

Maximize Your UHPLC Capability by Minimizing Extra-column Volume



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Advances in column technology for ultra-high-performance liquid chromatography (UHPLC) and the use of small particle-size stationary phase and shorter, smaller diameter columns have made minimizing extra-column volume critical in ensuring the accuracy of chromatographic separations. Connections that consistently eliminate extra-column volume when operating at ultra-high pressures (up to 19,000 psi) reduce the complexity and simplify the UHPLC workflow.

Importance of Minimizing Extra-column Volume in the System

UHPLC uses short columns with small internal diameters (e.g., 2 mm) packed with very small (1.3 μm to 2- μm) fully porous or superficially porous particles. These columns are increasing in demand and used in most instruments today, which are now capable of withstanding pressures of up to 18,000 psi.

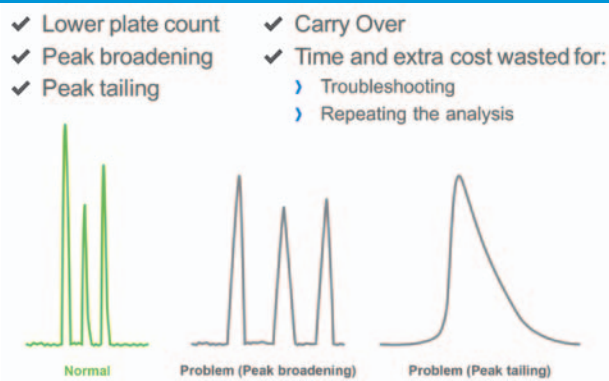
Because of the small volumes inherent in these newer columns, the extra-column volume of the instrument can become a significant source of dispersion, leading to extra-column effects, notably band broadening and peak tailing caused by carryover within dead spaces in a fitting system (**Figure 1**). These effects significantly limit the separation potential of columns and reduce the accuracy of separations.

The connections in a UHPLC system often are the one of the biggest contributors to peak broadening. Tracking down the source of these peak problems in HPLC tubing connections entails time and cost.

Sources of Extra-column Volume in the System

As illustrated in the diagram of a simple HPLC system in **Figure 2**, extra-column volume occurs between the point of injection and the detector cell. The total contribution to band broadening ($\sigma_{t,\text{tot}}^2$) is the sum of band spreading caused by the injector and the volume inherent in the column, tubing, and the detector. The tubing includes contributions from fittings, for example,

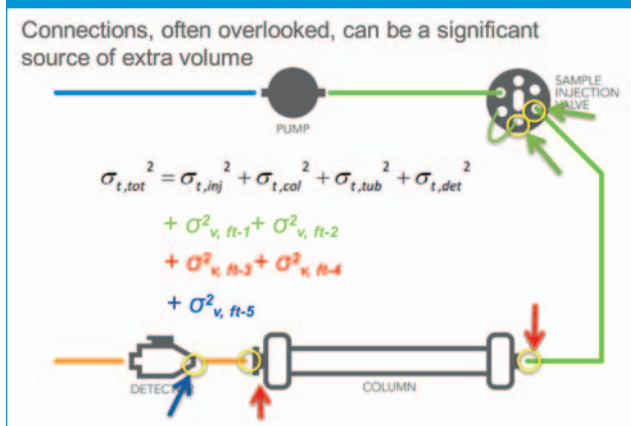
Figure 1: Why Extra Volume in the System is Critical



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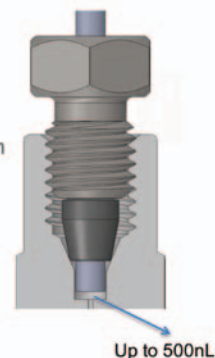
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Figure 2: Sources of Extra Volume in the System

Figure 3: How Connections Create Extra Volume

Majority of the connections in the market today require:

- ✓ Perfect insertion depth
- ✓ Perfectly cut tubing
- ✓ Perfect surface finish at the port bottom in order to make a good connection



at the outlet of the sample loop and the outlet of the valve as it transitions to the head of the column. Fittings at the head of the column and column outlet are also sources of extra-column volume, as is the detector itself.

The nature of band spreading varies in gradient chromatography and isocratic chromatography. A full discussion of extra-column contributions from fittings, tubing, and other system components to band broadening can be found in a paper published this year in the *Journal of Chromatography* by Vanderlinden et al. (*J Chromatogr A*. 2016 Apr 15;1442:73–82).

From the outlet of the HPLC column to the inlet of the detector, the fittings, the tubing diameter, and the connections should be chosen carefully relative to the sample size and flow rate to ensure good chromatography and minimal contribution to the band spreading of the eluting peaks.

Figure 4: Next Generation Connection Technology

MarvelX™ better, easier finger-tight connection system

- ✓ Seals up to 19,000psi (1,300 bar)
- ✓ Finger-tight, no tools required
- ✓ Reusable up to 200 times
- ✓ Zero Dead Volume assured



Creating Connections that Minimize Extra-column Volume

Creating proper connections in an HPLC system has been addressed extensively over the years, and information is readily available from a number of online sources.

The advent of short columns with 2- to 3-mm internal diameters (IDs) packed with sub-2- μm fully porous or superficially porous particles, has brought to light the importance of eliminating extra-column volume by shortening the tubing to the minimum lengths achievable in the instrument. Selecting the smallest-ID tubing with an acceptable backpressure will maximize the performance of the column.

Most every chromatographer has learned to make a proper connection by cutting $\frac{1}{16}$ -in stainless steel tubing, grinding and polishing the end (**Figure 3**), and inserting it into the fitting and ferrule system, making sure that it's seated snugly at the bottom of the port without a gap.

Improperly cut tubing, scratches, and other factors associated with standard ferruled connections can contribute as much as 500 nL to injections that should be in the submicroliter range. These volumes can affect band spreading than might be expected.

Ports are manufactured with space around the tubing, so extra-column volume exists between the sealed point on the ferrule and between the internal diameter of the tubing and outer diameter (OD) of the fitting.

MarvelX™ Technology to Minimize Extra Volume

IDEX Health & Science introduced the MarvelX Next Generation UHPLC face-sealing connection system (**Figure 4**) to address the shortcoming of traditional ferrule-style tubing connections. Next Generation technology provides fingertight connections with reliable elimination of the volume inherent in 10-32 ports. These fittings seal up to 1,300 bar (19,000 psi), can be re-used as many as 200 times, and have zero dead volume.

The MarvelX is a ferrule-free system that performs an automatic fitting and sealing function. In **Figure 5**, the green represents a $\frac{1}{16}$ -in OD tube that contains a microengineered fitting with a soft face seal sufficient to withstand pressures up to 23,000 pounds.

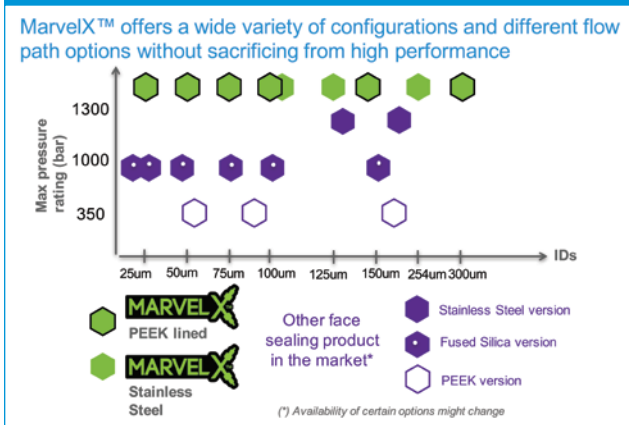
In the older stainless steel swaged ferrule systems, a nut and a ferrule engage the seat. A wrench is required at a force of 20-inch pounds to actually seat a stainless steel swaged

Figure 5: Minimize Extra Volume

MarvelX™ does all the work for you!

- ✓ Ferrule-free system
- ✓ Automatically seals at the bottom of the port
- ✓ No technique or lab prep required (micro-engineered fitting system)
- ✓ Adapts to the imperfections at the port bottom

Figure 6: Expanding Application Options



ferrule. Reusing the fitting is rarely possible because the ferrule is deformed in the process.

Later technology used a stainless steel collet and ferrule system, which required less force to seat the ferrule, and it also proved to be more reusable. More recently, face-sealing systems have become available from several manufacturers. Some stainless steel versions operate at pressures upwards of 1300 bar. A few silica versions of these face-sealing systems are available with an upper pressure limit of about 1000 bar. A PEEK version is available with a 350 bar (around 6000 psi) pressure rating.

MarvelX expands on these improvements with a fitting system that is even easier to install with a finger-tight operation. It is available in PEEK and stainless steel versions (Figure 6), both of which operate in excess of 1300 bar, eliminating any concern about selecting the proper material for a given application.

Making a good ferrule connection can be tricky, as most chromatographers know. Ferrules and tubing and port depth need to be compatible. Tubing can slip as you tighten the ferrule. In stainless steel swaged ferrule systems, it's possible to

overtighten the connection, especially if you need to remake the connection after the ferrule has become deformed. Breaking a 10-32 fitting off into a column or even in the port of a valve is not uncommon.

The more-recent stainless steel collet ferrule systems allow as many as 10 reconnections, but it also can be overtightened and still leaves the possibility that the tubing will back out of the ferrule as the connection is tightened. Even though it creates a leak-free connection, band spreading can still occur.

Other face-sealing connection systems, such as fused silica versions are fairly vulnerable to damage as it is easy to overtighten which limits the reusability of the fittings. The fused silica appears to fracture as a result of the pressure put on the end of the fitting when sealing against the port. These systems are also rated at the lower pressure than others. Both the PEEK and stainless steel versions of the MarvelX system have an extremely low possibility of overtightening, and they can be connected and broken 100 to 200 times.

PEEK-lined stainless steel MarvelX systems are suitable for a broad range of 1,300 bar applications and are available in a variety of lengths with internal diameters from 25 µm to 300 µm. The MarvelX stainless steel versions are available with internal diameters of 100 µm to 300 µm. These options provide a wide a range of choices for a variety of applications.

A unique feature of the MarvelX fitting system is the ease it provides in routing connections through an instrument because of the removable fitting. The nuts are removable from the tubing during or before installation. Tubing can be routed the through sheet metal, the nut reattached, and the fitting and tubing seated in the destination port. Because the fittings are removable, tubing of different types can be replaced or used independently of the nut itself. Waste is reduced because fittings don't need to be discarded.

Conclusion

The MarvelX fitting system is compatible with almost any UHPLC (or HPLC) instrument. Face-sealing systems ensure that the connections are reliable, and extra-column volume is minimized, allowing the operator to achieve the maximum capabilities of UHPLC system in a simplified workflow. The MarvelX fitting system works with any 10-32 port, and the fittings are truly fingertight. No tools are required.

This MarvelX Next Generation UHPLC connection system is currently available in PEEK and stainless steel flow path options with internal diameters ranging from 25 to 300 µm and in various lengths. With the capability of reusing these fittings as many as 200 times, it's entirely possible for a fitting to last as long as one year of changing columns as much as daily on an HPLC or UHPLC. The MarvelX fitting system operating at 1,300 bar can be expected to last a very long time.