C18 Flash Chromatography in Rapid Isolation of Organic Compounds

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Introduction

High performance reversed phase liquid chromatography has been widely used in isolation of organic product from reaction mixtures. We have used C18 Flash cartridges packed with 40-75 particle size media in rapid reversed phase preparative scale purification of a synthetic peptide and a biaryl compound. This poster describes method development process, scale-up and lifetime study of the C18 cartridges. Also the effect of different Sample loading techniques on peaks shapes and compound resolution are studied.

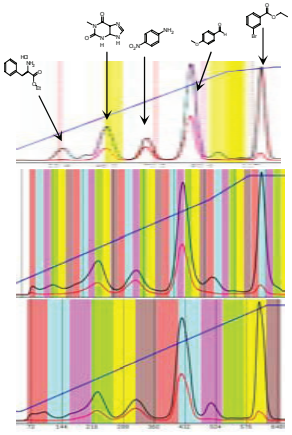
Results and Discussion

A short analytical column (4.6 x 50 mm) packed with the same media as C18 FLASH cartridges is used to find optimized separation conditions, maximum sample loading, flow rate and gradient conditions. The linear velocity of the analytical column was maintained through the FLASH cartridge using the following formulas to calculate flow rate and gradient duration (GD)[1].

$$\text{Flow Rate}_{Flash} = \text{Flow Rate}_{anal} \times \frac{(\text{Diameter } Flash)^2}{(\text{Diameter } anal)^2}$$

$$\text{GD}_{Flash} = \text{GD}_{anal} \times \frac{\text{Length}_{Flash}}{\text{Length}_{anal}} \times \frac{\text{Diameter}_{Flash}}{\text{Diameter}_{anal}} \times \frac{\text{Flow Rate}_{Flash}}{\text{Flow Rate}_{anal}}$$

The scalability was examined by isolating different amounts of a test mixture (H-Phe-OEt.HCl, theophylline, *p*-nitroaniline, 4-methoxy benzaldhyde and 3-ethyl-3-bromobenzoate) on different size C18 Flash cartridges. The small-scale of 60 mg, was isolated using Flash 12 + M. By keeping the same separation conditions and linear velocity on the larger diameter C18 cartridges (25 S and M), larger amount of test mixture (300 & 500 mg) was isolated with the same compound resolution and isolation order.



60 mg

Cartridge:	KP-C18-HS FLASH 12 + M
Dimensions	15 x 150 mm
Injection volume:	0.6 mL , 0.1 M
Mobil phase:	A: H_2O, B: MeOH
Gradient	10-100% B in 120 mL
Flow rate:	12 mL /min.
Collected fractions:	9 mL

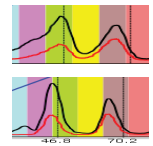
300 mg

Cartridge:	KP-C18-HS FLASH 25 + S
Dimensions	25 x 75 mm
Injection volume:	3 mL , 0.1 M
Mobil phase:	A: H_2O, B: MeOH
Gradient	10-100% B in 200 mL
Flow rate:	25 mL /min.
Collected fractions:	9 mL

500 mg

Cartridge:	KP-C18-HS FLASH 25 + M
Dimensions	25 x 150 mm
Mobil phase:	A: H_2O, B: MeOH
Gradient	10-100% B in 380 mL
Injection volume:	5 mL , 0.1 M
Flow rate:	25 mL /min.
Collected fractions:	50 mL

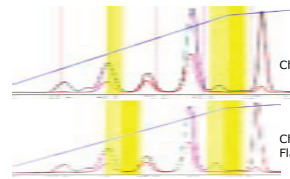
Sample can be introduced onto the *Flash* cartridges using two different techniques, direct injection or Samplet™ loading (pre-absorption of sample onto C18 media). To study the sample loading effects on the Flash chromatography, a mixture of theophylline and *p*-nitroaniline were isolated on *Flash* C18 using two different loading techniques.



Chromatogram Resulted from Direct Injection Technique

Chromatogram Resulted from Samplet loading Technique

Samplet™ loading technique resulted in tighter elution bands, maximized fraction purity and increased compound resolution. To study the lifetime and performance of C18 *Flash* cartridges the test mixture (H-Phe-OEt.HCl, theophylline, *p*-nitroaniline, 4-methoxy benzaldhyde and 3-ethyl-3-bromobenzoate) was injected onto C18 *Flash* cartridge using samplet loading technique this purification was repeated 25 times on the same cartridge.



Chromatogram of first injection

Chromatogram after 25 times injection on C18 Flash 12 + M

After 25 times use the C18 Flash cartridge resulted in consistent peak shape, peak retention and peak resolution.

C18 Flash Chromatography in Rapid Peptide Purification

A 10 amino acids sequence peptide (ACP) was prepared by (5+5) segment coupling, followed by removal of protecting groups[2]. The final product was isolated on C18 *Flash* chromatography.

Fmoc-Val-Gln(trt)-Ala-Ala-Ile-OH +H-Asp(t-Bu)-Tyr(t-Bu)-Ile-Asn(trt)-Gly-NHDMCP

HATU + HOAT — Segment coupling

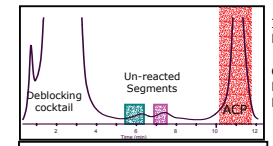
TAEA — Removal of Base sensitive protecting group

TFA, Triisopropylsilane, Phenol, H_2O — Removal of acid sensitive protecting groups

H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-NH_2

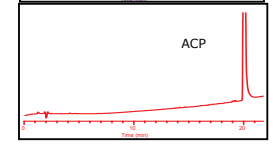
Side products + De-blocking cocktail

Side products and de-blocking cocktail (TFA, Triisopropylsilane, Phenol) were removed by C18 FLASH purification. Product was identified by H-NMR and molecular mass an the purity was determined using analytical reversed phase HPLC,.



Isolation of ACP on C18 FLASH 12+ M

Mobil phase:	A: H_2O + 0.1% TFA,
	B: ACN + 0.1% TFA
Gradient:	5- 45 %B in 18minutes
Flow rate:	12 ml/min
Detector:	UV @ 220 nm

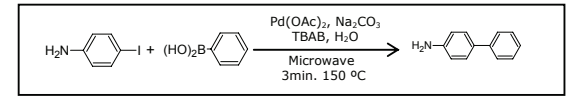


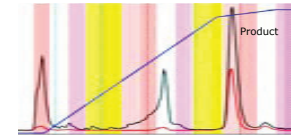
Analytical C18 HPLC Chromatogram

Column:	Vydac C18 4.6 x 250 mm
Mobil phase:	A: H_2O + 0.1% TFA
	B: ACN + 0.1% TFA
Gradient:	10-55% B in 33 minutes
Flow rate:	1 mL/min
Detector:	UV @ 220 nm

C18 Flash in Rapid Purification of Biaryl Prepared by Suzuki Reaction

A biaryl has been prepared in water using microwave assisted Suzuki reaction[3]. The resulting mixture was transferred onto a C18 Samplets, the direct addition of reaction mixture onto the Samplet eliminated tedious aqueous-aqueous extractions in work-up step. The pure product was isolated in 86%.





Isolation of Biaryl on C18 25+ S

Mobil phase	A: H_20, B: MeOH
Gradient:	2-100% in 200 mL
Flow rate:	9 ml/min
Detector:	UV @ 220, 254 nm
Injection volume:	3 mL , 0.1 M

Conclusion

C18 *Flash* cartridges can be used for rapid purification of peptide and biaryl. Introduction of sample by Samplet loading technique increased efficiency of Flash separation and also eliminates workup steps.

Samplet™ Cartridge Technique

SP1 Automated Purification System

References:

1- L. R. Snyder, J. J. Kirkland, Introduction to Modern Liquid Chromatography, A Wiley-Interscience Publication (1979), pp.168-245
2- L. A.Carpino, A. El-Faham, C. A. Minor, F. Albericio, " Advantageous Application of Azabenzotriazole (Triazolopyridine)-based Coupling Reagents to Solid-phase Peptide Synthesis" J.Chem.Soc. Chem. Comm. 201 (1994)
3- N. E. Leadbeater and M. Marco, Org. Lett., , 4, 2973 (2002)

http://www.biotage.com

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