

Troubleshooting UHPLC Connection Challenges

As UHPLC has gained a solid foothold in the market, some of the problems that have plagued chromatographers – like band broadening, split peaks, carryover, etc. – are beginning to occur with greater frequency in UHPLC. While there may be numerous causes contributing to these problems, one common issue is often overlooked: a possible problem with the system's tubing connections.

Problem #1 – Tubing Slippage

Because system pressures in UHPLC can exceed 15,000 psi (1,034 bar), the physical demands on the tubing connections can be very significant. Due to the higher pressures of UHPLC coupled with the pressure cycling that can occur in certain areas of the system (e.g., at the injection valve), one of the biggest problems UHPLC users can face is tubing slippage.

In the areas of the system where the highest amount of pressure is experienced, the conically-shaped fittings work to hold the tubing against the bottom of the receiving ports in each connection. However, at the same time, the fluid in the system's flow path fights against the holding power of the fittings and works to drive the tubing away from the bottom of the receiving port. Of course, any movement of the tubing away from the bottom of the receiving port can result in dead volume, which can lead to the chromatographic problems highlighted above.

Unfortunately, this can be very difficult to detect because the movement of the tubing often occurs slowly. It's possible for there to be no sign of problems with the flow path connections (e.g., no evidence of leaking, no loss of pressure, etc), while the chromatographic results continue to deteriorate due to the increasingly larger dead volume chamber being formed in the receiving port.

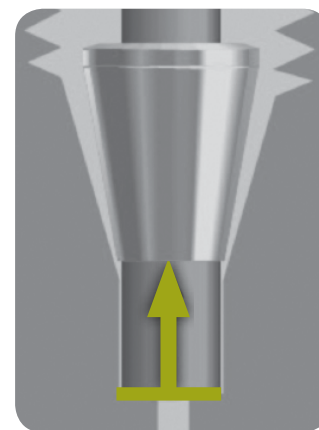


Diagram 1 – Tubing Slippage

Application Note: Dead Volume

In a standard 10-32 coned receiving port designed for 1/16" OD tubing, for every .001" the tubing slips past the tip of the ferrule, more than 50 nanoliters of dead volume is created.

Problem #2 – Tubing ID Compression

Because system pressures are so high – and because traditional polymer fittings can lead to tubing slippage at UHPLC pressures as described above – chromatographers typically use all-metal fittings in UHPLC applications to ensure the most reliable connections.

However, some of the most popular all-metal fittings require a high-torque load to ensure they swage properly on the tubing wall. When the need for a high-tightening torque is coupled with the axiom that says "you can never tighten too much," the stage is set to significantly compress the ID of the flow path tubing, as seen in *Diagram 2*.

Compression similar to that shown in *Diagram 2* can result in several problems:

- ▶ Higher system back pressures.
- ▶ A "throttling effect" on the fluid, resulting in increased turbulence and excessive mixing between the sample and mobile phase.
- ▶ Increased probability of tubing clogs due to suspended particulate matter.

Similar to the problem with tubing slippage, tubing ID compression is also difficult to detect. There are no leaks present and no loss of pressure, making this problem tough to troubleshoot and often resulting in unnecessary system down time and repair expenditures.

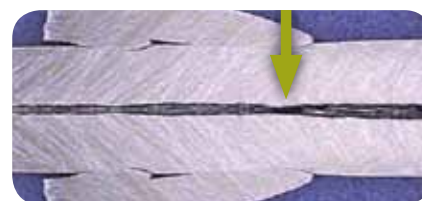


Diagram 2 – ID Compression, Competing Product

Problem #3 – Receiving Port Damage

The high torque required to successfully swage an all-metal fitting system on the flow path tubing doesn't just result in tubing ID compression. Depending upon the amount of tightening torque applied, it's possible for material to be ripped from the receiving port and transferred to the surface of the ferrule.

When galling occurs, the receiving port is damaged – possibly beyond use. In severe cases, the material that is ripped from the receiving port serves as a type of “glue” that forces the ferrule to stick in the receiving port. This makes it difficult to remove the fitting from the port and often results in a very expensive repair.

Even if galling doesn't occur, it's very typical for all-metal fittings to slightly damage the conical surface inside the receiving port. As seen in *Diagram 4*, it's possible for a ferrule to indent the conical surface by as much as 150 μ -inch.

The more often all-metal fitting systems are used in standard receiving ports, the more likely permanent damage of some sort will take place.

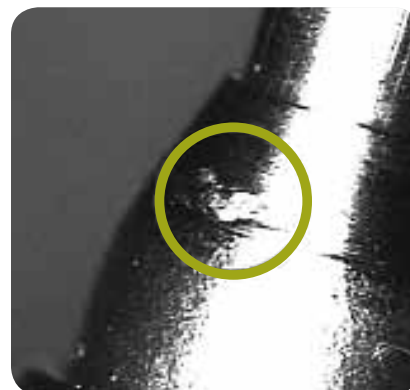


Diagram 3 – Galling, Competing Product

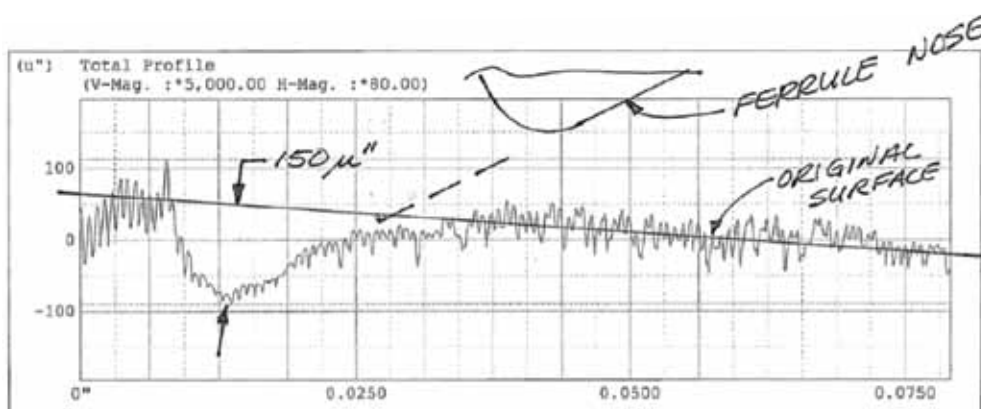


Diagram 4 – Profilometer Scan, Competing Product

Problem #4 – Injury From Failed Connections

Because of the tremendous pressures being applied to the fluid pathway in UHPLC applications, failed connections can lead to injury. When a fitting system fails, if the fluid is energized to 15,000 psi or greater, it is possible for the “jet” of fluid that rapidly exits the connection to damage soft tissue, like skin or the surface of the eye. As such, regardless of the fittings being used, IDEX Health & Science strongly recommends the use of proper protective gear when handling connections in UHPLC applications.

Overcoming Connection Problems with VHP Fittings

One very effective way to overcome the problems encountered in UHPLC connections is to switch to IDEX Health & Science's new “VHP” line of fittings. There are three fitting families with unique characteristics:

- ▶ The VHP-200 series is the highest rated at 30,000 psi (2,000 bar) and serves as a “drop-in” replacement for stainless steel two-piece ferrule systems.
- ▶ The VHP-300 and VHP-320 series offer the added benefit of reusability, allowing the same fitting to be used to connect tubing in different receiving ports over and over again without needing to replace the fitting each time.



Diagram 5 – VHP-200, VHP-300, VHP-320 & VHP-325

Through the use of a front ferrule manufactured from a proprietary PEEK-polymer blend, the VHP-300 and VHP-320 fittings ensure a biocompatible and inert fluidic seal without the risk of galling or damage occurring to the conical surface inside the receiving ports. Additionally, the internal ferrule – featuring patent-pending technology – helps hold the tubing in place securely without swaging onto the tubing. This feature allows these fittings to either connect the same tubing into multiple ports (e.g., when columns are changed on a system) or connect different tubes into the same port (e.g., when reducing the flow path tubing ID to account for lowering the system flow rate).

Please Note: Proper use of these fittings is paramount to obtaining optimal performance. Details on the proper use – including recommended tightening torque and number of times each fitting can be reused – are found on the product Spec Sheets, which are available upon request. While each fitting in the VHP fitting family has different features and benefits, all of the fittings share the following traits:

1. They prevent tubing slippage through the use of an innovative ferrule design

The IDEX Health and Science engineering team developed a measurement system to monitor tubing movement down to .0001" (2.5µm) as the fitting systems were stressed by a variety of conditions. The external stresses included the following: pressure dwell testing (monitoring pressure drop over an extended period of time), and pressure shock testing (repeated pressure cycling from ambient to above the stated pressure limit). The results showed that the VHP fittings did not move while competitive fittings showed movement before reaching their stated pressure limit.

2. They minimize tubing ID compression and help to ensure smoother flow

The VHP-200 fitting requires the most amount of tightening torque, yet it does not restrict the inner diameter of the tubing, as seen in *Diagram 7*.

Additionally, even though the VHP-300 and VHP-320 fittings employ the use of a stainless steel "back ferrule", the slotted design of this ferrule leaves a uniform fluid passage through the inner diameter of the tubing, even after multiple tightening cycles.

3. They reduce, or avoid completely, any damage to internal receiving ports

The strength of a connection relies partially on the quality of the receiving port. While competitive products can leave a damaging impression on the receiving port, as great as 150 µ-inch (as shown in *Diagram 4*, page 2), the VHP-200 fittings create less than half of the impression on the receiving port, as seen in *Diagram 8*.

Additionally, because the VHP-300 and VHP-320 fittings use a polymer ferrule for the forward sealing function, these fittings will not create any measurable impression on a stainless steel receiving port surface. This helps to increase the number of cycles a quality connection can be made into a receiving port.



Diagram 6 – VHP-320 Showing the Internal Compression Ferrule

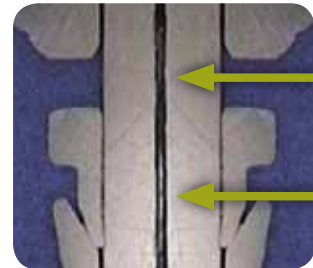


Diagram 7 – Uniform Tubing Inner Diameter after Using the VHP-200

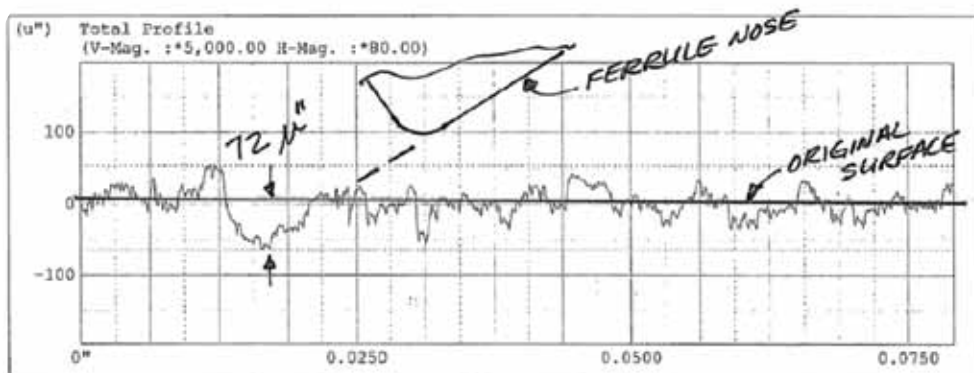


Diagram 8: Profilometer Scan VHP-200

Conclusion

While the challenges linked to UHPLC connections can be significant, it's important to remember that most can be traced back to the tubing connections. Therefore, before investing a small fortune in the replacement of expensive system components, take a little time to inspect the tubing connections and replace or update them to fittings from IDEX Health & Science's VHP product line. Doing so will be much less expensive and will typically result in less system down time while ensuring improved overall system performance.