Utilizing Sub-2µm Particulate HPLC Columns for High Resolution, Ultra Fast Chromatographic Methods

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OVERVIEW

Particle technology for HPLC columns has evolved from 10 µm irregular particles to 5 µm spherical particles and more recently to 3 µm spherical particles. Each of these advances has resulted in improved chromatographic performance in terms of resolution and speed of analysis. The next logical advance to sub-2 µm particles presents certain challenges in terms of column packing, bed stability, operating pressure and extracolumn effects. A new generation of HPLC columns that incorporate sub-2 µm particle size hybrid packing material will be discussed. These columns provide the ultimate in chromatographic performance, leading to faster methods development, more sensitivity and higher resolution.

INTRODUCTION

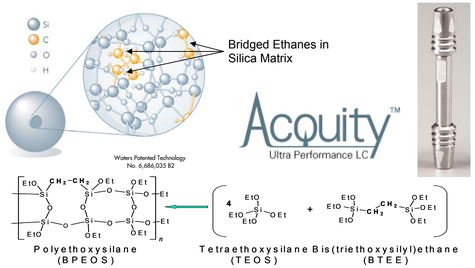
In order to reach the maximum efficiency of a chromatographic separation, the optimal linear velocity must be reached. Particle technology has evolved over the years producing smaller and smaller particle sizes. The most recent advance being sub-2 µm packings. These packings require a high linear velocity to reach their maximum efficiency, in return yielding the necessity to operate at higher pressures (up to 15,000 PSI). An extensive research program has been conducted to develop a particle that is mechanically strong enough to withstand these types of pressures. In addition to a new stationary phase, instrumentation was designed to meet the requirements of sub-2 µm packings. These requirements include low system volumes (to minimize dispersion), high pressure fluidic modules and high speed detectors. The integration of both chemistry and instrumentation has led to the development of the next generation of separation science, Ultra Performance LC™.

Ultra Performance LC™ is a new category of separation science that brings novel and powerful capabilities to the laboratory. **UPLC™** leverages the theories and principles of HPLC. It incorporates small particles, very low system volumes, and fast detection for increased throughput, sensitivity and peak capacity.

ACQUITY Ultra Performance LC™ Systems have been holistically designed to control and optimize all the parameters required to take full advantage of the benefits of UPLC in today's laboratory.

A NEW GENERATION OF HYBRID PACKINGS

Optimal linear velocities of sub-2 µm particles require operations at higher pressures. Inorganic-organic hybrid materials have demonstrated increased mechanical stability compared to traditional silica based materials*[1]. A 1.7 µm bridged ethyl-siloxane hybrid particle was designed to meet the demands of operation at pressures as high as 15,000 PSI, as well as mobile phase pH in the range of 1—12.



Improvements upon existing instrumentation had to be developed to meet the demands of these 1.7 µm particles. These improvements have led to the development of ACQUITY Ultra Performance LC™.

UPLC™ enabling technologies:

- Small particle size chemistries (1.7 µm)
- High pressure fluidic modules (up to 15,000 PSI)
- Low system volumes (150 µL)
- High speed, low dispersion optical detectors (up to 40 Hz)
- Minimized cycle times with minimal autosampler carryover
- High speed communication capabilities (Ethernet control)
- Comprehensive diagnostic suite
- Software optimized for system integration

OPTIMAL SEPARATION EFFICIENCY: van Deemter Equation

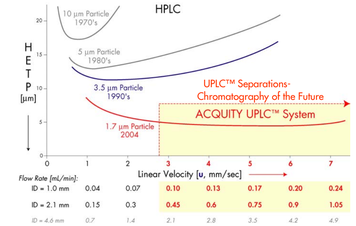
$$H = a(dp) + \frac{b}{u} + c(dp)^2u$$

A term + B term + C term

u = Linear Velocity (Flow Rate) dp = Particle Size

C term is impacted by Both

A term	Eddy Diffusion/ Interparticle channels. It is particle size dependant
B term	Molecular Diffusion (Axially). It is inversely proportional to velocity and insignificant in LC
C term	Mass Transfer Kinetics. It is directly proportional to velocity, and the particle size squared.



Properly designed small particles allow us to achieve higher speeds and improved resolution.

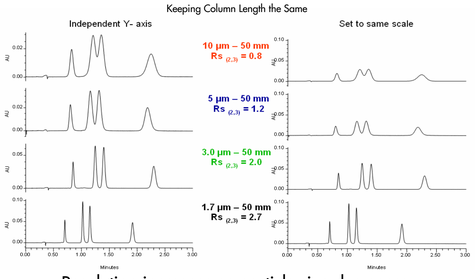
IMPROVED RESOLUTION BY REDUCING PARTICLE SIZE

- In UPLC™ systems, N (efficiency) is the primary driver
- Selectivity and retentivity are the same as in HPLC
- Resolution, Rs, is proportional to the square root of N

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k}{k+1}\right)$$

System Efficiency — Selectivity — Retentivity

If N ↑ 3X, Rs ↑ 1.7X



Resolution increases as particle size decreases.
Additionally, sensitivity increases as particle size decreases.

CONSTANT RATIO OF COLUMN LENGTH INVERSELY PROPORTIONAL TO PARTICLE SIZE

Productivity and speed at constant L/dp

Efficiency, N, is directly proportional to column length, L, and inversely proportional to particle size, dp

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

For same N and, therefore, same Rs,

N = 1X, Rs = 1X
dp ↓ 3X, L ↓ 3X, , F ↑ 3X, T ↓ 9X

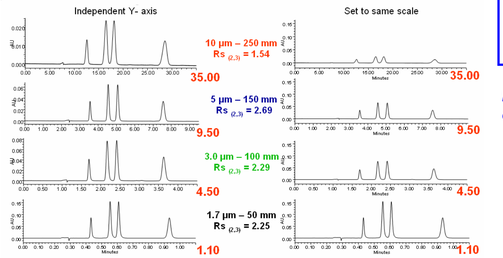
Sensitivity at constant L/dp

Assuming same efficiency, peak height is inversely proportional to column length, L

$$Height = \frac{1}{L}$$

For same efficiency, column length, L is decreased proportionally to particle size, dp (constant L/dp)

N = 1X, Rs = 1X
dp ↓ 3X, L ↓ 3X, , F ↑ 3X, T ↓ 9X
Sensitivity ↑ 3X



Resolution is maintained while decreasing analysis time.
Additionally, sensitivity increases as particle size decreases

APPLICATION OF 1.7 µm UPLC™ PARTICULATE COLUMNS

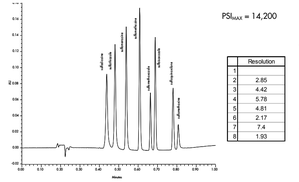
1.7 µm particulate columns offer significant benefits in terms of speed, sensitivity and resolution

Sub– 1 minute gradient separation of 8 analytes:
Achieve fast analysis without sacrificing resolution!

Chromatographic Conditions :
Columns: ACQUITY UPLC™ BEH C18 2.1 x 50 mm, 1.7 µm
Mobile Phase A: 0.1% FA in H2O
Mobile Phase B: 0.1% FA in MeOH
Flow Rate: 0.65 mL/min
Gradient: (Curve 3)

Time (min)	Profile %A	%B
0.0	85	15
1.0	20	80

Injection Volume: 0.5 µL
Sample: Sulfonamides
Sample Diluent: 85:15 water: methanol with 0.1% FA
Sample Concentration: 36 µg/mL
Temperature: 35 °C
Detection: UV @ 280 nm
Sampling rate: 40 pts/sec
Time Constant: 0.1
Instrument: Waters ACQUITY UPLC™, with TUV detector

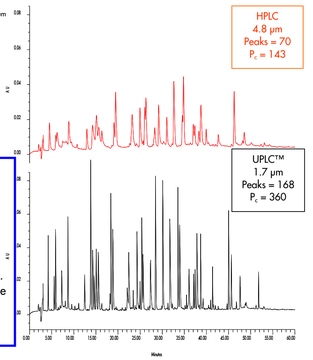


	Resolution
2	2.85
3	4.42
4	5.78
5	4.81
6	2.17
7	7.4
8	1.93

High resolution peptide mapping of enolase tryptic digest:

Achieve higher resolution and sensitivity by utilizing 1.7 µm UPLC™ columns compared to traditional 5 µm columns.

Chromatographic Conditions :
Columns: ACQUITY UPLC™ BEH C18 2.1 x 100 mm, 1.7 µm
BEH C18 2.1 x 100 mm, 4.8 µm
Mobile Phase A: 0.02% TFA in H2O
Mobile Phase B: 0.016% TFA in ACN
Flow Rate: 0.1 mL/min
Gradient: 5 – 40% B over 60 minutes
Injection Volume: 10.0 µL
Sample Diluent: 0.02% TFA in water
Sample Concentration: 100 pmol
Temperature: 38 °C
Detection: UV @ 214 nm
Sampling rate: 20 pts/sec
Time Constant: 0.1
Instrument: Waters ACQUITY UPLC™, with TUV detector



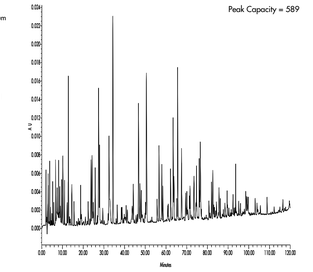
A 2.5X increase in sensitivity is observed on the 1.7 µm column compared to a traditional 5 µm column. Additionally, increased resolution is observed on the 1.7 µm column allowing for the identification of additional peaks. Utilizing UPLC™ improves both the quality and the quantity of information collected.

High resolution peptide mapping of phosphorylase b tryptic digest:
Get more information for complex mixtures!

Chromatographic Conditions :
Columns: ACQUITY UPLC™ BEH C18 2.1 x 100 mm, 1.7 µm
Mobile Phase A: 0.02% TFA in H2O
Mobile Phase B: 0.016% TFA in ACN
Flow Rate: 0.1 mL/min
Gradient:

Time (min)	Profile %A	%B
0.0	95	5
120.0	55	35

Injection Volume: 10.0 µL
Sample: MassPREP™ Phosphorylase b Digestion Standard
Sample Diluent: 0.02% TFA in water
Sample Concentration: 100 pmol
Temperature: 38 °C
Detection: UV @ 214 nm
Sampling rate: 20 pts/sec
Time Constant: 0.1
Instrument: Waters ACQUITY UPLC™, with TUV detector



CONCLUSIONS:

For more than 20 years scientists have been experiencing the same fundamental restrictions of HPLC theory and principles. ***ACQUITY UPLC™*** systems dramatically improve the success of scientists with the **next generation technology** of ***Ultra Performance LC™***. This enables unprecedented improvements in productivity, sensitivity and resolution allowing for the ability to get more information faster.

1. Wyndham, K.D., O/Gara, J.E., Walter, T.H., Glose, K.H., Lawrence, N.L., Alden, B.A., Izzo, G.S., Hudalla, C.J. and Iraneta, P.C. Anal, Chem. 2003, 75, 6781-6788