Maximizing Resolution or Minimizing Analysis Time? Comparing and Evaluating Column Choices for High Speed and High Resolution LC

Maureen Joseph, Brian Billington, William Long and William Barber
Agilent Technologies, 2956 Centerville Rd., Wilmington, DE 19808, USA
Pitcon 2008 New Orleans

Abstract
The typical approach to achieving high-speed and high resolution LC is to decrease the particle size and reduce the column length. This approach works well and is the basis of the increasing use of sub-2-micron particles.

Other separation and column particle parameters can impact the choice for maximum resolution. Temperature is one parameter that needs to be considered. The impact of temperature on a separation when using columns with a range of particle sizes where the pressure drop across the column varies with the particle size may influence column choice.

These parameters and others will be examined when comparing the performance of different column choices for speed and resolution.

Hi Speed & Hi Res Key Parameters
Parameters for Optimizing a Separation – Achieving Maximum Resolution

- Particle size
  - Smaller particles generate more resolution for the same column length
- Pressure considerations – particle size vs. pressure

Bonded phase selectivity
- Multiple choices maximize resolution
- Optimized retention and resolution can minimize analysis time

Operating conditions
- Temperature – changes in efficiency or selectivity

Particle Size Considerations

Increasing Resolution by Using Long RRHT, 1.8um Columns

Sample: Desacetylselenium & Impurities

RNST BR C18 3.2 x 50mm 1.8um (3.0um)

Resolution

- Longer column length increases resolution
- Resolution increases slowly over the particle size range
- Resolution remains high for a longer period of time

Pressure considerations

- The 2.2um, 250mm long column had resolution that exceeded that of the 100mm long column with 3.5um and 1.8um particle sizes

Eclipse Plus C18 Gradient Run on Different Column and Particle Sizes

Sample: Analgesics, Column: 4.6 x 50mm, 1.8um SB-C18

Resolution

- Higher pressure LC is required
- The 1.8um, 100mm column operated at 474 bar, requiring a higher pressure LC, but with a pressure limit of 450 bar was acceptable

Bonded Phase Selectivity

Typical Method Development Parameters:

- Effects of selectivity, efficiency and resolution

More RRHT Bonded Phase Choices Allow for Optimized Method Development

- 5 Micron RRHT Bonded Phases can be used for method optimization
- RRHT SB-C18 Phased Column – more resolution to the last time

Comparison of Optimal Conditions On Columns with Different Dimensions – Length, Particle Size, Resolution

Sample: 19 FAA

- Faster, higher resolution with 1.8um vs. 3.5um columns
- Practical resolution optimized for method development

Comparison of Columns with the Highest Efficiency

1,2,4,6,8,10,12,14,16

Reduce plate height, h

- N7 = 6,838
- N5 = 8,180
- N3 = 8,745
- N2 = 9,269

More RRHT Bonded Phase Choices Allow for Optimized Method Development

- 5 Micron RRHT Bonded Phases can be used for method optimization
- RRHT SB-C18 Phased Column – more resolution to the last time

Conclusion

- More RRHT Bonded Phase Choices Allow for Optimized Method Development
- 5 Micron RRHT Bonded Phases can be used for method optimization
- RRHT SB-C18 Phased Column – more resolution to the last time

- Temperature Impact

Van Deemter Plots

- Plot 1 – shows the change in the A, B and C terms expected when the temperature changes

Higher Column Temperature - Higher Throughput, Greater Efficiency

Sample: Analgesics, Column: 4.8 x 50mm, 1.8um SB-C18

Temperature Can Change Selectivity, Lower Pressure, Increase Sensitivity, Improve Peak Shape and Reduce Analysis Time: Estrogens

- Temperature impact on selectivity

- Faster, higher resolution with 1.8um vs. 3.5um columns

Sub 2-micron particles deliver higher efficiency and can be used effectively for high speed and high resolution separations.
- When comparing particle size choices the best choice may depend on the operating pressure of the separation and the availability of different LC’s.

- Method development parameters, like bonded phase selectivity, are still equally important to optimizing a method.
- At elevated temperature more efficient mass transfer occurs even when using sub-2-micron columns and can be used to reduce analysis time. But temperature should still be evaluated in terms of changes in selectivity and the impact on the overall method performance.