# Agilent Low-Volume Columns Operational Guidelines Datasheet

## General Considerations

lnterest has developed for columns with smaller internal volumes (shorter length, smaller internal diameters) designed for high performance/high speed separations. Anticipated uses for such columns include: (1) applications requiring enhanced sensitivity for small samples; (2) reduced mobile phase solvent consumption per analysis to decrease costs and environmental impact; (3) specialized techniques such as LC/MS; (4) high speed analyses with no loss in separation performance to improve analytical productivity. The purpose of this document is to provide the user of these columns with information targeted at optimizing the chromatographic utility of high performance, low internal-volume ZORBAX columns. The dimensional configuration of columns designed for the applications noted above results in decreased internal volumes as compared to commonly-used 4.6 mm ID x 150 mm columns. Peaks generated from these columns at reasonable k' values have considerably smaller volumes than those of standard 4.6 x 150 mm columns. This volume effect is shown in Table 1.

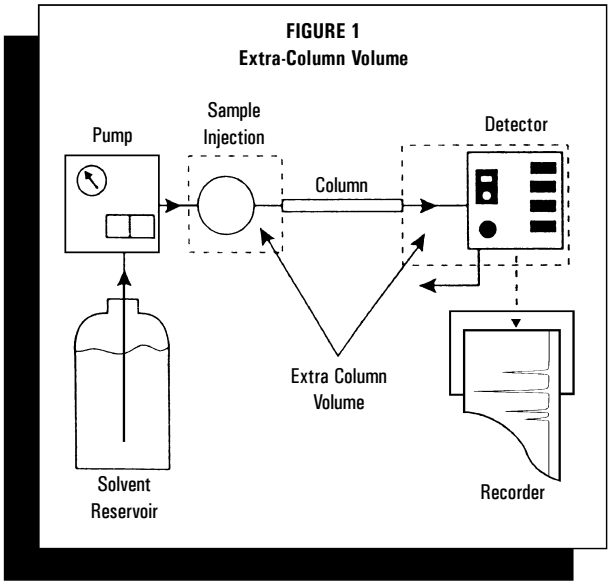



**FIGURE 1**
**Extra-Column Volume**

TABLE 1
**Volume Characteristics**

| Column | Internal Column Volume | Calculated Peak Volume (4s) |
|---|---|---|
| 1.0 x 150 mm | 0.09 mL | 13 µL |
| 2.1 x 150 mm | 0.35 mL | 52 µL |
| 3.0 x 150 mm | 0.70 mL | 112 µL |
| 4.6 x 75 mm | 0.80 mL | 120 µL |
| 4.6 x 150 mm | 1.60 mL | 260 µL |

The peak volumes listed in Table 1 are typical of columns having about 10,000 theoretical plates and peak elution with k' = 3. The very low-volume 1.0 and 2.1 x 150 mm columns require HPLC equipment specifically designed with low-volume components (injectors, detector cells, etc.) to achieve maximum performance. The larger volumes of the 3.0 x 150 mm and 4.6 x 75 mm columns allow them to be used in well-designed standard HPLC equipment. However, all low-volume columns perform best when used with proper attention to the factors discussed in the following sections.

## Extra-Column Volume Effects

Extra-column volume is defined as the HPLC system volume measured from the injector through the detector, exclusive of the column (Figure 1). The efficiency of separations achieved with low-volume columns is highly dependent on operating an HPLC unit with minimal extra-column volume. This effect is due to the small peak volumes exhibited by these columns (Table 1). Large extra-column volumes can significantly degrade the inherent performance of a low-volume column. Some HPLC systems that perform well with standard bore, 4.6 mm ID columns, can be poorly matched to the needs of narrow-bore columns or 4.6 mm ID columns shorter than 150 mm. For example, an HPLC system with 50 µL of extra-column volume (typical for some older standard systems) reduced the column efficiency of a 2.1 mm ID narrow-bore column by 45%, in contrast to an "optimized" HPLC system with only 8 µL of extra-column volume (Figure 2). Loss of column efficiency, and therefore, resolution, will be even more severe for earlier eluting peaks.

Clearly, it is very important to use appropriate equipment and to take special care in using any low-volume column to realize the full potential for high resolution afforded by these products.

## Special Considerations

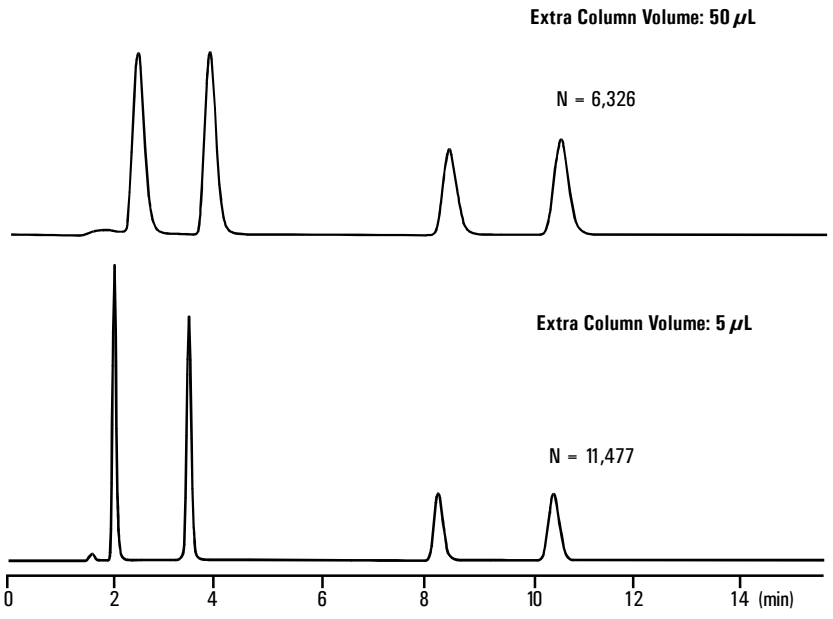
- Peak Retention – The volume of a peak increases as its retention is increased. Thus, extra-column volume is less of a factor if peak retention is increased for the first peak of interest.
- Detector – For best results, the detector must be designed for low-volume operation. The flow-cell volume should be minimized (preferably 2µL or less).
- Detector Performance – Peaks eluting from low-volume columns are often of very short duration. Therefore, detectors must be set for fast response times and computer data analysis programs must take at least 10 data points per second to properly sense these fast peaks.
- Injection System – The sample injector should be of a micro-design to minimize internal volumes.
- Connecting Tubing – Tubing of internal diameter of at most 0.010 inch (0.25 mm) is required, and the shortest possible lengths should be used for connection from the injector to the column and the column to the detector. Only zero dead-volume connectors should be used for any required connections. All tube ends must be flat and bottom out in the fittings.
- Sample Solvent

  Isocratic – The sample should be dissolved in the mobile phase or in a solvent that is weaker than the mobile phase.

  Gradient – Sample should be dissolved in the initial A solvent or in a solvent substantially weaker than the B solvent.
- Injection Volume Isocratic – The volume of sample injected must be kept as small as possible. A good guideline is to inject a volume less than 15% of the volume of the initial peak. Typical sample volumes are 5 µL or less, and usually < 2 µL.

  Gradient – Sample-volume requirements are less critical if the initial peak k' (isocratic) is high (e.g. 20). In these cases, it is possible to inject several column volumes of sample, concentrating it on the head of the column. The sample peaks then are eluted at appropriate gradient compositions with low peak volumes.
- Flow Rates – The flow rate for optimal column efficiency for narrow-bore columns is proportionately smaller than that of a standard bore column due to the smaller internal volume. Flow rates recommended for ZORBAX narrow-bore columns are 0.05 to 0.10 mL/min (1.0 mm ID), 0.15 to 0.25 mL/min (2.1 mm ID), and 0.4 to 0.5 mL/min for 3.0 mm ID columns.

**FIGURE 2**
**The Effects of Extra-Column Volume on Narrow-Bore (2.1x150 mm) Column Performance**

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