Technical Information

Column Handling

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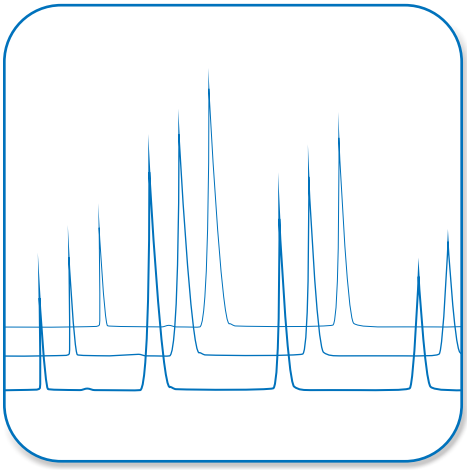
Troubleshooting

and more



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Technical Information

Introduction

Technical Information

YMC produces chromatography packing materials and HPLC columns under very strict Quality Control procedures and supplies to customers only those products which pass the strict Quality Assurance tests prior to shipment.
In order to ensure the best performance and long column life, the following instructions should be followed for all packed columns.

Column Handling

- ***Shipping solvent***
- ***Mobile phase considerations***
- ***Mobile phase replacement and column cleaning***
- ***Guard columns***
- ***Column back pressures***
- ***Temperature***

1. Shipping solvent

The solvent used for shipping the column is described on the COLUMN INSPECTION REPORT or in the COLUMN CARE AND USE INSTRUCTIONS. Please determine the miscibility of this solvent with the mobile phase used in your analysis to prevent immiscibility problems. If you intend to store the column for any length of time, you should replace the mobile phase in the column with the shipping solvent or solvent specified in the column inspection report.

2. Mobile phase considerations

Reversed phase columns can be used with both aqueous and nonaqueous solvents. However, repeated alternating between solvents with extremely different polarities can result in loss of column performance. Typical general organic solvents include acetonitrile, methanol and THF.
Cyano columns can be used in both normal and reversed phase modes. However, a column should be dedicated for use in only one separation mode and not switched between normal and reversed phase modes as this can result in loss of column performance. When using the column in a normal phase mode, replace the solvent in the column with isopropanol. (Make sure that the flow rate is set so that the pressure does not exceed 15 MPa during solvent exchange.) Silica columns are usually used with nonaqueous solvents such as n-hexane, chloroform or other weak solvents with the addition of isopropanol, ethyl acetate or similar as appropriate to allow elution of high polarity components.
All Amino columns (i.e. both YMC-Pack Polyamine II and YMC-Pack Amino) can be used with both aqueous and nonaqueous solvents. However, repeated alternating of solvents with extremely different polarities can result in loss of column performance.
Solvent should flow in the direction of the arrow (as indicated on the column label) for normal use, although reversed flow for washing will not affect column stability.
The pH ranges for stability of every type of column varies by product. For specific information please refer to COLUMN CARE AND USE INSTRUCTIONS, downloadable from www.ymc.eu, of each column.

3. Mobile phase replacement and column cleaning (general methods)

a) Reversed phase columns
When a mobile phase which contains no buffers or salts is used, wash the column with an eluent consisting of the same solvents as that of the mobile phase, but with a higher organic solvent concentration.
When a mobile phase containing buffers or salts is used, this should first be replaced with an eluent containing the same ratio of water and organic solvent as the mobile phase but which has no buffer or salt components. If the concentration of buffer or salts used is less than 100 mM, it can be replaced directly with approximately 60% acetonitrile in water. After using a column near the usable pH limit, washing the column with water alone may cause column deterioration. Instead wash the column with a mixture of water and organic solvent containing no buffer or salt components or alternatively 60% acetonitrile in water to remove the aggressive pH eluents.
Should the column back pressure increase, wash the column in the reverse direction (the opposite direction of the arrow shown on the column label) making sure that the detector is not in line with the solvent stream. A solution having the same composition as that of the mobile phase, but with a higher organic solvent concentration and no added salts or buffers is usually used as the cleaning solution. However consideration should be given to the characteristics of sample so that a solvent which easily dissolves the sample is chosen.

Column Handling

When macromolecules, including proteins and sugars, adsorb onto the column, it is usually difficult to wash them off with organic solvents. When columns are used to analyse samples containing such macromolecules, it is preferable to pretreat the sample and/or use a guard column.

b) Normal phase columns

Wash the column with a solution having the same solvents as that of the mobile phase, but with an increased content of high polar component concentration. If polar compounds absorb on the column, flush with isopropanol or similar solvent.

Before storing a column used with a mobile phase containing acid or alkali, replace the eluent with a simple solvent or solvent/water mixture (for example replace n-hexane/isopropanol/acetic acid (90/10/0.1) with n-hexane/isopropanol (90/10) for storage).

RECOMMENDED COLUMN CLEANING AND REGENERATING PROCEDURES

Use the cleaning routine that matches the properties of the column and what you believe is contaminating it. Flush columns with 20 column volumes (80 mL total for 4.6 x 250 mm column) of HPLC-grade solvents. Run columns in reverse flow direction, with the outlet disconnected from the detector. Cleaning efficiency is increased by increasing mobile phase temperature to 35-55°C. If the column performance is poor after regenerating and cleaning, please feel free to contact YMC directly either by phone (+49 (0) 2064 427-0), by mail (info@ymc.eu) or use online chat on our homepage (www.ymc.eu).

Silica-/Hybrid-/Core-Shell-based particles

Non-polar-bonded phases (Carotenoid, C18, Octyl, YMCbasic J'sphere, Phenyl, PFP, Butyl, TMS)

Polar Samples	Non-polar Samples	Proteinaceous Samples
1. Water	1. Isopropanol	Option 1: Inject repeated aliquots of DMSO
2. Methanol	2. THF	Option 2: Gradient of 10 to 90% B where:
3. THF	3. Dichlormethane	A = 0.1% TFA in water
4. Methanol	4. Hexane	B = 0.1% TFA in CH_2CN
5. Water	5. Isopropanol	Option 3: Flush column with 7M guanidine
6. Mobile phase	6. Mobile phase	HCl, or 7M urea

Polar-bonded phases (Cyano, Diol, Amino, PVA-Sil, Silica):

Polar Samples	Non-polar Samples
1. Water	1. Chloroform
2. Methanol	2. Methanol
3. THF	3. Dichlormethane
4. Methanol	4. Heptane or Isocyanate
5. Water	5. Isopropanol
6. Mobile phase	6. Mobile phase

Polymer-based particles: Polymer C18

1. Flush column with mobile phase but omit buffers or salts (i.e. just organic and water, acetonitrile is preferable)
2. Run a gradient to 100% organic
3. Flush with twenty column volumes of THF
4. Flush with twenty column volumes of acetonitrile
5. Run a gradient back to starting mobile phase conditions, ommitting buffers and salts
6. Re-equilibrate in mobile phase

4. Guard columns

YMC recommends that you always use a guard column with the same packing material and of the recommended inner diameter for your column (see table).

A YMC guard column is normally composed of a cartridge holder and a guard cartridge. The cartridge holder can be used repeatedly.

Where different cartridge lengths are available, only chose the longer cartridge when samples containing high levels of contaminants are present to increase the time between cartridge changes.

Guard cartridges should be changed frequently in order to maximise their protection of the main column. Cartridge holders should be connected to the main column using the shortest length of tubing possible. This tubing should be of an appropriate inner diameter for the flow rate and pressure to be used.

Samples containing particulate matter MUST always be pre-filtered (at least 0.45 µm but 0.2 µm is preferred and essential for UHPLC) before being injected onto a column.

Column ID [mm]	Recommended Guard Cartridge ID [mm]
1.0	2.1
2.0 / 2.1	2.1
3.0	3.0
4.0	4.0
4.6	4.0*
10	10
20	20
30	30

*As a result of intense testing of the compatibility of different hardware concepts no negative influence of a 4.0 mm ID guard cartridge combined with a 4.6 mm ID main column was observed.
Therefore, we recommend the use of 4.0 mm ID guards with a 4.6 mm ID analytical column.

Column Handling

5. Column Back Pressures

Column back pressure is a function of several parameters, including

- particle size and distribution
- packing porosity and bonded phase coating levels
- column length and inner diameter
- solvent flow rate, viscosity and temperature

Typically for a column packed with 12 nm, 5 µm ODS phase and pumped at ambient temperature with methanol:water (70:30) at 1 mL/min the back pressure should be less than 25 MPa (250 bar, 3,750 psi) for 250 x 4.6 mm ID.
For wide pore (20 or 30 nm) 5 µm ODS phase and pumped at ambient temperature with methanol:water (70:30) at 1 mL/min the back pressure should be less than 17 MPa (170 bar, 2,550 psi) for 250 x 4.6 mm ID.
We recommend using a column at below the maximum operating pressure to ensure maximum column life.

Column ID [mm]	Maximum Operating Pressure [MPa]	[bar]	[psi]
YMC-Triart 1.9 µm	100	1,000	15,000
YMC-Triart 3/5 µm[1]	45 (20/25)	450 (200/250)	6,525 (3,000/3,750)
Meteoric Core 2.7 µm	60	600	8,700
YMC UltraHT 2 µm	50	500	7,500
YMC-Pack Diol UHPLC 2 µm	45	450	6,525

Column ID [mm]	Maximum Operating Pressure [MPa]	[bar]	[psi]
0.075	55	550	7,975
0.1	55	550	7,975
0.3	55 / 60[2]	550 / 600[2]	7,975 / 8,700[2]
0.5	55 / 60[2]	550 / 600[2]	7,975 / 8,700[2]
1.0	20 / 25[3]	200 / 250[3]	3,000 / 3,750[3]
2.0 / 2.1	20 / 25[3]	200 / 250[3]	3,000 / 3,750[3]
3.0	20 / 25[3]	200 / 250[3]	3,000 / 3,750[3]
4.0	20 / 25[3]	200 / 250[3]	3,000 / 3,750[3]
4.6	20 / 25[3]	200 / 250[3]	3,000 / 3,750[3]
6.0	20 / 25[3]	200 / 250[3]	3,000 / 3,750[3]
8.0	20 / 25[3]	200 / 250[3]	3,000 / 3,750[3]
10	10	100	1,500
20 / 30	10	100	1,500
YMC-Actus 20 / 30	30	300	4,500
YMC-Actus 50	20	200	2,900

[1] previous hardware in brackets

[2] The first figure is for particle sizes of 2/3/5 µm, the second figure is for particle size of 1.9 µm.

[3] The first figure is for up to 150 mm column length, the second figure is for 250 mm column length.

6. Temperature

The upper temperature limit for silica and bonded phases is 50 °C (90 °C for YMC-Triart C18/C18 ExRS/C8/C4, 70 °C for Meteoric Core C18/C18 BIO at pH=7 or lower). However YMC recommends using columns between 20 and 40 °C because certain conditions of pH or mobile phase composition may affect column lifetime. For recommended column temperatures for other column types, please refer to the instruction manual included with each column.

Mobile Phases for RP Columns

Mobile Applications for reversed phase columns

The composition of mobile phase greatly affects the separation in HPLC. To optimise a separation, it is necessary to consider the interaction between the solutes, stationary (or solid) phase, as well as the mobile phase.

For reversed phase columns, the most commonly used in HPLC, various mobile phases are available. Attention needs to be paid to a number of points when deciding on the mobile phase composition.

The variable factors to be considered include:

- miscibility of solvents
- effects on detection methods (eg., UV or MS)
- effects on the column
- column deterioration due to pressure or pH)
- separation reproducibility
- stability of solutes

Typical solvents for ODS columns and some helpful tips for establishing optimum separation conditions are described below.

General solvents

Water, acetonitrile, methanol and tetrahydrofuran (THF) are the important solvents for use with reversed phase columns.
It is important to use high purity water purified by ion-exchange, distillation, reverse osmosis, etc. The presence of organic substances or ionic impurities may cause problems, including ghost peaks during short wavelength UV detection or high sensitivity gradient elution systems.
Acetonitrile is frequently used as an HPLC solvent, due to its low UV absorption and low viscosity. Methanol has a higher viscosity and often shows different separation selectivity to that obtained using acetonitrile. THF is used occasionally to influence selectivity in conjunction with acetonitrile and methanol, due to the cyclic ether structure of THF. THF has several adverse properties for a solvent for HPLC; it has:

- significant UV absorption
- high viscosity
- a tendency to form peroxides, especially as the use of antioxidants can give rise to ghost peaks.

Appropriate separating conditions can be obtained by using these three solvents plus water individually or in combination.

Buffers and reagents

Acetic acid, formic acid, phosphoric acid and trifluoroacetic acid (TFA) are generally used as acidic modifiers. The buffers normally used include phosphate and acetate buffers (sodium, potassium, ammonium). Monobasic phosphates provide a pH of 4.6 and are used as convenient pH adjusters rather than buffers.
In order to separate ionic compounds, such as amines and carboxylic acids, with good repeatability, the pH of mobile phase must be adjusted so that it is 1 (or preferably 2) units away from the pKa of the solute. At or near the pKa, peak broadening or splitting may be observed as the free acid/base and its salt coexist.
Most buffers are used at a concentration of about 10 mM. However, depending on dissociation of solutes and interactions with the stationary phase, this can be raised to 100 mM.

When acids or alkalis which degrade reversed phases are used, caution must be taken regarding their concentrations and pH. TFA and phosphoric acid are usually used at concentrations of 0.1% or less. Acetonitrile/water (approx 60/40) solution is a convenient storage solvent after use of acids or buffers (salts).
Tetrabutylammonium salts and sodium perchlorate may be used as ion pair reagents for retention of highly polar compounds on reversed phase columns or for improvement of separation and peak shape. When these additives are used, it is necessary to use a reagent with the shortest alkyl chains available. If sodium dodecylsulfate, (SDS; which contains long alkyl chains) is used, it may be retained on the column phase and can cause problems with repeatability.

Other solvents for HPLC

Ethanol, 2-propanol, ethyl acetate, or chloroform may be used in the mobile phase (particularly in normal phase separations) in order to improve retention or separation of solutes. In some cases, hexane is used as a mobile phase.

When a hydrophobic solvent is added to a mobile phase, care must be taken with regards to the miscibility with the mobile phase existing in the column and a separate wash stage should be included before changing the eluent.

HPLC Column Performance

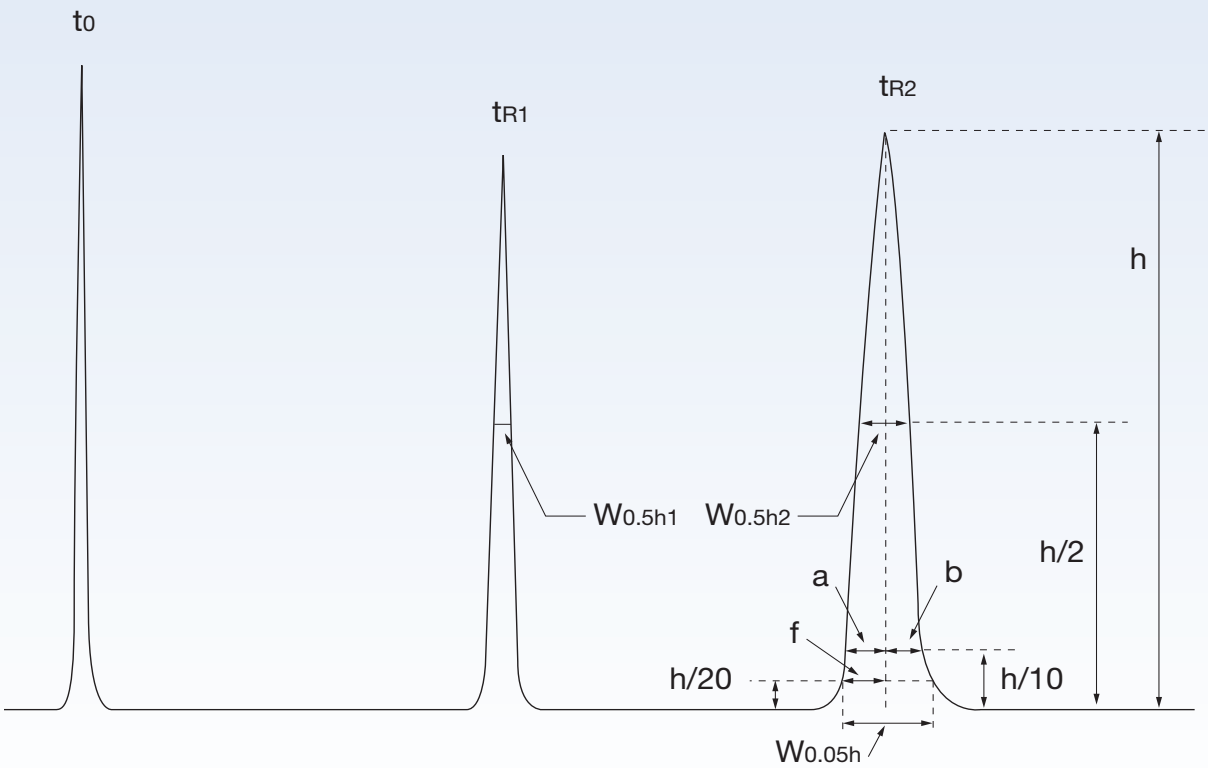
HPLC Column Performance

Important factors used to evaluate column performance include column efficiency, capacity, separation characteristics of solutes, peak shape and column pressure. The parameters used to assess column performance by YMC are defined below. Column efficiency, an important characteristic for evaluation of column performance, is generally measured in terms of theoretical plate number. This is calculated using peak width at half-height.
Narrower peak widths result in higher theoretical plate numbers. Longer columns and smaller packing material particle size also result in higher theoretical plate numbers. Due to a variety of factors, one column does not always show the same theoretical plate number. This may be caused by differences between linear velocity and solute diffusion in the column or because of interaction between solutes and the mobile phase or the stationary phase.

For these reasons, column efficiency is solute specific and the measurement of efficiency must be conducted under nearly identical HPLC conditions for results to be directly comparable.

Retention and separation characteristics for solutes on the column are evaluated by the capacity factor and separation factor values.
These values are indices of the packing material characteristics and, in contrast to the retention time, are independent of column inner diameter and length.

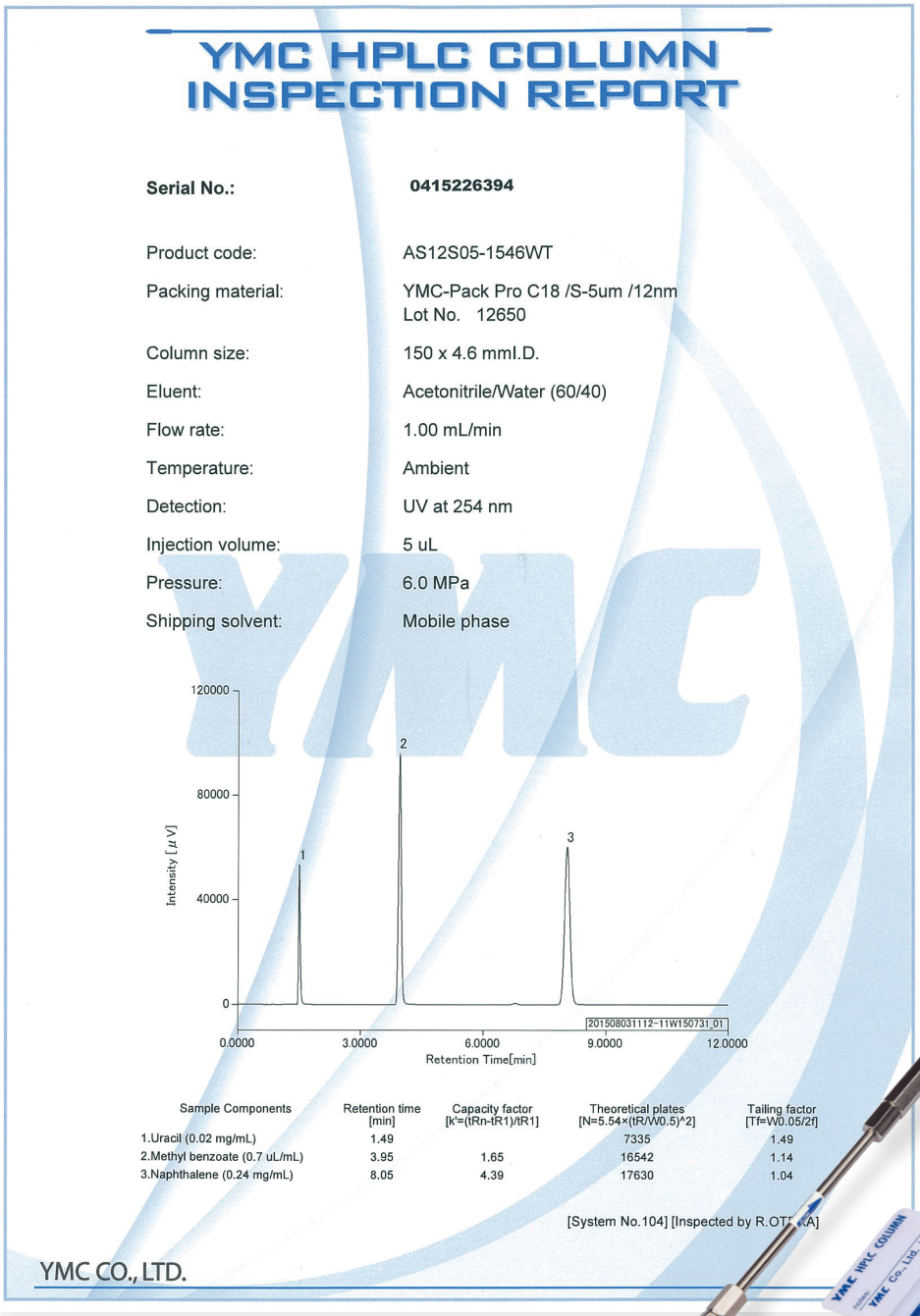
Elution peak shape is also an important factor for evaluation of column performance. The asymmetry factor is a relatively simple measurement, usually calculated at 10% of peak height.



t_0	Void volume, Column dead-time	
t_R	Retention time	
h	Peak height	
$W_{0.5h}$	Peak width at half-height	
N	Theoretical plate number	$N=5.54 \times (t_R / W_{0.5h})^2$
k'	Capacity factor	$k'=(t_R - t_0) / t_0$
α	Separation factor	$\alpha= k'_2 / k'_1$
R_S	Resolution	$R_S= 1.18 \times (t_{R2}-t_{R1}) / (W_{0.5h1} + W_{0.5h2})$
A_S	Asymmetry factor	$A_S=b / a$
T_f	Tailing factor	$Tf= W_{0.5h} / 2f$

Inspection Reports

Formation



YMC HPLC COLUMN
INSPECTION REPORT

Serial No.:	**0415226394**
Product code:	AS12S05-1546WT
Packing material:	YMC-Pack Pro C18 /S-5um /12nm Lot No. 12650
Column size:	150 x 4.6 mmI.D.
Eluent:	Acetonitrile/Water (60/40)
Flow rate:	1.00 mL/min
Temperature:	Ambient
Detection:	UV at 254 nm
Injection volume:	5 uL
Pressure:	6.0 MPa
Shipping solvent:	Mobile phase

Sample Components	Retention time [min]	Capacity factor [k'=(tRn-tR1)/tR1]	Theoretical plates [N=5.54×(tR/W0.5)^2]	Tailing factor [Tf=W0.05/2f]
1.Uracil (0.02 mg/mL)	1.49		7335	1.49
2.Methyl benzoate (0.7 uL/mL)	3.95	1.65	16542	1.14
3.Naphthalene (0.24 mg/mL)	8.05	4.39	17630	1.04

[System No.104] [Inspected by R.OT[illegible]A]

YMC CO., LTD.

YMC employs strict quality control of packing materials to ensure lot-to-lot and column-to-column reproducibility. All packed columns are subject to performance tests and only those columns which meet strict specifications are shipped to customers.
A test report (see below) is shipped with each column. The test method shown on this inspection report is not only a method used for column performance evaluation but can also be used as a test method for determination of column life. We provide full details of all the analytical conditions, used test method, including compounds analysed, sample concentration, eluent composition, etc. to allow the end user to reproduce these tests.

FAQ Frequently Asked Questions

What is "Endcapping"?

Conventional ODS (C18) packing materials are silica gel bonded with octadecyl groups. This is the result of reaction between silanol groups on the silica surface and octadecyl groups. However some active silanol groups remain after the reaction. It is impossible for all the silanol groups to react because of steric hindrance of octadecyl groups. Such residual silanol groups create a secondary interaction in chromatography, which, in many cases, affects on chromatograms by causing peak tailing of basic compounds or irreversible absorption to the column. Therefore, a secondary silanisation reaction with residual silanol groups using a small reagent (typically trimethylsilane) should be performed. This process is called "endcapping".

Are there ODS columns used with 100% aqueous mobile phase?

YMC-Triart C18, Hydrosphere C18 and YMC-Pack ODS-AQ columns can be used with 100% aqueous mobile phase. With conventional ODS columns, retention time becomes shortened due to the incompatibility between water molecules and the silica bonded surface with high hydrophobicity. Water tends to be expelled from the pores on material and the C18 chains "collapse" onto themselves. The retention time is hardly affected for YMC-Triart C18, Hydrosphere C18 and YMC-Pack ODS-AQ columns because the silica surface is capable of solvation between mobile phase and hydrophilic silica surface as a result of the reduced C18 functional group density and the proprietary modification process.

What is the upper limit of column pressure?

Column length of 150 mm or less and diameters less than 10 mm:
20 MPa (200 bar, 3,000 psi)
Column length of 250 mm or greater and diameters less than 10 mm:
25 MPa (250 bar, 3,750 psi)
UHPLC columns (1.9 µm):
100 MPa (1,000 bar, 15,000 psi)
Triart columns (3/5 µm; novel column hardware):
45 MPa (450 bar, 6,525 psi)
YMC-Pack Diol UHPLC (2 µm):
45 MPa (450 bar, 6,525 psi)

How should we store the columns?

When columns are not used for a long time, they should be stored in a cool place after replacing the eluent with the shipping solvent as described in the Inspection Report. Do not store the column in the mobile phase with salt or acid, even for very short times. Close the airtight stopper tightly to prevent the solvent from evaporating.

How can we evaluate the performance of columns?

Repeat the performance test using exactly the same conditions as the Inspection Report which accompanies the column at the time of purchase. Columns which show no change in retention time, theoretical plate number, peak asymmetry, etc are acceptable for further use.
Columns which show no change in these parameters after several years of use may, however, have changes in separation characteristics for certain types of compounds such as ionic species. It is advisable to avoid using such columns for method development as reproducibility compared to new columns may not be possible.

FAQ

How do I clean the columns?

1. To remove strongly adsorbed hydrophobic material; pump the column in the reverse direction with eluent with a greater elution ability than mobile phase. For example, for cleaning reversed phase columns, use an eluent with increased ratio of organic modifier and flush the column with at least 10 column volumes.
2. To recondition the gel surface condition caused by damage resulting in generation of active silanol groups, and observed as irregularities in peak asymmetry and retention time. Washing with acidic solvents can be effective in such cases. Typically a mixed solvent of 0.1% aqueous phosphoric acid solution and organic solvent (between 10 and 60% organic content) can return the silanol groups to the dissociation state.

Do we need guard columns?

YMC recommend the use of guard columns, particularly if the samples being analysed contain a high level of contaminants. This will extend the useful lifetime of a column, particularly if replaced at frequent intervals. We recommend that guard columns are packed with the same packing material as the analytical column. Guard columns with different material may cause abnormalities in peak asymmetries and reproducibility. YMC guard cartridges are particularly economic when frequent replacement is required.

What is required in system and flow rate for using semi-micro columns?

Recommended flow rates for semi-micro column (1.0 to 3.0 mm inner diameters) are:

1.0 mm ID	0.05 mL/min
2.1 mm ID	0.2 mL/min
3.0 mm ID	0.4 mL/min

This can be increased if the column length is short and the system back pressure is low. Such columns can be used in conventional HPLC systems but it is advisable to use short lengths of smaller diameter connection tubing and detector flow cells which are optimised for low flow rates.

How do I carry out a scale-up of a method?

Step 1: Determine separation conditions by using analytical columns.
Step 2: Study the preparative scale. Select the particle size of the packing material and the inner diameter of column appropriate for the sample volume.
Step 3: Optimise the separation conditions using analytical columns with inner diameter of 4.6 mm or 6.0 mm packed with the packing material selected for the preparative separation (scout column). If the particle size of the packing material is the same as in the Step 1, this process can be omitted. If the preparative column is more than 100 mm ID, it is advisable to insert another step with a scout column of 20 mm ID in order to accurately predict loadability and calculate the running costs.
Step 4: Proceed with the preparative separation.

FAQ Frequently Asked Questions

What should I do when the column pressure increases?

Depending on the reasons for increased pressure, the following procedures are recommended:
Blocked frits: Flush the column in reverse flow as described in "column handling". Reduce the flow rate in order to keep the column pressure within recommended limits whilst flushing the column.
Contamination of the packing material: Wash the column in reverse flow as described in "column handling".
If pressure increases occur frequently despite treatment as above, it is recommended that sample pretreatment or the use of guard columns is employed to prevent the problem occurring in the first place.

What are the solutions for poor peak shapes?

The following solutions are recommended, depending on the cause.
Inappropriate Mobile phase: If pKa of the analyte and pH of mobile phase are close for ionic analytes , it will result in poor peak shape. Set the pH of mobile phase at least 1 (or better 2) units from pKa.
Effect of solvent used to dissolve sample: If the dissolving solvent of sample and mobile phase are not the same, it causes defects in the peak shape. Dilute the sample solution with mobile phase or reduce the injection volume.
Overloading sample injection: Overloading the column will cause defects in the peak shape. Reduce injection volume and/or the sample concentration.
Insufficient equilibration time: When the difference in pH between the current and a previous mobile phase is wide or the buffer concentration of mobile phase is low, column equilibration may take some time
Column contamination and degradation: If the column is contaminated, wash the column as described in "column handling". If the column is degraded, it is not possible to regenerate it and it should be replaced.
System problems: Dispersion of the sample may occur within the tubing between the injector and the column or within the flow cell of detector which can result in peak tailing and/or broadening. Optimisation of the system for use with semi-micro use should be performed.

What are the solutions for ghost peaks?

The following solutions are recommended, depending on the cause.
Injector fouling: If the ghost peak(s) appears when injecting only mobile phase (no sample), wash the injector.
Gradient Analysis: When hydrophobic impurities are eluted by a stronger solvent, they appear as ghost peaks. Clean the column as described in the Instruction Manual. If this does not eliminate them, they are probably due impurities of solvent. Use a higher grade solvent, purified specifically for HPLC or alternatively install a guard column between the solvent delivery pump and the mixing chamber or injector.

What should I do if columns dry out?

Flush the column with a solvent such as methanol for all bonded phase silica or hexane for non bonded silica and remove trapped air using a flow rate such that the column pressure is about half that normally used for analysis. When all the air has been removed, check the column performance by running a test chromatogram under the conditions stated on the original Column Inspection Report.

FAQ

What should I do if the column fails to provide reproducibility?

This can arise for a number of reasons:
Inappropriate mobile phase conditions:
It may become difficult to obtain reproducibility when analysing ionic compounds, if the pH of mobile phase is not controlled or the buffer concentration is low. Increase the buffer concentration.
Retention time can fluctuate widely due to a slight variance of pH when the pH of a mobile phase is set too close to the pKa of analyte. Set the pH of the mobile phase to be at least 1 (or preferably 2) units away from the pKa.
System variance: It may be difficult to obtain reproducibility in chromatograms when using different HPLC systems. Where possible the manufacture of pumps, detectors and injectors should be the same, otherwise differences in extra column volume from mixing chamber, detector cell and plumbing will result in poor reproducibility between systems. Also, with column heaters from different manufacturers, there may be an effect on the retention time due to the set temperature being different between the 2 systems. Use of the same system throughout a sequence of analysis is recommendable.
Column histories: Reproducibility between chromatograms may not be obtained when using different columns of the same type. This is due to differences in the columns' prior histories. For example, changes in the chemistry of the surface of the packing material can arise by use of mobile phases containing ion pair reagents or when strongly hydrophobic material (especially proteins) becomes adsorbed on the column. Dedicating a column to a specific application is recommended.
Using 100% aqueous mobile phase: Reproducibility of chromatograms obtained on conventional ODS columns will not be obtained when using 100% aqueous mobile phase due to the short retention times obtained. Columns which can be used in 100% aqueous mobile phase are recommended. YMC recommends the use of either YMC-Triart C18, Hydrosphere C18 or YMC-Pack ODS-AQ which are designed to be used in 100% aqueous mobile phase.
Grade difference in mobile phase: Reproducibility between chromatograms may not be obtained when using different grade of solvent in a mobile phase. Impurities contained in a solvent can act like salts in mobile phase and affect the separation. Solvent in HPLC grade is recommendable.

I still have poor retention after adding ion pair reagent to mobile phase. Why?

This is caused by excess of ion pair reagent. In general, the higher the concentration of ion pair reagent, the greater the retention. However, if the concentration of ion pair reagent is above a certain level, the retention may become poor because of micelle formation. Good separations are achieved when the concentration of ion pair reagent is between 5 mM to 20 mM. YMC recommend that the lowest possible concentration is used to avoid short column life.

Please feel free to contact YMC with any issue either by phone (+49 (0) 2064 427-0), by mail (info@ymc.eu) or use our online chat on our homepage (www.ymc.eu).

Troubleshooting

1. Consideration of solvent grade for reversed phase LC

Reversed phase liquid chromatography frequently employs organic solvents such as methanol, acetonitrile or tetrahydrofuran. Although HPLC grade products of these types of solvents are available, it seems some users have trouble when using a reagent grade solvent instead of HPLC grade. This results in them wasting considerable amounts of time. How do the two solvent grades differ?

Methanol and acetonitrile

Reagent grade solvents contain larger amounts of UV absorbing impurities than HPLC grade solvents do, which makes it difficult to use them for gradient elution or trace analysis, especially when the detection requires short wavelength. This gives rise to significant increases in baseline noise or detection sensitivity.

In some cases (or at some wavelengths) it might be possible to use a reagent grade solvent, but we recommend the use of HPLC grade solvents whenever possible.

Tetrahydrofuran

Tetrahydrofuran easily generates peroxides. To prevent this, the solvent generally contains antioxidants which can cause ghost peaks. As a result, solvent containing no antioxidants should be used in HPLC.

The peroxides in tetrahydrofuran also have a marked effect on the baseline stability (with differences between grades and between different suppliers being greater than for other organic solvents), which leads to the recommendation that HPLC grade solvent with little or no impurities should be used.

2. Eluent conditions

Although a column is frequently thought to be the cause of HPLC analysis not providing the correct chromatogram, many failures can be attributed to causes other than the column, including improper maintenance operations. This discussion illustrates the case in which the grade of a solvent affects the peak shapes. In the chromatogram for basic compound analysis, using an eluent of acetonitrile/water, Peak 2 represents the basic compound.

The figures on the right show chromatograms from two identical separations except that the acetonitrile used was of different grades. One used HPLC grade (Figure 1); the other used reagent grade (Figure 2). While the peak shape was broadened with HPLC grade acetonitrile, it was much improved when using reagent grade. The differences in peak shapes which were observed were also found to be dependent on the different makers even though they were of the same specific grade. This may be the effect of traces of impurities contained in acetonitrile behaving in the same way as modifiers added to an eluent.

Replacing eluent with acetonitrile / 5 mM ammonium acetate produced the chromatogram shown in Figure 2 irrespective of the grade of solvent. To avoid the influence of different grades, solvents specifically made for HPLC must be used. Even compounds which have groups which can dissociate can be analysed with eluent containing no acid or salt, although eluents with additives such as salt must be used when reproducibility is important.

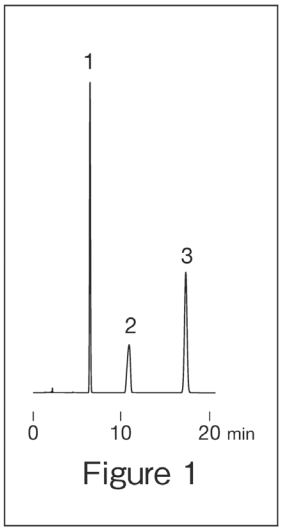


Figure 1

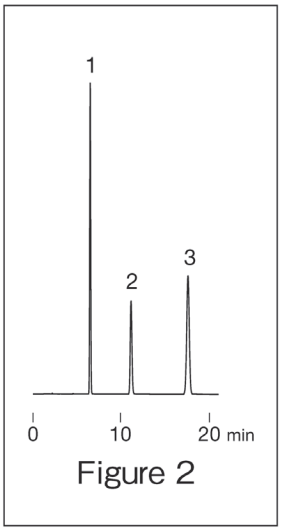
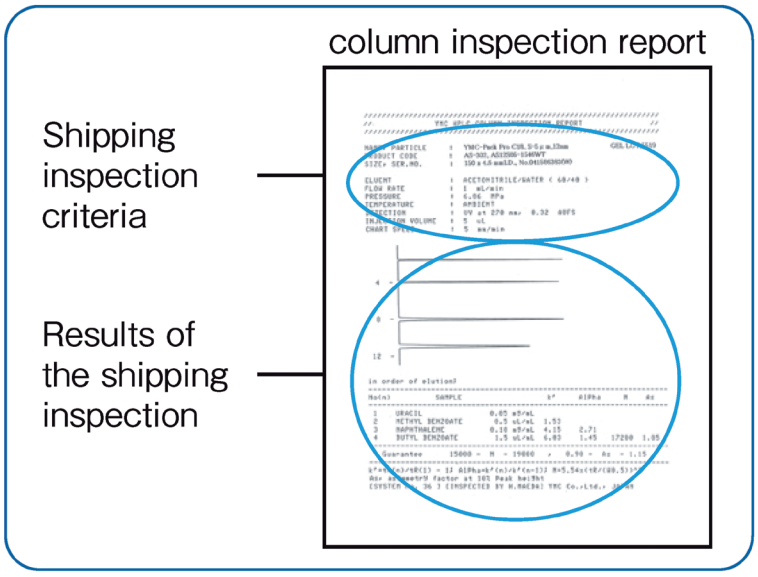


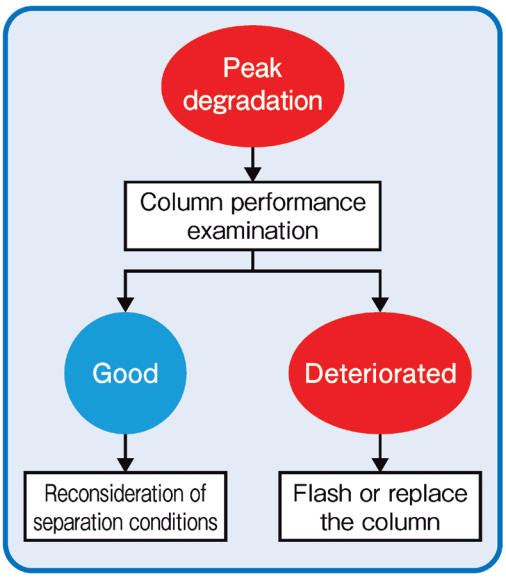
Figure 2

Troubleshooting

3. Peak shape anomalies

A common problem encountered during HPLC operations is peak shape anomalies such as peak tailing or split peaks. In order to remove these problems, the cause must be precisely determined. The majority of cases are the result of inappropriate conditions for the separation; including inappropriate selection of column or solvent, or use of an old column which has a void to the packing at the top of the packing. Here we discuss the method of determining the cause of the problem with peak shapes.
The simplest way is to test the column performance using the "shipping inspection criteria" as described in the Column Inspection Report which is included with every column. If the examination reveals no peak shape anomaly, then the cause will be the result of inappropriate selection of separation condition. The separation condition such as eluent selection must be reconsidered. If, on the contrary, the same examination reveals any anomaly, the column may be the problem. Flushing (to remove the impurity could have accumulated on the column) or replacement of the column is necessary. We recommend examining column performances on a regular basis and always under the identical conditions.





YMC provide sufficient analytical information, including sample concentration, in the Column Inspection Reports to allow customers to evaluate the performance of the column using standard compounds.

4. Column Pressure Increases

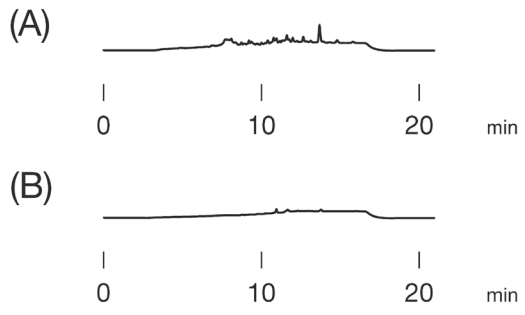
Pressure increase is a common problem in HPLC. Some of the reasons for pressure increases in reversed phase chromatography are discussed below.
If the system pressure increases, you should first disconnect the column and run the system to determine the line pressure. If the line pressure is high, the tubing may be clogged or damaged. If there is no excessive line pressure, then the column pressure may be high and the column needs cleaning. Cleaning by pumping in the reversed direction can be very effective. Generally, the relative proportion of the organic solvent in a mobile phase should be increased when washing, to speed up removal of bound material. However, the key consideration is to choose, in accordance with the characteristics of the sample, an appropriate solvent that will easily dissolve the adsorbed material and not cause precipitation. Reversed phase separations often cause protein to be adsorbed by the packing material which results in high pressure. This problem can be overcome effectively by gradient washing with acetonitrile/ water containing 0.1% TFA, rather than washing with an organic solvent. If the cause of high back pressure is believed to be the result of insoluble material in samples or precipitation of a sample during separation, washing or replacing the inlet frit may be successful.
However, once high back pressures occur, it frequently becomes difficult to restore performance despite washing, etc. It is far better to prevent increased column pressure from occurring by simple sample preparation such as protein removal or filtration and using a guard column to protect the analytical column.

Troubleshooting

5. The Cause of the Ghost Peaks

As part of a test of a gradient method a chromatogram was run without a sample being injected. A number of peaks were obtained, as in trace (A). When a similar test was performed, but with the column disconnected, the ghost peaks disappeared, as in trace (B). This led to the idea that the column was at fault.
However, despite flushing and replacing the column, the baseline could not be improved. Several other factors were then examined; the cause was found to be water used to prepare the mobile phase. Standard distilled water (which is inadequate for HPLC) had been mistakenly used. When HPLC grade distilled water was used, an excellent baseline as in trace (B) was obtained.
Water purity can have a great impact on gradient elution. Even HPLC grade distilled water will become contaminated with time, causing ghost peaks. This will have no significant influence on isocratic elution methods, but it will cause problems in gradient elution methods.
In gradient elution methods, a column is equilibrated with an eluent with low organic content. This allows impurities in the eluent to be adsorbed and concentrated in the column. After starting the analysis, the amount of organic solvent increases and impurities begin to elute from the column, resulting in ghost peaks.

The heights of the ghost peaks are dependent on the duration of equilibration (the amount of contaminant adsorbed during equilibration).
Such ghost peaks do not appear when the column is disconnected because there is nothing to adsorb and concentrate the impurities during the equilibration stage. Therefore, in gradient analysis, the grade and storage conditions of all solvents requires great care.



Eluent: A) water / TFA (100/0.1)
B) acetonitrile / TFA (100/0.1)
Gradient: 0-60% B (0-10 min), 60% B (10-15 min)
Detection: UV at 254 nm

6. pH Adjustment of Eluents

Analysis of ionic compounds by reversed phase HPLC has to be performed with the pH of eluent controlled using acid or buffering agent. However, separation at a pH which is not the optimum for the compound of interest can cause problems such as double peak or peak broadening. Even if the peak shape is satisfactory, retention time reproducibility may not be obtained in some cases. The relation between retention of benzoic acid and pH value is a good example; although the retention time (measured as k') varies little when the pH is in the range 2 - 3.5, it varies widely when the pH ranges is in the range 3.5 - 4.5. The pKa of benzoic acid is 4.2 and it is noticeable that the region where the retention time varies most widely is near the pKa. If the eluent pH is adjusted to a value near the pKa, the results may not be reproducible due to very small variations of the pH adjustment having a large impact on the retention time. In fact, the eluent pH variation of just 0.1 will affect the separation significantly. Therefore, it is recommended that the eluent pH should be more than 1 unit away from the pKa.
If the pKa of the analyte is unknown, the eluent pH should be adjusted to within the value where the impact on the separation seems minimal, after having evaluated the relation between the eluent pH and the retention time by using several eluents with their pH values adjusted to be slightly different from each other.

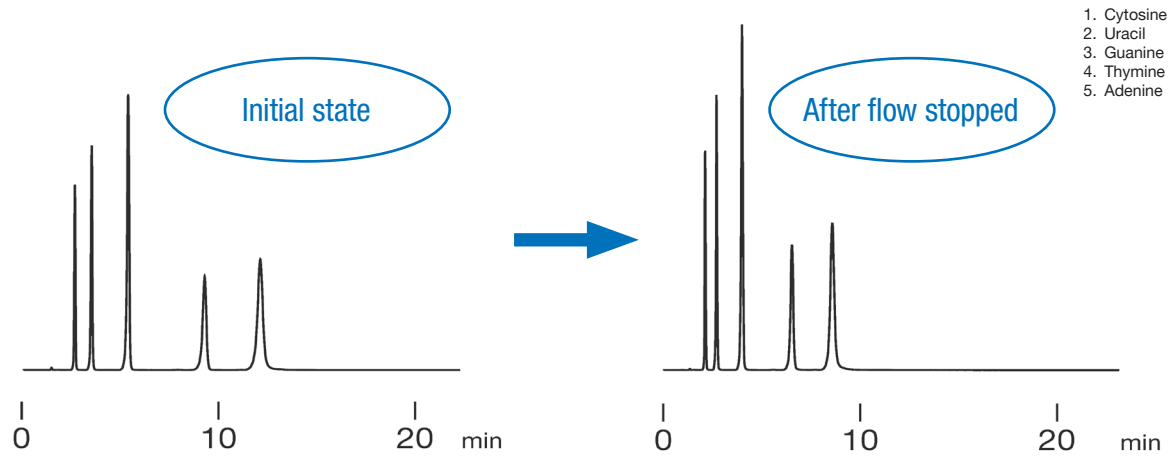
Troubleshooting

7. Regenerating Columns

In reversed phase HPLC, column deterioration can cause poor peak shapes and/or reduced retention times. The column deterioration is the result of changes in the packing material's structure, such as the loss of bonded phase (eg C18 chains) or dissolution of the silica gel base material. Should this occur, columns are difficult to restore and reuse.

If 100% aqueous mobile phase is used in an ODS column, a sharp reduction in retention times of compounds can arise (as in the figure below). Whilst some may think this reduction in retention time is due to column deterioration, this is not the case. In this case, the cause is due to the decrease of apparent hydrophobicity of the packing material due to polarity difference between the water and the C18 functional groups, leading the C18 chains to collapsing onto themselves. In some cases where this occurs, the initial retention times can be restored by flushing the column with 10 times its volume of mobile phase containing 50% organic solvent.

This decreases the repulsion between the eluent and the C18 chains and allows them to return to their normal pendant state. However, YMC recommend that columns specifically intended for 100% aqueous eluents should be used to prevent this problem arising.



After the first day´s analysis, the column was stored overnight with stopped flow. Reanalysis was conducted the day after.

Column:	150 x 4.6 mm ID
Eluent:	20 mM KH_2PO_4-K_2HPO_4 (pH 6.9)
Flow rate:	1.0 mL/min
Temperature:	37 °C
Detection:	UV at 254 nm

Essential Data

Conversion factors

Pressure

MPa	bar	psi	kgf/cm^2	atm
1	10	145.04	10.20	9.87
0.1	1	14.504	1.020	0.987
6.90x10^{-3}	0.069	1	0.070	0.068
0.0981	0.981	14.223	1	0.968
0.101	1.013	14.696	1.033	1

Length

m	in	ft	yd	mile
1	39.37	3.28	1.094	6.21x10^{-4}
0.025	1	0.083	0.028	0.15x10^{-4}
0.305	12	1	0.33	1.89x10^{-4}
0.91	36	3	1	5.68x10^{-4}
1609.3	63360	5280	1760	1

Weight

kg	oz	lb
1	35.274	2.204
0.0283	1	0.0625
0.454	16	1

Volume

l	gal(UK)	gal(US)
1	0.22	0.26
4.55	1	1.201
3.79	0.83	1

Temperature

K	°F	°C
0	-459.67	-273.15
255.37	0	-17.8
273.15	32	0
298.15	77	25
310.93	100	37.8
373.15	212	100

formula: °C=(°F-32)x5/9 °F=°Cx9/5+32

Ratio Scale

ppb	ppm	%
1	10^{-3}	10^{-7}
10^{3}	1	10^{-4}
10^{7}	10^{4}	1

SI Prefixes

da (deca)	h (hecto)	k (kilo)	M (mega)	G (giga)	T (tera)	P (peta)	E (exa)	Z (zetta)	Y (yotta)
10^{1}	10^{2}	10^{3}	10^{6}	10^{9}	10^{12}	10^{15}	10^{18}	10^{21}	10^{24}

d (deci)	c (centi)	m (milli)	μ (micro)	n (nano)	p (pico)	f (femto)	a (atto)	z (zepto)	y (yocto)
10^{-1}	10^{-2}	10^{-3}	10^{-6}	10^{-9}	10^{-12}	10^{-15}	10^{-18}	10^{-21}	10^{-24}

1 Å (ångström) = 0.1 nm = 10^{-10} m

Linear scale-up

Inner Diameter	1.0	2.0	3.0	4.6	10.0	20.0	30.0	50.0
Scale-Up factor	0.0473	0.189	0.425	1	4.73	18.90	42.53	118.15

Essential Data

	iso-Octane	n-Hexane	n-Heptane	di-Ethyl ether	Cyclohexane	Ethyl acetate	Toluene	Chloroform	Tetrahydrofuran	Benzene	Acetone	Dichloromethane	Dioxane	n-Propanol	Ethanol	Dimethylformamide	Acetonitrile	Acetic acid	Dimethyl sulfoxide	Methanol	Water
iso-Octane																					
n-Hexane	✔																				
n-Heptane	✔	✔																			
di-Ethyl ether	✔	✔	✔																		
Cyclohexane	✔	✔	✔	✔																	
Ethyl acetate	✔	✔	✔	✔	✔																
Toluene	✔	✔	✔	✔	✔	✔															
Chloroform	✔	✔	✔	✔	✔	✔	✔														
Tetrahydrofuran	✔	✔	✔	✔	✔	✔	✔	✔													
Benzene	✔	✔	✔	✔	✔	✔	✔	✔	✔												
Acetone	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔											
Dichloromethane	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔										
Dioxane	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔									
n-Propanol	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔								
Ethanol	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔							
Dimethylformamide	✗	✗	✗	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔						
Acetonitrile	✗	✗	✗	✔	✗	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔					
Acetic acid	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔				
Dimethyl sulfoxide	✗	✗	✗	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔			
Methanol	✗	✗	✗	✔	✗	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔		
Water	✗	✗	✗	✗	✗	✗	✗	✗	✔	✗	✔	✗	✔	✔	✔	✔	✔	✔	✔	✔	

✔ = miscible
✗ = immiscible

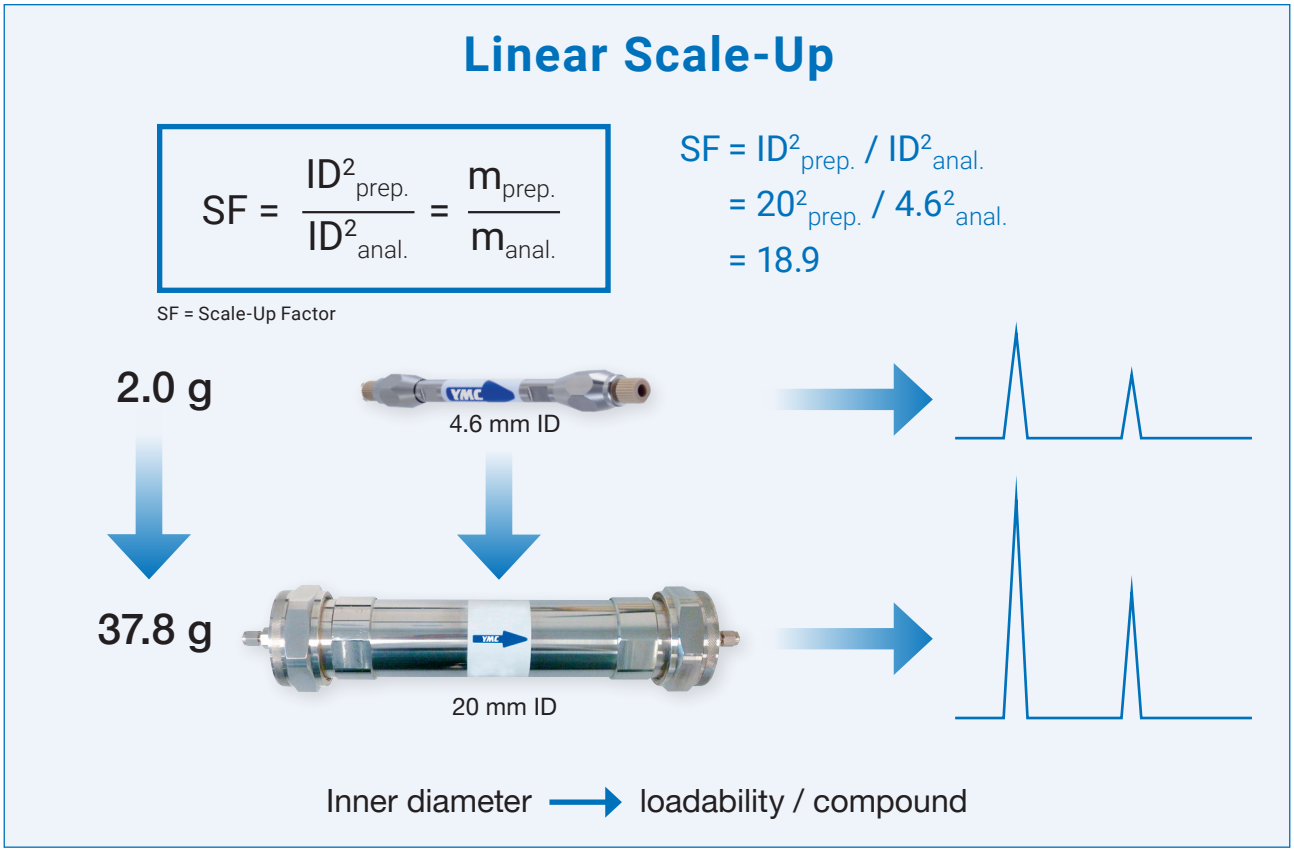
Linear Scale-Up

In order to simplify your scale-up the three most important scale-up factors are summarised.

Scalable factor SF	ID "Linear Scale-Up"	Column length	Column length and ID "Volume"
	$SF = \frac{ID^2_{prep.}}{ID^2_{anal.}}$	$SF = \frac{L_{prep.}}{L_{anal.}}$	$SF = \frac{ID^2_{prep.}}{ID^2_{anal.}} / \frac{L^2_{prep.}}{L^2_{anal.}}$
Impact	Flow rate Eluent composition	Retention time Cycle time Plate number	Amount of adsorbent

Linear Scale-Up

In most cases it is beneficial to develop a semi-preparative method on an analytical scale column. The analytical separation carried out on a 150 x 4.6 mm ID column has to be scaled up to 150 x 20 mm ID. Therefore the chromatographic parameters such as flow rate and column load have to be adjusted according to the following equation:



Guideline for Sample Load according to column ID

Column ID (mm)	Scale–Up factor	Loadability (mg)
4.6	1	1–4
10	4.7	5–20
20	18.9	20–80
30	42.5	40–160
50	118	80–350
75	266	270–980
100	472	470–1,900
150	1,060	1,000–4,200

Preparative Column Selection Guide

Optimisation of preparative chromatography

The main task for a preparative chromatographer is to find the suitable system. In order to simplify the considerations YMC developed a **"Preparative Column Selection Guide"**.

			Lab scale → Production scale								
Column inner diameter [mm ID]			**4.6**	10	20	30	50	100	200	500	1,000
Cross sectional area ratio			**1.0**	4.7	19	42	118	473	1,890	11,800	47,300
Example of calculation	**Flow rate** [ml/min]		**0.5**	2.4	9.5	21	60	235	950	6,000 (6 L)	24,000 (24 L)
			1.0	4.7	19	42	120	470	1,900	12,000 (12 L)	47,000 (47 L)
	Loading [mg]		**5**	25	100	220	600	2,500	10,000	60,000 (60 g)	240,000 (240 g)
Column efficiency, Pressure, Costs HIGH → LOW	**Particle size** [µm]	**5**	+++	+++	+++	+++	++	+	+		
		10	++	+++	+++	+++	+++	++	++	++	++
		10–20	+	++	++	++	+++	+++	+++	++	++
		15–30		+	+	+	++	+++	+++	+++	++
		50~					+	++	++	+++	+++

+++ Most appropriate, ++ Appropriate, + Depending on purpose

The **"Preparative Column Selection Guide"** will help to select:

1. the column ID for the required sample loading
2. the particle size for optimum efficiency
3. the column length for the necessary resolution

Scale-Up

The YMC Scale-Up is defined by 4 steps:

1. Analytical Scale: Method Development
Determine separation conditions by using analytical columns packed with different stationary phases and various conditions.

2. Study the preparative scale. Select the particle size of the packing material and the inner diameter of column appropriate for the sample volume.

3. Optimise the separation conditions and perform loadability studies using analytical columns with inner diameter of 4.6 mm or 6.0 mm packed with the packing material selected for the preparative separation (scout column). If the particle size of the packing material is the same as in the Step 1, this process can be omitted. If the preparative column is more than 100 mm ID, it is advisable to insert another step with a scout column of 20 mm ID in order to accurately predict loadability and calculate the running costs.

4. Proceed with the preparative separation with scale-up of chromatographic parameters such as flow rate/ column ID/ sample load as necessary. From all the given steps above the most demanding step will be the scale-up of the chromatographic parameters in order to meet the preparative demands. There are a number of scalable parameters: flow rate, column ID, sample load, tubing ID, sample injection concentration, volume of sample loop, consumption of solvent, dead volume, fraction mass, size of the detector cell.

YMC Order Guide

The product listing at the end of each chapter represent commonly used standard column dimension.
In order to identify any specific product version and part number, please see the example and the table below.

Full listing of all chemistries and dimensions

Gel Code								Hardware Code					
Chemistry code		Pore size [nm]		Particle shape		Particle size [µm]		Length [mm]		Inner diameter [mm]		Column type	
YMC Carotenoid	CT	6	06	**spherical**	**S**	**1.9**	**P9**	5	E5	0.075	E8	Parker type HPLC	PTH
YMC-Triart C18	**TA**	8	08			2.0	02	10	01	0.1	F0	**Parker type UHPLC**	**PT**
YMC-Triart Bio C18	TA	**12**	**12**			2.3	Q3	20	02	0.3	H0	Parker type metal-free	PTP
YMC-Pack Pro C18 RS	RS	16	16			2.7	Q7	30	03	0.5	J0	Parker type bioinert coated	PTC
Hydrosphere C18	HS	20	20			3.0	03	33	H3	1.0	01	Biocompatible PEEK	WP
Meteoric Core C18	CAS	25	25			4.0	04	**50**	**05**	**2.0**	**02**	Waters type	WT
Meteoric Core C18 BIO	CAW	30	30			5.0	05	75	L5	2.1	Q1	Analytical guard cartridges	GC
YMC-Pack ODS-A	AA	100	A0			6.0	06	100	10	3.0	03	Capillary 1/16”	AU
YMC-Pack ODS-AM	AM	proprietary	99			10	11	125	R5	4.0	04	Capillary 1/32”	RU
YMC-Pack ODS-AQ	AQ	non-porous	00			15	16	150	15	4.6	46	Actus semi prep. 20/30 mm ID	WX
YMC-Pack ODS-AL	AL					20	21	250	25	6.0	06	Actus semi prep. 50 mm ID, 1/8”	DX
J’sphere ODS-H80	JH					30	30	300	30	8.0	08	Actus semi prep. 50 mm ID, 1/16”	AX
J’sphere ODS-M80	JM					50	50	500	50	10	10	Semi prep. 10 mm ID/ UHPLC guard cartridges	CC
J’sphere ODS-L80	JL					60	60			20	20	Semi prep. guard cartidge 20/30 mm ID	CCN
YMC-Pack PAH	YP					75	75			30	30	Semi prep. guard	WTG
YMC-Pack PolymerC18	PC									50	53	Alcyon SFC	WTS
YMC-Triart C8	TO												
YMC-Pack Pro C8	OS												
Meteoric Core C8	COS												
YMC-Pack C8	OC												
YMCbasic	BA												
YMC-Triart Phenyl	TPH												
YMC-Pack Ph (Phenyl)	PH												
YMC-Triart PFP	TPF												
YMC-Triart Bio C4	TB												
YMC-Pack Pro C4	BS												
YMC-Pack C4	BU												
YMC-Pack Protein-RP	PR												
YMC-Pack TMS (C1)	TM												

Example

YMC-Triart C18		12 nm		Spherical		1.9 µm		50 mm		2.0 mm		Parker Type UHPLC	
	TA		12		S		P9		05		02		PT

Your part number: TA12SP9-0502PT (Example)

YMC Order Guide

Full listing of all chemistries and dimensions

Gel Code								Hardware Code					
Chemistry code		Pore size [nm]		Particle shape		Particle size [µm]		Length [mm]		Inner diameter [mm]		Column type	
YMC-Pack PVA-Sil	PV	6	06	**spherical**	**S**	1.9	P9	5	E5	0.075	E8	Parker type HPLC	PTH
YMC-Pack Polyamine II	PB	8	08			2.0	02	10	01	0.1	F0	Parker type UHPLC	PT
YMC-Pack NH2 (Amino)	NH	12	12			2.3	Q3	20	02	0.3	H0	Parker type metal-free	PTP
YMC-Triart SIL (SFC)	TS	16	16			2.7	Q7	30	03	0.5	J0	Parker type bioinert coated	PTC
YMC-Triart Diol-HILIC	TDH	20	20			3.0	03	33	H3	1.0	01	Biocompatible PEEK	WP
YMC-Pack Diol NP	DN	25	25			4.0	04	50	05	2.0	02	**Waters type**	**WT**
YMC-Pack SIL	SL	30	30			5.0	05	75	L5	2.1	Q1	Analytical guard cartridges	GC
CHIRAL ART Amylose-C	KAN	100	A0			6.0	06	100	10	**3.0**	**03**	Capillary 1/16"	AU
CHIRAL ART Amylose-C Neo	KBN	**proprietary**	**99**			10	11	125	R5	4.0	04	Capillary 1/32"	RU
CHIRAL ART Cellulose-C	KCN	non-porous	00			15	16	**150**	**15**	4.6	46	Actus semi prep. 20/30 mm ID	WX
CHIRAL ART Amylose-SA	**KSA**					20	21	250	25	6.0	06	Actus semi prep. 50 mm ID, 1/8"	DX
CHIRAL ART Cellulose-SB	KSB					30	30	300	30	8.0	08	Actus semi prep. 50 mm ID, 1/16"	AX
CHIRAL ART Cellulose-SC	KSC					50	50	500	50	10	10	Semi prep. 10 mm ID/ UHPLC guard cartridges	CC
CHIRAL ART Cellulose-SJ	KSJ					60	60			20	20	Semi prep. guard cartidge 20/30 mm ID	CCN
CHIRAL ART Cellulose-SZ	KSZ					75	75			30	30	Semi prep. guard	WTG
YMC-Pack Diol (SEC)	DL									50	53	Alcyon SFC	WTS
YMC-SEC MAB	DLM												
BioPro IEX QA	QA												
BioPro IEX SP	SP												
BioPro IEX QF	QF												
BioPro IEX SF	SF												
BioPro HIC HT	BHH												
BioPro HIC BF	BHB												

* for YMC-Triart Diol (SFC) add "B" at the end of the part number! Example: TDH12S03-1503PTH**B**

Example

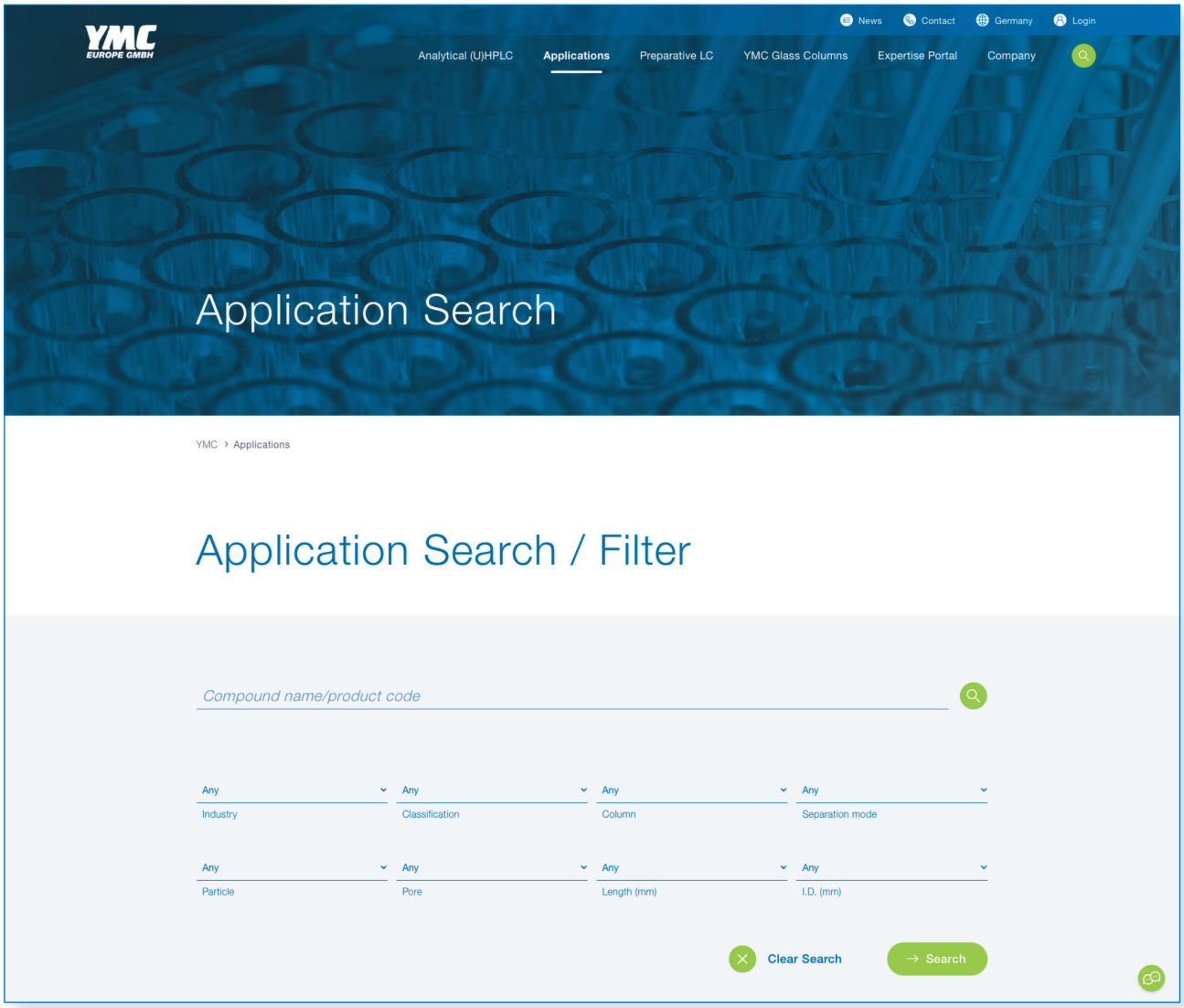
CHIRAL ART Amylose-SA		Proprietary		Spherical		3.0 µm		150 mm		3.0 mm		Waters Type	
	KSA		99		S		03		15		03		WT

Your part number: KSA99S03-1503WT (Example)

YMC Application Database

Application Search

In order to get full access to more than 900 YMC applications please visit our homepage www.ymc.eu/applications.html, where you can easily search for substances, colums, separation modes, etc.



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