Reversed-Phase Flash Purification

Organic Chemistray Application Development #1

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Reversed-Phase Flash: Beyond the limits of normal-phase chromatography

Normal-phase flash chromatography[1] has been widely adopted as the method of choice for separation of product mixtures and reaction by-products. One of the most significant developments in this area concerns the practical separation of polar molecules. Reversed-phase purification is a modification of normal-phase chromatography that provides an efficient mechanism for the separation of polar compounds.

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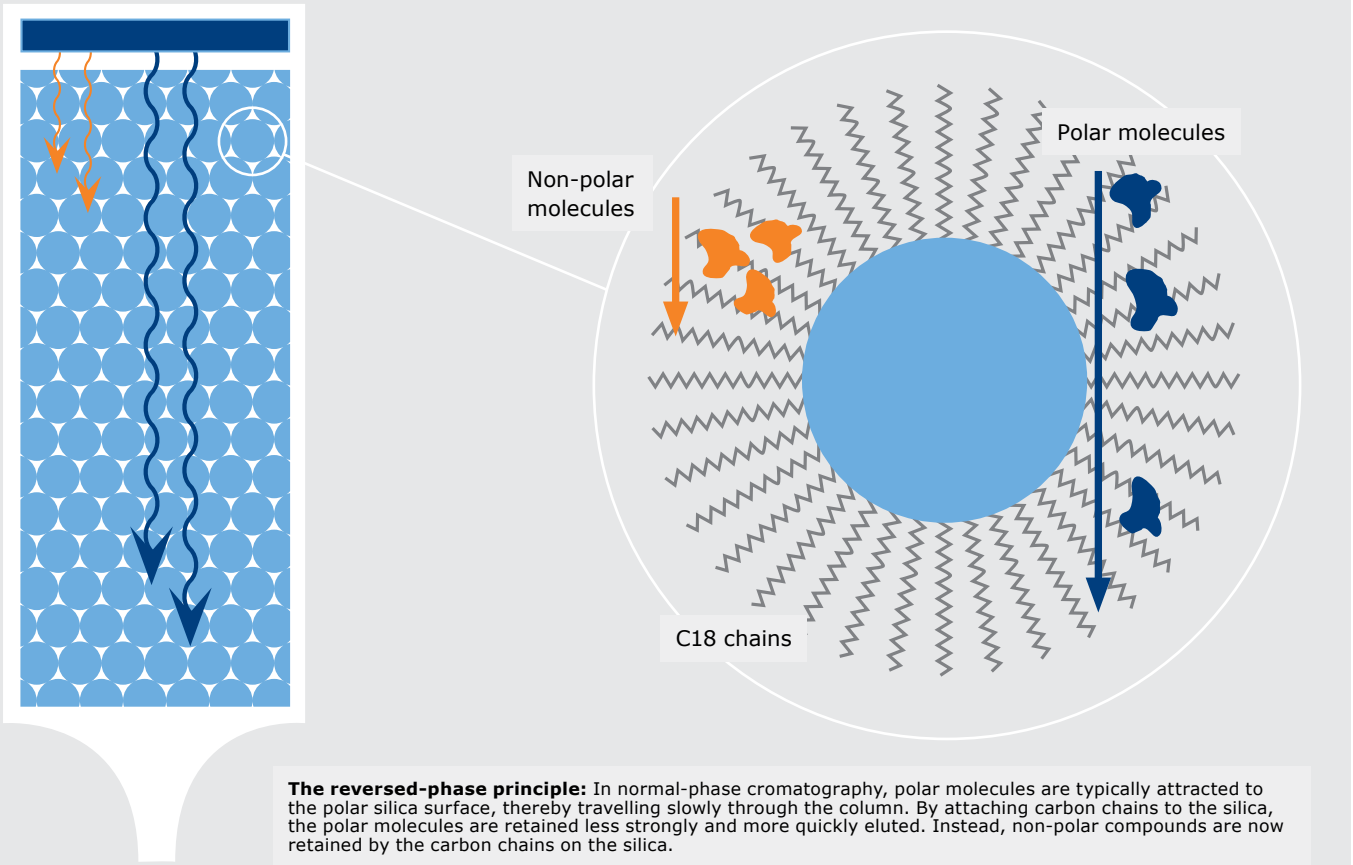
Efficient separation at low cost and minimum environmental impact is the holy grail for the chemistry industry. Reversed-phase chromatography provides a great leap forward, but this has been a relatively under-used technique due to lack of information and supporting data.

Polar, water soluble molecules are the focus of many pharmaceutical drug development programs and natural product research. These molecules make up the majority of the compounds involved in the fundamental chemistry of living organisms. Isolation of large quantities of such polar compounds has traditionally been carried out using expensive preparative HPLC (High Performance Liquid Chromatography) systems. These systems are typically dedicated to final product purification and are not economically viable options for many chemists for day to day purification needs. Reversed-phase chromatography[2,3] using pre-packed flash cartridges is a development that facilitates the isolation of milligram to multi-gram quantities of polar compounds from naturally occurring materials or from synthetic reaction products.

Reversed-phase flash chromatography uses larger particle media that lowers operating pressures and allows the use of larger pre-packed disposable columns for routine isolation of milligram to multi-gram quantities of material (Figure 1).

Figure 1: Biotage offers pre-packed columns for reversed-phase flash chromatography which can be effortlessly run using fully automated flash chromatography instruments such as the Biotage® Isolera™ Purification Instrument family.



The reversed-phase principle: In normal-phase cromatography, polar molecules are typically attracted to the polar silica surface, thereby travelling slowly through the column. By attaching carbon chains to the silica, the polar molecules are retained less strongly and more quickly eluted. Instead, non-polar compounds are now retained by the carbon chains on the silica.

Introduction

What is Reversed-Phase Flash Chromatography?

Reversed-phase flash chromatography differs from normal-phase chromatography in three key areas, 1) the stationary phase chemistry, 2) the solvents used and 3) the type of product being separated.

A variety of compounds
Given the diversity of chemical compound classes, it can sometimes be difficult to choose the most appropriate stationary phase and separation mode. A guide to the types of compounds amenable to separation using reversed-phase techniques is presented in Table 1 (overleaf). The following is a brief summary of the stationary phases and the solvent conditions used.

Structure of the stationary phase
Normal-phase silica has a polar surface, whereas reversed-phase silica has a surface modified with alkyl chains (e.g. C-18) making it much less polar and therefore less retentive toward polar compounds (Figure 2).

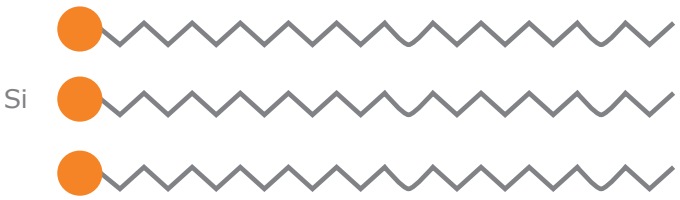


Figure 2: Representative view of a reversed phase silica phase. The C18 alkyl chain bonded to the silica surface increases the silica's hydrophobicity. (Backbone details not shown for clarity).

Solvents
In normal-phase chromatography, highly polar water soluble compounds are difficult to elute as they have strong affinity for the polar surface of silica. This strong affinity can make successful purification overly challenging, forcing the use of stronger (more polar) solvents in elution of products. Under normal-phase conditions, these solvents are less selective and cannot produce compounds of sufficient purity. However,

in reversed-phase purification, solvents with lower polarity can be used, since the polar compounds have less affinity for the hydrophobic stationary phase and can be eluted more effectively. So in practice, reversed-phase provides a more facile means of separation using inexpensive readily available solvents such as water, methanol or acetonitrile.

The separation mechanism is based on hydrophobic binding interactions (partitioning) of the solute between the mobile phase and the stationary phase. Throughout the chromatographic procedure, the mobile phase composition can be gradually modified (this is called a *gradient elution*) to favor desorption of the more retentive products back into the mobile phase, in order to control the separation and elution of sample mixture components.

Optimizing reversed-phase flash

Reversed-phase chromatography compliments normal-phase chromatography by extending the scope of separations possible using normal laboratory automated flash purification instruments. There are two principle options for implementing a reversed-phase separation protocol.

1. Allow by-products to pass through the column while the compound of interest is retained more strongly by the column, thereby teasing products away from the crude mixture.
2. Allow by-products to bind the column while the product of interest passes through.

It is usually more effective to bind the product of interest, since it can be released in a more concentrated state on elution. This also means that very dilute samples can be applied to the reversed-phase column, because compounds are concentrated by binding to the stationary phase. When the appropriate solvent gradient is later passed through the column, concentrated compounds can be released to the eluent in a tighter band resulting in a minimum fraction volume.

In this guide we have focused on the first method as this is one of the most popular and efficient ways to run reversed-phase flash chromatography.

Product	Examples	Normal- Phase Silica	Reversed-phase Silica	Amino Phase Silica
Alkaloids	Cocaine, morphine, nicotine, quinine	•	•	•
Amino acids			•	
Analgesics	Aspirin, acetaminophen, ibuprofen	•	•	
Aromatics		•	•	•
Basic drugs			•	•
Carbohydrates	Sugars		•	•
Flavanoids			•	
Glycosides			•	•
Lipids	Phospholipids	•	•	
Natural Products	Terpenes, saponins, polyphenols	•	•	
(Oligo)nucleotides			•	
Peptides (< 2k MW)		•	•	
Steroids		•	•	
Tannins			•	
Vitamins	Tocopherols (Vitamin E), retinol (Vitamin A), Vitamin D, Vitamin K	•	•	•

Table 1: Overview of chromatographic methods and application to a variety of product types

Applications

1) Natural Product Purification

Naturally occurring compounds are the basis for many commonly used pharmaceuticals and are also the backbone of the rapidly growing nutraceutical market. The feed materials for these natural products can be flowers, leaves, bark, root systems, fruit, or other natural substance. The challenge is extracting the compounds of interest from the natural matrix in which they occur. The Active Pharmaceutical Ingredients (API) in the extracts may only be present in small quantities (0.3 % to 3 %)[4]. Procedures for extraction vary widely and are dependent on the matrix as well as the compound of interest ranging from cold pressing to extraction, distillation and steam distillation methods. In this guide we used extraction methods.

Flash chromatography systems are typically equipped with an ultraviolet (UV) or UV-Vis (for visible light) detector. Natural products do not always absorb energy in the UV or visible region of the spectrum, so they may be missed by these visualization techniques. These may instead be visualized by TLC after reacting with a visualization reagent. However, a far better solution is the use of a detector designed to capture signals from weakly UV absorbing molecules, such as the Biotage Evaporative Light Scattering Detector (ELSD-1080). This detector vaporizes a portion of the flash cartridge effluent and is able to detect the particles left, making it universal in its application.

Cyclodextrins

The ELSD-1080 detector described above enables fractionation of compound classes with little or no UV or visible light absorption, for example lipids, terpenes, steroids and carbohydrates such as cyclodextrins, thus expanding the use of the Biotage® Isolera™ flash purification systems (Figure 3).

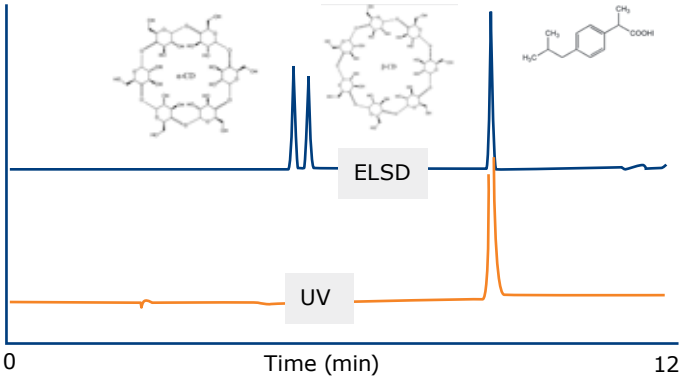


Figure 3: The ELSD revealed the true composition of a mixture of cyclodextrin and ibuprofen due to its sensitivity to compounds that possess weak or no UV chromophores.
Column: C18 5 µm, 150x4.6 mm, (such as Biotage® Resolux™).
Gradient conditions: eluent A: water; eluent B: acetonitrile.
Gradient: 50–95 % B in 5 min, flow rate: 1.0 mL/min, inj vol: 20 µL.
Detector: Biotage ELSD (neb=30°C, evap=50°C, gas=1.0 SLM.

Sweeteners

In another example, a reversed-phase mixture using commercially available Equal® (Aspartame®, dextrose, maltodextrin) was separated into its components (Figure 4).

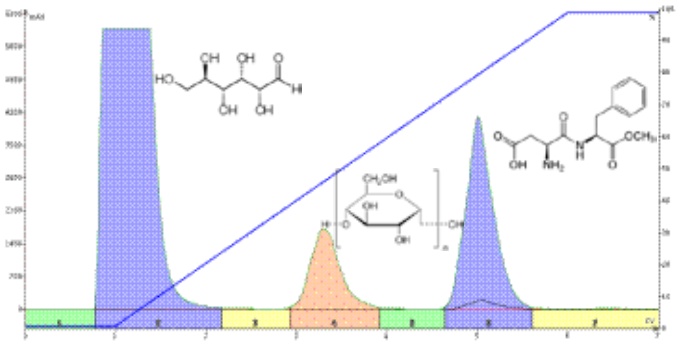


Figure 4: 1 packet (1 g) of Equal® was dissolved in 10 mL of water and separated using a reversed phase gradient with a pre-packed Biotage® SNAP KP-C18-HS cartridge.
Run conditions: Solvent A: Deionized water, Solvent B: Methanol.
Flow rate: 12 mL/min.
Equilibration: 5 CV @ 50 mL/min.
Gradient: 0 % B for 1 CV, 0 to 100 % B in 5 CV, 100 % B for 1 CV, injection 0.5 mL, Detection, UV1: 200, UV2: 205, ELSD, EVAP 40, NEB 40, GAS 2.5, LED 50, PMT 1, Smth 50, Threshold 50, Collection Mode: Collect all.

Tobacco Extract

Tobacco extract contains a lot of compounds, and its composition is subject of interest to a wide variety of researchers. In this example, commercially available tobacco was extracted and purified by reversed-phase flash chromatography using a 12 g Biotage SNAP KP-C18-HS flash cartridge (Figure 5).

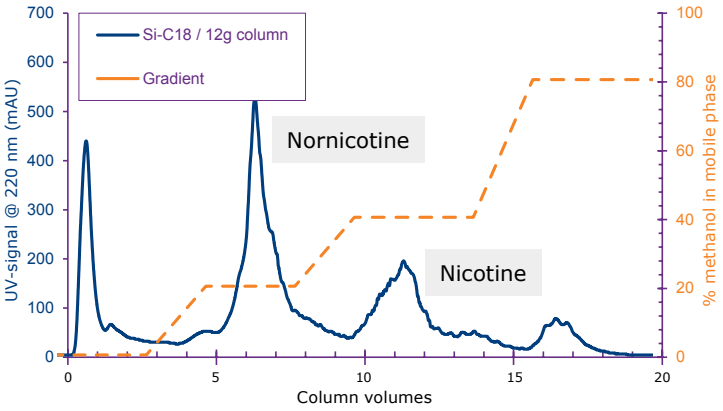


Figure 5: Chromatogram of crude tobacco extract. 1 g of commercially available tobacco was extracted with 10 mL of 20 mM ammonium acetate, pH 6.8 for 24 hours. The mixture was centrifuged to remove solids and purified by reversed phase flash chromatography using a pre-packed 12 g SNAP KP-C18-HS column. The identity of all peaks was not confirmed, but the major constituents (nornicotine, anabasine, anatabine, nicotine and flavorings) confirmed.

2) Peptide Purification

Fully protected peptides can be rather large (2–3 kDa) and hydrophobic, but may still be fairly easily isolated using reversed-phase flash chromatography. This approach overcomes the problems encountered in traditional normal phase gravity glass column chromatography by providing better resolution, faster separation times, increased sample recovery, and greatly reduced solvent and adsorbent consumption.

The peptide ACP 65-74 (Figure 6) is a good example of a sequence that benefits from the combined application of normal- as well as reversed-phase flash chromatography at different stages in the peptide synthesis. Traditionally this has been categorized as a difficult sequence to synthesize, but the recent advent of microwave peptide synthesizers (such as the Biotage® Syro *Wave*™) makes synthesis of this sequence much easier, resulting in higher purity crude products.

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

Figure 6: Acyl Carrier Protein (ACP 65-74).

Peptide yields are generally improved by preventing the formation or development of internal secondary structures during chain extension. In this example, ACP 65-74 was prepared using a solution phase (5+5) segment condensation. The fully protected peptide segments are purified prior to segment condensation using normal phase flash chromatography. The final deprotected peptide was further purified by reversed-phase flash chromatography. This simple, two-step purification approach eliminated the traditional costly HPLC purification step (Figure 7).

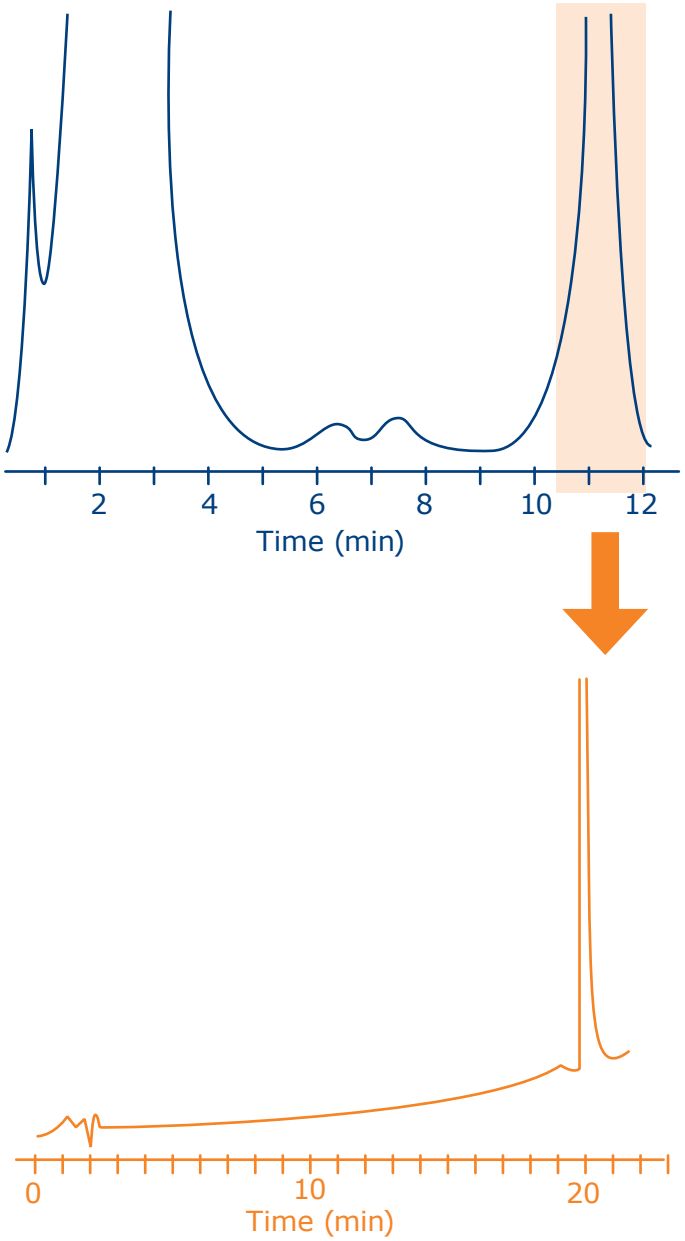


Figure 7: Reversed flash purification. Solvent A: Deionized or HPLC-grade water + 0.05 % TFA. Solvent B: Acetonitrile + 0.1 % TFA. Flow rate: 8 mL/min. Equilibration: 5 CV @ 50 mL/min. Gradient: 10 % B to 100 % B in 15 min, Detection, UV220nm. Confirmed by HPLC Solvent A: Deionized or HPLC-grade water + 0.05 % TFA, Solvent B: Acetonitrile + 0.1 % TFA, Flow rate: 1.5 mL/min, Gradient: 0-100 % B in 40 minutes, Detection, UV 220 nm. Column C18, 5 um (4.6 x 250mm).

3) Desalting

Desalting is a general term applied to the separation of (typically) low molecular weight additives/by-products from higher molecular weight products. This is associated with natural products since amine/peptide synthesis usually employs reagents which generate low molecular weight species as well as target products. This process is also called buffer exchange and may be carried out by size exclusion (gel filtration) methods. Size exclusion chromatography is a mild separation method, but it suffers from being less economical than flash chromatography methods. Furthermore, it is not always predictably scaled up and can dilute the original sample concentration significantly, resulting in the need to extract product from a much larger volume of solvent.

Desalting via reversed-phase flash methods allows samples to be recovered in much smaller solvent volumes, even though very large volumes of product solution may be used. Since compounds have an affinity for the stationary phase, they are essentially concentrated on the column and the product may be eluted using a smaller volume of lower polarity mobile phase such as acetonitrile, which may be evaporated for easier product isolation later.

The ACP 65-74 peptide was synthesized by conventional SPPS methods and Figure 8 shows the result of desalting. The peptide as isolated from a traditional crude synthesis was approximately 45 % pure by HPLC. Following desalting using a Biotage SNAP KP-C18-HS 12 g cartridge, the product purity increased to approximately 90 %.

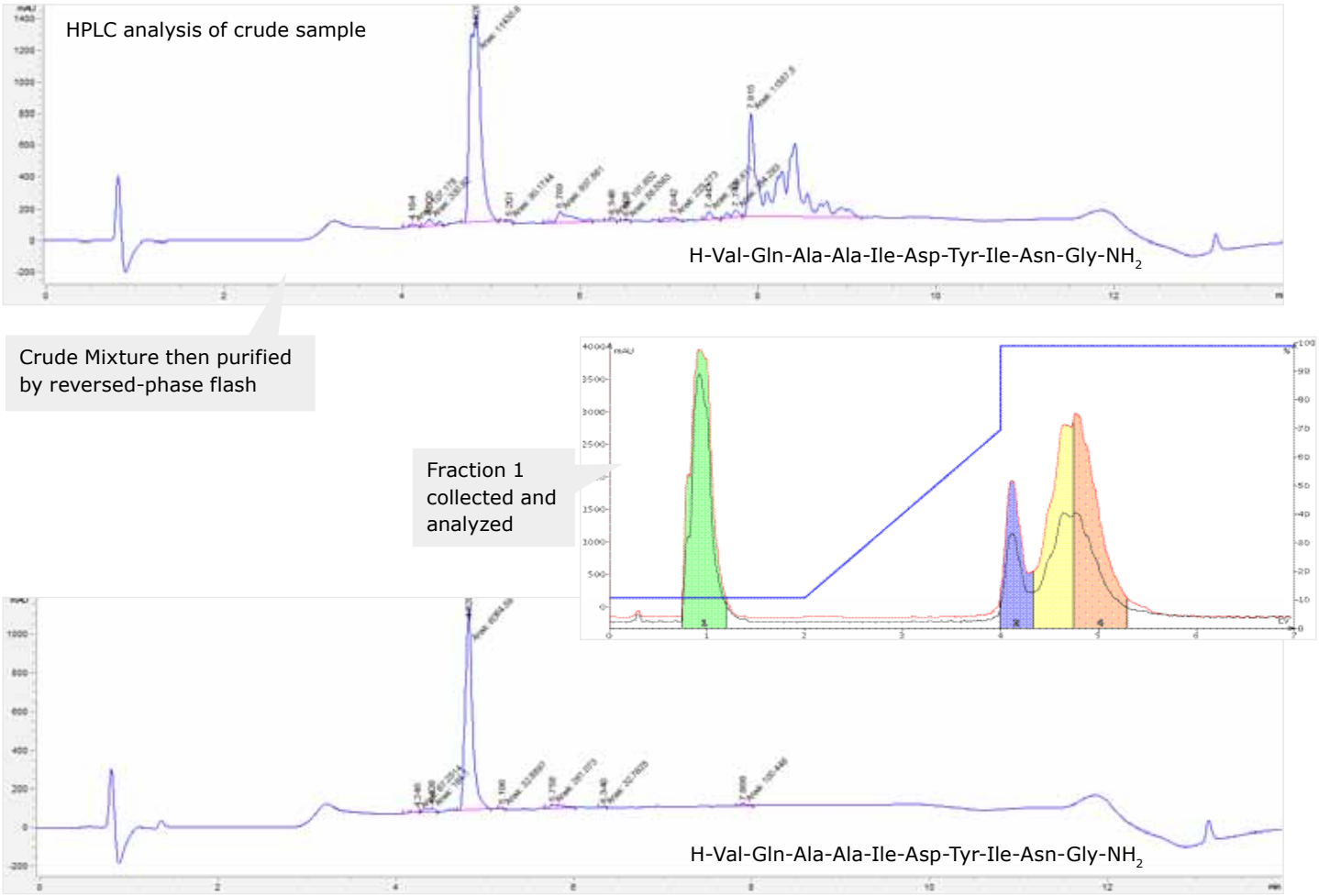


Figure 8: ACP 10-mer peptide sequence HPLC (top). This was applied to reversed-phase purification using a 12 g Biotage SNAP KP-C18-HS cartridge (right). Fraction 1 (10 mL) was then collected and analyzed by HPLC (lower).

4) pH dependency

Sometimes it is necessary to conduct flash purification under conditions of high pH. It is generally accepted that silica can chemically break down under highly alkaline conditions. Silica that has been modified with C18 chains is however more resistant to chemical attack and may therefore be used under certain conditions of alkaline pH.

Propanolol is a non-selective beta blocker used to treat hypertension, anxiety and panic symptoms. Amitriptyline is a tricyclic antidepressant which also eases migraines, tension headaches, anxiety attacks, and some schizophrenic symptoms. Both of these molecules are bases, and are ionized at low pH. Since the main mechanism of interaction in reversed-phase flash chromatography is hydrophobic interactions, the presence of formal charges in substrates can affect the chromatography. Species that are charged during the gradient will have very low affinity for the C18 chains and are therefore poorly retained, eluting quickly in very few column volumes (CV) (Figure 9).

5) Recycling

While it is much more efficient and economical not to re-use normal phase flash silica columns (deactivation of the retentive silica surface and cross-contamination is always a significant risk), many chemists do consider re-using their reversed phase flash cartridges. The risk of failure is perceived to be lower since the bonded silica is more resistant to conditions of the flash chromatography, and it has been normal practice to re-use analytical reversed phase HPLC columns for many years.

Biotage KP-C18-HS silica has been designed to be effective for reversed phase purifications, and while it is not considered normal procedure to expose the reversed phase silica to extreme conditions of pH, Biotage has demonstrated that the silica is very resistant to chemically harsh conditions. As an indication of stability we subjected the same 12 g KP-C18-HS SNAP cartridge to constant aqueous exposure at pH 11 for 16 hours. Aliquots of a stock propanolol and amitriptyline solution mixture at pH 11 was repeatedly added to the column and run under isocratic conditions (to maintain the pH). The plate count and chromatography of the SNAP cartridge was excellent showing very stable peak shape and profile. 10 successive runs were carried out, with the 11th run performed after 16 hours of exposure to the buffer. Although these results were satisfying, the user is similarly encouraged to proceed with caution if considering purification under extreme pH conditions.

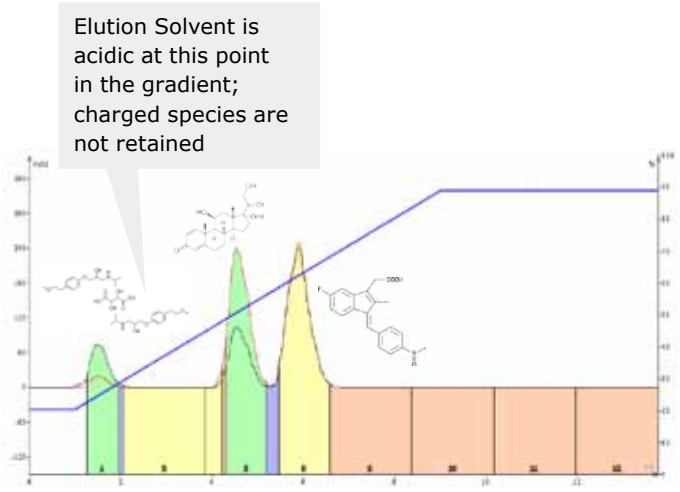


Figure 9: Gradient elution of acidic, basic, and a neutral molecules. Early in the gradient, the solvent was predominantly acidic under these conditions so the basic compound was charged and therefore eluted faster than expected.
Run conditions: Mobile Phase: A: 0.1 % formic acid in H2O. B: acetonitrile. Flow: 20 ml/min, Load: 0.5 % (60 mg total load in 0.5 ml acetonitrile). Wavelength: 254 nm (collection) 275 nm (monitoring). Gradient: 20 % B – 1CV, 20-90 % B – 8CV, 90 % B - 4CV.

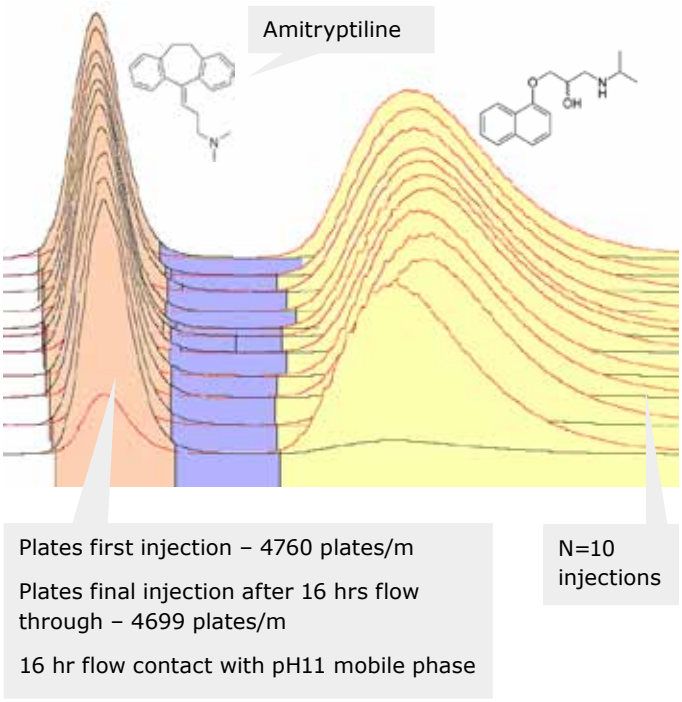


Figure 10: At pH 11, a Biotage SNAP KP-C18-HS 12 g cartridge showed reproducible chromatography for 8 hours, and also retained good selectivity after 16 hours of constant exposure to high pH elution solvents. (Note an isocratic, *not* gradient solvent system was used in order to maintain the high exposure of the cartridge to aggressive solvents).
Mobile Phase: Methanol: 20 mM. Phosphate buffer 80:20 v/v adjusted with ammonium hydroxide to pH 11. Load 1 % load/g in Methanol.

How To-Guide to Reversed-Phase Flash Purification

Reversed-Phase Silica

Biotage KP-C18-HS silica was developed for the purification of small organic molecules and has also been widely used for the purification of low molecular weight, chemically synthesized peptides. C18 bonded silica is produced as porous particles that are stable in a range of pH's, and compatible with all reversed-phase solvents; thus an ideal choice as a generic media. Biotage has optimized essential physical properties for a number of silicas such as porosity, surface area, loading capacities, and distribution of surface groups, resulting in enhanced separating efficiency. Most of this selectivity depends on the bonded silica phase and the solvent conditions employed during a purification run.

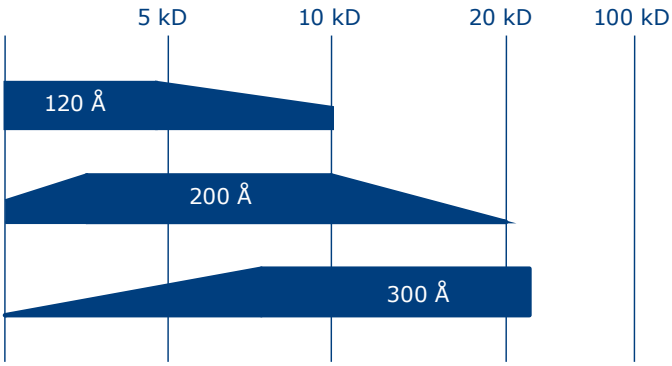


Figure 11: Generally accepted MW cut offs for silica and size exclusion. In flash chromatography, a standard 120 Å pore size media is capable of separating products up to 10,000 Daltons.

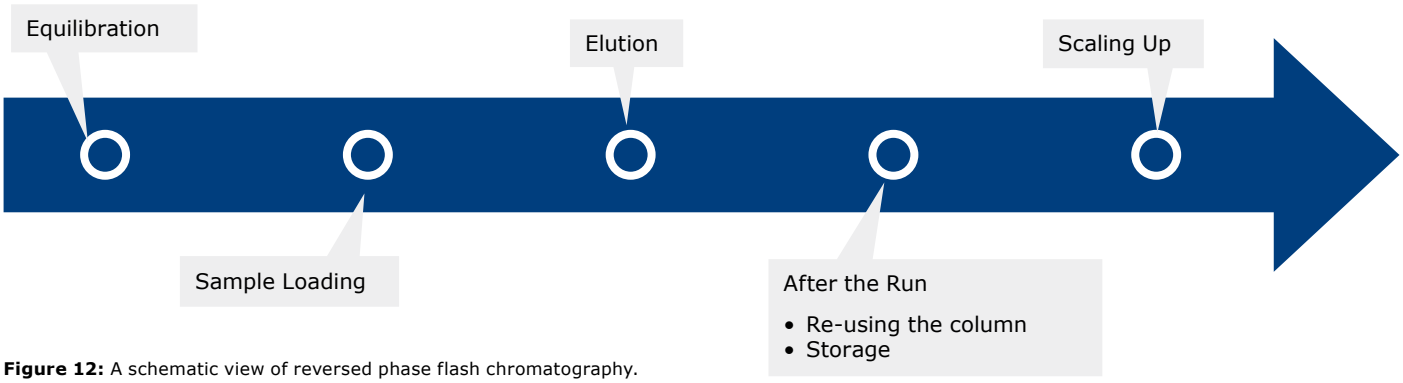


Figure 12: A schematic view of reversed phase flash chromatography.

Silica with a pore size of approximately 120 Å is used predominately for small organic molecules and peptides, while silica with a pore sizes of 300 Å or greater is typically used for the purification of larger molecules such as peptides and proteins. Figure 11 shows generally accepted cut off points for pore size and molecular weight.

Running Reversed-phase Chromatography

Just like normal-phase flash, reversed-phase separations consist of a number of discrete stages (Figure 12).

1) Equilibration
The first step is to equilibrate the reversed-phase column using the three steps below under suitable initial conditions of pH, ionic strength, and polarity. The polarity of the mobile phase is controlled by adding organic modifiers such as acetonitrile and buffers. Modifiers/ion-pairing buffering agents (for example TFA) may also be added. The polarity of the initial mobile phase must be low enough to dissolve the partially hydrophobic product but high enough to ensure that the product can bind to the RP matrix.

Nominal Cartridge Size (g)	Mass of Media (g)	Column Volume (mL)	Recommended Load Range for ΔCV 0.1–4	Flow Rate (mL/min)
10	12	15	60-120 mg	5-20
25	30	33	150-300 mg	15-40
50	60	66	300-600 mg	25-50
100	120	132	600-1.2 g	25-50
340	400	510	2-4 g	50-100
750	950	990	4.5-9 g	100-500
1500	1850	1980	9-18 g	300-500

Table 2: Loading table for Biotage SNAP reversed-phase flash cartridges. The separation using the scaling column will dictate sample loading on the Biotage SNAP cartridge. A separation in which there is clear and large baseline separation will be able to tolerate a much higher initial sample loading amount.

Recommended equilibration steps

1. 100 % organic (5 column volumes)
2. 50 % organic/50 % aqueous (5 column volumes)
3. Starting conditions (5 column volumes)

2) Sample Loading
After equilibration, the sample can be applied. Typically, the sample is dissolved in the initial gradient or solvent conditions for the run. The amount of sample that can be loaded varies with column size, separation difficulty and column efficiency.

Another technique used for loading difficult-to-dissolve samples is dry loading. A crude sample may be soluble only in a strong solvent like DCM or acetone, which if injected into a highly aqueous solvent system can cause precipitation or poor chromatography. When dry loading Biotage® SNAP* cartridges, Biotage recommends Samplet® cartridges. Samplet cartridges are inserted into the Biotage SNAP cartridge after the sample is added and dried to remove the solvent. Samplet cartridges will also act as a guard cartridge protecting the main flash cartridge, therefore further extending the life and usability of the SNAP cartridge.

For convenient reference, see the sample loading table for reversed-phase flash columns (Table 2).

3) Elution
When the polarity of the mobile phase is decreased, product bound to the reversed-phase media elutes from the column. In reversed-phase chromatography this means increasing the percentage of organic solvent (methanol or acetonitrile). Typically, a gradient increasing from 5 % organic in water to 100 % organic is "swept" over a number of column volumes in order to elute the product efficiently from the column.

Although a large variety of organic solvents can be used, in practice only a few are routinely employed. The two most widely used are methanol and acetonitrile. Isopropanol can also be used, but is more viscous than acetonitrile or methanol, resulting in higher back-pressures. All of these solvents are UV transparent below 254 nm, which is important because product elution is typically monitored using UV detection.

*For reference, see Snap Users Guide. Biotage 2011, available on www.biotage.com

In practice, acetonitrile is mostly used when separating peptides, since it provides a lower baseline response for the low UV region (under 220 nm) than other solvents. however, recent advances in automated flash purification, such as the advanced baseline correction algorithm used by Biotage® Isolera™ Spektra further alleviate the problems of drifting baselines, increasing reversed phase flash solvent scope.

Amino (NH_2) groups in peptides and proteins are charged below pH 7.5, whereas carboxylic acid groups become neutral as pH decreases. Mobile phases in reversed-phase chromatography are generally prepared with a small proportion (0.1 %) of strong acid such as TFA or formic acid. The benefit of charge suppression is elimination of mixed mode retention effects due to ionizable silanol groups on the silica gel surface. Mixed mode retention may increase retention times and cause significant peak broadening and distortion.

4) After the Run

The reversed-phase flash column should be washed with 3–5 CV of mobile phase B (the organic phase) after completed run to remove remaining contamination and sample components. If the product elution gradient did not reach 100 % of solvent B, a further gradient running 100 % B should be used. After this the column can be re-equilibrated into a higher proportion of solvent A by a gradient. Just like HPLC, large solvent changes should be avoided to preserve the integrity of the stationary phase.

Re-using the column

Typically, a reversed-phase column will be re-used. It is important to employ good housekeeping in order to preserve the life and integrity of the flash column. Particulate matter or bi-products from runs must be removed to avoid issues of increasing backpressure, loss of resolution or column discoloration. Peak broadening is an indication of this. Samplet cartridges used in association with Biotage SNAP cartridges can minimize this issue (see *Sample Loading* above).

To clean a used reversed-phase cartridge, flush with 3 to 5 CV of 100 % B solvent (MeOH or MeCN) and repeat the equilibration process from the Equilibration section. For heavily contaminated cartridges, flushing with stronger solvents (acetone, DCM) will sometimes remove more lipophilic contamination. (For storage, please see next section).

When completed successfully, reversed-phase cartridges can be re-used (Figure 10) without ill-effect, as shown by the recycling experiment conducted above.

5) Scaling Up

In normal-phase chromatography, it is fairly typical to first run TLC plate experiments in order to optimize a flash chromatography run. In reversed phase flash chromatography, this can sometimes be problematic resulting in generation of non-representative Rf data from reversed-phase TLC plates on the market.

Method optimization is possible directly from Biotage SNAP cartridges, by first using a 0–100 % gradient to determine product fraction elution, but for routine method development leading to scale up, Biotage offers dedicated scaling columns. Scaling columns use the same media used in flash cartridges packed into a 4.6x250 mm HPLC column, making them excellent method development tools.

One of the key features of the Biotage SNAP cartridge is the ability to open the cap and load sample into the cartridge via the use of SNAP Samplet cartridges as previously mentioned. Figure 14 shows a very good correlation between the Samplet loading method and expected position of peaks (based on HPLC comparison) in the separation of a mixture of antibacterial small molecule drug analogues. A HPLC gradient method was

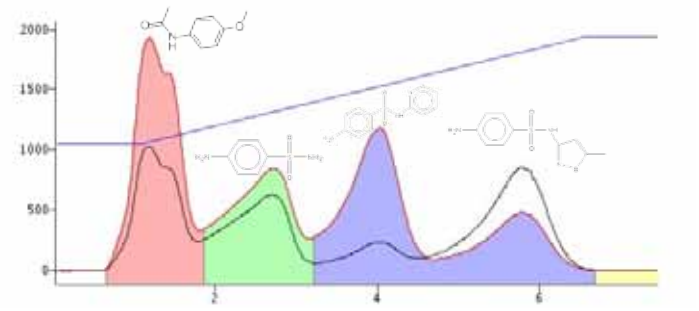


Figure 14a (above): A Biotage SNAP KP-C18-HS 60g cartridge (x-axis shows column volumes). The sample, 0.5 % load/g (300 mg) of 4 component Antibacterial analyte suite, was loaded by liquid injection onto the column.

Figure 14b (top right): A Biotage SNAP KP-C18-HS 60g cartridge using a Samplet loaded with 300 mg of the 4 component antibacterial suite. (x-axis shows column volumes). Flash Run Conditions: Solvent A: Deionized or HPLC-grade water + 0.1 % formic acid, Solvent B: Acetonitrile, Flow rate: 30 mL/min, Equilibration: 20 % B for 1 CV, 20 % to 35 % B in 5 CV, 35 % B for 1 CV, Injection 0.5 wt % load, 111 mg/mL, Detection, UV1 254 nm.

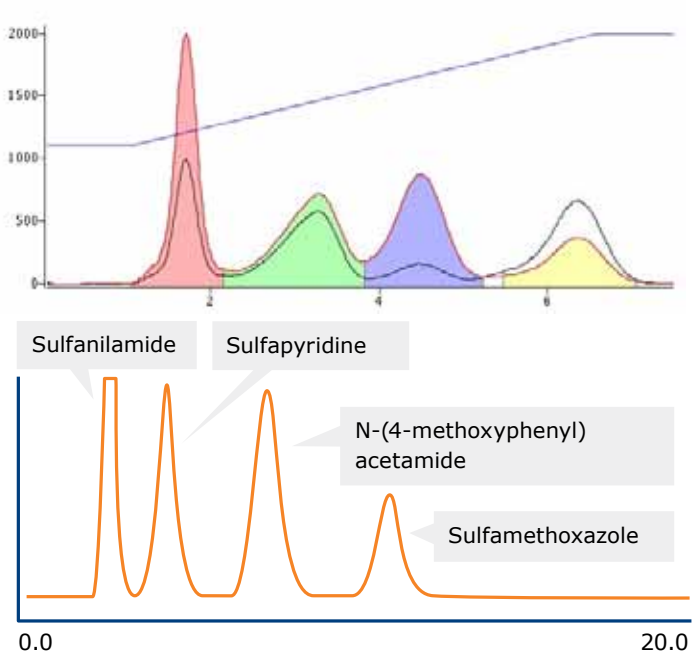


Figure 14c (low right): HPLC confirmation of mixture.

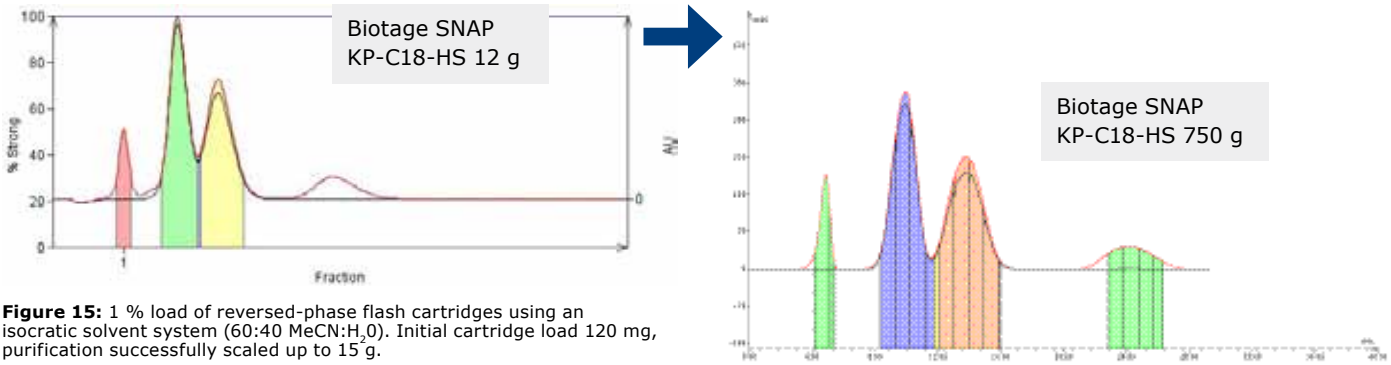


Figure 15: 1 % load of reversed-phase flash cartridges using an isocratic solvent system (60:40 MeCN:$H_2$0). Initial cartridge load 120 mg, purification successfully scaled up to 15 g.

developed (and used to confirm purity) for the separation of the suite of antibacterial compounds using an HP1100 HPLC system and a Biotage KP-C18-HS, 250x4.6 mm, 35-70 um scaling column with 0.5 % load of 4 component antibacterial suite.

The Samplet loading method produced better chromatography than traditional liquid load onto the column.

Once a small scale (12 g cartridge) reversed phase purifications has been optimized, reversed-phase flash chromatography is just as predictable for scale up as normal-phase flash chromatography. Figure 15 shows a 125x fold scale-up of a purification using 15 g of a three component test mix.

From the natural product extraction (Figure 5), scale up was demonstrated in two ways. Figure 16 shows the loading of increasing amounts of sample onto the reversed phase Biotage SNAP KP-C18-HS 12g cartridge. Even at 15 times the initial sample load, the separation is still effective without significant risk of overloading. In this example the same 12 g flash cartridge was re-used, showing excellent reproducibility and reliability.

Figure 17 shows the effect of scale up using a 2.5wt % sample load of the tobacco extract, first on a Biotage SNAP KP-C18-HS 12 g cartridge and then a Biotage SNAP KP-C18-HS 120 g cartridge. The separation performance is identical in both cases indicating predictable reversed-phase flash scale-up.

Storage

Long term, reversed-phase cartridges should be stored in organic solvent such as isopropanol.

References

1. Still, W. C., Kahn, M. and Mitra, A. J. *Org. Chem*. 1978, 43(14), 2923-2925.
2. Molnar, I. and Horvarth, C. *Clin. Chem.* 1976. 22 1497.
3. Howard, G. A. and Martin, A. J. P. The separation of the C12-C18 fatty acids by reversed-phase partition chromatography. *Biochem. J.* 1950, 46, 532.
4. Wiley, V.C.H., Weinheim, B., Hans-Jörg/Pilz, Stephan (eds.). 2011. Industrial Scale Natural Products Extraction 1st edition.

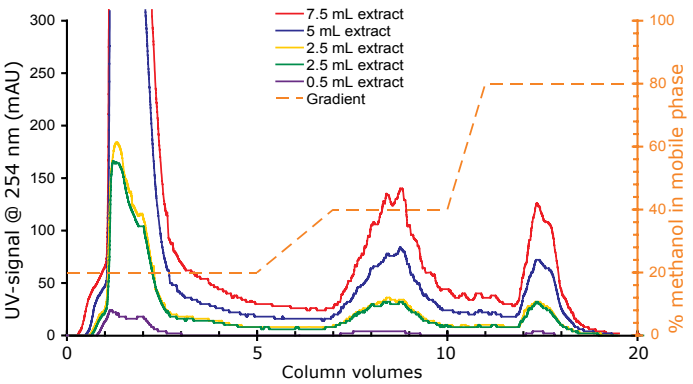


Figure 16: Separation of increasing sample loads of tobacco extract using a Biotage SNAP KP-C18-HS 12 g flash cartridge.

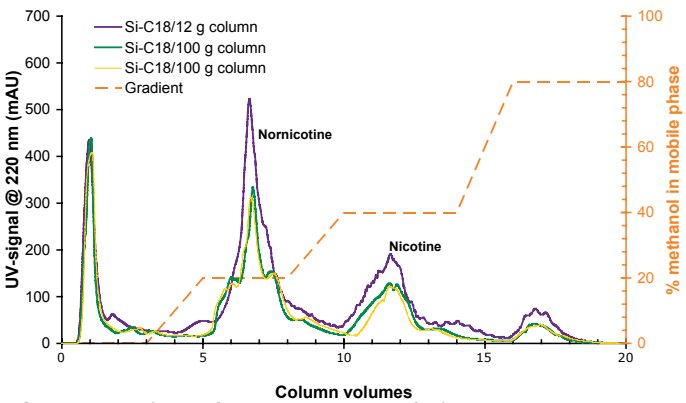


Figure 17: Scale-up of a 2.5wt% injection of tobacco extract using a Biotage SNAP KP-C18-HS 12 g and 120 g cartridges.

Part Number References

The following items were used in the making of this brochure. For more information on these, please contact 1-pt@biotage.com.

Product	Part Number
Biotage SNAP KP-C18-HS cartridges (12–1500 g)	
SNAP 12g KP-C18-HS cartridge, 2/cs	FSL0-1118-0012
SNAP 30g, KP-C18-HS cartridge, 2/cs	FSL0-1118-0030
SNAP 60g KP-C18-HS cartridge, 2/cs	FSL0-1118-0060
SNAP 120g KP-C18-HS cartridge, 2/cs	FSL0-1118-0120
SNAP 400g KP-C18-HS cartridge, 1/cs	FSL0-1118-0400
SNAP 950g KP-C18-HS CARTRIDGE	FSL0-1118-0950
SNAP 1850g KP-C18-HS CARTRIDGE	FSL0-1118-1850
Isolera Part Numbers	
Biotage Isolera Spektra	ISO-PDA
Biotage Isolera Spektra upgrade and license for existing Isolera systems	SER-SPKUPG
Isolera One with single fraction collector bed and fixed 254 nm detector. Includes four 16 x 150 mm racks and a starter set of SNAP cartridges	ISO-1SF
Isolera One with single fraction collector bed and variable wavelength detector. Includes four 16 x 150 mm racks and a starter set of SNAP cartridges	ISO-1SV
Isolera One with expanded fraction collector bed and fixed 254 nm detector. Includes four 16 x 150 mm racks and a starter set of SNAP cartridges	ISO-1EF
Isolera One with expanded fraction collector bed and variable wavelength detector. Includes for 16 x 150 mm racks and a starter set of SNAP cartridges	ISO-1EV
Isolera One with single fraction collector bed and dual-wavelength UV-VIS detector. Includes four 16 x 150 mm racks and a starter set of SNAP cartridges	ISO-1SW

Product	Part Number
Isolera One with expanded fraction collector bed and dual-wavelength UV-VIS detector. Includes four 16 x 150 mm racks and a starter set of SNAP cartridges	ISO-1EW
Isolera Four with single fraction collector bed and fixed 254 nm detector. Includes four 16 x 150 mm racks and a starter set of SNAP cartridges	ISO-4SF
Isolera Four with single fraction collector bed and variable wavelength detector. Includes four 16 x 150 mm racks and a starter set of SNAP cartridges	ISO-4SV
Isolera Four with expanded fraction collector bed and fixed 254 nm detector. Includes four 16 x 150 mm racks and a starter set of SNAP cartridges	ISO-4EF
Isolera Four with expanded fraction collector bed and variable wavelength detector. Includes for 16 x 150 mm racks and a starter set of SNAP cartridges	ISO-4EV
Isolera Four with single fraction collector bed and dual-wavelength UV-VIS detector. Includes four 16 x 150 mm racks and a starter set of SNAP cartridges	ISO-4SW
Isolera Four with expanded fraction collector bed and dual-wavelength UV-VIS detector. Includes four 16 x 150 mm racks and a starter set of SNAP cartridges	ISO-4EW
Isolera Prime system with 254nm detector and single collector bed	ISO-PSF
Isolera Prime system with 200-400nm variable detector and single collector bed	ISO-PSV
Isolera LS with extended fraction collector bed and variable 200-400 nm wavelength detector. Includes two 240 mm bottle racks and a starter set of SNAP 50g cartridges	ISO-1LSV
Isolera LS with extended fraction collector bed and variable UV-VIS 200-800 nm wavelength detector. Includes two 240 mm bottle racks and a starter set of SNAP 50g cartridges	ISO-1LSW
Samplets	
SNAP 1g KP-C18-HS samplet cartridges, 20/cs	SAS-1118-0012
SNAP 3g KP-C18-HS samplet cartridges, 20/cs	SAS-1118-0030
SNAP 12g KP-C18-HS samplet cartridges, 20/cs	SAS-1118-0120
SNAP 40g KP-C18-HS samplet cartridges, 6/cs	SAS-1118-0400
ELSD	
Isolera ELSD-1080	ISO-ELSD-1080
Scaling Columns	
Scaling column, KP-C18-HS, 4.6 x 250 mm, 1/pk	S1L0-1118-93050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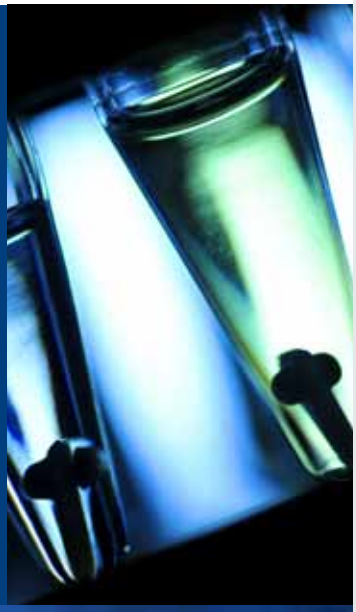

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