Advances in the Optimization of a Micro-flow HPLC System

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Abstract

Current trends in HPLC system development have embraced higher pressures, higher temperatures and lower flow rates to increase separation efficiency and analytical throughput. By combining recent advances in micro-scale fluid delivery, small particle (~ 3μm) stationary phases, high temperature separations and chip-based UV absorbance detection to produce a fully integrated microflow gradient HPLC system, the overall chromatographic performance has been optimized for short cycle times, high resolution and good detection sensitivity.

Results of tests using several pharmaceutical formulations indicate that the system design optimization enables separation efficiencies approaching that of UHPLC systems without the requisite ultra-high pressures.

Introduction

While the recent advances in HPLC systems using sub-2μm particles appear to offer high separation efficiency (~ 150,000 N/m) column pressures become a limiting factor. In order to achieve high efficiency without ultra-high pressures, a fully integrated micro-flow HPLC system has been designed to utilize columns containing 3 μm particles at moderate (40-60 ºC) temperatures. In this way separation efficiency is maintained without the associated ultra-high system pressures.

The three most important aspects of this design are accurate flow rate control, close-coupling of flow path components and detection flow cell optimization. The first is accomplished by employing an inline flow module with rapid (~5Hz) feedback to a pneumatically amplified syringe pump (figures 1 & 2).

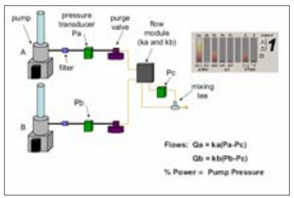

Figure 1 – microfluidic flow control

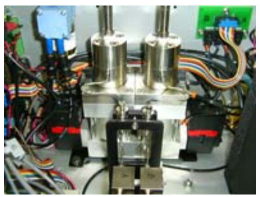

Figure 2 – Express pumping system

Close coupling (figure 3) of the injection valve, column and detection flow cell allows flexibility of operation without introducing extra-column dispersion which would compromise separation efficiency such as found in larger scale systems UPLC.

Perhaps the most important aspect of system performance is the optical detection system. Clearly, reducing the flow cell's dimensions preserves chromatographic resolution. However this change in the flow cell design is unavoidably accompanied by a reduction in light transmission which increases short-term noise. This reduction in signal-to-noise ratio can compromise the system's performance. To overcome this limitation optical fibers and chip-based technology have been employed. The detector flow cell (figure 4) incorporated in the expressLC-100 has an internal volume of only 45 nL with an optical path length of 4mm.

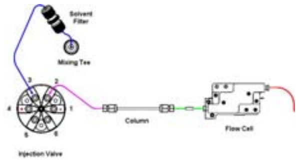

Figure 3 – close coupling of key components

Figure 4 – chip-based UV detector flow cell

Experimental

To verify separation efficiency, a solution of standards were separated isocratically and the plate count calculated (figure 5). The plate count for the 15 cm column ranged from 19,000 to 20,300. This equates to nearly 135,000 plates/meter.

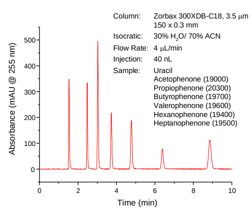

Figure 5 – isocratic separation

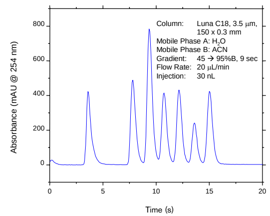

Figure 6 – gradient separation

This separation was then tested for flexibility by changing the separation from isocratic conditions to a very fast gradient of less than 10 seconds. The result (figure 6) shows that baseline resolution is maintained while decreasing the run time to less than 20 seconds.

One procedure commonly used by the pharmaceutical industry to characterize a drug candidate's lipophilicity by measuring its chromatographic hydrophobicity index (CHI). This HPLC analysis is designed to correlate the retention of the drug candidate on a reverse phase column with its theoretical lipophilicity. Normally, this gradient separation results in a run time on the order of 6 – 10 minutes. Here (figure 7) we show the same analysis completed in under 1 minute by reducing the gradient down to 45 seconds . Despite the extremely fast gradient, the correlations of results at three different pH's with that of a conventional system (figure 8) are quite good.

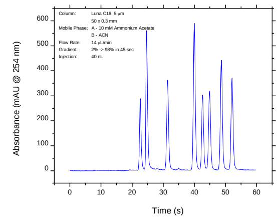

Figure 7 – CHI separation

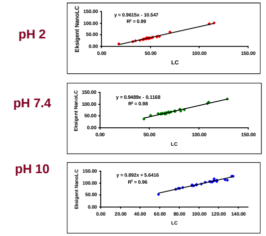

Figure 8 – CHI correlation

Our final example shows a gradient separation of an analgesic tablet containing three active ingredients: aspirin, acetaminophen and caffeine. This separation (figure 9) is accomplished using the ExpressLC-100 (figure 10) in less than 1.3 minutes using a 40-second gradient following a 30 second initial isocratic step.

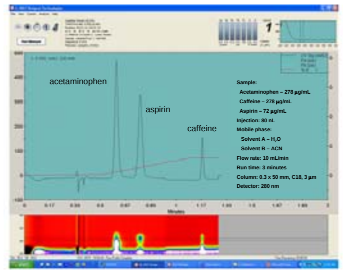

Figure 9 – separation of analgesics tablet

Figure 10 – ExpressLC-100

Conclusions

From these results it can be clearly seen that by combining the inherent attributes of micro-flow HPLC with optimized hardware design, highly efficient separations can be achieved without the necessity of operating at ultra-high pressures. In addition, high separation efficiency can lead to high throughput by reducing gradient duration. The expressLC-100 also offers the benefit of reduced solvent consumption and waste generation making it a "green" addition to the analytical lab.

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