Chromatography Solutions

Knowledge note #0006

UHPLC Column Connections

INTRODUCTION

To obtain good performance from UHPLC systems and columns, it is helpful to minimise peak dispersion. Dispersion may be introduced in various ways that include unwanted system dead volume or poor connections. This Knowledge Note explains how poor connections can impact UHPLC performance and explains how problems can be avoided.

COLUMN CONNECTIONS

The performance of UHPLC instruments can be reduced through the introduction of extra column volume (dead volume) within the flow path. The high efficiencies possible with sub-2 micron particles result in narrow peak widths and smaller peak volumes which will be more affected. An often overlooked potential source of extra column volume is through poorly made connections between tubing and system components including the UHPLC column. When installing any UHPLC column, it is important to ensure that the inlet and outlet tubing are fitted into the column ports to the correct depth to avoid the introduction of any extra column volume.

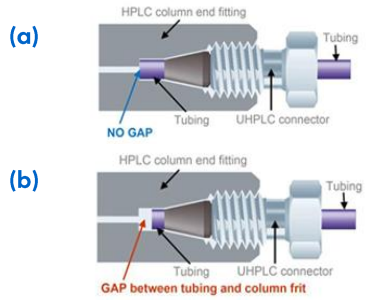

Figure 1: a) Illustration of tubing installed correctly b) Incorrect installation of tubing, resulting in the introduction of dead volume.

Figure 1 illustrates tubing that has been (a) correctly and (b) incorrectly fitted. In Figure 1a, the tubing is correctly seated in the port, resulting in no extra column volume being introduced.

Figure 2 demonstrates the effect of introducing such dead volume at the column inlet connection. By introducing just a 0.5 mm gap, a 23% loss in efficiency and 11% loss in peak asymmetry is observed, highlighting the importance of making good connections.

It is important to remember that all connections within the system must be correctly made in order to minimise dead volume, including the column outlet and any tubing connections to system components such as valves, needle seats and detectors etc.

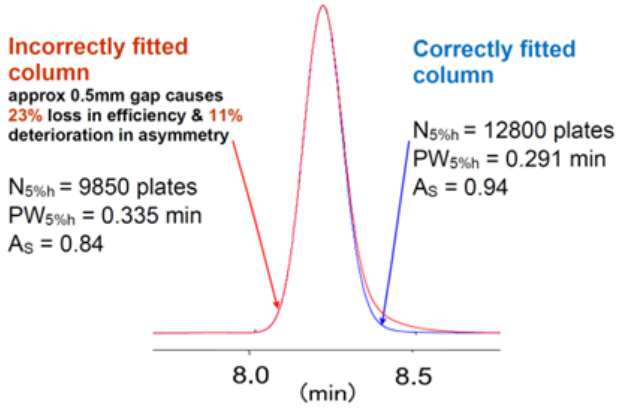

Figure 2: The measurable effect of poor column connections via incorrect positioning of the inlet tubing at the column inlet.

HOW TO CORRECTLY INSTALL A UHPLC COLUMN

When installing a UHPLC column, a "fresh connection" should always be made between the ferrule and tubing. The nut and ferrule should slide freely on the tubing prior to fitting. Avoid UHPLC connectors that have already been swaged onto the tubing and do not move, as these do not allow correct positioning of the ferrule on the tubing relative to the column port depth. This may lead to a gap, introducing extra column volume (dead volume) into the system.

For a "fresh connection" every time, we recommend the use of Avantor® ACE® UHPLC reusable column connectors (Figure 3). These fittings are designed to connect your UHPLC column to your UHPLC system (and make other similar connections). They feature a compact, one-piece design, are simple and easy to use and are suitable for use with 1/16" o.d. tubing and virtually all manufacturers' UHPLC systems and all brands of UHPLC columns. These fittings are rated for use up to 25,000psi (1,720 bar) and are also suitable for use up to 100°C. Correctly used, these fittings will provide a "fresh connection" for approximately 10 column installations.

For optimum connector lifetime and to avoid accidental swaging of the connector to the tubing by overtightening, the use of an Avantor® ACE® Torque Wrench is required. The Torque Wrench delivers exactly the required tightening torque to produce a good seal, without the risk of over tightening.

INSTALLATION USING AVANTOR® ACE® UHPLC REUSABLE COLUMN CONNECTORS (Part no. EXL-CC)

1. Slide the fitting onto the UHPLC tubing (approximately 5 mm (1/4") from the end).

2. Insert the assembly onto the receiving female port of the column, pushing the tubing in until it 'bottoms out'.

3. Whilst maintaining pressure on the tubing (to ensure that it continues to 'bottom out'), finger tighten the fitting into the column until snug.

4. Using the Avantor® ACE® Torque Wrench (Part no. EXL-TW), tighten the fitting to the correct torque (while continuing to maintain pressure on the tubing). Instructions for the correct use of the Torque Wrench are provided with the Torque Wrench.

UHPLC COLUMN OUTLET END CONNECTIONS

Avantor® ACE® UHPLC reusable column connectors are suitable for use at both the column inlet and the column outlet, to make good UHPLC connections. Alternatively, at the column outlet end, an ACE reusable PEEK finger-tight column connector (ACE-CC, suitable for use up to 5,000 psi, 345 bar) can be used. To avoid extra column volume and unwanted peak dispersion, it is additionally important to make good connections at the outlet end of your UHPLC column. Similar principles as previously described for the inlet end connection should be employed.

AVANTOR® ACE® UHPLC REUSABLE COLUMN CONNECTORS

- Compact, one-piece, easy to use connector
- Pressure rated to 25,000 psi (1,720 bar)
- Usable at elevated temperatures (up to 100°C)
- Fits virtually all manufactures' UHPLC systems
- Fits all brands of UHPLC columns
- Reusable, non-permanent swaging design
- 10 make and break cycles

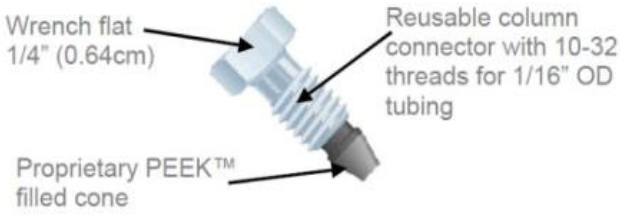

Figure 3: Avantor® ACE® UHPLC reusable column connectors.

UHPLC COLUMN INLET / OUTLET CONNECTORS

Description	Details	Part number
Avantor® ACE® UHPLC reusable column connectors	Suitable for use up to 25,000psi (1,720 bar)	EXL-CC10 (10/pk)
Avantor® ACE® Torque Wrench		EXL-TW
Avantor® ACE® UHPLC reusable column connectors starter kit	Contains 1 x EXL-TW and 4 x EXL-CC	EXL-CCSK

UHPLC COLUMN OUTLET CONNECTORS

Description	Details	Part number
Avantor® ACE® reusable PEEK finger-tight column connector	Suitable for use up to 5,000psi, 345 bar	ACE-CC10 (10/pk)