Improved Resolution of Closely Related Organic Compounds with Reveleris Flash Cartridges

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Abstract

The need for high purity compounds in drug discovery continues to expand as higher throughput purification techniques encourage better ways to discover new drugs and other materials. Flash chromatography is one well-accepted purification technique that can produce high purity compounds. The silica packed in Reveleris™ cartridges is specifically designed to increase cartridge efficiency and therefore resolution of closely eluting compounds, in some cases greater than two times better resolving power over typical packings. Greater efficiency enables more peaks to be baseline resolved and results in increased sample purity and decreased fraction volumes. Additionally, by increasing sample loads on a given cartridge size and elevated pressure capability, sample throughputs and yields increase and overall purification costs are lowered.

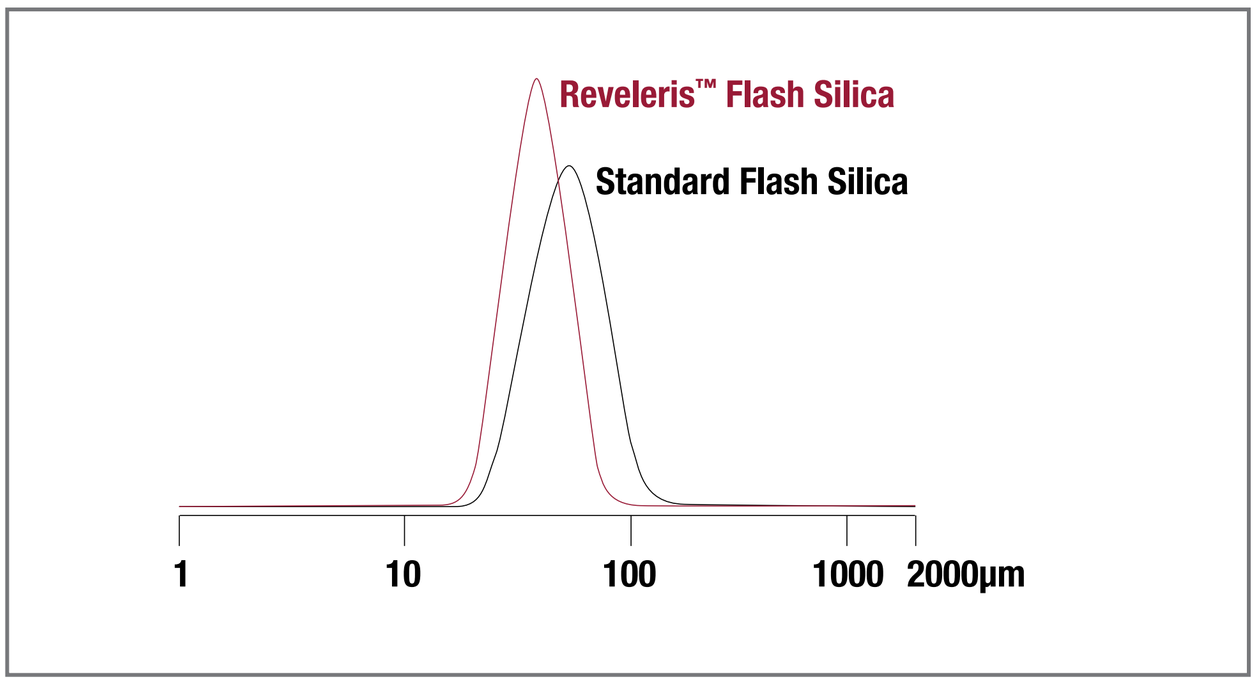
Introduction

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, irregular shaped silica 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 Reveleris™ cartridges is smaller in particle size, centered around 40µm, 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. The narrow particle size distribution (**Figure A**) reduces fines (small particles less than 5µm), which keeps backpressure low. The Reveleris™ 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 – Reveleris™ Silica 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 instrument.

Figure B – Reveleris™ Silica Purity

Percentage of Metal Content				
Silica	**Fe_2O_3**	**Na_2O**	**Al_2O_3**	**SO_4**
Reveleris™	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 Reveleris™ cartridges to leading competitive cartridges. Experiments designed to test sample resolution, loading capacity, recovery, and throughput were performed.

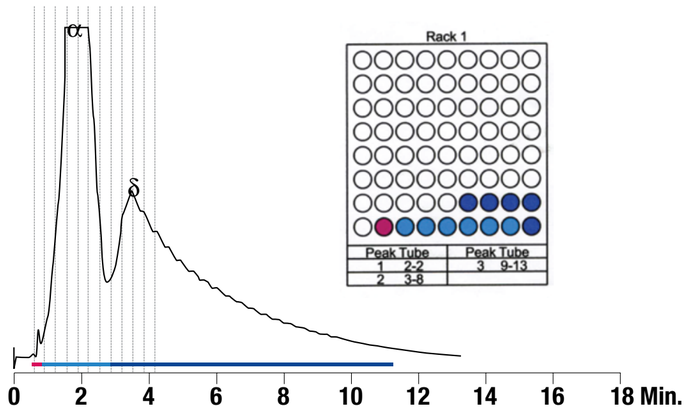
Results and Discussion

The Reveleris™ cartridge's superior resolution of closely eluting compounds dramatically increases the yield of high purity fractions as demonstrated in **Figure 1**, 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 2**). The combination of higher resolution and loading capacity also can enable chemists to increase flow rate while still maintaining baseline resolution, which saves time (**Figure 3**). To demonstrate the higher resolution of Reveleris™ flash cartridges improvements on speed, we compared Reveleris™ flash cartridges to a leading competitive cartridge. A tighter particle size distribution and increased capacity for Reveleris allows a 3x increase in flow rate while maintaining resolution of >1 (**Figure 4**).

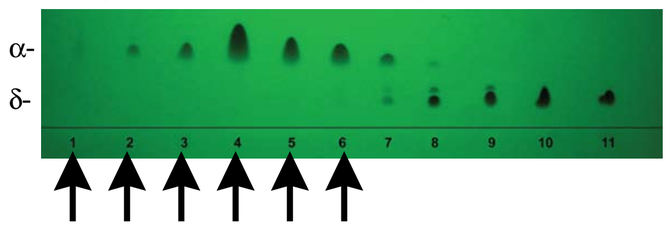
Figure 1

Purification of α-tocopherol from δ-tocopherol

Reveleris™ Cartridge

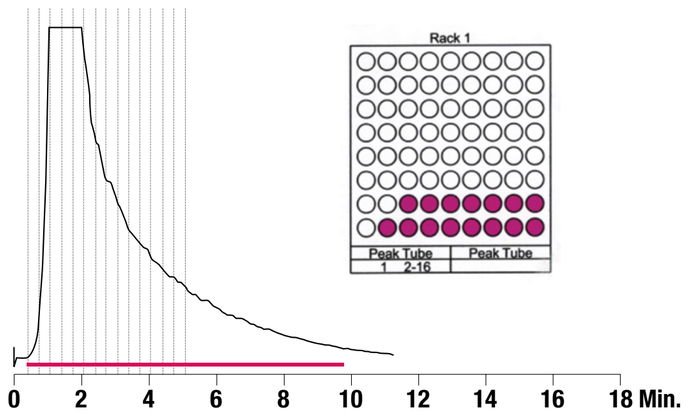


Purity Analysis of Fractions by TLC

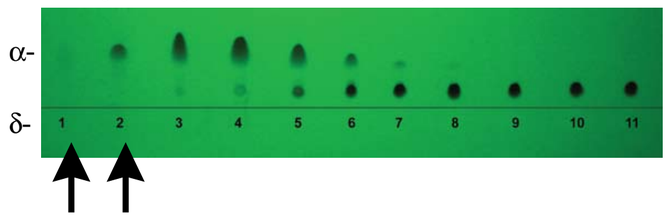


Number of Pure Fractions: 6

Competitor A Cartridge

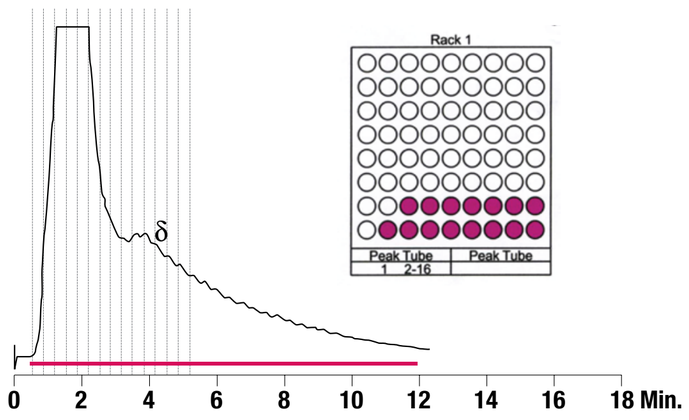


Purity Analysis of Fractions by TLC

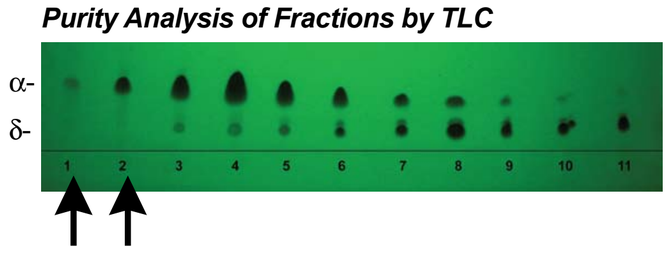


Number of Pure Fractions: 2

Competitor B Cartridge



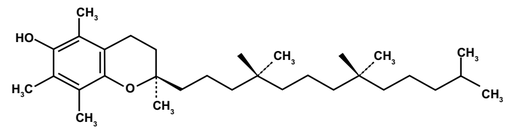
Purity Analysis of Fractions by TLC



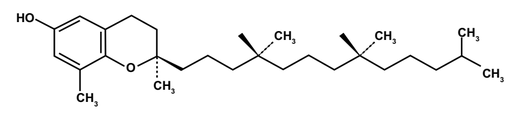
Number of Pure Fractions: 2

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



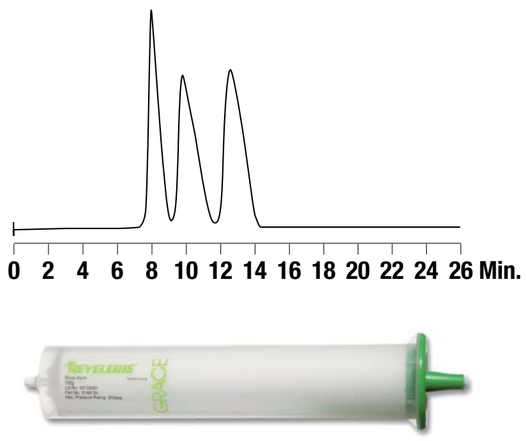
Recover more pure fractions using Reveleris™ cartridges

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

Figure 2

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

Reveleris™ 40g Cartridge

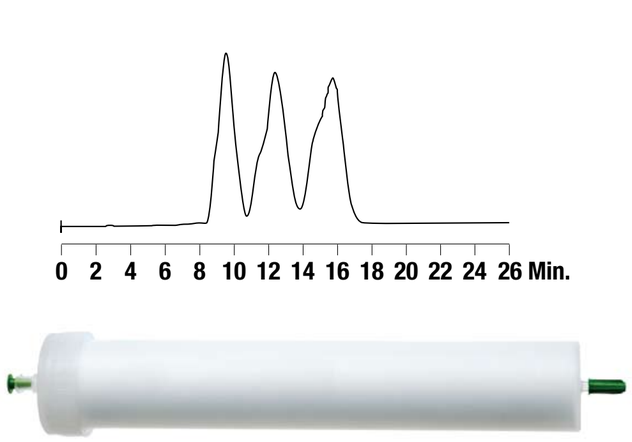


Instrumentation: CombiFlash® Companion (Teledyne Isco®)
Flash cartridge: Reveleris™ 40gram
Wavelength: 254nm
Gradient: **Solvent A:** Hexane, **Solvent B:** Ethyl Acetate

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

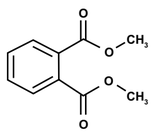


Instrumentation: CombiFlash® Companion (Teledyne Isco®)
Flash Cartridge: Competitor 80gram
Wavelength: 254nm
Gradient: **Solvent A:** Hexane, **Solvent B:** Ethyl Acetate

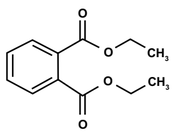
Time:	0	3	19	20
%B:	0	0	25	25

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

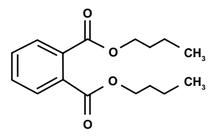
Dimethyl Phthalate



Diethyl Phthalate



Dibutyl Phthalate



As demonstrated above, the higher loading capacity of the Reveleris™ cartridge allows users to purify amounts previously requiring a 80g cartridge on a 40g Reveleris™ 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%

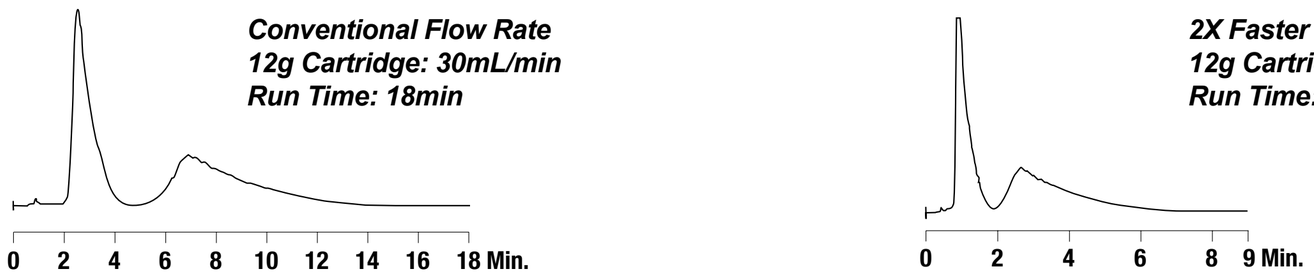
Cartridge cost based on list price found at www.fishersci.com

Reduce costs using smaller Reveleris™ cartridges

3.36g (8.4%) loading on 40 gram Reveleris™ 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 3

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



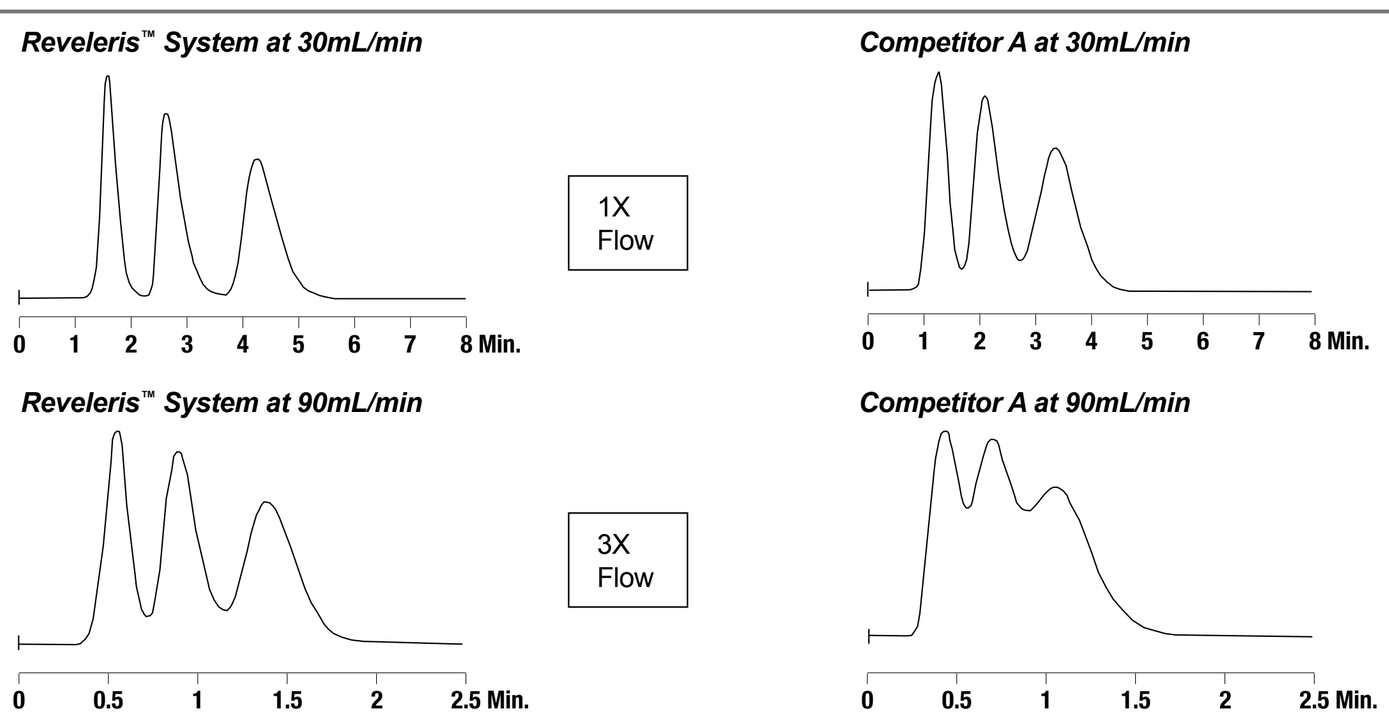
Instrumentation: CombiFlash® Companion Touch screen (Teledyne Isco®)
Flash cartridge: Reveleris™ 12 gram
Wavelength: 295nm
Isocratic: **Solvent A:** Hexane 97%
Solvent B: THF 3%
Sample: 500mg/mL
Sample Inject Volume: 2mL

Save time with faster purifications using Reveleris™ cartridges

Higher resolution of Reveleris™ 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.

Figure 4

High speed separations.



Cartridge: 40g
Sample: Di-Methyl Phthalate, Di-Ethyl Phthalate, Di-Butyl Phthalate
Isocratic: **Solvent A:** Hexane 90%
Solvent B: Ethyl Acetate 10%
Wavelength: 254nm
Sample Inject Volume: 4mL

The improved efficiency and capacity of Reveleris™ cartridges increases initial resolution compared to leading competitive cartridges. By increasing flow rate, analysis times are shortened while still maintaining resolution.

Conclusion

Reveleris™ 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 Reveleris™ cartridges:

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

The benefits of the Reveleris™ cartridges can be further maximized when used in combination with the new Reveleris™ Flash Chromatography System.

- Reveleris™ cartridges incorporate Reveal**X**™ Technology (patents pending) – A universal detection system for better compound detection
- State of the art fraction collection