

# Scaling Up Purifications using Reversed Phase Flash Chromatography

Panagiotis Ioannidis, Biotage

This study shows how to scale-up purifications using reversed phase flash chromatography. We demonstrate a 33-fold scale increase in both column and loading quantity.

## Introduction

Reversed phase flash chromatography is a very effective purification technique. Its main application areas include polar, ionizable and highly lipophilic compounds which cannot easily be separated by normal-phase techniques.

Unlike normal phase chromatography, reversed phase uses a hydrophobic stationary phase (e.g. C18 or ODS) and hydrophilic mobile phases (methanol/water, acetonitrile/water). By converting the silica's active, polar silanols sites to neutral, lipophilic sites, compounds that will either aggressively stick to silica or not stick at all can be retained, separated and eluted using water-based solvent systems. Thus KP-C18-HS is more like RP-HPLC than traditional glass column flash chromatography.

For that reason, it is sometimes difficult to visualize the best conditions to run or scale-up reversed phase systems, especially when transferring purification methods, where normal phase methods are typically used.

## Experimental Summary

Experiments were conducted with sample loadings ranging from 80 mg to 2.7 g using three different flash cartridge sizes (Table 1). They were first equilibrated with 4 CV (20% Acetonitrile). The solvent gradient used was 1 CV 20% Acetonitrile, then 10 CV 20–70% Acetonitrile, and finally 1 CV 70% Acetonitrile.



Biotage® SNAP KP-C18-HS is the flash cartridge used in this study.

## Results and Discussion

This study shows scale-up purifications using reversed phase flash chromatography. We have demonstrated a 33-fold scale increase in both column and load during the reversed phase purification run, which enabled the separation of 80 mg–2.7 g. Typically reversed phase flash columns are loaded to 0.5–1.0% wt, but this is dependent upon sample composition. For easily resolved compounds, it is possible to increase loading to more than 1% by weight of silica.

Experiment ID	Cartridge	Total Load	Flow Rate mL/min
1	SNAP KP-C18-HS, 12 g	0.8 mL load, ~0.08 g	12
2	SNAP KP-C18-HS, 120 g	8.0 mL load, ~0.80 g	50
3	SNAP KP-C18-HS, 400 g	27.0 mL load, ~2.7 g	100

**Table 1.** Conditions for the experiments. See next page for results.

A typical starting point for reversed phase flash chromatography is a binary gradient, going from a low to high fraction of organic, typically with water as co-solvent. Before the C18 chains can be used effectively it is important to equilibrate the column, transitioning out of the dry state or organic storage solvents, and into a higher percentage of aqueous solvents to start the run.

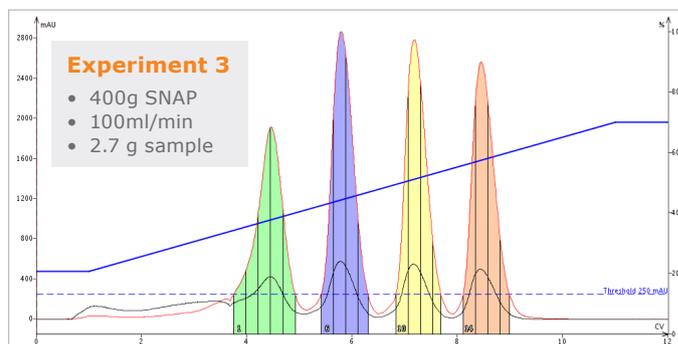
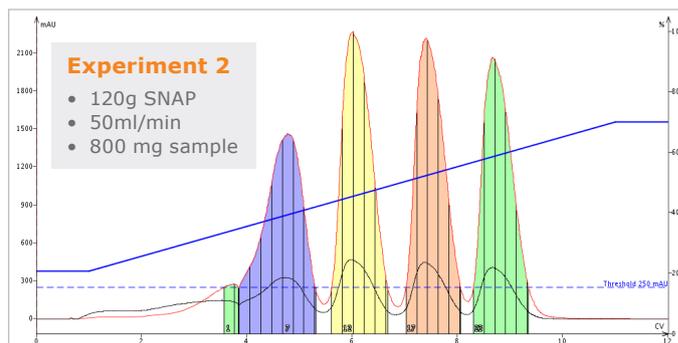
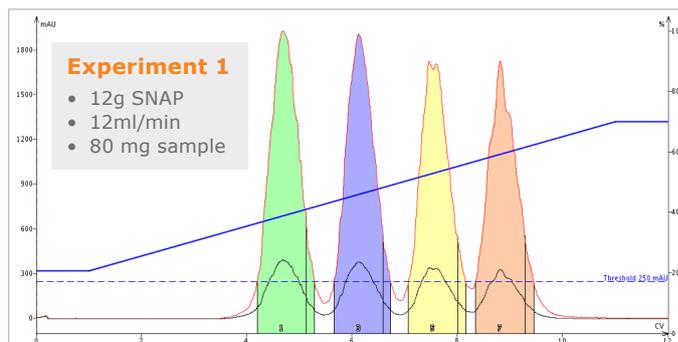
In this example 80 mg of a 4 component test mix was separated using a 12 g Biotage® SNAP KP-C18-HS cartridge. After equilibration of the column (using 4 CV of 20% acetonitrile in water), a standard reversed phase gradient was run using acetonitrile/water. The same gradient and conditions were applied to larger cartridges on scale up (1 CV 20% Acetonitrile, 10 CV 20–70% Acetonitrile, 1 CV 70% Acetonitrile), using larger loads and with similar results.

## Conclusion

This study shows the conditions used to scale-up purification using reversed phase flash chromatography. We have demonstrated a 33-fold scale increase in both column size and loading during reversed phase flash purification which can be used as a template for further scale-up.

## Ordering Information

Part No.	Description
<b>FSL0-1118-0012</b>	SNAP Cartridges, KP-C18-HS 12g, 2/cs
<b>FSL0-1118-0030</b>	SNAP Cartridges, KP-C18-HS, 30g, 2/cs
<b>FSL0-1118-0060</b>	SNAP Cartridge, KP-C18-HS 60g, 2/cs
<b>FSL0-1118-0120</b>	SNAP Cartridge, KP-C18-HS, 120g, 2/cs
<b>FSL0-1118-0400</b>	SNAP Cartridges, KP-C18-HS, 400g, 1/cs
<b>FSL0-1118-0950</b>	SNAP 950g KP-C18-HS CARTRIDGE
<b>FSL0-1118-1850</b>	SNAP 1850g KP-C18-HS CARTRIDGE
<b>SAS-1118-0012</b>	SNAP Samplet Cartridges, KP-C18-HS, 1g, 20/cs
<b>SAS-1118-0030</b>	SNAP Samplet Cartridges, KP-C18-HS, 3g, 20/cs
<b>SAS-1118-0120</b>	SNAP Samplet Cartridges, KP-C18-HS, 12g, 20/cs
<b>SAS-1118-0400</b>	SNAP Samplet Cartridges, KP-C18-HS, 40g, 6/cs



### EUROPE

Main Office: +46 18 565900  
 Toll Free: +800 18 565710  
 Fax: +46 18 591922  
 Order Tel: +46 18 565710  
 Order Fax: +46 18 565705  
 order@biotage.com  
 Support Tel: +46 18 56 59 11  
 Support Fax: + 46 18 56 57 11  
 eu-1-pointsupport@biotage.com

### NORTH & LATIN AMERICA

Main Office: +1 704 654 4900  
 Toll Free: +1 800 446 4752  
 Fax: +1 704 654 4917  
 Order Tel: +1 704 654 4900  
 Order Fax: +1 434 296 8217  
 ordermailbox@biotage.com  
 Support Tel: +1 800 446 4752  
 Outside US: +1 704 654 4900  
 us-1-pointsupport@biotage.com

### JAPAN

Tel: +81 3 5627 3123  
 Fax: +81 3 5627 3121  
 jp\_order@biotage.com  
 jp-1-pointsupport@biotage.com

### CHINA

Tel: +86 21 2898 6655  
 Fax: +86 21 2898 6153  
 cn\_order@biotage.com  
 cn-1-pointsupport@biotage.com

To locate a distributor,  
 please visit our website at  
[www.biotage.com](http://www.biotage.com)

### Part Number: AN083

© 2014 Biotage. All rights reserved. No material may be reproduced or published without the written permission of Biotage. Information in this document is subject to change without notice and does not represent any commitment from Biotage. E&OE. Product and company names mentioned herein may be trademarks or registered trademarks and/or service marks of their respective owners, and are used only for explanation and to the owners' benefit, without intent to infringe. For more information visit [www.biotage.com](http://www.biotage.com).