Shalini S .et al. / Vol 4 / Issue 3 / 2014 / 200-205.

International Journal of Pharmaceutical Research & Analysis

e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

www.ijpra.com

FLASH CHROMATOGRAPHY: AN OVERVIEW

S. Shalini*, A. Ajitha , V. Uma Maheshwara Rao

Department of Pharmaceutical Analysis and Quality Assurance, CMR College of Pharmacy, Medchal Road, Kandlaykoya, Hyderabad, India.

ABSTRACT

Flash chromatography is a rapid purification technique used to purify compounds of wide ranging polarity; often reaction mixtures, where the target (synthesized) molecule must be separated from excess reagents and reaction by-products. It is an established technique that can have a very positive impact on productivity in the pharmaceutical research and development chemistry laboratory . Finding the optimal conditions for a normal phase or reverse phase Flash-LC separation can be a laborious process. The aim of this work was to develop a screening system to enable automated, rapid and reliable method development for preparative Flash chromatography.

Keywords: Flash chromatography, Flash Columns, Solvents, etc.

INTRODUCTION

The concept of FC as originally described by still, kahn and mitra. The technique was considerably step forward in preparative chromatography compared to previously used open columns driven by gravity. The resolution was improved and the separation times were drastically shortened. Briefly, the original method typically employed columns with a diameter of 1-5cms and a sample loading 0.1-2.5g.The columns were dry filled with silica gel(40-63)to a length of about 15cms and flow rates of up to about 100ml/min were obtained by application of compressed air via a needle valve. Samples were introduced in solution and the eluents (ethyl acetate, petroleum ether) were selected with the aid of TLC.A solvent mixture giving the compound of interest an Rf value of 0.35 was used for isocratic elution.

The different preparative liquid chromatographic techniques are commonly classified according to the pressure employed for the separation.

- Flash chromatography, 1-2 bars
- Low pressure LC, <5 bars
- Medium pressure LC, 5-20 bars
- High pressure LC, >20 bars
- UPLC, up to 1000 bars

As in standard liquid chromatography, the separation takes place in a column packed with stationary phase (sorbent). The sample is applied to the head of the column, and the components are separated as the mobile phase (solvent) flows through the column.

ISOLUTE Flash columns are polypropylene columns, pre-packed with ISOLUTE Flash sorbents. Normal phase sorbents (e.g. Si, NH2), reversed phase sorbents (e.g. C18) and ion exchange sorbents (e.g. SCX-2) are available. This technical note describes the use of normal phase (silica) flash columns [1].

OBJECTIVE

- Flash chromatography is often employed in the isolation of constituents from plant sources, to separate reaction mixtures and plant products.
- In general, the higher the resolution, lower the sample capacity becomes. For purification, the sample amounts are quite high &therefore FC is the technique of choice.

ADVANTAGES

- Flash is dominant in organic labs because it's fast, adaptable, and cheap.
- Flow rates are high.
- Time saving, fast (1-15 mins).
- Large quantities of samples can be separated (0.5-2 g).
- Higher loading capacity and high fraction purity.

Corresponding Author:-S. Shalini Email:- sithale12@gmail.com

➢ Eliminates the need for post purification analysis by recording, displaying and saving the uv spectrum for each eluting compound.

If high resolution is required, FC is carried out before HPLC to avoid contamination of experimental plates.

PRINCIPLE

➢ In the traditional column chromatography system, the user fills the glass columns with silica gel.ths is not desirable because of serious health concerns regarding breathing of silica gel dust.

➢ The sample is placed on the top of the column. liquid is passed through column to elute the sample. exposure to organic solvents is uncommon and not desirable

➢ The separation is very slow(typically many hours) and is restricted to an isocratic solvent mixture

➢ At the end of the run the silica gel must be removed, cleaned, dried and repacked. This is both time consuming and hazardous.

FLASH CHROMATOGRAPHY

➢ The main principle involved in this is adsorption in which the mobile phase is passed through the solid stationary phase (silica gel).

➢ In the modern FC system, the glass columns are replaced with prepacked plastic cartridges which are much safer and also more reproducible.

➢ The Sample loading capacity is high.

➢ Solvent is pumped through the cartridges which is much quicker and more reproducible systems may also be linked with detectors and fraction collectors providing automation

➢ The introduction of gradient pumps means quicker separation, less solvent usage and greater flexibility [2].

INSTRUMENTATION

Instrumentation includes the following.

1) The flash columns and cartridges

➢ **Flash columns**

- These columns are used for flash purification organic compounds with sample loading up to 10% packed mass. flash column phase is selected as follows
- If the sample has charged, acidic or basic properties then the column phase is selected as

a. charged compound-ion exchange reverse phase-silica,amine

b. acidic compound-both normal and reverse phase-silica,C-18

c. basic compound-normal phase-silica, amine ,alumina basic but if the sample is neutral, it is selected based on the polarity as

a. Low to medium polarity- normal phase- cyano, amine, silica

b. High polarity- reverse phase- C18,cyano

columns are classified as:

1) Normal phase columns

a. NP silica flash columns(40-60µm)

Eg.supersep TM standard silica

b. NP highperformancesilicaflashcolumns(20-30µm)

Eg.Supersep TM high performance

2) Reverse phase columns- c18,cyano columns

Cartridges

- These are used for solid phase extraction mostly.
- They are used to improve the separation efficiency and compound purity for low solubility samples.
- Low solubility samples are dissolved in supporting media and the slurry is loaded to empty cartridges.
- Prior to cartridge loading, vaccum vapourisation of the solvent typically improves the purification efficiency.
- They are packed with silica, alumina, c18, celite, cyano etc.

Eg. superSep Solid Load Cartridges (compatible with ISCO)

2) Solvents

Solvents used in flash chromatography are generally polarized and cheap. Three binary mixes are sufficient for large variety of compounds .They are

a. Dichloromethane / methanol – for polar compounds and compounds poorly soluble in ethyl acetate

b. Petroleum ether / diethyl ether – perfect for heat sensitive substances when this mixture is boiled at around 40°c

c. Hexanes / ethyl acetate – for nonpolar compounds stable at least 80°C

3) Gradients

A binary solvent works well when two compounds of roughly similar polarity has to be separated . But it is inefficient when there are four or more compounds in the sample .Hence gradients are used . The methodical gradient column approach is to use mixture in which the least polar compound has an Rf of 0.3

4) Filtration Products

Luknova supplies a variety of high quality syringe filters for sample preparation and solvent filtration to remove particles from a sample prior to chromatography, filtration of gases, and for the removal of bacteria from a sample, including:

• Nylon, PTFE , PVDF, PES, and MCE (mixed cellulose acetate and cellulose nitrate)

• Popular 13mm and 25mm diameters and 0.22µm and 0.45µm pore sizes

• Minimum extractables and maximum chemical compatibility

• High through put and flow rates, low hold-up volume, and minimum adsorption

❖ **TYPES OF FLASH CHROMATOGRAPHY**

There are different types of FC methods. They are

1. '**Off-line' flash chromatography**

In this mode, ISOLUTE Flash columns are processed using a vacuum manifold such as the Flash Vac, in a similar way to solid phase extraction (SPE) columns.

The columns should be attached to the manifold, and the sample applied. Elution solvent is then applied in successive aliquots to selectively elute the compounds of interest into suitable collection vessels. The elution solvent can be isocratic, where the successive aliquots applied consist of the same solvent mixture each time, or a 'step gradient' where the successive aliquots applied consist of solvents of increasing polarity, and can elute compounds that are more strongly retained by the sorbent.

2.'On-line' flash chromatography

In this mode, the columns are mounted on a system that allows an external liquid pump to be connected to the column. A continuous flow of elution solvent is pumped through the columns, and depending on the capability of the pump, the solvent composition can be isocratic (a single solvent or solvent mixture) or a gradient with an increasing proportion of stronger solvent (either in a step gradient or a linear gradient).

3.Normal phase flash chromatography

Traditionally, normal phase flash chromatography has been most popular.

Sorbents: polar (e.g. Silica, NH2)

Elution solvents: non-polar (e.g. hexane, heptanes, dichloromethane), sometimes modified with small amounts of more polar solvents such as isopropanol)

4. **Reverse phase flash chromatography**

Reversed-phase flash chromatography is growing as an alternative to expensive reversed-phase HPLC. Today's improved reversed-phase flash cartridges separate reaction mixtures and natural product extracts effectively, providing higher loading capacity and high fraction purity [3].

METHOD DEVELOPMENT USING ISOLUTE FLASH COLUMNS

1. **Column equilibration**

Prior to sample loading, the column should be prepared for the separation by equilibration (pre-wetting) with a suitable solvent

Off-line

Apply the equilibration solvent to the top of the column and allow to flow through the column under gravity.

On-line

We recommend that flash columns to be used on-line for the separation should be equilibrated in the off-line mode using a vacuum manifold such as the FlashVac®.

Alternatively, the column should be mounted on the instrument, and a suitable volume of equilibration solvent pumped through the column.

Typical equilibration solvents

For normal phase flash chromatography using Silica or NH2 columns, the optimum results are achieved when the column is pre-wetted. Suitable solvents are non-polar, for example, hexane or pentane. Normal phase flash columns can be used without pre-wetting, but some column to column variation may be experienced.

2. Sample application

There are two popular approaches to loading the sample in flash chromatography.

a) Wet loading

Load the liquid sample directly onto the top frit of the column, and allow to percolate through to the top of the sorbent bed. For best results, load sample onto a pre-wetted column, in a non-polar solvent for e.g. **Sorbent-si**

Typical solvent- Hexane or other non-polar solvent or solvent mixture (as normal phase HPLC)

Practical tips for wet-loading:

- Dissolve the sample in as non-polar a solvent as possible.
- If the compounds are not easily soluble in a non-polar solvent, either dissolve in a small volume of polar solvent, and dilute with a non-polar solvent to reduce the elution strength, or, consider dry loading of the sample
- Position your column on a suitable vacuum manifold (e.g. FlashVac or VacMaster, equipped with PTFE stopcock needles.
- Apply the sample evenly over the entire area of the top frit. To promote this, seal the column by closing the stopcock, then apply the sample so that it forms a 'pool' on top of the frit. When the stopcock is opened, the sample will load evenly.
- Samples can alternatively be loaded onto the column in 'on-line mode' when Susing the Flash module system, via a 3-way injection valve

b) Dry loading

Pre-absorb the reaction mixture onto a small amount of bulk material of the chosen sorbent. Evaporate off the majority of the solvent, leaving the compounds bound to the surface of the sorbent. Add this blend to the top of the pre-packed (and pre-wetted) flash column, settle, and add a further top frit to secure the blend in place. The top frit can be placed using a suitably sized frit inserter. This is the loading method of choice for reaction mixtures consisting of polar solvents when loading onto a silica or other normal phase column .A popular alternative sorbent for dry loading using the flash sorbent is to use

adiatomaceous earth such as ISOLUTE HM-N. This can be used in the same way asthe flash sorbent, but has several advantages including more efficient desorption of the compounds into the mobile phase.

Practical tips for dry loading

- Dissolve the sample initially in a suitable solvent, ensuring complete dissolution if possible. Use the smallest volume possible.
- Add the bulk material of choice. The ideal proportion of sample to bulk material is 1:1 to 1:3 by volume.
- Evaporate off the residual solvent using a rotary evaporator to ensure even adsorption of the sample on the bulk material.
- Pack the dry blend on top of the flash column (above the top frit) and add another frit. Push down to the new surface to prevent movement of the blend.
- When loading with ISOLUTE HM-N, ensure that the material is not crushed at this stage

OPTIMISATION OF FLASH SEPARATION

Prediction of elution profile using TLC

In normal phase flash chromatography using silica columns, a popular method development approach is to use silica TLC plates to predict the best mobile phase composition. TLC data can be used to predict column elution behavior using the relationship

$$CV = 1 / Rf$$

Where,

Rf is the retention factor for a particular component, and can be calculated as follows:

Rf = distance between origin and component distance between origin and solvent front

CV is the number of column volumes required to elute the component from the column using that solvent system, regardless of column dimensions.

CV can be defined as the interstitial volume of the sorbent bed, i.e. (bed volume) – (volume of packing material)

Table 1. Rf vs CV

Rf	CV
0.90	1.10
0.70	1.40
0.50	2.00
0.30	3.33
0.10	10.00

i.e. For a particular set of separation conditions, a fast eluting component (weakly retained) with an Rf value of 0.9 can be eluted in just over 1 column volume, whereas a more slowly eluting component (strongly retained) with an Rf of 0.10 requires 10 column volumes to complete elution.

For transfer of a separation from TLC to flash chromatography, the solvent system should be optimized in terms of solvent strength and selectivity so that Rf values of between 0.15 and 0.35 are achieved for each component. This will allow isocratic elution within 7 column volumes

For non-optimized TLC separations, a better separation can generally be achieved using a less polar solvent system (OR decreasing the proportion of polar modifier)

Limitations of TLC in separation prediction

TLC is a fast, effective and useful tool for prediction of the chromatographic behavior of the sample on a normal phase flash column. However there are some limitations:-

- Accuracy of prediction can be reduced if the mobile phase contains very polar modifiers such as methanol or acetonitrile, or very volatile modifiers such as diethyl ether
- The activity (or moisture content) of the plate used can be different from the separation column
- The binding agent used in the TLC plate may affect the separation

As well as being a useful tool for determining isocratic elution conditions, TLC can be used for prediction of separation conditions for mixtures that require gradient elution conditions for efficient separation

Step gradient

Step gradients provide controlled elution with discrete changes in eluent strength. Each step is optimized to elute only those components that have ‘solubility’ in that eluent. This technique can be applied to both off-line and on-line flash chromatography.

1. Use TLC to determine suitable solvent strengths to elute components at discrete intervals, choosing different solvent mixtures that elute each component separately with an Rf = 0.2 to 0.5.
2. Calculate the volume of solvent required to elute each component using CV = 1/Rf. Column volumes for each column configuration are listed in table 1.
3. Apply between 2 and 5 column volumes of solvent for each step, starting with the solvent with weakest solvent strength.
4. Collect the eluent from each step in a separate vessel

Linear gradient

Linear gradients are a quick way of separating complex mixtures, reducing the complexity of the subsequent purification of the fractions collected. This technique is suitable for on-line flash chromatography.

1. Use TLC to find both the ‘weakest’ and ‘strongest’ elution solvents. The ‘weak’ solvent (solvent A) should give retention of the majority of components (Rf of <0.1). The ‘strong’ solvent (solvent B) should allow elution of all of the components of interest (Rf > 0.5).
2. Run a gradient starting with 100% solvent A and ending with 100% solvent B.
3. Collect the eluent at regular intervals.

Method development can also be performed without TLC. This can be a useful approach to method

development, particularly for non-silica based flash chromatography (e.g. reversed phase) where suitable TLC plates are not available [4].

OPTIMISATION OF FLOW RATE

The optimum flow rate for a flash separation is related to the particle size and dimensions of the column. Theoretical optimum flow rates for flash columns of different dimensions can be predicted. However, in practice, increasing the flow rate has not been found to significantly affect the separation, and has important productivity advantagesOther factors also affect the flow rate that can be used, such as composition of mobile phase and back pressure.

Table 2. Recommended flow rates

Column diameter (configuration)	Recommended flow rate range
16 mm (D)	5-25 ml / min
20 mm (E)	5-25 ml / min
27 mm (F)	10-30 ml / min
40 m(j)	20-50 ml / min
40 mm (V, W, X)	20-50 ml / min

FRACTION COLLECTION

Once separated on the flash chromatography column, purified fractions / components must be collected for further processing and / or testing [5].

'Off-line' flash chromatography

When performing flash chromatography on a vacuum manifold such as the FlashVac, successive fractions can be collected as follows:

- Load collection rack with vials of a suitable volume in each position.
- Place a single flash column in position 1, and apply the first solvent aliquot.
- Collect the aliquot in the vial in position 1 of the collection rack.
- Move the column to position 2, and apply the second solvent aliquot.
- Collect the aliquot in the vial in position 2 of the collection rack.
- Continue until all the components of interest have been collected.

Alternatively, multiple columns can be processed by replacing collection vials at each elution step. A typical volume for each fraction is 2 column volumes.

'On-line' flash chromatography

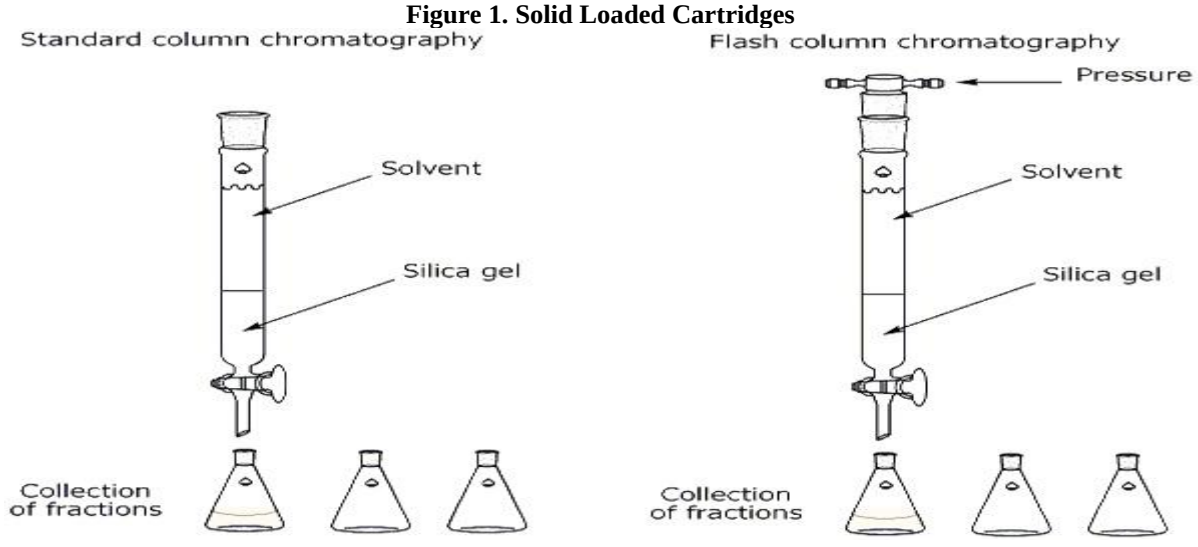
Using an automated system equipped with a fraction collector, fractions can be collected in a variety of ways, for example

- Fixed volume fractions can be collected
- Individual peaks can be collected – trigger collection via detector

Table 3. Comparision between Flash and MPLC

Characteristics	Flash Chromatography	MPLC
Resolution	Low to moderate	Moderate to high
Loading capacity	Very high	High
Column packing	Requires experience	Easy to pack
Reproducibility of packing and separation	Low	High

Figure 1. Solid Loaded Cartridges



APPLICATIONS

Separating low solubility sample

- Low solubility samples can cause some differences in flash system.it is necessary to dissolve them in a solvent matrix that may not be compatible with separation solvents.
- In this case, sample is dissolved in a strong solvent ,mixed with silica and then evaporated to dryness. placing the silica or sample matrix in a solid sample introduction module(SSIM)allows the sample to be eluted on to the top of the separation column using this technique can improve the separation dramatically.

1. Separation of pyridine derivatives
2. Separation of polar compounds
3. Natural products purification
4. Isolation of pure compounds from plants can be done.
5. Purification of reaction mixtures
6. Real time fraction purity analysis using reverse phase FC:

Pure fractions are in high demand. Impurities mean more work after purification. With new technology, fraction purity can be digitally analyzed directly during chromatography to reveal any problems on the fly [6].

CONCLUSION

Flash chromatography technique is thus very simple and time saving process which utilizes low pressures and large quantities sample of crude or semi purified samples can be separated in a single run

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