HPLC Tubing and Fittings "Hints & Tips for the Chromatographer"

By

William S. Letter   (Chiralizer Services, L.L.C.)

INTRODUCTION:

Setting up a high pressure liquid chromatography (HPLC) system to run trouble-free over time takes some patience and a strong set of troubleshooting skills. The patience aspect has usually worn out with most of us and the troubleshooting skills often come from years of tinkering and practical experience. As a consultant who works with chromatographers on a daily basis, I have found that most chromatographers share many of the same basic HPLC hardware problems. Most of these problems are either caused by a failure to logically troubleshoot a problem from scratch or by overlooking seemingly minor changes that have been made to the system over time. One common area that is often overlooked in the area of HPLC is that of connection fittings and tubing selection. Selection and installation of the correct HPLC fittings and tubing can help you avoid future problems while allowing your system to run at peak performance. Common types of high pressure chromatography fittings and tubing found in the laboratory will be discussed in this report.

FITTINGS:

We often inspect an HPLC system only to find a half dozen problems related to the fittings and tubing choices. Examples of problems found would include; mismatched male nuts and ferrules, incorrect thread pitch and tubing sizes that are either too long or are of the wrong internal diameter for the application. Unfortunately for chromatographers, the various manufacturers have not standardized their fitting sizes and this can present serious problems when you work in a laboratory which employs several different brands of equipment. Many of the fittings are not interchangeable, and if used in the wrong combinations can add delay volumes, mixing chambers, leaks and other undesirable problems to your system. Even if the correct fittings and tubing are chosen for the application there still exists a potential problem of not swaging the fittings to the tubing properly. Once swaged, the fitting/tubing combination should not be used in other applications unless the tubing's internal diameter, fitting type and ferrule seating depth exactly match that of the mating fitting (In our laboratory, once a ferrule is permanently swaged onto a line, it is not reused again).

The three most important things to remember when selecting fittings are to select a fitting with the correct thread size (pitch); the correct overall length and the correct threaded

length. The three most common thread sizes in use today are 10-32, 1/4-28 and the metric M6. If in doubt as to the fitting type and thread pitch used on your equipment then please consult with the instrument manufacturer of your system. Once you are sure of the thread type (most systems use the 10-32 size for 1/16" OD tubing) then be sure to select the correct brand of nuts and ferrules to use. Some of these parts are interchangeable while others are not. Most of the male nuts sold by Upchurch®, Valco®, Swagelok®, Parker® and Hewlett-Packard® are interchangeable with each other. They principally differ in their overall threaded length (between 0.210 and 0.300") and use of one or two piece ferrules. Be sure to use a nut that has long enough threads or it will not seal properly inside of the fitting. Most Water's and Rheodyne brand fittings should not be interchanged and should be used with their equipment only. Water's uses a very long threaded nut to seal their fittings (shorter nuts like some from Parker and Swagelok can bottom out and will not seal a Water's brand connection properly). Rheodyne manufacturers several nuts in a variety of lengths to accommodate the very tight spaces found around their valves as well. Rheodyne also supplies special ferrules for use with their valves and nuts as well. Always use the correct ferrule designed to seal the fitting inside the union or fitting.

SWAGING FITTINGS:

With the proper nut and ferrule selected, the fitting must be properly swaged onto the tubing (defined as tightening the ferrule to the outside wall of the tubing such that it is crimped to the tubing without decreasing the internal ID. of the tubing). Most manufacturers have a suggested procedure for swaging their fittings onto 1/16" steel tubing and you should follow their advice. Usually the procedure consists of sliding first the nut then the ferrule (some use two piece ferrules !) onto the tubing, bottoming out the tubing into the end of the connector that you are attaching the nut to, then finger tightening the fitting followed by wrench tightening (between 1/8 to 1/2 turn more for most). Always hold the end of the tubing flush to the inside of the fitting while wrench tightening and do not over-tighten. Most problems start off from fittings that have been over or under tightened. The depth that the ferrule is swaged onto the tubing is the most important aspect covered so far and is usually where most of the problems start (the distance from the end of the tubing to the base of the ferrule is usually greater than 0.080" and less than 0.170"). If the tubing that now contains the swaged nut/ferrule combination is re-used on another part of the system it is imperative that the seating depth be the same on the other connector. If the seating depth is too deep then the fitting will not seal inside of the fitting and the end of the tubing could be damaged while tightening. If the depth is too short, the end of the tubing will not fit flush with the connector and an additional mixing chamber will have been created in the system. If in doubt, fabricate a new fitting/ferrule combination for the part. We try not to reuse tubing/fitting combinations except in emergencies and prefer to fabricate fresh tubing/fitting assemblies as needed. These connections are critical when they are formed between the injector and column as well as the column and detector. Ideally, once formed, they are not manipulated again and become a static part of the HPLC.

PLASTIC UNIVERSAL FITTINGS:

A partial solution has been found that can solve some of the seating depth problems as well as the problem of identifying the correct brand of fitting for each part. Most commonly available in PEEK™, Teflon® and Delrin® polymeric materials, one piece plastic "Fingertight" fittings solve the problem of seating depth and thread length. *Two piece fingertight fittings are also available, but we find that it is too easy to loose the separate plastic ferrule that comes with them! The easier to use one piece fittings are easily slid on and off of stainless and polymeric tubing and most can universally fit many brands of column end-fittings and union connectors. After tightening by hand, many can hold between 4000 and 6000 psi of pressure and are resistant to attack by many solvents. Newer versions can be found with ratings of over 10,000 psi as well which can prove useful in UHPLC applications. These fingertight fittings are relatively low in cost and can be re-used many times with great success. They are especially useful as column inlet/outlet fittings since they will allow connection to several different brands of column end fittings while reducing the normal wear and tear associated with steel fittings during column installation and removal. We still recommend that steel fittings be used in all areas where you are unlikely to disconnect and reconnect fittings often and use the fingertight style fittings only for other applications such as column connections. We have these plastic fittings for many years and can highly recommend them for many applications. One caution worth noting here is that when polymeric fittings are employed inside of column ovens (heated or cooled), the resulting temperature changes can cause them to loosen and leak. It is advisable to regularly check them for a proper seal when first using them.

TUBING:

Lastly, tubing internal diameter is very critical to an HPLC system. If you don't already, try to get into the habit of filtering your samples through 0.45 or 0.20 micron syringe filters. This minor delay in sample preparation can minimize plugs and other problems that will foul your column and/or plug your injector or associated tubing. If an analytical system (for 2.1 mm ID mini-bore and/or 4.6 mm ID standard analytical columns) is assembled with tubing with an I.D. of 0.007" (0.17 mm) after the injector than the band broadening will be minimal and the interconnecting tubing's volume will minimally affect the chromatography. Note: For applications where ultra-low flow rates (< 0.2 ml/min) *or* HPLC columns with very small volumes are to be used (< 0.5 ml/min), you may want to use even smaller diameter tubing such as 0.005" ID (0.12 mm) to reduce total delay volume. Keeping the total length of all the tubing used to a minimum will also help. If desired, the tubing that leads from the pump to the injector can be of larger internal diameter (i.e. 0.010" / 0.25 mm) to lower the system operating pressure, but it will increase the delay volume of the pumping system. The larger the I.D. of the tubing used the longer the gradient delay will be for the system. The tubing which is often supplied with new HPLC systems is often not optimized for any particular application. Manufacturer's often supply extra long lengths of tubing (to accommodate the different arrangements of the system) and larger I.D. bores on some of the lines (to reduce the chance of clogging the lines). Time spent re-plumbing your HPLC system with optimized lengths and I.D.'s of new capillaries can both reduce the total delay volume of the system and reduce the total analysis time. Make sure that the tubing that you are using is appropriate for your system (again, consult with the manufacturer or an expert in system optimization). A basic table of how tubing ID relates

to tubing volume is shown below. Note how reducing the tubing ID from 0.010" to 0.007" cuts the internal volume by half!

Narrow bore 0.005" ID tubing reduces the volume by another factor of two from 0.007" ID. These very narrow capillaries are more susceptible to plugging or clogging though so please be sure and filter all sample and mobile phases before use.

Capillary Tubing I.D. vs. Internal Volume Table:

I.D. inches (mm)		Volume (ul/cm)		Volume (ul/inch)
0.005" (0.12)		0.127		0.323
0.007" (0.17)		0.249		0.632
0.010" (0.25)		0.507		1.288
0.020" (0.51)		2.026		5.146
0.040" (1.02)		8.103		20.581

CUTTING TUBING:

The best method to cut stainless steel chromatography tubing in house is to not cut it at all. Ideally, purchase pre-cut lengths of tubing from one of the many major chromatography supply houses where are prefinished and polished. Many use a professional electrolytic cutting process to cut each capillary length to size. The tubing is cleaned; the ends are squared and polished, optimizing your chances of making a clean connection to your system. We strongly suggest you purchase tubing this way. However, if you require custom lengths or need the tubing now, there is a second option. You can purchase a chromatography tubing cutter and cut it yourself. Should you choose to cut the tubing to length yourself, then make sure and select one of the professional powered saw type tubing cutters to slice through the 0.020 and smaller I.D. stainless steel tubing. Conventional "plier type" cutters often cut the tubing at an angle and leave a crimped edge on the tubing. Hand cutters, while useful for very larger ID tubing, often leave a ring around the center of the smaller ID (1/16" OD) tubing where it sliced through. This ring causes the tubing to be slightly convex and does not allow a clean, square surface for connection to the instrument. For 1/16" and 1/8" O.D. analytical grade tubing with ID's of less than 0.020" invest in a professional power cut-off saw with a sharp deburring tool to obtain the best cut. These units cost several hundred dollars each, but they will pay for themselves in the time you save by using these devices to make quality cuts. Don't forget to clean and debur the ends after cutting using a sharp deburring tool. Sometimes the use of very fine wet sandpaper (600 grit) or a very fine diamond grit file is useful to remove any burs or edges. Check the result with a low powered magnifier. Finally, flush the tubing out with filtered solvent before you install it in the system.

Recommendations:

We suggest you maintain a custom kit of tubing and fittings suitable for use on your specific instrument(s) at all times. The ideal kit would consists of various lengths and

sizes (I.D.) of stainless steel tubing as well as at least ten stainless steel male nuts of various lengths and ferrules for use with your HPLC system. Additionally, a small assortment of one or two piece polymeric fingertight fittings and a spare ¼" open ended fitting wrench and medium adjustable wrench should be on hand. If you have a place in your laboratory where you have stored all of the used tubing with pre-swaged connectors of various types and sizes attached, then please donate them all to the trash can and only use new tubing and new fittings, custom swaged to the exact fitting you require. This will save a lot of time and get you back to work and reviewing data much faster.

NOTES:

• **HPLC Column Volume & Capillary Volume Tables: Includes the formula for calculating true column void volume. [http://www.hplctools.com/tablescharts.htm]**