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Grace Davison Discovery Sciences

Unique Selectivity Improves Separation of Polar Compounds by HPLC and UHPLC

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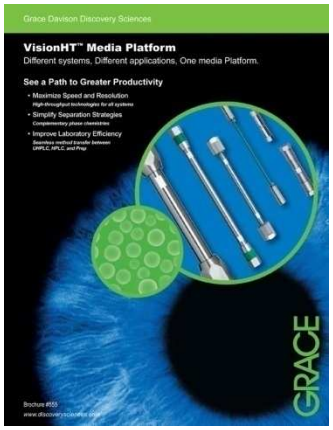
Enriching Lives, *Everywhere.*®

Outline

- **Modern Challenges**
- **VisionHT™ Reversed-Phase Media**
- **Consistent Manufacturing Process**
- **Unique Selectivity for Polar and Basic Compounds**
- **Versatile Column Formats**
- **Method Transfer**



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Today's Challenges

- **Demand for More Productivity**
  - Process more samples in less time
  - Obtain more information
  - Faster method development

- **Complex and Challenging Separations**
  - Resolution of critical compounds
  - Stricter reporting requirements
  - Retain polar compounds

- **Need for Optimal Laboratory Organization**
  - Fast LC replacing standard LC systems
  - Make best use of available resources

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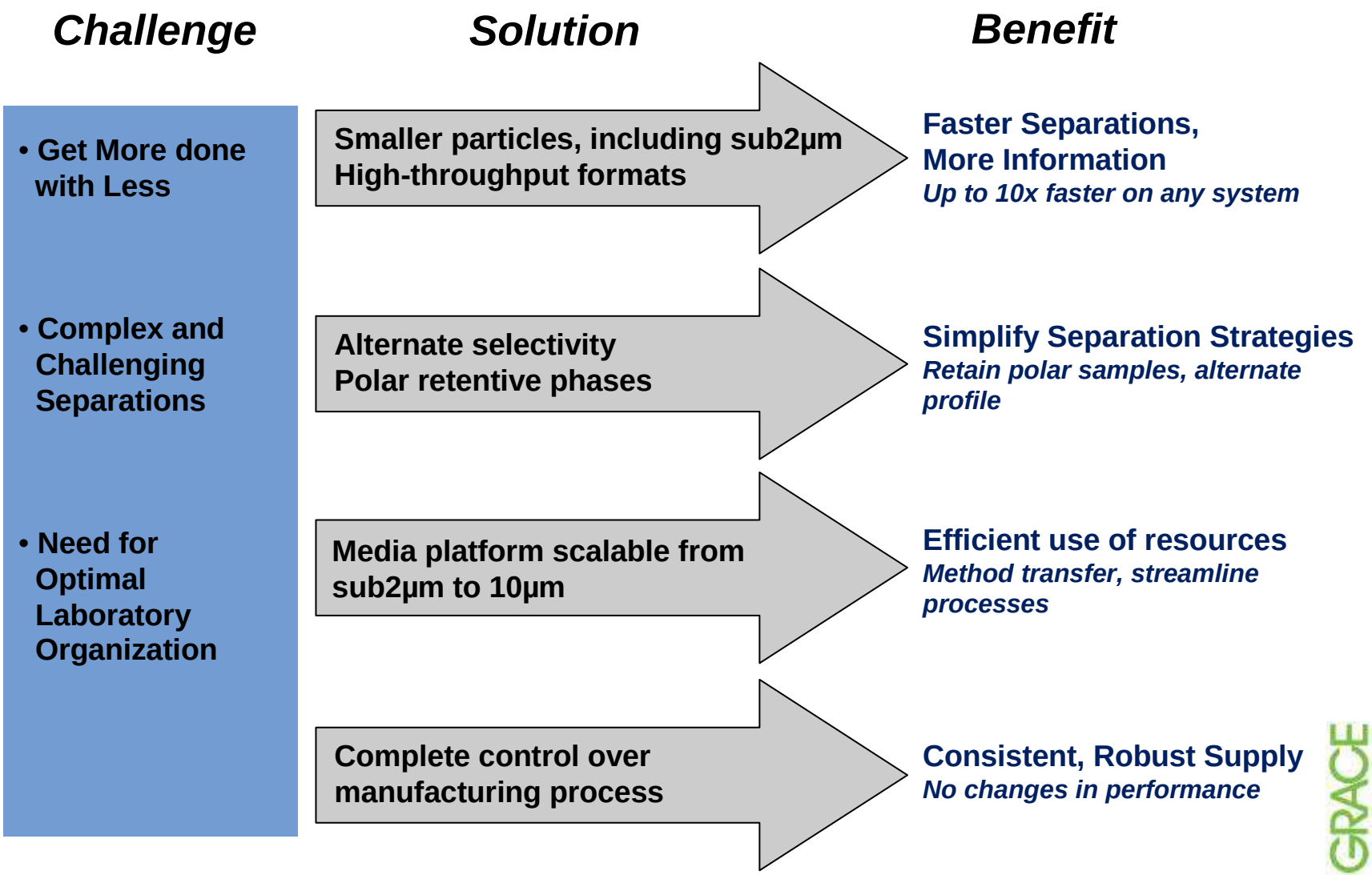
The Right Column Technologies Help Address These Challenges



VisionHT™ High Purity Media

VisionHT™ High Purity Silica

1.5-, 3-, 5-, 10µm particle sizes

Four Reversed-Phase Chemistries

C18 High Load

- **Carbon load:** 11%
- **Pore size:** 120Å
- **Surface area:** 220m^2/g
- **Endcapping:** Yes
- **pH range:** 1 - 10

C18 Classic

- **Carbon load:** 6%
- **Pore size:** 100Å
- **Surface area:** 200m^2/g
- **Endcapping:** Yes
- **pH range:** 1 - 10

C18 Basic

- **Carbon load:** 5%
- **Pore size:** 120Å
- **Surface area:** 220m^2/g
- **Endcapping:** No
- **pH range:** 1 - 10

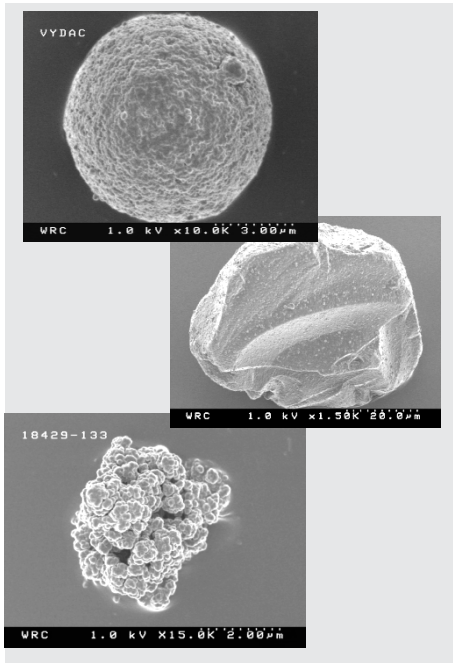
C18 Polar

- **Carbon load:** 5%
- **Pore size:** 100Å
- **Surface area:** 200m^2/g
- **Endcapping:** No
- **pH range:** 1 - 10

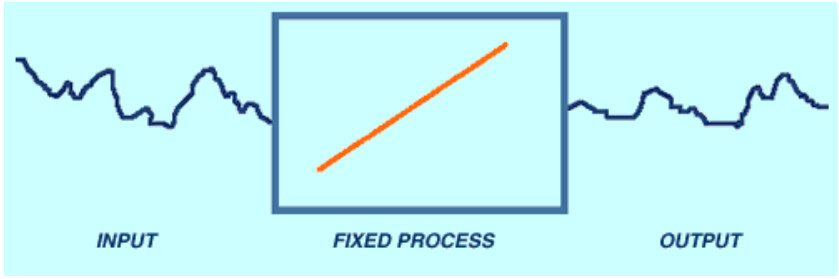
Classic and unique selectivity phases for polar and basic compounds

Silica Manufacturing Process

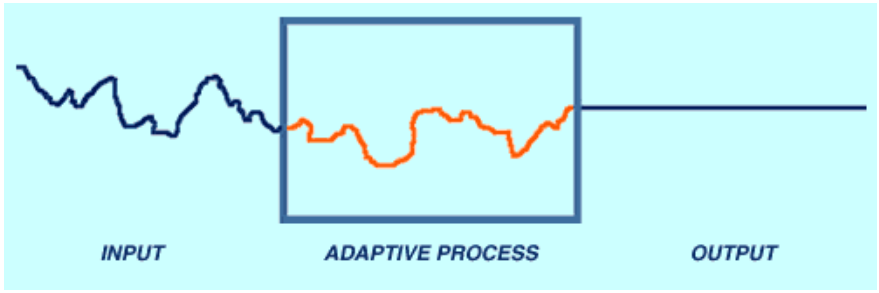
- **Grace has 80 years of experience manufacturing silica and is one of the leading silica producers in the world**



Conventional Manufacturing Process



VisionHT™ Manufacturing Process

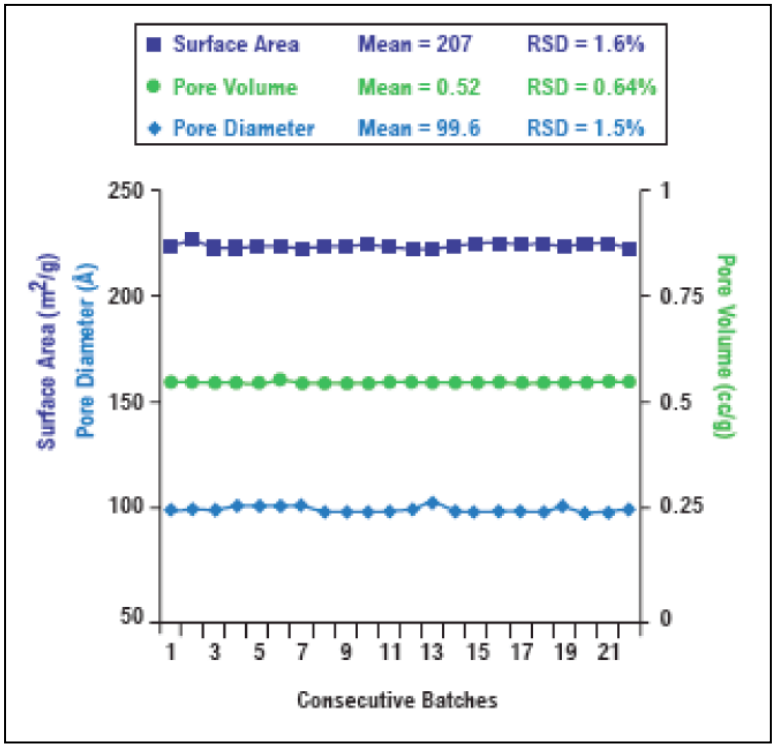


- **Adaptive manufacturing process creates highly reproducible end product**

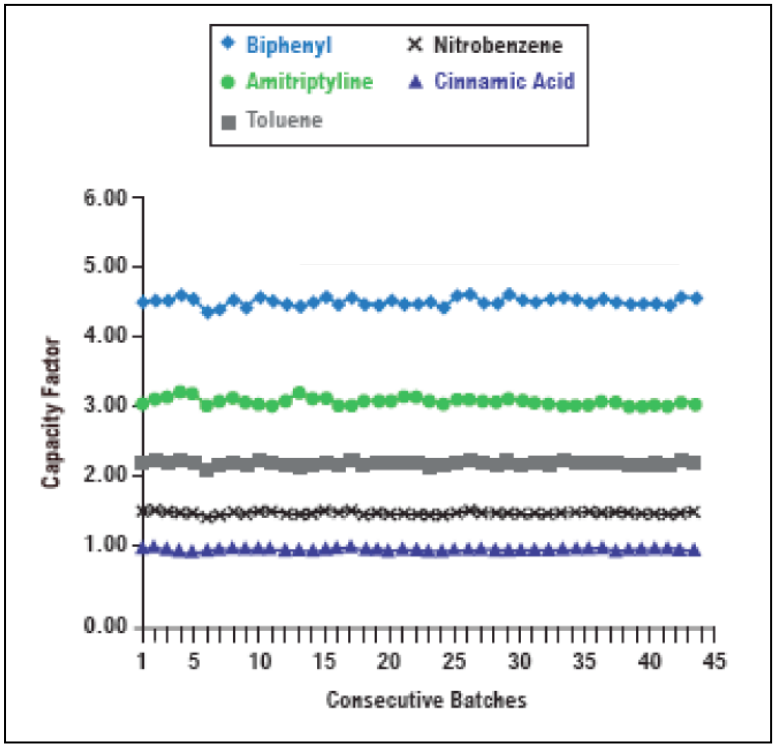
Consistent, Reliable Media

- **Highly consistent silica surface area, pore volume, and diameter**
- **Excellent reproducibility for acids, bases, and neutrals**

Reproducible Particle Technology



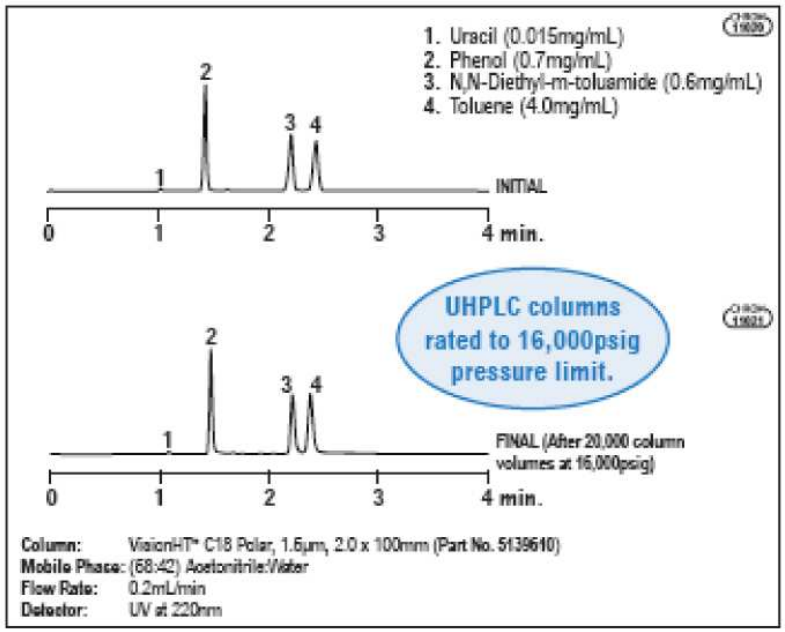
Consistent Batch-to-Batch Performance



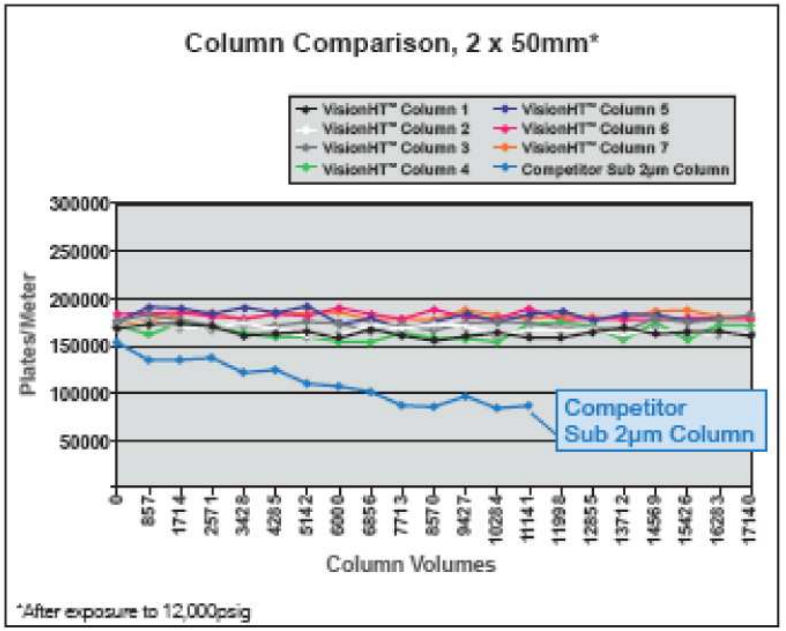
Grace's manufacturing process minimizes variations in capacity and selectivity

Mechanically Strong and Stable for Long Column Life

Consistent performance after 20,000 column volumes at 16,000 psig pressure



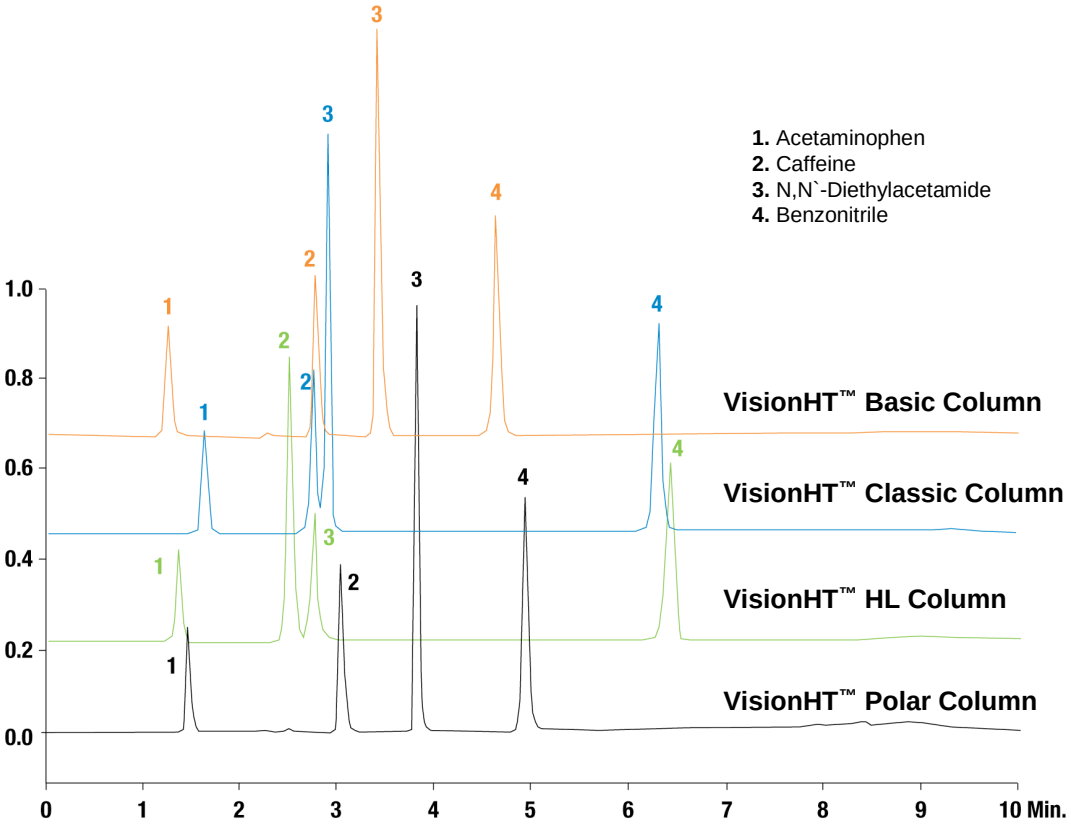
Column efficiencies of seven different VisionHT™ columns remained stable over 20,000 column volumes



Consistent performance at high pressures

Unique Approach to Phase Chemistries

- **Most commercial reversed-phase columns thoroughly cover the silica surface to minimize interactions**
- **VisionHT™ controlled silica exposure gives unique mixed-mode separations**



Column: 50 x 2.1mm, 1.5µm
Mobile Phase: Water:Acetonitrile
Injection Volume: 2.0µL

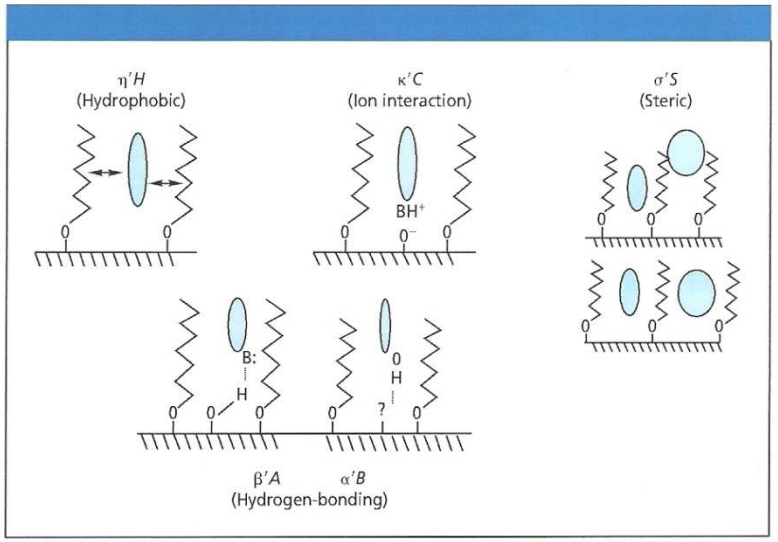
Dramatically different selectivity compared to traditional highly endcapped phases

Characterizing Selectivity – The Snyder/Dolan/Carr Approach

The Hydrophobic Subtraction Model[1,2]

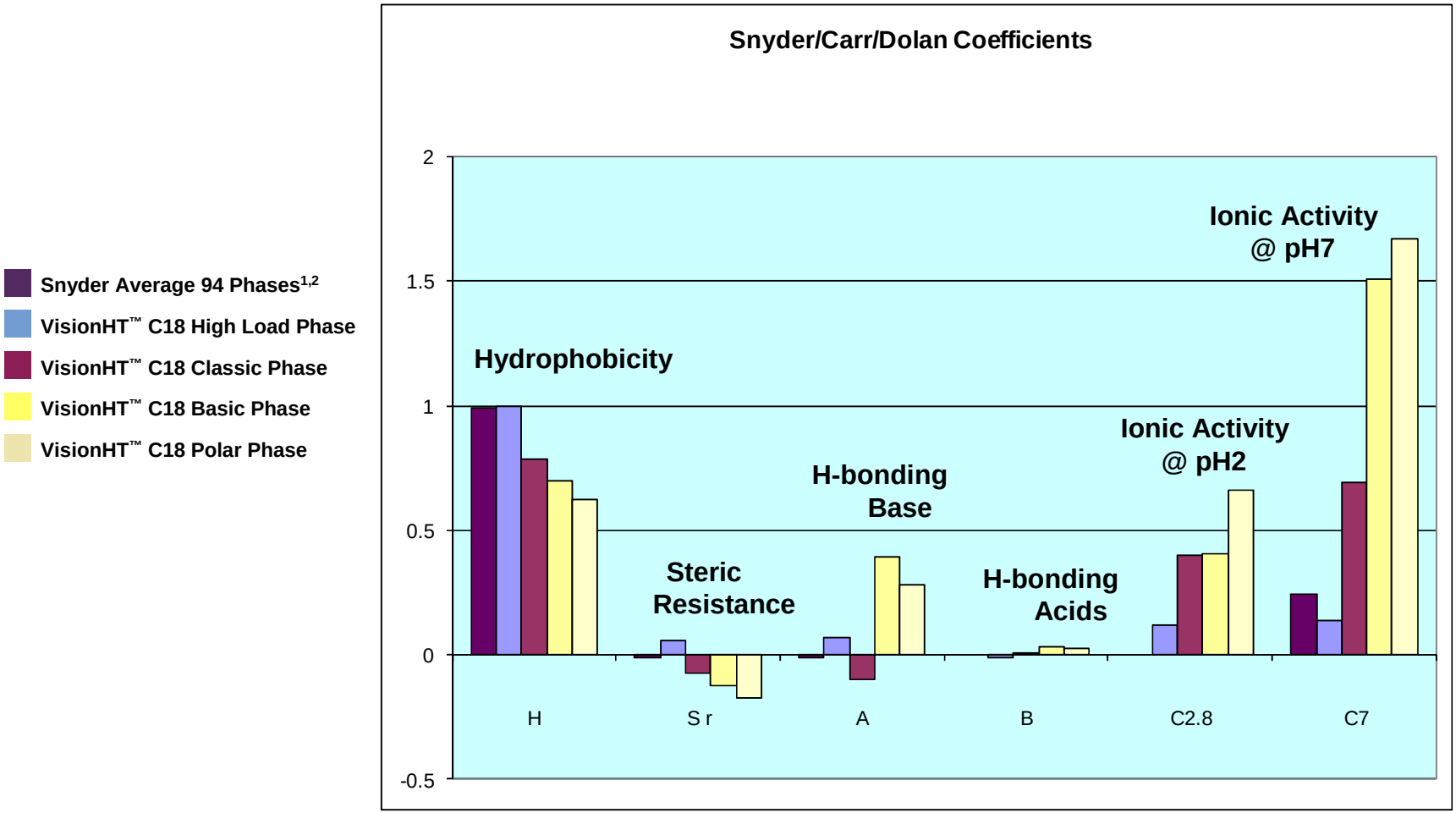
Log α = η'H - σ'S + β'A + α'B + κ'C

- H Hydrophobicity
- S Steric Resistance
- A Hydrogen bonding of bases
- B Hydrogen bonding of acids
- C Coulombic



1. "The "Hydrophobic-subtraction" Model of Reversed-Phase Column Selectivity", L.R. Snyder, J.W. Dolan and P.W. Carr, J. Chromatogr. A, 1060 (2004) 77 -116.
2. "A New Look at the Selectivity of Reversed-phase HPLC Columns", L.R. Snyder, J.W. Dolan and P.W. Carr, Anal. Chem., 79 (2007) 325 -3262.

The VisionHT™ C18 Phases Exhibit Different Selectivity Compared to Traditional Highly Endcapped C18 Phases



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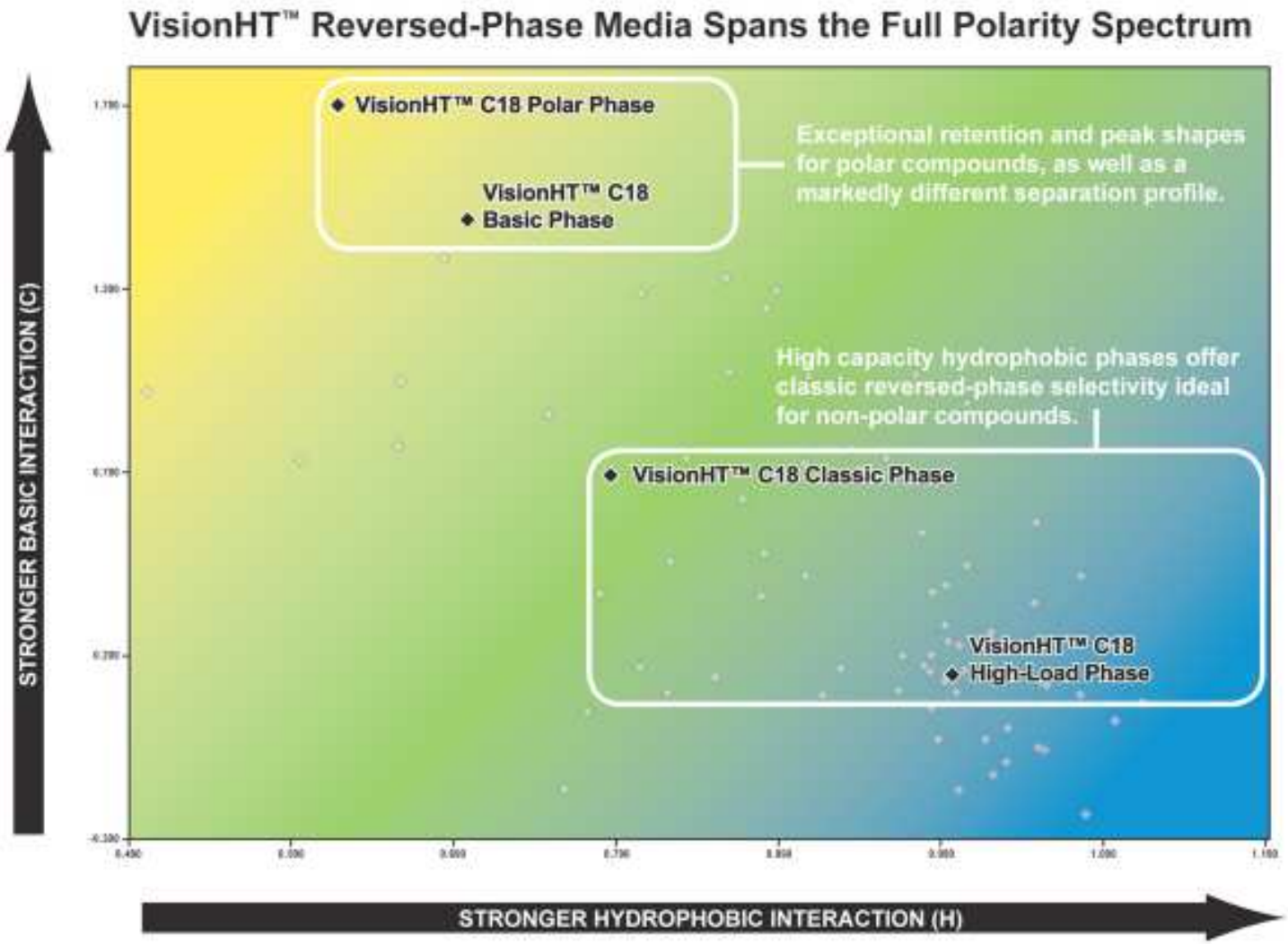
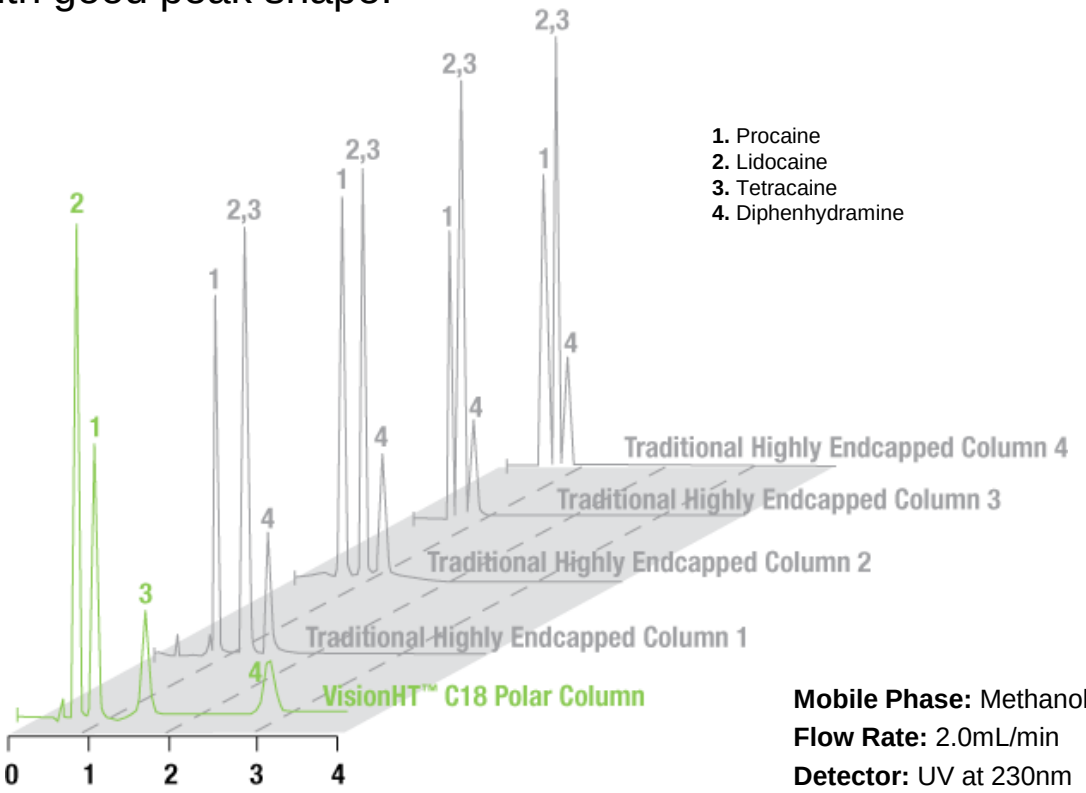
Broad Selectivity Options



Better Resolution for Polar Pharmaceuticals

The Grace® VisionHT™ C18 Polar column shows an increase in retention and change in selectivity from the 4 traditional highly endcapped columns. The difficult pair of lidocaine and tetracaine is fully resolved away from the solvent front and with good peak shape.



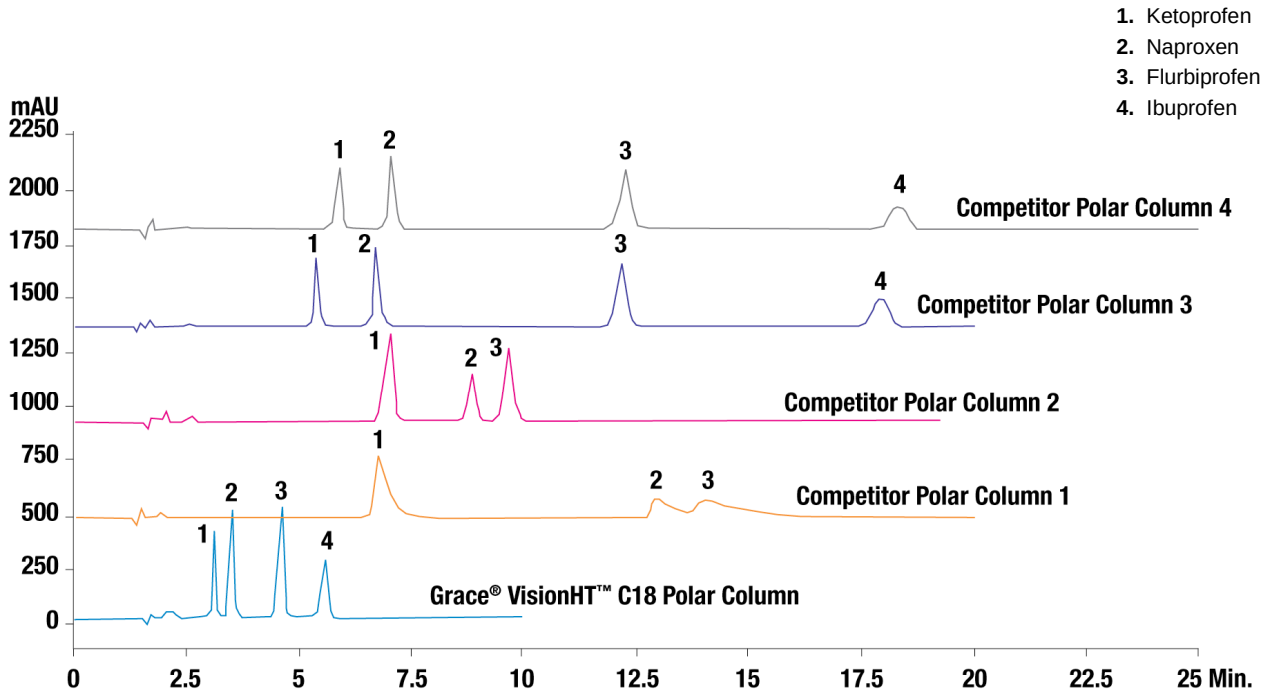
1. Procaine
2. Lidocaine
3. Tetracaine
4. Diphenhydramine

Mobile Phase: Methanol:0.025M K_2HPO_4, pH 7.0 (85:15)
Flow Rate: 2.0mL/min
Detector: UV at 230nm

The Grace® VisionHT™ C18 polar column separates highly polar pharmaceuticals better than traditional highly endcapped C18 phases

VisionHT™ C18 Polar Column vs Other Polar Phases

The VisionHT™ C18 polar column separates anti-inflammatories faster than other polar phases

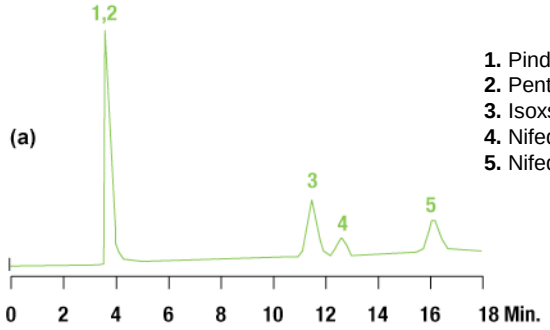


Column: Grace® VisionHT™ C18 Polar, 4.6 x 100mm, 5µm
Mobile Phase: Methanol:25mM KH_2PO_4, pH 3 (65:35)
Flow Rate: 1mL/min
Detector: UV at 210nm

Simplify Separation Strategies

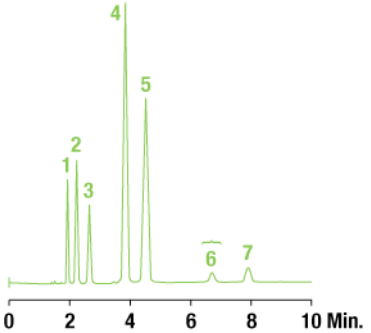
- **See peak reversal with orthogonal phase chemistries**
- **Get different retention times and selectivities for the same sample**

VisionHT™ C18 Classic Column



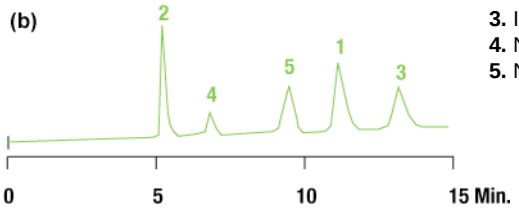
1. Pindolol
2. Pentoxifylline
3. Isoxsuprine
4. Nifedipine Degradant
5. Nifedipine

VisionHT™ C18 Classic Column



1. Barbital
2. Phenobarbital
3. Butalbital
4. Oxazepam
5. Triazolam
6. Unknown
7. p-Xylene

VisionHT™ C18 Polar Column



1. Pindolol
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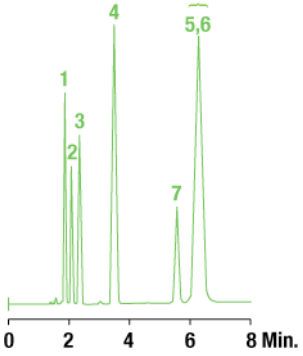
VisionHT™ C18 Polar Column

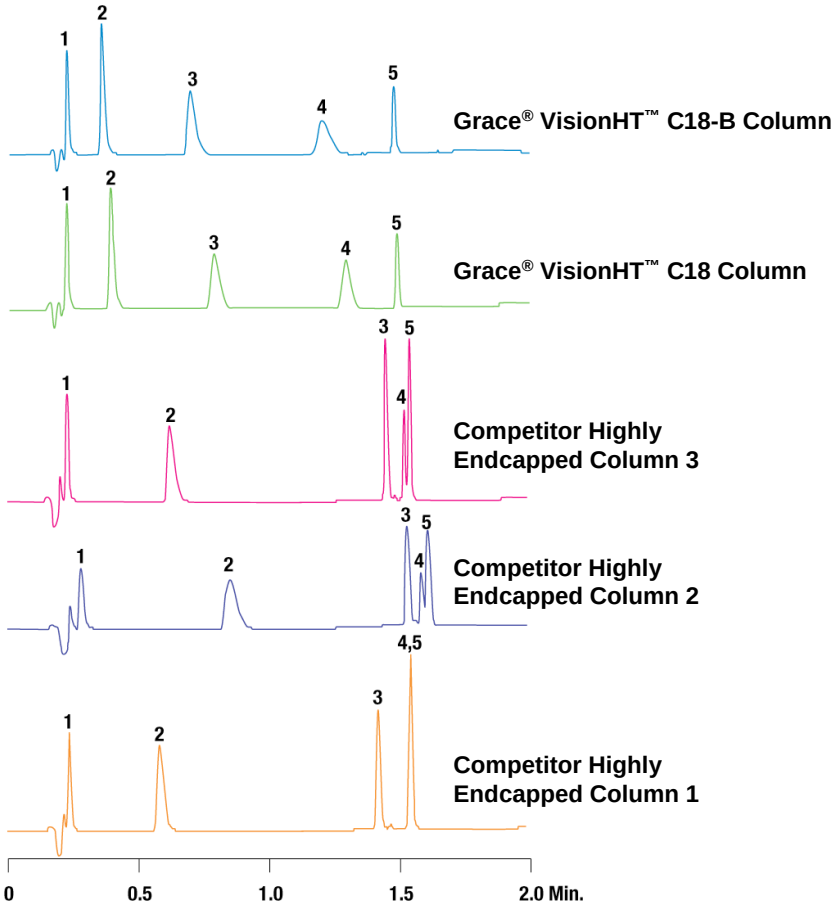


1. Barbital
2. Phenobarbital
3. Butalbital
4. Oxazepam
5. Triazolam
6. Unknown
7. p-Xylene

When one phase doesn't work, the other likely will

1.5µm Particles Increase Speed While Maintaining Resolution

The VisionHT™ C18 and C18-B columns show better resolution for the five peptides compared to the traditional highly endcapped columns, indicating a potential for increased resolution with complex samples.

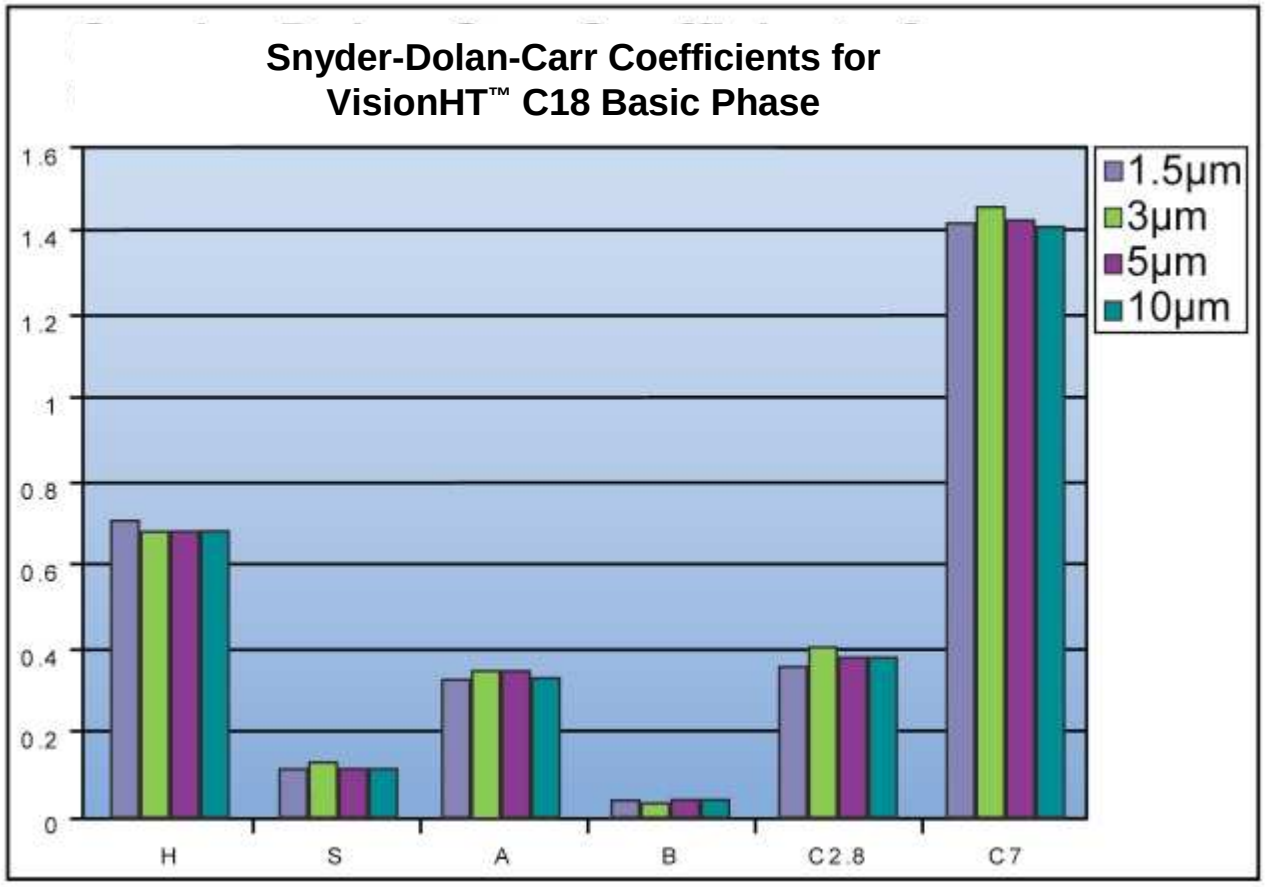


1. GY (238 Da)
2. VYV (379 Da)
3. Met Enkephalin (YGGFM, 573 Da)
4. Leu Enkephalin (YGGFL, 555 Da)
5. Angiotensin II (DRVYIHPF, 1045 Da)

Column Dimension:	1.5µm, 2.1 x 50mm
HPLC System:	Agilent 1200 RRLC
Mobile Phase A:	0.1% TFA in Water
Mobile Phase B:	0.085% TFA in 80:20 Acetonitrile:Water
Gradient (min,%B):	(0.0, 5), (1.0,100), (2,100)
Flow Rate:	0.8mL/min.
Detector:	UV at 215nm
Injection Volume:	10µL
Column Temperature:	45℃
Column Backpressure:	508bar

Equivalent Performance Across All Particle Sizes

Identical selectivity across the particle size range for VisionHT™ C18 basic phase



Versatile Column Formats

Apply VisionHT™ Media to multiple column formats

UHPLC	Rocket™ column	Expedite™ column	Analytical	Prep
UHPLC system	Traditional system	Low Volume system	Traditional system	Preparative system

High Throughput Technologies can help maximize speed and resolution from any system

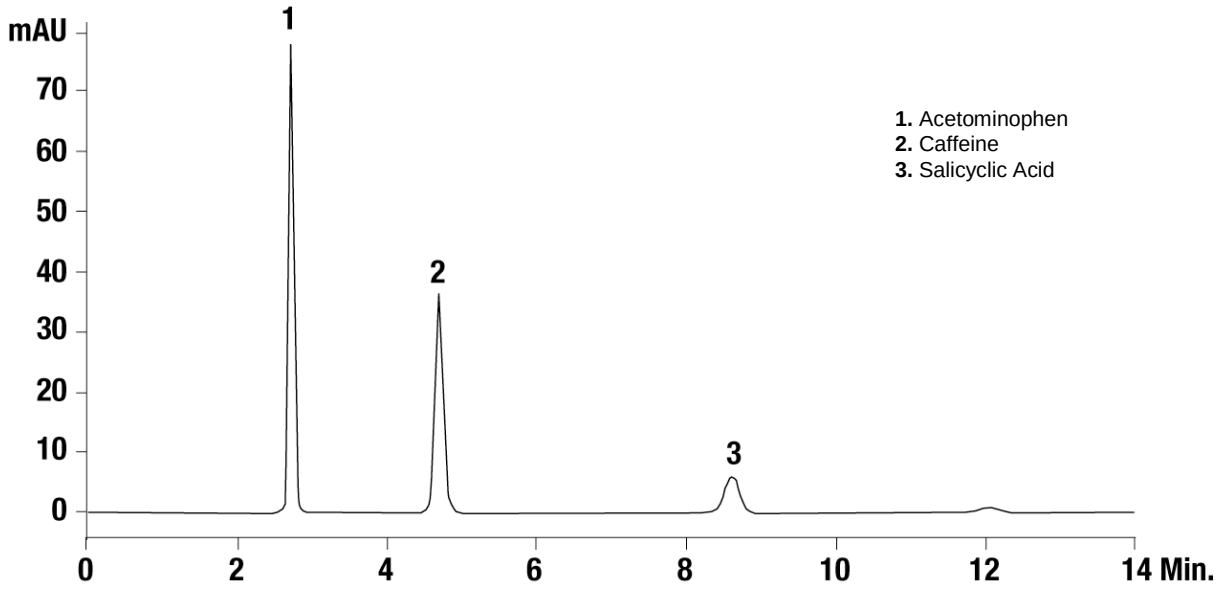
Standard Analytical meets existing needs

Highly efficient Lab-scale prep up to 2” i.d.

Variety of column formats to suit the system type and goals of the separation

Transfer Methods to Other Particle Sizes and Formats

Original Method



Column:	VisionHT™ C18, 5µm, 4.6 x 150mm
Mobile Phase:	Water:Methanol:Acetic Acid (68:29:3)
Flow Rate:	1.0mL/min
Detector:	UV at 280nm

Transfer Method to Higher Throughput Formats

System Type 1

Ultra High-Pressure LC System
(>10,000psig Pressure Limitation)

Column Format: VisionHT™ UHPLC
Length: 20, 30, 50, 100mm
i.d.: 1.0 and 2.0mm

System Type 2

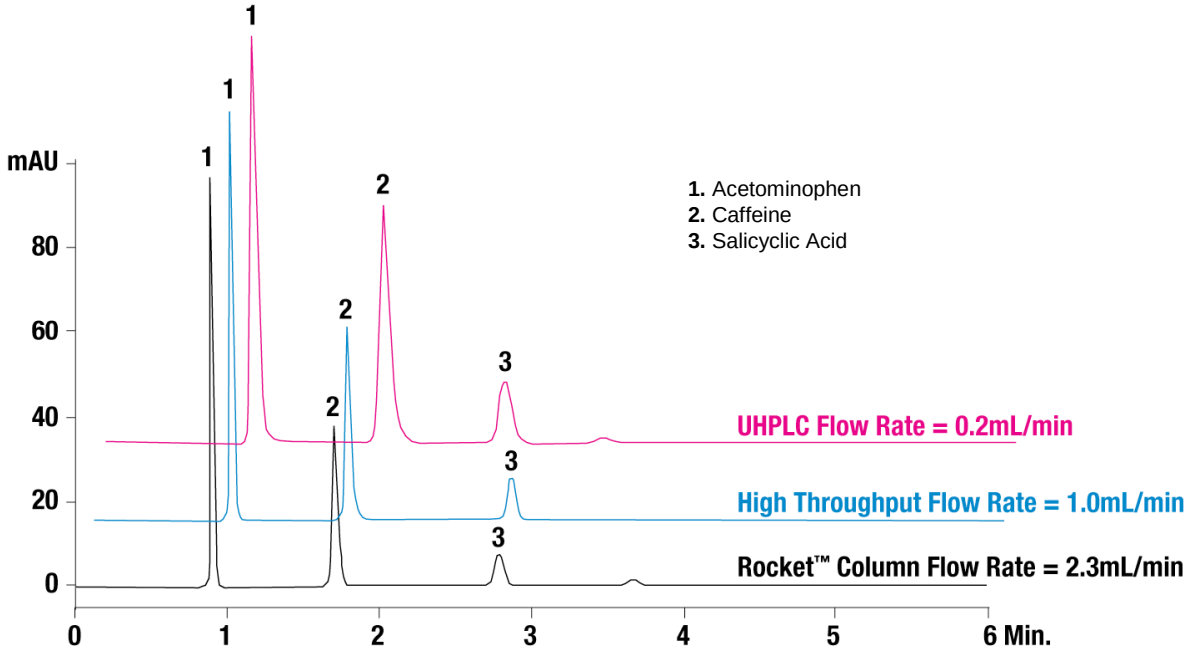
Low Volume, High Throughput (HTP)
(<10,000psig Pressure Limitation)

Column Format: Expedite™, Analytical
Length: 10, 20, 30, 50mm
i.d.: 2.1 or 4.6mm

System Type 3

Traditional LC System
(>10,000psig Pressure Limitation)

Column Format: Rocket™
Length: 33 or 53mm
i.d.: 7mm



Method Transfer Calculator

Calculation of Up-Scaling

1st Column Given Parameters

Length cm	ID mm
10.0	4.000

Flow rate mL/min	µL/min	Linear Flow cm/min
1.000	1000.00	6.250

Pressure bar	Mpa	Psi
30.0	3.0	435

Particle Size µm
5.0

Injection Volume mL	Loading mg	Concentration mg/mL
0.010	0.005	0.500

2nd Column Calculated Parameters

Length cm	ID mm
20.0	20.0

Flow rate ml/min	L/h	Linear Flow cm/min
25.000	1.500	6.250

Pressure bar	Mpa	Psi
15.0	1.5	218

Particle Size µm	Up-Scaling factor
10.0	35.4

Injection Volume mL	Loading mg	Concentration mg/mL
0.250	0.125	0.500

g
0.000

Conclusion

VisionHT™ Media Platform

- **Simplify challenging separations**
  - Four phases span the polarity spectrum – if one doesn't work, another likely will
  - Unique selectivity for polar compounds
- **Speed up separations**
  - 1.5µm particles
  - High throughput formats
- **Transfer Methods efficiently**
  - Selectivity consistent across particle sizes
  - Transfer calculator helps eliminate guesswork
- **Ensure batch-to-batch consistency**
  - Complete control of manufacturing process

High performance particle technology solves the challenges of modern laboratories

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