



Modifying an existing HPLC method for micro HPLC can be very simple. Since the column stationary phase and the mobile phase remain the same, for isocratic separations all that needs to be changed is the flow rate. To maintain the same linear velocity of the mobile phase in columns of differing internal diameter (ID) the following simple equation can be used:

$$\text{Flow (new column)} = \text{Flow (old column)} \times \frac{\text{ID}^2 \text{ (new column)}}{\text{ID}^2 \text{ (old column)}}$$

For example, when changing an isocratic method from a 4.6mm ID column to a 2mm ID column the original flow rate of 1mL/min would have to be modified to:

$$1 \times 2^2 / 4.6^2 = 0.189 \text{ mL/min or } \sim 0.2 \text{ mL/min}$$

Therefore, when smaller column IDs are used, large solvent savings are made possible as shown in Table 1.

Table 1.

Column ID	Column Flow ¹	Solvent Use ²	Solvent Saving ³
4.6mm	1mL/min	600mL	—
4.0mm	0.75mL/min	450mL	150mL
2.0mm	0.20mL/min	120mL	480mL
1.0mm	0.05mL/min	30mL	570mL

1. Based on HPLC methods providing the same separation as a 4.6mm ID column operated at a flow rate of 1mL/min.
2. Solvent use for 10 hours (1 day) of HPLC operation.
3. Solvent saving compared to the operation of a HPLC system at a flow rate of 1mL/min.



Isocratic Methods

Due to improvements in modern HPLC systems, micro HPLC has become easily accessible to chromatographers worldwide. Modern pumps can easily deliver the 0.05mL/min flows required. A main consideration is that gradient micro HPLC does require special approaches such as dedicated equipment or flow splitting. Isocratic micro HPLC is, however, easy with current HPLC systems and the only considerations are detector flow cells and connecting tubing. Standard detector flow cells can still be used with 2.0 mm ID columns though micro flow cells are recommended. The reason for this is that the residence time of the mobile phase is much higher with the low flows used for micro HPLC. Micro flow cells are available for most detectors from the vendors, and these are designed with low illuminated volumes so that there is no band broadening at low flow rates. Micro flow cells typically have a volume of 1µL compared with 14µL for standard flow cells to optimize peak shape. The final equipment requirement is low volume connecting tubing. This is readily available as short lengths of small ID (0.1mm ID) pre-cut PEEKsil™ HPLC tubing. Many chromatographers prefer long lengths of connecting tubing to enable easy connection of columns. This approach is unsuitable for micro HPLC as unnecessary tubing can lead to high extra column volume and poor chromatography. Of course, 5µL or smaller sample loops are advisable to minimize band broadening and also to ensure that sample overload does not take place as micro columns have lower sample capacities than standard HPLC columns.



For further information on PEEKsil HPLC tubing, please request Publication No. PD-0106-H.

Gradient Methods

Gradient methods are more difficult to modify for smaller bore HPLC columns. This is not because of the column or mobile phase chemistry, but rather due to the design of HPLC pumps. Typical HPLC pumps of the last few years have been built with minimal internal tubing but gradient pumps also often include a solvent mixer after the proportioning valve which all adds to the volume of the system between the proportioning valve and the column. This volume is known as the delay volume, which needs to be flushed before the solvent gradient actually enters the column. This delay volume is usually about 2mL which means there is a delay of about 2 minutes before the gradient would get to a HPLC column operated at a flow of 1mL/min. A 2 minute delay is not significant for many analyses but for small bore columns the delay volume becomes a problem. For example if a 2mm ID column was run on a typical HPLC system with a 2mL delay volume at a flow of 0.2mL/min it would take 10 minutes before the gradient entered the column making the separation very different from one run on a similar 4.6mm ID column. Unfortunately there is no easy way around this problem, as the HPLC pump really needs to have its internal tubing and mixing chamber modified to better operate at lower flow rates.