Operating Parameters

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Mobile phase compatibility	All commonly used reversed-phase mobile phases, including formic acid/acetonitrile (check solubility of buffer components and system pressure)
pH stability	2 to 13 (1 to 14 short term)
Operating temperature	20 – 80 °C
Operating pressure	< 50 bar (1,500 psi)
Maximum pressure	100 bar (3,000 psi)
Working flow rate	0.05 to 0.5 mL/min
Working at extremes of the operating parameters is likely to reduce column lifetime.	

A note about pressure: the instrument will only report the total system backpressure (which will always be > 100 bar) rather than the pressure experienced by each individual column. Almost all of the system backpressure will be dissipated across the analytical column; as long as the AdvanceBio Desalting-RP column is the second column, it will experience minimal backpressure, regardless of the reported system backpressure.

Recommended Starting Conditions

		Gradient:	Time (min)	%B
Mobile phase A:	0.1% formic acid in water			
Mobile phase B:	0.1% formic acid in acetonitrile	0		5
Flow rate:	0.4 mL/min	0.5		5
		3.0		80
		4.0		80
		4.1		5
		6.0		5

The sample is loaded after the column and is equilibrated in 5% mobile phase A.
The cartridge will retain the protein and the salts are eluted to waste.
The protein is then eluted by increasing the percentage of mobile phase B at which time it will be in mobile phase compatible with MS detection – 0.1% formic acid in water/acetonitrile. After the protein has eluted, the column is then conditioned and equilibrated with 5% mobile phase A to desalt the next sample.

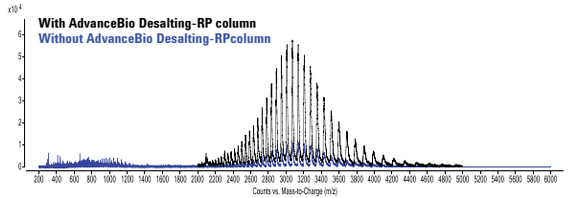

Figure 4: *Effective desalting by AdvanceBIO RP desalt column. 2nd dimensional mass spectrum profiles with and without* ***AdvanceBio Desalting-RP column.***

Column Care

If there is an increase in operating pressure, the cartridge can be flushed with a strong organic, such as propanol, to remove any strongly retained material and then conditioned and equilibrated using the method recommended for a new cartridge. When the performance of the AdvanceBio Desalting-RP cartridge starts to deteriorate, it is recommended to replace it with a new one – the cartridge holder can be re-used multiple times.

Recommended Storage Conditions

The AdvanceBio Desalting-RP cartridge is packed with polymeric particles, which are chemically and thermally stable under the conditions used for LC analysis of biomolecules. However, Agilent does not recommend that the cartridge be flushed or stored in 100% aqueous as this can cause a loss in efficiency. When not being used for a short period of time, such as overnight, the cartridge can be left on the LC system and continuously flushed with mobile phase at low flow rate. For short term storage of less than one week, store the column in the mobile phase. For extended storage, it is recommended to remove any mobile phase additives and to store the cartridge in a 50:50 mix of the organic used for the analysis, for example, acetonitrile and water. The cartridges should be sealed to prevent evaporation of the mobile phase. The cartridges can be stored at room temperature or refrigerated (4 to 35 °C).

Ordering Details

Part Number	Description
PL1612-1102	AdvanceBio Desalting-RP, 2.1 x 12.5 mm, 3/pk
820999-901	Cartridge holder

www.agilent.com/chem/store

Published in the USA, July 1, 2016
Part Number: 820120-012

Agilent Technologies

USER GUIDE

Agilent AdvanceBio Desalting-RP Cartridges

Reversed-phase desalting cartridge for online removal of salt ions prior to MS detection.

Agilent Technologies

Agilent AdvanceBio Desalting-RP cartridges are designed and manufactured by Agilent for online removal of salt ions prior to MS detection in the LC analysis of biomolecules. The cartridges contain 10 µm reversed-phase polymeric particles that effectively remove the salt and improve the quality of the MS data generated.

Getting Started

The AdvanceBio Desalting-RP cartridges are used with a cartridge holder (p/n 820999-901). The holder can be reused multiple times as the cartridge can be replaced with a new one when the performance starts to deteriorate.

If you have specific questions, contact Technical Support at **agilent.com/chem/techsupport**

Important Safety Considerations

- All points of connection in an LC system are potential sources of leaks. Users of LC instruments should be aware of the potential toxicity or flammability of the mobile phases.
- Do not break apart the cartridge. Doing so will expose AdvanceBio Desalting-RP particles, causing irreversible damage to the cartridge.

Characterization of proteins, including monoclonal antibodies, requires multiple techniques due to the complexity of the molecules. Techniques such as affinity, ion exchange and size exclusion chromatography are used as part of the workflows. However, the mobile phases are typically aqueous based and contain nonvolatile salts, which are not compatible with MS detection. To enable MS analysis of the sample heterogeneity, the salt ions must be removed either offline using a manual SPE device or online using an inline desalting cartridge.

The inline desalting cartridge can be used either on its own to desalt collected fractions, or as part of a 2D-LC system, such as the Agilent 1290 Infinity II 2D-LC solution, where the first dimension can be any LC technique and second is the desalting cartridge. A typical schematic is shown below.

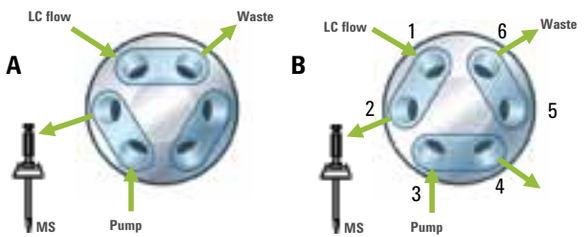

Figure 2: *MS valve switching. (A) Sample loading and desalting, (B) Elution to MS*

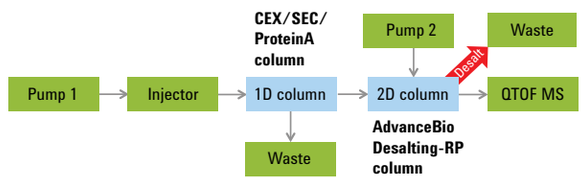

Figure 3: *2D LC/MS configuration*

Using Your Column

Installation

The AdvanceBio Desalting-RP cartridges are supplied as a pack of three in a sealed plastic bag. Remove one of the cartridges from the bag and reseal the bag containing any remaining cartridges and store in the original shipping box. The cartridges do not have a flow direction arrow and can be installed in either direction. Unscrew the two parts of the guard cartridge holder, p/n 820999-901, make sure the holder is empty and insert the inline desalting cartridge. Assemble the cartridge holder and tighten. The assembly is now ready for installing in the LC system prior to the MS detector in the direction of the flow arrow marked on the cartridge holder. Use 0.12 mm PEEK tubing to make the connection.

Column Conditioning

The cartridges are shipped without solvent and must be wetted and conditioned before use. AdvanceBio Desalting-RP cartridges contain a hydrophobic polymer material that must be first wetted by flushing for approximately 10 column volumes with 90 to 100% of the organic component in the mobile phase. The cartridges can then be equilibrated with 95% aqueous and a blank gradient run before being used for the first time.

During conditioning and equilibration, check all the connections for leaks.

Instructions for Use

The AdvanceBio Desalting-RP cartridges are compatible with all common mobile phases used for reversed-phase chromatography, including acetonitrile with formic acid. The polymeric media is both chemically and thermally stable and does not degrade during use to produce leachables that could interfere with the analysis. It is recommended to prepared fresh mobile phases using high-purity components and ultrahigh purity water suitable for use with MS detection. Filter buffers through a 0.2 or 0.45 µm filter and degas prior to use. This will remove particulates and help reduce the risk of bacterial growth, which will interfere with MS detection. When desalting samples that contain high levels of salt, it is recommend to equilibrate the desalting cartridge with 95% aqueous prior to use to reduce the risk of salt precipitation.

It is not recommended to flush the AdvanceBio Desalting-RP cartridge with 100% aqueous mobile phase – always keep a minimum of 5% organic.