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Preparative HPLC and its Applications: A Review

[1]Aarti Patil, [2]Saurabh Chavan

[1]M Pharmacy Student, PES Modern College of Pharmacy (For Ladies), Moshi, Pune.
[2]M Pharmacy Student, SCES Indira College of Pharmacy, Tathawade, Pune.

ABSTRACT:

Preparative chromatography is only one of many vital techniques that can be used effectively as a precursor to determine the structures of unknown impurities or clean up the sample to meet specification. Dealing with these unknown impurities, for example in finished products, stability samples or discovered compounds, can be very challenging, as failure to identify or remove them could interrupt product supply, delay clinical trials, or disrupt with toxicology studies. Very often there is a regulatory requirement to disclose the structure of new impurities above a certain limit. Financial loss to the pharmaceutical, agrochemical, food and consumer and fine chemical industries can be huge.

Introduction:

The term preparative HPLC is usually associated with large columns and high flow rates. However, the preparative HPLC experiment does not determine the size of the instrumentation or the amount of mobile phase pumped by the system, but rather the goal of the separation. The goal of preparative HPLC is the isolation and purification of a valuable product. Since preparative HPLC is a relatively expensive technique compared to traditional purification methods such as distillation, crystallization or extraction, it has only been used for rare or expensive products. With the increasing demand to produce highly pure compounds in various quantities for activity, toxicology and pharmaceutical screenings, the scope of preparative HPLC is changing.

Objectives of Preparative HPLC:

Three important parameters used to assess the outcome of a preparative run are product purity, yield, and power. Since the parameters are interdependent, it is not possible to optimize the preparative HPLC method with respect to all three parameters.

Principle of Preparative HPLC:

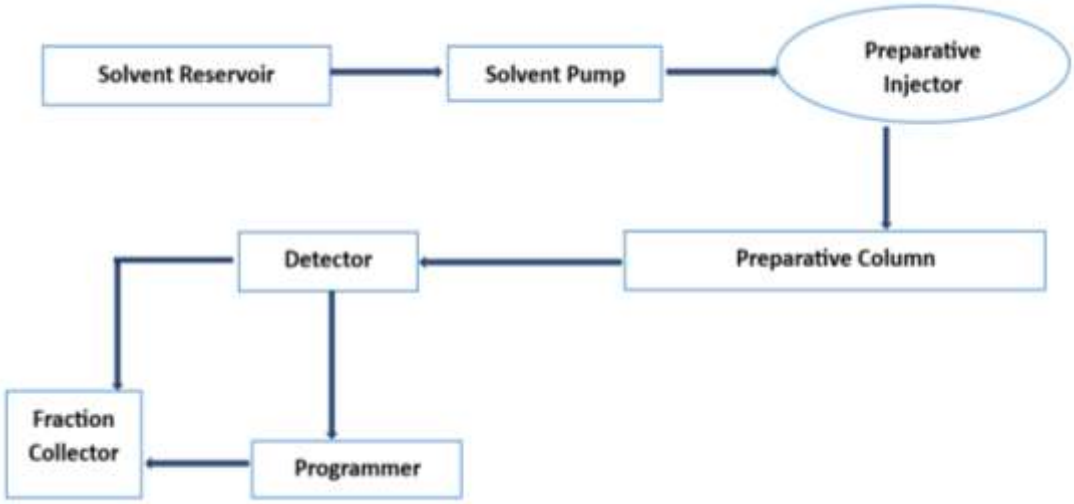
The working principle of prep. HPLC is the same as analytical HPLC, which involves separation based on adsorption. In addition to analytical HPLC components, prep. The HPLC fraction collector is located behind the detector. In prep. In HPLC, the monitored analyte is collected in the fraction collector after detection by the detector. By purification, we can reuse this sample for detection if the experiment fails under any conditions.

Comparison between analytical and preparative HPLC:

Parameter	Analytical	Preparative
Sample collection	Sample goes from detector to waste	Sample goes from detector to fraction collector
Column Size (mm)	120 - 250 x 2-4.6	120 - 250 x 20-62
Particle size (µm)	Upto 5	>10
Stationary Phase (g)	Upto 5	50-450
Flow rate (ml/min)	0.1-2	100-1000
Sample size (mg)	0.01-2	1-700
Sample concentration	High	Low

Solubility of Sample	Solubility of Sample in mobile phase usually not important	Solubility of Sample in mobile phase is very important
Capacity of solvent reservoir	In litre	Several gallons
Diverter Valve	Absent	Present
Fraction Collector	Absent	Present
Injector Capacity	Upto 100µl	0.1-100 ml
Pump Capacity	Low	High
Sensitivity of detector	High	Low
Analytical Use	For Quantitation and/or identification of compound	For isolation and/or purification of compound

Instrumentation:



1. Solvent reservoir:

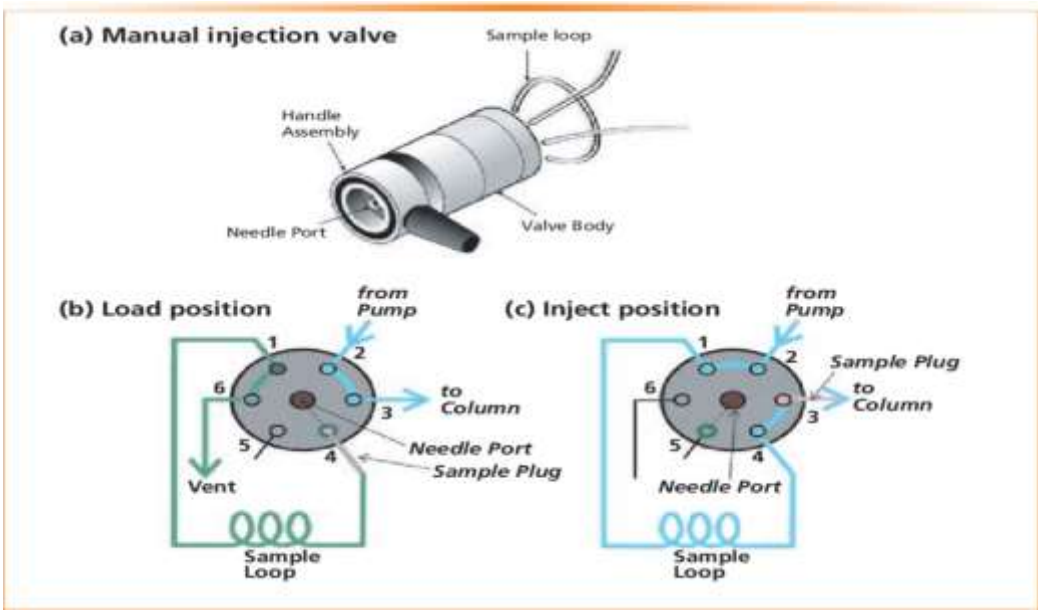
Large capacity (up to several gallons) containers made of glass or stainless steel are used in preparation. HPLC. The material of the structure varies according to the type of material. A biocompatible material is used for biologically sensitive material.

2. Pump:

Prep. HPLC requires a high eluent flow rate and the inner diameter of the column used in the prep. HPLC is usually larger. Required flow rate for prep. HPLC is usually between 10 to 100 ml/min (larger columns may require a flow rate of 500 ml to 1 liter/min). To prepare. HPLC, analytical pumps are designed for high pumping speeds and large volumes. The main modification that is required to work at a flow rate of 10-100ml/min is a larger piston head with a larger volume of liquid filled chamber.

3. Preparative Injector:

The Rheodyne injector should be capable of injecting a sample in the range of 0.1 to 100 mL volume under high pressure (up to 4000 psi). Also referred to as a rotary sampling valve. It involves four steps of operation.The preparative rheodyne injector and the rheodyne injector positions are shown in the figure



4. **Preparative Columns:** The role of the column is very important in the development of a reproducible preparation. HPLC method. Preparative columns should be able to withstand the high inlet pressure necessary to achieve the desired flow rate. The particle size of the packing material used for the column defines the performance of the prep. column. Because the preparative columns are wide, a sample distribution plate is placed to distribute the sample across the column. The distributor plate consists of a disk with radial slots.

5. **Packing of preparative column:**

To pack the prep. HPLC columns efficiently, the pressure upto 650 bar or 9500 psi is necessary.

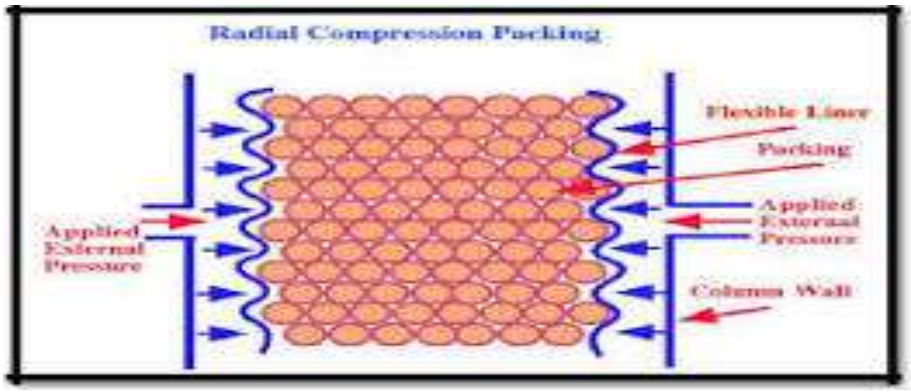
1. Dry Packing
2. Slurry Packing

1. **Dry Packing:** Dry packing is used when the particle size of the filling material is greater than 20 µm. It can be charged with knocking or sonic vibrations.

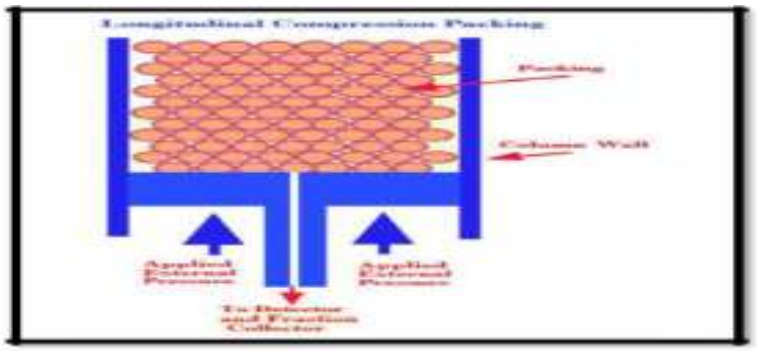
2. **Slurry Packing:** Liquid chromatography columns are packed with slurry when the particle size of the packing is less than 20 µm. The slurry is prepared by adding silica (2.2 g) in suitable solvents such as dibromomethane, tetrachloroethylene, carbon chloride, diiodomethane, etc.

3. **Column packing techniques:**

1. **Radial compression packing:**



2. **Longitudinal Compression Packing:**



6. **Detector:** Prep. chromatographic detectors can have very limited specifications. It may not be sensitive because the sample size and the solute concentration in the eluent are very large. Analytical detectors can be used for preparative purposes. In prep. The HPLC sample should be diluted with a large volume of mobile phase and then passed through the detector.

7. **Fraction collector:**

In prep. The HPLC diverter valve is used to divert the sample flow either to the waste or to the desired portion of the sample injected into the fraction container using the fraction collection needle.

Different fraction collection methods:

1. Mass collection of fractions

2. Collection of fractions based on vertex

3. Collecting factions based on time

4. Manual collection of fractions

APPLICATIONS OF PREPARATIVE HPLC:

- Preparative HPLC is used in the pharmaceutical industry, to study drug stability and for quality control analysis.
- Preparative HPLC is used in the purification of compounds in natural product chemistry.
- Micropurification and purity analysis of several different classes of compounds can be performed using preparative HPLC and pure compounds can be further used for advanced studies.
- Preparative HPLC is used in the purification of by-products arising from the analysis of impurities.
- Preparative HPLC is used in clinical testing for antibodies, urea and bilirubin.
- Preparative HPLC is used to determine contamination and water pollution in environmental analysis.
- Preparative HPLC is used in forensic analysis to quantify drugs and poisons in body fluids.
- Reported studies are as follows:

Sr No.	REPORTED STUDIES	CHROMATOGRAPHIC PARAMETERS	RESULTS
1	Isolation and purification of heroin from heroin street sample	1. Mobile phase: Methanol 20%- Trifluroacetic acid in water 0.05% 2. Column: C18 (250mm x 20mm, I.D. 15µm) 3. Flow rate: 20 ml/min. 4. Detector: SPD-M20A Photodiode- array detector	About 110.7mg of heroin HCl was obtained from 180mg of heroin street sample which contains 156.15mg of heroin HCl with the purity of about 99.52%
2	Resolution of Gossypol: Analytical and Large-Scale Preparative HPLC on Non-Chiral Phases	1. Mobile phase: MeCN: 0.01 M phosphate buffer (82: 18 v/v) 2. Column: C18(33 cm x 22 mm I.D.) 3. Flow rate: 8.5 ml/min 4. Detector: Variable wavelength detector	Multi-gram of Gossypol enantiomers are formed with high purity

3	Identification, isolation and characterization of potential degradation product in lansoprazole drug substance	1. Mobile phase: Water and acetonitrile 80:20 (v/v) 2. Column: C18 (250mm long×9.4mm I.D.) 3. Flow rate: 10 ml/min. 4. Detector: Photodiode-array detector	The isolation of impurity which is obtained as red color solid and the chromatographic purity was 96.0% by area percentage
4	Preparative high-performance liquid chromatographic purification and structural determination of 1-O-α-dglucopyranosyl-d-fructose (trehalulose)	1. Mobile phase: Water 2. Column: C18 columns (30 x 5.8 cm I.D.) 3. Flow rate: 0.1 ml/min. 4. Detector: Refractive-index detector	With 98% purity, gram quantities of trehalulose can be conveniently obtained using preparative HPLC
5	Improved gram-quantity isolation of malto-oligosaccharides by preparative HPLC	1. Mobile phase: Acetonitrilc- H2O (11:9) 2. Column: Aminopropyl silica gel column (21.4 and 41.4 mm I.D. X 250 mm) 3. Flow rate: I3 mL/min 4. Detector: Refractive index detector	Isolation of 1 g of oligosaccharide per hour. The purity of isolated malto-oligosaccharides was found to be at least 98%

Conclusion:

Preparative liquid chromatography is widely accepted as an excellent purification technique. Prep-HPLC with the same parts as analytical HPLC has some advantageous features compared to "low pressure" column chromatography systems, including particle size (prep-HPLC smaller, 3–10 mm) and large surface area results in a system with high resolution performance and can also be connected to some detectors such as diode array, refractive index, fluorescence, etc.

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