



# **Application of Flash Chromatography in Purification of Natural and Synthetic Products**

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# Abstract

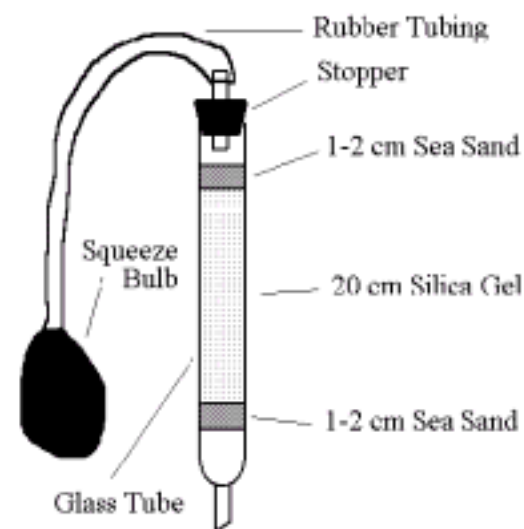
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**Flash chromatography is widely used for purification of low molecular weight natural compounds and products of organic synthetic reactions. Modern flash techniques include the use of convenient disposable flash cartridges instead of glass columns. Flash purification systems allow users to speed up the purification process for quicker results and higher throughput. A new High Throughput Flash Purification (HTFP) system - VersaFlash has been used to purify natural plant pigments, synthetic organic products and synthetic peptides.**

# Introduction

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**Glass column packed with silica gel is widely used to purify synthetic compounds in many labs. It is simple and easy to use, but it is time-consuming, irreproducible and difficult to pack a good column.**





# Disposable Cartridge – Time-Saving Flash Chromatography

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- In 1978 Clark Steel demonstrated that purification in glass columns may be carried out at high flow rates – up to 5cm/min. This approach was named flash chromatography (1).
- As a result, purification becomes much faster. Bottleneck in flash chromatography was repacking of glass columns
- Situation has been changed with the introduction of disposable plastic cartridges in combination with appropriate holders



# VersaFlash Stand and VersaPac Cartridges

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**VersaFlash is a convenient and flexible system for modern flash chromatography**

- **One stand fits cartridges of four different sizes – from 75 x 40mm up to 300 x 80mm, from 50 up to 700g of silica, from milligrams to tens of grams of sample (1300g silica cartridge will be available by end of June)**
- **Scale-up is possible in seconds**
- **Cartridges are bi-directional – RevElution allows to decrease elution volume and sample concentration time**

# VersaFlash Stand and VersaPac Cartridges



**VersaFlash Stand**



**VersaPac Cartridges**

# VersaFlash Stand and VersaPac Cartridges (cont.)

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- Cartridges are stackable
- Different chemistries may be combined in a single run
- Specific separated zone may be collected on smaller cartridge and further purified under different conditions

# VersaFlash - Sample Loading Options

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- Direct injection using Luer syringe (1)
- Loading of diluted sample through pump
- Loading multiple cartridges using VersaVac station (2)
- Solid sample cartridges for samples diluted in strong solvents (3)

1



2



3

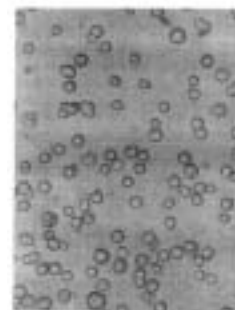


# VersaPac Cartridges – Spherical Silica, High Capacity

- **VersaPac spherical silica provides high capacity and low backpressure**

40 x 75mm cartridges packed with Supelco, competitors and bulk silica	Capacity, g of benzyl alcohol per cartridge in break-through test
Supelco	4.6
Competitor 1	3.0
Competitor 2	3.6
Competitor 3	3.3
Bulk silica 1	3.4
Bulk silica 2	4.6

**Supelco VersaPac Silica**



**Competitor 1 Silica**

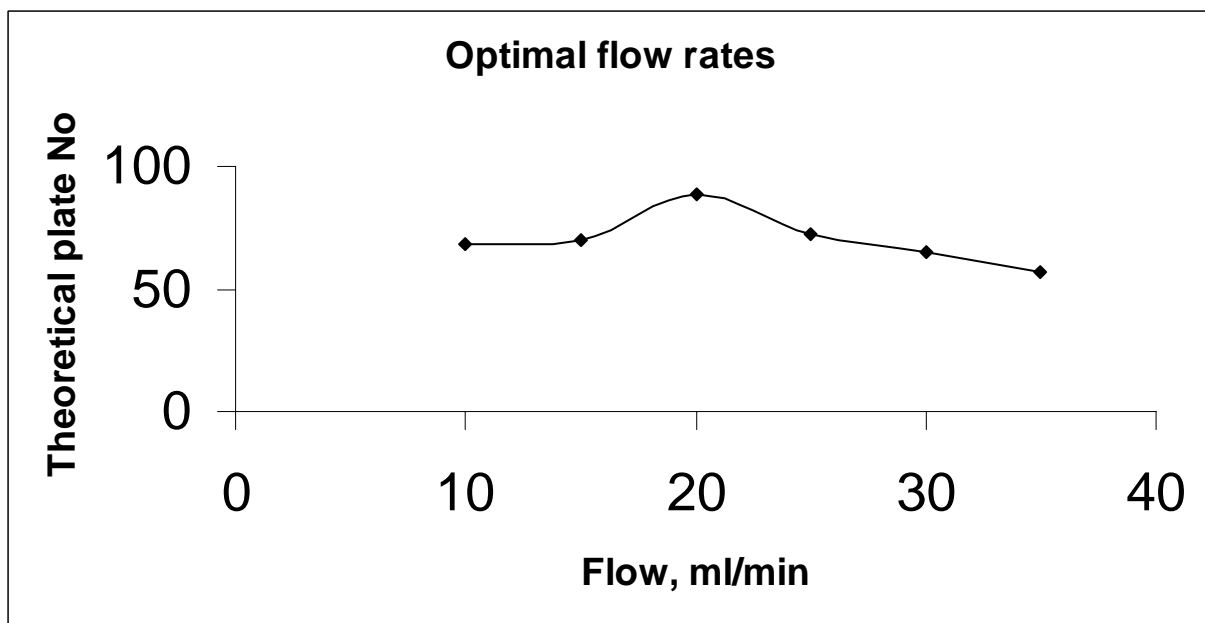


**Competitor 2 Silica**



# Selection of Optimal Flow Rate

- Optimal linear flow rate is 2cm/min.
- Practically it means 20mL/min for 40mm ID cartridges and 80mL/min for 80mm ID cartridges.
- Flow rate may be increased up to 80mL/min for 40mm ID cartridges and up to 300mL/min for 80mm ID cartridges for fast separation.



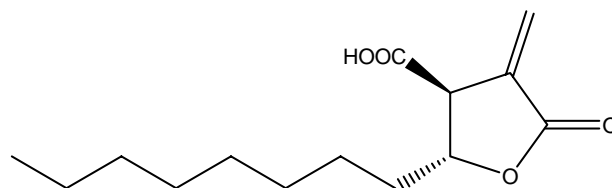
Data obtained using  
75 x 40mm ID Si  
cartridge

Mobile Phase:  
MeOH/CH<sub>2</sub>Cl<sub>2</sub>, 1/10

Sample: benzyl  
alcohol

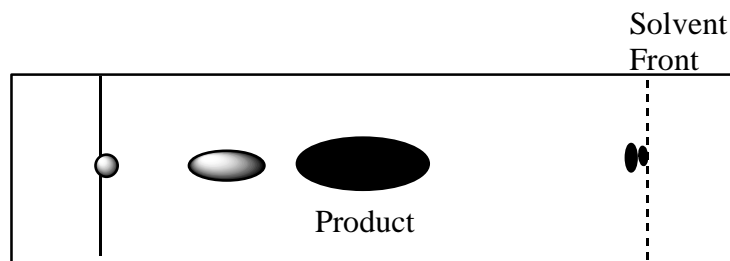
# Application 1.

## Purification of Synthesized Compounds



VersaFlash Cartridge	40 x 150mm Silica
Amount of Crude Loaded	3.7g
Solvent System = isocratic	Hex:EtOAc:HOAc (80:20:1) 50mL/min
Amount of Product Recovered	2.3g
Purity of Product Recovered	~85%
Time Start to Finish	3 hours to run, check & combine

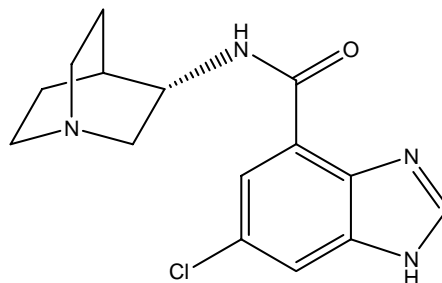
**TLC Plate**  
(visualization by iodine)



Baseline  
Impurity

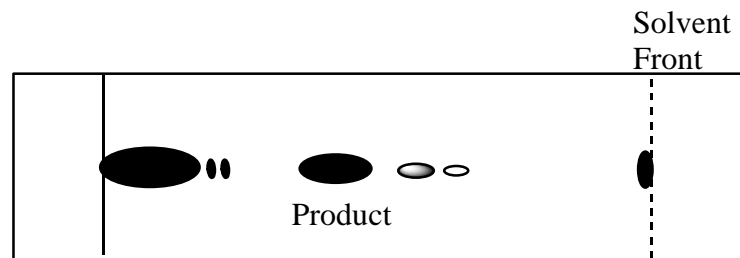
# Application 1.

## Purification of Synthesized Compounds (contd)



VersaFlash Cartridge	40 x 75mm Silica
Amount of Crude Loaded	0.3g
Solvent System – Step Gradient, 50mL/min	DCM:MeOH(100:0) to DCM:MeOH:NH <sub>4</sub> OH (85:15:1)
Amount of Product Recovered	0.1g
Purity of Product Recovered	~90%
Time Start to Finish	2 hours to run, check & combine

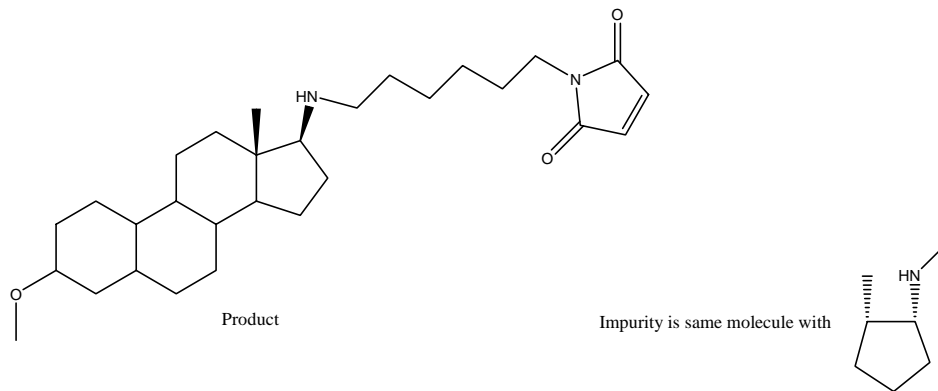
TLC Plate



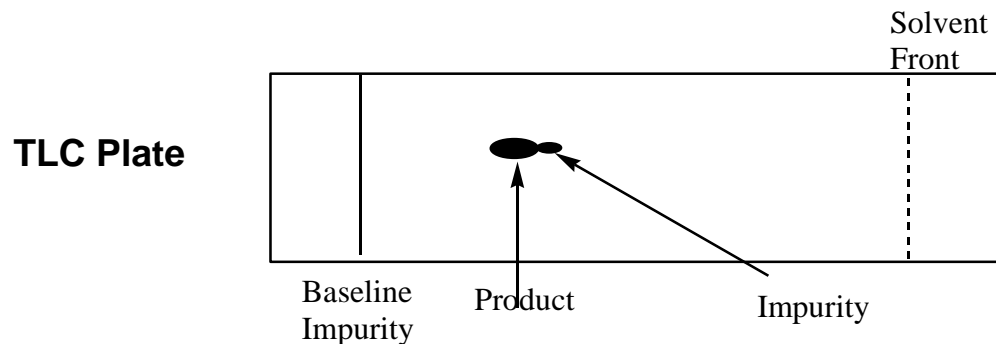
Baseline  
Impurity

# Application 1.

## Purification of Synthesized Compounds (contd)

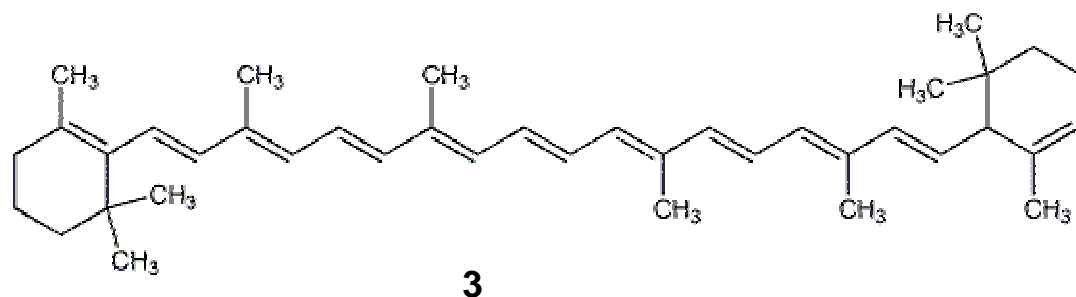
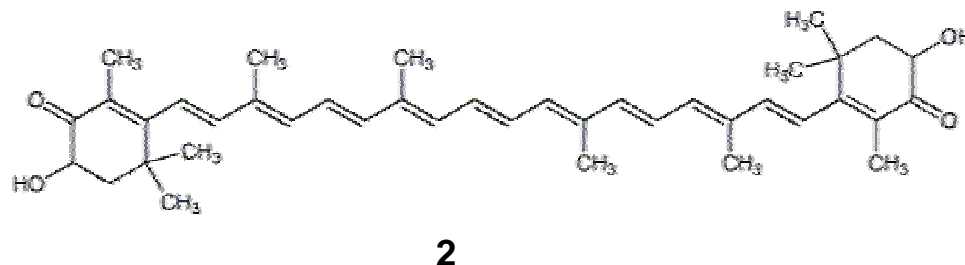
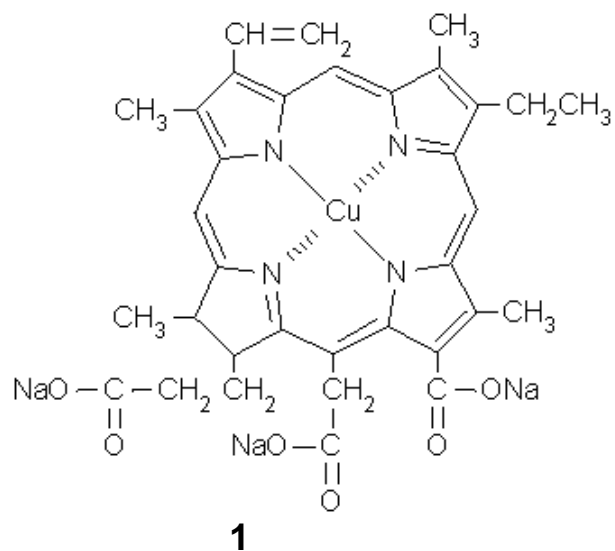


VersaFlash Cartridge	300 x 80mm Silica
Amount of Crude Loaded	~ 20g
Solvent System Isocratic , 50mL/min	3% (9:1 MeOH, NH <sub>4</sub> OH) DCM
Amount of Product Recovered	~ 2g of pure, ~ 7g isomer mix
Time Start to Finish	6 hrs



# Application 2. Natural Plant Pigments - Carotenoids & Chlorophylls

- Chlorophylline (1), astaxanthene (2), and carotene (3) represents typical groups of plant pigments. Mixture of these compounds was separated by flash purification on a C18 VersaPak cartridge.



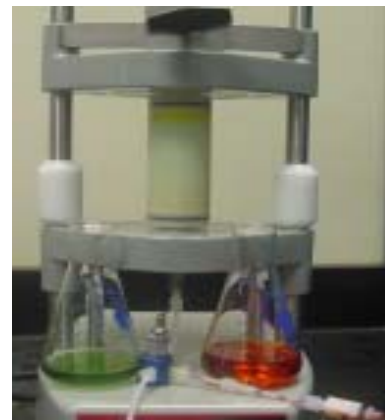
## Application 2 (contd)

- A 75 x 40mm C18 flash cartridge was pre-wetted with water. The sample (0.5mL) was injected. The green chlorophylline band was eluted with ~120mL of 60% methanol in water, and astaxanthene was eluted with ~140mL of 100% methanol, while the carotene band stayed essentially at the origin. It was eluted by reversing the cartridge and then using ~100mL of methylene chloride as mobile phase.



Chlorophylline (green - 1) & Astaxanthene (red - 2) eluted - Carotene (yellow - 3) remains at the origin.

Invert  
Cartridge



Cartridge is inverted 180° to elute carotene (yellow - 3) more quickly.



## **Application 3. Synthetic Peptide Purification**

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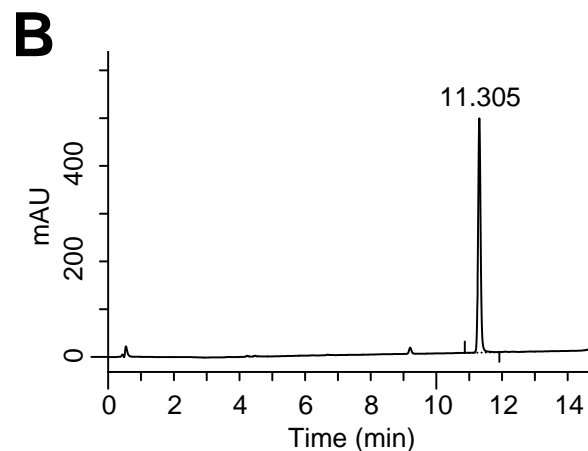
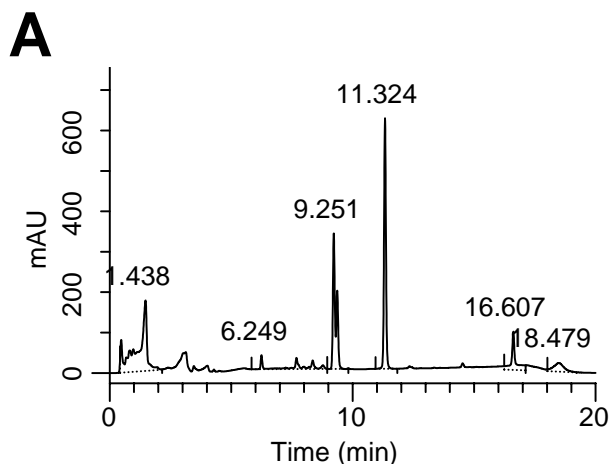
**Synthetic peptide V Q A A I D Y I N G was separated on a 75 x 40mm C18 cartridge using step gradient:**

- 1. 150mL water**
- 2. 150mL 60% acetonitrile**
- 3. 100mL 100% acetonitrile in RevElution mode**

**Fractions were collected, evaporated and analyzed by reversed-phase HPLC.**

# Application 3 (contd)

HPLC analysis of initial and purified peptide demonstrate 95+% purity and >75% yield



Initial (A) and purified (B) peptide

Column: Discovery C18, 50cm x 2.1mm ID

Flow Rate: 0.5mL/min

Detection: UV 254nm, eluent A – water, eluent B – 100% acetonitrile

Linear gradient from 0 to 100% B in 15 min.



# Conclusions

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- **The new flash system with bidirectional cartridges provides reverse-direction for faster elution of highly retained compounds.**
- **Scale-up on larger cartridges can be accomplished using the same flash station.**
- **Stacking capability allows one to increase system performance or combine cartridges with different sorbents (bi-modal NP and RP).**
- **Cartridges can be changed in and out in less than 10 seconds in this novel design**
- **Spherical silica allows cleaner separation.**

## Reference

1. **W.C. Still, M. Kahn, and A. Mitra, *J. Org. Chem.*, 1978, 43, 2923-2925.**