APPLICATIONS

Conventional Low Volume Stainless Steel Tubing Connection Compared to Stainless Steel Zero Dead-Volume SecurityLINK™ Connection

Genevieve Hodson and Jason Anspach
Phenomenex, Inc., 411 Madrid Avenue, Torrance, CA 90501 USA

Genevieve Hodson
Technical Specialist
Genevieve loves to pick up a round of Skee-Ball, watching lighting storms in the distance, lazy summers in the Texas Hill Country and drinking a good cup of coffee with a book of equal good-ness.

Background

When using a UHPLC system, narrow column ID, or sub-2 µm particle column, overall system dead-volume is an important variable to minimize. A bad connection can contribute greatly to system-dead-volume and result in poor peak shape and lower overall efficiency.

Introduction

The minimization of dead-volume within a UHPLC system is an important consideration. Additional dead-volume in a system will lead to more diffusion, thus causing poor peak shape and potential failing results during method transfers or system suitability testing. When utilizing a UHPLC system, even small amounts of extra dead-volume created by poor connections can contribute greatly to diffusion, due to the overall decrease in the systems dead-volume. An increase in the dead-volume of a system, for both UHPLC or HPLC, can also cause chromatography problems when columns with smaller inner diameters or core-shell/sub-2 µm particles are used. An incorrect, or poor, connection can be defined as any connection where the base of the tubing used does not sit flushed up against the inlet of the column and subsequently contributes to the dead-volume of the system. Although most chromatographers consider the connection of the column as the largest potential location for an incorrect connection, poor connections can happen in many places throughout the system, including connections from the autosampler to the column heater.

There are two main approaches to creating a zero dead-volume connection. The first involves swaging your stainless steel tubing at the exact depth of the columns inlet. While effective, this only allows for the connection to be used with one specific vendors columns and relies on the scientist's proper measurements before the tubing in swaged together. If the distance was measured or implemented incorrectly then the result is a permanent poor connection. The second approach involves the use of a zero dead-volume connection where the connection is not permanently swaged and allows for a perfectly flush connection independent of the columns inlet depth.

This technical note investigates the effects of poor and zero dead-volume connections using a sub-2 µm particle column on a UHPLC system.

Experimental Conditions

Two different connections were used on an Agilent® 1290 Infinity II system. The tubings were connected directly from the autosampler to a Luna® Omega 1.6 µm 50 x 2.1 mm column. The same sample was injected using the same run conditions, the only difference being the type of inlet connection to the column. The connection from the columns outlet to the detector remained unchanged throughout the experiment. The poor connection was made by swaging a ferrule to stainless steel tubing with the inner diameter of 1/16 inch at a depth that was too short for a Phenomenex column endfitting. The second connection was made with a SecurityLINK stainless steel 100 µm x 250 mm connection.

Figure 1.
Ideal Flush Connection

Figure 2.
Incorrect/Poorly Made Connection

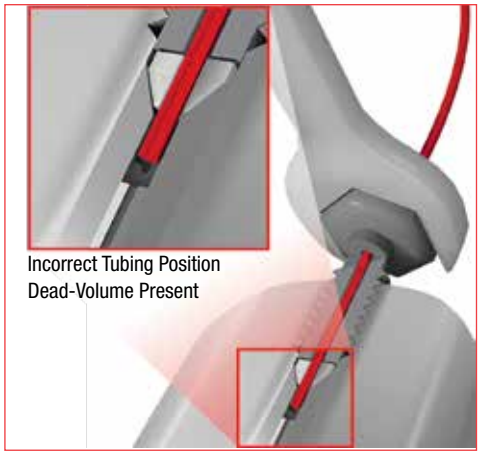
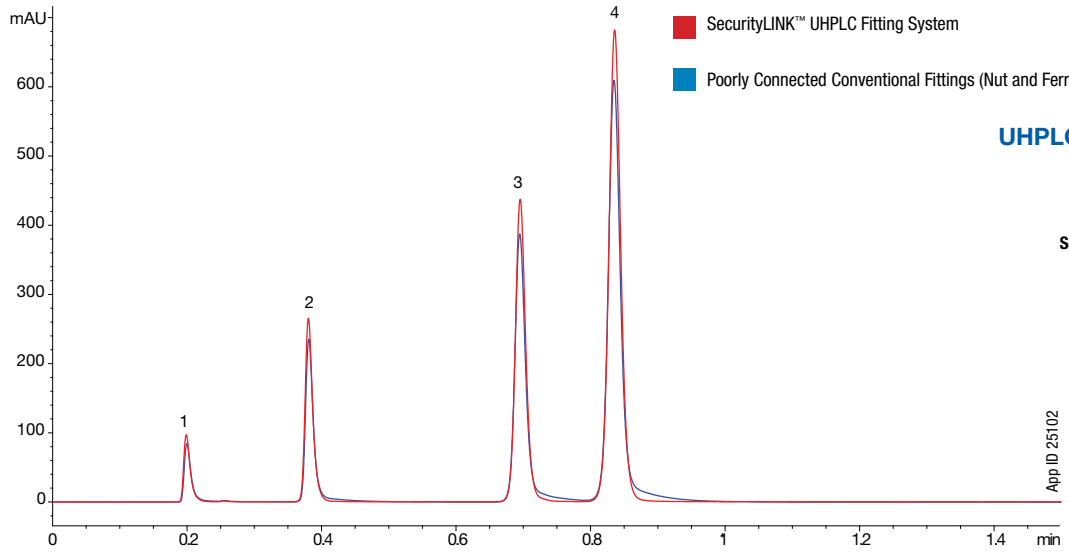


Figure 3.
Chromatogram of Poor Connection and Good Connection Overlay



UHPLC Conditions

Column:	Luna® Omega 1.6 µm C18
Dimensions:	50 x 2.1 mm
Part No.:	00B-4742-AN
SecurityLINK Part No.:	AJ1-1441
Mobile Phase:	Water/Acetonitrile (35:65)
Injection:	0.2 µL
Flow Rate:	0.5 mL/min
Temperature:	25 °C
Detection:	UV @ 254 nm
Sample:	1. Uracil
	2. Acetophenone
	3. Toluene
	4. Naphthalene

Figure 4.
Zoom In of Poor Connection and Good Connection Overlay

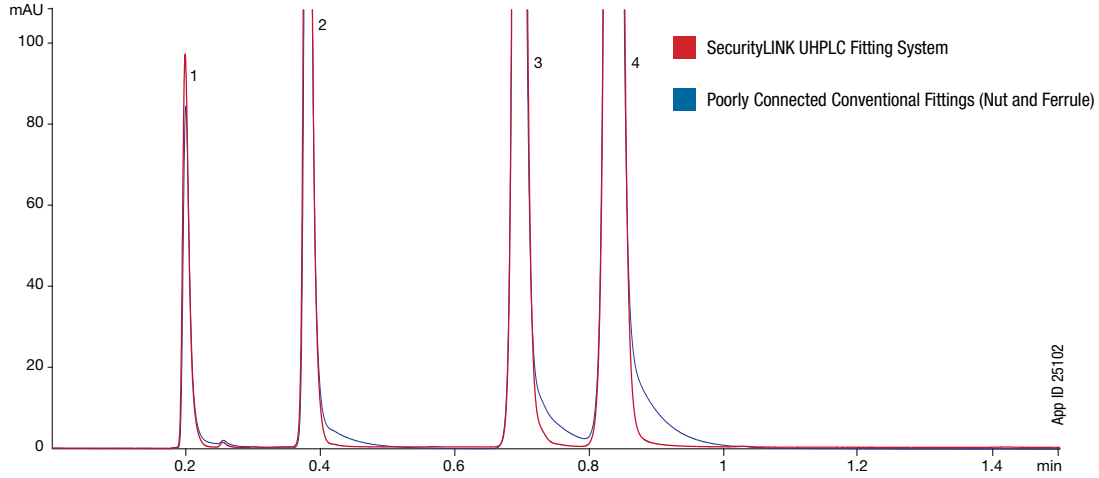


Table 1.
Asymmetry

Peak	Analyte	SecurityLINK Connection	Poor Connection
1	Uracil	1.72	2.00
2	Acetophenone	1.39	1.72
3	Toluene	1.22	1.41
4	Naphthalene	1.11	1.33

Results & Discussion

The first connection, using the conventional stainless steel tubing and swaging the ferrule at a designated distance, provided an asymmetry value for naphthalene of 1.33. It can be observed that, aside from the unretained Uracil peak, the retained peaks have noticeable tailing when this connection was used. The SecurityLINK™ connection provided an asymmetry value for naphthalene of 1.11, which is a significant improvement to the previously collected asymmetry value. A noticeable improvement can be seen in all the peaks in the chromatogram, in terms of width and tailing. All data was collected in triplicate to confirm peak shape/tailing was due to poor connection and not an improperly equilibrated column.

It should be noted that the conventional connection required the use of two wrenches to be installed. Extra time was needed for this installation to ensure that no leaking at the connection site was observed. The SecurityLINK connection employs a responsive feedback that 'Clicks' when the connection is complete and the proper amount of torque has been applied to ensure no leaking will occur. This enabled the second connection to be made quickly and with the use of no extra tools.

Conclusion

These results demonstrate the importance of the column connection with regards to peak shape. The SecurityLINK connection featured in this technical note provided a zero dead-volume connection, which minimized the amount of potential diffusion in a system thus providing good, consistent peak shapes.

SecurityLINK™ Ordering Information

PEEKsil™

PEEKsil Double-Sided 10-32 Fittings with 1/16 in. OD tubing

Part No.	ID (µm)	Length (mm)
AJ1-2111	25	100
AJ1-2121	25	150
AJ1-2141	25	250
AJ1-2151	25	300
AJ1-2171	25	500
AJ1-2191	25	750
AJ1-21A1	25	1000
AJ1-2211	50	100
AJ1-2221	50	150
AJ1-2231	50	200
AJ1-2241	50	250
AJ1-2251	50	300
AJ1-2271	50	500
AJ1-2291	50	750
AJ1-22A1	50	1000
AJ1-2321	75	150
AJ1-2341	75	250
AJ1-2371	75	500
AJ1-23A1	75	1000
AJ1-2411	100	100
AJ1-2421	100	150
AJ1-2441	100	250
AJ1-2471	100	500
AJ1-24A1	100	1000

Stainless Steel

Stainless Steel Double-Sided 10-32 Fittings with 1/16 in. OD tubing

Part No.	ID (µm)	Length (mm)
AJ1-1421	100	150
AJ1-1441	100	250
AJ1-1461	100	350
AJ1-1471	100	500
AJ1-1481	100	600
AJ1-1521	125	150
AJ1-1541	125	250
AJ1-1561	125	350
AJ1-1571	125	500
AJ1-1581	125	600
AJ1-1621	254	150
AJ1-1641	254	250
AJ1-1661	254	350
AJ1-1671	254	500
AJ1-1681	254	600

PEEKsil

PEEKsil Single-Sided 10-32 Fitting with 1/16 in. OD tubing

Part No.	ID (µm)	Length (mm)
AJ1-2224	50	150
AJ1-2274	50	500
AJ1-2294	50	750
AJ1-22A4	50	1000

PEEK-Lined Stainless Steel

PEEK-Lined Stainless Steel Double-Sided 10-32 Fittings with 1/16 in. OD tubing

Part No.	ID (µm)	Length (mm)
AJ1-3121	25	150
AJ1-3141	25	250
AJ1-3161	25	350
AJ1-3171	25	500
AJ1-3181	25	600
AJ1-3221	50	150
AJ1-3241	50	250
AJ1-3261	50	350
AJ1-3271	50	500
AJ1-3281	50	600
AJ1-3321	75	150
AJ1-3341	75	250
AJ1-3361	75	350
AJ1-3371	75	500
AJ1-3381	75	600
AJ1-3421	100	150
AJ1-3441	100	250
AJ1-3461	100	350
AJ1-3471	100	500
AJ1-3481	100	600

APPLICATIONS

Australia
t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 800-852-3944
sginfo@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 61 692 20 20
swissinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
info@phenomenex.com

All other countries
Corporate Office USA
t: +1 (310) 212-0555
info@phenomenex.com

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at international@phenomenex.com

TN64611018_W