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YMC COLUMNS

I. INTRODUCTION

Thank you for purchasing a YMC™ high-performance liquid chromatography (HPLC) column. We are sure you will find that YMC's built-in quality helps solve many of your challenging separation problems. We strive to provide products with the highest degree of lot-to-lot and column-to-column reproducibility to minimize variations in your chromatographic results. YMC manufactures each packing material and packed column under highly controlled conditions. Each must pass a series of stringent tests before being accepted for shipment. Included with each column is the final Quality Control Report.

CONTENTS

YMC SEPARATION TECHNOLOGY

a. Column Installation

Note: The flow rates given in the procedure below are for a typical 5 µm packing in a 4.6 mm i.d. column. Scale the flow rate up or down accordingly based upon the column i.d., length, particle size and back-pressure of the YMC column being installed. See "Scaling Up/Down Isocratic Separations" section for calculating flow rates when changing column i.d and/or length. See "Connecting the Column to the HPLC" for a more detailed discussion on HPLC connections

1. Purge the pumping system of any buffer-containing mobile phases and connect the inlet end of the column to the injector outlet. An arrow on the column identification label indicates the correct direction of solvent flow.
2. Flush column with 100% organic mobile phase (methanol or acetonitrile) by setting the pump flow rate to 0.1 mL/min. and increase the flow rate to 1 mL/min over 5 minutes.
3. When the mobile phase is flowing freely from the column outlet, stop the flow and attach the column outlet to the detector. This prevents entry of air into the detection system and gives more rapid baseline equilibration.
4. Gradually increase the flow rate as described in step 2.
5. Once a steady backpressure and baseline have been achieved, proceed to the next section.

Note: If mobile phase additives are present in low concentrations (e.g., ion-pairing reagents), 100 to 200 column volumes may be required for complete equilibration. In addition, mobile phases that contain formate (e.g., ammonium formate, formic acid, etc.) may also require longer initial column equilibration times.

b. Column Equilibration

YMC columns are delivered in the test solvent shown on the performance report, with the exception of J'sphere™ and Cartenoid™ columns, which are shipped in 100% ACN and 70%/30% ACN/H_2O respectively. It is important to ensure mobile phase compatibility before changing to a different mobile phase system. Equilibrate the column with a minimum of 10 column volumes of the mobile phase to be used (refer to Table 1 for a listing of empty column volumes).

To avoid precipitating out mobile phase buffers on your column or in your system, flush the column with five column volumes of a water/organic solvent mixture, using the same or lower solvent content as in the desired buffered mobile phase. (For example, flush the column and HPLC system with 60% methanol in water prior to introducing 60% methanol/40% buffer mobile phase).

c. Initial Column Efficiency Determination

1. Perform an efficiency test on the column before using it in the desired application. It is recommended to use a suitable solute mixture, as found in the Quality Control Report to analyze the column upon receipt.
2. Determine the number of theoretical plates (N) and use this value for periodic comparisons.
3. Repeat the test at predetermined intervals to track column performance over time. Slight variations may be obtained on two different HPLC systems due to the quality of the connections, operating environment, system electronics, reagent quality, column condition and operator technique.

Table 1: Empty Column Volumes in mL (multiply by 10 for flush solvent volumes)

	Column internal diameter (mm)						
Column Length (mm)	**1.0**	**2.0**	**3.0**	**4.6**	**10**	**20**	**50**
20	–	0.06	0.14	0.33	–	–	–
30	–	0.09	0.21	0.50	2.4	8.5	–
50	0.04	0.6	0.35	0.83	3.9	16	98
100	0.08	0.31	0.71	1.7	7.8	31	–
150	0.12	0.47	1.0	2.5	12	47	294
250	–	0.79	1.8	4.2	20	79	490

II. COLUMN USE

To ensure the continued high performance of YMC columns, follow these guidelines:

a. Guard columns

Use a YMC guard column of matching chemistry and particle size between the injector and main column. It is important to use a high-performance matching guard column to protect the main column while not compromising or changing the analytical resolution. Guard columns need to be replaced at regular intervals as determined by sample contamination. When system backpressure steadily increases above a set pressure limit, it is usually an indication that the guard column should be replaced. A sudden appearance of split peaks is also indicative of a need to replace the guard column.

b. Sample Preparation

1. Sample impurities often contribute to column contamination. One option to avoid this is to use Waters Oasis® solid-phase extraction cartridges/columns or Sep-Pak® cartridges of the appropriate chemistry to clean up the sample before analysis.
2. It is preferable to prepare the sample in the operating mobile phase or a mobile phase that is weaker (less organic modifier) than the mobile phase for the best peak shape and sensitivity.
3. If the sample is not dissolved in the mobile phase, ensure that the sample, solvent and mobile phases are miscible in order to avoid sample and/or buffer precipitation.
4. Filter sample with 0.2 µm filters to remove particulates. If the sample is dissolved in a solvent that contains an organic modifier (e.g., acetonitrile, methanol, etc.) ensure that the filter material does not dissolve in the solvent. Contact the filter manufacturer with solvent compatibility questions. Alternatively, centrifugation for 20 minutes at 8,000 rpm, followed by the transfer of the supernatant liquid to an appropriate vial, could be considered.

c. Recommended pH Ranges for YMC Column Chemistries

Recommended pH ranges for solvent and buffer combinations are between 2 and 7 for most YMC columns, unless other wise indicated. A pH less than 2 may cause hydrolysis of the bonded phase. At a pH greater than 7, the alkaline solvent buffers will attack the silica substrate resulting in void formation in the column as the silica solubilizes.

Table 2: Recommended pH Limits for YMC Columns Chemistries

Product	pH Range (room temp)
Pro C18™	2-8
Pro C18 RS™	1-10
J'sphere™	1.1-9.3(H) 2-7(M,L)
ODS-AM™	2-7
ODS-A™	2-7
ODS-AL™	2-6
ODS-AQ™	2-6.3
PolymerC18™	2-12
YMCbasic™	2-7
C8 (Octyl)	2-7
C4 (Butyl)	2-7
Protein-RP™	1.5-7
C1 (TMS)	2-7
Phenyl	2-7
CN (Cyano)	2-7
*The Carotenoid Column™	2-6
*PVA-Sil™	2-9.5
Diol	2-7
Aqueous GPC/SEC	2-7
Silica	NA
NH_2 (Amino)	2-7
*PA gel™	2-9
*Polyamine II™ (PBMN)	2-9
Hydrosphere™ C_{18}	2-8

d. Solvents

To maintain maximum column performance, use high quality chromatography grade solvents. Filter all aqueous buffers prior to use. Pall Gelman Laboratory Acrodisc® filters are recommended. Solvents containing suspended particulate materials will generally clog the outside surface of the inlet distribution frit of the column. This will result in higher operating pressure and poor performance. Degas all solvents thoroughly before use to prevent bubble formation in the pump and detector. The use of an on-line degassing unit is also recommended. This is especially important when running low pressure gradients since bubble formation can occur as a result of aqueous and organic solvent mixing during the gradient.

e. Pressure

The column backpressure is a function of several parameters.

- Particle size and distribution
- Packing porosity and bonded phase coating levels
- Column dimensions (diameter and length)
- Solvent viscosity, flow rate, and temperature

a. 120Å pore size, 5 micron ODS in a 4.6 x 250 mm column should be less than 250 kg/cm^2 or 3750 psi at 1 mL/min with 70/30 MeOH/water at ambient temperature.

b. 200Å and 300Å wide-pore, 5 micron ODS in a 4.6 x 250 mm column should be less that 170 kg/cm^2 or 2550 psi at 1 mL/min with 70/30 MeOH/water at ambient temperature.

c. For columns 1 mm i.d. to 30 mm i.d., the operating pressure should be as follows:

For i.d. < 6 mm and lengths ≤ 150 mm the operating pressure should be kept under 200 kg/cm^2 or 3000 psi.

For i.d. < 6 mm and lengths of 250 mm the operating pressure should be kept under 250 kg/cm^2 or 3750 psi.

For i.d. > 10 mm the operating pressure should be kept under 100 kg/cm^2 or 1500 psi

PolymerC18 has a pressure limit of 145 kg/cm^2 or 2000 psi.

For 50 mm i.d. and larger, operating pressure needs to be less than 1050 psi if it is packed with a 5 µm particle (unless certified for 2000 psi operation). If it is packed with ≥ 10 µm particle then the operating pressure should be kept under 20-30 kg/cm^2 or 300-600 psi.

Note: It is recommended that all YMC semiprep columns of 20 mm i.d. and larger be operated at pressures below 2400 psi. High pressure may result in a loss of performance.

f. Temperature

Temperatures between 20 °C – 50 °C are recommended for operating YMC columns in order to enhance selectivity, lower solvent viscosity and increase mass transfer rates. However, any temperature above ambient will have a negative effect on lifetime which will vary depending on the pH and buffer conditions used.

III. SCALING UP/DOWN ISOCRATIC METHODS

The following formulas will allow scale up or scale down, while maintaining the same linear velocity, and provide new sample loading values:

If column i.d. and length are altered:

$$F_2 = F_1 (r_2/r_1)^2$$

$$Load_2 = Load_1 (r_2/r_1)^2 (L_2/L_1)$$

$$Injection\ volume_2 = Injection\ volume_1 (r_2/r_1)^2 (L_2/L_1)$$

Where: r = Radius of the column

F = Flow rate

L = Length of column

1 = Original, or reference column

2 = New column

IV. TROUBLESHOOTING

Changes in retention time, resolution, or backpressure are often due to column contamination. See the Column Cleaning, Regeneration and Storage section of this Care and Use Manual. Information on column troubleshooting problems may be found in HPLC Columns Theory, Technology and Practice, U.D. Neue, (Wiley-VCH, 1997), the Waters HPLC Troubleshooting Guide (Literature code # 720000181EN) in the Waters Application Library or visit the Waters Corporation website for information on seminars (www.waters.com).

VI. COLUMN CLEANING, REGENERATION AND STORAGE

a. Cleaning and Regeneration

Changes in peak shape, peak splitting, shoulders on the peak, shifts in retention, change in resolution or increasing backpressure may indicate contamination of the column. Flushing with a neat organic solvent, taking care not to precipitate buffers, is usually sufficient to remove the contaminant. If the flushing procedure does not solve the problem, purge the column using the following cleaning and regeneration procedures.

Use the cleaning routine that matches the properties of the samples and/or what you believe is contaminating the column (see Table 4). Flush columns with 20 column volumes each of HPLC-grade solvents (e.g., 80 mL total for 4.6 x 250 mm column) listed in Table 4. Increasing mobile phase temperature to 35-50 °C increases cleaning efficiency. If the column performance is poor after cleaning and regeneration, call your local Waters office for additional support.

Table 4: Cleaning and Regeneration Sequence or Options

Polar Samples	Non-polar Samples	Proteinaceous Samples
1. water	1. isopropanol (or anappropriate isopropanol/water mixture*)	Option 1: Inject repeated aliquots of dimethyl sulfoxide (DMSO)
2. tetrahydrofuran (THF)	2. methanol	Option 2: gradient of 10% to 90% B where: A = 0.1% trifluoroacetic acid (TFA) in water B = 0.1% trifluoroacetic acid (TFA) in acetonitrile (CH_3CN)
3. tetrahydrofuran (THF)	3. dichloromethane	
4. methanol	4. hexane	
5. water	5. isopropanol (followed by an appropriate isopropanol/water mixture*)	Option 3: Flush column with 7M guanidine hydrochloride, or 7M urea
6. mobile phase	6. mobile phase	

* Use low organic solvent content to avoid precipitating buffers.

b. Storage

For periods longer than four days at room temperature, store the column in 100% acetonitrile. Immediately after use with elevated temperatures and/or at pH extremes, store in 100% acetonitrile for the best column lifetime. Do not store columns in highly aqueous (<20% organic) mobile phases, as this may promote bacterial growth. If the mobile phase contained a buffer salt, flush the column with 10 column volumes of HPLC grade water (see Table 1 for common column volumes) and replace with 100% acetonitrile for storage. Failure to perform this intermediate step could result in precipitation of the buffer salt in the column or system when 100% acetonitrile is introduced. Completely seal column to avoid evaporation and drying out of the bed.

Note: If a column has been run with a mobile phase that contains formate (e.g., ammonium formate, formic acid, etc.) and is then flushed with 100% acetonitrile, slightly longer equilibration times may be necessary when the column is re-installed and run again with a formate-containing mobile phase.

V. CONNECTING THE COLUMN TO THE HPLC

a. Column Connectors and System Tubing Considerations

Tools needed:

- 3/8 inch wrench
- 5/16 inch wrench

Handle the column with care. Do not drop or hit the column on a hard surface as it may disturb the bed and affect its performance.

1. Correct connection of 1/16 inch outer diameter stainless steel tubing leading to and from the column is essential for high-quality chromatographic results.
2. When using standard stainless steel compression screw fittings, it is important to ensure proper fit of the 1/16 inch outer diameter stainless steel tubing. When tightening or loosening the compression screw, place a 5/16 inch wrench on the compression screw and a 3/8 inch wrench on the hex head of the column endfitting.

Note: If one of the wrenches is placed on the column tube flat during this process, the endfitting will be loosened and leak.

3. If a leak occurs between the stainless steel compression screw fitting and the column endfitting, a new compression screw fitting, tubing and ferrule must be assembled.
4. An arrow on the column identification label indicates correct direction of solvent flow.

Correct connection of 1/16 inch outer diameter stainless steel tubing leading to and from the column is essential for high-quality chromatographic results. To obtain a void-free connection, the tubing must touch the bottom of the column endfitting. It is important to realize that extra column peak broadening due to voids can destroy an otherwise successful separation. The choice of appropriate column connectors and system tubing is discussed in detail below.

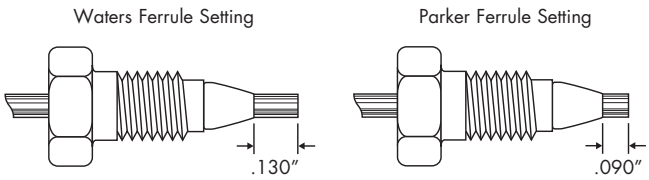

Figure 1: Waters and Parker Ferrule Types

Note: YMC columns are supplied with Waters-style endfittings.

Due to the absence of an industry standard, various column manufacturers have employed different types of chromatographic column connectors. The chromatographic separation can be negatively affected if the style of the column endfittings does not match the existing tubing ferrule settings. This section explains the differences between Waters style and Parker style ferrules and endfittings (Figure 1). Each endfitting style varies in the required length of the tubing protruding from the ferrule. The YMC column is equipped with Waters style endfittings that require a 0.130 inch ferrule depth. If a non-Waters style column is presently being used, it is critical that ferrule depth be reset for optimal performance prior to installing a YMC column. In a proper tubing/column connection (Figure 2), the tubing touches the bottom of the column endfitting, with no void between them.

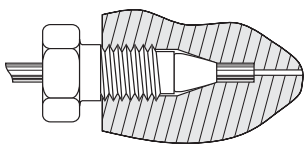

Figure 2: Proper Tubing/Column Connection

The presence of a void in the flow stream reduces column performance. This can occur if a Parker ferrule is connected to a Waters style endfitting (Figure 3).

Note: A void appears if tubing with a Parker ferrule is connected to a Waters style column.

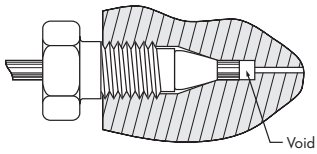

Figure 3: Parker Ferrule in a Waters Style Endfitting

There is only one way to fix this problem: Cut the end of the tubing with the ferrule, place a new ferrule on the tubing and make a new connection. Before tightening the screw, make sure that the tubing bottoms out in the endfitting of the column. Conversely, if tubing with a Waters ferrule is connected to a column with Parker style endfitting, the end of the tubing will bottom out before the ferrule reaches its proper sealing position. This will leave a gap and create a leak (Figure 4).

Note: The connection leaks if a Waters ferrule is connected to a column with a Parker style endfitting.

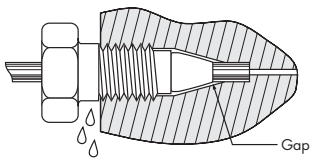

Figure 4: Waters Ferrule in a Parker Style Endfitting

There are two ways to fix the problem:

1. Tighten the screw a bit more. The ferrule moves forward, and reaches the sealing surface. Do not overtighten since this may end in breaking the screw.

2. Cut the tubing, replace the ferrule and make a new connection. Alternatively, replace the conventional compression screw fitting with an all-in-one PEEK fitting (Waters part number PSL613315) that allows resetting of the ferrule depth. Another approach is to use a Keystone, Inc. SLIPFREE® connector to always ensure the correct fit. The fingertight SLIPFREE® connectors automatically adjust to fit all compression screw type fittings without the use of tools (Figure 5).

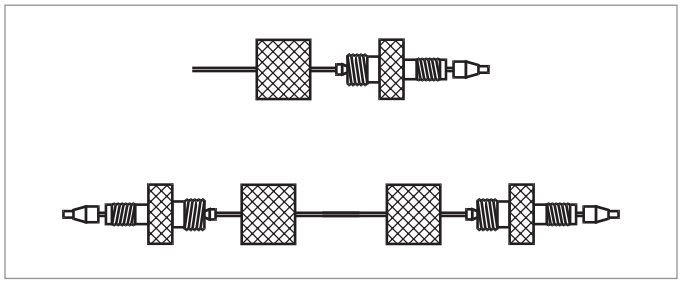

Figure 5: Single and Double SLIPFREE® Connectors

SLIPFREE® Connector Features:

- Tubing pushed into endfitting, thereby guaranteeing a void-free connection
- Connector(s) come(s) installed on tubing
- Various tubing IDs and lengths available
- Fingertight to 10,000 psi – Never needs wrenches
- Readjusts to all column endfittings
- Compatible with all commercially available endfittings
- Unique design separates tube-holding function from sealing function

Table 5: Waters Part Numbers for SLIPFREE® Connectors

SLIPFREE® Type	Tubing Internal Diameter		
Tubing Length	0.005"	0.010"	0.020"
Single 6 cm	PSL 618000	PSL 618006	PSL 618012
Single 10 cm	PSL 618002	PSL 618008	PSL 618014
Single 20 cm	PSL 618004	PSL 618010	PSL 618016
Double 6 cm	PSL 618001	PSL 618007	PSL 618013
Double 10 cm	PSL 618003	PSL 618009	PSL 618015
Double 20 cm	PSL 618005	PSL 618001	PSL 618017

Band Spreading Minimization

Figure 6 shows the influence of tubing internal diameter on system band spreading and peak shape. As can be seen, the larger tubing diameter causes excessive peak broadening and lower sensitivity.

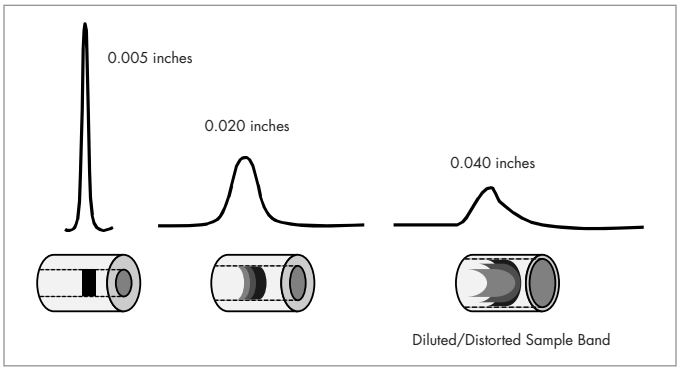

Figure 6: Effect of Connecting Tubing on System

b. Measuring System Bandspreading Volume and System Variance

This test should be performed on an HPLC system with a single wavelength UV detector (not a Photodiode Array (PDA)).

1. Disconnect column from system and replace with a zero dead volume union.
2. Set flow rate to 1 mL/min.
3. Dilute a test mix in mobile phase to give a detector sensitivity of 0.5 - 1.0 AUFS (system start up test mix can be used which contains uracil, ethyl and propyl parabens; Waters part number WAT034544).
4. Inject 2 to 5 µL of this solution.
5. Measure the peak width at 4.4% of peak height (5-sigma method):

   5-sigma Bandspreading (µL) = Peak Width (min) x Flow Rate (mL/min) x (1000 µL/1 mL) System Variance (µL2) = (5-sigma bandspreading)2 / 25

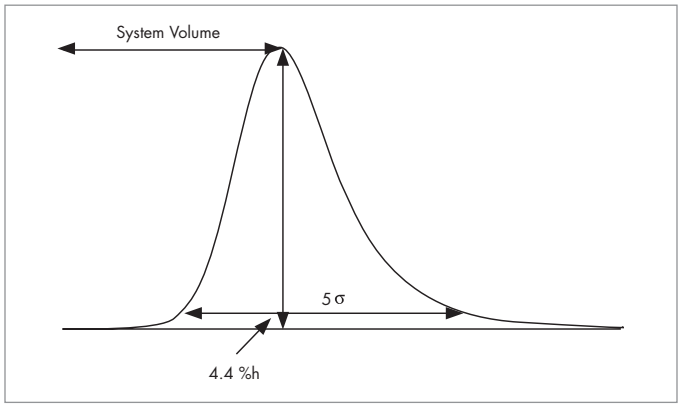

Figure 7: Determination of System Bandspreading Volume Using 5-Sigma Method

In a typical HPLC system, the Bandspreading Volume should be no greater than 100 µL ± 30 µL (or Variance of 400 μL^2 ± 36 μL^2).

In a microbore (2.0 mm i.d.) system, the Bandspreading Volume should be no greater than 20 to 40 µL (or Variance no greater than 16 μL^2 to 64 μL^2).

c. Measuring Gradient Delay Volume (or Dwell Volume)

For successful gradient-method transfers the gradient delay volumes should be measured using the same method on both HPLC systems. The procedure below describes a method for determining the gradient delay volumes.

1. Replace the column with a zero dead volume union.
2. Prepare mobile phase A (pure solvent, such as methanol) and mobile phase B (mobile phase A with a UV absorbing sample, such as (v/v) 0.1% acetone in methanol).
3. Equilibrate the system with mobile phase A until a stable baseline is achieved.
4. Set the detector wavelength to the absorbance maximum of the probe (265 nm for acetone).
5. Program a 0-100% B linear gradient in 10 min at 2 mL/min (the exact conditions are not critical; just make sure the gradient volume is at least 20 mL) with a hold at 100% B.
6. Determine the dwell time by first locating the time at the midpoint of the formed gradient (t1/2) (half the vertical distance between the initial and final isocratic segments as shown in Figure 8).
7. Subtract half the gradient time (1/2 tg) (10 min/2 = 5 min in this example) from the gradient midpoint (t1/2) to obtain the dwell time (tD).
8. Convert the dwell time (tD) to the dwell volume (VD) by multiplying by the flow rate (F). Dwell Volume VD = (t1/2—1/2 tg) x F For fast gradient methods, the gradient delay volume (or dwell volume) should be less than 1 mL. If the gradient delay volume is greater than 1 mL, see System Modification Recommendations section on how to reduce system volume.

VII. ADDITIONAL INFORMATION

a. Use of Narrow-Bore (3.0 mm i.d.) Columns

This section describes how to minimize extra column effects and provides guidelines on maximizing the performance of a narrow-bore column in an HPLC system. A 3.0 mm i.d. narrow-bore column usually requires no system modifications. A 2.0 mm i.d. column, however, requires modifications to the HPLC system in order to eliminate excessive system bandspreading volume. Without proper system modifications, excessive system band spreading volume causes peak broadening and has a large impact on peak width as peak volume decreases.

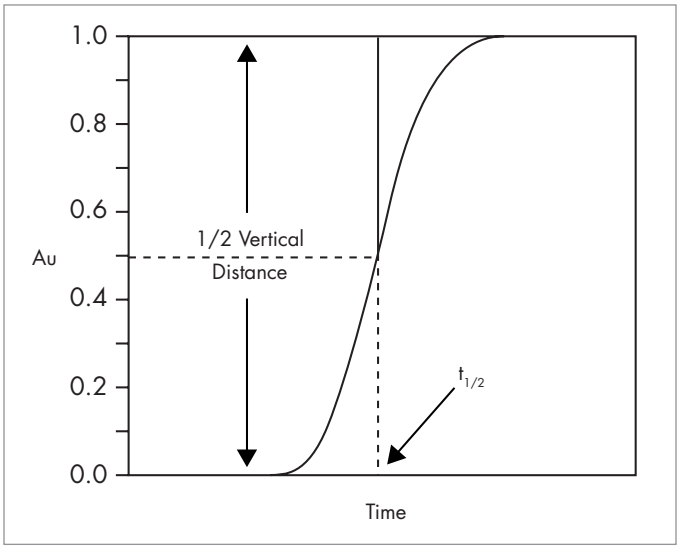

Figure 8: Determination of Gradient Delay Volume

b. Impact of Bandspreading Volume on 2.0 mm i.d. Column Performance

System with 70 µL bandspreading: 10,000 plates

System with 130 µL bandspreading: 8,000 plates (same column)

Note: Flow splitters after the column will introduce additional bandspreading.

System optimization, especially in a system that contains a flow splitter, can have dramatic effects on sensitivity and resolution. Optimization includes using correct ferrule depths and minimizing tubing inner diameters and lengths. An example is given in Figure 9 where system optimization resulted in a doubling of sensitivity and resolution of the metabolite in an LC/MS/MS system.

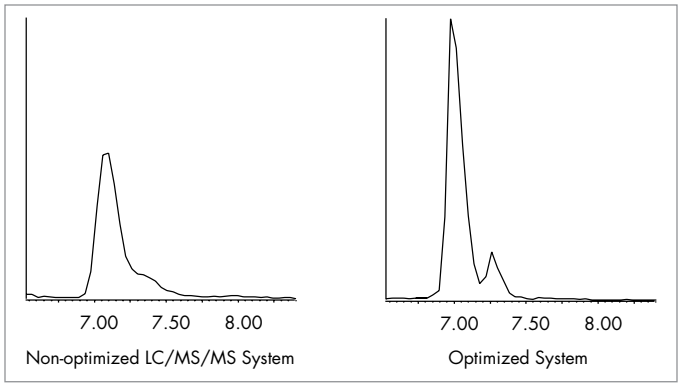

Figure 9: Non-Optimized vs. Optimized LC/MS/MS System

c. Non-Optimized vs. Optimized LC/MS/MS System: System Modification Recommendations

1. Use a microbore detector flow cell with 2.0 mm i.d. columns.

Note: Detector sensitivity is reduced with the shorter flow cell path length in order to achieve lower bandspreading volume.

2. Minimize injector sample loop volume.
3. Use 0.009 inch (0.25 mm) tubing for rest of connections in standard systems and 0.005 inch (0.12 mm) tubing for narrowbore (2.0 mm i.d.) systems.
4. Use perfect (pre-cut) connections (with a variable depth inlet if using columns from different suppliers).
5. Detector time constants should be shortened to less than 0.2 seconds.

d. Instructions for YMC-Ultra-HT *Pro* C18 Columns

1. Recommendations for column connection, detector settings and data processing considerations

- When the tubing connection results in unnecessary extra-column volume, the user may experience a decrease in the number of theoretical plates and/or increase in peak shape distortion. Please make sure of the integrity of fluidic connections to the column.
- Fluidic connections between the column, injector, and detector should be as short as is practical and extra-column volumes minimized by using small inner diameter tubing (less than 0.15mm).
- Please use a detector flow cell designed for use with smaller particle columns (for example a semi-micro or for micro-bore flow cell). If the volume of the detector flow cell is too large, the sample band expands and peak performance degrades considerably. Optimize the response of detector (time constant) and the data-sampling speed of data-processing equipment in order to accept peaks having short band widths.

2. Mobile phase and sample solvent

- The solvent enclosed at shipment is acetonitrile/water (60/40). If you intend to store the column for a long time replace the mobile phase in the column with shipping solvent.
- Solvent should flow in the direction of the arrow as indicated on the column label.
- Aqueous to nonaqueous solvents are applicable. However continuous replacement of solvents, which have wide differences in polarity may degrade the column performance.
- When preparing mobile phase, the pH should be kept within the range of 2.0 to 8.0. When using mobile phase pH near the upper and lower limits, mobile phase containing 10% concentration of organic solvent should be used. The column durability will shorten under certain conditions by temperature and mobile phase composition.
- When replacing the mobile phase, solvents need to be miscible. Deposition of salts should be carefully monitored and salts should be washed from the column before storage.

- When possible, the sample should be dissolved in a solvent in the same composition as the starting mobile phase composition. Using solvent compositions that are stronger than the mobile phase for sample dissolution may result in distortion of peak symmetry and/or degradation of peak resolution and hence an decrease in analytical reproducibility.
- In order to prevent exposure of the column to excessive pressures, the mobile phase should be filtered and sample solution should be filtered through an a Pall Gelman Laboratory Acrodisc folter (0.2 µm) before injection.

3. Column cleaning (general method)

- When a mobile phase containing neither buffer nor salts is used and you are trying to remove substances that show great retention on the column, wash the column with a solvent composition of higher organic solvent concentration to remove substances that otherwise would remain in the column. When a lipophilic component is stubbornly retained on the column, the addition of THF to the column wash solvent may be effective in removing strongly retained substances.
- When a mobile phase containing buffer or salts is used, wash as above after replacement of the mobile phase with a mixture of water and organic solvent (at the same ratio as the mobile phase but containing neither buffer nor salts). When a mobile phase containing buffer or salts is used at about 100 mM, the mobile phase can be replaced directly with about 60% acetonitrile aqueous solution.
- After using a column near the usable pH limit, washing the column with water alone may cause column deterioration. Use the mixture of water and organic solvent described above or use about a 60% acetonitrile aqueous solution to replace the mobile phase.
- When macromolecules including protein and sugars adsorb to the column, it is usually difficult to wash out the macromolecules with organic solvents. When a sample containing macromolecules is analyzed, it is preferable to conduct sample pretreatment prior to introducing the sample to the column.

4. Other environments

- The upper limit of column pressure is about 50 Mpa (7,250 psi).
- The upper limit of column temperature is 50°C. However, we recommend using the column between 20°C and 40°C because some conditions of usage, such as operating at the extremes of pH of the mobile phase, may shorten the column lifetime especially at the upper temperature limit of the column.

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