Reduce Tubing Volume to Optimize Column Performance

Application Note

General Analysis

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Abstract

A comparison of the effects of extra-column volume on 50 and 150 mm columns in 2.1 and 4.6 mm internal diameters was made using a range of capillary tubing volumes installed between the autosampler and column. Red tubing (0.12 mm id), green tubing (0.17 mm id) and blue tubing (0.25 mm id) were used for this study. For a fixed length of tubing, the volume inside green tubing is twice as large as that inside red tubing, and the volume inside blue tubing is slightly more than twice as large as that inside green tubing. Decreases in efficiency were measured between 1 and 31%, depending upon the volume of tubing installed and the dimension of the column installed. The larger decreases in efficiency are found when 2.1-mm id columns are used, while the 4.6 mm columns appear nearly immune to the extra column volume effects. In this work, the 1.8 µm and 5 µm columns show the same percent decrease in efficiency as that caused by the extra column volume.

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Introduction

In order to increase productivity and reduce solvent costs, analysts are using shorter columns packed with smaller particles, which can maintain the required separation efficiency while yielding significant solvent and time savings [1-6]. Other investigators are choosing to use more narrow columns packed with the same particle size material in order to save solvent, or accommodate mass spectrometers and evaporative light scattering detectors [7,8]. However, these method changes can come at the price of robustness. In many cases, failure to make accommodations for narrower peaks generated by the smaller columns can lead to an apparent loss of column efficiency. In some cases, simply optimizing detector acquisition rate can correct the apparent efficiency deficit [9]. However the problem in many cases is caused by the extra column volume in a "non-optimized" HPLC system. By replacing parts such as tubing, injector seats, column heaters or flow cells with parts more suitable to an analysis, it becomes possible to achieve the best performance of a column. This note will show the effect of various lengths and volumes of tubing on column quality control test performance, and will hopefully guide future use of the column.

Experimental

In this work an Agilent 1200 Series High Performance LC SL was used. The performance of this system was already optimized. The objective was to determine how much extra-column volume (as capillary tubing) would be required to compromise system performance and the performance of the column. Detector acquisition was set to the second fastest data collection rate for the narrowest peak. Using the 2.1 mm × 50 mm column and the shortest length of tubing, the acquisition rate was set at 0.01 s, or approximately 40 Hz. A 2-µl flow cell was used for all testing. These are consistent with recommendations for best column performance. The column was directly connected to the autosampler valve using a reusable Polyethlyether Ketone (PEEK) connector, p/n 5046-4426, for pressures over 400 bar, we recommend Polyketone p/n 5042-8957. This ensures the best possible connection to the column by providing a perfect fit to the column with each piece of tubing. The isocratic test mixture used contains uracil, and acetophenone, 1 chloro-nitrobenezene and naphthalene at the concentrations listed on the column QC report. Samples were prepared in 50:50 acetonitrile/water and shown in Figure 4. The efficiency of the column was calculated and compared with various lengths of tubing installed between the autosampler and the column.

Discussion

In the system diagram shown in Figure 1, extra-column volume is in any tubing or connector in the system, other than the column, where the sample peak could broaden and efficiency could be reduced. It includes the volumes of the sample loop or injector, connecting tubing, fittings, and detector cell. All of these should be minimized on columns 100 mm or shorter, and must also be minimized when peaks elute early in the run on a 2.1-mm column. When using small volume columns, detector cell volume of 2 µL or less, reduced injection size (usually less than 5 µL), capillary tubing with 0.12 mm inside diameter and unions with zero dead volume should be used.

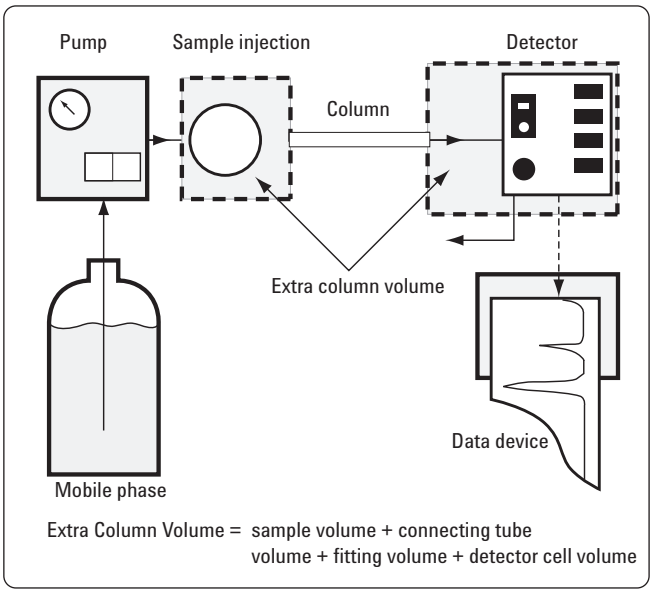

Figure 1. The instrument schematic above depicts where extra-column volume can occur, thus effecting instrument and column performance.

In reference [4], recommendations are shown using similar methodology. A comparison of varied column lengths was made in showing a decrease of between 1 and 31% depending upon whether a 280-mm red (0.12 mm id) or green (0.17 mm id) capillary is used in the testing. The difference between the two capillaries is a doubling of volume. The larger differences are found when 2.1-mm columns are used.

In this work tubing connections are made using tubing as short as 70 mm in length with a 0.12 mm inside diameter and up to 1 m in length. Tubing with different inside diameters is also used: "red" 0.12 mm, and "green" 0.17 mm. The volume of the tubing is calculated using the formula of a cylinder. Tubing volume verses efficiency is plotted for all columns. Table 1 lists the volumes of the four column sizes used in this

study. Column volume is calculated as the volume of a cylinder less the space occupied by the packing material. Since the Agilent ZORBAX Eclipse Plus C18 packing material occupies 40% of the column, the remaining 60% of the cylinder should be considered as column volume. As can be seen, the 2.1 mm id columns have approximately 20% of the volume of an equivalent length 4.6 mm id column. Table 2 lists the part number, length and volume for ten commonly-used flexible capillary connectors. As can be noted the "green" capillaries have twice the volume of a "red" capillary of equivalent length.

Figure 2 compares the efficiency of each of these four columns with various volumes of tubing attached. Efficiency loss on the smallest column begins at approximately 2 µl of additional volume in the system which corresponds to between 150 and 200-mm length of 0.012 mm id tubing. At 7 µL, which amounts to 10 % of the column volume, a significant decrease in efficiency is noted on the 2.1 mm id columns. In addition it should be noted that very little efficiency drop occurs for the 4.6 mm id columns.

Figure 3 compares the loss of efficiency for a 2.1 x 50 mm column packed with either 1.8 or 5 µm packing. Figure 3a shows the efficiency loss with added volume. In both cases efficiency is lost with added tubing, but since the column packed with smaller particles delivered more theoretical plates, even with extra tubing it was more efficient than the larger particle column with the same amount of tubing. Figure 3b shows the efficiency normalized to 100% with the shortest tubing for columns. In the normalized graph it is evident that the percent loss for both 1.8 and 5 µm particle columns is the same. As can be seen, the decrease in efficiency is more a function of column dimension rather than particle size. A small particle sized column compromised with extra tubing will still be more efficient than a similarly sized column with larger particles.

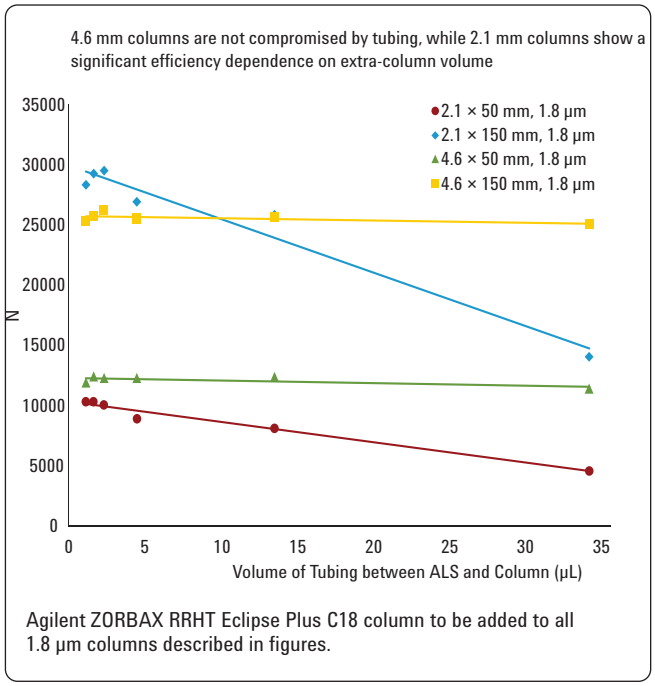

Figure 2. The above scatter plots compare the effects of column length and internal diameter on extra-column volume; results indicate length has only a slight effect, while internal diameter is greatly affected by extra-column volume.

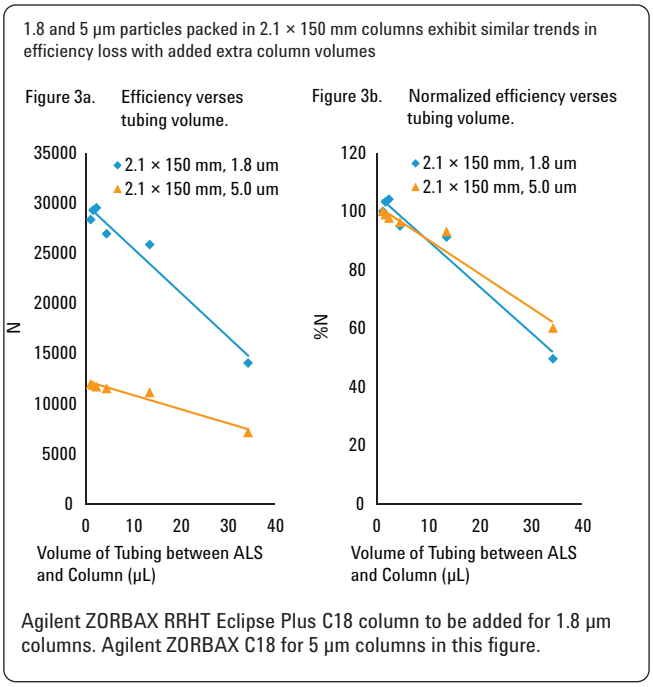

Figure 3. The above scatter plots show the negative effects of extra tubing volume on naphthalene peak efficiency with both 1.8 and 5 µm particles.

While Figures 2-3 show the effects of extra-column volume as a scatter plot, Figure 4 shows this phenomenon chromatographically. Figure 4 shows an Agilent ZORBAX RRHT Eclipse Plus C18 column with minimal tubing and with the maximum tubing used in this study. The effects are broader peaks and reduced efficiency on the peak of interest, naphthalene, when significant extra-column volume is present.

Conclusions

In order to achieve the best results, it is necessary to minimize extra column volume, especially when using small volume columns. Select the narrowest diameter and shortest length tubing your application and system allows to prevent peak dispersion or loss of resolution. The use of smaller id tubing (0.12 mm) is recommended for all small column applications. It is important to minimize connecting tubing as part of instrument optimization.

Table 1. Volume of Various HPLC Columns

$l \times (r)2 \times \pi = l \times (D/2)2 \times \pi$ = Volume

1 cubic mm = 1µL

Length (mm)	Diameter (mm)	Volume (µl)	60% (µl)
50	2.1	173	104
150	2.1	519	312
50	4.6	831	498
150	4.6	2492	1496

Table 2. Description of Common Connecting Tubing with Volume

Color Code	Red 0.012 mm id		Green 0.017 mm id	
Length mm	**Part number**	**Volume (µl)**	**Part number**	**Volume (µl)**
105	5021-1820	1.2	5021-1816	2.4
150	5021-1821	1.7	5021-1817	3.4
200	5065-9935	2.3	5065-9931	4.6
280	5021-1822	3.2	5021-1818	6.4
400	5021-1823	4.5	5021-1819	9.1

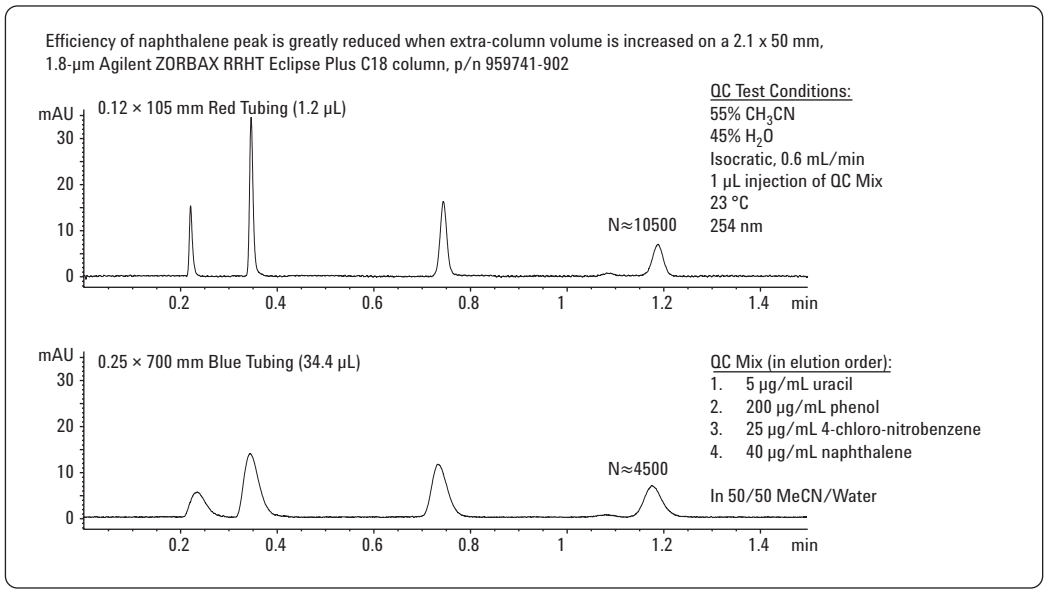

Figure 4. QC test of a 2.1 × 50 mm, 1.8-µm Agilent ZORBAX Eclipse Plus C18 showing the peak broadening when larger volume tubing is installed between the autosampler and column.

References

1. John W. Henderson Jr. “Plug and Play”, Fast and Ultra-Fast Separations Using 3.5 µm Rapid Resolution and 1.8 µm Rapid Resolution High Throughput Columns. 5989-2908 EN May 2005.
2. John W. Henderson Jr, William J. Long, and Cliff Woodward, “Scalability of Short RRHT Columns to longer 3.5 µm and 5-µm Columns”, 5989-6169EN, February 2007.
3. LC Columns for Reducing Solvent Use and Waste, Technical Overview 5995-3772EN, April 2009.
4. Alan D. Broske, Robert D. Ricker, Bernard J. Permar, Wu Chen, and Maureen Joseph, ”The Influence of Sub-Two Micron Particles on HPLC Perfomance”, 5989-9251EN, May 2003.
5. John W. Henderson Jr, and William J. Long, “Optimize Data Sampling Rate to Take Advantage of RRHT Columns”, 5989-5810EN , November 2006.
6. John W. Henderson Jr, William J. Long, “Exploiting RRHT Columns with Different C18 Selectivities to Quickly Develop Methods for Endocanabanoids”, 5989-6128EN, January 2007.
7. John W. Henderson Jr and Judy Berry, “Stevia Sweetener Analyses Using an Agilent ZORBAX Eclipse Plus Phenyl-Hexyl Column”. LC GC Magazine, The Application Notebook, Jun 1, 2009.
8. Rong-Jie Fu, “Analysis of Saponins in Spine Date Seed using Agilent ZORBAX Solvent Saver HT (1.8 µm) Columns”, 5990-4176EN, June 16, 2009.

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