EVOSPHERE COLUMN CARE GUIDE

Fortis™ Technologies

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INTRODUCTION

All Evosphere columns shipped are manufactured to the highest possible quality standard and should reach you in this condition. Each column is individually tested using Quality Assured (QA) conditions.

Each column is a precision instrument which if cared for properly will provide significant performance, lifetime and repeatability. This column care guide is designed with these factors in mind allowing you to extract the utmost from your HPLC column. It is designed to help alleviate problems before they occur and covers a range of topics. It is not designed to replace good lab practise merely to aid in this for your chromatography.

If you would like help in developing a method on a Evosphere column then please feel free to contact our technical support group who will be happy to assist you in this:

technicalsupport@fortis-technologies.com

If you do have an issue with a Evosphere column please contact our technical team who will assist in troubleshooting with you, please have to hand your part number, serial number and supporting chromatography.

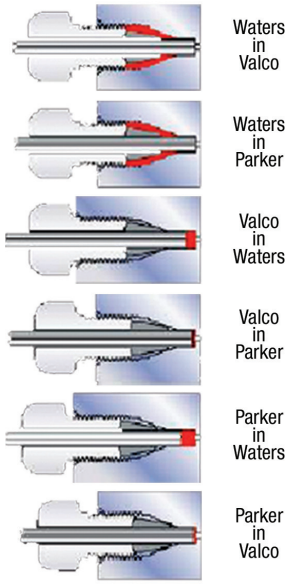
Upon receipt of your new column it should immediately be tested as per our QC conditions to verify the performance and quality. After 30days columns will only be accepted as returns with a valid authorisation number from our technical support team.

Column Installation

All Fortis columns come in 316L stainless steel (SS) hardware. A SS female endfitting houses a SS and Peek frit system which retains the stationary phase in place and stops packed bed disturbance. The SS endfitting will accept a male 10-32 threaded nut with standard 1/16th OD tubing.
Care must be taken to ensure that the tubing fits correctly against the frit cap, failure to do this can result in a dead volume which can lead to poorer efficiency and peak shapes. Swaged ferrule's can differ between manufacturers instruments.

Choice of tubing and ferrule material is generally restricted to three materials: PEEK for general use, most solvents and normal pressures. Stainless Steel for more aggressive solvents, buffers and pressures, and Titanium for biocompatibility and solvent range.

If PEEK can be utilised then this will aid in ensuring the correct depth in the female endfitting, as is unfixed and variable in its depth.
Stainless steel ferrules are fixed once they are tightened the first time, and if a column brand is changed the depth of tubing must correspond to the new column endfitting.



RECOMMENDED TUBING

If the tubing and fittings are not optimised this can lead to bandbroadening and poor peak shapes. The following are guidelines on the diameter of tubing which will give optimum results in chromatography. All tubing is suggested as 1/16th OD tubing.

Tubing ID (inch)	Column ID (mm)	Typical Flow rate (ml/min)
0.002	0.32	0.001 – 0.02
0.005	1.0 – 2.1	0.02 – 0.4
0.007	2.1 – 4.6mm	0.02 – 2.0
0.010	4.6 – 7.8mm	0.4 – 5.0

0.005" is optimal for most general analytical dimensions and flow rates. Keep tubing lengths as short as practically possible.

EQUILIBRATION

- All solvents used in the HPLC columns should be of HPLC grade or MS grade quality.
- Mobile phase should be filtered and de-gassed before use
- Flush the solvent to be used through the system before installing the column
- Connect the column taking care to match the arrow to the direction of flow
- Slowly ramp the flow rate to that required, ensuring no pressure surges occur. Analytical columns should be kept below 400bar. Evosphere UHPLC (Ultra high pressure liquid chromatography) columns can be run up to 1000bar.
- Flush the column with 20-30 column volumes of mobile phase.
- Column temperature should be kept below <60°C for optimum lifetime, columns used above this temperature are at the customers discretion, lifetime will be affected.
- pH of mobile phases should ideally be kept between 2-8 for optimum performance of phases. Some stationary phases will operate at pH 1-11. Refer to physical specifications table on the Fortis website.
- Use of organic buffers such as formic acid, ammonium acetate will enhance lifetime over inorganic buffers such as phosphate
- Use of guard system or in-line filters will aid column lifetime

For full information on pH ranges and buffer choice contact please Fortis Technologies technical support department

If using columns in Normal Phase (NP) mode then use a miscible solvent such as acetone to move from RP to NP solvent system. All Evosphere columns are shipped in RP solvents.

To calculate column volume a rough approximation is:

$$V_m \approx 0.5 L d_c^2$$

Where:
V_m - Column volume
L - Length of column (cm)
d_c - Internal diameter of the column (cm)

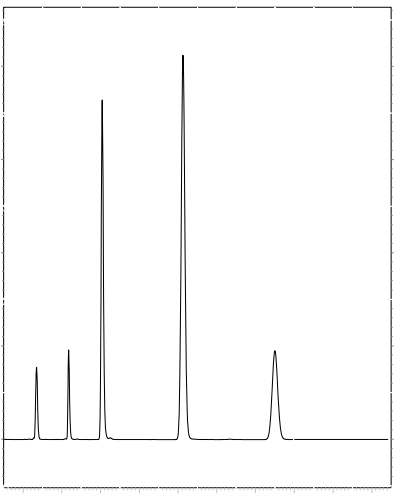
TESTING THE COLUMN

Column performance can be replicated using the test conditions within the enclosed QC certificate.

All results are subject to subtle changes due to system differences. Evosphere column testing is performed under optimum conditions to minimise band dispersion. Any differences in extra-column effects from injector, connecting tubing or flow cell will affect the result that is achieved. Pumps and mixing cells can affect pressure and retention times.

If any changes are noted that are outside of expected "experimental error" they should be discussed with your local distributor or Fortis Technologies representative.

Fortis QC Test



Column: Evosphere C18/AR 100x4.6mm 5µ
p/n: EV018AR-050505
Mobile Phase: 60:40 ACN:H_2O

Flow: 1.0ml/min
Temp: 25°C
Wavelength: 254nm

1. Uracil
2. Phenol
3. Propiophenone
4. Butyrophenone
5. Napthalene

CALCULATE EFFICIENCY

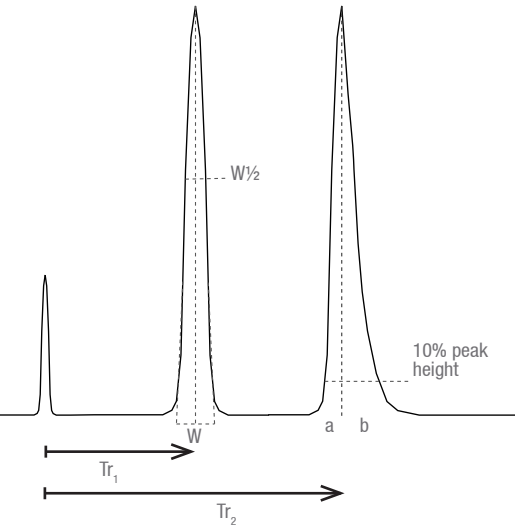
Column efficiency can be calculated by using the following equation:

$$N = 5.54 \times \left(\frac{Tr_1}{W½} \right)^2$$

CALCULATE PEAK SHAPE

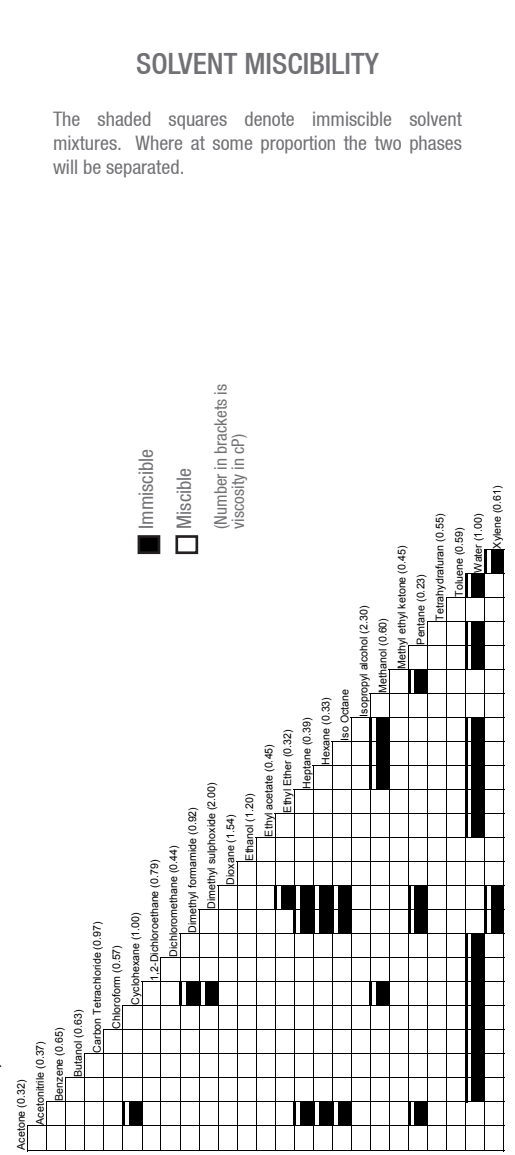
Column peak shape can be calculated by using the following equation:

$$Tf = \frac{(a+b)}{2a}$$



SOLVENT MISCIBILITY

The shaded squares denote immiscible solvent mixtures. Where at some proportion the two phases will be separated.



BUFFER CHOICE

Under most conditions stationary phases are highly stable bondings. However excessive use of pH can cause damage to the stationary phase. At low pH hydrolysis of the siloxane link can occur leading to loss of the stationary phase ligand. At high pH dissolution of the silica template can occur leading to voids and reduced retention. Careful choice of buffer and pH should be made to ensure the maximum possible lifetime of your Fortis column. Organic buffers such as formic and acetate will be 'softer' on the stationary phase than inorganic phosphate type buffers, as well as being more MS friendly.

Guard columns should always be used as sacrificial columns when pH is being used at extremes.

Buffer	pKa	pH range
Trifluoroacetic acid	<< 2	1.5 -2.5
KH_2PO_4 / Phosphoric acid	2.12	1.1 - 3.1
Tri-K-Citrate / hydrochloric acid 1	3.06	2.1 - 4.1
Potassium formate / formic acid	3.8	2.8 - 4.8
Tri-K-Citrate / hydrochloric acid 2	4.7	3.7 - 5.7
Potassium acetate / acetic acid	4.8	3.8 -5.8
Tri-K-Citrate / hydrochloric acid 3	5.4	4.4 - 6.4
Ammonium formate	3.8 & 9.2	2.8-4.8 & 8.2-10.2
Bis-tris propane.HCl/Bis-tris propane	6.8	5.8 - 7.8
Ammonium acetate	4.8 & 9.2	3.8-5.8 & 8.2-10.2
KH_2PO_4 / K_2HPO_4	7.21	6.2 - 8.2
Tris.HCl / Tris	8.2	7.3 - 9.3
Bis-tris propane.HCl/Bis-tris propane	9.0	8.0 - 10.0
Ammonium hydroxide / Ammonia	9.2	8.2 - 10.2
Borate (H_3BO_3 / $Na_2B_4O_7.10H_2O$)	9.24	8.2 - 10.2
Glycine.HCl / glycine	9.8	8.8 - 10.8
1-methylpiperidine	10.1	9.1 - 11.1
Diethylamine.HCl / diethylamine	10.5	9.5 - 11.5
Triethylamine.HCl / triethylamine	11.0	10.0 - 12.0
Pyrollidine.HCl/pyrollidine	11.3	10.3 - 12.3

STORAGE OF COLUMNS

- Buffers should be removed from the column. The column should be stored in at least 40% organic solvent.
- Endfittings should be fitted to ensure no evaporation of solvent and consequent column 'dry-out'.
- Columns should be stored at room temperature free from vibration
- If a column is to be stored for any period of time ideally column cleaning should take place as per guidelines

Columns are shipped initially in 60:40 ACN:Water this is an ideal storage solvent.

COLUMN CLEANING

Columns should be flushed free from buffer to start any cleaning procedure. High concentrations of water should be used to do this. It is useful if prior knowledge of what the contaminants are, and what they are most likely to be soluble in.

Columns can be flushed in the reverse direction to ensure minimal wash path and also to ensure no blockage of the frits.

Reversed Phase (RP) Wash

20 column volumes should be used for each wash stage:
95 : 5 Water: ACN (Removal of buffer)
100% ACN
THF (optional)
50 : 50 Water: ACN

Strong RP Wash

In the event of very highly retained compounds or matrix on the column then an additional step of DMSO or THF injections can be added:

95 : 5 Water: ACN (Removal of buffer)
100% ACN
Inject DMSO or THF 5 x 20µl
50 : 50 Water: ACN

Normal Phase (NP) Wash

20 column volumes should be used for each wash stage:
THF
Chloroform
Methylene Chloride
Hexane

HILIC Wash

20 column volumes should be used for each wash stage:
95 : 5 ACN: Water (Removal of buffer)
50 : 50 ACN : Water
50 : 50 Methanol : Water
95 : 5 ACN: Water

Protein Wash

If biological molecules proteins/peptides are suspected to be retained then you can use a mixture of :
Trifluoroacetic acid (TFA), MeCN and IPA (1:2)
or
Large 100ul injections of Trifluoroethanol

To calculate column volume a rough approximation is:

$$V_m \approx 0.5Ld_c^2$$

Where:
V_m - Column volume
L - Length of column (cm)
d_c - Internal diameter of the column (cm)

TROUBLESHOOTING

Columns can be affected in several ways and stability can be compromised for several reasons:

» Partially blocked (plugged) frit or column bed
» Adsorbed sample impurities
» Mechanical or thermal shock creating voids
» Chemical attack on the support or stationary phase

Retention and Resolution	
Causes:	Solutions:
Loss of bonded phase	Replace column or guard
Build up of contamination	Wash as per cleaning guidelines
Slow Equilibration	Equilibrate in mobile phase for longer
Normal Phase – moisture in the mobile phase	Ensure dry solvents, or use 2% water in the mobile phase
Backpressure - Change/Increase	
Causes:	Solutions:
Blocked frit	Reverse column and clean as per guidelines
	Replace frit or guard column
Contaminated phase	Reverse column and clean as per guidelines
Precipitation of buffer due to high concentration of organic modifier	Flush with high water (>90%) concentration
Tailing or Split peaks	
Causes:	Solutions:
Void	Replace column or guard
Blocked Frit	Reverse flush the column
Sample Overload	Dilute sample
Build up of contamination	Clean column as per guidelines
Extra column broadening	Check all tubing connections, injector seat for leaks
Poor sample solubility	Modify injection solvent or mobile phase composition

GUARDS and FILTERS

Guard columns provide extra protection for the analytical column, giving increased lifetimes and added value. Fortis Guards are 10mm in length, which adds enough protection to the system without adding any notable change in retention or selectivity. They are quickly changeable cartridges in order to keep downtime to a minimum.

Guards for any Fortis stationary phase are available in the format to match your analytical column.

Description	Part Number
F18-040003G/2	10x4mm Fortis C18 3µm Guard pk 2
F18-040003G/4	10x4mm Fortis C18 3µm Guard pk 4
F18-020003G/2	10x2mm Fortis C18 3µm Guard pk 2
F18-020003G/4	10x2mm Fortis C18 3µm Guard pk 4
F18-040005G/2	10x4mm Fortis C18 5µm Guard pk 2
F18-040005G/4	10x4mm Fortis C18 5µm Guard pk 4
F18-020005G/2	10x2mm Fortis C18 5µm Guard pk 2
F18-020005G/4	10x2mm Fortis C18 5µm Guard pk 4

Another alternative to a guard column is a frit filter system. This adds an extra frit into the flow path ensuring that any particulate matter is trapped and does not reach the analytical column.
These in-line filters are available for LC 400bar systems or 1000bar UHPLC systems.
Analytical frits are 2um pore size

UHPLC frits are 0.5um in size and are made entirely from stainless steel.

Description	Part Number
2-SAV5	2µm In-line filter pk 5
2-SAV10	2µm In-line filter pk 10
UHPSAV2	UHPLC In-line filter pk 2
UHPSAV4	UHPLC In-line filter pk 4

HPLC Column Warranty

All Fortis Technologies columns shipped are manufactured to the highest possible quality standard and should reach you in this condition. Each column is individually tested using Quality Assured conditions. These conditions should be reproducible in your laboratory. All columns, when received, should provide the same quality and performance as originally shown, if tested under identical conditions. Columns which perform to the original quality standard will be deemed fit for purpose. Fortis Technologies does not accept responsibility for the results of a column performing a specific customer application or for the length of time that the column will last under any given conditions.

In order for a column to be returned the following criteria must be met:
Proof of conditions used must be provided to the technical department of Fortis Technologies, due care shown in use must also be in evidence. Any column supplied by but not manufactured by Fortis Technologies is subject to the original manufacturer's warranty. Tampering with or removing end-fittings invalidates the column warranty. Conditions used should not exceed those implied or stated by Fortis Technologies; use of the column outside of these limits will lead to the warranty being invalidated.

- Maximum warranty period is limited to 30days commencing on the date of receipt.

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