Liquid Chromatography Problem Solving and Troubleshooting

Question:

I know that system dead volume can broaden peaks and contribute to the destruction of column efficiency. But can a "modern" HPLC detector cause a difference in a column's true performance? I was told that all modern detectors have been optimized and, therefore, should not contribute negatively to the column's performance.

Answer:

The instrument can contribute significantly to the column's performance through dead volume; and, to answer your question, the detector as part of the system can reduce the column's innate performance. The concern of the detector's contribution to the plate count of a column was actively focused upon during the early years of HPLC instrumentation. Currently, however, this concern is not generally mentioned, probably because the columns that are available are significantly superior in their efficiencies such that a small loss of plates from the manufacturer's plate count specifications still results in a very large number of plates. With the recent focus on smaller column lengths and smaller particle sizes, the issue of the instrument's contribution to performance is resurfacing. However, the performance of all columns, not just small volume ones, can be impacted. Clearly, minimization of the instrument's band spreading is one of many important concerns that should be understood when setting up and/or optimizing a particular HPLC.

The role that the detector has in contributing to band broadening can be illustrated by data recently shared with me by a reader. Table I shows the theoretical plates calculated for the same column placed into five different HPLC systems. The reproducibility on each instrument was within a range of ±250 plates, but it was clear that there was a big difference between one unit and the others.

Table I. Effect of Detection on Column Performance

HPLC	Theor. plates	Asymmetry
System A	11,795	1.12
System B	10,000	1.19
System C	10,900	1.16
System D	10,780	1.17
System E	14,132	1.11

In another experiment, the role of the time constant of the detector was evaluated. The data are shown in Table II. System B was used at the time constant set by the factory and then varied to the "faster" setting as denoted by the arbitrary numbers going from a slow setting, 4, to a faster setting, 1. Visually, differences in the peak shapes could not be easily distinguished; yet, from the calculated plate count, it can be seen that using a faster time constant increased the plate number to 40% more than the original test indicated using the standard cell. This effect is not a result of the dead volume. The lower plate count is due to electronic smoothing of the detector's response and, in essence, is an electronic broadening of the peak.

Table II. Effect of Time Constant on Column Performance Using Detector B

Cell type	Time constant	Theor. plates	Asymmetry (10%)
Standard	4	9865	1.19
Standard	3	12,695	1.10
Standard	2	14,114	1.08
Standard	1	14,355	1.08
Small	1	16,478	1.05

Also, in this table, the result obtained with a standard detector cell was compared with that obtained with a small cell (both using the fastest time constant). Interestingly, the use of the small cell resulted in the plate number increasing 15% (from 14,355 to 16,478 plates) over the standard cell. This may be due to reduced dead volume in the small cell.

To further verify the role of the detector in column performance, several configurations were investigated using both a standard and a small cell. The data are summarized in Table III. The same pump, injector, and column were used; different detectors were inserted into the system. In this case, the time constant was arbitrarily set at a response factor of 3. In this experiment, there was a small

Table III. Effect of Detector Flow Cell on Column Performance

System	Cell type	Time constant	Theor. plates	Asymmetry (10%)
Detector B	Standard	3	12,695	1.10
Detector C	Standard	3	11,696	1.10
	Small	3	14,067	1.06
Detector D	Standard	3	11,775	1.08
	Small	3	14,455	1.06
Detector E	Standard	3	12,833	1.09

difference in the column's performance between detectors. This very small difference is probably within the reproducibility of a detector manufacturer. More importantly, it can be seen that the smaller cell increased the column's performance slightly from 12,000 to 14,000 plates.

These data are not part of an optimization study nor should this discussion be interpreted to imply that modern detectors are not adequate. What the data does show is that the HPLC and, specifically, the detector can make a big impact on the measured performance of a column. Therefore, individuals who wish to obtain the true or innate column performance from a system should concern themselves with doing some experimental testing of the variables that are within their control.

The purpose of *Chromatography Problem Solving and Troubleshooting* is to have selected experts answer chromatographic questions in any of the various separation fields (GC, GC–MS, HPLC, TLC, SFC, HPTLC, open column, etc.). If you have questions or problems that you would like answered, please forward these to the *Journal* editorial office with all pertinent details: instrument operating conditions, temperatures, pressures, columns, support materials, liquid phases, carrier gas, mobile phases, detectors, example chromatograms, etc. In addition, if you would like to share your expertise or experience in the form of a particular question accompanied by the answer, please forward to JCS Associate Editor, *Chromatography Problem Solving and Troubleshooting,* P.O. Box 48312, Niles, IL 60714. All questions/answers are reviewed to ensure completeness. The *Journal* reserves the right not to publish submitted questions/answers.

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