interchim®

Technical Information

Contents

Introduction

Technical Information

YMC produces chromatography packing materials and HPLC columns under very strict quality control procedures and delivers to customers only those products that 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 leaflet which is included with each column. Please determine the miscibility of this solvent with the mobile phase to 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 (ie both 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 the instruction manual included with each column. Should this instruction manual be available, please contact YMC or your local distributor. A general overview of the characteristics of each phase is shown on the inside of the envelope.

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.

Column Handling

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.

When macromolecules, including proteins and sugars, adsorb to 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 is used, 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, call us.

Silica-based particles

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

Polar Samples	Non-polar Samples	Proteinaceous Samples
1. Water	1. Isopropanol	Option 1: Inject repeated allqouts 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) before being injecting onto a column.

Column ID (mm)	Recommended Guard Cartridge ID (mm)
1.0	1.0
2.1	2.1
3.0	3.0
4.0	4.0
4.6	4.0* (4.6*)
10	10
20	20
30	30

* Formerly, external guard columns of 4.6 mm ID exhibit dead volume resulting in poor performance of the column system. Additionally, these guards were rather expensive.
In the course 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.
Thus, we recommend to use 4.0 mm ID guards with a 4.6 mm ID main 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, 3750 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, 2550 psi) for 250 x 4.6 mm ID.

We recommend using a column at below the maximum operating pressure to ensure maximum column life.

	Maximum Operating Pressure		
	(MPa)	(bar)	(psi)
YMC-Triart 1.9 µm	100	1000	15000
YMC UltraHT 2.0 µm	50	500	7500

Column ID (mm)	Maximum Operating Pressure		
	(MPa)	(bar)	(psi)
1.0	20/25[1]	200/250[1]	3000/3750[1]
2.1	20/25	200/250	3000/3750
3.0	20/25	200/250	3000/3750
4.0	20/25	200/250	3000/3750
4.6	20/25	200/250	3000/3750
6.0	20/25	200/250	3000/3750
8.0	20/25	200/250	3000/3750
10	10	100	1500
20	10	100	1500
30	10	100	1500
50 and above	7.0/10	70/100[2]	1000/1500[2]
YMC-Actus 20/30	30	300	4500
YMC-Actus 50	10	100	1500

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

[2] depending on certification of the column; >10 µm packings should be kept below 3 MPa (30 bar, 450 psi)

Note: PolymerC18 has a pressure limit of 14.5 MPa (145 bar, 2000 psi)

6. Temperature

The upper temperature limit for silica and bonded phases is 50°C (70°C for YMC-Triart 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 phases 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 variables 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 tot 50 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 perchloric acid 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 the miscibility with the mobile phase existing in the column and a separate wash stage 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.

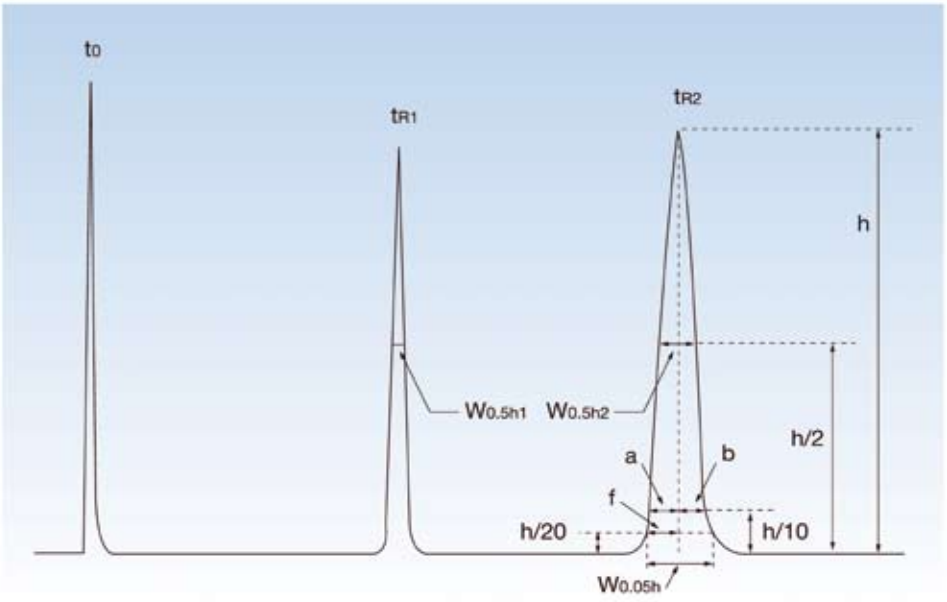
Column efficiency, an important characteristic for evaluation of column performance, is generally measured in terms of theoretical plate number. This is calculated using peak width at half-height. Narrower peak widths result in higher theoretical plate numbers. Longer columns and smaller packing material 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.

Theses 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.



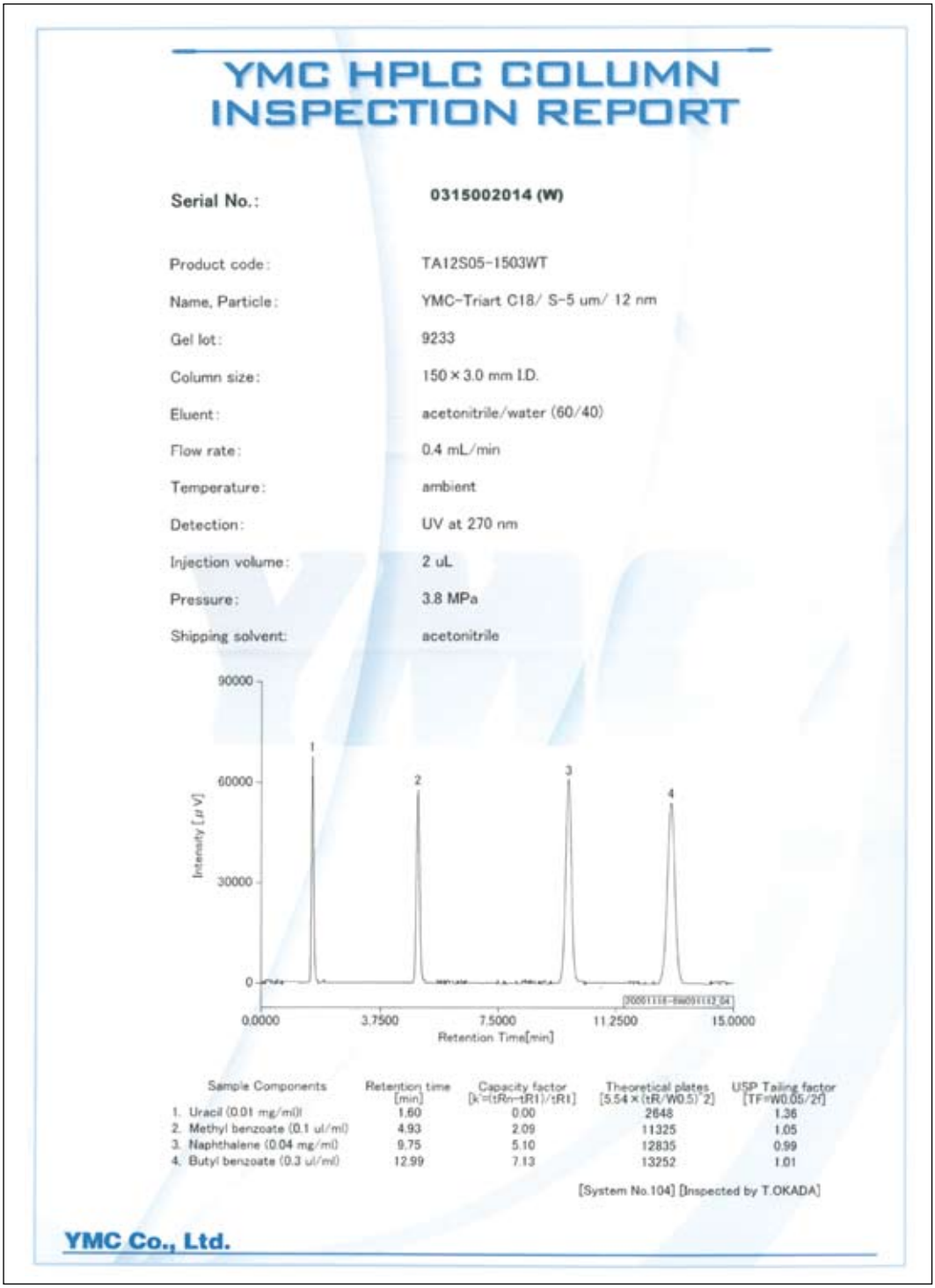
t_0	Void volume, Column dead-time	
t_R	Retention time	
h	Peak height	
$W_{0.5h}$	Peak width at half-height	
N	Theoretical plate number	$N=5.54\times (t_R/W_{0.5h})^2$
k'	Capacity factor	$k'=(t_R-t_0)/t_0$
α	Separation factor	$\alpha=k'_2/k'_1$
R_s	Resolution	$Rs=1.18\times (t_{R2}-t_{R1})/(W_{0.5h1}+W_{0.5h2})$
A_s	Asymmetry factor	As=b/a
Tf	Tailing factor	$Tf=W_{0.05h}/2f$

Inspection Reports

Formation

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.



YMC HPLC COLUMN
INSPECTION REPORT

Serial No.: 0315002014 (W)

Product code:	TA12S05-1503WT
Name, Particle:	YMC-Triart C18/ S-5 um/ 12 nm
Gel lot:	9233
Column size:	150 × 3.0 mm I.D.
Eluent:	acetonitrile/water (60/40)
Flow rate:	0.4 mL/min
Temperature:	ambient
Detection:	UV at 270 nm
Injection volume:	2 uL
Pressure:	3.8 MPa
Shipping solvent:	acetonitrile

Sample Components	Retention time [min]	Capacity factor [k'=(tRn-tR1)/tR1]	Theoretical plates [5.54×(tR/W0.5)^2]	USP Tailing factor [TF=W0.05/2f]
1. Uracil (0.01 mg/ml)]	1.60	0.00	2648	1.36
2. Methyl benzoate (0.1 ul/ml)	4.93	2.09	11325	1.05
3. Naphthalene (0.04 mg/ml)	9.75	5.10	12835	0.99
4. Butyl benzoate (0.3 ul/ml)	12.99	7.13	13252	1.01

[System No.104] [Inspected by T.OKADA]

YMC Co., Ltd.

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?

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 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 derivatisation 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, 3000 psi)

Column length of 250 mm or greater and diameters less than 10 mm:
25 MPa, (250 bar, 3750 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.

Frequently Asked Questions

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 of gel surface condition caused as a 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 mixedsolvent 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 materials 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.

Frequently Asked Questions

What should I do when the column pressure rises up?

Depending on the reasons for increased pressure, the following procedures are recommended:-

Blocked frits: Flush the column in reverse flow as described on page 342-343. 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 on page 218-219.

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 on page 225. 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.

Frequently Asked Questions

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 to be 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 Hydrosphere C18 or 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.

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 quantity of impurities UV absorbing 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 trace, many failures are 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.

Figures right show chromatograms from two identical operations except that the acetonitrile used was of different grades. One was HPLC grade (Figure 1); the other was 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 dependant 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

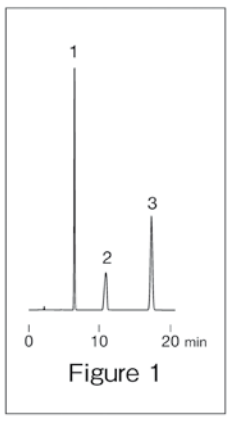


Figure 1

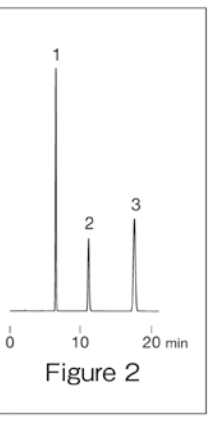


Figure 2

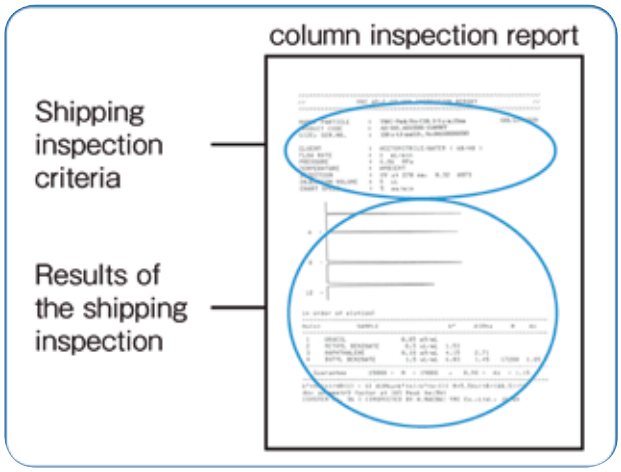
the chromatogram shown in Figure 2 irrespective of the grade of solvent. To avoid the influence of different grades, solvents specifically made for HPLC must be used. Even compounds which have groups which can dissociate can be analysed with eluent containing no acid or salt, although eluents with additives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

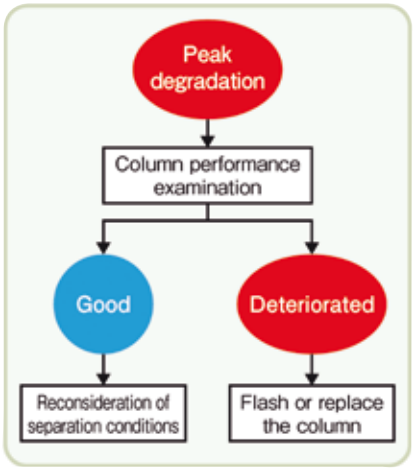
Troubleshooting

3. Peak shape anomaly

A common problem encountered during HPLC operations is peak shape anomalies such as peak tailing and double 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.

* Application data by courtesy YMC Co., Ltd.

Troubleshooting

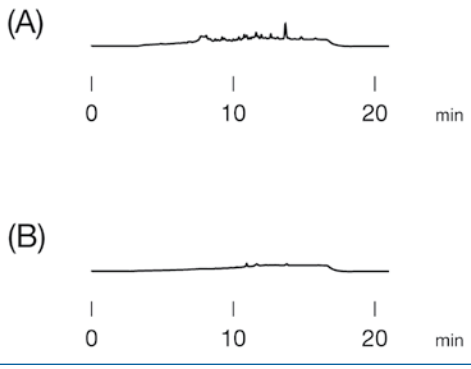
5. The Cause of the Ghost Peaks

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).

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)
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 shown in the figure below. 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.

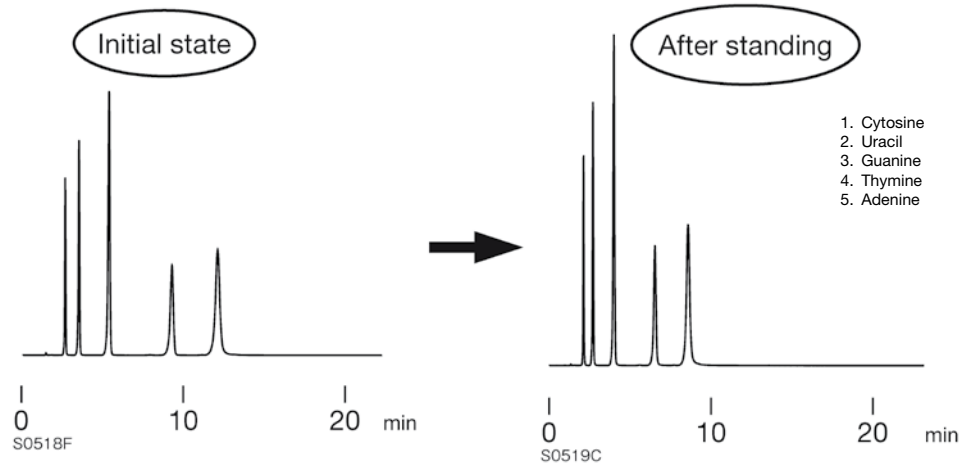
Troubleshooting

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

* Application data by courtesy YMC Co., Ltd.

Basic Data

Conversion factors

Pressure

MPa	bar	psi	kgf/cm²	atm
1	10	145.04	10.20	9.87
0.1	1	14.504	1.020	0.987
6.90×10^{-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	6.21×10^{-4}
0.025	1	0.083	0.028	0.15×10^{-4}
0.305	12	1	0.33	1.89×10^{-4}
0.91	36	3	1	5.68×10^{-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

Column Area Ratio

Inner Diameter	1.0	2.0	3.0	4.6	10.0	20.0	30.0	50.0
Ratio	0.0473	0.189	0.425	1	4.73	18.90	42.53	118.15

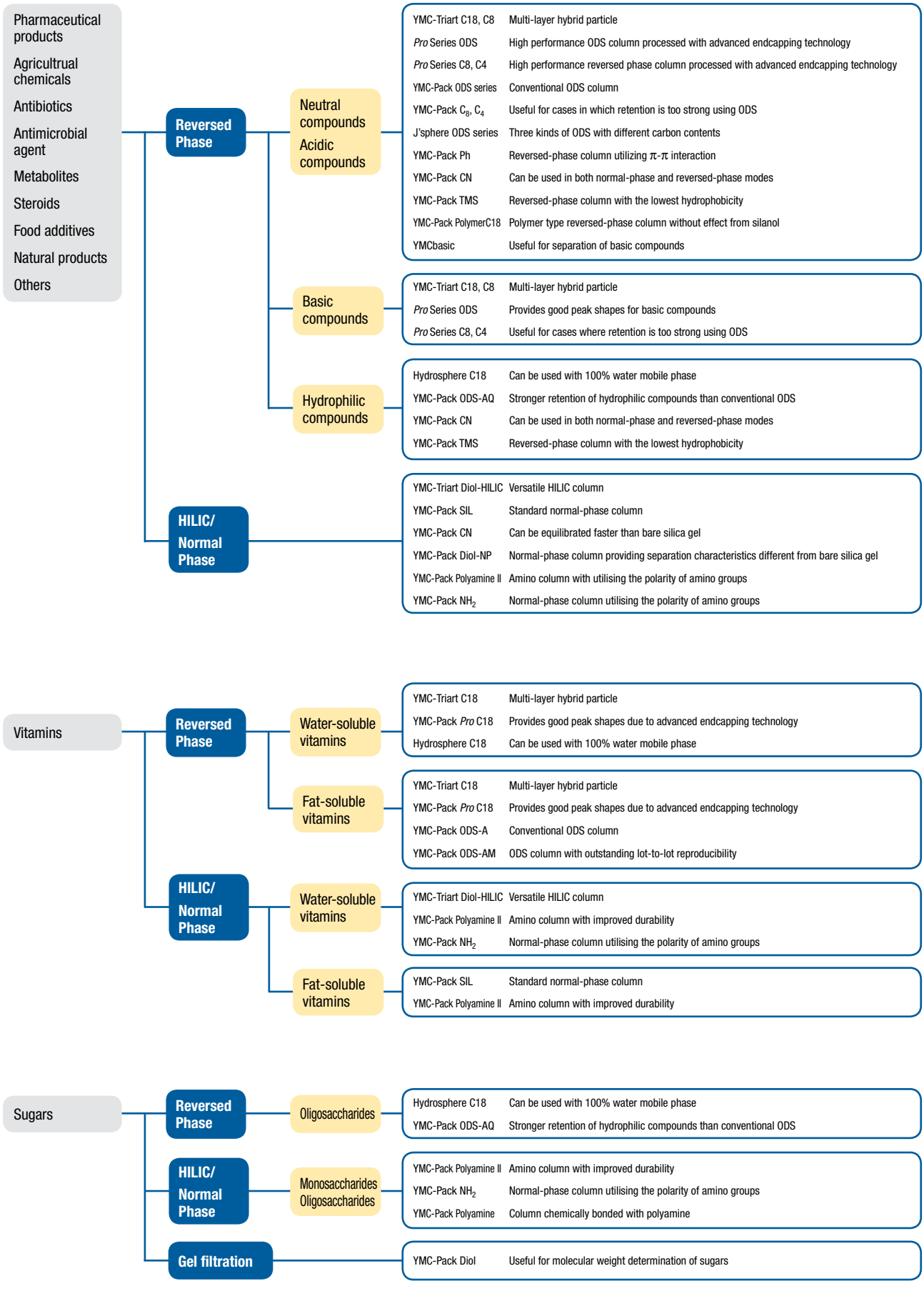
Solvent Miscibility Table

✔ = miscible

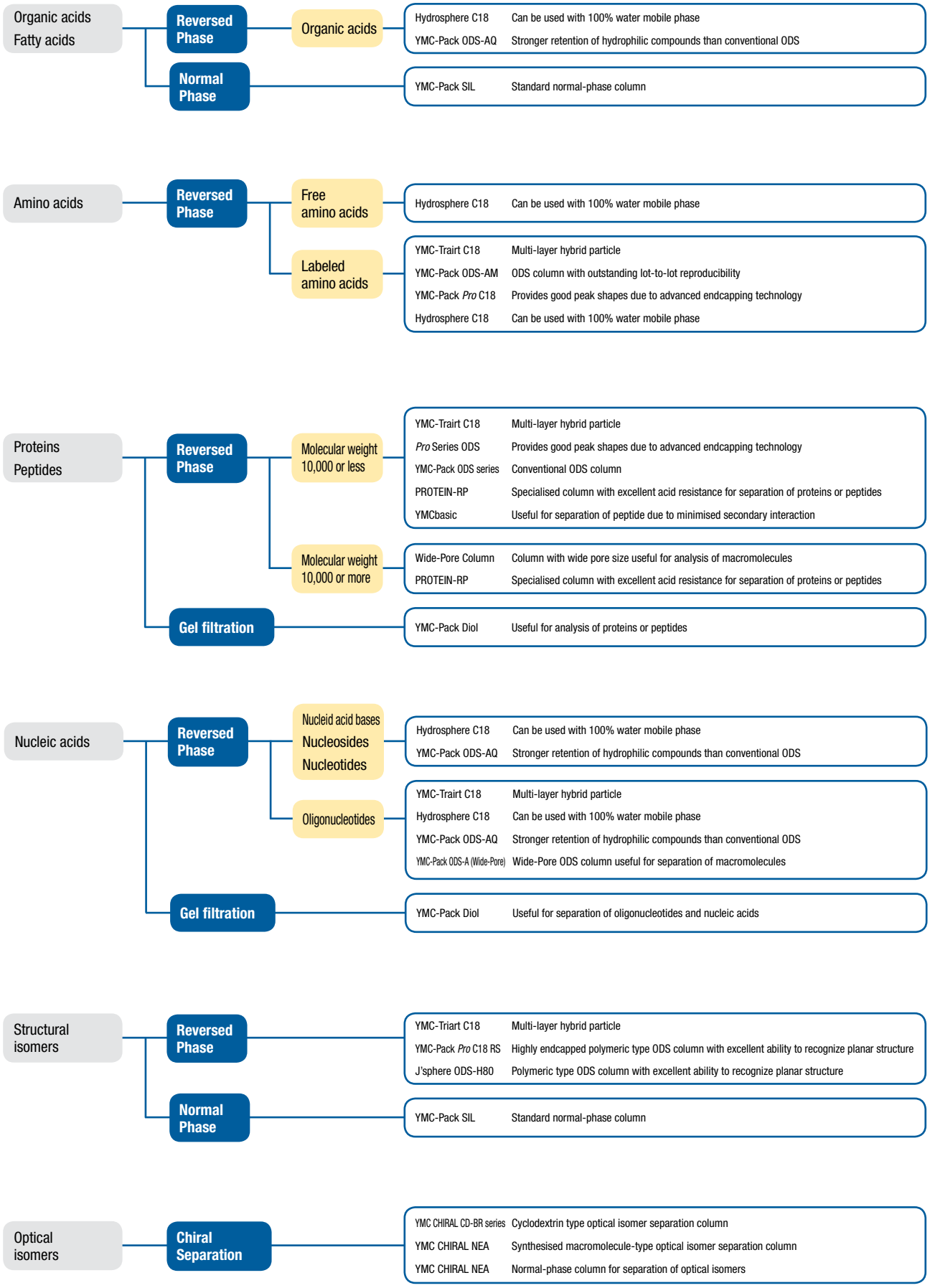
X = immiscible

	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	X	X	X	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔						
Acetonitrile	X	X	X	✔	X	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔					
Acetic acid	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔				
Dimethyl sulfoxide	X	X	X	X	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔			
Methanol	X	X	X	✔	X	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔		
Water	X	X	X	X	X	X	X	X	✔	X	✔	X	✔	✔	✔	✔	✔	✔	✔	✔	

Column Selection Guide



Column Selection Guide



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"
	$SF = r^2_{ID,\ prep} / r^2_{ID,\ anal.}$	$SF = l_{ID,\ prep} / l_{ID,\ anal.}$	$SF = (r^2_{ID,\ prep} / r^2_{ID,\ anal.}) / (l_{ID,\ prep} / l_{ID,\ anal.})$
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:

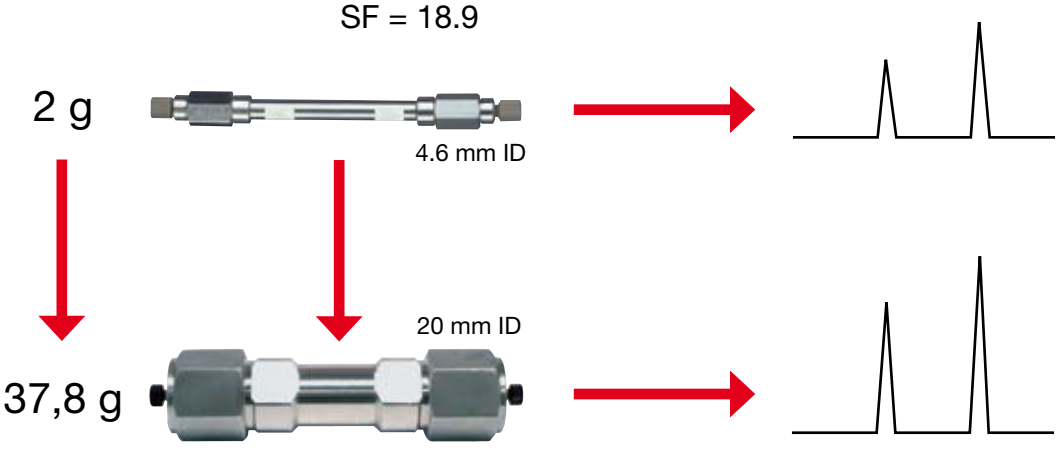
Linear Scale-Up

$$SF = \frac{\Pi^2_{ID,prep}}{\Pi^2_{ID,anal.}} = \frac{m_{prep}}{m_{anal.}}$$

$$SF = r^2_{ID,\ prep} / r^2_{ID,\ analytical}$$

$$SF = 20^2_{ID,\ prep} / 4.6^2_{ID,\ analytical}$$

$$SF = 18.9$$



Inner diameter ⟶ loadability / compound

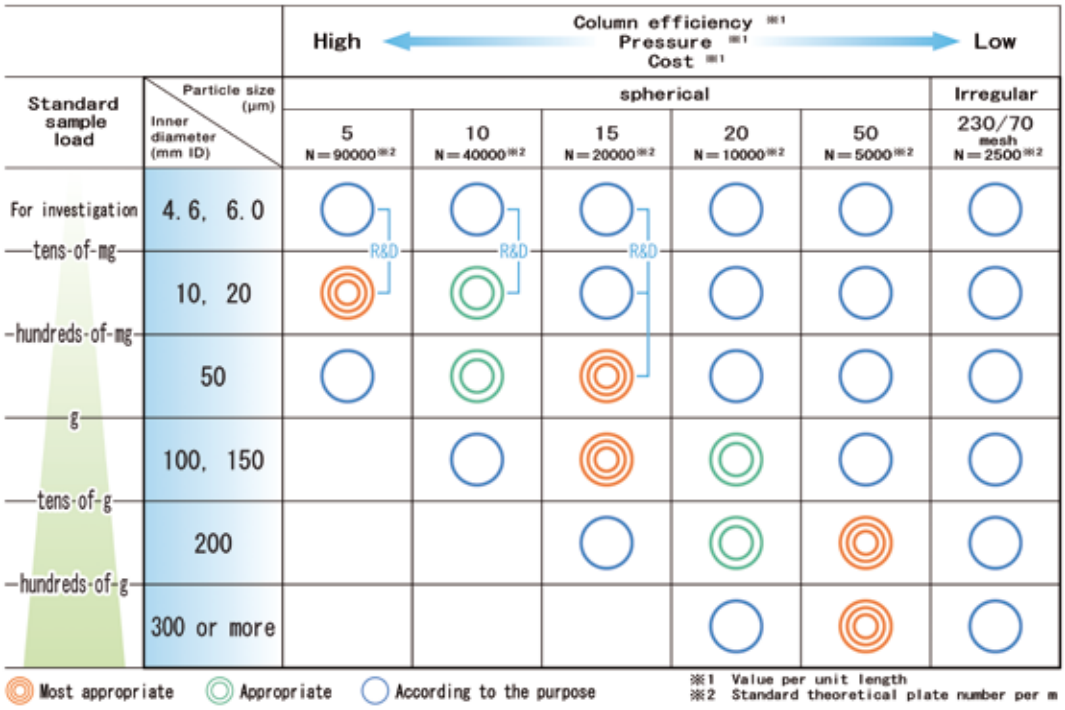
Guideline for Sample Load according to column ID

Column ID (mm)	Scale-Up factor	Loadability (mg)
4.6	1	1-4
10	4.7	5-20
20	18.9	20-80
50	118	80-350
75	266	270-980
100	472	470-1900
150	1060	1000-4200

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”.



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.

* Application data by courtesy YMC Co., Ltd.

Substance Index

Substance Index

Substance Index

Substance Index

Substance Index

Substance Index

Substance Index

Substance Index

Substance Index

Ordering Information

The previous product listing represents commonly used standard column dimension. In order to identify any specific product version and order 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	
YMC30	CT	6	06	spherical	S	3	03	10	01	0.05	E5	Quick Seal	QT
Triart C18	TA	8	08			4	04	20	02	0.075	E8	Cartridge	QC
Pro C18	AS	12	12			5	05	33	03	0.1	F0	Waters type	WT
Pro C18 RS	RS	20	20			6	06	50	05	0.2	G0		
Hydrosphere C18	HS	30	30					75	L5	0.3	H0		
ODS-A	AA	100	A0			10	11	100	10	0.5	J0		
ODS-AM	AM	proprietary	99			15	16	125	R5	0.8	M0		
ODS-AQ	AQ	non-porous	00			20	21	150	15	1.0	01		
J'sphere ODS-H80	JH					50	50	250	25	2.1	02		
J'sphere ODS-M80	JM					75	75			3.0	03		
J'sphere ODS-L80	JL							300	30	4.0	04		
ODS-AL	AL					63/210	A4	500	50				
PAH	YP					150	A5	1000	A0	4.6	46		
PolymerC18	PC									6.0	06		
Triart C8	TO									8.0	08		
Pro C8	OS									10	10		
C8 (Octyl)	OC									20	20		
YMCbasic	BA									30	30		
Ph (Phenyl)	PH												
Pro C4	BS									50 (2000 psi)	52		
C4 (Butyl)	BU									70 (2000 psi)	72		
Protein-RP	PR									100 (2000 psi)	A2		
TMS (C1)	TM									150 (2000 psi)	B2		
PVA-Sil	PV									200 (2000 psi)	C2		
Polyamine II	PB												
NH_2 (Amino)	NH												
CN (Cyano)	CN												
Triart Diol-HILIC	TDH												
Diol	DL												
SIL (Silica)	SL												
BioPro-QA	QA												
BioPro-SP	SP												
BioPro-QA-F	QF												
BioPro-SP-F	SF												
Chiral NEA (R)	NR												
Chiral NEA (S)	NS												
Chiral CD BR α	DA												
Chiral CD BR β	DB												
Chiral CD BR γ	DG												
Chiral Prep CD ST	ST												
Chiral Prep CD PM	PM												

Example

Choose your column and fill in the "Gel and Hardware Code" or detailed description (The part number consists of the "Gel Code" and the "Hardware Code").

YMC-Pack ODS-A	12 nm	spherical	3 µm	250 mm	1.0 mm	Quick Seal
AA	**12**	**S**	**03**	**25**	**01**	**QT**

Your column part number: AA12S03-2501QT (Example)

Please note that combinations of features cannot be selected at random, but only from the possible specifications for a chosen stationary phase. These can be determined from the individual product sections in this catalogue or from our homepage www.ymc.de.

For more details

contact your local distributor or

YMC Europe GmbH, Schöttmannshof 19, D-46539 Dinslaken, Phone: +49 (0) 2064 / 427-0,
Fax +49 (0) 2064 / 427-222, e-mail: info@ymc.de, homepage: www.ymc.de

The following brands, trademarks, or service marks are the property of the listed company and/or its subsidiaries. Every effort has been taken to ensure this list is accurate at the time of printing this brochure.

SureFit is trademark of IDEX Corporation.

Capcell Pak MG-II, Capcell Pak C18 AQ are trademarks of Shiseido Co., Ltd.

Intertsil ODS-3V, Intertsil ODS-3, Intertsil ODS-4, Inertsil C8-3 are trademarks of GL Sciences, inc..

Acquity UPLC BEH C18, Acquity UPLC CSH C18, Atlantis dC18, Atlantis T3, SunFire C18, Symmetry C18, Symmetry C8, X-Bridge C18, XTerra MS C18, OBD are trademarks of Waters Corp.

Gemini, Gemini NX, Kinetex C18 are trademarks of Phenomenex, Inc.

Hypersil GOLD and ProPac WCX-10 are trademarks of Thermo Fisher Scientific.

Kromasil Eternity is a trademark of EKA Chemicals AB.

TSK-gel ODS-100S, TSKgel DEAE-NPR, TSKgel SP-NPR, TSKgel BioAssist Q and TSKgel BioAssist S are trademarks of Tosoh Bioscience LLC.

Zorbax Extend-C18, Zorbax StableBond, Zorbax Eclipse Plus, Zorbax Eclipse XDB-C18, Zorbax SB-C18 and Zorbax XDB-C18 are trademarks of Agilent Technologies, Inc..

L-column is a trademark of Chemicals Evaluation and Research Institute.

Mini Q, Mono Q and Mono S are trademarks of General Electric Company as GE Healthcare.

ReproSil-Pur C18-AQ is a trademark of Dr. Maisch GmbH.

ECO, ECOplus and Kronlab are trademarks of YMC Europe GmbH.

Bio-Rad is a trademark of Bio-Rad Laboratories Inc..

GE is a trademark of General Electric Company.

Pall is a trademark of Pall Corporation.

Tosoh is a trademark of Tosoh Corporation.

Analytical stationary phases routinely available from YMC

		PRODUCT	PAGE	PHASE (silica-based unless stated)	END-CAPPED	USP CLASS NO.	PARTICLE SIZE (µm spherical)	PORE SIZE (nm)	CARBON LOAD (%C)	pH	TYPICAL APPLICATIONS	PAGE	PRODUCT
Reversed Phase	C30	C30	192	proprietary polymeric bonding chemistry	—	—	3, 5	proprietary	—	2.0-7.5	isomeric carotenes, retinols, steroids, fat-soluble vitamins	192	C30
	C18	Triart C18	13-40 216	multi-layer hybrid particle	yes	L1	1.9, 3, 5	12	20	1.0-12.0	acid, neutral, basic and chelating compounds, metabolites, "versatile" stationary phase	13 216	Triart C18
		Pro C18	70	very low residual non-specific interactions	yes	L1	3, 5	12	17	2.0-8.0	fat-soluble vitamins, antioxidants, metabolites, acidic, neutral, basic and chelating compounds	70	*Pro* C18
		UltraHT	54	2 µm *Pro* C18 for fast and ultra fast separations			2					54	UltraHT
		Pro C18 RS	76	high carbon load with polymeric bonding C18	yes	L1	3, 5	8	22	1.0-10.0	acidic and basic compounds	76	*Pro* C18 RS
		Hydrosphere C18	80	can be used in 100% aqueous eluent	yes	L1	3, 5	12	12	2.0-8.0	strong polar compounds, antibiotics, nucleic acids, water-soluble vitamins, acidic, neutral, basic and chelating compounds	80	Hydrosphere C18
		UltraHT	54	2 µm Hydrosphere C18 for fast and ultra fast separations			2					54	UltraHT
		ODS-A	90	one of the YMC's international bestsellers	yes	L1	3, 5	12, 20, 30	17, 12, 7	2.0-7.5	general purpose phase	90	ODS-A
		ODS-AM	92	high performance C18 column for validated methods operation	yes	L1	3, 5	12	17	2.0-7.5	purines, phenols, PTC-amino acids, angiotensins, alkaloids	92	ODS-AM
		ODS-AQ	86	"hydrophilic" endcapping, for 100% aqueous eluent systems	yes	L1	3, 5	12, 20	14, 10	2.0-7.5	strong polar compounds	86	ODS-AQ
		J'sphere	196	C18-family with differently controlled hydrophobicity for method development	yes	L1	4	8	22, 14, 9 (JH, JM, JL)	1.0-9.0 (JH) 2.0-7.5 (JM+JL)	positional isomers, complexing agents, pharmaceuticals	196	J'sphere
		ODS-AL	94	traditional C18 for "mixed mode" separations	no	L1	3, 5	12	17	2.0-7.5	tocopherols, fat-soluble vitamins, disinfectants	94	ODS-AL
		Polymer C18	96	polymethacrylate matrix, wide pH applicability	—	—	6	proprietary	C18 equivalent 10%	2.0-13.0	phenols, anilines, peptides in high pH, pharmaceuticals, quaternary amines	96	Polymer C18
	C8	Triart C8	13-40 216	multi-layer hybrid particle	yes	L1	1.9, 3, 5	12	17	1.0-12.0	acid, neutral, basic and chelating compounds, metabolites, "versatile" stationary phase	13 216	Triart C8
		Pro C8	72	C8, with very low residual non-specific interactions	yes	L7	3, 5	12	10	2.0-7.5	acidic, neutral, basic and chelating compounds, drugs and metabolites	72	*Pro* C8
		C8 (Octyl)	100	traditional C8	yes	L7	3, 5	12, 20, 30	10, 7, 4	2.0-7.5	proteins and peptides, estrogens, general purpose phase	100	C8 (Octyl)
		YMCbasic	98	monomeric bonded chains of C8 and smaller	—	L7	3, 5	proprietary	8	2.0-7.5	basic molecules w/o modifiers, anilines, alkaloids, antidepressants	98	YMCbasic
		Ph (Phenyl)	102	monomeric bonded phenyl	yes	L11	3, 5	12, 30	9, 3	2.0-7.5	phenols, fullerenes, sweeteners	102	Ph (Phenyl)
	C4	*Pro* C4	74	C4, with very low residual non-specific interactions	yes	L26	3, 5	12	8	2.0-7.5	polar acidic, neutral, basic and chelating compounds, polar peptides	74	*Pro* C4
		C4 (Butyl)	104	traditional C4	yes	L26	3, 5	12, 20, 30	7, 5, 3	2.0-7.5	biological separations, polar compounds	104	C4 (Butyl)
		PROTEIN-RP	158	high stability, good recovery rates	yes	L26	5	proprietary	—	1.5-7.5	proteins, peptides	158	PROTEIN-RP
		YMC-PAH	194	proprietary bonding chemistry	—	—	3, 5	—	—	2.0-8.0	polyaromatic hydrocarbons	194	YMC-PAH
Normal Phase / HIILIC		TMS (C1)	106	trimethyl silane	—	L13	3, 5	12, 30	4, 3	2.0-7.5	water-soluble vitamins	106	TMS (C1)
		PVA-SIL	118	polyvinyl alcohol bonded on silica support	—	L24	5	12	—	2.0-9.5	proteins, phospholipids, retinoids, lipids	118	PVA-SIL
		Polyamine II (PBMN)	124	mixed secondary and tertiary amino derivative	—	—	5	12	—	2.0-7.5	malto-oligosaccharides, tocopherols, nucleotides, sugars	124	Polyamine II (PBMN)
		NH_2 (Amino)	126	primary amino derivate	—	L8	3, 5	12	3	2.0-7.5	sugars, nucleotides, water-soluble vitamins	126	NH_2 (Amino)
		CN (Cyano)	120	useful for SFC applications	yes	L10	3, 5	12, 30	7, 3	2.0-7.5	proteins, steroids, catechols	120	CN (Cyano)
		Triart Diol-HILIC	32	versatile HILIC column	—	L20	1.9; 3; 5	12	—	2.0-10.0	peptides, proteins, malto-oligosacchardes	32	Triart Diol-HILIC
		Diol	122 146	versatile alternative to silica for normal phase separations	—	L20	5	6, 12	—	2.0-7.5	peptides, proteins, malto-oligosacchardes	122 146	Diol
		SIL (Silica)	116 214	ultra high purity, high mechanical stability	—	L3	3, 5	6, 12, 20, 30	—	2.0-7.5	small oganic molecules, fat-soluble vitamins, tocopherols	116 214	SIL (Silica)
IEX		BioPro QA / SP	136	high ion exchange capacity, porous hydrophilic polymer	—	—	5	100	—	2.0-12.0	proteins, peptides, nucleotides	136	BioPro QA / SP
		BioPro QA-F / SP-F	141	high ion exchange capacity, non-porous hydrophilic polymer	—	—	5	—	—	2.0-12.0	proteins, peptides, nucleotides	141	BioPro QA-F / SP-F
SEC		Diol-60, -120, -200, -300	122 146	versatile phase for gel filtration separations	—	L20	5	6, 12, 20, 30	—	5.0-7.5	peptides, proteins, malto-oligosacchardes	122 146	Diol-60, -120, -200, -300

ANALYTICAL SCIENCES

interchim

211 bis, avenue JF Kennedy
BP 1140 - 03100 Montluçon
Fax 33 4 70 03 82 60

Hotline +33 4 70 03 73 09

interchrom@interchim.com