PolyLC INC.
9151 Rumsey Road, Suite 180 • Columbia, MD 21045 USA
Phone: (410) 992-5400 • FAX: (410) 730-8340 • e-mail: POLYLC@aol.com

Instructions for: Capillary and Microbore Columns

Dead Volume:
It is critical to keep this to a minimum. Use connecting tubing of 0.002 - 0.003" i.d. For capillaries < 200 µm i.d., use connecting tubing of 50 µm i.d. Upchurch Scientific sells PEEK tubing in these sizes; an alternative is SGE's PEEKsil material. Keep connections short. This tubing has the following drawbacks:

1) Backpressure: A 15-cm length of 0.003" tubing confers @ 500 psi (35 bar) backpressure at a flow rate of 1.0 ml/min.

2) Tubing this narrow tends to clog easily. Use inline filters to minimize this tendency.

Detectors:
Flow cells should be no larger than 0.5 µl for microbore columns, and cells for packed capillaries should be 0.1 µl.

Flow Rates:
Assuming a flow rate of 50 µl/min for a column of 1.0-mm i.d., corresponding flow rates for packed capillaries would be as follows:

Capillary ID	Flow rate	Recommended load or peptides per injection (SCX)
300 µm	5.0 µl/min	14 µg
220 µm	2.4 µl/min	7 µg
175 µm	1.5 µl/min	4 µg
100 µm	0.5 µl/min	1.4 µg

While one can load 2x these amounts per run, the resulting peaks may not be as sharp as would be optimal for proteomics applications. Loading levels in HILIC are about ¼ of those of SCX.

Sample Injections:
It is advisable to use either a valve designed for submicroliter sample volumes (*e.g.*, Valco's) or else sample loading via a pressure bomb (@ Lin, Alpert & Yates, *Amer. Genomic/Proteomic Technol.* [Aug. 2001; in press]).

Conditioning, Maintenance, and Storage:
Use the same protocols as with the corresponding columns of conventional dimensions.

Air Bubbles:
It is more difficult to clear these from the detector flow cells than with conventional size columns, owing to the slow flow rates. You can minimize this problem with the following procedures:

1) Degas mobile phases.

2) Mobile phase should flow from bottom to top into the flow cell.

3) Consider installing a flow restrictor after the detector to confer modest additional back pressure.

Death by Mass Spectroscopy:
There is considerable electrical potential at the orifice to a mass spectrometer. The mobile phase can conduct current back to the HPLC system; potentials of 600 V have been measured at injector valves! Be sure to ground the system somewhere between the outlet of the HPLC and the inlet of the MS.