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## Desalting RP Columns

### Column Information

Desalting RP columns are specifically designed for removing salts from aqueous biomolecule samples such as peptides, proteins and DNAs while enabling online MS analyses of the biomolecules. The packing support is composed of porous, spherical, and highly cross-linked poly(styrene/divinylbenzene) (PS/DVB) beads with a particle size of 5  $\mu\text{m}$ . The column is compatible with most aqueous buffers, such as acetate, phosphate, tris, etc.

Though usually run as a stand-alone column for quick LC/MS analysis, the Sepax Desalting RP column can run as a second dimension with SEC, IEX, affinity and others. After the salt is washed out of the column, the bio analytes can be eluted and further analyzed.

### Safety Precaution

Desalting RP columns are normally operated under high pressure. Loose connections will result in leaking of mobile phases and injected samples, all of which should be treated as hazardous. Proper gloves should be worn in the case of leaking. When opening the columns, proper protection should be used to avoid inhalation of the small polymer particles.

### Column Installation and Operation

Columns are always capped at both ends while being shipped or held in storage. During column installation, first remove the end caps. Unless the user has a specific need for reverse flow, for example, the removal of inlet pluggage, follow the flow direction marked on the column. Make the flow direction as marked on the column. Column connections are an integral part of the chromatographic process. If ferrules are over tightened, not set properly, or are not specific for the fitting, leakage can occur. Set the ferrules for column installation to the HPLC system as follows:

(a) Place the male nut and ferrule, in order, onto a 1/16" o.d. piece of tubing. Be certain that the wider end of the ferrule is against the nut.

(b) Press tubing firmly into the column end fitting. Slide the nut and ferrule forward, engage the threads, and fingertighten the nut.

(c) While continuing to press the tube firmly into the endfitting, use a 1/4" wrench to further tighten.

(d) Repeat this coupling procedure for the other end of the column.

**Shipping Solvent** New columns are usually shipped in a mixture of acetonitrile and water.

### Technical Specifications

Packing	Highly cross-linked PS/DVB resin support grafted with a densely packed, nanometer thick amphiphilic coating.
Particle size	5 $\mu\text{m}$
Pore structure	Porous
pH stability	2-13 (working pH)
Operating temperature limit	80 °C
Operating pressure limit	200 bar
Mobile phase compatibility	Compatible with aqueous buffer solutions: phosphate, tris and acetate, as well as a mixture of water and acetonitrile (ACN), isopropanol (IPA), acetone, or methanol.
Flow rate	0.1-0.5 mL/min is typical for a 2.1 mm I.D. column

### Samples and Mobile Phases

To avoid column clogging, all samples and solvents including mobile phases should be filtered through 0.2  $\mu\text{m}$  filters before use. It is also strongly recommended to use a pre-column filter (0.5 $\mu\text{m}$ ) to protect the column. The desalting RP columns are compatible with an aqueous mobile phase or a mixture of organic and water, such as methanol or IPA and water. Typical LC/MS compatible mobile phases can be applied to this column, such as Formic acid, TFA, ACN, IPA, etc. Always use an inline degassor or degas the mobile phase prior to use. A simple way for degassing is to sonicate it for 5 minutes under water pumped vacuum.

**First-time use** During stocking and shipping, the packing may dry out. It is recommended to equilibrate the columns with 10 column volumes of the 90% organic component in the mobile phase. Then, condition the column with the mobile phase. It is always recommended to apply a gradual increase of the flow rate from 0.1 mL/min to your operation condition, until the baseline is stable. Clean the desalting columns with high organic content.

**pH** To ensure optimum performance and a long lifetime, the pH should remain between 2-13.

**Pressure** Even though the Desalting RP columns can operate at a pressure of up to 200 bar, the normal operation is usually under

UM007, Ver.20170117

100 bar. Continuous use at high pressure may eventually damage the column. Since the pressure is generated by the flow rate. The maximum flow rate is limited by the backpressure. It is expected that the backpressure might gradually increase with its service. A sudden increase in backpressure suggests that the column inlet frit might be plugged. In this case, it is recommended to flush the column with reverse flow direction in an appropriate solvent. It is recommended to wait until the pressure drops to zero to safely disconnect the column from the testing apparatus at the end of the test.

**Temperature** The maximum operating temperature is 80 °C. Continuous use of the column at higher temperatures (>80 °C) can damage the column, especially under extreme pH (>13 or <2.0).

**Flow rate range** Normal operation is 0.1-0.5 mL/min for 2.1 mm I.D. columns.

**Storage** When not in use for extended time, store the desalting column in 50% organic component in use with water. Then seal both ends with the removable end plugs provided with the column, to prevent mobile phase evaporation.

**Column clean-up** Used columns can be cleaned with organic solvents, such as IPA, THF or a mixture of organic solvent in water. A caustic 250 mM NaOH solution can also be applied to clean the column if the above procedures fail to provide a satisfactory result.