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Transitioning Existing LC Methods to New Technologies

Choosing a Liquid Chromatography Platform

INTRODUCTION

Analytical laboratories face the continual challenge of balancing investment in new technology—to improve throughput and performance—with the need to run existing methods and tests to support current production. High Performance Liquid Chromatography (HPLC) is one of these key analytical technologies that has become a major tool for food safety testing, life science studies, and pharmaceutical, environmental and chemical analysis.

- In the pharmaceutical industry, HPLC is employed throughout the whole drug analysis process, including drug discovery screening, raw material analysis, impurity profiling, stability studies, pharmacokinetic studies and final product testing.
- HPLC has found great application in the environmental and chemical analysis arena, where it has been employed for testing water quality, soil samples and product quality.
- Food safety analysis has also benefited from the capabilities of HPLC either alone or in combination with mass spectrometry (MS), allowing food to be rapidly assessed for contaminants such as pesticides or veterinary drugs.

The popularity of liquid chromatography is due to its speed of analysis, compatibility with samples to be analyzed, ease of use, simple interfacing with a wide range of detectors (optical, electrochemical, radiochemical, evaporative light scattering, NMR and MS), wide range of stationary phases, high resolving power and easy scale-up to preparative chromatography.

Several instrument manufacturers have introduced liquid chromatography systems that are designed to take advantage of columns packed with smaller particles. Compared to traditional LCs, these instruments are capable of higher pressure operation, have reduced system volumes, use faster autosamplers and employ detectors with much higher data capture rates.

All of these elements are required to leverage the benefits of higher resolution, sensitivity and throughput offered by columns packed with smaller particles. R&D and method development laboratories have extensively adopted these new LC platforms, as they greatly improve the efficiency of product development.

Methods for products advancing through the pharmaceutical pipeline are now being developed with these new LC platforms. Thus, quality control laboratories must also make similar technology investments to enable them to run the methods that will be required to release new products. Furthermore, many QC labs are in the process of replacing traditional instruments purchased five to seven years ago that have fully depreciated.

The convergence of these two trends—new methods being developed on new LC platforms and the need to replace large numbers of legacy instruments—leaves QC lab managers at a critical decision point with respect to investing in a platform for the future.

Such a long-term view makes selecting one of the new LC platforms very attractive. However, given that the chosen platform will predominantly be running legacy methods during its early lifecycle, it is absolutely critical that it can effectively run these methods. At the same time, it must be fully capable of running methods being developed today with small particle columns—as well as methods developed in future years that will utilize next-generation columns.

In this paper, we demonstrate that a new LC platform, using Ultra Performance LC® (UPLC®) Technology, can be used to run these legacy methods. We will then demonstrate the stepwise transfer of a legacy method to a short, small particle column for greatly improved throughput with equivalent resolution. Finally, we will demonstrate transfer of the legacy method to a long, small particle column for improved throughput with increased resolution.

DISCUSSION

The increased chromatographic performance obtained from the Waters® ACQUITY UPLC® System, when used in combination with the 1.7 µm stationary phase, is illustrated in the analysis of Omeprazole. Omeprazole is in a class of drugs called proton pump inhibitors (PPI), which blocks the production of acid by the stomach.

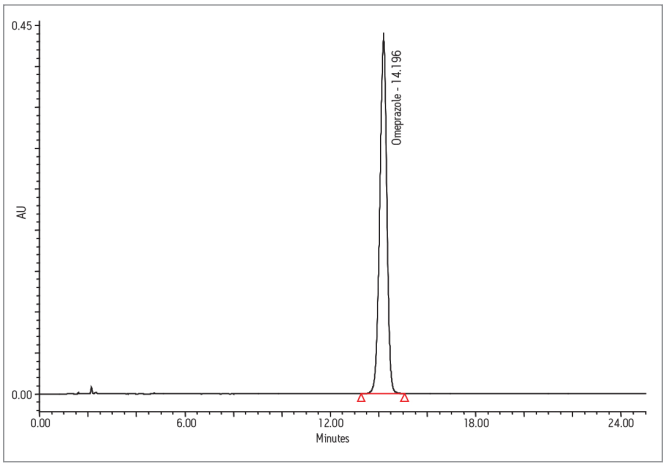


Figure 1.

The first chromatogram, Figure 1, illustrates the chromatography obtained on a 4.6 x 150 mm, 5 µm C_8 column eluted under isocratic conditions with a phosphate buffer: acetonitrile (3:1) mobile phase at a flow rate of 0.8 mL/min.

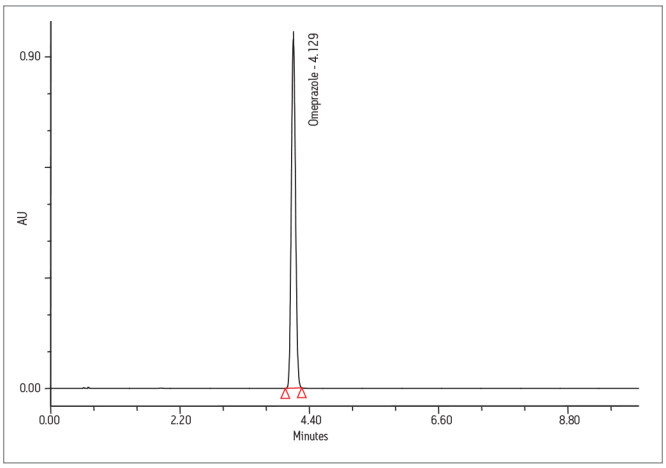


Figure 2.

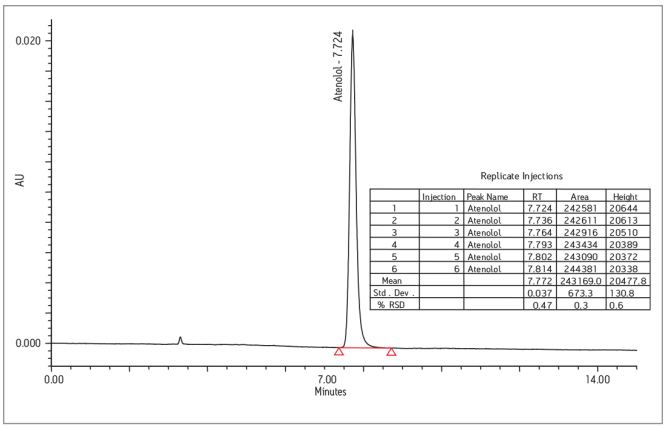
We can see from this data that the major peak elutes at a retention time of 14.2 minutes and peak width of nearly 1 minute at the base. The small peaks at the front of the chromatogram are the observed impurities in the sample. The data displayed in Figure 2 shows the same separation again performed on the ACQUITY UPLC System, but this time employing an ACQUITY UPLC BEH 2.1 x 150 mm, 1.7 µm C_8 column, using the same mobile phase conditions. The injection volume and flow rate were scaled to reflect the change in column internal diameter and particle size, with the flow rate now being 0.5 mL/min.

We can see from the data that the retention factors are similar, but the peak width is much reduced, now being only 13 seconds wide at the base, giving a plate count of 15,300 compared to the value of 8,704 obtained by the HPLC method. The analysis time is also significantly reduced, from 25 minutes to 8 minutes.

Thus this data illustrates that, when scaled correctly and the same column length is used, the UPLC solution produces superior peak shape and sensitivity. One other major benefits of increased performance are the sharper peaks; they not only increase resolution and sensitivity but also simplify the task of peak integration, reducing the need for manual reintegration.

One of the major advantages of using UPLC in the field of product release testing is the ability to shorten analysis time without reducing peak resolution. This is achieved by scaling the separation from the existing LC methodology to UPLC by keeping the ratio of column length to particle size (L/dp) constant. This increases throughput without compromising analytical performance.

Figure 3 shows the separation of the common beta1-selective (cardioselective) adrenoreceptor blocking agent Atenolol, using a 3.9 x 300 mm, 5 µm C_{18} column. The column was eluted at a flow rate of 0.52 mL/min with a mobile phase of 70% phosphate buffer (pH 3.0) and 30% methanol. Detection was performed by UV absorbance at 226 nm.



	Injection	Peak Name	RT	Area	Height
1	1	Atenolol	7.724	242581	20644
2	2	Atenolol	7.736	242611	20613
3	3	Atenolol	7.764	242916	20510
4	4	Atenolol	7.793	243434	20389
5	5	Atenolol	7.802	243090	20372
6	6	Atenolol	7.814	244381	20338
Mean			7.772	243169.0	20477.8
Std . Dev .			0.037	673.3	130.8
% RSD			0.47	0.3	0.6

Figure 3.

The inset table in Figure 3 shows the assay reproducibility of the separation performance. Here we can see that the retention time variation was 0.47% RSD and the peak area deviation calculation returned a value of 0.3%.

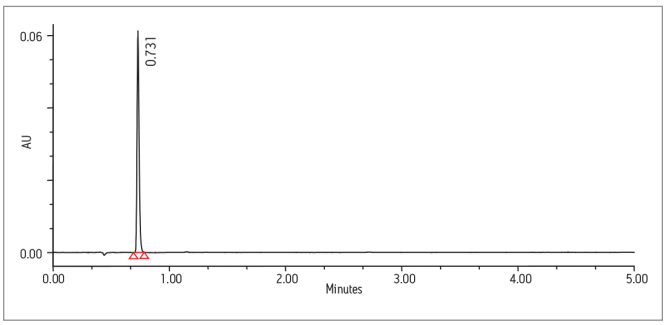


Figure 4.

The assay was repeated on an ACQUITY UPLC BEH 2.1 x 100 mm, 1.7 µm C_{18} column, with the column operated at a flow rate of 0.52 mL/min. In this assay, the particle size was reduced by a factor of three, from 5 µm to 1.7 µm, while the column length was also reduced by a factor of three, from 300 mm to 100 mm, thus keeping the ration of L/dp constant. The result of scaling this separation to ACQUITY UPLC technology can be seen in Figure 4. The analysis time has been significantly reduced—from 7.8 minutes to under 1 minute—while the assay performance remains unchanged in terms of plate count.

This magnitude of improvement in throughput and performance can result in a reduced time for product release and/or a reduction in the number of instruments required within the laboratory.

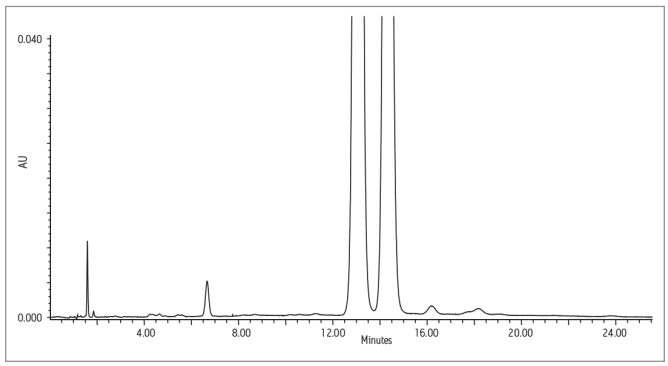


Figure 5.

UPLC can both reduce analysis times and increase chromatographic performance. One way this can be achieved is by reducing the column length by a factor of two while reducing the particle size by a factor of three, from 5 µm to 1.7 µm. The flatter nature of the van Deemter plot for the 1.7 µm material allows a faster mobile phase linear velocity to be employed, further speeding up the analysis.

This approach is illustrated in the analysis of Budesonide, a common inhaled steroid; the chromatogram shown in Figure 5 depicts the separation of the two components on an ACQUITY UPLC System using a 4.6 mm x 250 mm, 5 µm C_{18} column with a mobile phase of 68% 20 mM ammonium formate buffer (pH 3.2) and 32% acetonitrile. Detection was performed by UV absorbance at 240 nm.

In this figure, we can see that there are small low-level impurities as well as the R- and S-epimers of the active component. The assay was transferred to a ACQUITY UPLC BEH 2.1 x 100 mm, 1.7 µm C_{18} column operating with the same mobile phase and a flow rate of 0.6 mL/min. The resulting chromatogram is displayed in Figure 6.

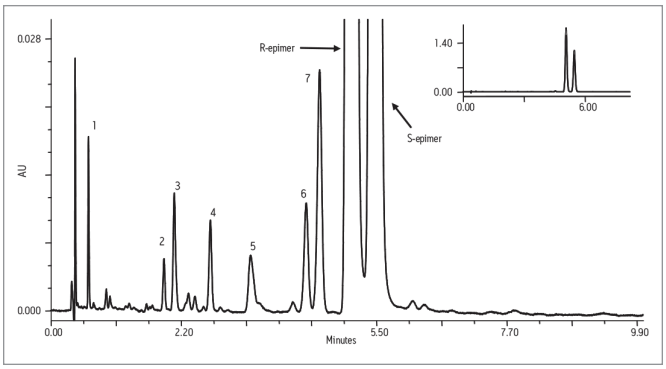


Figure 6.

The analysis time has been reduced from 25 minutes to 10 minutes; the chromatographic performance has also improved, producing sharper peaks. The extra chromatographic resolution and increased sensitivity with the ACQUITY UPLC System has allowed the detection of more impurities in less time, while the resolution of the two major peaks has been maintained. Using this approach, the method was simply transferred to UPLC with both improved throughput and assay performance.

CONCLUSIONS

Laboratories making investments in new LC platforms must take into account their ability to run legacy methods, while also preparing themselves for future methods that will employ new column technologies.

UltraPerformance LC is a new category of separation science that builds upon the well-known and established principles of liquid chromatography. UPLC leverages the performance of sub-2 µm particles to provide increased resolution, sensitivity and throughput.

The ACQUITY UPLC System has been specifically designed to exploit the capabilities of these new chromatographic stationary phases, with detailed attention focused on controlling system volumes and peak dispersion. Although this system has been designed to work with these new small particles, it also provides an excellent platform for easily transferring existing HPLC methods to the new UPLC methods. Thus, this new technology is allowing users to confidently move from existing LC methods to the new technology of UPLC.

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