Reversed Phase Flash Chromatography Purification of Peptide-Peptoid Hybrids

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Chromatography Application Note AN115

*Vera D'Aloisio[1] and Christopher R. Coxon[2]**

Application Overview

Peptide-peptoid hybrids or 'peptomers' are oligomeric synthetic polymers, in which one or more amino acids is replaced with a peptoid submonomer. The key structural feature is the relocation of the amino acid side chain from the α-carbon to the adjacent nitrogen atom of the peptide bond (see scheme in Fig. 1). Generally, these species exhibit physicochemical properties similar to the parent peptides.

Purification of peptide-peptoid hybrids can be obtained by reversed phase chromatography with excellent recovery and resolution. In general, for optimising the separation of a mixture of peptides and similar compounds, it is best to consider parameters, such as sample loading and stationary phase particle size. In this application note, the separation of a peptomer from a peptide side-product using Teledyne ISCO's Redi*Sep*® C18 reversed phase columns as the stationary phase will be shown.

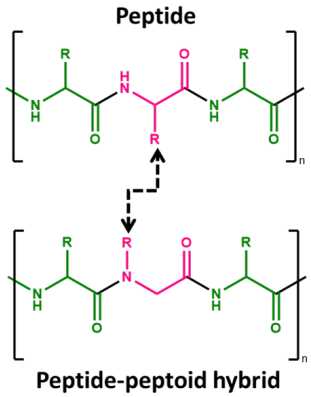

Figure 1. Peptides and peptomers

Experimental Section

The separation of a mixture of one peptomer and one close-running peptide (retention times 9.34 and 8.06 min, respectively over a 20 min gradient H_2O/MeCN as shown in Fig. 3) was achieved with both Redi*Sep*® C18 4.3 g and Redi*Sep* C18 Gold® 5.5 g, using the Teledyne ISCO Combi*Flash*® NextGen 300+. Table 1 shows the sequences of the peptomer and its side-product, which appears as a deletion of the parent compound. Parameters kept constant in this study are listed in Table 2, while the parameters that have been modified are showed in Table 3.

Entry	Sequence
Peptomer	VTPDGVPFA**F**-NH_2
Side-product	VTPDGVPFA-NH_2

*Table 1. Peptomer sequence and side-product sequence. The letter **F** highlighted in bold is the amino acid replaced with its peptoid equivalent.*

Instrumentation	Teledyne ISCO Combi*Flash* NextGen 300+	
Wavelengths	214 nm (red) 254 nm (purple)	
Mobile phases	Solvent A: Purified H_2O Solvent B: Methanol	
Flow Rate	13 mL/min	
Equilibration Volume	28.7 mL	
Gradient	**% Solvent B** 10 100 100 90 90	**Minute** Initial 5 7.6 8 8.8
Run Length	8.8 min, not including equilibration time	

Table 2. Fixed parameters used in Flash chromatography separation

Run N.	Column	Amount loaded (liquid loading)
1	Redi*Sep* C18 4.3 g	0.5 mL (8 mg/mL)
2	Redi*Sep* C18 5.5 g GOLD	0.5 mL (8 mg/mL)
3	Redi*Sep* C18 4.3 g	1 mL (8 mg/mL)
4	Redi*Sep* C18 5.5 g GOLD	1 mL (8 mg/mL)

Table 3. Parameters changed in different runs.

Characterization of crude and purified peptomer was performed with a low resolution LCMS Waters TQD mass spectrometer equipped with an Agilent ZORBAX SB-C18 Stable Bond Analytical column (5 µm particle size, 4.6 x 150 mm) with a binary eluent system comprising MeCN/H_2O (20 min gradient: from 90% H_2O/10% MeCN to 100% MeCN with 0.1 % formic acid) as mobile phases. Electrospray ionisation mass spectrometry was conducted in positive ion mode (m/z range: 600–1900) using a cone voltage of 50 V, desolvation temperature of 350 °C and source temperature of 100 °C.

Results

When a low amount of peptide-peptomer mixture (8 mg) was loaded on C18 reversed phase 4.3 g Redi*Sep* column (Run 1), flash chromatography successfully separated the product using water/methanol as the mobile phase (Figure 1). No ion pairing reagent (e.g. TFA) was required for the separation. Higher peak resolution was obtained when the same amount of initial mixture (8 mg) was loaded on a C18 reversed phase 5.5 g Redi*Sep* Gold column (Figure 2, Run 2).

Loading a higher amount of crude mixture (16 mg) incurred a loss of resolution when a normal Redi*Sep* C18 4.3 g was used (Run 3). In contrast, the successful separation at this scale when using a Redi*Sep* Gold C18 5.5 g (Run 4) highlights the importance of the use of a column with lower particle size when the separation is scaled up. The gold column stationary phase composition exhibits a patented spherical Flash media, which affords the benefits of tighter packing without increasing back pressure. The resolving power is doubled compared with a standard flash chromatography column, allowing the separation of difficult compounds with low ΔR_f, such as a parent peptomer and truncation side-product and, at the same time, allowing the loading of twice as much compound without losing resolution of the peaks.

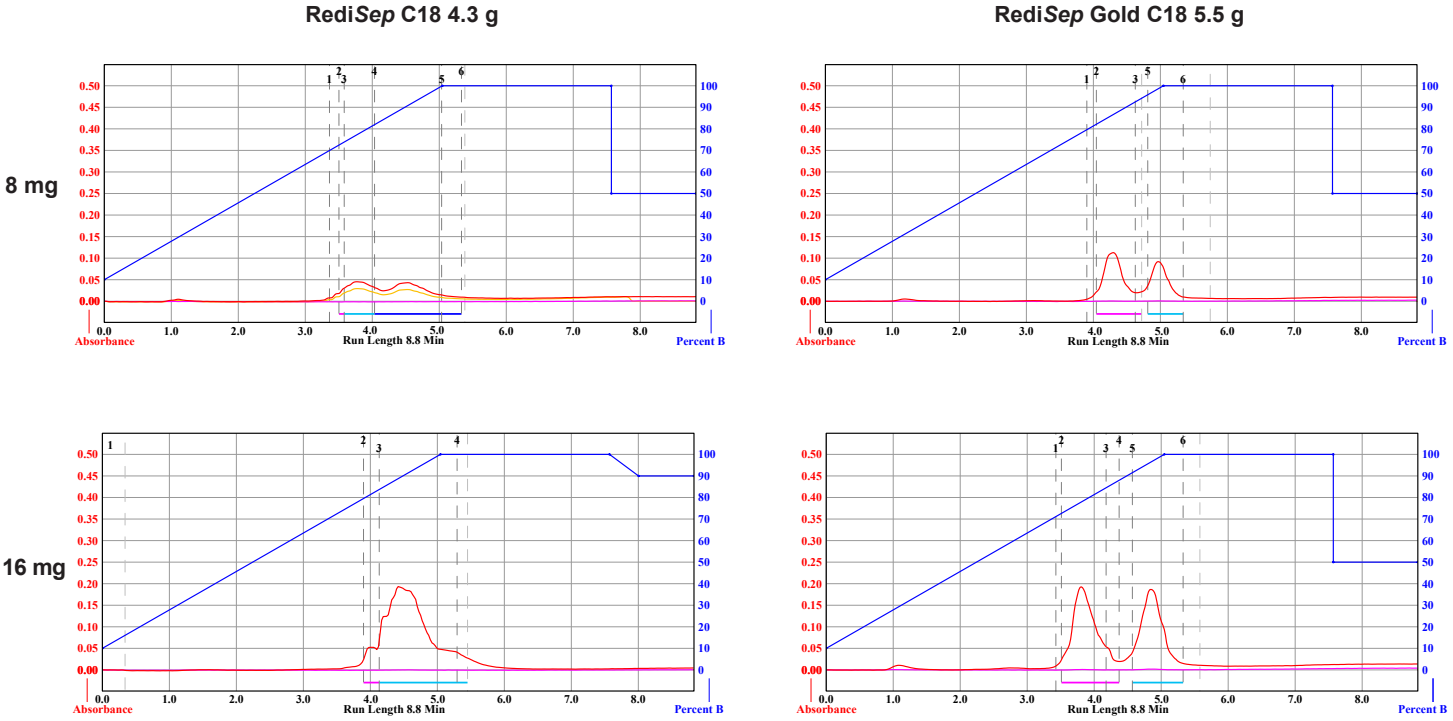

Figure 2. Chromatograms from separations obtained by varying the sample amount and the column media.

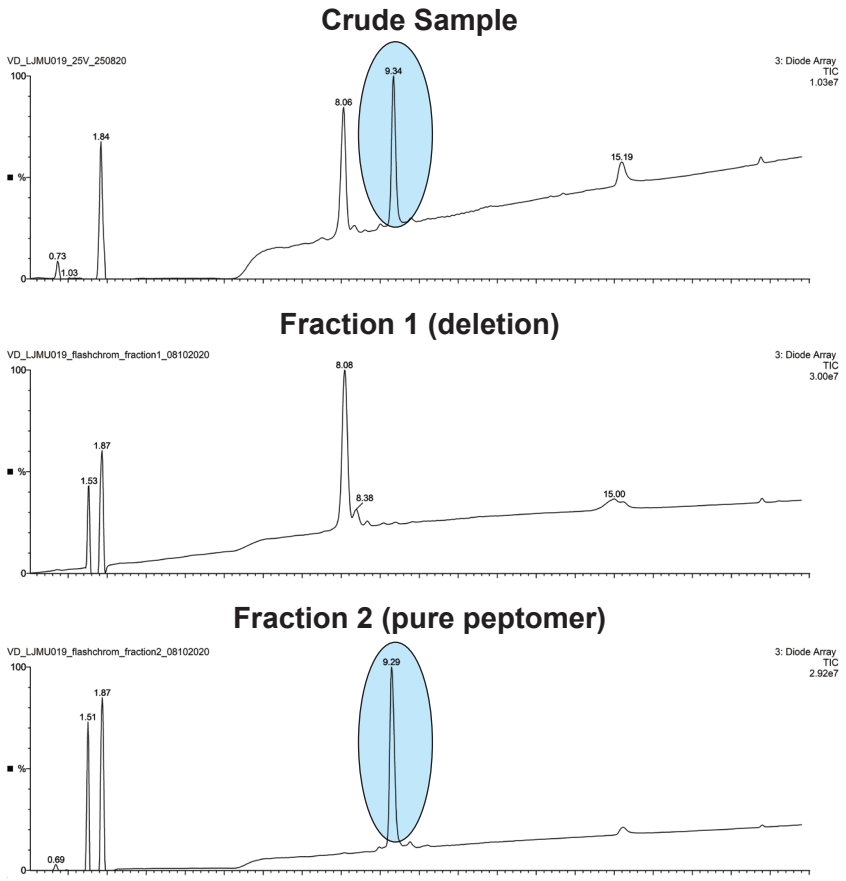

Figure 3. HPLC chromatograms before and after separation. Fraction 1 contains the side-product of the parent sequence, while Fraction 2 contains the desired purified peptomer.

Conclusions

The separation of polar compounds such as peptides and peptidomimetics is usually achieved by reversed phase preparative-HPLC due to the typically higher resolving power compared to traditional flash chromatography. Separating closely related peptides, differing by one or two amino acids, can be challenging even when using reversed-phase liquid chromatography. The use of preparative-HPLC is also time-consuming, requires expensive columns, with the possibility of loading only small amounts of sample in each run, meaning that multiple injections are required. Flash chromatography using pre-packed C18 reversed-phase columns provides convenient and comparable preparative separations with automated flash chromatography instrumentation.

In this work, separation of a peptomer from its side product was successfully achieved using flash chromatography pre-packed columns. This allowed the introduction of higher crude sample amounts and achieved purity comparable with preparative-HPLC but requiring a shorter run time (8.8 min). The peak resolution was clearly influenced by the column particle size and the loading, suggesting that for higher loading and difficult separation, the extra resolution of the Redi*Sep* Gold C18 technology is key for successful separation.

[1] *School of Pharmacy and Biomolecular Sciences, Faculty of Science, Liverpool John Moores University, Liverpool, United Kingdom, L3 3AF*

[2]**Corresponding author, Institute of Chemical Sciences, School of Engineering and Physical Sciences, Heriot-Watt University, Edinburgh, United Kingdom, EH14 4AS*