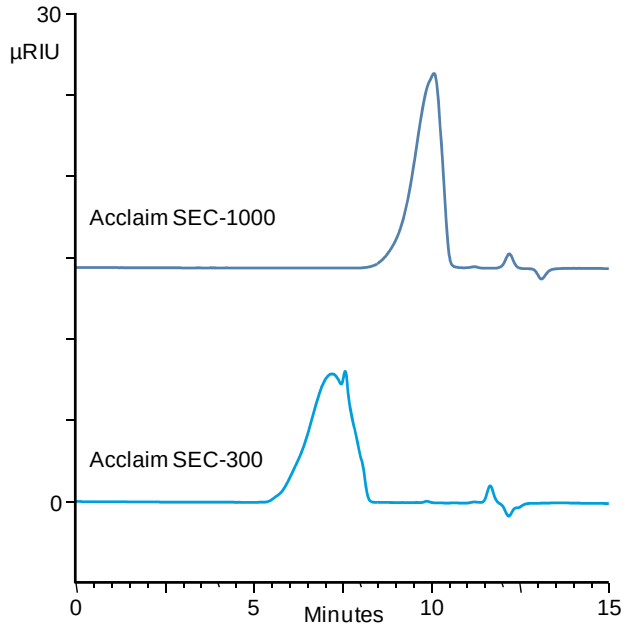
Column:	Acclaim SEC-1000
Dimension:	4.6x300 mm
Mobile Phase:	100 mM ammonium acetate pH5.0
Temperature:	25°C
Flow Rate:	0.35 mL/min
Injection Volume:	5 µL
Detection:	Corona *ultra*
Sample:	MALTRINs, 5 mg/mL each



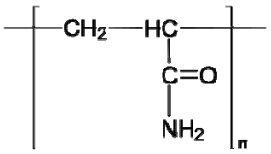
5.5 Polyacrylamide

Polyacrylamide is a polymer formed from acrylamide monomer. It is highly water-absorbent, forming a soft gel when hydrated, used in such applications as polyacrylamide gel electrophoresis and in manufacturing soft contact lenses. The straight-chain polyacrylamide is often used as a thickener and suspending agent. Figure 8 exhibits elution profiles of a polyacrylamide polymer with an average molecular weight of 10,000 Dalton on both Acclaim SEC-300 and Acclaim SEC-1000 columns.

Figure 8 Polyacrylamide: Acclaim SEC-300 vs. Acclaim SEC-1000



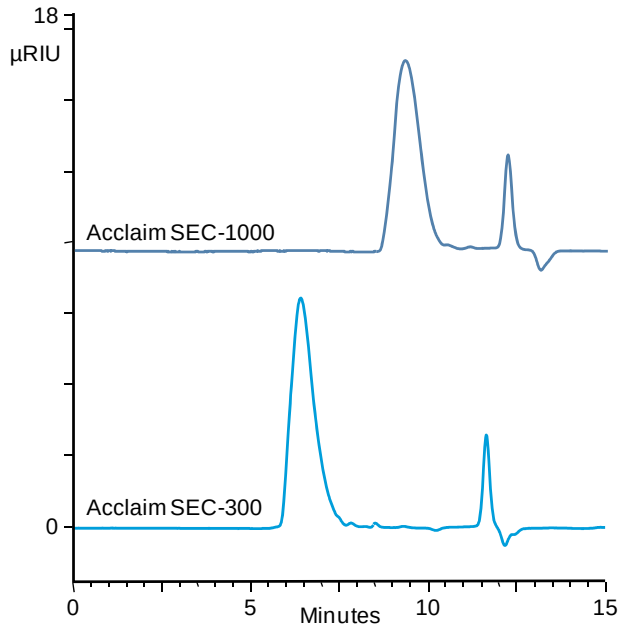
Columns:	Acclaim SEC-300 Acclaim SEC-1000
Dimension:	4.6 x 300 mm
Mobile Phase:	10 mM sodium perchlorate
Flow Rate:	0.35 mL/min
Temperature:	30 °C
Injection Volume:	5 µL
Detection:	RI
Samples:	Polyacrylamide, MW 10,000 (5 mg/mL in mobile phase)



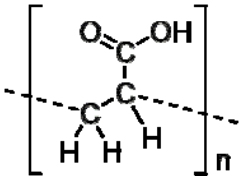
5.6 Polyacrylic Acid

Polyacrylic acid (PAA) is generic name for synthetic polymers from acrylic acid monomer. In a water solution PAA is an anionic polymer, which makes PAA a polyelectrolyte, with the ability to absorb and retain water and swell to many times their original volume. Thus it is widely used in disposable diapers, and as thickening, dispersing, suspending and emulsifying agents in pharmaceuticals and cosmetics. Note that the SEC stationary phase has a small amount of carboxylate groups on its surface. As the result, the elution times of PAAs are usually shorter than those of neutral water soluble polymers with the similar size due to the electrostatic repulsion between anionic polymers like PAAs and the stationary phase. This feature makes PAA peak free from interference from the void, resulting in accurate measurement of total quantity of PAA in the sample. Figure 9 shows the elution profiles of a PAA (MW 10,000) on both Acclaim SEC-300 and Acclaim SEC-1000 using an un-buffered salt solution. For the purpose of molecular weight determination, a buffered mobile phase would be needed.

Figure 9 Polyacrylic Acid: Acclaim SEC-300 vs. Acclaim SEC-1000



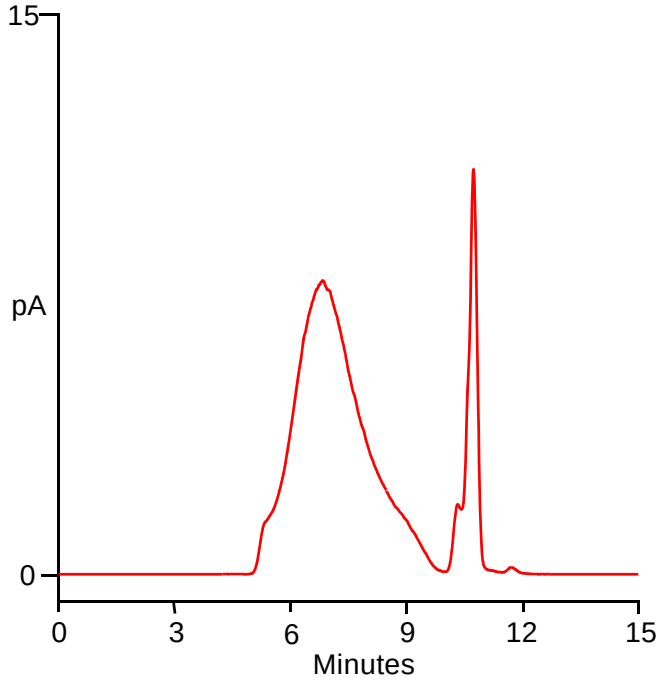
Columns:	Acclaim SEC-300 Acclaim SEC-1000
Dimension:	4.6 x 300 mm
Mobile Phase:	10 mM sodium perchlorate
Flow Rate:	0.35 mL/min
Temperature:	30 °C
Injection Volume:	5 µL
Detection:	RI
Samples:	Polyacrylic acid, MW 50,000 (5 mg/mL in mobile phase)



5.7 Polyquaternium-10 (Quaternized hydroxyethylcellulose)

Polyquaternium-10 is a polymeric quaternary ammonium salt of hydroxyethyl cellulose that is positively charged. At high pH, the presence of hydroxyl groups may reduce the normally high water solubility of quaternary ammonium compounds. The charge on Polyquaternium-10 makes this compound an antistatic agent which helps prevent the condition commonly known as fly-away hair. It is also used as a film former and a hair fixative. Due to the presence of small amount of carboxylate groups in the stationary phase, an acidic buffered mobile phase is required to suppress electrostatic attraction between cationic water-soluble polymers like Polyquaternium-10 and the stationary phase, as shown Figure 10

Figure 10 Polyquaternium-10 (Quaternized hydroxyethylcellulose)

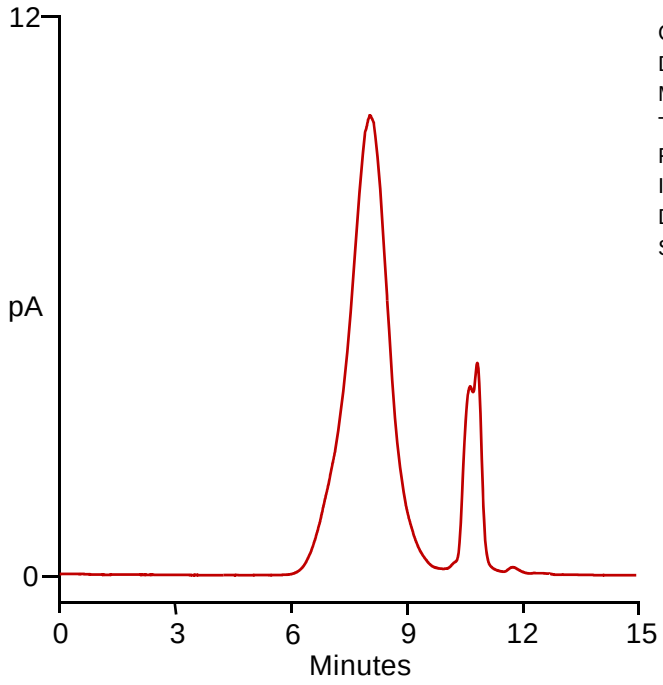


Column:	Acclaim SEC-1000
Dimension:	4.6x300 mm
Mobile Phase:	50 mM ammonium formate pH3.5
Temperature:	25°C
Flow Rate:	0.35 mL/min
Injection Volume:	5 µL
Detection:	Corona *ultra*
Sample:	Polyquaternium-10, 5 mg/mL

5.8 Gum Arabic

Gum Arabic is a complex mixture of glycoproteins and polysaccharides. It is used primarily in the food industry as a stabilizer. Gum Arabic is a key ingredient in traditional lithography and is used in printing, paint production, glue, cosmetics and various industrial applications. As shown in Figure 11, the Acclaim SEC-1000, combined with a charged aerosol detector (CAD) and a volatile mobile phase, provides a viable solution to determine the molecular weight distribution of Gum Arabic.

Figure 11 Gum Arabic



Column:	Acclaim SEC-1000
Dimension:	4.6x300 mm
Mobile Phase:	100 mM ammonium acetate pH5.0
Temperature:	25°C
Flow Rate:	0.35 mL/min
Injection Volume:	5 µL
Detection:	Corona *ultra*
Sample:	Gum Arabic, 5 mg/ mL