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Nota applicativa

Measuring the Performance of Sub-2 µM Particle Columns: Impact Of Extra Column Band Spreading and LC Operating Pressure

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Introduction

Since their commercial introduction in the mid-1990s, sub-2 µm particle columns have grown in popularity due to their potential for more chromatographic separating power and higher throughput. However, simply using these small particle columns in conjunction with conventional chromatographic systems does not produce the efficiencies and resolving power predicted by theory. Operating sub-2 µm particle columns at their optimal linear velocities generates pressures that are not achievable with conventional HPLC instrumentation. Therefore, sub-optimal flow rates are used, which deteriorates separations due to the influence of analyte diffusion. Furthermore, sub-2 µm particle columns are more susceptible to extra-column effects and band spreading.

Recently published reports, presentations, and marketing literature have claimed that HPLC columns containing particles in the 2–3 µm range can be used with existing HPLC instrumentation to achieve the speed and efficiencies seen with UPLC® technology.[1–3] These claims are made based on data comparing their performance to that of 1.7 µm particle columns on conventional HPLC systems. The disadvantages to this approach are that these systems have significant dwell volumes and band spreading, and they cannot reach the pressures required to operate 1.7 µm particle columns at their optimum linear velocity.

In this work, the effects of extra-column band spreading and LC system operating pressure were investigated for sub-2 µm particle columns. The system contribution to band spreading and pressure limitations of conventional HPLC instrumentation were found to be the main reasons for the sub-optimal performance of 1.7 µm particle columns. Conventional instrumentation was found to be incapable of operating at the pressures required to use 1.7 µm particle columns at their optimal flow rates, resulting in a 55% reduction in the measured column efficiency (plate count).

Experimental

LC Conditions

UPLC system:	ACQUITY UPLC® System with an ACQUITY UPLC PDA detector
HPLC system:	Alliance® 2695 Separations Module with a 2998 PDA detector
Columns:	ACQUITY UPLC BEH C_{18}, 2.1 x 50 mm, 1.7 µm XBridge™ C_{18}, 2.1 x 50 mm, 2.5 µm. p/n: 186003085
Column temperature:	30 ˚C
Flow rate:	0.05–1.0 mL/min
Mobile phase:	65/35 ACN/H_2O (isocratic)
Detection:	254 nm
Sampling rate:	40 Hz
Time constant:	no filter

Sample:	0.01 mg/mL thiourea and 0.2 mg/mL acenaphthene in 65/35 ACN/H_2O
Injection volume:	1 µL (PLNO mode; 2 µL sample loop)

The system volume and extra-column band spreading values were measured for each LC instrument used in this study. The measured system volume of the ACQUITY UPLC System is 105 µL, and the measured system volume of the Alliance 2695 is 876 µL. Extra-column band spreading measured at 5σ is 14 µL for the ACQUITY UPLC System and 36 µL for the Alliance 2695 system.

Results and Discussion

All HPLC columns have a theoretical limit for performance. In other words, there is a maximum efficiency/resolving power that each column can achieve. This limit is typically determined by the column dimensions (diameter, length), particle size, and packing process. However, experimental determination of a column's resolving power by HPLC can impact the efficiency measurement as well. Extra-column band spreading (also called band broadening or dispersion) is the unwanted widening of a chromatographic peak due to the HPLC system, which results in observable sub-optimal performance of an HPLC column. There are two sources of extra column band spreading. The first one is volumetric in nature and derives from the injection volume, the detector volume, and the volume of the tubing connecting the injector to the detector. The second contribution stems from time-related events such as the sampling rate or the detector time constant. This technical note will focus on the volumetric sources of extra-column band spreading.

The impact of fixed extra-column band broadening on the observed column efficiency becomes more important for columns with a smaller diameter. For a column with a diameter of 4.6 mm, the negative effect of a particular band broadening value may be small. However, for a column with a diameter of 2.1 mm (~5-fold reduction in column volume from 4.6 mm *i.d.*), this same band broadening value will have a much larger effect.[4] Figure 1 shows the impact of column diameter on the observed efficiency. As column diameter decreases, the observed column efficiency decreases as well.

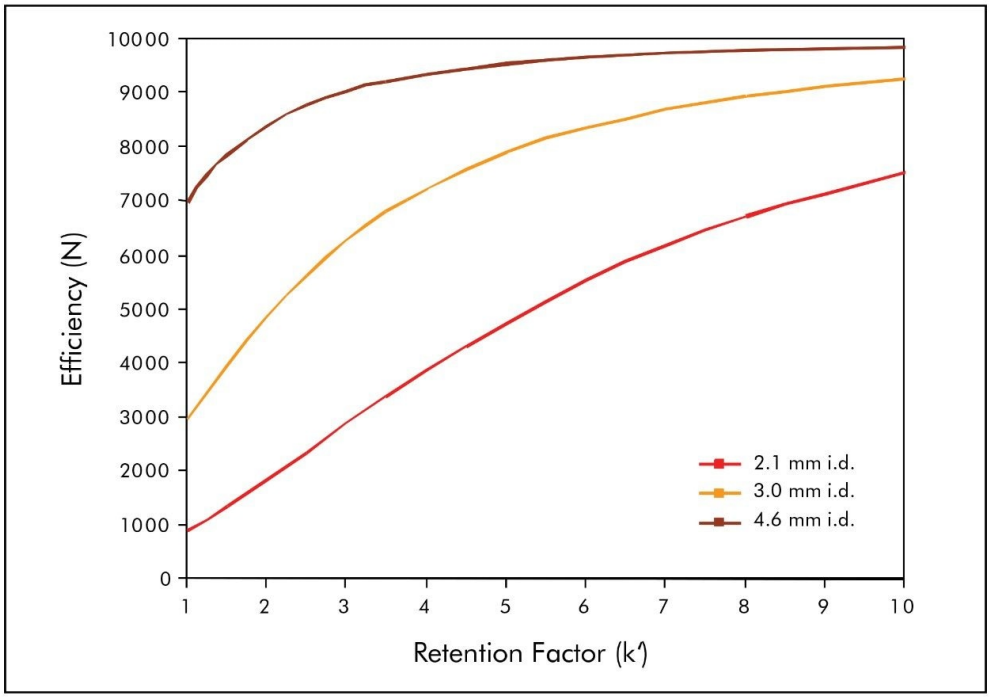


Figure 1. Calculated effect of column diameter on observed efficiency using a conventional HPLC system. Calculations were made assuming a plate count of 10,000 on 50 mm length columns.

For an analyte with a retention factor of 5, the observed efficiency on a 2.1 mm i.d. column is half that of a 4.6 mm i.d. column. This effect is even more pronounced for analytes that are less retained. It is clear from Figure 1 that as the column diameter decreases, the influence of extra-column band spreading becomes larger, negatively impacting the observed column efficiency.

However, column diameter is not the only parameter affecting column efficiency. Smaller particle columns are also more susceptible to band spreading than columns containing larger particles. There are two reasons for this. The first one is that the true performance of columns packed with smaller particles is higher than that of columns packed with larger particles. Thus, the peaks are narrower, and a given extra-column band broadening value will have a larger impact on the narrower peaks. Therefore, the performance is deteriorated more for columns packed with smaller particles.

Second, shorter columns packed with smaller particles can be used to achieve the same column efficiency as

longer columns packed with larger particles. A shorter column has a smaller column volume, and at equal efficiency, the impact of extra-column band spreading increases for the shorter column.

Furthermore, the optimum linear velocity increases with decreasing particle size. Sub-2 µm particle columns generally cannot be operated at their optimum linear velocity on conventional HPLC instruments. As a compromise, sub-optimal flow rates are used, resulting in column efficiencies that are much lower than expected. The solution to achieving maximum efficiency on smaller particle (sub-2 µm) columns is to use a properly designed LC system that has minimal extra-column band spreading and the ability to operate at the pressures necessary for these columns (ACQUITY UPLC System).

Figure 2 shows the difference in calculated efficiency for 1.7, 2.5, and 5 µm particle columns run on a conventional HPLC system and the ACQUITY UPLC System.

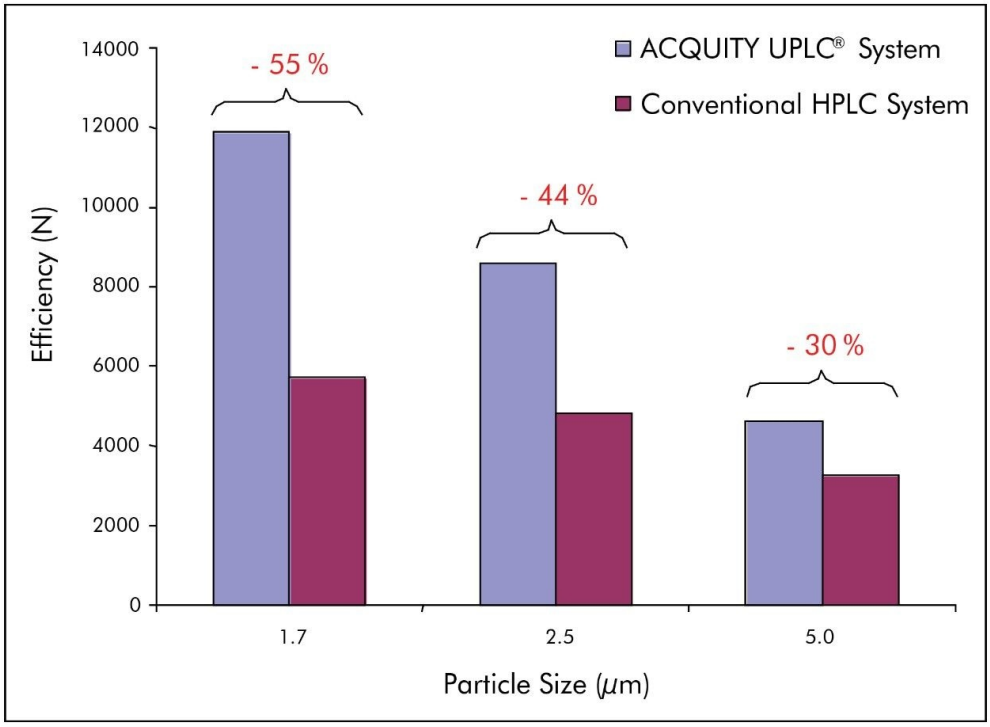


Figure 2. Calculated maximum achievable efficiency of 1.7, 2.5, and 5 µm particle columns (2.1 x 50 mm) on a conventional HPLC System and an ACQUITY UPLC System. Plate counts shown are for an analyte with a k' of 5. The number shown in red is the percentage of efficiency lost when using the same column on a conventional HPLC system.

As particle size decreases, the difference between the efficiency measured on both LC systems increases. The result is more than a 50% decrease in efficiency for a 1.7 µm particle column used on a conventional HPLC system when compared to the ACQUITY UPLC System. It is also interesting to note that the 2.5 and 5 µm particle columns also benefit (although to a lesser extent) from being run on the ACQUITY UPLC System, indicating that this system can be used not only for UPLC applications, but can improve existing HPLC applications as well. A practical example of the theoretical concepts depicted in Figures 1 and 2 is shown by comparing the experimentally measured van Deemter curves for acenaphthene on 1.7 and 2.5 µm particle columns using UPLC and conventional HPLC systems (Figure. 3).

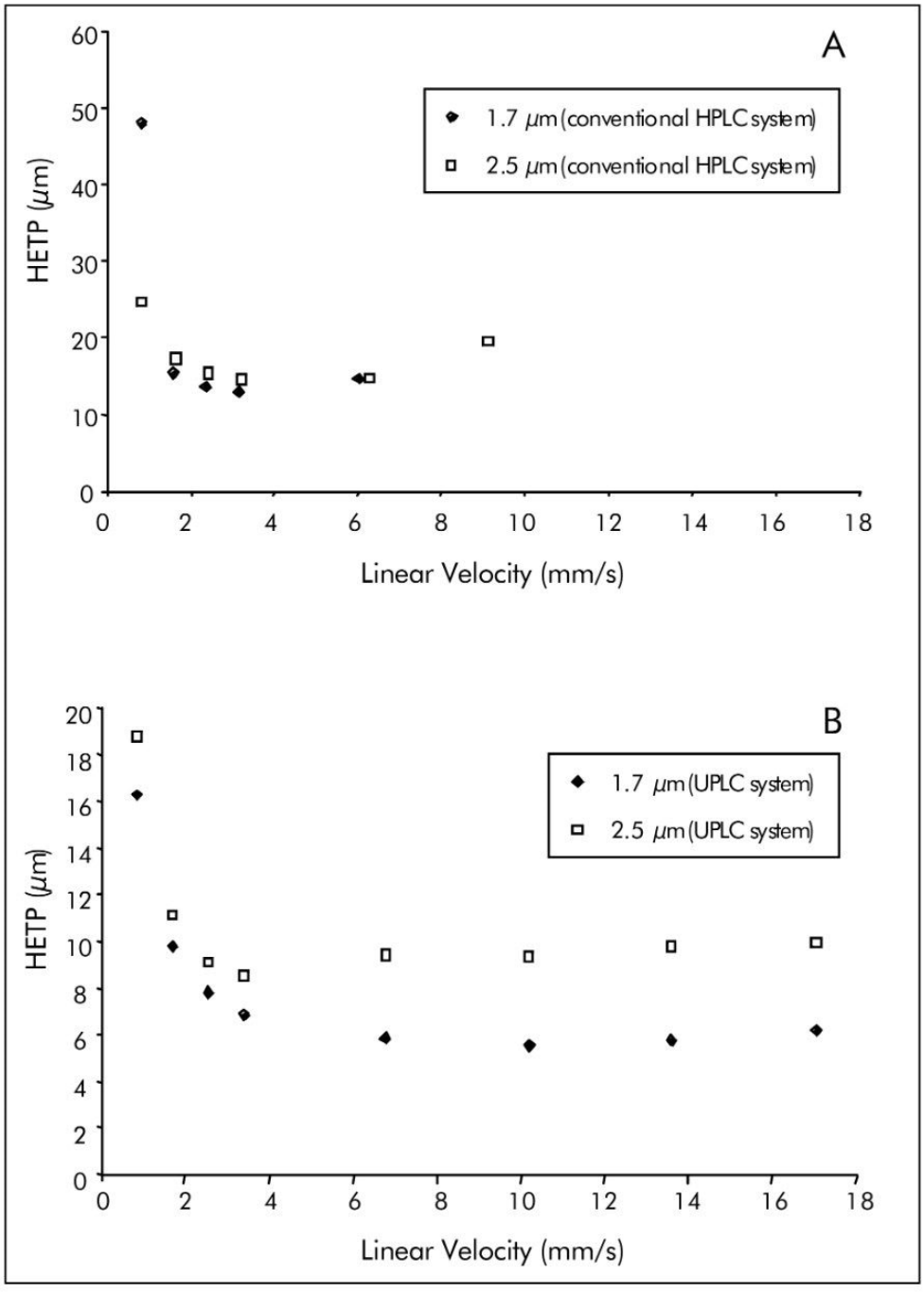


Figure 3. Van Deemter curves for 1.7 µm and 2.5 µm particle columns on (A) a conventional HPLC system, and (B) an ACQUITY UPLC System. Acenaphthene was used as the test probe (k' ~3.6). Efficiency was calculated using peak width measured

at 4σ.

There is no observable benefit of using the 1.7 µm particle column on a conventional HPLC system (Fig. 3A). It is only when both columns are analyzed using an ACQUITY UPLC System that there is a clear difference in performance. The maximum efficiency that was achieved for each column on the different LC systems is shown in Figure 4. The 2.5 µm particle column showed a 42% loss in plate count when used on the conventional HPLC system, and the 1.7 µm particle column showed a 57% loss in efficiency. This correlates well with the calculated trends shown in Figure 2.

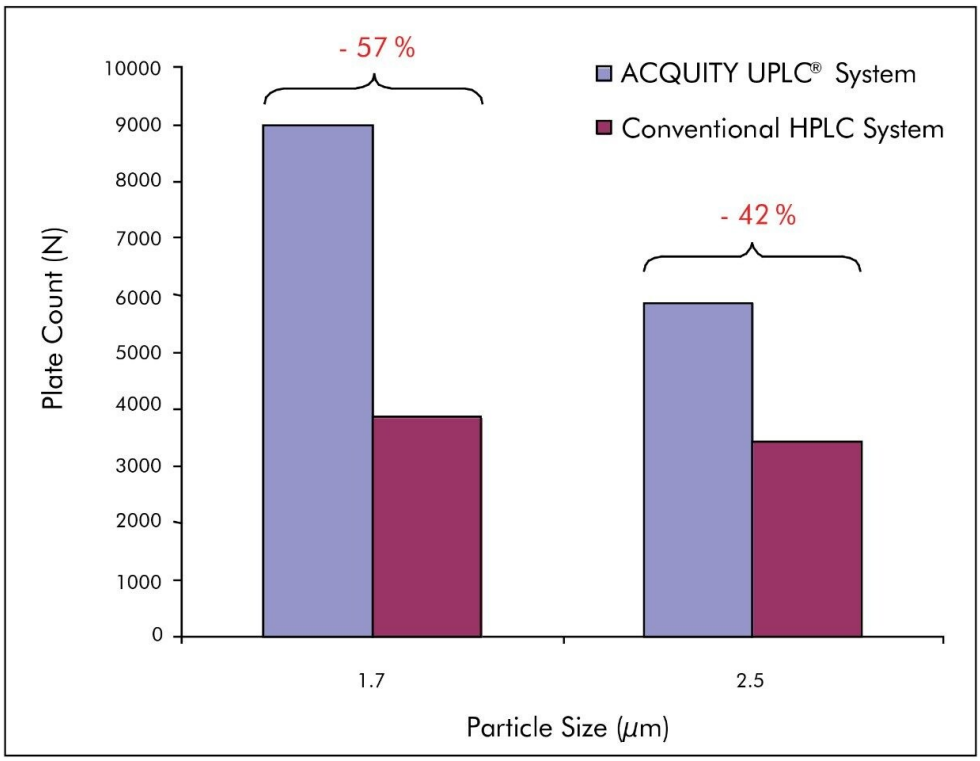


Figure 4. Comparison of the maximum measured column efficiency as a function of particle size and LC system. Acenaphthene was the test probe. Plate counts were calculated using the peak width at 4σ.

There are two reasons for this dramatic improvement in efficiency on the 1.7 µm particle column. First, the system contribution to band spreading is about 2.5 fold larger on the conventional HPLC system than on the ACQUITY UPLC System. This causes peaks to be much broader and can cause severe tailing (Figure. 5).

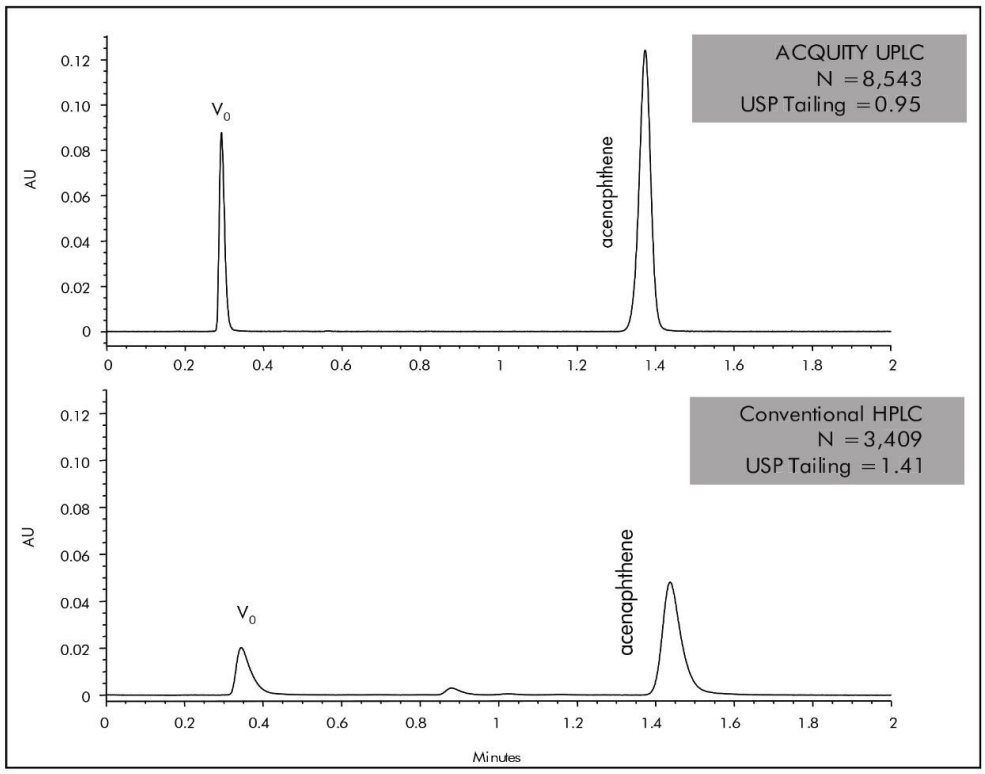


Figure 5. Isocratic separation of acenaphthene on an ACQUITY UPLC System and a conventional HPLC System. The column is an ACQUITY UPLC BEH C_{18}, 2.1 x 50 mm, 1.7 µm. Flow rate is 0.4 mL/min.

Second, the optimum linear velocity for sub-2 µm particle columns usually generates pressures that are above the limit of conventional instrumentation. Therefore, one must operate these columns at sub-optimal flow rates in order to remain within the pressure constraints of conventional HPLC systems. This can be seen in Figure 3, where the optimum linear velocity for the 1.7 µm particle column is 3 mm/s (0.2 mL/min) on the HPLC system and 10 mm/s (0.6 mL/min) on the UPLC System. While it is possible for some HPLC systems to achieve these higher flow rates on sub-2 µm particle columns for a limited set of conditions, the performance of these systems still suffer from large extra-column band broadening. Since the ACQUITY UPLC System can routinely operate at

pressures up to 15,000 psi (~1,000 bar) and has low system band spreading, sub-2 µm particle columns can be operated at their optimum linear velocity to yield highly efficient separations in a much shorter time.

Conclusion

- The system contribution to band spreading and pressure limitations of conventional HPLC were found to be the main reasons for the misconception that 2–3 µm particle columns perform equally well or better than sub-2 µm particle columns
- The performance of sub-2 µm particle columns cannot be accurately determined using conventional HPLC instrumentation due to pressure restrictions and extra-column effects. This is especially true for columns with diameters less than 4.6 mm
- The efficiency of a 1.7 µm, 2.1 x 50 mm column was 130% greater when used on the ACQUITY UPLC System rather than a conventional HPLC system
- Reducing the column packing particle size is limited by the pressure capability of the instrument, and no benefit is observed when using sub-2 µm particle columns on a conventional HPLC instrument
- This study provides clear evidence that sub-2 µm columns must be used with a high pressure instrument that has minimal extra-column band spreading in order to achieve the efficiency and resolution that theory predicts for these particles

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