**Biotage® SNAP Bio Flash Cartridges**

**Useful Hints for Isolera™ Set-up**

**Method Set-up**

1. At the **Method** tab, open a method by pressing Open. Select User and in Method select Reversed Phase.
2. At the **Parameters** tab, select the solvents, cartridge type and size, rack type, rack size and fraction volume. For cartridge type selection, select the similar size Biotage® SNAP Ultra C18 cartridge. Run with the recommended flow rates for Biotage® SNAP Ultra C18 cartridges.

<table>
<thead>
<tr>
<th>SNAP Bio Cartridge</th>
<th>Cartridge Type</th>
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</thead>
<tbody>
<tr>
<td>SNAP Bio C18/C4 10 g</td>
<td>SNAP Ultra C18 12 g</td>
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<tr>
<td>SNAP Bio C18/C4 25 g</td>
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**Note:** In some cases if an oscillating baseline is observed this can be eliminated by increasing the flow rate. Higher flow rates are adopted to enable efficient column conditioning, the increased backpressures that are generated at higher flow ensure the larger pores and therefore column bed are completely solvated to allow efficient and stable chromatography.

3. At the **Collection** tab, select the collection and fractionation parameters.
   a. Collection by λ-all is very useful for peptide purification as there are few UV absorbing side chains – set the end wavelength to about 290 nm for maximal signal to noise.
   b. The peptide bond absorbs well in the 214–215 nm wavelength range (UV absorption).

4. At the **Gradient** tab.
   a. Adjust default gradient.
   b. The most commonly used mobile phase is aqueous acetonitrile solution (+0.1% TFA) with gradient elution generally preferred for peptide purification.
   c. The concentration of CH$_3$CN required for peptide elution correlates similarly to data from analytical HPLC.
   d. Note if using DMF or DMSO for peptide dissolution, extend the initial starting condition hold to ~3 CV to ensure complete clearance (elution) from the cartridge before starting the gradient.
   e. Method development strategies employed in RP-HPLC of peptides can be also be used to improve separation by flash chromatography.
**Equilibrating Cartridges**

Reversed phase chromatography cartridges require several equilibration steps to ensure they become wet with solvent, transitioning out of the dry state or organic storage solvents, and into a higher percentage of aqueous solvents to start the run.

For optimal purification with Biotage® SNAP Bio cartridges follow these steps:

1. Flush with 100% acetonitrile (CH₃CN) using 3 to 5 column volumes (CV).
2. Flush with 3 to 5 CV 50% aqueous acetonitrile (+0.1% TFA or 0.1% formic acid).
3. Flush with 3–5 CV of your initial gradient conditions.

**Loading Samples**

**Syringe Liquid Injection**

Dissolve the peptide in a minimum volume of solvent either mobile phase, DMF or DMSO. Using a syringe to load a cartridge is straightforward. Biotage cartridges have Luer inlet fittings for connection to a Luer syringe.