Biotage Flash Cartridge User Guide

The Definitive Guide to Flash Chromatography

Biotage®

Biotage Flash Cartridges

Loading, Flexibility and Performance

CONTENTS

Leading Innovation

In 1994, Biotage invented automated flash chromatography with pre-packed cartridges, and continues to be the innovation leader with superior designs such as Biotage ZIP®, Biotage® SNAP and Isolera™ Dalton chromatography systems.

Quality and Testing

Biotage SNAP flash cartridges are manufactured from polypropylene and polyethylene that meet or exceed the extractable requirements in US 21 CFR 177.1520 and are packed using proprietary methods that strictly adhere to ISO 9001:2008 quality standards. Every batch is tested and undergoes a stringent series of tests including shock resistance and chromatography testing for maximum quality. SNAP cartridges are laser-etched with a unique lot number to ensure traceability.

Which Cartridge Should I Use?

From Entry-Level to High-End

SNAP Family Loading Options

- Samplet
- Solid Loading
- Direct Pour-on
- Liquid Injection
- 3-Way Valve
- Dry Load Vessel
- Pump Injection

ZIP Family Loading Options

- Liquid Injection
- 3-Way Valve
- Dry Load Vessel
- Pump Injection

Performance

Biotage® SNAP Ultra

- Ultra Resolution
- Versatile Loading Options
- Lowest Pressure for Increased Speed
- Lowest Cost per Gram Purified
- Particle Size: 25 µm
- Surface Area: 700 m^2/g
- Silica Type

Biotage ZIP® Sphere

- Silica Type
- Improved Resolution
- Simple Loading Options
- Low Pressure for Increased Speed
- Low Cost per Gram Purified
- Particle Size: 60 µm
- Surface Area: 700 m^2/g

Biotage® SNAP

- Good Resolution
- Versatile Loading Options
- Intermediate Cost per Column
- Particle Size: 45–50 µm
- Surface Area: 500 m^2/g
- Silica Type

Biotage® ZIP

- Silica Type
- Good Resolution
- Simple Loading Options
- Low Cost per Column
- Particle Size: 45–50 µm
- Surface Area: 500 m^2/g

Pre-Adsorption Technique

Materials with Low Solubility are Adsorbed Before Loading

Figure 1. Left: Pre-adsorption on silica gel (3 g, part number K0-1107-05000). Right: Pre-adsorption on Biotage HM-N diatomaceous earth (3 g, part number 9800-1000).

Poorly soluble materials are adsorbed on solid materials such as silica gel or Biotage HM-N and then applied onto a cartridge. When the amount of adsorbent is small, materials for purification are not uniformly adsorbed but agglomerated. The use of a large amount of silica gel for prevention of agglomeration may result in diffuse bands and poor separation. When a large amount of silica is needed, the use of Biotage HM-N is recommended.

Unlike silica gel, HM-N is a refined diatomaceous earth tailored to the needs of flash chromatography that has no interaction with reaction products and prevents band diffusion. Because HM-N is hygroscopic, a small amount is enough for pre-adsorption.

Pre-Adsorption Comparison

Silica Gel

Biotage HM-N

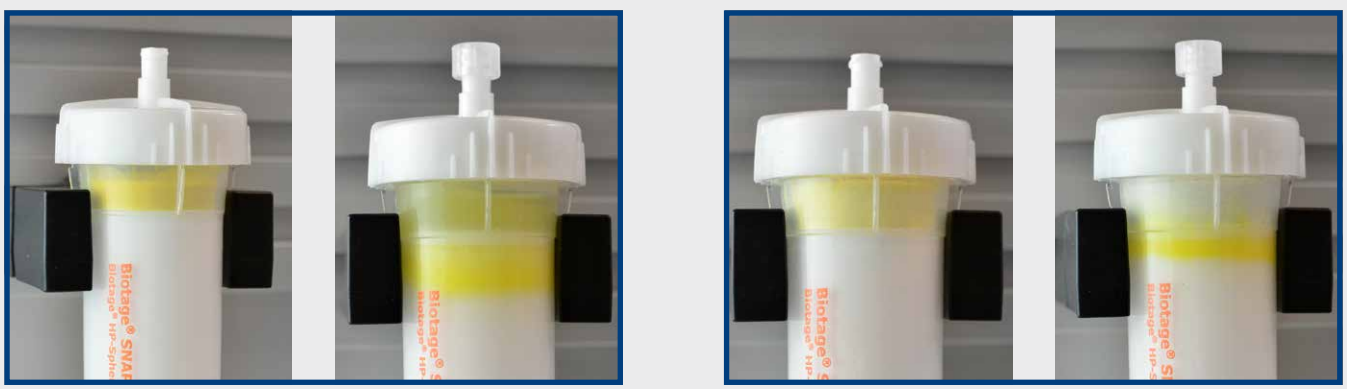


Figure 2. Biotage® SNAP Ultra cartridge loaded with a sample pre-adsorbed on silica gel (left) and Biotage HM-N (right). The pre-adsorption on the HM-N renders much tighter bands.

Preparation

Connecting Your Cartridge

1. Place the cartridge in the appropriate holder.

25 g Biotage® SNAP Ultra cartridge.

2. Remove the top Luer plug cap.

Top plug cap.

3. Remove the bottom Luer plug cap.

Bottom plug cap.

4. Attach the lower Luer fitting to the cartridge.

Twist the Luer fitting on securely to the bottom of the cartridge.

5. Attach the upper luer fitting to the cartridge.

Figure 3. Biotage ZIP® cartridges have a push-in luer tip connection on the low pressure (lower) end of the cartridge. Press the fittings together and seal with a slight twist.

Loading Selection Guide

Find the Best Method for Your Needs

Sample Characteristics	In Which Solvent?	Loading Recommendation	Page
Soluble	In weak solvent*	Samplet internal dry load Internal liquid load Syringe liquid load External liquid load	10–13 15 16–17 17
	In strong solvent*	Samplet internal dry load Internal dry load External dry load Liquid load pump	10–13 14 18 21
Not very soluble	In weak solvent*	Samplet internal dry load Internal liquid load Syringe liquid load	10–13 15 16–17
Very low solubility	In any solvent	Internal dry load External dry load	14 18
Specials			
Large volume		Internal dry load External dry load Liquid load pump	14 18 21
Not stable on silica		External dry load	18
Oil		Internal dry load Internal liquid load Syringe liquid load External liquid load External dry load	14 15 16–17 17 18

*Strong solvent = EtOAc, MeOH etc. Weak solvent = Hexane, toluene

Cartridge Size and Loading Selection Guide

Type of Column	Dimensions (mm)	Column Volume	Flow Rate (mL/min)	Load ΔCV = 0.1-1.9	Load ΔCV = 2.0-3.9	Load ΔCV = 4.0+
Biotage SNAP Ultra 10 g	21 x 55	17 mL	10-50	<200 mg	200-1000 mg	1-2 g
Biotage SNAP Ultra 25 g	30 x 72	45 mL	20-100	<500 mg	500-2500 mg	2.5-5 g
Biotage SNAP Ultra 50 g	39 x 81	85 mL	30-150	<1 g	1-5 g	5-10 g
Biotage SNAP Ultra 100 g	39 x 157	164 mL	30-150	<2 g	2-10 g	10-20 g
Biotage SNAP Ultra 340 g	71 x 168	582 mL	65-325	<6.8 g	6.8-34 g	34-68 g
Biotage SNAP 10 g	21 x 55	15 mL	10-20	<100 mg	100-500 mg	500-1000 mg
Biotage SNAP 25 g	30 x 72	33 mL	20-40	<250 mg	250-750 mg	750-2500 mg
Biotage SNAP 50 g	39 x 81	66 mL	30-50	<500 mg	500-2500 mg	2.5-5 g
Biotage SNAP 100 g	39 x 157	132 mL	30-50	<1 g	1-5 g	5-10 g
Biotage SNAP 340 g	71 x 168	470 mL	65-100	<3.4 g	3.4-17 g	17-34 g
Biotage SNAP 750 g	82 x 291	990 mL	100-300	<7.5 g	7.5-40 g	40-75 g
Biotage SNAP 1500 g	107 x 328	1980 mL	300-500	<15 g	15-80 g	80-150 g
Biotage ZIP Sphere 5 g	15.5 x 61	10 mL	5-25	<75 mg	75-325 mg	325-1000 mg
Biotage ZIP Sphere 10 g	20 x 69	19 mL	10-50	<150 mg	150-750 mg	750-3000 mg
Biotage ZIP Sphere 30 g	27 x 116	58 mL	20-100	<450 mg	450-2250 mg	2.25-4.5 g
Biotage ZIP Sphere 45 g	32 x 107	75 mL	30-150	<600 mg	800-4000 mg	4-8 g
Biotage ZIP Sphere 80 g	38 x 130	129 mL	30-150	<1.2 g	1.2-6 g	6-12 g
Biotage ZIP Sphere 120 g	42 x 176	213 mL	50-200	<1.8 g	1.8-10 g	10-20 g
Biotage ZIP 5 g	15.5 x 61	8 mL	5-20	<50 mg	50-250 mg	250-500 mg
Biotage ZIP 10 g	20 x 69	15 mL	10-20	<100 mg	100-500 mg	500-1000 mg
Biotage ZIP 30 g	27 x 116	45 mL	20-40	<300 mg	300-1500 mg	1.5-3 g
Biotage ZIP 45 g	32 x 107	60 mL	30-50	<450 mg	450-2250 mg	2.25-4.5 g
Biotage ZIP 80 g	38 x 130	102 mL	30-50	<800 mg	800-4000 mg	4-8 g
Biotage ZIP 120 g	42 x 176	170 mL	50-75	<1.2 mg	1.2-6 mg	6-12 g
Flash 75S	75 x 90	300 mL	100-250	<2 g	2-10 g	10-20 g
Flash 75M	75 x 150	500 mL	100-250	<4 g	4-20 g	10-40 g
Flash 75L	75 x 350	1000 mL	100-250	<8 g	8-40 g	40-80 g
Flash 150M	150 x 300	4.3 L	500-1000	<25 g	25-125 g	125-250 g
Flash 150L	150 x 600	8.6 L	500-1000	<50 g	50-250 g	250-500 g
Flash 400M	400 x 300	28 L	7000	<200 g	0.2-1.0 kg	1-2 kg
Flash 400L	400 x 600	56 L	7000	<400 g	0.4-2.0 kg	2-4 kg

Table 1. Cartridge size and loading selection guide

Seven Ways to Load a Cartridge

Options for Any Scenario

Internal Loading – Exclusive to Biotage® SNAP

1. Samplet® Loading (p. 10-13)
Samplets offer a convenient way to introduce materials to be purified which are dissolved in any volatile solvent. Add the dissolved material to a Samplet and allow the solvent to evaporate. The Samplet is placed inside the SNAP cartridge which maximizes the amount of media available for purification and eliminates and precipitation risk.

2. Internal Dry Loading (p. 14)
Sometimes, it is more convenient to adsorb materials for purification onto a solid support such as Biotage HM-N (diatomaceous earth) or silica. For this approach the internal loading directly into a SNAP cartridge is ideal. Mix a small amount of sorbent with the material and evaporate the solvent. Dried support is then placed inside the SNAP cartridge and retained with a top frit.

3. Direct Pour-on (p. 15)
For retentive compound purification where cartridge pre-equilibration is not required, liquids can be added directly onto the opened SNAP cartridge eliminating the need to inject via a syringe.

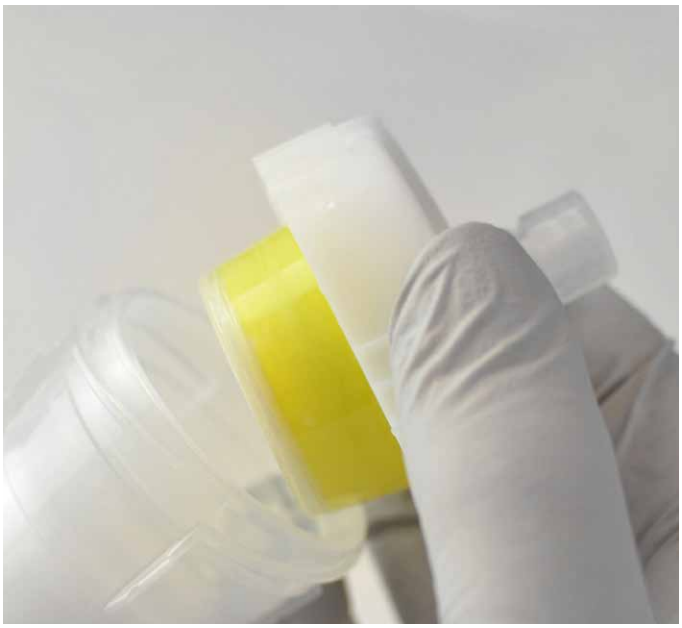


Figure 4. Samplets are placed inside of Biotage® SNAP cartridges which maximizes the amount of media available for purification and eliminates and precipitation risk.

External Loading

4. Direct-to-Cartridge, Syringe Liquid Injection (p. 16-17)
Using a syringe to load a cartridge is straightforward. Biotage cartridges have Luer inlet fittings for connection to Luer syringe.

5. 3-Way Syringe Injection, Valve (p. 17)
For chemists who are used to HPLC and injecting samples through a valve, this will be very familiar. Injection through the valve requires that the solubility of reaction products be considered or risk plugging.

6. Dry Load Vessel (p. 18-20)
Sometimes sample sizes require more sorbent volume then the Samplet cartridges can provide. Compounds that are soluble only in large volumes of volatile solvents or in a strong solvent (e.g. methanol) are best loaded using external dry loading. This technique uses an adsorbent media mixed with reaction products and solvent. After evaporation of the solvent the dried powder is then placed into an empty dry load vessel which is connected to a cartridge.

7. Liquid Injection with Pump (for Isolera™ LS, p. 21)
For larger scale purification, typically gram to multi-gram, the Biotage Isolera LS includes a mounted peristaltic pump. Liquid reaction mixtures are conveniently pumped onto a cartridge prior to purification.

Figure 5. After evaporation of the solvent, dried adsorbent is poured into empty Dry Load Vessels which can be connected to any Biotage cartridge.

Liquid Loading – Methods & Techniques

» The dissolution solvent should be as weak as possible, ideally the weaker solvent of the elution mixture. For optimal performance, the ideal volume should be less than 10% of the cartridge column volume (see table on p. 7).

» If a polar dissolution solvent (strong solvent) must be used it is recommended that a Samplet®, dry load vessel or internal dry loading is used. If this is not practical use the minimum volume of polar solvent to dissolve materials to be purified thereby minimizing the effect of the polar solvent on the purification. Alternatively, a weaker solvent can be used to dilute the polar solvent but care must be taken to ensure precipitation does not occur.

» If a solvent other than one used for the elution is used it is best to check its effect on the separation system first by running a TLC plate with the anticipated solvent blend. If the R_f of the desired component is 0.1 or lower, the dissolution solvent will have little effect on the separation; a good example of such a solvent is dichloromethane.

» If a different dissolution solvent is used, often it is a good idea to "sandwich" the reaction products between two layers of the dissolution solvent thereby minimizing the chance for precipitation, which may affect the separation or block the cartridge. Usually ¼ of a column volume (CV) is injected before loading followed by ¼ CV after.

Dry Loading – Methods & Techniques

» A Biotage Samplet can be loaded and left to dry overnight in a fume-cupboard, provided materials are stable. Samplets loaded with reaction products can be placed in vacuum desiccators or ovens to speed the process.

» Adsorbing liquids onto silica or other media and then drying, is a common technique. Drying methods include spin drying on the Biotage® V-10, air drying in a fume hood, vacuum oven, or rotary evaporation.

» Biotage dry load vessels (DLV) are useful for external dry-loading with Biotage ZIP® cartridges or any time a larger mass of reaction products are to be separated.

Samplet® Loading

Simple Rapid Dry-Loading

Samplets offer a convenient way to introduce soluble samples dissolved in a volatile solvent. Pour the dissolved material on a Samplet and allow the solvent to evaporate. The Samplet is placed inside of the SNAP cartridge which maximizes the amount of media available for purification and eliminates precipitation risk.

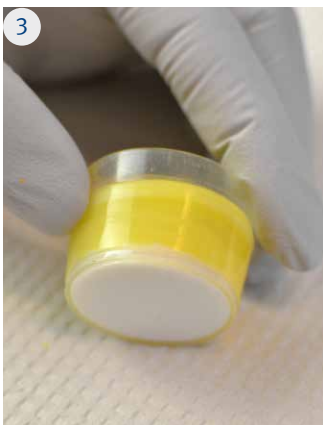
1. Place the Samplet on a flat, clean surface.

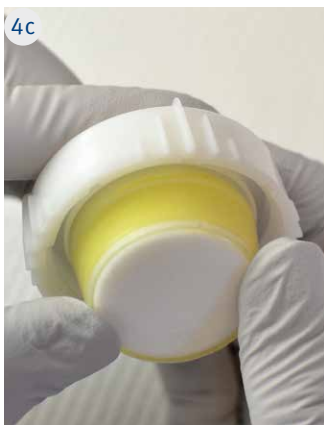
2. Add the liquid reaction products in a single smooth pouring operation to ensure the most uniform distribution of material and best chance of high purity.

Single Samplet on tissue ready for addition liquid for purification.

3. With the correct volume, no breakthrough is seen on the bottom frit or on the tissue, and the Samplet is now ready for drying. See drying times and loading volumes in Table 2, 3 and 4.

4. Once the Samplet is ready to load into the cartridge, remove the cap and dispose of the SNAP insert . Insert the Samplet into the cap groove as shown. Ensure that the lip of the Samplet fits snugly into the groove of the cartridge cap.





5. Insert the cap with Samplet® into the cartridge top and tighten firmly. Ensure the cap is even and fully tightened down, there should be no more than a 1 mm gap between the cap and the mounting fin as shown.

6. The purification is now ready to run using the chosen method.

Cap fully tightened showing 1 mm gap between the cap and mounting fin.

Figure 6. If several Samplet® cartridges are to be prepared at the same time, labels or descriptions can be written on the cardboard shipping material by each Samplet®.

Samplet Loading Capacity

Samplet® Cartridge	Usable Volume (mL)	Silica Mass (g)
SNAP 1 g	0.5	1.0
SNAP 3 g	1.3	2.5
SNAP 10 g	5	10
SNAP 34 g	18	34

Table 2. Sample loading capacity.

Drying Times

Samplet® Size (g)	Solvent Vapor Pressure ≥ 100 mm Hg	Solvent Vapor Pressure < 100 mm Hg
1	20 min	30 min
3	25 min	35 min
10	30 min	45 min
34	45 min	60 min

Table 3. Drying times.

Vacuum Oven Settings

Solvent Vapor Pressure	Vacuum	Heat
>300 mm Hg	<100 mm Hg	None
100 mm Hg solvent VP 300 mm Hg	50-150 mm Hg	None
<100 mm Hg	100-200 mm Hg	With heat*

**Set the temperature 15 °C to 20 °C below the solvent boiling point*

Table 4. Vacuum oven settings.

Ordering Information

Samplet to Fit Biotage SNAP Cartridge	Ultra Silica	KP-SIL Silica	Amino KP-NH Silica	Reversed Phase, C18, KP-C18-HS
Fits SNAP 10 g cartridge	SAS-0442-0010	SAS-0909-0011	SAS-0909-0011	SAS-1118-0012
Fits SNAP 25 g cartridge	SAS-0442-0025	SAS-0909-0028	SAS-0909-0028	SAS-1118-0030
Fits SNAP 50 and 100 g cartridge	SAS-0442-0100	SAS-0909-0110	SAS-0909-0110	SAS-1118-0120
Fits SNAP 340 g cartridge	SAS-0442-0340	SAS-0909-0375	SAS-0909-0375	SAS-1118-0400

Table 5. For complete ordering information, please refer to page 26-27.

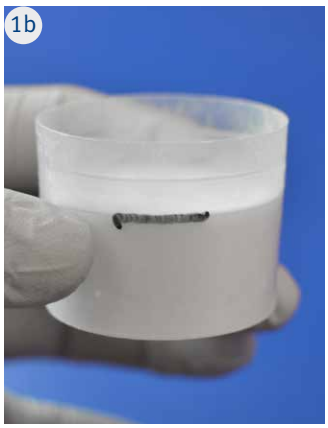
Dry-Loading an Empty Samplet®

Using Bulk Powder or Adsorbents

This technique is useful for larger volumes of dissolved reaction products and when the volume is too large for a pre-filled Samplet.

1. Fill an empty Samplet® with the media to the fill line highlighted in the photo below. Add the media to the round bottom flask.
2. Dissolve the materials in a suitable solvent with an evaporation system such as a Biotage® V-10 centrifugal evaporator. Or in a round bottom flask (if a rotary evaporator will be used) or a scintillation vial (20 or 30 mL).
3. Evaporate the solvent to leave a free flowing powder scraping the sides of the vessel as needed.

Empty Samplet®.

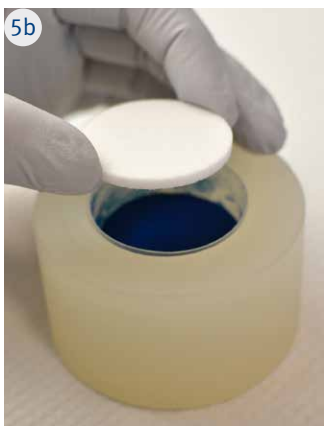


Correctly filled Samplet®.

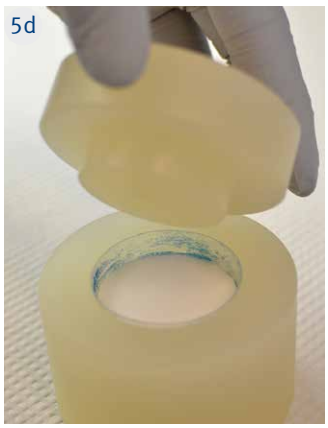
Round bottom flask.

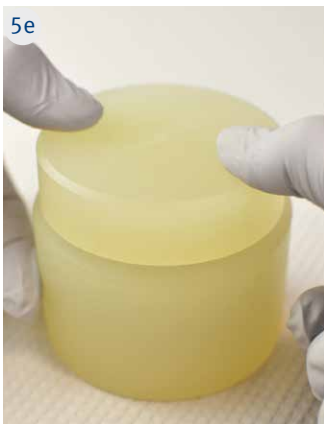
Biotage® V-10 scintillation vial.

4. Place the free flowing powder into an empty Samplet.
5. Once the powder with adsorbed materials is in the Samplet, gently tap it on a flat surface to level the bed. Place a frit using the Frit Insertion tool.



6. Once the Samplet® is ready, follow the instruction from steps 4-5 in the Samplet Loading section (p. 10-11) to insert the Samplet into a cartridge and run the purification.





Using the Frit Insertion Tool Kit

1. Place the filled Samplet in the holder body.
2. Insert a top frit and press it into place with the Frit insertion tool.

Ordering Information – Empty Samplets

Samplet to Fit Biotage SNAP Cartridge	Samplet Frit Insertion Tool	Empty Samplets
Fits SNAP 10 g cartridge	SFS-0010	SES-0010
Fits SNAP 25 g cartridge	SFS-0025	SES-0025
Fits SNAP 50 and 100 g cartridge	SFS-0100	SES-0100
Fits SNAP 340 g cartridge	SFS-0340	SES-0340

Table 6. For complete ordering information, please refer to page 26-27.

Internal Dry Loading

Maximizes Cartridge Loading Capacity

Sometimes, it is more convenient to adsorb materials for purification onto a solid support such as Biotage HM-N Diatomaceous Earth or silica. For this approach the internal loading directly into a SNAP cartridge is the preferred approach. Mix a small amount of sorbent with the material to be purified and evaporate the solvent. Dried sorbent and material is then placed inside the SNAP cartridge and retained with a top frit.

This method is similar to 'Dry-Loading an Empty Samplet®' described on page 12-13, except that the space in the top of the cartridge is utilized. As there is no Samplet to use, it does allow slightly more sorbent to be used but is less convenient.

1. Remove the cartridge cap and SNAP insert.
2. Add materials for purification adsorbed onto dry media into the space at the top of the cartridge to the fill line.
3. Pour the absorbed materials into the recess at the head of the cartridge.
4. Place a dry-load cartridge frit and press into place with a frit insertion tool.
5. Screw the cap back on, ensuring it is even and fully tightened. There should be no more than a 1 mm gap between the cap and the mounting fin.
6. The purification is now ready to run.

NOTE: Fill line shown in black for clarity only.

Dry Loading Capacities of Biotage® SNAP and Biotage® SNAP Ultra Cartridges

Cartridge	Usable Volume (mL)	Silica Mass (g)
SNAP 10 g	3	1.5
SNAP 25 g	11	5.5
SNAP 50 g	28	14
SNAP 100 g	28	14
SNAP 340 g	115	57

Table 7. Dry loading capacities of Biotage® SNAP and Biotage® SNAP Ultra cartridges.

Ordering Information – Frits and Insertion Rods for SNAP Cartridges

Samplet to Fit Biotage SNAP Cartridge	Dry Load Kit, 100 Frits with Insertion Rod	Dry Load Top Frits (20 ea)	Insertion Rod
Fits SNAP 10 g cartridge	SLF-0010-R	SLF-0010	SFR-0010
Fits SNAP 25 g cartridge	SLF-0025-R	SLF-0025	SFR-0025
Fits SNAP 50 and 100 g cartridge	SLF-0100-R	SLF-0100	SFR-0100
Fits SNAP 340 g cartridge	SLF-0340-R	SLF-0340	SFR-0340

Table 8. For complete ordering information, please refer to page 26-27.

Internal Liquid Loading

Direct-to-Cartridge, Pour-on Liquid Loading

For retentive compound purification where cartridge pre-equilibration is not required, liquids can be added directly onto the opened SNAP cartridge eliminating the need to inject via a syringe.

Prepare materials for liquid injection in a solvent that is as non-polar as possible, using a minimum volume.

1. Remove the top of the SNAP cartridge and remove the SNAP insert (shown on page 10).
2. Liquids materials can simply be poured into the recess at the top of the cartridge.
3. Press the inlet tube stub of the SNAP insert into the center hole of the cartridge cap and screw the cap back onto the cartridge. Ensure the cap is even and fully tightened down. There should be no more than a 1 mm gap between the cap and the mounting fin as shown.
4. The purification is now ready to run using the chosen method.

Cap screwed back onto the cartridge.

Cap fully tightened showing 1 mm gap between the cap and mounting fin.

NOTE: This technique can lead to uneven sample band loading which may result in poorer separations.

Direct-to-Cartridge: Syringe Liquid Injection

Ideal for Compounds Soluble in Weak Solvents

Using a syringe to load a cartridge is straightforward. Biotage cartridges have Luer inlet fittings for connection to Luer syringe.

Prepare materials for liquid injection in a solvent that is as non-polar as possible, using a minimum volume.

1. Remove the plunger from the syringe barrel and attach the barrel to the top of the cartridge. Then carefully pour the liquid into the barrel. This approach avoids losses of materials often experienced when aspirating liquids with a syringe.
2. When all liquid has been dispensed, either rinse the flask and add the wash to the syringe barrel or retain the remainder as a reference. Replace the syringe plunger and dispense onto the cartridge using smooth, even pressure.
3. You should observe a uniform band at the head of the cartridge.
4. Now attach upper Luer fitting and begin separation.

Sample loaded as a uniform band.

Tip – Column Loading and Software: Isolera "Load Sample" Screenshot

Figure 7. To load, press the "Load Sample" button when using an Isolera system (marked with orange circle).

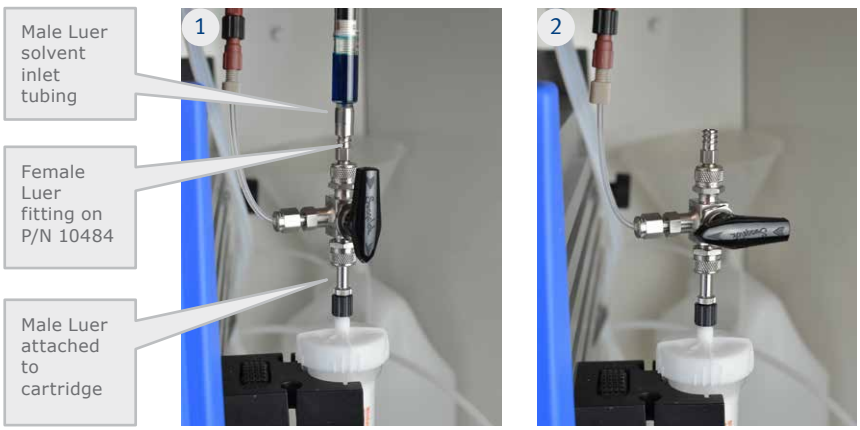
3-Way Syringe Injection with Valve

Convenient Liquid Injection

For chemists who are used to HPLC and injecting samples through a valve, this will be very familiar. And similarly, this type of injection requires fully soluble samples.

Biotage's 3-way valve facilitates liquid injections on to Biotage ZIP® or Biotage® SNAP cartridges.

1. Attach to the Isolera male Luer solvent inlet tubing and the male Luer on the bottom of the valve attaches to the cartridge as shown.
   Attach syringe barrel to female Luer on top of the 3-way valve, follow the procedure to inject with a syringe (page 16).
2. Turn valve handle clockwise 90° to open the solvent inlet.
3. The purification is now ready to run using the chosen method.



Valve handle in the load position.

Ensure the "Sample Load" button has been pressed prior to injection.

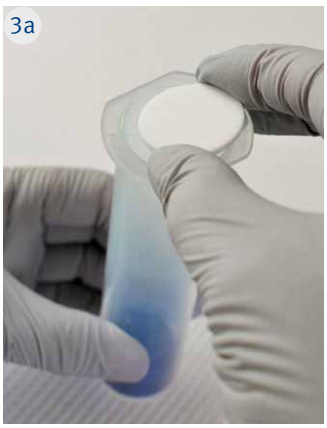
Figure 8. Isolera 3-way inject valve, part number 413027. For complete ordering information, please refer to page 26-27.

Dry Load Vessel

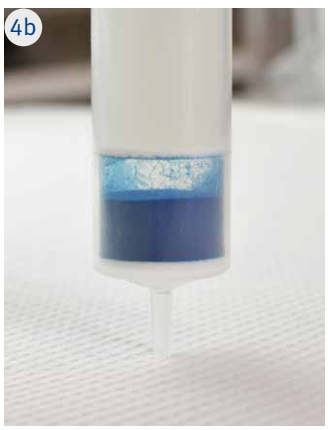
For Larger Amounts of Sample

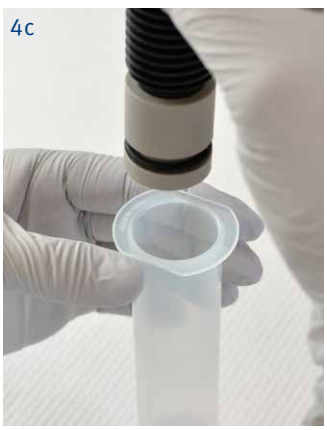
Sometimes samples sizes require more sorbent volume than can be provided by the Samplet cartridges (p. 10). Compounds that are soluble only in large volumes of volatile solvents or in a strong solvent (e.g. methanol) are best loaded using external dry loading. This technique uses an adsorbent media mixed with reaction products and solvent. After evaporation of the solvent the dried powder is then placed into an empty Dry Load Vessel which is connected to a cartridge. Cartridges pre-loaded with HM-N Diatomaceous Earth, silica and a broad variety of other adsorbants are available from Biotage.

1. Dried materials can be poured into the empty disposable cartridge.
2. Settle and level the silica bed by tapping on the side of the cartridge.
3. Insert the frit into the disposable cartridge just beyond the cartridge rim. Do not let the frit come in contact with the silica bed.

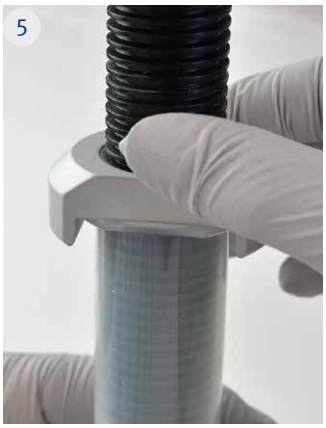


4. Insert the DLV plunger into the disposable cartridge.





5. Lock the plunger to the cartridge by turning the plunger head until its edges and the edges of the cartridge rim are aligned.
6. Turn the lower nut on the plunger clockwise to push the frit onto the silica bed.



7. Turn the upper nut on the plunger clockwise to lock the o-ring. Finger-tighten.

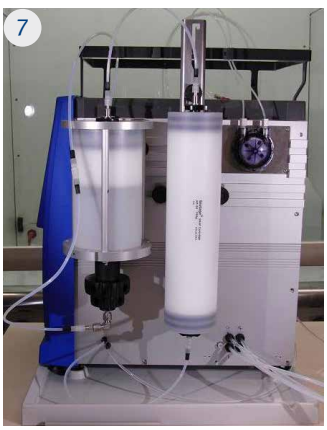
8. Lock the DLV in position using the locking pin.
9. Connect the cartridge inlet tube to the DLV and the cartridge outlet tube to the Biotage® SNAP or Biotage ZIP® cartridge.

For ordering information, please refer to page 26-27.

Dry Load Vessel (DLV-500)

Large Scale Purification with Isolera™ LS

1. Pour dried material into the disposable DLV.
2. Settle the bed by tapping the cartridge five to ten times on a lab bench. Level by tapping on the side of the cartridge.
3. Insert the frit on an angle as shown in the picture to just beyond the cartridge rim. Once in the cartridge, level the frit. Make sure it is at least 30 mm below the cartridge rim but not in contact with the bed.
4. Put the cartridge into the DLV cage. Attach the DLV head by placing it in the slots of the cage top flange and turn the head into its locked position. Turn the large nut clockwise to push the frit onto the silica bed.
5. Place the DLV onto the two guide pins on the DLV bracket with the threaded part of the DLV facing up.
6. To ensure a liquid-tight seal, insert the handle (rod) into one of the holes in the large nut and turn it firmly clockwise. Remove the handle from the large nut and insert it into the small nut. Turn the small nut about ¾ of a turn to ensure that main sealing is tight and will remain tight if the silica bed does collapse. Seal tightness can also be checked by visually inspecting the o-ring through the cartridge wall. The o-ring should be seen as a consistent black ring with no white spots. White spots indicate that the o-ring is not in contact with cartridge wall and leaks can occur in the main sealing.
7. For operation, flip the DLV over with the threaded part facing down, as seen in picture. Connect tubing as shown in the photo.



Ordering Information

Capacity	Dry Load Vessel Kits	Empty Barr	Qty.	Top Frit	Qty.
500 g	DLV-500	DLV-505	4/pk	DLV-TF	10

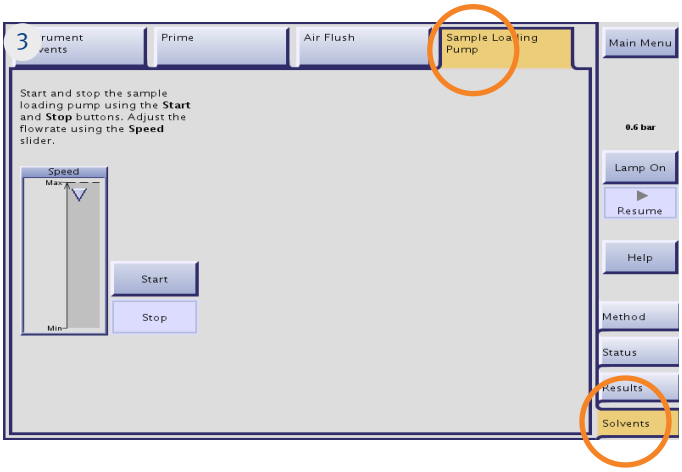
Table 9. For complete ordering information, please refer to page 26-27.

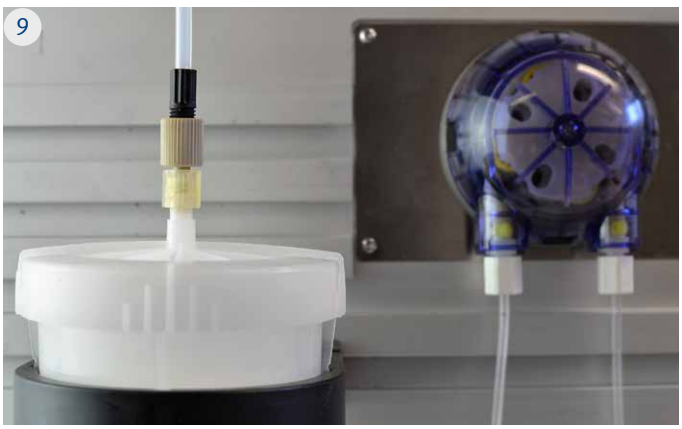
Liquid Injection with Pump

Loading Large Amounts of Liquid on Isolera™ LS

For larger scale purification, typically gram to multi-gram, the Biotage Isolera LS includes a mounted peristaltic pump. Liquid reaction mixtures are conveniently pumped onto a cartridge prior to purification.

1. Place the pump's inlet line into the liquid to be loaded on the cartridge.
2. Connect the pump's outlet line to the cartridge's inlet.
3. In the Isolera LS software select the Solvents tab in the right-hand panel then the Sample Loading Pump tab.
4. To start the pump, press Start. Adjust the flow rate using the Speed slider. So long as the amount of air is 10 mL or less, there is no adverse effect to the purification. When all liquid is loaded, press Stop.
5. When the liquid has been delivered to the cartridge, allow a small amount of air to be drawn through the pump and onto the cartridge.
6. Remove the cap and replace the pump tubing.
7. Place the pump's outlet line into a waste reservoir.
8. Select the Solvents tab in the right-hand panel (in the software) and then the Sample Loading Pump tab.
9. Press Start to start the pump and draw air into the inlet line. When the pump tubing is empty, press Stop.





NOTE. To reuse the peristaltic pump tube used in the previous run, flush and fill the tubing with a solvent suitable for the next purification.

Ordering Information

Product	Qty.	Part Number
Replacement peristaltic pump tubing for Isolera™ LS	1	412481

Table 10. For complete ordering information, please refer to page 26-27.

Theory You Need to Know

Principle of Flash Chromatography

1.1 Adsorption of Silica Gel

Silica gel is extensively used for purification by flash chromatography. Unlike the absorption of compounds onto pores of activated carbon, adsorption is based on the interaction between the surface of silica gel (hydroxyl group [-OH] for normal-phase, octadecyl group [C18] for reverse-phase), compounds, and the mobile phase.

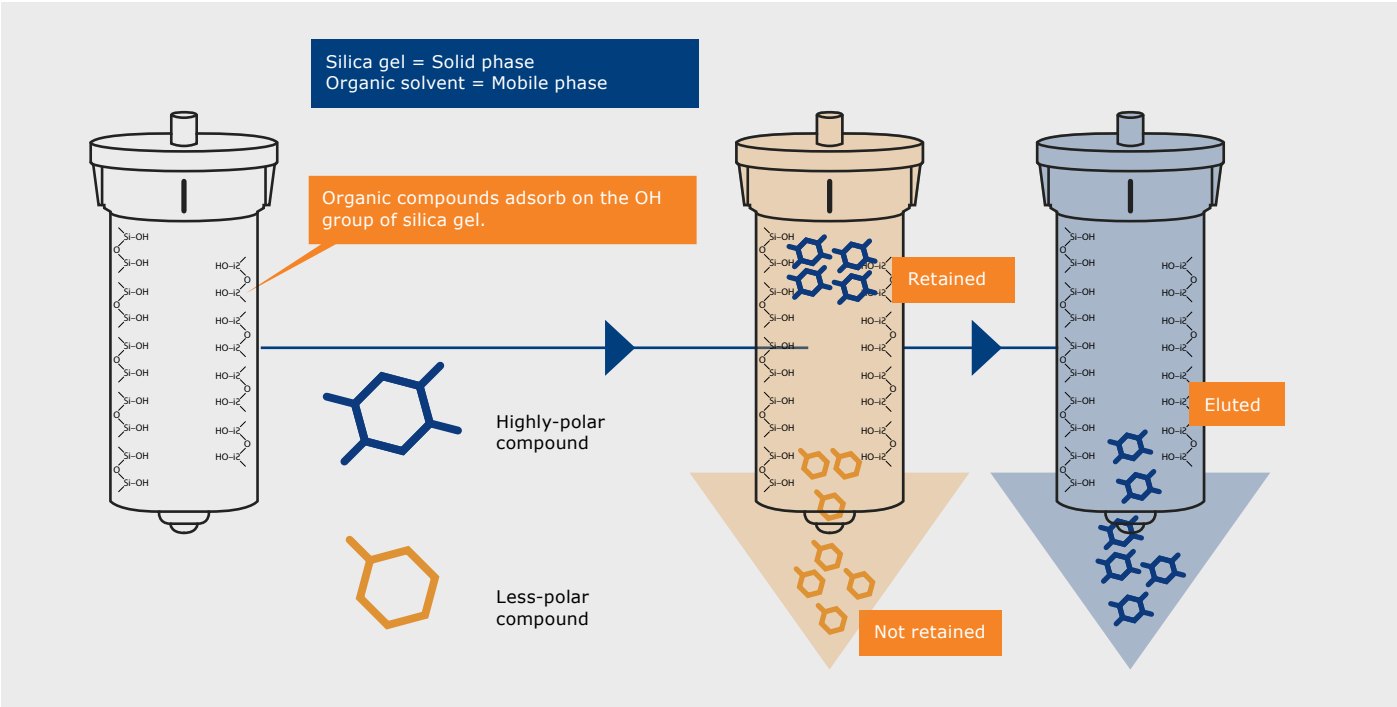


Figure 9. Interaction among the surface of silica gel, compounds, and organic solvent.

1.2 Mobile Phase

Silica gel is the stationary phase, whereas liquid flowing through the column is called the mobile phase. For normal phase chromatography, the mobile phase consists of hydrophobic solvent and hydrophilic solid phase is generally used.

For example, when a combination of ethyl acetate and hexane is used as the mobile phase in normal-phase chromatography, hydrophobic compounds are eluted from a column with a low concentration of ethyl acetate (hydrophilicity).
Typically the concentration of ethyl acetate is gradually increased until the concentration of ethyl acetate detaches the compounds from the stationary phase silica gel.

However, attention should be paid to hydrophobic and hydrophilic interactions as well as the influence of solvent properties on elution. For example, the use of dichloromethane (DCM) may produce completely different chromatograms.

1.3 Column Volume

The column volume indicates a real and fillable micro space in a packed column. The volume of the mobile phase required to fill this space is called a column volume. Chromatography can be described by column volumes of solvent passing through the cartridge.

1.4 Relationship between the R_f Value and the Column Volume Value

The column volume (CV) depends on the mass of stationary phase silica in the column. The CV value is used to help predict the solvent conditions needed to elute a compound of interest. Thin layer chromatography (TLC) results are expressed in terms of retention factor or R_f. Since CV = 1/R_f, the retention factors determined in TLC can be used to predict ideal conditions to elute a compound using flash chromatography.

Figure X shows the correlation between the R_f value and the CV value. For example, a compound with an R_f value of 0.5 has a CV value of 2 and is eluted with two column volumes. For example a cartridge with a CV of 10 mL will require 20 mL of solvent to elute a compound with an R_f of 0.5. Elution of a compound with an R_f value of 0.2 requires 5 CV.

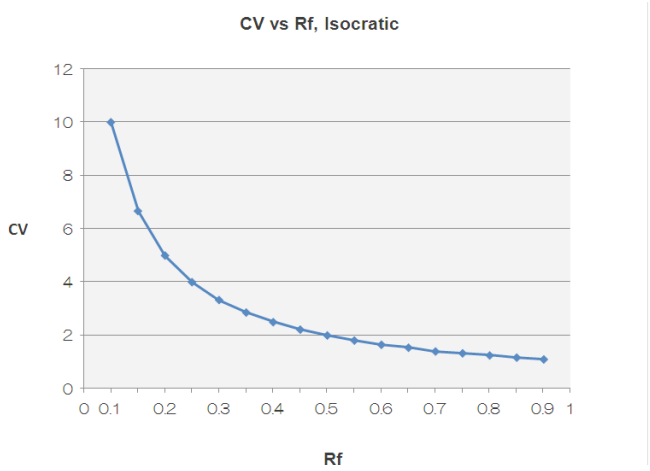


Figure 10. Correlation between the R_f Value and the CV Value.

R_f Value	0.90	0.85	0.80	0.75	0.70	0.65	0.60	0.55	0.50	0.45	0.40	0.35	0.30	0.25	0.20	0.15	0.10
CV	1.10	1.17	1.25	1.33	1.40	1.54	1.65	1.81	2.00	2.22	2.50	2.86	3.33	4.00	5.00	6.67	10.10

Table 11. Correlation between the R_f Value and the CV Value.

Thin Layer Chromatography (TLC) Plate

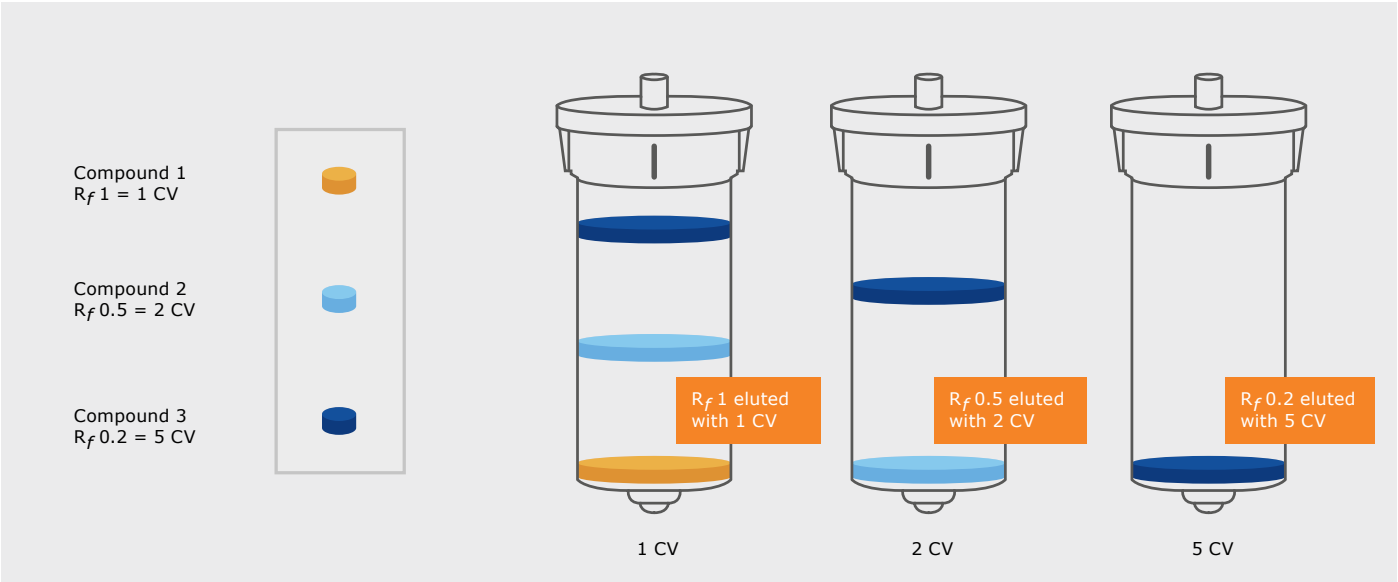


Figure 11. Conversion from TLC to Column Chromatography. Compound 1 has an R_f of 1, and since Cv=1/R_f, the a single column volume of solvent is all that is needed to elute Compound 1. Compound 2 has an R_f of 0.5 and since CV=1/0.5 = 2, Compound 2 will elute in two column volumes. Finally Compound 3 has an R_f of 0.2 resulting in a CV of 5 meaning that it will take 5 column volumes of solvent to elute Compound 3.

1.5 Prediction of Chromatograms from R_f Values

When predicting chromatograms from R_f values, people tend to attach importance to the distance between spots. In TLC, the solvent ratio that maximizes the distance between spots provides the best conditions for separation. However, the condition cannot be used for flash chromatography. When TLC spots are located close to the original spot, the distance between peaks is large in flash chromatography.

For satisfactory separation, it is important to modify the composition of mobile phase and adjust the R_f value of analytes in the range of 0.15 to 0.35.

You may think the mixture ratio of 6:4 appropriate because spots are well distributed on the TLC plate however, satisfactory separation is not achieved. Also, when spots are too close to the origin, in our example where the ratio is 8:2, peaks are diffuse and separated from each other, and the fourth peak is not visible.

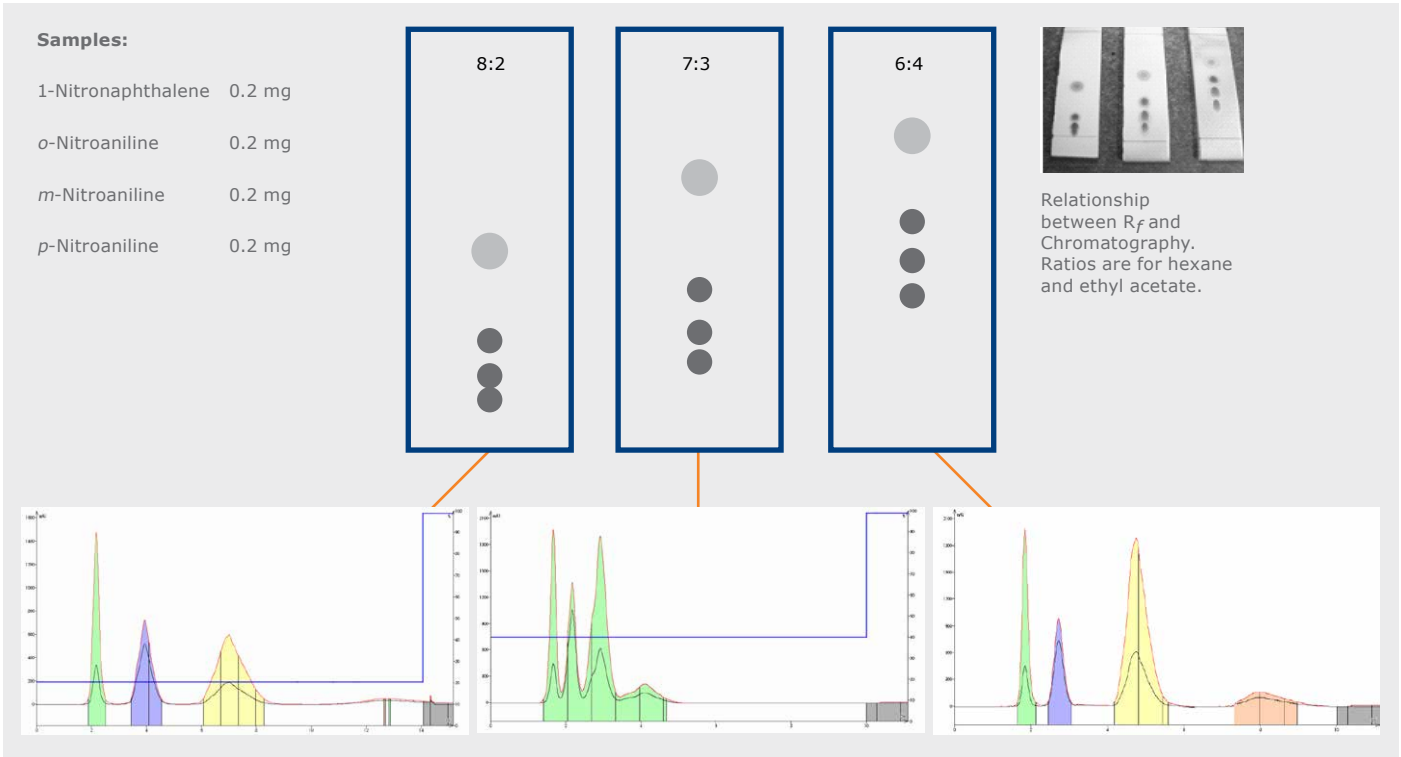


Figure 12. Prediction of chromatograms from R_f values.

1.6 Prediction of Chromatograms from CV Values

The CV values calculated from TLC are used to calculate the elution time of compounds and the volume of solvent required for purification.
Note that Biotage Isolera™ Flash Chromatography systems predict chromatographic conditions and separation based on TLC results. An example of the use of a Biotage® SNAP 25 g Cartridge is provided to the right.

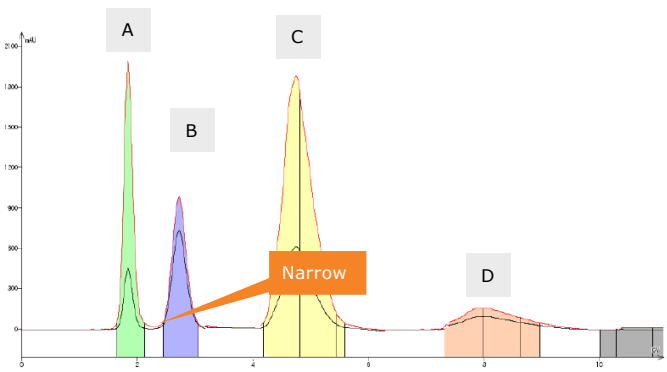


Figure 13. 25 g flash chromatography run with corresponding TLC plate.

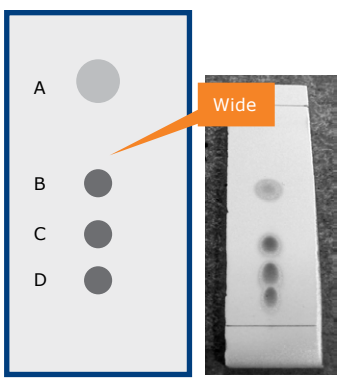


Figure 14. Chromatography and Solvent Volume.

1.7 Gradient Formation

There are three categories of solvent gradients:

Isocratic
Where the blend of mobile phase solvents is held constant throughout the separation. This approach is most like the conditions in a TLC plate or traditional glass column and requires that you through trial and error, determine the ideal solvent that produces an R_f of approximately 0.3 for your target compound. Any flash chromatography system can be run in this manner. Biotage Isolera systems use TLC data to predict ideal gradients based on the results from one or more TLC experiments.

Linear Gradient
Linear gradients are created where the solvents are varied throughout the separation. This approach is ideal for situations when optimal peak shapes are desired for all compounds in the mixture. Often this is not needed because, only one compound is of interest and excess solvent and time is used up purifying contaminants in the mixture.

Step Gradient
Step-gradients are often short and powerful processes providing separations for one or more compounds, but are also challenging to develop and optimize manually. Isolera™ Spektra provides gradient optimization through, patent-pending TLC-to-Step Gradient technology. Using solvent and TLC R_f data values, Isolera Spektra builds a step gradient to separate up to 6 compounds in the sample. This new technology will also provide cartridge selection guidance based on cartridge loading capacity and purification speed. The step gradient can also be used to isolate a targeted compound.

Gradients calculated by Biotage Isolera Spektra routinely achieve 30-50% reduction of solvent usage compared to manually calculated runs from TLC.

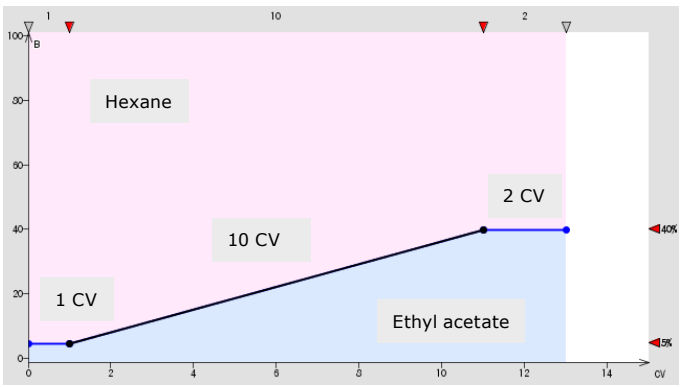


Figure 15. Example of Gradient Formation.

Ordering Information

Biotage® SNAP Ultra

Product	Qty.	Part Number
Cartridge		
Biotage SNAP Ultra cartridge, 10 g	20	FSUL-0442-0010
Biotage SNAP Ultra cartridge, 25 g	20	FSUL-0442-0025
Biotage SNAP Ultra cartridge, 50 g	20	FSUL-0442-0050
Biotage SNAP Ultra cartridge, 100 g	20	FSUL-0442-0100
Biotage SNAP Ultra cartridge, 340 g	6	FSUL-0442-0340
Samplet		
Biotage SNAP Ultra Samplet, 1 g	20	SAS-0442-0010
Biotage SNAP Ultra Samplet, 3 g	20	SAS-0442-0025
Biotage SNAP Ultra Samplet, 10 g	20	SAS-0442-0100
Biotage SNAP Ultra Samplet, 34 g	6	SAS-0442-0340

Biotage® SNAP KP-Sil

Product	Qty.	Part Number
Cartridge		
Biotage SNAP Cartridge, silica, 10 g	20	FSKO-1107-0010
Biotage SNAP Cartridge, silica, 25 g	20	FSKO-1107-0025
Biotage SNAP Cartridge, silica, 50 g	20	FSKO-1107-0050
Biotage SNAP Cartridge, silica, 100 g	20	FSKO-1107-0100
Biotage SNAP Cartridge, silica, 340 g	6	FSKO-1107-0340
Biotage SNAP Cartridge, silica, 750g	2	FSKO-1107-0750
Biotage SNAP Cartridge, silica, 1500g	2	FSKO-1107-1500
Samplet		
Biotage SNAP Samplet, 1 g	20	SAS-1107-0010
Biotage SNAP Samplet, 3 g	20	SAS-1107-0025
Biotage SNAP Samplet, 10 g	20	SAS-1107-0100
Biotage SNAP Samplet, 34 g	6	SAS-1107-0340
Bulk		
KP-Sil, bulk 5 kg	1	K0-1107-05000

Biotage® SNAP KP-C18-HS

Product	Qty.	Part Number
Cartridge		
Biotage SNAP Cartridge, 12 g	2	FSL0-1118-0012
Biotage SNAP Cartridge, 30 g	2	FSL0-1118-0030
Biotage SNAP Cartridge, 60 g	2	FSL0-1118-0060
Biotage SNAP Cartridge, 120 g	2	FSL0-1118-0120
Biotage SNAP Cartridge, 400 g	1	FSL0-1118-0400
Biotage SNAP Cartridge, 950 g	1	FSL0-1118-0950
Biotage SNAP Cartridge, 1850 g	1	FSL0-1118-1850
Samplet		
Biotage SNAP Samplet 1 g	20	SAS-1118-0012
Biotage SNAP Samplet 3 g	20	SAS-1118-0030
Biotage SNAP Samplet 12 g	20	SAS-1118-0120
Biotage SNAP Samplet 40 g	6	SAS-1118-0400

Product	Qty.	Part Number
Bulk		
KP-C18-HS, bulk 100 g	1	L0-1118-00100
KP-C18-HS, bulk 1 kg	1	L0-1118-01000
KP-C18-HS, bulk 5 kg	1	L0-1118-05000

Biotage® SNAP HP-Sil

Product	Qty.	Part Number
Cartridge		
Biotage SNAP Cartridge, 10 g	20	FSHP-1207-0010
Biotage SNAP Cartridge, 25 g	20	FSHP-1207-0025
Biotage SNAP Cartridge, 50 g	20	FSHP-1207-0050
Biotage SNAP Cartridge, 100 g	20	FSHP-1207-0100
Biotage SNAP Cartridge, 340 g	6	FSHP-1207-0340
Samplet		
Biotage SNAP Samplet 1 g	20	SAS-1207-0010
Biotage SNAP Samplet 3 g	20	SAS-1207-0025
Biotage SNAP Samplet 10 g	20	SAS-1207-0100
Biotage SNAP Samplet 34 g	6	SAS-1207-0340

Biotage® SNAP KP-NH

Product	Qty.	Part Number
Cartridge		
Biotage SNAP Cartridge, 11 g	10	FSN0-0909-0011
Biotage SNAP Cartridge, 28 g	10	FSN0-0909-0028
Biotage SNAP Cartridge, 55 g	10	FSN0-0909-0055
Biotage SNAP Cartridge, 110 g	10	FSN0-0909-0110
Biotage SNAP Cartridge, 375 g	1	FSN0-0909-0375
Biotage SNAP Cartridge, 900 g	1	FSN0-0909-0900
Biotage SNAP Cartridge, 1800 g	1	FSN0-0909-1800
Samplet		
Biotage SNAP Samplet 1 g	20	SAS-0909-0011
Biotage SNAP Samplet 3 g	20	SAS-0909-0028
Biotage SNAP Samplet 11 g	20	SAS-0909-0110
Biotage SNAP Samplet 37 g	6	SAS-0909-0375
Bulk		
KP-NH, bulk 100 g	1	N0-0909-00100
KP-NH, bulk 250 g	1	N0-0909-00250
KP-NH, bulk 500 g	1	N0-0909-00500
KP-NH, bulk 1 kg	1	N0-0909-01000
KP-NH, bulk 5 kg	1	N0-0909-05000

Biotage ZIP®

Product	Qty.	Part Number
Cartridge		
Biotage ZIP cartridge, 5 g	20	440-0500-DZ-20
Biotage ZIP cartridge, 10 g	20	440-1000-EZ-20
Biotage ZIP cartridge, 30 g	20	440-3000-FZ-20
Biotage ZIP cartridge, 45 g	20	440-4500-SZ-20
Biotage ZIP cartridge, 80 g	20	440-8000-JZ-20
Biotage ZIP cartridge, 120 g	20	440-120G-UZ-20

Biotage® ZIP Sphere

Product	Qty.	Part Number
Cartridge		
ZIP Sphere Silica cartridges, 5 g	20	445-0500-DZ-20
ZIP Sphere Silica cartridges, 10 g	20	445-1000-EZ-20
ZIP Sphere Silica cartridges, 30 g	20	445-3000-FZ-20
ZIP Sphere Silica cartridges, 45 g	20	445-4500-SZ-20
ZIP Sphere Silica cartridges, 80 g	20	445-8000-JZ-20
ZIP Sphere Silica cartridges, 120 g	20	445-120G-UZ-20

ISOLUTE® HM-N Diatomaceous Earth

Product	Qty.	Part Number
Cartridge		
ISOLUTE HM-N, 0.3 mL	100	800-0040-BM
ISOLUTE HM-N, 1 mL	100	800-0100-CM
ISOLUTE HM-N, 3 mL	100	800-0220-DM
ISOLUTE HM-N, 5 mL	100	800-0350-EM
ISOLUTE HM-N, 10 mL	50	800-0700-EM
ISOLUTE HM-N, 20 mL	50	800-1300-FM
Tab-Less Columns for High Throughput Applications		
ISOLUTE HM-N, 0.3 mL, tab-less	100	800-0040-BMG
ISOLUTE HM-N, 1 mL, tab-less	100	800-0100-CMG
Bulk		
ISOLUTE HM-N, bulk 1 kg	1	9800-1000
ISOLUTE HM-N, bulk 5 kg	1	9800-5000

Accessories

Product	Qty.	Part Number
Injection Valve Adapter		
Adapter to attach a Biotage 3-way injection valve to a Biotage SNAP Cartridge	1	411081
Empty Samplets		
Empty Samplet kit for 10 g Cartridge	20	SES-0010
Empty Samplet kit for 25 g Cartridge	20	SES-0025
Empty Samplet kit for 50 and 100 g Cartridge	20	SES-0100
Empty Samplet kit for 340 g Cartridge	6	SES-0340
Dry Load Frits and Insertion Tools		
Frit insertion tool for 1 g empty Biotage SNAP Samplet cartridges	1	SFS-0010
Frit insertion tool for 3 g empty Biotage SNAP Samplet cartridges	1	SFS-0025
Frit insertion tool for 10 g empty Biotage SNAP Samplet cartridges	1	SFS-0100
Frit insertion tool for 34 g empty Biotage SNAP Samplet cartridges	1	SFS-0340
Frit insertion rod for SNAP 10 g cartridge	1	SFR-0010
Frit insertion rod for SNAP 25 g cartridge	1	SFR-0025
Frit insertion rod for SNAP 50 and 100 g cartridge	1	SFR-0100
Frit insertion rod for SNAP 340 g cartridge	1	SFR-0340
SNAP cap wrench, 50 and 100 g	1	SFW-0100
SNAP cap wrench, 340 g	1	SFW-0340
Dry load frits for SNAP 10 g cartridge	20	SLF-0010
Dry load frits (100) and insertion rod (1) set for SNAP 10 g cartridge	1	SLF-0010-R
Dry load frits for SNAP 25 g cartridge	20	SLF-0025
Dry load frits (100) and insertion rod (1) set for SNAP 25 g cartridge	1	SLF-0025-R
Dry load frits for SNAP 50 and 100 g	20	SLF-0100
Dry load frits (100) and insertion rod (1) set for SNAP 50 and 100 g cartridge	1	SLF-0100-R
Dry load frits for SNAP 340 g cartridge	6	SLF-0340
Dry load frits (6) and insertion rod (1) set for SNAP 340 g cartridge	1	SLF-0340-R
Dry Load Vessel (DLV)		
Dry load vessel kit with holder, 20 empty cartridges & frits, and plunger, 30 g	1	DLV-030
Dry load vessel kit with holder, 20 empty cartridges & frits, and plunger, 70 g	1	DLV-070
Dry load vessel kit with holder, 20 empty cartridges & frits, and plunger, 500 g	1	DLV-500
Dry load vessels and frits, 30 g	20	DLV-035
Dry load vessels and frits, 70 g	20	DLV-075
Dry load vessels and frits, 500 g	4	DLV-505

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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
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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
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