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# Cautions on Connecting HPLC/UHPLC Column End Fittings

# Features of the MarvelX and MarvelXACT Tubing

## 1. Introduction

HPLC/UHPLC systems consist of pumps, autosamplers, injectors, columns, and detectors, which are connected with 1/16” stainless-steel tubing. It is well known that the volume of tubing and dead volume generated at the connecting points greatly affects the column performance. In this article, I will discuss mistakes made when connecting the column end fittings, how to avoid issues, and introduce tubing that can be connected regardless of the end fitting standard.

## 2. Connection Using Setscrews and Ferrules

When Liquid Chromatography (LC) evolved, it was called high pressure liquid chromatography (HPLC), which literally meant LC operated at high pressure. Therefore, stainless-steel (SUS316) materials were used for columns and tubing as stainless-steel can handle high pressure and conical metal ferrules and setscrews were used to connect column ends and the 1/6” tubing as shown in Figure 1. Turning the setscrew applies pressure to the ferrule firmly fastening it and the ferrule changes shape preventing the mobile phase from leaking by adhering closely to the end of the column. Figure 1(A) is an illustration and Figure 1(B) shows a metal ferrule and setscrew. The metal ferrule is not firmly fastened and has not changed shape. Figure 1(C) shows connection with a colored resin ferrule and setscrew to better convey the concept visually.

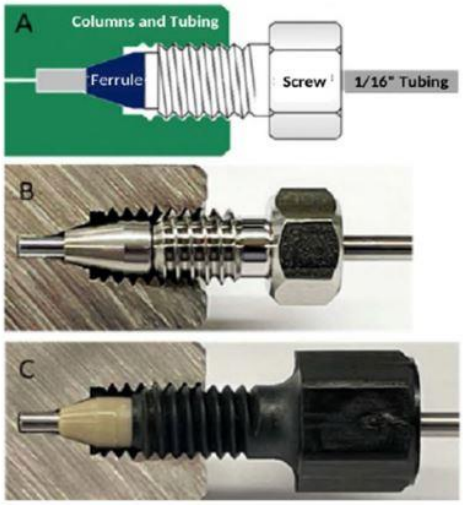



*Figure 1. Connection using setscrews and ferrules*

In the 1970s, during the early days of HPLC, hexagon cap nuts were used instead of setscrews. There were also various styles of end fittings, such as sizes of setscrews both using inches and millimeters and ferrules that were connected in reverse. After the 1980's, hexagon cap nuts were no longer used and the size of setscrew was standardized using inch standard No.10-32UNF. However, today, every HPLC/UHPLC system manufacturer has their own length of tubing (1/16" tubes) that protrudes out of the ferrule and there are several different end fitting standards from 1.6 mm to 3.5 mm, which are the lengths of the tubing that protrude out of the ferrule. A few major column packing manufacturers offer columns with different kinds of end fittings while many of them offer columns that are compatible with only one or two kinds of fitting standards that are popular with consumers, such as the Company W type or the Parker type.

## 3. Problems Caused by Different End Fitting Standards

As stated earlier, a metal ferrule that is used for connection is firmly fastened and is secured by the tip penetrating into the tubing. Figure 2 shows problems when fittings with different tubing lengths are used for connection. Figure 2(A) shows a significant dead volume between the tubing and column as the length of the tubing is short. Diffusion of sample components occurs here, resulting in broad peaks or tailing. The tubing shown in Figure 2(B) is too far from the ferrule tip and therefore the ferrule does not contact the end of the column closely, resulting in leaks. Figure 2(C) shows the optimal length of the tubing from the ferrule tip. As a result, there is no dead volume generated and no effect on the peak shapes. Each column has different end fitting standards and the users have to decide

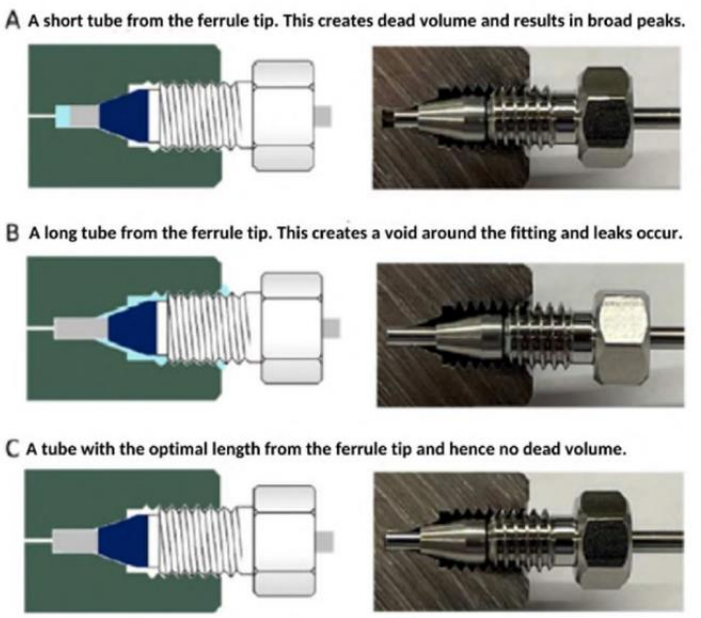



*Figure 2. Fittings for column tubing*
*(A) A short tube from the ferrule tip, which creates dead volume and results in broad peaks, (B) A long tube from the ferrule tip, which creates a void around the fitting and leaks occur, (C) A tube with the optimal length from the ferrule tip and hence no dead volume.*

where to secure the ferrule in accordance with the column. When metal ferrules are used, the users have to change the tubing for each end fitting standard. Further, metal ferrules will deform gradually with continual use even with columns that have the same end fitting standard, and it eventually becomes ineffective. While this degenerated tubing should be replaced with new tubing, if users fasten the setscrew too tightly trying to avoid leaks, the setscrew will break and the tubing will no longer be able to be returned to its original form. Many experienced chromatographers have probably experienced this phenomenon.

A functional resin, Polyether Ether Ketone (PEEK), which became available in the 1980s, is a thermoplastic resin that is heat resistant, has mechanical strength and superior chemical resistance, and came to be used in parts for HPLC. PEEK also came to be used as the ferrule to connect columns and tubing. As a material, PEEK was used as ferrules alone and integrated connectors that consist of ferrules and setscrews were also developed. Unlike metal ferrules, the PEEK ferrule is not fixed to the stainless-steel tubing, so the length of the tubing on the ferrule tip can be changed for each connection. Therefore, users were able to connect end fittings with different standards by using PEEK ferrules or connectors. The drawback of a PEEK ferrule is the pressure at the connection points, which is 20 MPa. For this reason, it could not be used with HPLC systems that require high pressure resistance; however, the integrated ferrule of metal and PEEK was developed to improve pressure resistance. As UHPLC has declined in popularity since 2004 and requires higher than 100 MPa of pressure resistance, metal ferrules are being used once again with UHPLC.

## 4. Compatibility with Different End Fittings

As discussed in the previous paragraph, all the end fittings that are currently used with HPLC/UHPLC columns have the same nut standard of No. 10-32UNF. Moreover, the tapering angle of the ferrules and ferrule inlets (column ends) is 40°, which applies to most of the columns; however, the tapering angle of UHPLC columns from Company W was 36°. I cut the tubes on commercially- available columns at the center and measured the parts as shown in Figure 3 (below). Figure 3(a) shows the fitting points of Company W's column and a Parker-type column. Both column tubes have a two-piece construction with separate threaded sections and tapered sections to receive the ferrules. The depth of the tapered area of Company W's is 4.2 mm while the Parker type is 1.9 mm, which is significantly different. Additionally, the length of the insert tube from the taper tip of Company W was 1.6 mm while the same for the Parker type was 2.0 mm. Figure 3(b) shows the end of each

column when secured with metal ferrules and stainless-steel tubing. Each ferrule has an angle of 36° and 40° respectively. Figure 3(c) shows the end of a column produced by Company W that is connected with a Parker-type tube and the end of a Parker-type column that is connected with a tube for Company W. When connecting a Parker-type tube to the end of Company W's column, there will be a void as the ferrule tip does not contact the column end securely and the insert tube is long; however, the mobile phase does not leak as the length of tapered area is long and tapering angle is small with Company W's column end resulting in the ferrule end securely contacting the column end. There is also no void between the tube tip and column end hence no broad peaks or peak tailings as a result. On the other hand, when connecting Company W's tube to the end of a Parker-type column, there will be a great void in the column end as the length of the tube from the ferrule tip is short although there will be no leaks as the ferrule tip is securely contacting the column end. This void creates broad peaks and causes tailing. As this experiment shows, UHPLC columns of Company W not only fit their own UHPLC systems, but also other UHPLC systems that have Parker-type end fittings without issue; however, columns with Parker-type end fittings do not fit Company W's UHPLC systems properly. Simply put, the end fitting standard of

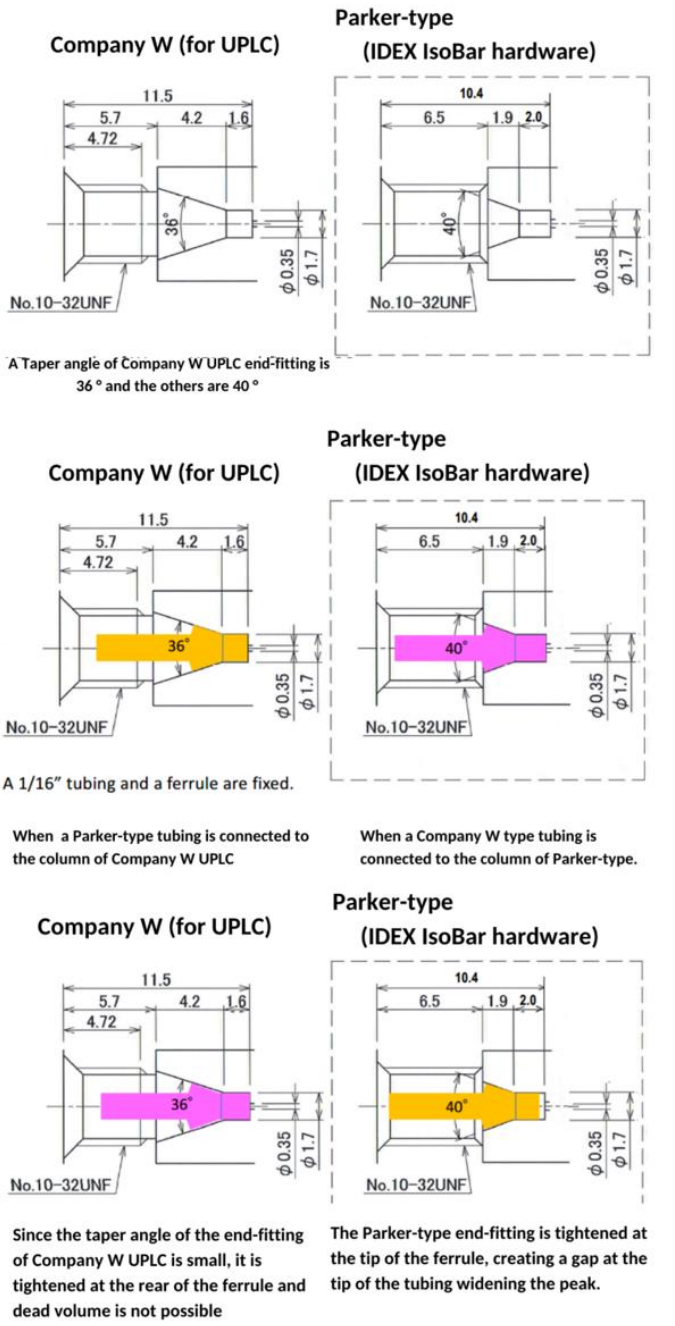



*Figure 3. Compatibility between Company W and Parker-type column end fittings (a) shows the structure of column end fittings, (b) shows the tube to secure ferrules for each end fitting, and (c) shows end fittings to mount different column tube ends.*

Company W is unique with a 36° tapering angle and a deep tapered area to accept ferrules. This is quite different compared to other end fitting standards and this uniqueness enables Company W's columns to fit Parker-type end fittings. Suppose a user thinks that Company W's columns have Parker-type end fittings and columns with Parker-type end fittings fit Company W's UHPLC systems without issue. When this user compares the column performance of Company W's column and other column brands with Parker-type end fittings on Company W's UHPLC system, other column brands show broad peaks and peak tailings while a column made by Company W shows better peaks; therefore, this is not a comparison that truly shows the performance of each column.

## 5. Connections That Do Not Use Ferrules

If there is a connection method for HPLC/UHPLC columns that does not use a ferrule, users will not have to worry about the various end fitting standards that currently exist with different lengths of tubes protruding out of ferrule tips. The MarvelX tubing manufactured and sold by IDEX Health & Science since 2016 is a ferrule-free connector as shown in Figure 4. Figure 4(A) is the MarvelX tubing. Both ends of the MarvelX are 1/16" stainless-steel tubes while the tubing itself is a 1/32" stainless-steel tubing. The nut has a slit so the tubing can be detached. There is 0.1 mm of PEEK that is protruding from the end and this PEEK adheres to the column end fitting firmly. Figures 4(B) and 4(C) show the tip of the MarvelX connected to the column end. Figure 4(B) shows the MarvelX before it is tightened and Figure 4(C) shows the tip of the MarvelX adhering to the column end although it is not fully tightened yet. The MarvelX does not require a wrench to ensure a pressure of 130 MPa with hand-tightening being sufficient. Six different tubing internal diameters (IDs) are available, which are: 25 µm, 50 µm, 75 µm, 100 µm, 125 µm, 254 µm and six different lengths: 70 mm, 150 mm, 250 mm, 350 mm, 500 mm, and 600 mm. Also, there are two

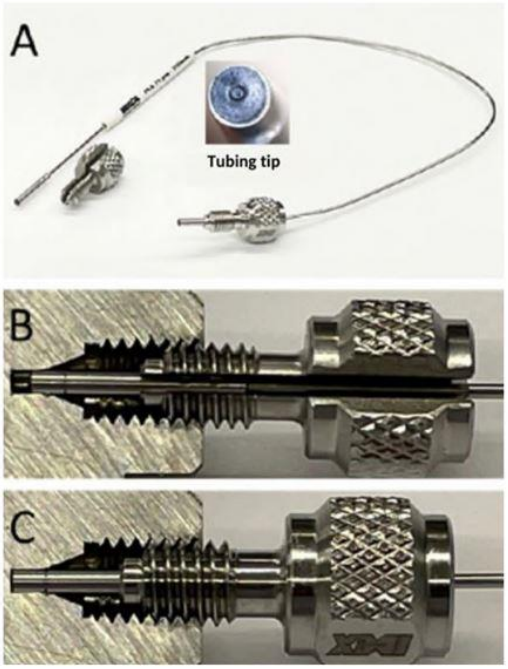



*Figure 4. MarvelX tubing and column end fitting attachment (A) shows a MarvelX tubing and its tip, (B) shows a MarvelX tubing being connected halfway to a column end, and (C) shows the completed procedure*

kinds of tubing materials available: one is a biocompatible PEEK-Lined stainless-steel with PEEK resin inside the tubing and the other is standard stainless-steel tubing only. In general, resin materials change form gradually by being pressured and this lowers the pressure resistance at the tubing connections; however, the MarvelX is covered with stainless-steel on the outside of the tubing and the PEEK on the contact surface does not spread outward in response to the pushing force on the tubing. This makes it possible to repeatedly use the MarvelX. In addition, MarvelXACT was introduced to the market as a tubing system that can be tightened in the same way by anyone. Figure 5 is a photo showing how it looks. Although the setscrew is secured on the tubing, a mechanism to prevent over-tightening is provided by allowing the setscrew to run idle at a certain torque.

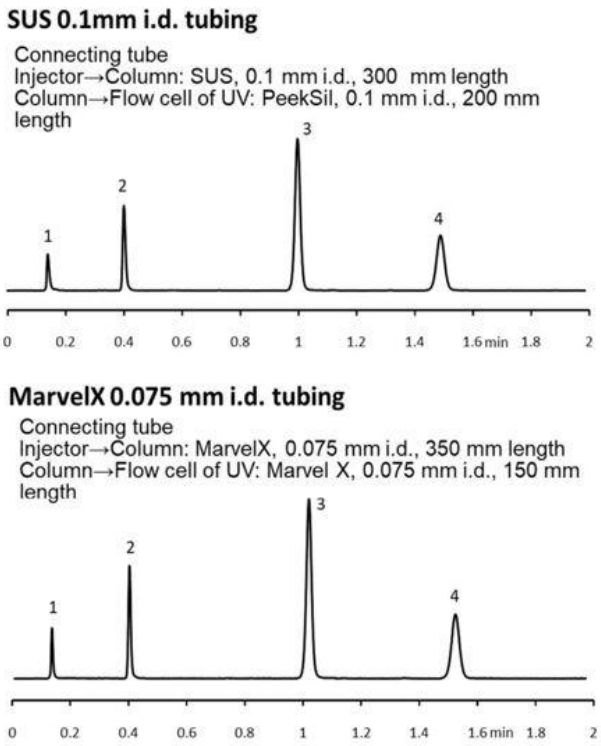
*Figure 5. MarvelXACT tubing*

It is important that the tubes used in HPLC/UHPLC have clean-cut cross-sectional tips and that the tips are not crushed or deformed. The same applies to MarvelX and MarvelXACT tubing. At ChromaNik Technologies, we take pictures of all the MarvelX and MarvelXACT's tips to ensure that there are no clogs or burrs attached, as shown in Figure 4, before shipping them to our customers.

## 6. Example of Using MarvelX Tubing

Since UHPLC is operated under high pressure conditions, high pressure-resistant tubing is required. The smallest ID of general stainless-steel tubing is 0.1 mm, while MarvelX and MarvelXACT offer an ID as small as 0.025 mm. Figure 6 shows the change in peak shape by reducing the inner diameter of the tubing that comes after the injector. I used a packed coreshell column C18 (SunShell C18) 2 um ID 2.1 x 50 mm. The upper chromatogram shows peaks by using an ID 0.1 mm stainless-steel (SUS) and inner-fused sil tubing (PEEKSil) and the bottom chromatogram shows peaks using ID 0.075 mm MarvelX (PEEK-Lined Stainless-steel). The length of both tubing is 500 mm. The theoretical plate number of the first elution of uracil (peak 1) was increased from 1208 to 3593 by decreasing the inner diameter of the pipe from 0.1 mm to 0.075 mm. The peak half-width also narrowed by 0.0043 min from 0.0094 min to 0.0051 min. Similarly, the theoretical plate number of acenaphthene (Peak 3) improved by 12%, from 13589 to 15153. All four peaks showed increases in theoretical plate numbers and decreases in tailing factor. This was especially

significant for the peaks with smaller peak volumes that eluted earlier. It is believed that the reduced volume of the tube besides the column suppressed the diffusion of components in this tubing, narrowing the peak width and suppressing tailing at the same time. Usually 0.1 mm ID tubing is used with ID 2.1 mm columns; however, 0.1 mm ID tubing is too large for a column with a length of 50 mm that achieves nearly 15,000 theoretical plate numbers and the use of 0.075 mm ID tubing will bring the column closer to its original performance. Although I used MarvelX tubing of 0.075 mm ID here and MarvelX tubing is available in sizes up of 0.025 mm ID, the back pressure on the tubing alone is inversely proportional to the fourth power of the inner diameter and, therefore, changing the inner diameter from 0.075 mm to 0.025 mm, which is by 1/3, would increase the back pressure on the tubing 81 times. Note that if the inner diameter is too small to reduce the volume of the tubing, the back pressure on the tubing may become too large. The bottom chromatogram in Figure 6 with the 0.075 mm ID tubing shows 7.8 MPa of back pressure just on the 500 mm long tubing. If tubing with a 0.025 mm ID is used, 81 times the back pressure, which results in 631 MPa, would be applied only to the tubing.




Peaks, 1=Uracil
2=Ethylbenzene
3=Acenaphthene
4=Butylbenzene

SunShell C18 2 µm, 50 x 2.1 mm

| | Peak No. | SUS | MarvelX | |
|---|---|---|---|---|
| Theoretical plate | 1 | 1208 | 3593 | 197% up |
| | 2 | 7720 | 12625 | 64% up |
| | 3 | 13589 | 15153 | 12% up |
| | 4 | 13936 | 14733 | 6% up |
| Tailing factor | 1 | 2.326 | 1.445 | |
| | 2 | 1.401 | 1.286 | |
| | 3 | 1.048 | 1.006 | |
| | 4 | 0.997 | 0.972 | |
| Peak width, $h_{0.5}$ (min) | 1 | 0.0094 | 0.0051 | |
| | 2 | 0.0107 | 0.0083 | |
| | 3 | 0.0201 | 0.0194 | |
| | 4 | 0.0297 | 0.0295 | |

*Figure 6. Relationship of Tubing ID to Plate Numbers*
*Column SunShell C18, 2 µm 50 x 2.1 mm ID; Mobile Phase, Acetonitrile/Water = 60/40; Flow Rate 0.6 mL/min; Column Temperature, Ambient Temperature; Detector, UV250 nm; Injection Volume, 0.4 µL; Peaks, 1 = Uracil, 2 = Ethylbenzene, 3 = Acenaphthene, 4 = Butylbenzene, Tubing ID is shown in the Figure.*

## 7. Conclusion

As described in this paper, it is important to make tubing connections that meet the column end fitting specifications in order to achieve the best column performance. It is also important to fully understand the differences between the end fitting standards of different companies, which are not always easy to see. Ferrule-free tubing, such as MarvelX, does not depend on column end fitting standards, and MarvelXACT is a convenient tubing system that can be tightened to the same torque by anyone. Since dead volume significantly affects the column performance, especially on 2.1 mm ID columns, it was confirmed that the use of MarvelX or MarvelXACT with an ID of 0.075 mm, which can be used under high pressure, enabled the columns to perform at their full potential.

**Reference**

N. Nagae, Wisdom for LC and LC/MS **2**, 6-25 (2021).