

Advances in Medium Pressure Liquid Chromatography

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Abstract

Advances in medium pressure liquid chromatography (MPLC) column design and particle morphology allow higher loading capacities and higher throughput as well as increased compound purity in both normal and reverse phase chromatography. Example purifications are presented with comparisons to currently existing MPLC columns.

Background

Chromatography has recently trended towards smaller particle size to improve resolution and reduce run time. Medium Pressure Liquid Chromatography (a.k.a. Flash Chromatography) has been slower to adapt smaller particle sizes due to pump and backpressure limitations. The single-use columns are made of inexpensive material with limited pressure capability. The pumps also need to deliver high volume while maintaining an accurate gradient and appropriate backpressure.

Spherical silica allows the use of smaller particles with a narrower particle size range which improves resolution with a minimal increase in back pressure with improved resolution. This improved resolution can be used to purify closely eluting compounds and can be critical when the desire is to save time or solvent.

Experimental

All experiments were run with a CombiFlash Rf MPLC system (Teledyne Isco, Lincoln, NE, P/N 68-5230-006). Columns used were irregular shaped particles for standard flash columns; RediSep Rf Gold columns (20–40 μ , spherical particle) were used as high performance columns except as noted. Other parameters are as noted in the examples.

Conclusion

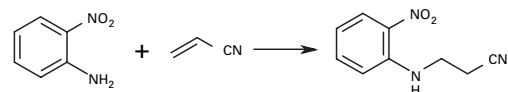
A combination of smaller particle size and spherical particles allows a choice of higher resolution or increased speed when using silica. When using small, spherical particles for reverse phase, a combination of higher resolution and capacity provide product purity near that of prep HPLC.

Example 1

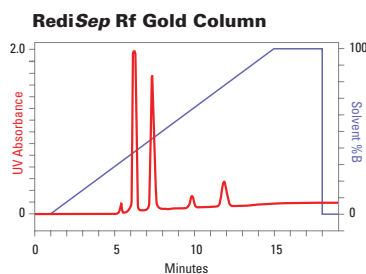
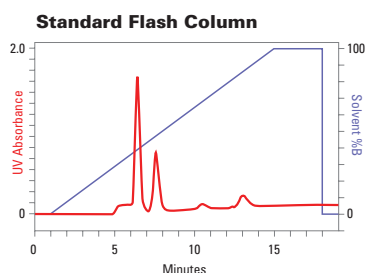
Standard silica gel purification of 3-(2-nitrophenyl amino) propionitrile. This example shows that smaller, spherical particles exhibit higher resolution than standard Flash-grade silica gel. The enhanced resolution causes sharper, taller peaks enabling minor components to be collected more easily.

Run Conditions

Column Size: 40 g
Sample Load: 400 mg
Solvents: Hexane : Acetone
Gradient: 0 – 100%
Flow Rate: 40 mL/min
Run Time: 19 min
Wavelength: 229 nm



The use of spherical media provides twice the resolution compared to standard Flash-grade silica gel columns.

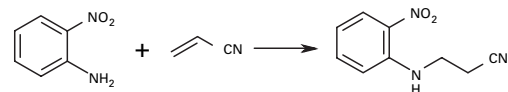


Example 2

Silica gel purification of 3-(2-nitrophenyl amino) propionitrile using conditions optimized for speed and solvent usage.

Run Conditions

Column Size: 40 g
Sample Load: 400 mg
Solvents: Hexane : Acetone
Gradient: 0 – 100%
Flow Rate: 40 mL/min
Run Time: 19 min
Wavelength: 229 nm

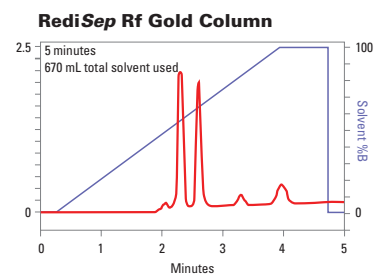
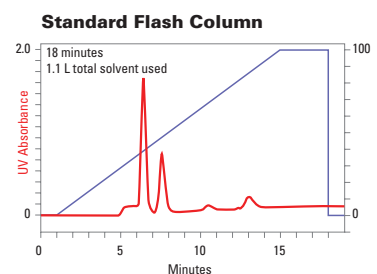


When compared to standard Flash-grade silica and its supporting method, the RediSep Rf Gold column and optimized method demonstrated:

- 60% time savings,
- >30% solvent savings,
- no loss in compound purity.

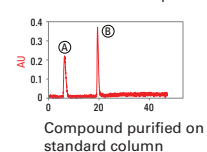
Run Conditions

Column Size: 40 g
Sample Load: 400 mg
Solvents: Hexane : Acetone
Gradient: 0 – 100%
Flow Rate: 80 mL/min
Run Time: 5 min
Wavelength: 229 nm

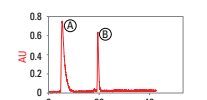


Analytical HPLC

A: Acetone
B: Peak 2 compound



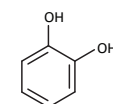
Compound purified on standard column



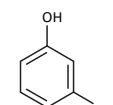
Compound purified on RediSep Rf Gold column
Analytical HPLC showed >98% purity after fast MPLC purification.

Example 3

Comparison of spherical silica to similar size irregular silica under conditions of increased speed.



Catechol

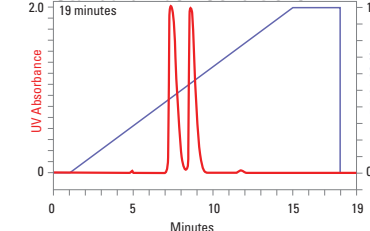


Resorcinol

Run Conditions

Column Size: 40 g
Sample Load: 400 mg
Solvents: Hexane : EtOAc
Gradient: 0 – 100%
Flow Rate: 40 mL/min
Run Time: 19 min
Wavelength: 229 nm

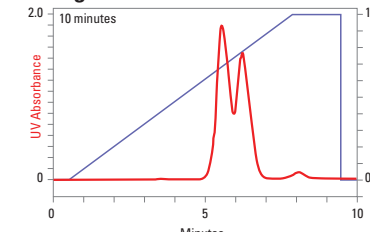
Standard Flash Column, Standard Flash Conditions



Run Conditions

Column Size: 40 g
Sample Load: 400 mg
Solvents: Hexane : EtOAc
Gradient: 0 – 100%
Flow Rate: 40 mL/min
Run Time: 10 min
Wavelength: 229 nm

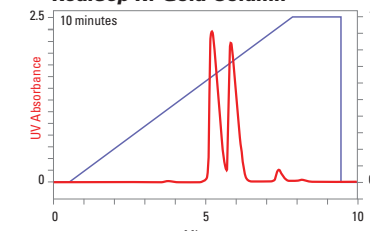
Irregular Shape, Small Particle High Performance Flash Column



Run Conditions

Column Size: 40 g
Sample Load: 400 mg
Solvents: Hexane : EtOAc
Gradient: 0 – 100%
Flow Rate: 40 mL/min
Run Time: 10 min
Wavelength: 229 nm

RediSep Rf Gold Column

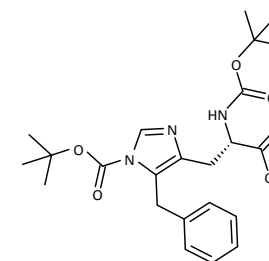


Spherical silica allows improved purification under conditions optimized for higher speed compared to irregular silica (15–40 μ). Catechol and resorcinol can be difficult to resolve¹.

¹ Optimized flash chromatography purification: From TLC to large scale in three steps, Thomason, V., presented at American Chemical Society meeting, Spring 2006.

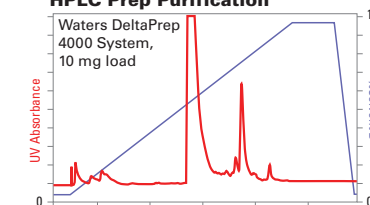
Example 4

C18 purification of (S)-3-[5-benzyl-1-(tert-butoxycarbonyl)-1H-imidazol-4-yl]-2-(tert-butoxycarbonyl-amino)propanoic acid².



HPLC Prep Purification

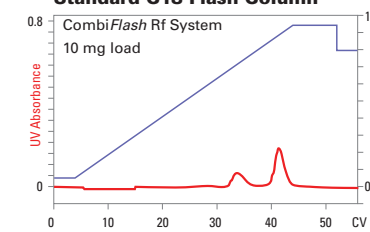
Column: Vydac 10x250 mm, 5 μ
Sample Load: 10 mg
Solvents: H₂O : ACN in 0.1% TFA
Gradient: 5 – 95%
Flow Rate: 5 mL/min
Run Time: 60 min
Wavelength: 214 nm



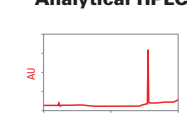
CombiFlash Rf Run Conditions

Column: 13g, (Std)
Sample Load: 10 mg (Std)
Solvents: H₂O : ACN in 0.1% TFA
Gradient: 5 – 95%
Flow Rate: 35 mL/min
Run Time: 20 min
Wavelength: 214 nm

Standard C18 Flash Column



Analytical HPLC

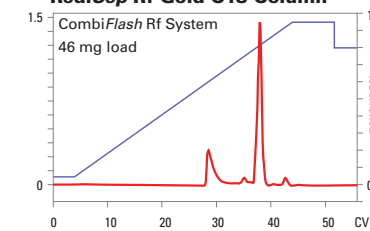


Compound purified by RediSep Rf Gold C18

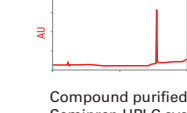
CombiFlash Rf Run Conditions

Column: 15.5g (Gold)
Sample Load: 46 mg (Gold)
Solvents: H₂O : ACN in 0.1% TFA
Gradient: 5 – 95%
Flow Rate: 35 mL/min
Run Time: 20 min
Wavelength: 214 nm

RediSep Rf Gold C18 Column



Analytical HPLC



Compound purified on Semiprep HPLC system

The smaller, spherical particles in the RediSep Rf Gold C18 allow twelve times the productivity of a similar size prep HPLC column. Four times the material can be loaded and run in one-third of the time to obtain product with similar purity.

²The assistance of Dr. D. Smith of Creighton University is gratefully acknowledged.