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FLASH CHROMATOGRAPHY AND ITS ADVANCEMENT: AN OVERVIEW

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ABSTRACT

Flash chromatography is widely used for purification of low molecular weight natural compounds and products of organic synthetic reactions. Modern flash techniques include the use of convenient disposable flash cartridges instead of glass columns. Flash purification systems allow users to speed up the purification process for quicker results and higher throughput. In this review, we present the introduction of FC, the principle involved, instrumentation, general procedure and advancement in Flash chromatography along with its applications.

Keywords: Flash cartridges, Molecular weight and Modern flash.

INTRODUCTION

All chromatographic methods –with the exception of TLC- use columns for the separation process. Column chromatography has found its place in many laboratories for preparative purposes as well as for reaction control in organic synthesis [1]. The importance of column chromatography is mainly due to following factors like Low expense for instrumentation, Simple packing procedure, Low operating pressures. Column chromatography is separated into two categories, depending on how the solvent flows down the column. If the solvent is allowed to flow down the column by gravity, or percolation, it is called Gravity column chromatography. If the solvent is forced down the column by positive air pressure, it is called Flash chromatography [2]. In traditional column chromatography a sample to be purified is placed on the top of a column containing some solid support, often silica gel. The rest of the column is then filled with a solvent (or mixture of solvents) which then runs through the solid support under the force of gravity. The various components to be separated travel through the column at different rates and then can be collected separately as they emerge from the bottom of the column. Unfortunately, the rate at which the solvent percolates through the column is slow. In flash chromatography however air pressure is used to speed up the flow of solvent, dramatically decreasing the time needed to purify the sample, therefore making the column and running the separation could take less than 10-15 minutes.Flash chromatography is basically an air pressure driven hybrid of medium pressure and shorter column chromatography which has been optimized for particularly rapid separation. Flash chromatography is a technique used to separate mixtures of molecules into their individual constituents, frequently used in the drug discovery process. Flash chromatography, also known as medium pressure chromatography, was popularized several years ago by Clark Still of Columbia University, as an alternative to slow and often inefficient gravity-fed chromatography. Flash chromatography differs from the conventional technique as Slightly smaller silica gel particles (250-400 mesh) are used. Due to restricted flow of solvent caused by the small gel particles, pressurized gas (10-15 psi) is used to drive the solvent through the column of stationary phase. The net result is a rapid ("over in a flash") and high resolution chromatography. Automated flash chromatography systems include components normally found on more expensive HPLC systems such as a gradient pump, sample injection ports, a UV detector and a fraction collector to collect the eluent. Typically these automated systems separate samples from a few milligrams up to an

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industrial kg scale and offer much cheaper and quicker solution to doing multiple injections on prep-HPLC system [3]. The software controlling an automated system coordinate the components, allow a user to only collect the factions that contain their target compound and help the user to find the resulting purified material within the fraction collector. The software also saves the resulting chromatograph from the process for archival and/or later recall purposes.

ADVANTAGES

- Speed in purifying milligrams to grams of material.
- Typical purity of greater than 90% on a single run.
- Operation costs lower than HPLC like,
- Less acquisition cost.
- Higher sample loading.
- Less column costs.
- Minimal method development time.
- Well packed cartridges ensure better resolution and separation than manual column chromatography.
- Pressure and flow rates are controlled and optimal for individual runs.
- Less solvent consumption, shorter purification times due to minimal setup needs.
- Pure samples are obtained in fewer fractions, more concentrated products requiring less drying.
- Multiple detection allows non chromophoric components to be easily identified and isolated.

PRINCIPLE

The principle is that the eluent which is a liquid, under gas pressure (normally nitrogen or compressed air) rapidly pushed through a short glass column. The glass column is packed with an adsorbent of defined particle size with large inner diameter. The most used stationary phase is silica gel 40 – 63 μm, but obviously packing with other particle sizes can be used as well. Particles smaller than 25 μm should only be used with very low viscosity mobile phases, because otherwise the flow rate would be very low. Normally gel beds are about 15 cm high with working pressures of 1.5 – 2.0 bars. Originally only unmodified silica was used as the stationary phase, so that only normal phase chromatography was possible. In the meantime, however, and parallel to HPLC, reversed phase materials are used more frequently in flash chromatography [4-6].

INSTRUMENTATION

- Solvent reservoir
- Pumps
- Cartridges
- Sample injector
- Detectors
- Fraction collection trays
- Display for monitoring
- Waste collection

Solvent reservoir

- It is used for the storage of solvents.
- It is located back side of the display.
- Solvent lines are dipped into the solvent bottles.
- For each solvent line has solvent sensor to remind when solvent bottle is empty [7].

Pumps

- It has quaternary pump.
- It can pump four different solvents at a time.
- The pumps work on suck and pump principle i.e first it sucks the solvent and then pump [8].

Cartridges

- Separation of the various components in the sample takes place on the stationary phase.
- Choice of stationary phases:
- **Silica**: slightly acidic medium. Best for ordinary compounds, good separation is achieved(230-400 mesh, 40-63um, is the most commonly used)
- **Florisil:** mild, neutral medium. 200 mesh can be effective for easy separation.
- **Alumina:** basic or neutral medium. Can be effective for easy separation, and purification of amines
- **Reverse phase silica:** best for highly polar compounds. The most polar compounds elute faster than the non polar compounds [9].

Different sizes of cartridges

Cartridge size	Easy separation (10% loading)	difficult separation (1% loading)
4g	400mg	40mg
12g	1.2g	120mg
400mg	40g	4.0g
80g	8.0g	800g
120g	12g	1.2g
330g	33g	3.3g

Sample injector

- This is used for the introduction of sample into the cartridge.
- Sample can be injected into the cartridge as liquid injection or dry sample [10].

Sample loading techniques in flash chromatography

- Loading in normal phase flash;
  - Solid loading.
  - Liquid injection.

- Loading in reverse phase flash:
  - Solid loading;
  - Liquid injection

Sample loading in normal phase Flash

Solid loading

For solid loading these are two components useful

1. Solid load cartridge
2. Cap

Solid load cartridges are two types;

1. User packed solid load cartridge
2. Pre packed solid load cartridge

User packed solid load cartridges

- Also called empty solid load cartridges

Procedure

- Weigh sample to be separated.
- Determine amount of sorbent,
  - 20% load for silica.
  - 10% load for celite.
- Dissolves sample completely in less strong solvent.
- Mix with media.
- Dry on rotary evaporator.
- Place in cartridge.
- Add top frit.
- Insert solid load cartridge cap.

This loading technique gives

- Best separation
- We can match sample size to silica for loading
- Allows user to use strong solvent to dissolve component
- Act as pre column
- Prevents clog from precipitated samples

Pre packed solid load cartridge

- Dissolve sample
- Add to solid load cartridge
- Dry if desired by,
  - Vacuum dryer (or)
  - Vacuum oven
- Insert solid load cartridge cap.

This loading technique gives

- Act as pre column.
- Increased column length due to extra silica.

Liquid loading:

- Dissolve sample in large volume of weak solvent and inject directly in to the injection port.
- Liq. injection is fast and easy.
- Sample is dissolved in minimum strong solvent, gives better resolution.
- Sample is dissolved in large strong solvent, gives poor resolution.

Loading in reverse phase Flash

Same as normal phase loading except that c18 silica or celite is used to load sample with 30% of water.

Detectors

Two types of detectors available

1. Uv detector.
2. ELSD (evaporating light scattering detector).

Uv detector

Principle: molecules have either n, pi, sigma or combination of these electrons. These bonding and nonbonding electrons absorb the characteristic radiation and undergoes transition from ground state to excited state. By the characteristic absorption peaks, the nature of the electrons present and molecular structure can be elucidated.

Disadvantage: it can't detect non uv active compounds [9].

ELSD detector

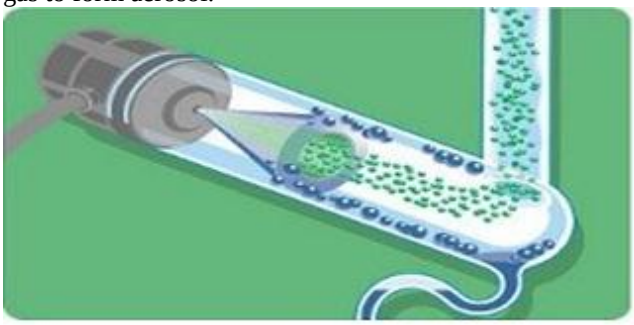
Principle of operation,

It is a three step process.

1. Nebulization of the column effluent to form an aerosol.
2. Solvent evaporation with in heated drift tube.
3. Detection of the non volatile solute particles in the scattering cell.

Nebulization

- The column effluent from flash separation enters the nebulizer where it mixed with steady stream of nebulizing gas to form aerosol.

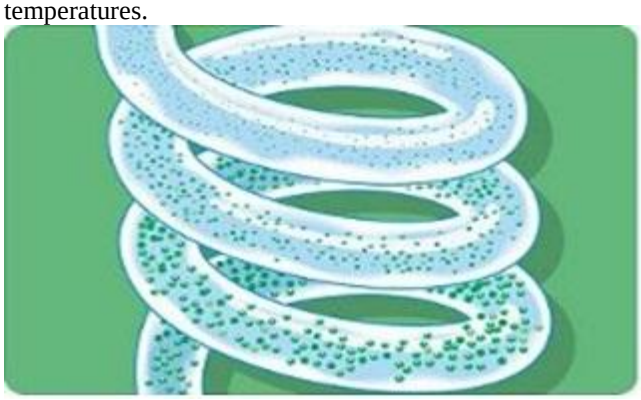


- The aerosol consists of uniform distribution of droplets whose size dependent on the gas flow rate.
- The lower the gas flow rate used for the formation of larger droplets.
- Larger droplets scatter more light and increase sensitivity of the analysis, but they are also more difficult to evaporate in the drift tube.
- So optimal flow rate of each method will produce highest signal to noise ratio [10].

Evaporation

- Evaporation of the volatile components in the aerosol occurs in heated, stainless steel drift tube.

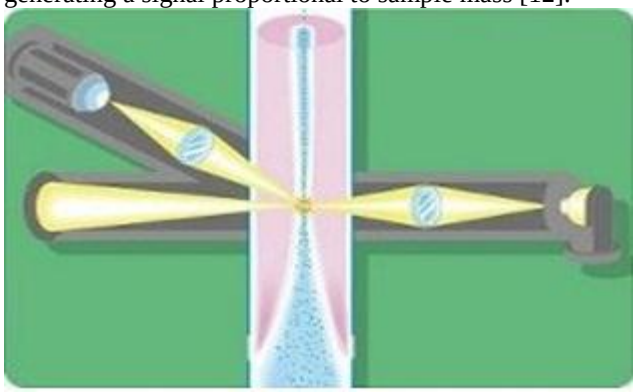
- Drift tube temperature depend on mobile phase composition. Mobile phase flow rate, sample volatility.
- Organic mobile phases require lower drift tube temperatures.



- A stain less steel impactor is located three inches from the nebulizer.
- The plate is perpendicular to the aerosol path so the aerosol contacts the plate, larger droplets exit through the drain tube on the side panel.
- The remaining droplets pass around the impactor and travel through drift tube to the optical cell for detection [11].

Detection

- The non volatile sample particles emerge from the drift tube in the mobile phase vapor and enter the light scattering cell.
- In the optical cell, sample particles scatter light emitted by laser light source.
- The scattered light is detected by a silicon photodiode, generating a signal proportional to sample mass [12].



Disadvantage: volatile compounds can't be detected by this detector.

Display

This is used for the entering the run conditions.

- Monitor the signals obtaining from the detecto

Touch pad solvent section start and stop

Fractions collection

Fraction collection trays are available to collect fractions.

Waste collection

The remaining solvent goes in to the waste collection.

Procedure

- Select the mobile phases for the separation of given compound.
- Insert in solvent lines into the solvent bottles.
- Before placing the solvent lines into the solvent bottles make sure that the solvent level sensors are at zero, otherwise make it zero by selecting zero button.
- After placing solvent lines in solvent bottles prime the solvent lines.
- This priming helps to remove previous run solvents and air present in lines.
- Make the gradient method, by entering the appropriate conditions for the separation of given mixture.
- Install the cartridge by lifting the cartridge arm.
- Prepare the sample as liquid or as solid.
- Run the method by selecting start button.
- The mobile phase passes from the cartridge and separate the mixture.
- The separated pure fractions collected in fraction collection trays.
- Remaining impure fractions goes in to the waste collection
- The detectors help in identifying and collection of compounds by giving sign [13].

Differences between Flash Chromatography and HPLC

Flash Chromatography	**HPLC**
Low pressure(less than 200psi)	High pressure (greater than 1000psi).
Lower efficiency columns	High efficiency columns.
Focus on purification and speed	Focus on qualitative analysis
High flow and high sample loading	Low flow and low sample loading
Irregular shape of silica particlesparticles used.	Spherical or angular shape of silica used
Less solvent consumption	High solvent consumption
Typically silica based normalPhase	Typically modified silica basedReverse Phase

Advanced Detection Techniques for Flash Chromatography

UV detection is the traditional method used in Flash chromatography to monitor and fractionate peaks during the purification process. There are few detection options available in Flash chromatography for compounds that lack chromophores, and thus cannot be detected by UV. These invisible compounds may not be detected with

UV due to the absence of UV chromophores, or their absorbance may be "lost" in that of the solvents used in Flash chromatography. In other cases, the compounds' absorption spectrum may be unknown and or detection was at a sub-optimal wavelength. Additionally, the UV absorption of the necessary mobile phase may interfere with the λ-max of the compound in question. In other cases, the absorption spectrum of the compound of interest or co-eluting impurities may not be known and therefore not detected. These advanced detection techniques allow users to easily fractionate compounds without the need for follow-up TLC and subsequent staining to determine where the purified compound eluted.Evaporative Light Scattering Detection (ELSD) has long been used for High Performance Liquid Chromatography, but has only recently been employed for Flash chromatography. All-Wavelength Collection allows the collection of compound with unknown absorbance or collection in the presence of interfering solvent absorbance [13].

Green Flash Chromatography

Green Flash Chromatography is the ultimate flash chromatographic technologythat achieves most efficient sample purification.The sample run is always carried out with minimum eluting volume.It minimizes run time and solvent use while achieving a good separation.It is Eco-friendly. Optimum method will be developed automatically based on the true theory of the flash chromatography, with the simple input of the TLC results, which allows easy sample purification.

Features of Green Flash Chromatography

- Optimal parameters for flow rate, run time, fraction volume, etc. will be calculated and set automatically upon selecting a column on "Green Flash" software. The default parameters will be shown in System Setting window.
- Software provides the maximum sample load information for the selected column.
- State-Of-The-Art Software Based On True Theory of Chromatography.
- Sample Eluting Position and Resolution Can Be Fully Controlled for Systems.
- Automatic Method Setup for Reverse Phase Chromatography.
- Parallel Detection of UV Detector and RI Detector or ELSD [14].

Flash cromatographywithtlc image reader

The system is equipped with a built-in UV light source and a camera. By shooting the TLC plate and clicking the target compound on the TLC plate, the Rf value of the target compound will be calculated and the optimized chromatography method will be developed automatically. The TLC plate is displayed on the screen during run. Compound spots on the TLC plate and the compound peaks both are displayed on the screen. Both the photographic image of the TLC plate and the purification are saved as a data file. Click the target compound and the nearest impurity on the TLC plate, and the maximum sample load for each column will be automatically calculated. Thus, enabling the chemists to choose the best suited column for their sample [15].

Advantages

Fast and economic methods for the synthesis laboratory.Ideal for the separation of compounds up to gram quantities. No expensive equipment required. In an ideal way transfers results from TLC to CLC. Automated changes between normal phase and reversed phase chromatography.

APPLICATIONS OF FLASH CHROMATOGRAPHY

Natural Products/Nutraceuticals Application:

- Separation and Isolation of α-Santalol and β-Santalol from Sandalwood Extraction
- Isolation and Purification of Chromophoric and Nonchromophoric Compounds in Giant Knotweed Rhizome.
- Isolation and Purification of Flavonoids from Ginkgo Biloba Leaves Extract.
- Isolation and Purification of Ginsenosides from Red Panax Ginseng Extract.
- Isolation and Purification of Catechins from Green Tea Extract
- In Purification of GallaChinensis
- .Purification of Ferulic Acid in RhizomaChuanxiong Extract [16].

Carbohydrate Application

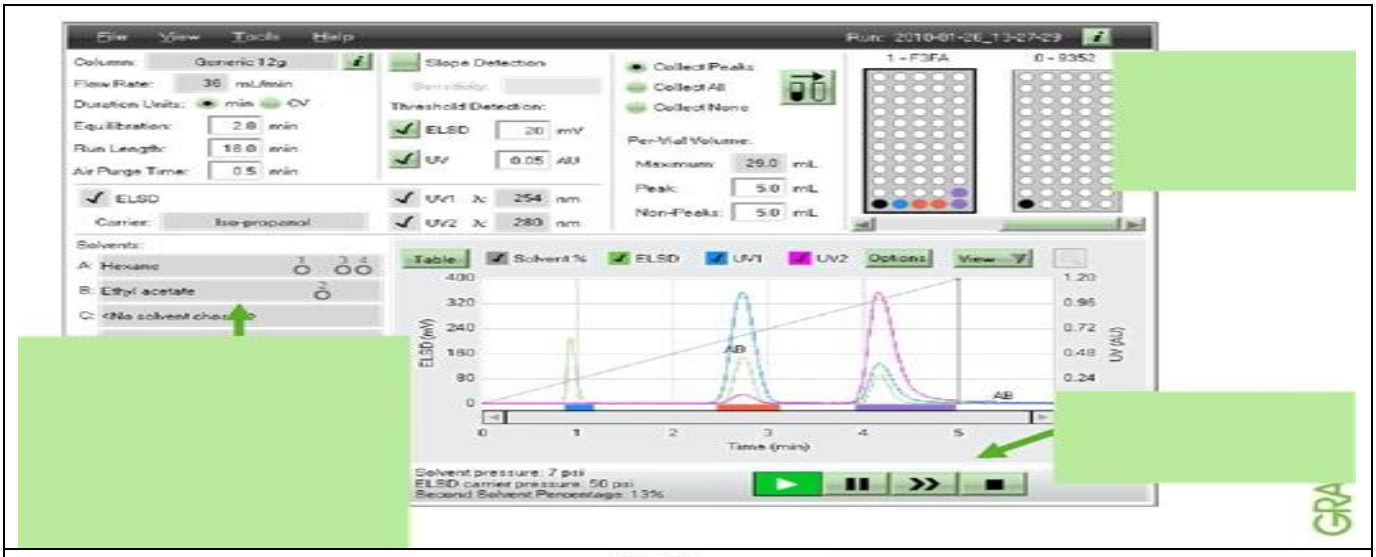
- Purification of Conjugated Quercetin and Rutinose.
- Impurity Isolation of Valproic Acid from CyclodextrinvDuring Encapsulation.
- Isolation ofAminosugar and Acarbose
- Flavanone Glycoside Purification.
- Isolation of Aminoglycoside Antibiotics.

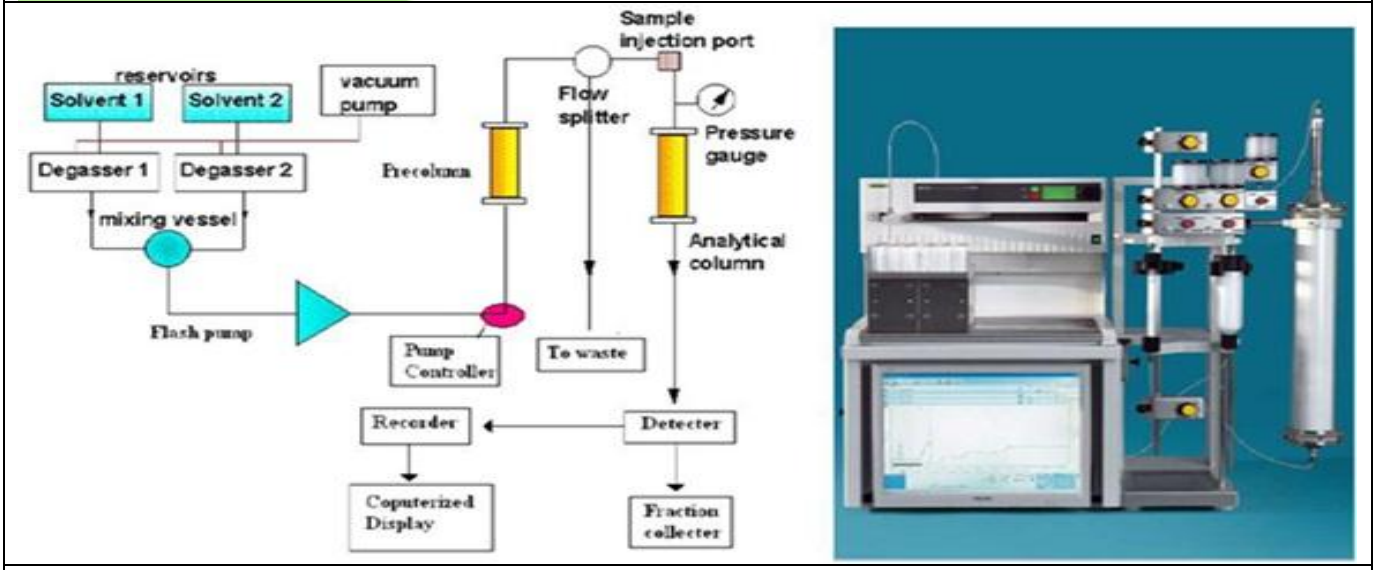
Lipids Application

- Purification of Fatty Acid Methyl Esters (FAMEs).
- Purification of a Mixture of Glycerides, Mono-, Di-, and Tristearin.
- Purification of Sterols.

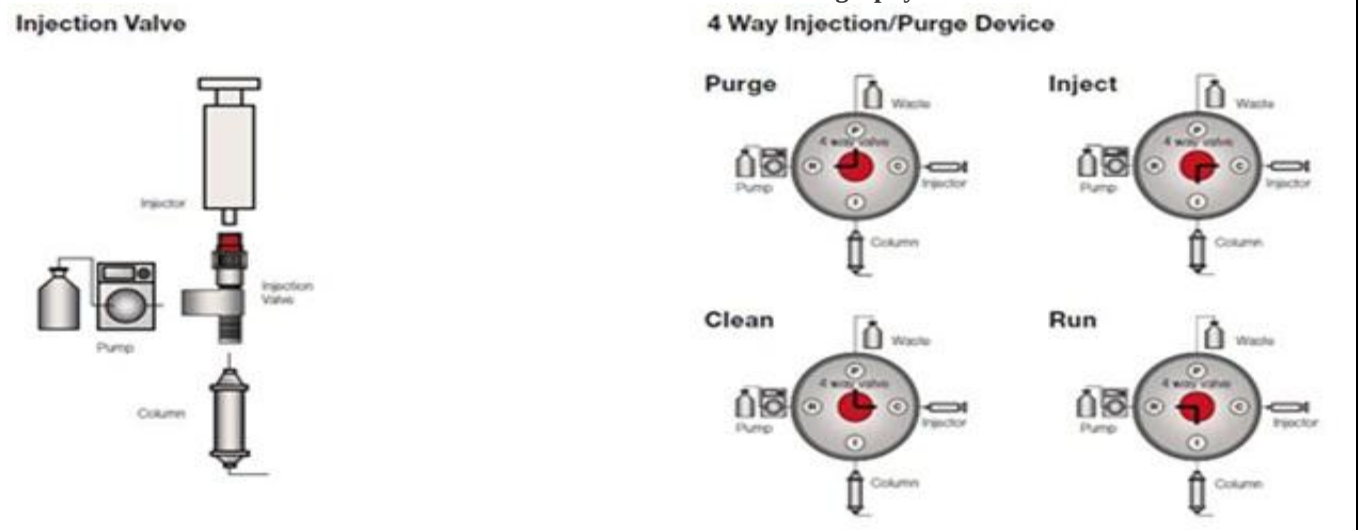
Pharmaceutical/Small Molecules Application

- Bile Acid Purification During Lead Generation in Drug Discovery.
- In Impurity Isolation During Drug Purification.
- Mestranol Purification During Chemical Synthesis.
- In Anti-malarial Drug Purification in Drug Discovery [17].





Instrumentation of Flash Chromatography



CONCLUSION

- The flash purification system provides time saving technology that increases productivity in the drug discovery process.
- It has an advantage of rapidly isolating and purifying compounds in less than 10 minutes.
- Compounds that cannot be separated on the same RP-HPLC chromatographic column due to their diverse chemical nature can be separated by using flash cartridges.
- Flash Chromatography can be alternative to preparative HPLC as it saves time and solvent.
- Modern Flash chromatography with disposable cartridges and advanced detection techniques is applicable to a wide range of compounds

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