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STYRAGEL COLUMN

I. INTRODUCTION

This manual covers the care and use of the Waters Styragel® HR, HT, and HMW families of Gel Permeation Chromatography (GPC) columns. Please take a few moments to read this manual carefully. Follow the recommendations in this manual to prolong column life and enhance chromatographic reproducibility.

This introduction describes the three families of Waters Styragel columns:

- Styragel HR
- Styragel HT
- Styragel HMW

Waters Styragel columns are packed with high-performance, fully porous, highly cross-linked styrene-divinylbenzene copolymer particles. Their different characteristics allow you to choose the column optimally suited to your application. Styragel columns are shipped in three solvents: toluene, tetrahydrofuran (THF), and dimethylformamide (DMF).

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a. Waters Styragel HR

Use Waters Styragel HR columns for the high-resolution analysis of low-molecular weight polymers, oligomers and additives. Packed with 5 µm particles, Waters Styragel HR columns provide the high plate counts necessary for this type of analysis.

b. Waters Styragel HT

Use Waters Styragel HT columns for the analysis of polymers with mid-range molecular-weight distributions. They are the most versatile columns for molecular-weight analysis. Waters Styragel HT columns are packed with 10 µm particles to provide dependable performance over a wide range of temperatures and solvents.

c. Waters Styragel HMW

Waters Styragel HMW columns are designed for the molecular-weight analysis of ultrahigh-molecular-weight polymers. Their 20 µm particle size together with the nominally 10 µm HMW frit design prevents the breakdown of ultrahigh molecularweight polymers due to shear, which can occur with smaller particles.

This manual covers both column sizes 7.8 x 300 mm columns, and the solvent efficient 4.6 x 300 mm columns. In sections with recommend flow rates or spare parts, the 4.6 mm column conditions, or spare part follow immediately after the 7.8 mm recommendations.

II. INSTALLING THE COLUMN BANK

This chapter describes:

- Preparing the GPC/HPLC system
- Installing the columns
- Repairing damaged compression screw assemblies
- Equilibrating the column

a. Preparing the GPC/HPLC System

Before attaching the columns in the flow path on a GPC/HPLC system, you must first prepare the system:

1. Directly connect the system injector to the detector by replacing the old columns with a zero-dead-volume union.
2. Convert the system to the solvent in which the columns have been stored. For a new column set, this is the shipping solvent.
3. Flush the system to remove any microparticulates and old solvents. Flush the injector loop if applicable.

Band spreading

The connection tubing and fittings in any chromatographic system contribute to extra-column band spreading. Before installing the column, me sure your system instrument band spreading (see Section on Testing Instrument Band Spreading). If this test is not possible with your system, refer to your system operator's manual.

Narrow-Bore Chromatography for 4.6 mm Solvent Efficient Columns

The peak volume in a narrow-bore system is so small, it is critical to minimize band spreading. Use the shortest tubing possible for all connections. It is not necessary to use a microbore flow cell in your detector or to change your conventional HPLC system in any way. Use 0.009-inch (0.25 mm) i.d. tubing throughout the system.

b. Installing the Columns

When connecting columns in series, use the 0.009 inch (0.25 mm) i.d. U-shaped column-joining tube supplied with each column.

Sequence of columns in a column bank

Generally, the results of an analysis are independent of the sequence in which a column bank is arranged. However, to improve resolution and column life, arrange the columns in order of decreasing pore size, with the column with the largest pore size closest to the injector. This is recommended because:

- The columns with the larger pore sizes are more rugged and are better able to tolerate the accumulation of extraneous materials.
- The species with the highest molecular-weight in the sample contributes the most to the viscosity of the sample. If the largest species is separated first, the viscosity decreases more quickly, placing less strain on the column bank. In the case of ultrahigh MW polymers, there is less shear on the sample.

Installing columns in a column bank

To install your columns:

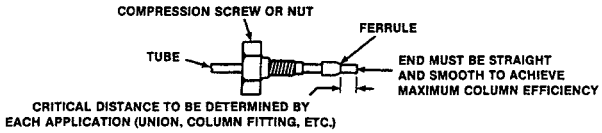
1. Remove the end plugs from each column and save them.
2. Connect the first column to the injector outlet tubing. Ensure that solvent flow is in the direction shown by the arrow on the column label. Finger-tighten the fittings, then tighten the fittings with a wrench by another 1/4 turn.
3. Connect the next column to the previous column using the U-tubes supplied with the columns. Ensure that solvent flow is in the direction shown by the arrow on the column label. Thread the inlet and outlet fittings of the U-tube until finger tight, then tighten the fittings by another turn with a wrench.
4. Repeat step 3, until all columns are connected.
5. Connect the last column to the detector inlet tubing using steps 1 through 4.

c. Repairing Damaged Compression Screw Assemblies

To remove a damaged compression screw or a worn ferrule assembly:

1. Scribe the circumference of the tubing at the desired break point using a tube cutter or a file with a cutting edge.
2. Grasp the tubing on both sides of the scribe mark with cloth-covered pliers (to prevent marring the tube surface). Gently work the tube back and forth until it separates at the scribe mark. Ensure that the tubing end is straight, open, and free of burrs.
3. Slide the compression screw, followed by the ferrule (large end of the taper first), over the tube. Properly bottom the tubing in the fitting seat. If the tubing is not completely seated, the resulting dead volume can lead to poor chromatographic results.

Figure 1. Ferrule and Compression Screw Assembly



Ferrule 0.009-inch I.D. Tubing (0.25 mm) 0.130-inch gap (3.3 mm)

The distance between the end of the ferrule and the end of the U-tube may differ for different column types. If you have used columns from another manufacturer, it may be necessary to reset the ferrules, or make up a new fitting (see Figure 1). Use 0.009-inch (0.25 mm) i.d. tubing for all lines between the injector and detector. For Waters columns, the critical distance is 0.130 inch (2.25 mm).

d. Equilibrating the Column Bank

Equilibrate the columns when you install them and when you use them after they have been stored. To equilibrate your column bank:

1. Set the pump flow rate at 0.0 mL/min, then turn on the pump.
2. Increase the flow rate by 0.1 mL/min at 15 second intervals until you reach the final flow rate.
3. Purge the columns with the shipping solvent until you obtain a stable baseline.

Flow rate and backpressure for 4.6 mm Solvent Efficient Columns

For best resolution and maximum column life, do not allow the flow rate to exceed 0.3 mL/min or the backpressure to exceed 3.5 MPa (500 psi, 35 atm) per column.

Please note: The flow rate recorded on the Certificate of Analysis, included with the column, may be higher than the guidelines provided. For maximum column life in your lab, please follow the flow and back pressure guidance we provide.

When using the HR 0.5, HR 1, and HR 2 columns, increase the flow rate in 0.1 mL/min increments at 30-second intervals until you reach 0.3 mL/min.

Defective columns

One or more defective columns in a series may cause the entire set of columns to appear defective. One defective column can cause peak spreading that cannot be overcome by any number of good columns. See Table 4, Table 5, or Table 6 for column efficiency data.

Initial efficiency tests

Test your system and columns before the first analysis. Run a test sample using the recommended parameters for your system and columns, and record the results. These results serve as a baseline to compare future performance. See Section on Efficiency Testing, for the procedures to determine the efficiency of your system and columns.

Save the chromatograms from these tests. For the column-efficiency test, record the retention times, system settings, and all experimental conditions so they may be reproduced exactly for future comparison.

III. PREPARING SOLVENT AND SAMPLES

This chapter describes:

- Preparing the solvent
- Changing solvents
- Converting columns to high temperature solvents
- Preparing the sample

a. Preparing the Solvent

Use clean solvent for reproducible results and maintenance-free operation. Use solvents of LC-grade or better, filtered to remove micro particulate matter larger than 0.45 µm. Refer to Waters catalog, filtration section for filter choice and solvent compatibility chart.

b. Changing Solvents

Solvent compatibility

Waters Styragel columns are shipped in the solvent of your choice: toluene, THF or DMF. Some applications require a different solvent. Changing solvents works best between compatible solvents. Refer to the table below for solvent compatibilities.

Note: The use of highly aqueous mobile phases may damage the resin and is not recommended.

Table 1. Solvent Conversion Table

To convert to:	Use columns shipped in:	To convert to:	Use columns shipped in:
o-Dichlorobenzene	Toluene		
Trichlorobenzene	Toluene	Hexafluoroisopropanol	THF
Phenol/TCB	Toluene	N-Methyl pyrrolidone	DMF
y-Butyrolactone	THF	m-Cresol	DMF

To use Waters Styragel columns for high-temperature chromatography in solvents like trichlorobenzene (TCB) or orthodichlorobenzene (ODCB), you must convert the column to the selected solvent at elevated temperature.

High-temperature conversion procedure

To convert the column bank to high-temperature operation:

1. Convert the system to the column shipping solvent at room temperature.
2. Purge solvent efficient columns at 0.1 mL/min while gradually increasing the temperature to 90 °C over a minimum of 3 hours.

Solvent Efficient Columns

(Purge solvent efficient columns at 0.1 mL/min while gradually increasing the temperature to 90 °C over a minimum of 3 hours.)

Set the system to 55 °C when converting columns from THF to HFIP or Y _ Butyrolactone.

3. With the system at 90 °C, convert to the high-temperature solvent at 0.1 mL/min using 12 mL per column. Then purge the system for a minimum of four column volumes at 0.2 mL/min.

   When using a bank of columns, multiply the number of column volumes specified in the procedure by the number of columns being used.

4. Convert to the high-temperature solvent using a flow rate of 0.1 mL/min. Use at least 20 mL per column.

5. Increase the temperature to the final conditions over a minimum of four hours while continuing to purge the column at 0.1 mL/min. Never exceed 150 °C.

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6. Adjust the flow rate to the final operating conditions. The optimal flow rate is 0.3 ml/min.

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Returning to room temperature

To return the columns to room temperature, reverse the above procedure. Alternatively, set the flow rate to 0.1 mL/min and reduce the temperature by 10 °C every 30 minutes.

Restarting the column

To restart the column, maintain a flow rate of 0.1 mL/min and increase the temperature to the desired temperature over 10 hours. Then program the desired operating flow rate.

For maximum column life, avoid temperature cycling. Maintain operating temperature but reduce flow rate to 0.1 mL/min when columns are not in use.

c. Preparing the Sample

Good sample preparation prolongs column life and ensures reproducible results. Take into account factors such as the capacity of the column, sample viscosity, and the type and sensitivity of the detector. Remove micro particulates with a 0.45 µm filter. Refer to Waters catalog for filter choice and solvent compatibility chart.

Reactive polymers

Some reactive polymers (such as epoxies) may "condition" the column. Improve column life and reproducibility by dedicating columns to specific classes of reactive polymers.

Sample concentration

Sample concentration affects both viscosity and injection volume. While small sample amounts produce narrower peaks, viscous samples may require larger, more dilute samples. Table 2 lists the recommended concentration of sample for optimal results.

High-molecular-weight polymers

High-molecular-weight polymers are especially susceptible to viscosity problems. When analyzing high-molecular-weight polymers, use the concentrations indicated in Table 2. Run narrow-distribution polymers, such as polystyrene standards, with an injection volume of 50 µL per column (20 µL per column for solvent efficient columns) at a concentration of 0.02 percent.

Polystyrene standards with molecular weights of approximately four million or greater become increasingly susceptible to degradation by shearing in solution. Shearing is indicated by molecular-weight distributions that are broader than expected. With proper handling, polymers with molecular weights as high as 20 million can be handled successfully.

Table 2. Recommended Sample Concentrations

Molecular-weight Range	Sample Concentration
0 to 25,000	<0.25%
25,000 to 200,000	<0.1 %
200,000 to 2,000,000	<0.05%
Above 2,000,000	<0.02%

Ultrahigh-molecular-weight polymers

Shearing is also a factor to be considered with most ultrahigh-molecular-weight polymers. The effect of shearing may not be as easily observed as it is with narrow molecular-weight standards. Ultrahigh-molecular-weight polyolefin fractions are subject to possible shearing and incipient precipitation. Use especially dilute solutions of these fractions (for example, 0.02 percent) to minimize precipitation.

IV. USING THE COLUMN

This chapter describes:

- Chromatography guidelines
- Calibrating the column

a. Chromatography Guidelines

Gel permeation chromatography columns have a finite lifetime directly related to their care and use. Column life is reduced by contamination from samples and eluents, frequent solvent changeover, improper handling and storage, and temperature cycling.

Guidelines for column use

When using Waters Styragel columns, observe the following guidelines:

- For best resolution and maximum column life, do not exceed a flow rate of 1.0 mL/min or the backpressure exceed 3.5 MPa (500 psi, 35 atm) per column (corrected for system backpressure).
- For solvent efficient columns, best resolution and maximum column life, do not let the flow rate exceed 0.3 mL/min or the backpressure exceed 3.5 MPa (500 psi, 35 atm) per column (corrected for system backpressure). Normal flow rate for these columns is 0.3 mL/min.
- Protect the column from vibration and mechanical shock.
- Minimize temperature cycling.
- Protect the column from rapid changes in pressure that can result from rapidly changing the composition of the solvent.
- When changing to a solvent with a different viscosity, it may be necessary to adjust the flow rate to stay below the operating backpressure specification of 3.5 MPa (500 psi, 35 atm) per column.
- Avoid precipitation by dissolving samples in the mobile phase.
- Always use high-quality HPLC solvents.
- Dedicate columns to specific applications. Frequent switching of samples and solvents accelerates column deterioration and loss of resolution.
- Using highly aqueous mobile phases may damage the resin and is not recommended.

b. Calibrating the Column

Whenever you replace a single column or a complete column bank, generate a new calibration curve to ensure the reproducibility of your application. Figure 2, Figure 3, and Figure 4 show typical calibration curves by column family. The calibration curves were obtained with polystyrene standards.

Figure 2. Calibration Curves for Waters Styragel HR Columns

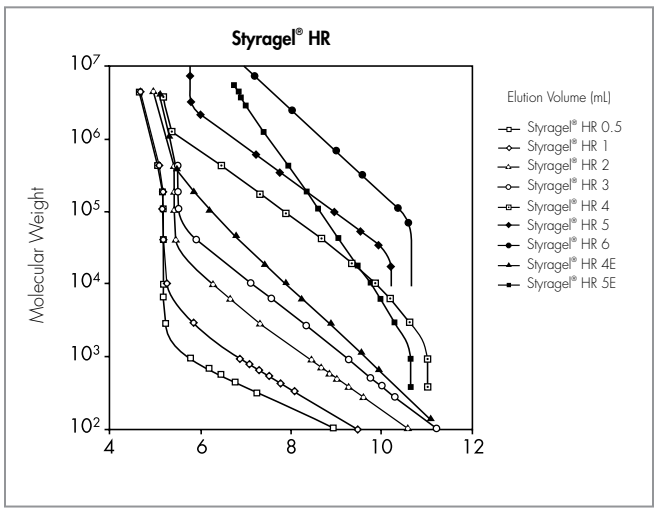


Figure 3. Calibration Curves for Waters Styragel HT Columns

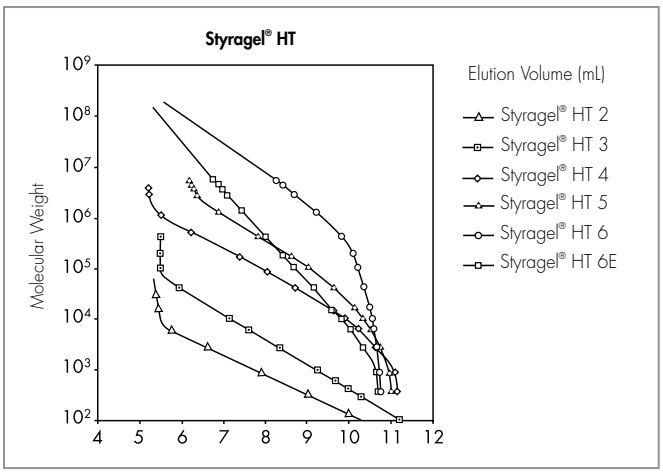
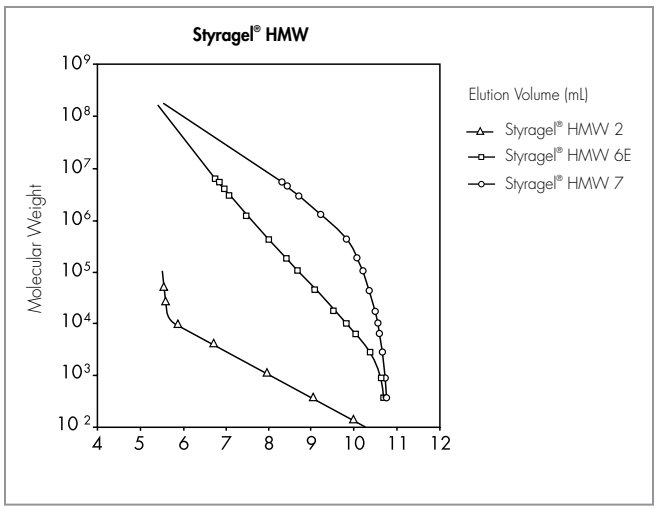


Figure 4. Calibration Curves for Waters Styragel HMW Columns



V. CARE AND MAINTENANCE

This chapter describes:

- Troubleshooting
- Storing the column
- Efficiency testing

a. Troubleshooting

Table 3 describes specific problems, causes, and corrective actions.

Table 3. Column Troubleshooting

Problem	Cause	Corrective Action
Buildup in system operating pressure	Inlet filter insert (from the first column only) plugged with particulates Injector and pump seal shedding	Replace the inlet filter insert. Install a Styragel Guard column. Fit an in-line filter between the pump and the first column.
	Clogged tubing	Replace the tubing
Loss of resolution, broad peaks, low plate counts	Filter inserts partially blocked	Replace or clean inlet and/or outlet filter inserts
	Failing injector	Repair the injector
	Insufficient equilibrium	Continue equilibrium
	Column damaged	Replace column

b. Storing the Column

If you will be using the column again in less than 24 hours, special storage procedures are unnecessary. However, be sure that the columns never dry out. For longer storage periods, return the column to its box with the end plugs firmly in place for storage. Do not leave a column at elevated temperatures without solvent flow.

For maximum column life, avoid temperature cycling. Maintain operating temperature and reduce flow rate to 0.1 mL/min when columns are not in use.

To restart the column, maintain a flow rate of 0.1 mL/min and, if applicable, increase the temperature gradually over 10 hours. Then set the flow rate to the desired operating flow.

c. Efficiency Testing

Waters columns are tested for adherence to our specifications. Slight variations may occur depending on:

- The type and condition of equipment used
- The nature of the sample
- Instrument settings

Perform your own efficiency tests. To test both your system and each column in the system, carry out both of these tests:

- Instrument band-spreading test
- Column-efficiency test

When to perform efficiency tests

Perform efficiency tests each time you add columns or otherwise change your system. Then, as you use the system, conduct efficiency tests on a regular schedule.

If problems occur during normal operation of the column, repeat the conditions for the initial efficiency tests and compare the results. Monitor instrument band spreading by performing a column efficiency test without the column in line. Resolve excessive band spreading before installing columns.

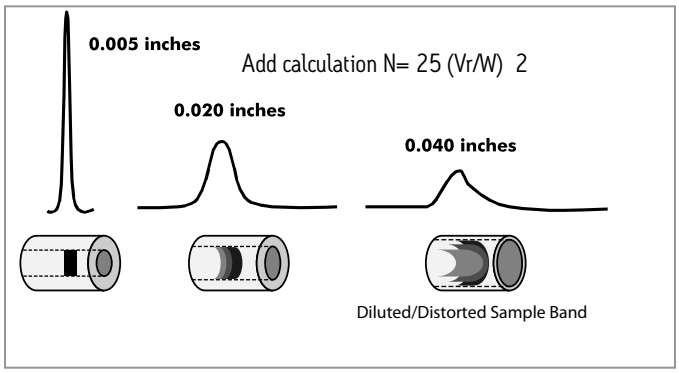
1) Testing Instrument Band Spreading

Test procedure

The band spreading of a properly operating system should be less than 150 µL (75 µL for solvent efficient columns). To determine the band spreading of your system:

1. Remove the column(s) from the system.
2. Connect the inlet and outlet tubing with a zero dead-volume union.
3. Set the flow rate to 1.0 mL/min.
4. Use a fast chart speed to obtain a peak of easily measurable width. If you use a data system, set the sampling rate to at least 10 data points per second. You may need to adjust the detector sensitivity to keep the peak on scale.

Figure 5. Method for Calculating Band Spreading



5. Inject the same sample as for a plate count determination (see Table 4, 5, or 6).
6. Measure the width of the resulting peak at 4.4% of the peak height to obtain a value in µL.

Figure 5 illustrates the 5 sigma method, in which the width of the peak (w) is measured at 4.4% of the peak height (h).

2) Column Efficiency Test

The column-efficiency test described below may be used to calculate a theoretical plate count, which is a measurement of column efficiency within your system.

Solvents

It is not necessary to use the solvent that your column is shipped in to determine the efficiency of your column. Initial plate counts that are determined using different solvents will have different values. Test columns individually using your normal operating solvent.

Test procedure

To perform a column efficiency test:

1. Slowly increase the flow rate to 1.0 mL/min for the HR 0.5, HR 1, HR 2, and HR 4E columns, or to 2.0 mL/min for all other columns.
2. Adjust the detector to an attenuation that achieves a peak of 70 percent full scale (noise level <0.5 percent full scale).
3. Set the recorder chart speed to 50 mm/min, or the data system sampling rate to at least 5 data points per second.
4. Inject up to 20 µL of marker solution. Use a solution of up to 10 percent marker in solvent. Use up to 5 µL for solvent efficient columns.

Refer to Table 4, Table 5, or Table 6 for the marker to use when performing the efficiency test.

5. Record the retention time, instrument settings, and column configuration so you can reproduce them exactly for comparison in the future.
6. Compute the plate count using the tangent method (see Figure 6). Use these results for comparison throughout the life of your column.
7. Replace any column that exhibits a plate count more than 30 percent below the original value.

Table 4. Conditions and sample for 7.8 mm for Column Efficiency Test: HR Columns

Column Type	Toluene	THF	DMF	Flow Rate mL/min
HR 0.5	Acetone	Propylbenzene	Ethyleneglycol	1.0
HR 1	Acetone	Propylbenzene	Ethyleneglycol	1.0
HR 2	Acetone	Propylbenzene	Ethyleneglycol	1.0
HR 3	ODCB	Acetone	Acetone	2.0
HR 4	ODCB	Acetone	Acetone	2.0
HR 4E	Acetone	Propylbenzene	Ethyleneglycol	1.0
HR 5E	DCHP[2]	Acetone	Acetone	2.0

[1] Dicyclohexylphthalate

Table 5. Conditions and Sample for 4.6 mm Column Efficiency Test: HR Columns

Column Type	Toluene		THF		DMF	
	Marker	Flow Rate (mL/min)	Marker	Flow Rate (mL/min)	Marker	Flow Rate (mL/min)
HR 0.5	ODCB	0.3	Acetone	0.3	Acetone	0.3
HR 1	ODCB	0.3	Acetone	0.3	Acetone	0.3
HR 2	ODCB	0.3	Acetone	0.3	Acetone	0.3
HR 3	ODCB	0.3	Acetone	0.3	Acetone	0.3
HR 4	ODCB	0.3	Acetone	0.3	Acetone	0.3
HR 4E	ODCB	0.3	Acetone	0.3	Acetone	0.3
HR 5E	DCHP[2]	0.3	Acetone	0.3	Acetone	0.3

Table 6. Conditions and sample for 7.8 mm for Column Efficiency Test: HT Columns 1

Column Type	Toluene	THF	DMF	Flow Rate (mL/min)
HT 3	ODCB	Acetone	Acetone	2.0
HT 4	ODCB	Acetone	Acetone	2.0
HT 5	ODCB	Acetone	Acetone	2.0
HT 6	DCHP	Acetone	Acetone	2.0
HT 6E	DCHP	Acetone	Acetone	2.0

Table 7. Conditions and sample for 4.6 mm for Column Efficiency Test: HT Columns 1

Column Type	Toluene		THF		DMF	
	Marker	Flow Rate (mL/min)	Marker	Flow Rate (mL/min)	Marker	Flow Rate (mL/min)
HR 3	ODCB	0.3	Acetone	0.3	Acetone	0.3
HR 4	ODCB	0.3	Acetone	0.3	Acetone	0.3
HR 5	ODCB	0.3	Acetone	0.3	Acetone	0.3
HR 6	DCHP	0.3	Acetone	0.3	Acetone	0.3
HR 6E	DCHP	0.3	Acetone	0.3	Acetone	0.3

Figure 6. Tangent Method for Calculating Column Efficiency

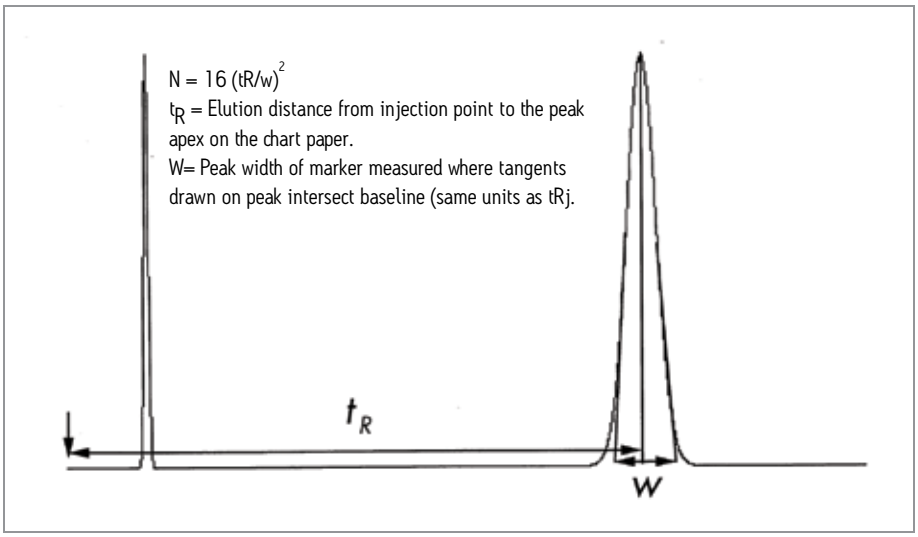


Table 8. Test Condition and Sample for Efficiency Test 7.8 mm HMW Columns

Column Type	Toluene	THF	DMF	Flow Rate (mL/min)
HMW 7	DCHP	Acetone	Acetone	2.0
HMW 6E	DCHP	Acetone	Acetone	2.0

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