Performance PerSPECtives

Waters

Waters® CapLC™ System

Reproducible Capillary Separations by Design:

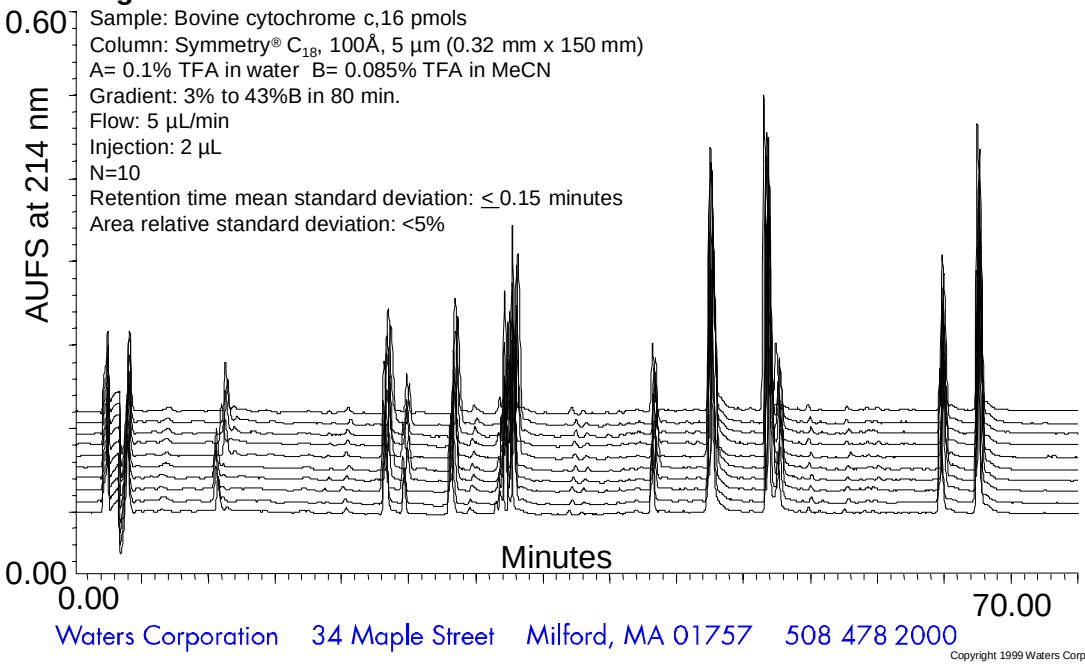
Traditional approach to capillary column chromatography:

A growing number of analytical laboratories require high quality separations from mass limited samples. To meet this demand, methods have been developed using capillary columns (internal diameter 0.1 to 1 mm) that are capable of producing high resolution separations at flow rates suitable for mass spectrometric analysis. Traditional HPLC instrumentation often use pre-injector split flow technology to deliver solvent at suitable flow rates to capillary columns, typically 1-20 µL/minute. These pre-injector devices work by splitting solvent flow based on the relative backpressure produced by the two flow paths. As long as the ratio remains constant, net flow to the column is consistent and will provide reproducible results. However, with time the backpressure on the column will increase, resulting in a decrease in net flow to the column. This change in column backpressure can occur rapidly, especially when analyzing biologicals or when samples contain particulates. To date, analysts have had no choice but to accept significant run-to-run variations when capillary separations are performed on split-flow systems.

Technology driven improvement in capillary separations:

The Waters CapLC System was specifically designed, tested and optimized to provide capillary scale chromatography with PDA detection. Use of Continuous Positive Displacement Flow and injection synchronization with the XYZ sample manager represent major improvements in solvent flow control that are essential for optimal isocratic and gradient chromatographic performance at flow rates ranging from 200 nL/min. for isocratic to 1 to 40 µL/min. for gradient analysis. In addition, the integrated design minimizes system volume in order to control undesired bandspread. Figure 1 shows the overlay results of 10 capillary gradient separations. Note the excellent retention time and area reproducibility obtained on the Waters CapLC System.

Figure 1



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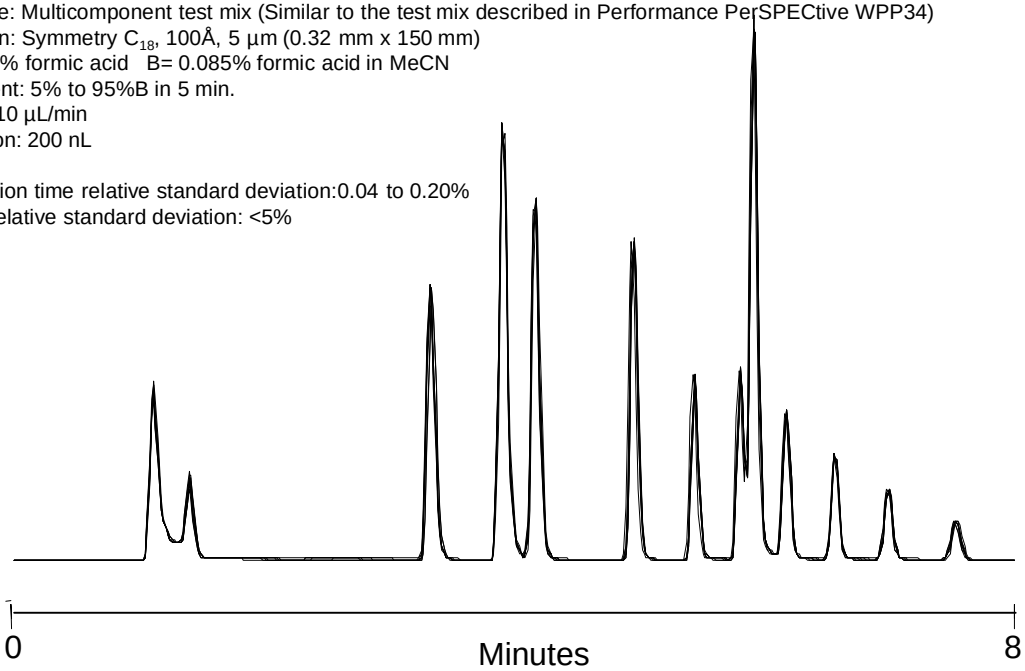
Results normally seen with high performance analytical systems:

Figure 2 is representative of results obtained for the rapid "generic" gradient separation of a multicomponent test mix separated on the Waters CapLC System. In this study of eight consecutive injections, the standard deviation for retention time of each resolved peak was less than 1 second, yielding results which are superior to any previously generated with a capillary HPLC system. Excellent peak area reproducibility is also evident. In addition to providing the benefits of highly reproducible results, the Waters CapLC System was also designed to maintain the sensitivity enhancements possible when using capillary columns (see Performance PerSPECtives WPP401).

Figure 2

Sample: Multicomponent test mix (Similar to the test mix described in Performance PerSPECtive WPP34)
Column: Symmetry C_{18}, 100Å, 5 µm (0.32 mm x 150 mm)
A= 0.1% formic acid B= 0.085% formic acid in MeCN
Gradient: 5% to 95%B in 5 min.
Flow: 10 µL/min
Injection: 200 nL
N=8
Retention time relative standard deviation:0.04 to 0.20%
Area relative standard deviation: <5%



Summary:

• The challenges of designing a gradient HPLC system capable of performing reproducible, efficient and sensitive capillary column separations are numerous. Each system component, including solvent delivery, automated injection, column design, fluidic connections, detection, and interfacing must be specifically engineered to provide the low total system volume required for true capillary gradient analysis.

•Continuous Positive Displacement Flow and injection synchronization with the XYZ sample manager utilized in the Waters CapLC system represent two major improvements in instrument design compared with traditional, "split flow" capillary systems. The result is excellent retention time and area reproducibility.

• The Waters CapLC System provides scientists with an unprecedented level of confidence in the results obtained from capillary column separations performed in HPLC or LC/MS applications.

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