INCREASING CHROMATOGRAPHIC PRODUCTIVITY AND REDUCING SOLVENT CONSUMPTION WITH ACQUITY UPLC

In today's economic climate, the need to produce more results with fewer resources, faster is a theme that is heard regularly in the world of analytical chemistry. Liquid chromatography has become the mainstay of many quantitative and qualitative analyses in areas such as pharmaceutical drug analysis, environmental monitoring, food testing, and water quality monitoring. The throughput, selectivity, and specificity achieved with the ACQUITY UPLC® System provides improved chromatographic results while consuming less solvent than traditional HPLC – for more efficient laboratory operations.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

Analytical HPLC often uses columns with dimensions of 4.6 mm x 150 mm or 250 mm, packed with particles of 3.0- to 5.0-µm material, operating with flow rates in the range of 1.0 to 1.4 mL/min. This results in HPLC analysis times in the region of 15 to 60 min/sample. With typical batches taking 10 to 20 hours to complete, analytical results are not available for decision making on batch release, next clinical dose, plant cleanliness, etc., until the next day. At a flow rate of 1.4 mL/min, these HPLC systems consume about 1.5 L of solvent per 20-hour day, of which typically 50% is organic solvent. This solvent has to be purchased and disposed of, all of which adds cost to the overall analysis. Over an average 250-day working year this can add up to nearly 200 L of organic solvent; at nearly $100 dollars per liter for acetonitrile, this is a $20,000 dollar consumable cost. Given the recent increase in acetonitrile costs, as well as shortages reported in certain areas of the world, there is a great need for more efficient use of this toxic solvent. An example of HPLC analysis used in the pharmaceutical industry, a representative chromatogram for the impurity analysis of quetiapine, is shown in Figure 1; here we can see that the gradient analysis time is 51 minutes with 16 minutes required for column re-equilibration.

The advent of sub-2 µm porous column particles and instrumentation designed to take advantage of the benefits of these particles has significantly improved the productivity of chromatographic analysis. The increased chromatographic efficiency of the sub-2 µm particles allows for a significant reduction in analysis time. As the chromatographic resolving power of a column is proportional to the column length and inversely proportional to the column particle diameter, the increased performance of the sub-2 µm material allows the column length to be reduced for the same performance. The column performance can be evaluated by comparing the l/dp ratio (column length/particle size); for a 150-mm column using 5.0-µm particle size, the l/dp ratio is 150/5, which gives a value of 30. When moving to a 1.7-µm chromatographic particle from a 5.0-µm particle, a column length of 50 mm can be employed without reducing the assay resolution. As the column length is reduced by a factor of three for the assay, time will also be reduced by a factor of three, improving throughput. When reducing the particle size, the optimal mobile phase linear velocity increases inversely with the particle size. Therefore when moving to a 1.7-µm material from a 5.0-µm material, the mobile phase velocity increases three-fold; this combined with the three-fold reduction in column length can give up to a nine-fold reduction in analysis time.

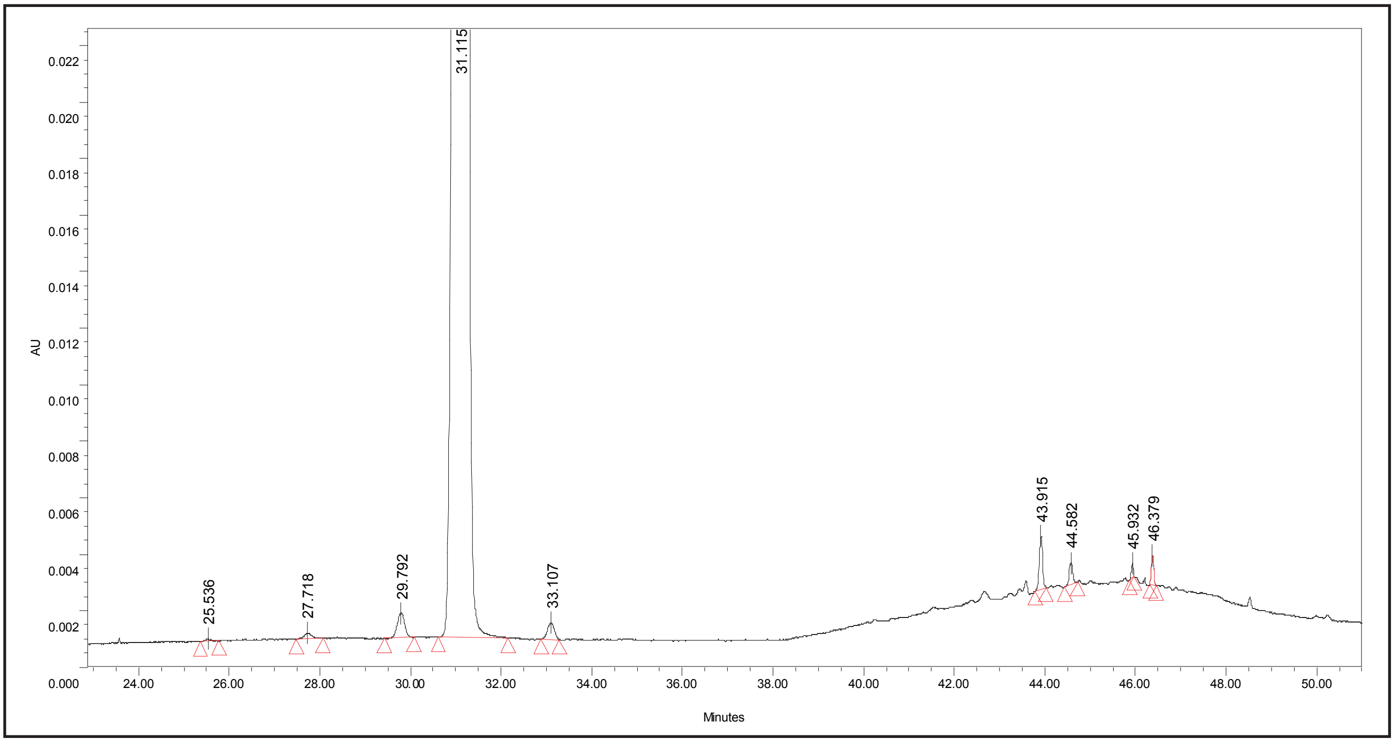

Figure 1. Reversed-phase HPLC separation of quetiapine.

The data shown in Figures 2A and 2B illustrate the reduction in analysis time achieved for the analysis of didanosine and its impurities when moving from a 5.0-µm separation to a 1.7-µm separation. In this example, we can see that assay performance and resolution for the 1.7-µm material is very comparable to that obtained from the 5.0-µm material and the resolution factors are very similar.

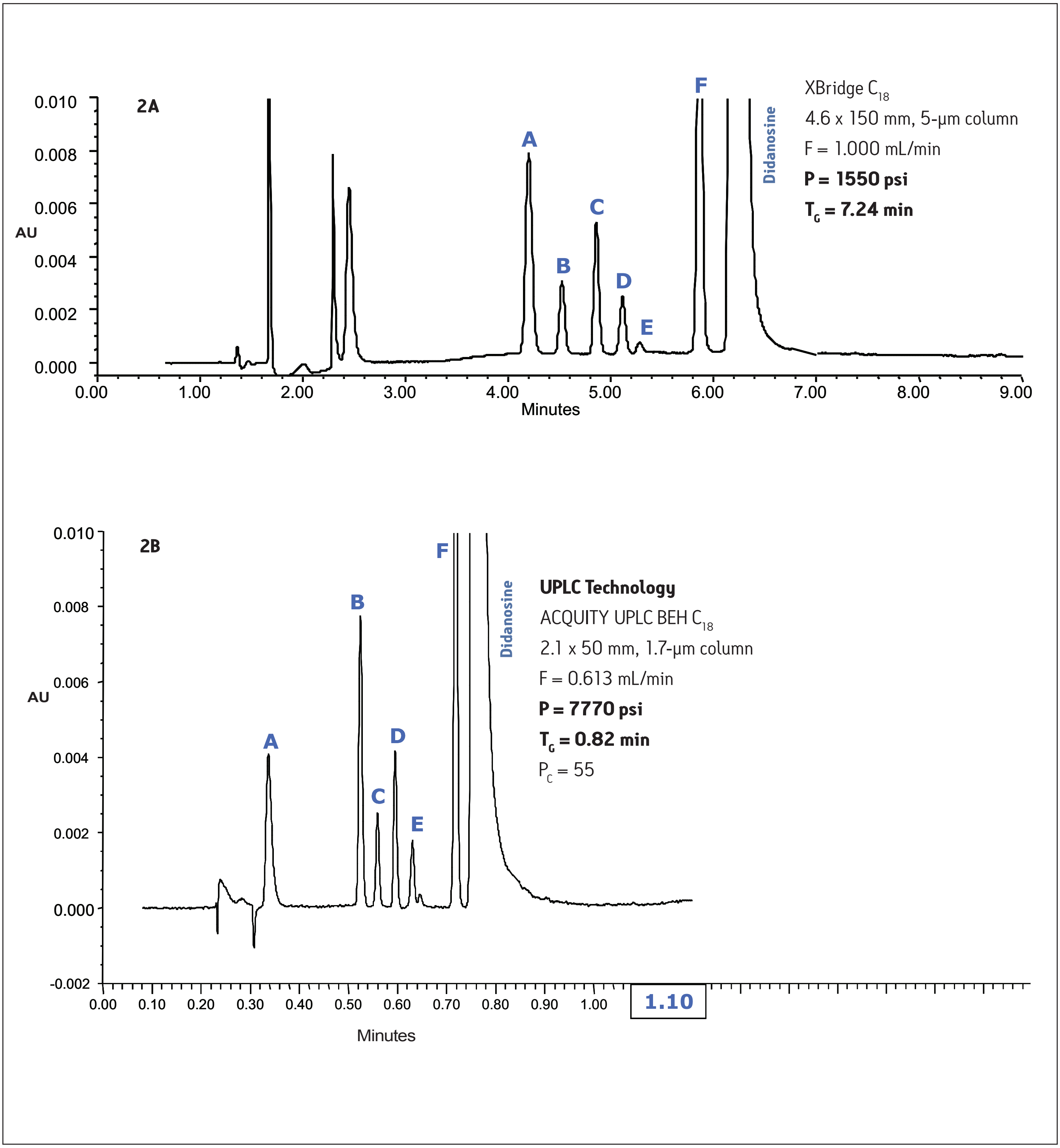

Figure 2. HPLC and UPLC separations of didanosine and its impurities (labeled A-F). 2A. HPLC separation with 5.0-µm particle size. 2B. UPLC separation with 1.7-µm particle size.

In practice, when using UPLC®, many scientists tend to trade off the increased throughput for increased resolution and superior chromatographic peak separation, and hence greater method robustness. This increased chromatographic performance obtained from the sub-2 µm particle material combined with the shorter column lengths required by UPLC produces sharper peaks and also more concentrated peaks, thus simplifying assay development, in bioanalysis, for example. The increased sensitivity and faster analysis time has been exploited by the mass spectrometry community to improve both UV and LC/MS assay sensitivity.

In order to realize the chromatographic performance of these sub-2 µm particles, it is necessary to carefully control both pre- and post-column band broadening; this is particularly important when running in isocratic mode. The ACQUITY UltraPerformance LC® System has been specifically designed to exploit the chromatographic performance available from sub-2 µm particle LC, with a pre-column volume of approximately 80 µL and extremely low dispersion characteristics. Without this careful control of dispersion, peak shape would be severely compromised and result in wider, tailing peaks. The transfer of the impurity method for quetiapine to a sub-2 µm particle (2.1 mm x 100 mm, 1.7 µm) C_{18} UPLC methodology is shown in Figure 3A. Here, we can see that the analysis time has been reduced to just 20 minutes, while the resolution of the peaks has not been reduced. The small impurities around the major peak are well defined and resolution from the major API peak is comparable to that obtained from the HPLC separation. When moving from HPLC to a 2.1-mm UPLC Column, the solvent saving was determined to be 82% due to both the faster analysis time and lower volumetric flow rate for the 2.1-mm column. Assuming that the HPLC system is operating for 20 hours per day and 5 days a week with 52 operational weeks a year, the instrument will make 4477 injections in this time and consume 419 liters of solvent. This can be compared with the UPLC System that, for the same number of injections, would consume just 71 L of mobile phase. As the mobile phase is approximately 50% acetonitrile, over the course of the analysis, this represents a saving of 174 L of acetonitrile at a cost of $100 per liter, for a fiscal **savings of $17,000 per year**.

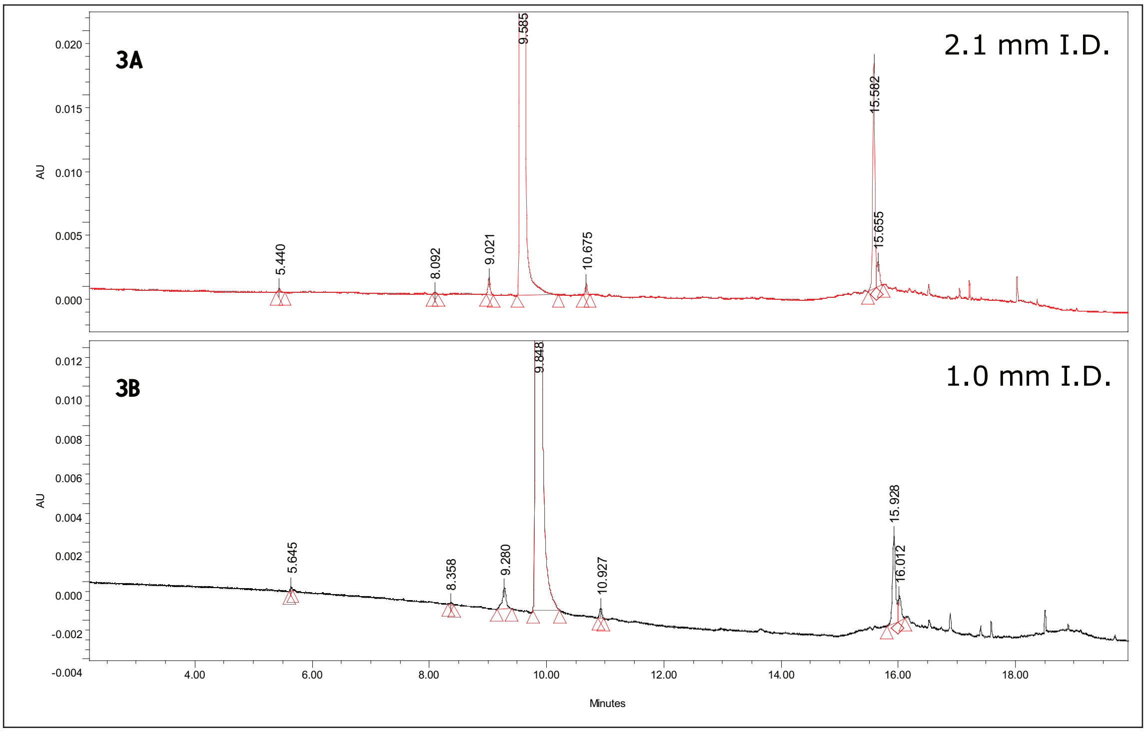

Figure 3. UPLC reversed-phase separation of quetiapine and its impurities. 3A. 2.1-mm column I.D. 3B. 1.0-mm column I.D.

The unique design of the ACQUITY UPLC System, which minimizes both the delay volume of the system and dispersion of the system, allows for the use of **1.0-mm columns** packed with sub-2 µm material. The data displayed in Figure 3B show the same analysis of quetiapine impurities operated on an ACQUITY UPLC BEH C_{18} 1.0 mm x 100 mm, 1.7-µm column. The sample was eluted under the same gradient conditions with the flow rate scaled to 0.181 mL/min; this represents a 77% reduction in solvent consumption compared to that of the 2.1-mm scale HPLC separation. Therefore to perform the same 4477 injections per year, this 1.0-mm scale separation would consume just 16.30 L of solvent and hence just 8.15 L of acetonitrile, which represents a **97% reduction** in solvent consumption. When compared to HPLC analysis, this 1.0-mm scale UPLC analysis would cost just $800 for acetonitrile solvent, which represents a **$20,000 saving over HPLC** for the same number of analyses. The data displayed in Figure 4 illustrates the solvent and cost savings of UPLC in 1.0-mm and 2.1-mm column scale compared to aid visualization; the solvent cost and savings are given in hundreds of dollars. From this data, we see that UPLC is not only more productive but more economical, as this method consumes hundreds of liters less solvent per year.

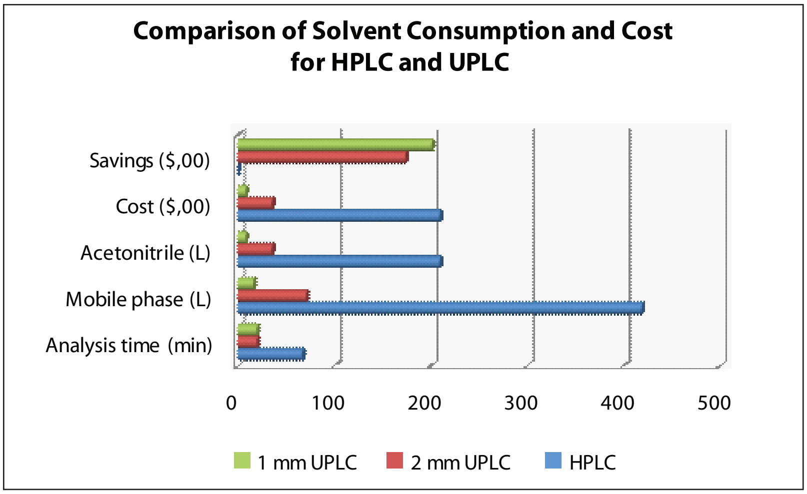

Figure 4. Solvent and cost savings of UPLC in 1.0-mm and 2.1-mm column scales vs. HPLC.

CONCLUSIONS

The advent of commercially available sub-2 µm particle LC columns and instrumentation that can exploit the chromatographic potential of these columns has allowed the chromatographer and LC/MS scientist to improve the sensitivity, quality, and speed of their analyses. The unique design of the ACQUITY UPLC System allows the scientist to obtain these results in a more productive manner than ever before. Not only is the system more productive, it also consumes significantly less solvent than HPLC or "fast LC" (operated at elevated flow rates with 4.6-mm columns) or monolithic stationary phases. The examples shown in this white paper illustrate that by moving to the UPLC method at the 2.1-mm scale, a solvent savings of 82% can be achieved; by moving to the UPLC method at the 1.0-mm scale, a **solvent savings of 97%** can be achieved, adding up to tens of thousands of dollars per year in savings. This cost savings makes good business sense and the use of less solvent and generation of less waste is beneficial for the environment.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

February 2009 720002964EN AG-PDF

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com