

APPLICATIONS

Assessing the Impact of SecurityLINK™ PEEKsil™ UHPLC Fingertight Fitting System on Increased Peak Efficiency and Reduction in Peak Asymmetry on an Optimized HPLC/UHPLC Instrumentation



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Background

When using a UHPLC system, narrow column ID, or sub-2µm 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 peak efficiency.

Figure 1.
Peak Asymmetry

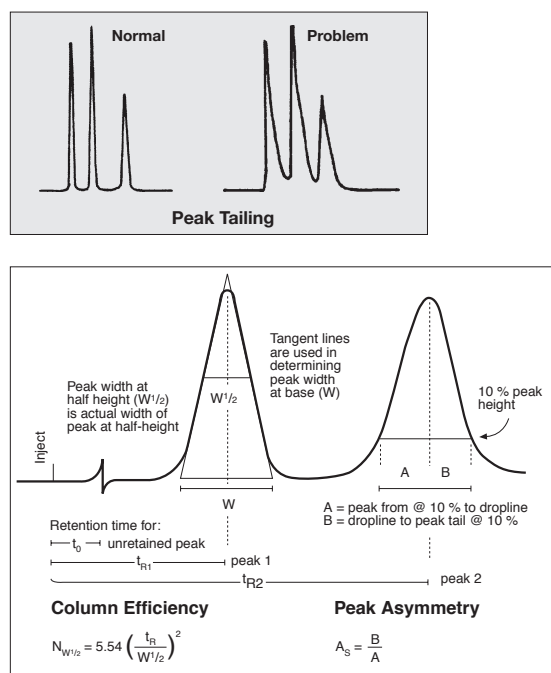


Figure 2.
Effect of Dead-Volume

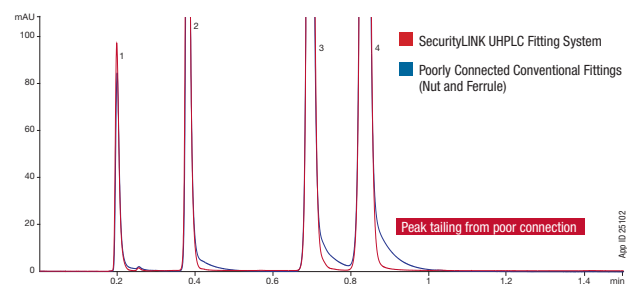


Figure 3.
Ideal Flush Connection (SecurityLINK)

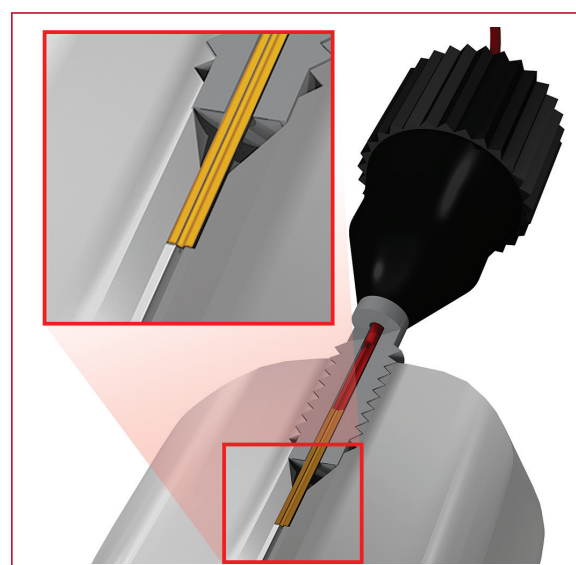
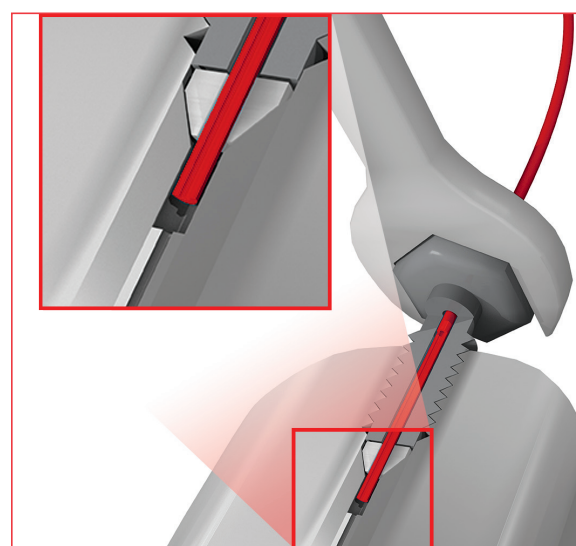


Figure 4.
Incorrect/Poorly Made Connection (Conventional Nut and Ferrule Fitting)



Introduction

To ensure that any HPLC or UHPLC system provides high efficiency and good peak shape it is essential to minimise dead-volume within the system. A common contributor to additional dead-volume are fitting connections routinely made to the column inlet and outlet which can lead to lower than expected efficiency, peak tailing or misshaped peaks. The impact of dead-volume is even more pronounced in columns with small internal volumes (narrow or short columns) and those packed with high efficiency sub-2 μ m or core-shell particles. Because of the small volumes found in these columns, the extra volume can become a source of dispersion. This can produce band broadening and peak tailing caused by carryover within dead spaces in a fitting, significantly limiting the efficiency and accuracy of the UHPLC columns. Peak asymmetry thus reduces the quality of an analysts chromatography while also decreasing the ability to quantify components in the sample.¹ Additionally, due to peak purity and proper quantification, the accuracy of any resulting data is diminished when separations contain severely tailing or asymmetrical peaks, leading to costly time delays and the need for rework.¹

PEEKsilTM tubing is manufactured with extremely high levels of precision in terms of ID and is also machine cut to provide connections with minimal dwell volume. As such it has become the tubing of choice where connections close to zero dwell volume are required. This technical note explores the difference observed in both peak efficiency and asymmetry when a conventional PEEKsil nut and ferrule fitting system is replaced with a SecurityLINKTM PEEKsil UHPLC fingertight fitting system.

Experimental Conditions

Two different column dimensions were tested, 30x2.1 mm and 100x2.1 mm, of which both columns were Kinetex[®] core-shell 1.7 μ m XB-C18.

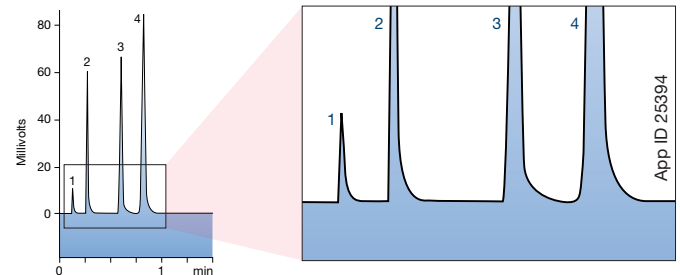
UHPLC Conditions

Column:	Kinetex 1.7 μ m XB-C18	
Dimensions:	30 x 2.1 mm	100 x 2.1 mm
Part No.:	00A-4496-AN	00D-4496-AN
SecurityLINK Part No.:	AJ1-2441	AJ1-2441
PEEKsil Part No.:	AT0-8896 (Tubing)	AT0-8896 (Tubing)
	AQ0-8503 (Fitting)	AQ0-8503 (Fitting)
Mobile Phase:	Acetonitrile/Water (50:50)	Acetonitrile/Water (65:35)
Flow Rate:	0.5 mL/min	0.5 mL/min
Temperature:	Ambient	Ambient
System:	Agilent [®] 1260 Infinity	Agilent 1260 Infinity

Results and Discussion

Figure 5.

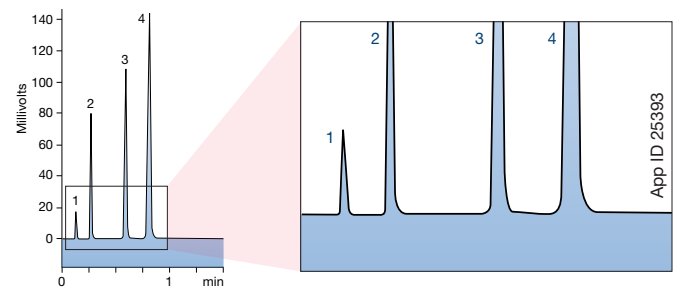
Conventional PEEKsil Nut and Ferrule Fitting System Connections
Kinetex 1.7 μ m XB-C18 30 x 2.1 mm



Peak	Analyte	Time	Area	k Factor	Width	Eff.	Asym.
1	Uracil	0.13	9	0.000	0.013	788	2.00
2	Acetophenone	0.27	55	1.110	0.014	2847	1.86
3	Toluene	0.60	97	3.725	0.022	4909	1.42
4	Naphthalene	0.81	163	5.452	0.029	4995	1.31

Figure 6.

SecurityLINK PEEKsil UHPLC Fingertight Fitting System Connections
Kinetex 1.7 μ m XB-C18 30 x 2.1 mm



Peak	Analyte	Time	Area	k Factor	Width	Eff.	Asym.
1	Uracil	0.13	13	0.000	0.011	802	1.56
2	Acetophenone	0.26	64	1.085	0.012	2852	1.46
3	Toluene	0.59	136	3.620	0.019	5273	1.16
4	Naphthalene	0.80	243	5.326	0.026	5433	1.12

Moving from a conventional PEEKsil™ nut and ferrule fitting system to a SecurityLINK™ PEEKsil UHPLC fingertight fitting system results in an improvement from 1.31 to 1.12 in terms of peak asymmetry for naphthalene (Δ 0.19 or 14.5%) and an increase in plate count on column from 4995 to 5433 (438 plates or 8.8%).

Figure 7.
Conventional PEEKsil Nut and Ferrule Fitting System Connections
Kinetex 1.7 μ m XB-C18 100 x 2.1 mm

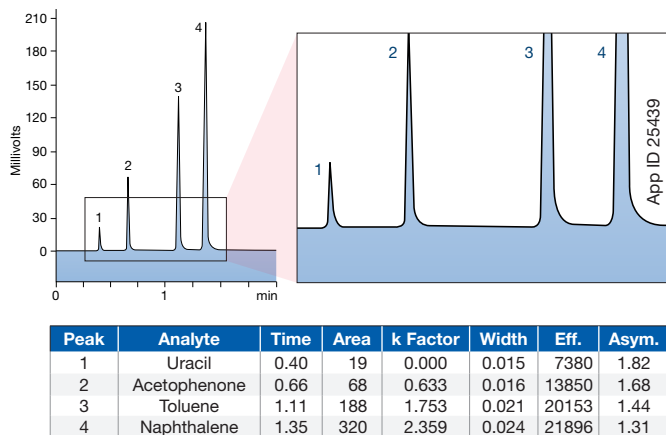
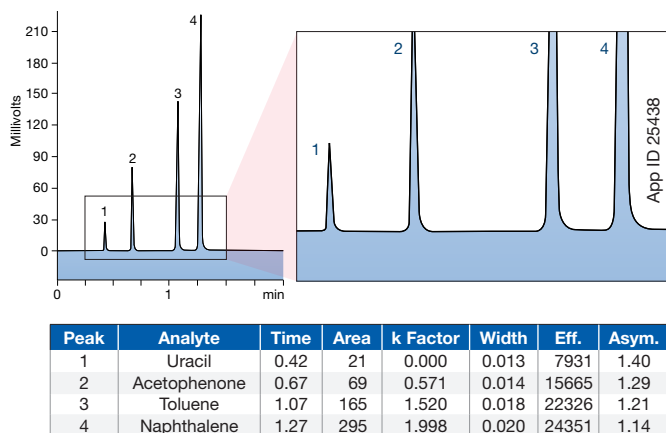


Figure 8.
SecurityLINK PEEKsil UHPLC Fingertight Fitting System Connections
Kinetex 1.7 μ m XB-C18 100 x 2.1 mm



The experiment conducted in **Figure 7 and 8** utilizes a longer column (Kinetex® 1.7 μ m XB-C18 100 x 2.1 mm). Moving from a conventional PEEKsil nut and ferrule fitting system to a SecurityLINK PEEKsil UHPLC fingertight fitting system resulted in a change from 1.31 to 1.14 in terms of peak asymmetry for naphthalene (Δ 0.17 or 12.9%) and a plate count on column from 21896 to 24351 (2455 plates or 11.2%). The increase in plate count is more significant than that seen in **Figure 5 and 6** when a smaller column (Kinetex 1.7 μ m XB-C18 30 x 2.1mm) was used. This reflects the fact that the column volume to dead-volume ratio is lower and the performance overall is less impacted by the amount of dead-volume in the system. The fact that the improvement in asymmetry is almost the same for both columns suggests that this is most likely impacted by removal of any potential mixing in the column outlet connection via the use of a SecurityLINK fittings.

In both cases, the original PEEKsil tubing was 200 mm long, the PEEKsil SecurityLINK used was 250 mm long, both were 100 μ m internal diameter. Theoretically increasing tubing length would result in an increase in volume with a corresponding decrease in performance. The fact that performance is enhanced when using SecurityLINK is evidence that performance issues can be directly linked to extra volume in the connections made when using regular fittings with standard PEEKsil tubing.

In this study both columns displayed an increase in peak efficiency, and a reduction in peak asymmetry when the conventional PEEKsil connecting tubing was replaced with SecurityLINK. This would indicate a reduction in overall system dead-volume, thus reducing the level of diffusion within the chromatographic pathway and improving overall column performance.

Conclusion

Even when moving from a robust PEEKsil system to SecurityLINK, improvements can be seen in terms of efficiency and peak asymmetry, indicating that the dead-volume of the system has been further reduced by making the change to SecurityLINK column/system fittings.

Sources

1. J.W. Dolan, Why Do Peaks Tail? BASI Northwest Laboratory, McMinnville, Oregon, USA.

SecurityLINK™ Ordering Information

SecurityLINK PEEKsil™ Double-Sided 10-32 Fittings for 1/16 in. Ports



Part No.	ID (µm)	LENGTH (mm)	Fitting Size Top (in.)	Fitting Size Bottom (in.)
AJ1-2111	25	100	1/16	1/16
AJ1-2121	25	150	1/16	1/16
AJ1-2141	25	250	1/16	1/16
AJ1-2151	25	300	1/16	1/16
AJ1-2171	25	500	1/16	1/16
AJ1-2191	25	750	1/16	1/16
AJ1-21A1	25	1000	1/16	1/16
AJ1-2211	50	100	1/16	1/16
AJ1-2221	50	150	1/16	1/16
AJ1-2231	50	200	1/16	1/16
AJ1-2241	50	250	1/16	1/16
AJ1-2251	50	300	1/16	1/16
AJ1-2271	50	500	1/16	1/16
AJ1-2291	50	750	1/16	1/16
AJ1-22A1	50	1000	1/16	1/16
AJ1-2321	75	150	1/16	1/16
AJ1-2341	75	250	1/16	1/16
AJ1-2371	75	500	1/16	1/16
AJ1-23A1	75	1000	1/16	1/16
AJ1-2411	100	100	1/16	1/16
AJ1-2421	100	150	1/16	1/16
AJ1-2441	100	250	1/16	1/16
AJ1-2471	100	500	1/16	1/16
AJ1-24A1	100	1000	1/16	1/16

PEEK-Lined Stainless Steel Double-Sided 10-32 Fittings for 1/16 in. Ports



Part No.	ID (µm)	LENGTH (mm)	Fitting Size Top (in.)	Fitting Size Bottom (in.)
AJ1-3121	25	150	1/16	1/16
AJ1-3141	25	250	1/16	1/16
AJ1-3161	25	350	1/16	1/16
AJ1-3171	25	500	1/16	1/16
AJ1-3181	25	600	1/16	1/16
AJ1-3221	50	150	1/16	1/16
AJ1-3241	50	250	1/16	1/16
AJ1-3261	50	350	1/16	1/16
AJ1-3271	50	500	1/16	1/16
AJ1-3281	50	600	1/16	1/16
AJ1-3321	75	150	1/16	1/16
AJ1-3341	75	250	1/16	1/16
AJ1-3361	75	350	1/16	1/16
AJ1-3371	75	500	1/16	1/16
AJ1-3381	75	600	1/16	1/16
AJ1-3421	100	150	1/16	1/16
AJ1-3441	100	250	1/16	1/16
AJ1-3461	100	350	1/16	1/16
AJ1-3471	100	500	1/16	1/16
AJ1-3481	100	600	1/16	1/16



**Stainless Steel Double-Sided
10-32 Fittings for 1/16 in. Ports**



Part No.	ID (µm)	LENGTH (mm)	Fitting Size Top (in.)	Fitting Size Bottom (in.)
AJ1-1421	100	150	1/16	1/16
AJ1-1441	100	250	1/16	1/16
AJ1-1461	100	350	1/16	1/16
AJ1-1471	100	500	1/16	1/16
AJ1-1481	100	600	1/16	1/16
AJ1-1521	125	150	1/16	1/16
AJ1-1541	125	250	1/16	1/16
AJ1-1561	125	350	1/16	1/16
AJ1-1571	125	500	1/16	1/16
AJ1-1581	125	600	1/16	1/16
AJ1-1621	254	150	1/16	1/16
AJ1-1641	254	250	1/16	1/16
AJ1-1661	254	350	1/16	1/16
AJ1-1671	254	500	1/16	1/16
AJ1-1681	254	600	1/16	1/16

**PEEKsil™ Single-Sided Fittings
1/32 in. OD PEEKsil Tubing with one 10-32 fitting
for 1/16 in. ports, and one side with no fitting**



Part No.	ID (µm)	LENGTH (mm)	Fitting Size Top (in.)	Fitting Size Bottom (in.)
AJ1-2224	50	150	1/16	None
AJ1-2274	50	500	1/16	None
AJ1-2294	50	750	1/16	None
AJ1-22A4	50	1000	1/16	None

Phenomenex Column/Tubing ID Recommendation Chart

	Nano	Microbore	Analytical			Semi-Prep		
Column ID	0.05 - 0.1 mm (50 µm - 100 µm)	0.3 - 0.5 mm (300 µm - 500 µm)	1 mm	2.1 mm	3 mm	4.6 mm	7.8 mm	9.0 - 16.0 mm
Tubing ID	25 µm	50 µm	50 µm - 75 µm	100 µm	100 µm	100 µm	120 µm	254 µm

Reorder Tip



SecurityLINK™ tubing material includes a sleeve that provides: ID, length and part number information.

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