Onyx Monolithic Capillary HPLC Columns

Use and Operation Addendum

How to connect an Onyx capillary to your system

Adjusting flow rates: loading time vs. resolution vs. sensitivity

Helpful hints for capillary chromatography

Onyx capillaries as a trap column

Onyx C18 150 x 0.1mm: Connecting the column to the system

Onyx capillary columns (150 x 0.1 mm) are a useful solution for many capillary LC/MS/MS applications where low flow rates, high peak capacity, and increased resolution are desired. The wide flow flexibility, low carryover, and excellent performance of these columns have generated significant interest in monolith column technology, however because of its unique nature many questions have arisen regarding connecting the Onyx capillaries to a microbore HPLC system.

Unlike other capillary columns, which use standard tubing filled with particulate medium, the Onyx capillary column is manufactured by the monolithic silica matrix being polymerized directly in a (100µm ID x 360µm OD {0.004"ID x 0.0145" OD}) glass capillary. Standard capillary columns typically use a female threaded endfitting to connect the capillary column to the HPLC. Onyx, being directly polymerized in a capillary tube without frits, can use a variety of different solutions to connect the column to a microflow HPLC. Included with the column are sleeves and a single-piece nut and ferrule to connect the capillary to a HPLC or LC/MS system (see **Figure 1**).

Geometry of Capillary Endfitting Included With Column

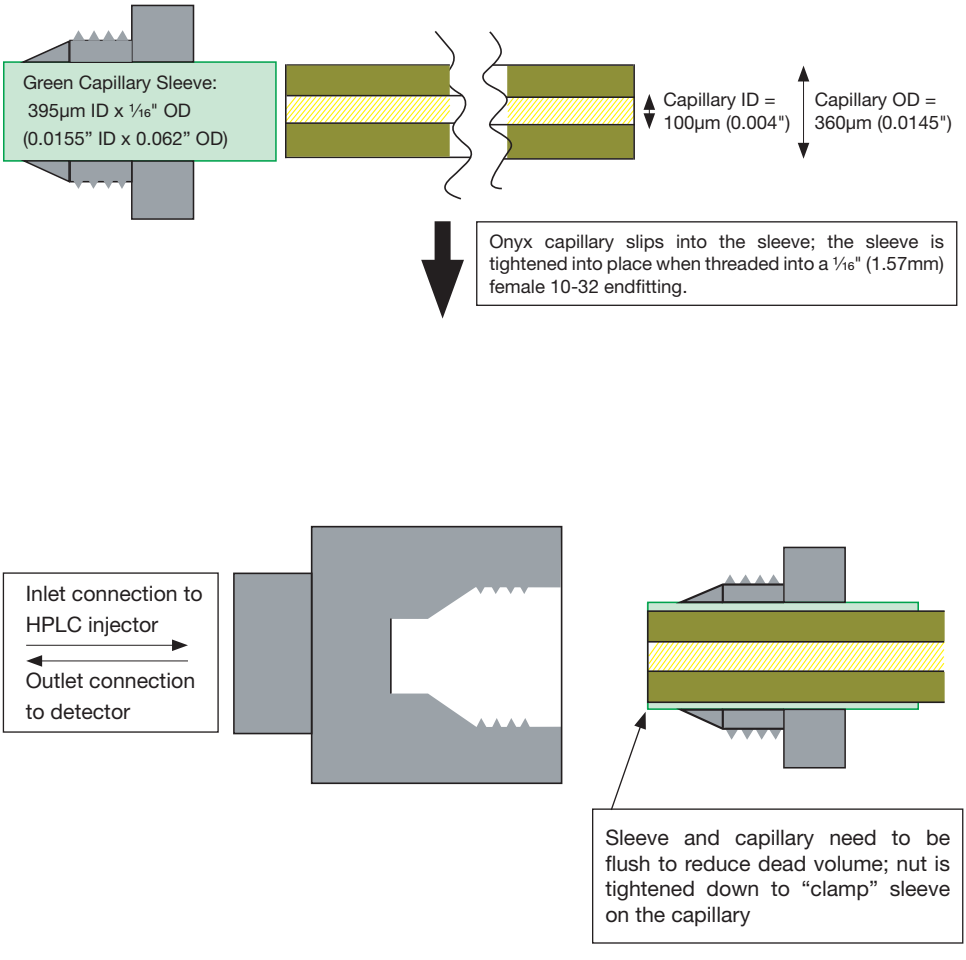

Figure 1: Diagram of Onyx capillary column and included capillary sleeve and single piece ferrule and nut. To connect the Onyx capillary to a HPLC, the sleeve and ferrule/nut are placed on the end of the capillary keeping the sleeve flush with the end of the capillary. Tightening the ferrule/nut into a female 1/16" fitting secures the sleeve in place on the column.

Capillary Chromatography Tips

- Reduce ID and length of tubing whenever possible
- Use correct size (low volume) flow cell
- Use injector program or trap column to reduce sample loop volume
- Minimize number of connections and fittings to reduce extra-column volume

Because of the low flow rate and minimal column volume, “perceived” column performance is strongly affected by the quality of the apparatus used for the separation. Reducing system extra-column volume is crucial in improving performance. With flow rates in the low and sub-microliter range any improper seated connections that generate a few microliters of dead volume can result in minutes of gradient delay that results in peak broadening.

Reducing system volume ahead of the column with smaller ID tubing and injector programs to remove sample loop volume is key to reducing gradient lag and reducing run times. Extra column volume after the column dramatically affects the efficiency, resolution, and peak capacity due to diffusion increasing the peak volume. The large volume in an electrospray MS interface can also have the same effect; reducing the resolution of any capillary separation.

Ideally, the inlet of the Onyx capillary column should be connected directly to the HPLC injector and the outlet should be connected to the detector flow cell. Obviously, this is not always practical; if faced with a choice it is better to reduce post column dead volume since such post column dilution effects degrade “apparent” column performance. If long distances need to be connected between the injector and detector, narrow bore capillary tubing (≤50µm I.D. {0.002"}) and a zero dead volume (ZDV) union (Phenomenex P/N AQ0-4092 or AQ0-4091) should be used in addition to the Onyx column to span the distance.

Utilizing Flow Rates for Performance Optimization

Because of its high porosity and reduced flow restriction, Onyx capillaries have a wide range of usable flow rates. One can vary the flow going through the capillary based on specific requirements for their application. This is different compared to particle-based capillary columns that have a narrow range of operating flows. Onyx capillaries can operate at flow rates up to 10 µL/min to reduce the amount of time it takes to load a sample on the column (far outside its optimal operating range of 1-3 µL/min). For improved sensitivity, flow rates can be reduced to as low as 400 nL/min and still deliver reasonable chromatographic performance. Such flexibility allows one to cater their operating conditions based on other performance criteria; improved separation power at higher flow rates (2-3 µL/min) or improved sensitivity at lower flow rates (0.5-1.5 µL/min). Higher flow rates (up to 3 µL/min) give improved performance by minimizing the effects of extra column volume; lower flow rates give improved sensitivity by increasing analyte concentration.

Onyx™ Finish First

Onyx Capillary Columns: Additional Notes and Applications

While the Onyx capillary is a well-endcapped C18 phase using type-B silica, it still has some limitations including a narrow pH stability range from pH 2.0 to 7.5. In addition, while the matrix is rugged and stable to pressures up to 300 bar, the column should not be disconnected from the HPLC system under pressure. Depressurize the column to below 5 bar (70 psi) before removal from the HPLC system.

The Onyx capillary column demonstrates low carryover and reduced non-specific binding to the media. Onyx capillaries also do not use inlet or outlet frits thus reducing carryover. Column cleaning can be performed with 95% acetonitrile. While not recommended, some users have recovered plugged columns by cutting off the inlet end of the column with a capillary cutter.

Several customers are using Onyx as a trap loading column ahead of a smaller packed capillary spray tip. For such applications, many have cut the Onyx capillary into smaller trap columns using a capillary cutter. While this is not a supported application for the Onyx capillary, many find the high porosity, low carryover, and fritless design of the Onyx monolith ideal for such applications.

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