



Evaluation of sub 2 μm Particle Packed Silica Columns in SFC

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Introduction

The need for greater speed and efficiency has driven the advent of UPLC as well as the evolution of smaller particle sized packing materials to effect separation, but at a cost of increased back-pressure and specialized equipment (UPLC) to maintain classical flow rates. In an attempt to further reduce analysis times, and more recently with the industry shortage of acetonitrile, it is of particular interest to determine if there are similar advantages of small particle technology in SFC as shown by UPLC, while offering a more "green" approach to separations.

To capitalize upon CO₂'s intrinsic properties of low viscosity and high diffusivity, we will show preliminary evaluations of resolution (Rs), run-time, and plate efficiency (N) to determine if sub 2 mm particle columns exhibit advantages in SFC as in HPLC using standard SFC equipment and conditions, as an orthogonal approach to UPLC in the analysis of compounds related to drug discovery.

Experimental

ES Industries EPIC HILIC Silica 2.1 x 100mm, 1.8μm packing

- Berger Analytical SFC Conditions - 8% MeOH, 100 bar, 40 C, 0.3-1.25 mL/min, 1 μL of Mixed Standard
- Mixed Standard - Caffeine, Theobromine, Acetaminophen
- Triplicate injections made at each flow rate

ES Industries EPIC HILIC Silica 2.1 x 100mm, 5μm packing

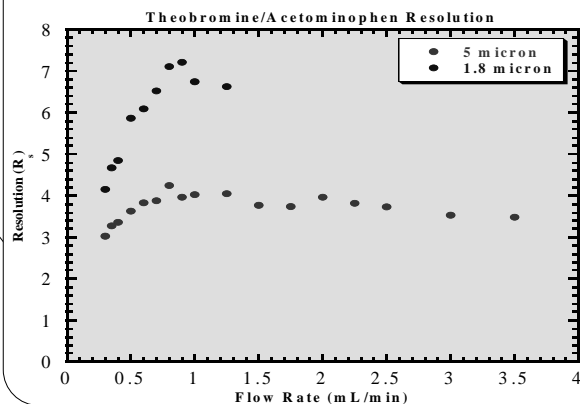
- Berger Analytical SFC Conditions - 8% MeOH, 100 bar, 40C, 0.3-3.0 mL/min, 1 μL of Mixed Standard
- Mixed Standard - Caffeine, Theobromine, Acetaminophen
- Triplicate injections made at each flow rate

Objectives

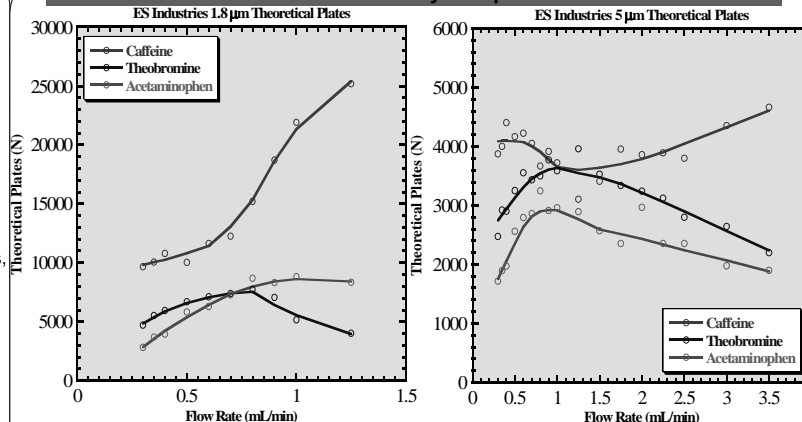
- Investigate the use of smaller particle size columns with SFC to gain faster methods and run-times
- Compare column efficiency, resolution, and peak symmetry
- Determine advantages of small particles columns with SFC

Results

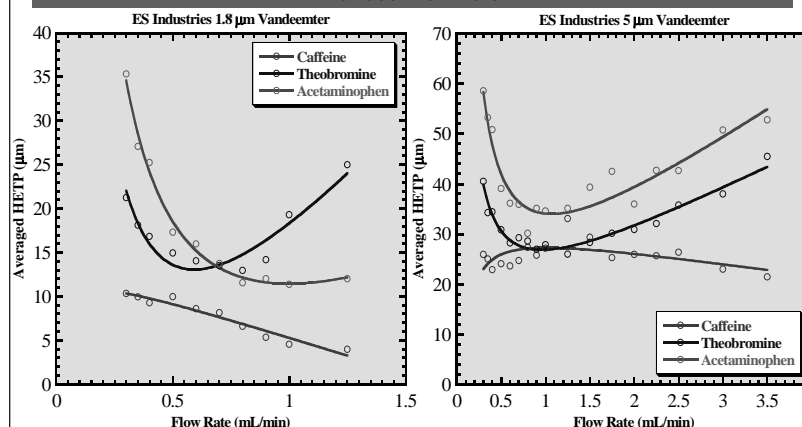
Resolution Between Theobromine and Acetaminophen



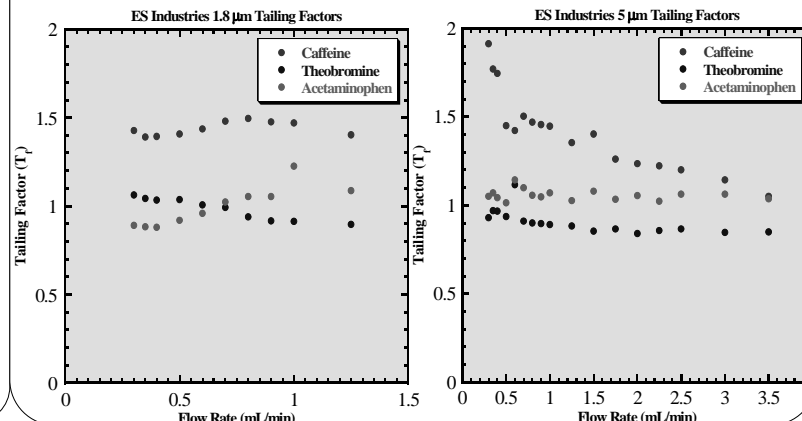
Column Efficiency Comparison



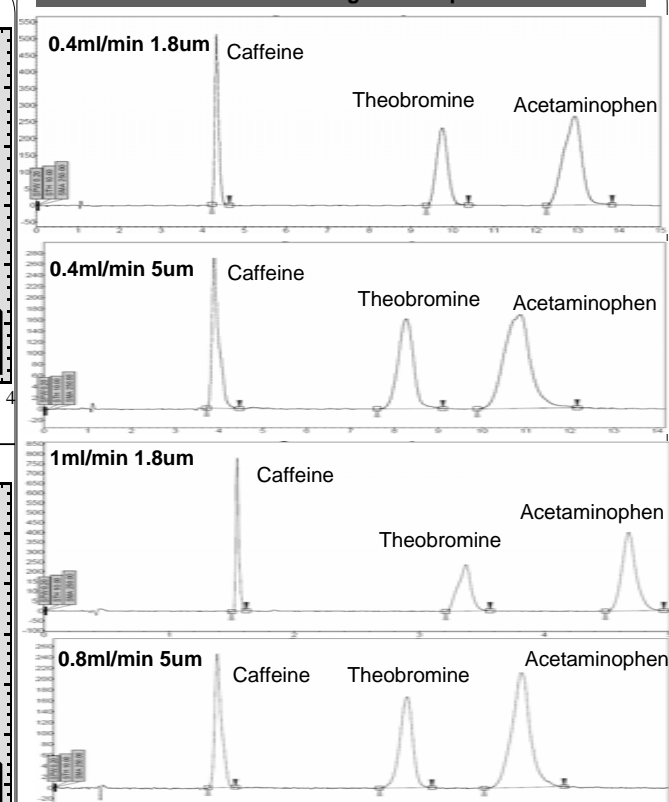
Vandemeter Plots



Peak Symmetry



Flow Rate Chromatogram Comparisons



Discussion and Conclusion

The resolution between theobromine and acetaminophen were relatively consistent across flow rates for the 5μm column, whereas the 1.8μm column reached a maximum Rs value between 0.7-0.8ml/min. Tailing factors indicated that both particle sizes afforded equally good peak symmetry for the given standard components. The Vandemeter curves exhibit a classical, flatter shape for small particle columns at increased flow rates and with greater efficiencies. As expected, our experimental results for column efficiency clearly demonstrates the number of theoretical plates (N) of the 1.8μm column to be much greater than the 5μm column. The maximum N for the 5μm column for two of the three selected standard components was exhibited between flow rates of 0.8-0.9ml/min, which would equate to ~4ml/min on a standard 4.6mm i.d. column typically used for our analytical analyses. Representative chromatograms at 0.4 ml/min for both columns demonstrate the chromatography at typical analytical flows for a 4.6mm diameter column. The 1.0ml/min, 1.8μm chromatogram and the 0.8ml/min, 5μm chromatogram, represent flow rates at maximum N for each column. The flow rates for the <2μm column did not exceed 1.25ml/min due to a pressure drop of ~160 bar across the column. One must keep in mind that a 1.25ml/min flow on a 2.1mm, 1.8μm column would be comparable to the flow rate of 6ml/min when scaling up to 4.6mm i.d. column; thereby, allowing the potential for greater speed without forsaking resolution and efficiency. Preliminary investigations have demonstrated that smaller particle columns offer higher performance as in HPLC. Our evaluation affords slight advantages of the 1.8μm column over the 5μm when applied to SFC. Further evaluation is needed to determine if the advantages of the 1.8μm column outweigh the performance of the 5μm column in regards to pressure, pressure drop, and column life.

Acknowledgements

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