Setup and Measurement of Flow Rate for Online Nanobore LC-MS

Eluent flow rate provides crucial implications for configuring your nanobore ESI application. The flow rate dictates recommended tubing and tip inner diameters (IDs) for standard LC columns, PicoTip® online emitters, and PicoFrit® columns. Figure 1 displays recommended tip and column dimensions for given flow rates.

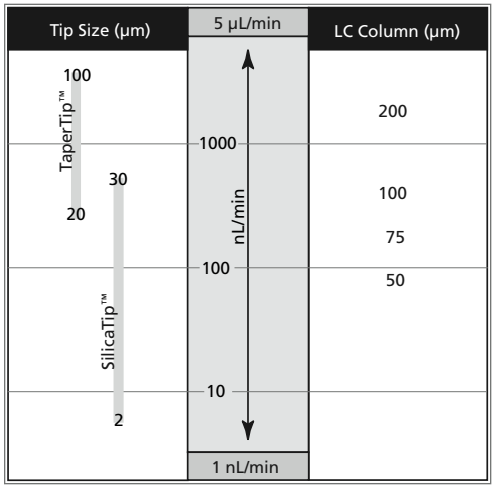

FIGURE 1

While many LC pumps accommodate flow in the µL/min range, few are specifically designed for the nL/min level. Employing a flow-splitter (Figure 2) between the LC pump and column helps to significantly reduce flow rate to accommodate nanobore LC. A flow-splitter is comprised of a "T" junction where the fused silica LC line from the pump is plumbed immediately opposite the LC line leading to the column. A third fused-silica line (waste tubing) is plumbed orthogonally to the flow path, providing a second mobile phase outlet (Figure 2 - Outlet 2).

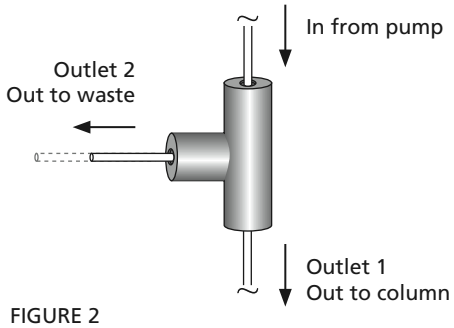

FIGURE 2

In general, the shorter the piece of fused silica for Outlet 2, above, the greater the flow rate reduction at the tip. Best practice involves starting with a long piece of fused silica for Outlet 2, measuring the flow rate at the column tip, and systematically reducing outlet 2 length with an appropriate fused-silica cutter (refer to Technical Note FS-1) to attain the desired flow rate. Installation of an inline microfilter immediately before the flow-splitter and an inline nanofilter between the flow-splitter and column helps minimize clogs for this configuration. Loading a sample onto the column by sample trap injection further purifies the sample and prevents the entry of particulates into the system. See Technical Note IF-3 for information of effective sample trap use.

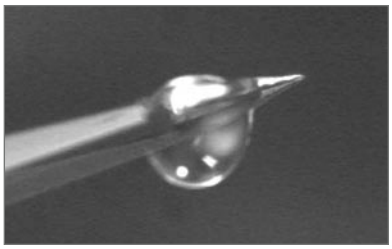

FIGURE 3

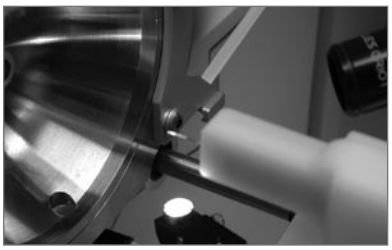

FIGURE 4

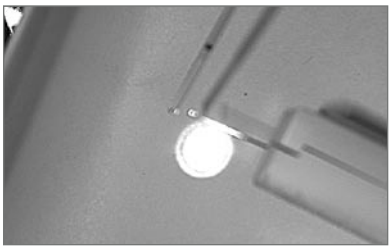

FIGURE 5

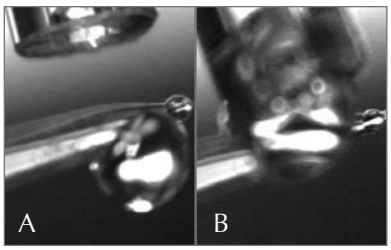

FIGURE 6

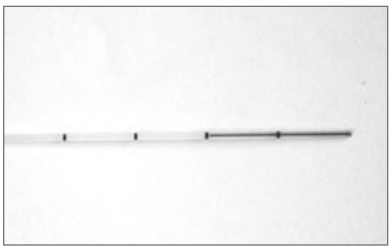

FIGURE 7

Flow-Rate Measurement

New Objective provides Calibrated Micropipettes (Order Number NO2-000-001) designed to measure flow rate at the tip of columns or online emitters. This technical note describes how to use these micropipettes to measure flow rate while minimizing tip damage.

WARNING: Electrospray ionization involves the use of potentially lethal high-voltage electrical current. Observe all manufacturers' safety recommendations in the use of such equipment. No equipment modifications should be made except by trained personnel using methods approved by the manufacturer in accordance with all safety requirements. Installation of equipment should be performed by qualified personnel in accordance with all applicable electrical codes.

CAUTION: Handling of fused-silica tubing and emitters can result in serious personal injury, including skin and eye injury. Use safety glasses or goggles meeting ANSI Z87.1-1989 requirements or the equivalent. Puncture- and chemical-resistant gloves should be worn at all times.

1. Following recommended procedures from your mass spectrometer manufacturer, turn off the voltage supply to your nanospray source
2. Activate your pump to begin eluent flow
3. Allow a droplet to form at the emitter tip (Figure 3)
4. From a distance of approximately 2 cm, aim the nozzle of canned air spray at the emitter tip (Figure 4)
5. With a stop watch ready, spray the canned air directly on the tip to remove the initial droplet
6. Immediately upon droplet removal, start the stopwatch and allow 4-5 minutes to elapse as a new droplet forms.
7. As the end of the 4-5 minute measurement interval approaches, hold the calibrated micropipette so the end points toward the growing droplet (Figure 5)
8. Immediately upon concluding the 4-5 minute measurement interval, lightly touch the tip of the micropipette to the droplet and collect fluid (Figures 6A - 6B)
9. Remove the micropipette from the tip and measure the fluid volume in the pipette
10. Use the formula below to calculate flow your rate:

$$\text{Eluent flow rate (µL/min)} = \frac{\text{Fluid volume in micropipette (µL)}}{\text{Total time of fluid collection (min)}}$$

11. Flow rate can be adjusted by increasing/decreasing the diameter/length of the waste tubing at the flow splitter. Depending on mobile phase composition and the inner diameters of both the tubing and emitter tip, typical nanospray flow rates range between 100 - 500 nL/min.