Increase Sample Purity with GraceResolv™ High Resolution Flash Cartridges

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GRACE

Abstract

The demand for fast and easy purification techniques is increasing. Flash chromatography can be a fast, yet crude methodology for preparative separations. To satisfy the need for flash cartridges with better resolution and higher loading capacity, GraceResolv™ high-resolution flash cartridges have been developed by Grace Davison Discovery Sciences. The silica packed in GraceResolv™ cartridges was specifically designed (patent pending) to increase cartridge efficiency (plates/meter) and reduce unwanted mixed-mode interactions thereby increasing resolution of closely eluting compounds. This results in an increase in sample recovered at the desired purity level. The increase in resolution also enables a significant increase in loading capacity, over two times greater in some cases, versus traditional flash cartridges. By purifying greater amounts on a given cartridge size, users can reduce purification costs and increase productivity.

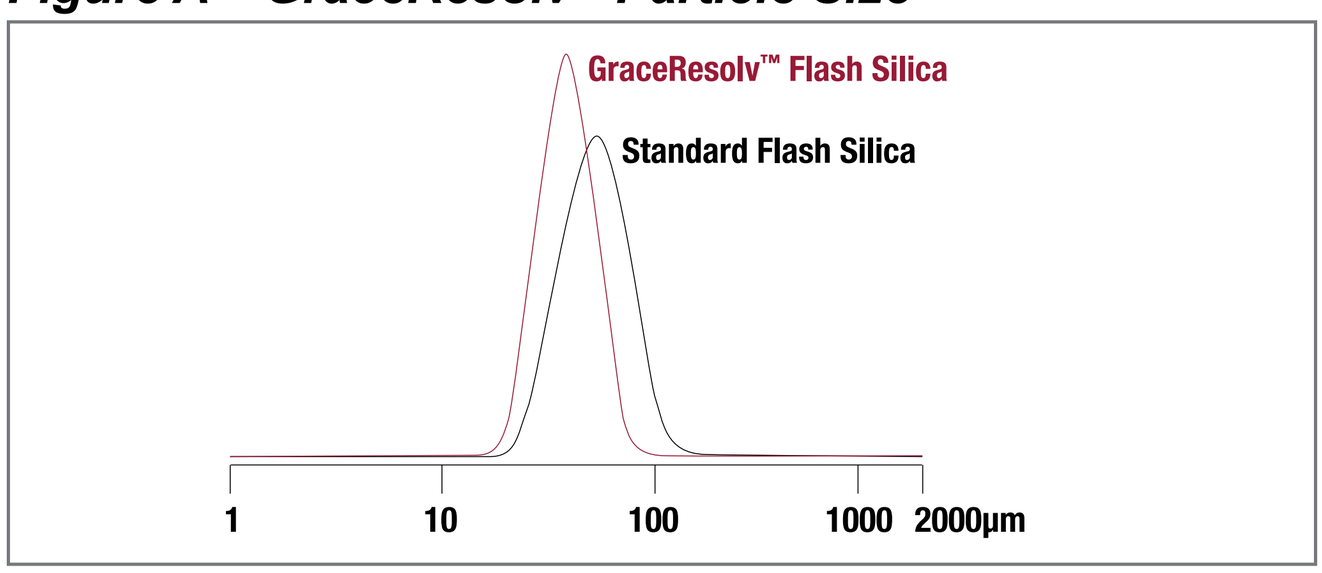
Introduction

Flash chromatography, also known as medium pressure chromatography was popularized several years ago as an alternative to slow and often inefficient gravity-fed chromatography. Today, flash chromatography has advanced considerably by introducing automated instruments using pre-packed flash chromatography cartridges eliminating the time-spent packing. The one area of flash chromatography that has seen little advancement is the heart of the separation, the silica.

Most cartridges today are packed with the same 40-60µm irregular shaped silica that was used for self-packed glass columns in the 1970s. The benefit of this type of silica is that it is inexpensive and generates low backpressure however it only provides a marginal level of resolution. Grace, as a silica manufacturer, and with extended chromatography knowledge is applying new technologies to the field of flash chromatography.

The silica used in GraceResolv™ cartridges is smaller in particle size, centered around 40um,with a more narrower particle size distribution than standard flash silica. The smaller particle size increases the efficiency of the cartridge resulting in higher resolution. While the narrow particle size distribution (**Figure A**) reduces fines (small particles less than 5um) which keeps backpressure low. The GraceResolv™ silica also undergoes a proprietary process to remove metals from the silica surface resulting in less peak tailing especially for metal chelator compounds (**Figure B**).

Figure A – GraceResolv™ Particle Size



A smaller particle size increases resolution. Tighter particle size distribution and minimal fines (small particles) keep backpressure low. Measured by Malvern® Mastersizer® 2000.

Figure B – GraceResolv™ Silica Purity

Percentage of Metal Content				
Silica	**Fe_2O_3**	**Na_2O**	**Al_2O_3**	**SO_4**
GraceResolv™	0.001	0.01	0.02	0.001
Competitive Standard	0.005	0.07	0.05	0.01

Grace's flash silica undergoes a proprietary process to reduce metals from the silica surface by over 50%. Metals cause peak tailing and reduce resolution especially for metal chelator compounds.

Purpose

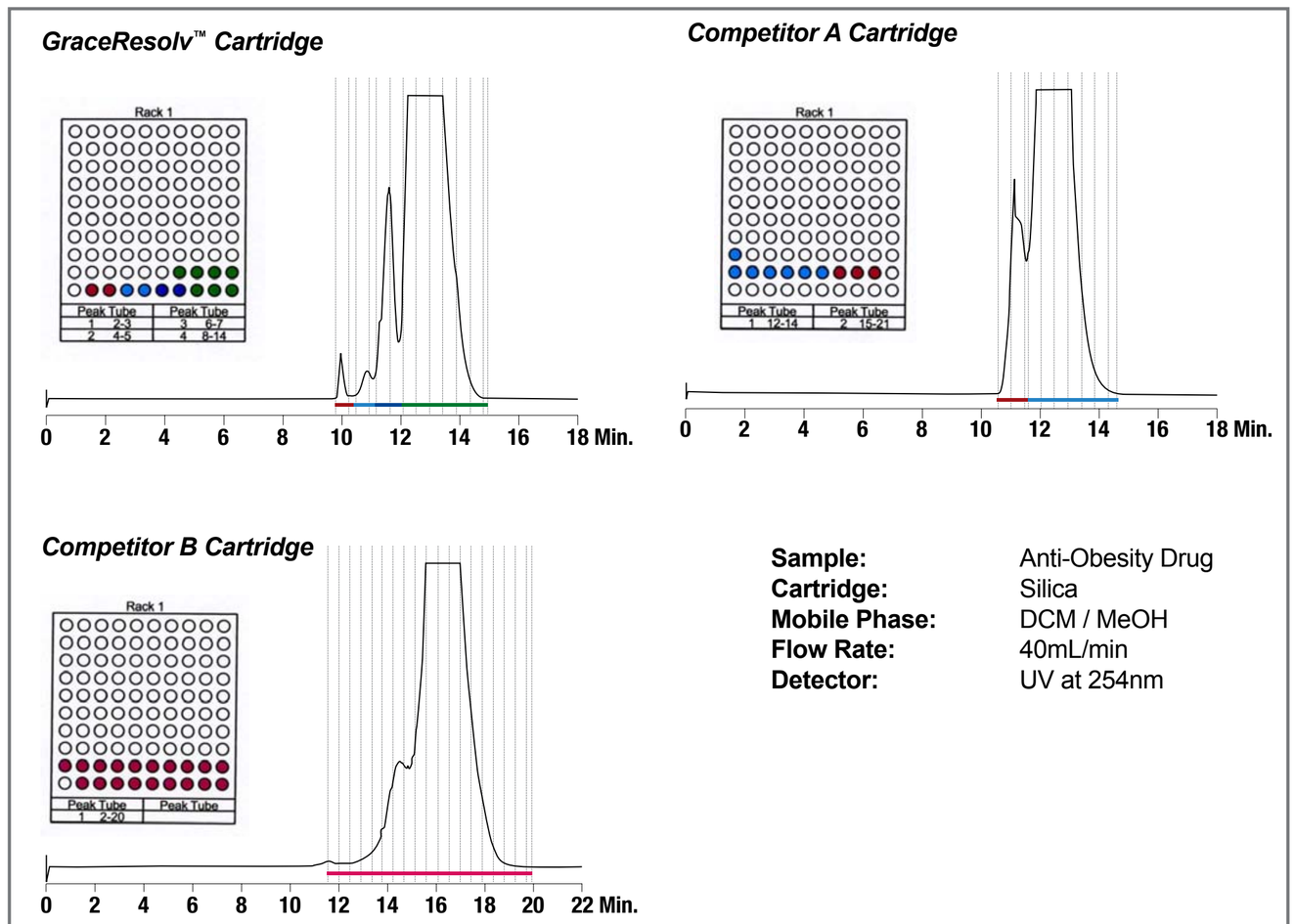
To demonstrate the advantages of higher resolution flash cartridges we compared GraceResolv™ cartridges to leading competitive cartridges. Experiments designed to test sample resolution, loading capacity, recovery, and throughput were performed.

Results and Discussion

Figure 1 demonstrates GraceResolv™ cartridges superior resolution of closely eluting impurities, separating two additional impurity compounds from the target drug and better resolving the main impurity verses leading competitor cartridges. This can dramatically increase the yield of high purity fractions as demonstrated in **Figure 2**, the purification of α from δ tocopherol. The increase in resolution also allows for higher loading capacity while maintaining base-line separation. This can enable chemists to use smaller cartridge sizes to purify the same amount of material typically purified on larger competitor cartridges reducing consumable costs (**Figure 3**). The combination of higher resolution and loading capacity also can enable chemists to increase flow rate and still maintaining baseline resolution, saving time (**Figure 4**).

Figure 1

Stage 1 Anti-obesity compound purified from synthetic byproducts (Data courtesy of Cambridge Biotechnology Ltd, part of the Biovitrum Group)



	Grace	Comp A	Comp B
Number of compounds resolved from target drug (computer calculated)	3	1	0
Number of fractions to analyze for purity	7	7	19

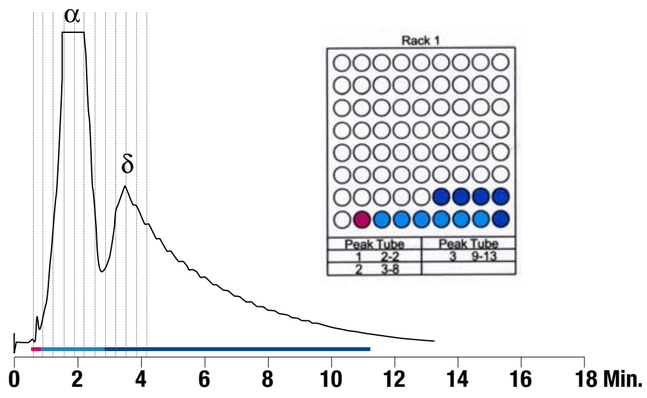
Increase sample purity using GraceResolv™ cartridges

Comparison of GraceResolv™ 40g flash cartridges to Competitor A 40g cartridge and Competitor B 50g cartridge demonstrates the increase in resolution possible with GraceResolv™ cartridges. The GraceResolv™ cartridge was able to separate 3 distinct impurity peaks and trigger 4 different fraction sets. The Competitor A cartridge could only separate 1 distinct impurity peak and trigger 2 fraction sets, while the Competitor B cartridge could not trigger a separate fraction collection from the main peak.

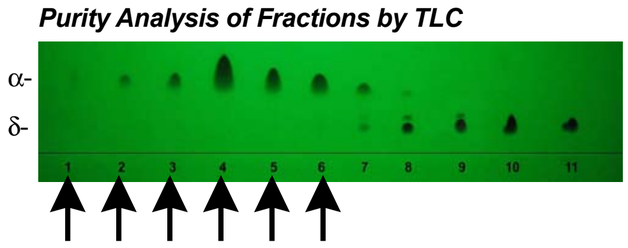
Figure 2

Purification of α-tocopherol from δ-tocopherol

GraceResolv™ Cartridge

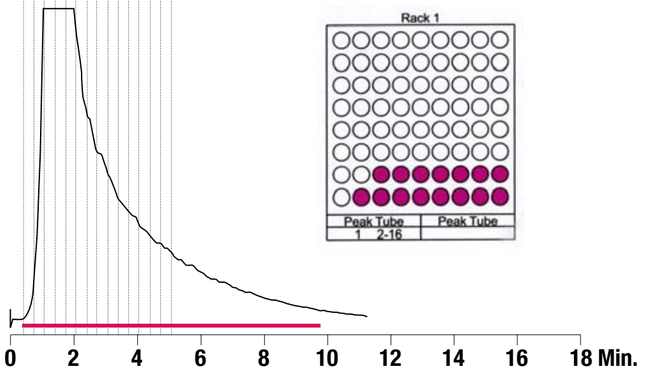


Purity Analysis of Fractions by TLC

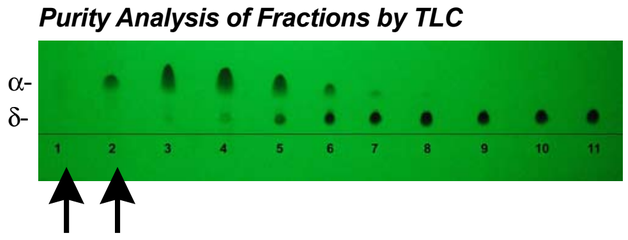


Number of Pure Fractions: 6

Competitor A Cartridge

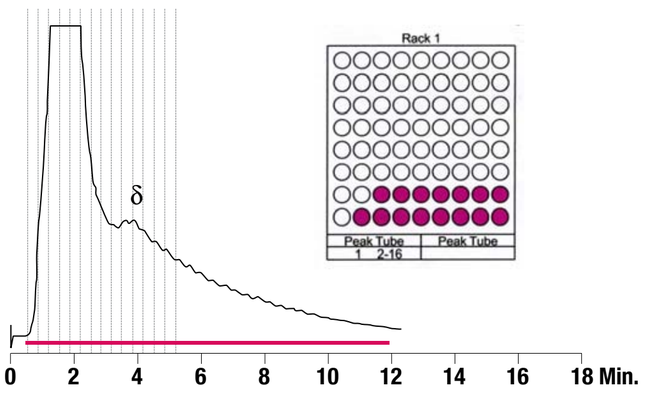


Purity Analysis of Fractions by TLC

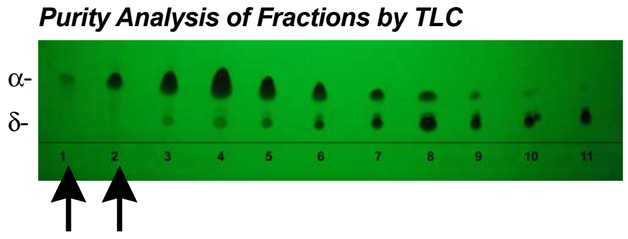


Number of Pure Fractions: 2

Competitor B Cartridge



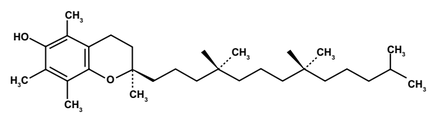
Purity Analysis of Fractions by TLC



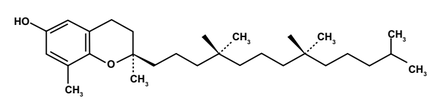
Number of Pure Fractions: 2

Instrumentation:	CombiFlash® Companion (Teledyne Isco®)
Flash Cartridge:	12 gram, silica
Wavelength:	295nm
Isocratic:	**Solvent A:** Hexane 97% **Solvent B:** THF 3%
Sample:	500mg/mL
Sample Inject Volume:	4mL (16.8% Loading)
Flow Rate:	30mL/min

α-Tocopherol



δ-Tocopherol



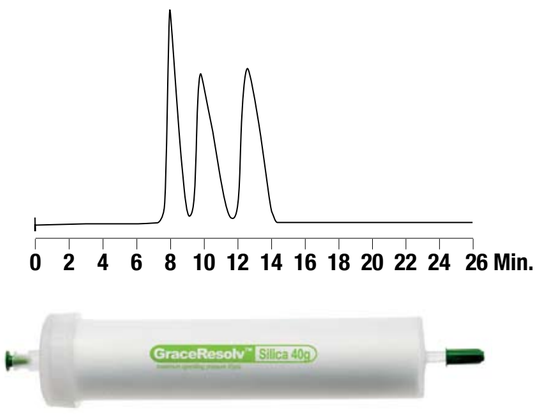
Recover more pure fractions using GraceResolv™ cartridges

Higher resolution and greater loading capacity enables the GraceResolv™ 12g cartridge to purify a 2g injection of α-tocopherol from δ-tocopherol. 6 pure fractions (as analyzed using TLC) were recovered from the GraceResolv™ cartridge compared to only 2 pure fractions from the Competitor A and Competitor B cartridge.

Figure 3

Loading comparison of a 3.35g Phthalate mixture: GraceResolv™ 40g cartridge vs Competitor 80g cartridge.

GraceResolv™ 40g Cartridge

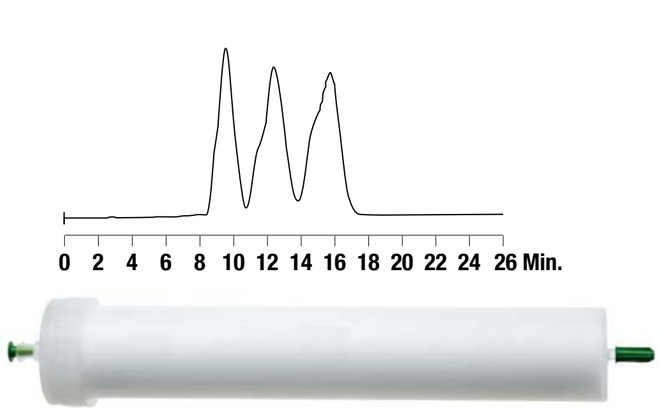


Instrumentation: CombiFlash® Companion (Teledyne Isco®)
Flash cartridge: GraceResolv™ 40gram
Wavelength: 254nm
Gradient: **Solvent A:** Hexane, **Solvent B:** Ethyl Acetate

Time:	0	2	18	20
%B:	0	0	25	25

Sample: 500mg/mL Containing: Di-Methyl Phthalate, Di-Ethyl Phthalate, Di-Butyl Phthalate
Sample Inject Volume: 6.7mL (8.4% Loading)
Flow Rate: 40mL/min

Competitor 80g Cartridge

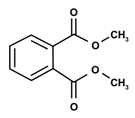


Instrumentation: CombiFlash® Companion (Teledyne Isco®)
Flash Cartridge: Competitor 80gram
Wavelength: 254nm
Gradient: **Solvent A:** Hexane, **Solvent B:** Ethyl Acetate

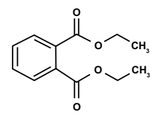
Time:	0	3	19	20
%B:	0	0	25	25

Sample: 500mg/mL Containing: Di-Methyl Phthalate, Di-Ethyl Phthalate, Di-Butyl Phthalate
Sample Inject Volume: 6.7mL (8.4% Loading)
Flow Rate: 60mL/min

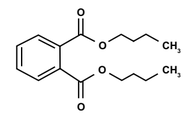
Dimethyl Phthalate



Diethyl Phthalate



Dibutyl Phthalate



As demonstrated above, the higher loading capacity of the GraceResolv™ cartridge, allows users to purify amounts previously requiring a 80g cartridge on a 40g GraceResolv™ cartridge. The ability to use smaller cartridges results in significant savings. Typical costs were tabulated below to demonstrate the savings a user would likely experience. The cost savings associated with using a smaller cartridge size not only include the savings of using a less expensive smaller cartridge, but also the reduction in solvent and time. This downsize of cartridge size saves 30% in solvent and cartridge costs. A 38% reduction in the volume of solvent requiring disposal is an additional benefit not included in this calculation.

Table 1

Cost Calculation

	40g Flash Cartridge		Competitor 80g Flash Cartridge		Cost Savings	
	Amount	Cost (USD)	Amount	Cost (USD)	$ (USD)	% Savings
Solvent Cost (equilibration & run time)	869mL	$37.37	1, 407mL	$60.50	$23.13	38%
Cartridge Cost*	1 Each	$22.71	1 Each	$25.50	$2.79	11%
Total Cost to Run Sample	4g	$60.08	4g	$86.00	$25.92	30%

Cartridge cost based on list price found at www.fishersci.com

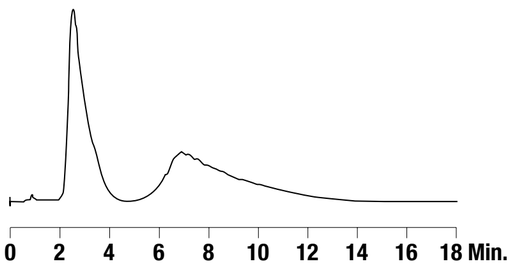
Reduce costs using smaller GraceResolv™ cartridges

3.36g (8.4%) loading on 40 gram GraceResolv™ cartridge has a better resolution then an identical loading of 3.36g (4.2%) on an 80 gram Competitor A cartridge. This indicates a >2X loading capacity and the ability to down size the cartridge reducing cartridge costs and solvent consumption.

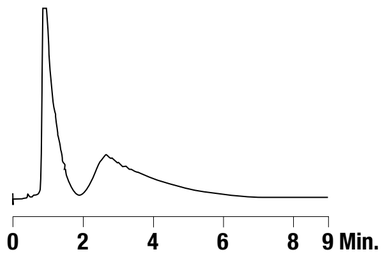
Figure 4

Flow rate comparison in the purification of α from δ-tocopherol.

Conventional Flow Rate
12g Cartridge: 30mL/min
Run Time: 18min



2x Faster Flow Rate
12g Cartridge: 60mL/min
Run Time: 9min



Instrumentation:	CombiFlash® Companion Touch screen (Teledyne Isco®)
Flash cartridge:	GraceResolv™ 12 gram,
Wavelength:	295nm
Isocratic:	**Solvent A:** Hexane 97% **Solvent B:** THF 3%
Sample:	500mg/mL
Sample Inject Volume:	2mL

Save time with faster purifications using GraceResolv™ cartridges
Higher resolution of GraceResolv™ cartridges enables the flow rate to be increased 2X while maintaining base-line separation. For this application, that results in over a 40% time savings per run.

Conclusion

GraceResolv™ cartridges are packed with a new silica (patent pending) that results in superior resolution and loading capacity versus the leading competitors. Increased resolution and loading capacity give GraceResolv™ cartridges:

- 3X increase in pure compound recovered
- 2X faster purification maintaining baseline resolution
- 30% reduction in consumable costs