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

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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 choices for maximum resolution. Temperature is one parameter than 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 evaluated 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 particle sizes 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

Experimental Comparison:

- A mixture of 16 PAH's (polynuclear aromatic hydrocarbons) was separated on ZORBAX Eclipse PAH columns with 5um, 3.5um and 1.8um particle sizes.
- The 5um column was 250mm in length. Columns with 100mm length and 3.5um and 1.8um particles were also compared.

Particle size vs. resolution

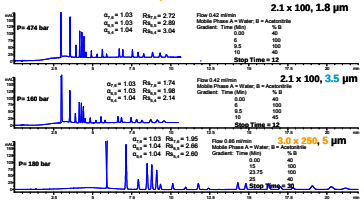
- The 5um, 250mm long column had resolution that exceeded that of the 100mm long, 3.5um column, but less resolution than the 100mm,1.8um column.
- The resolution increased 50% between the 3.5um and 1.8um columns in the same 100mm length and with the same analysis time.

Pressure considerations

- The 3.5um and 5um columns operated below 400 bar, making them suitable for any type of LC.
- The 1.8um, 100mm column operated at 474 bar, requiring a higher pressure LC, but one with a pressure limit of 600 bar was acceptable.

Choose column ID, length and particle size for Rs and Time – Note Pressure choices

Sample: 16 PAH's



Sub 2-micron particles deliver efficiency and productivity

This is the basic premise from which we operate.

Plates Selectivity Retention

$$R_s = \frac{\sqrt{N}}{4} \bullet (\alpha\text{-}1) \bullet \frac{k'}{k'+1}$$

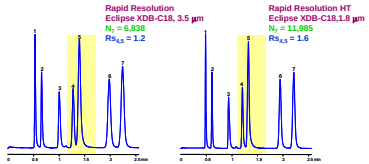
$$N \propto \frac{L}{d_p}$$

To Maintain Rs:
e.g.: L/2 ⇨ d_p/2

Column Length = ⇩ N
Particle Size = ⇧ N

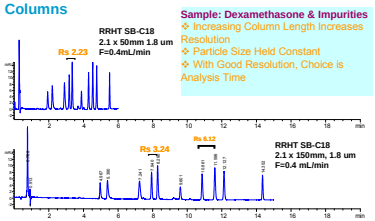
Particle Size Comparison - Smallest Particle Size for Highest Resolution and Efficiency

Conditions: Column: Eclipse XDB-C18, 4.6 x 50 mm Mobile Phase: 73% Methanol, 23% 20mM Phosphate Buffer, pH 7 Flow Rate: 1 mL/min, Temperature: Ambient Injection Volume: 1.8 µL Inj. Detection: UV 254nm Sample: 1) Uracil, 2) Naproxen, 3) Mefenamic Acid, 4) Butyl Paraben, 5) Propranolol, 6) Dipropyl Phthalate, 7) Naphthalene.



• Resolution improves and efficiency nearly doubles using the 1.8 um particles of the Rapid Resolution HT column.

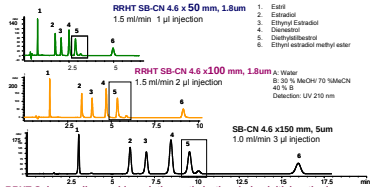
Increasing Resolution by Using Long RRHT, 1.8um Columns



Sample: Dexamethasone & Impurities

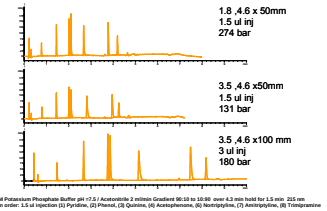
- Increasing Column Length Increases Resolution
- Particle Size Held Constant
- With Good Resolution, Choice is Analysis Time

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



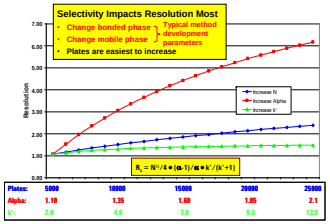
RRHT Columns allow rapid resolution optimization during initial method development. Column lengths compared quickly.

Eclipse Plus C18 Gradient Run on Different Column and Particle Sizes



Bonded Phase Selectivity

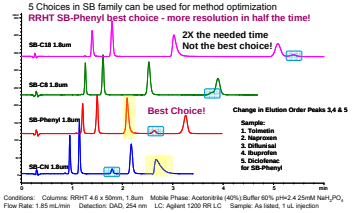
Typical Method Development Parameters: Effects of Selectivity, Efficiency and Retention



More RRHT Bonded Phase Choices Allow for Optimized Method Development

5 Choices in SB family can be used for method optimization

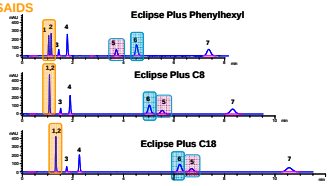
RRHT SB-Phenyl best choice - more resolution in half the time!



Conditions: Columns: RRHT 4.6 x 50mm, 1.8um Mobile Phase: Acetonitrile (40%):Buffer 60% pH=2.4 25mM NaH_2PO_4 Flow Rate: 1.85 mL/min Detection: DAD, 254 nm LC: Agilent 1200 RR LC Sample: As listed, 1 uL injection

Comparison of Eclipse Plus Family – C18, C8, Phenylhexyl Changes in Selectivity Occur

NSAIDS



Mobile Phase 40 % ACN 60 % 25 mM Sodium Phosphate Buffer pH= 2.4 Flow Rate= 1.5 ml/min 4.6 x 50mm UV 210 nm 2µl Elution order for Eclipse Plus Phenyl Hexyl: (1) Piroxicam, (2) Sulindac,(3) Tolmetin, (4) Naproxen, (5) Ibuprofen, (6) Diclofenac, (7) Celebrex (equal portions of approximately 1 mg/ml solutions

Temperature Impact

Van Deemter Plots

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

- A term – Eddy Diffusion –decreases with increasing temperature
- B term – Longitudinal Diffusion –increases with increasing temperature
- C term – Mass Transfer –decreases with increasing temperature

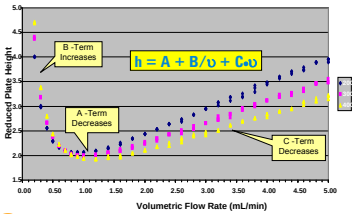
Plot 2 – 4.6 x 50mm,1.8um compares the reduced plate height of the column at increasing temperature vs. linear velocity.

- The reduced plate height should be close to 2 for a well packed column.
- The minima of the curve should shift out slightly with increasing temperature and the curves should overlay at the far ends, unless frictional heating is impacting performance.

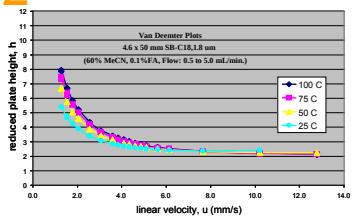
Plot 3 – 2.1 x 50mm,1.8um compares the reduced plate height vs. the linear velocity with increasing temperature

- The reduced plate height should be close to 2 for a well packed column
- Narrow bore columns are often harder to pack and the reduced plate height is closer to 3

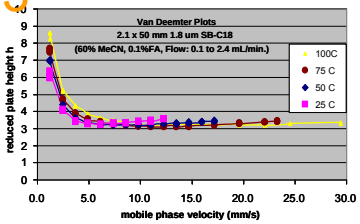
1



2

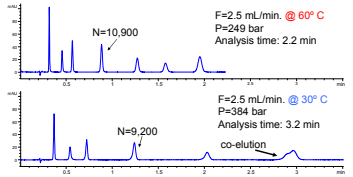


3

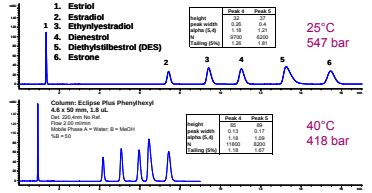


Higher Column Temperature - Higher Throughput, Greater Efficiency

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



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



Conclusion

- 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.