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Review Article

A Comprehensive Review for the Learners and Users: Preparative High Performance Liquid Chromatography

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ABSTRACT

Preparative High Performance Liquid Chromatography (Prep. HPLC) is an important quantitative technique which is generally used for the isolation and purification of variety of chemicals including pharmaceutical compounds, natural products and biological samples. It is the most versatile and safest chromatographic technique which is used for the quality control of drug components. The attempt was to review different aspects of preparative HPLC. In this article, preparative HPLC is discussed with respect to instrumentation in comparison with analytical HPLC, hyphenation with other techniques, reported studies, applications and uses.

Keywords: Preparative HPLC , Purification, Preparative column, Fraction collector Hyphenation.

1. INTRODUCTION

Preparative chromatography is powerful technique used for the isolation and purification of variety of compounds. The aim of prep. HPLC experiment is to isolate the maximum amount of analyte of interest at a desired purity in short period of time. Prep. HPLC typically involves, working with high concentration and volume which is required for analytical purposes therefore detector selectivity and sensitivity are less important parameters. As the demand for the production of highly pure compounds increasing day by day, the field of operation for prep. HPLC is also changing.

The working principle of prep. HPLC is same as that of analytical HPLC which involves separation on the basis of adsorption. In addition to the components of analytical HPLC, in prep. HPLC fraction collector is placed after the detector. In prep. HPLC the analyte of interest is collected in fraction collector after the detection by detector. By purification we can use this sample again for detection if experiment fails in any condition. [1-2]

The basic comparison in operational and instrumentational parameters between conventional and prep. HPLC are

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2. INSTRUMENTATION

The basic components of preparative HPLC instrument are shown in figure 1.

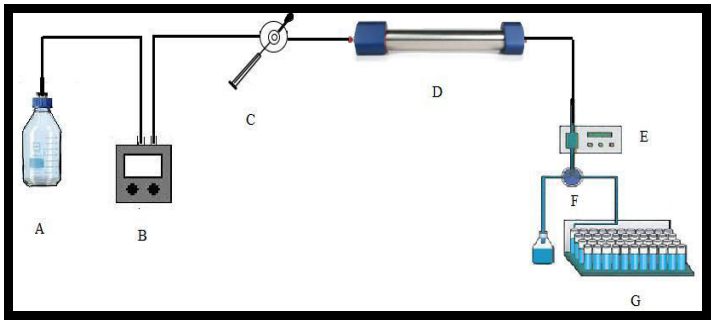

Figure 1: Schematic representation of components of prep. HPLC

A. Solvent reservoir
B. Pump
C. Preparative injector
D. Preparative column
E. Detector
F. Diverter valve
G. Fraction collector

The basic comparison in operational and instrumental parameters between conventional and preparative HPLC are summarized in Table 1.

Table 1: Operational and instrumentational parameters of HPLC Versus Preparative HPLC[2-5]

PARAMETERS		HPLC	PREPARATIVE HPLC
Operational	Sample size	0.01-2mg	1-500000mg
	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
	Recovery of mobile phase	Recovery is not possible	Recovery is not possible with the help of fraction collector
	Capacity of solvent reservoir	In liter	Several gallons
Instrumentational	Pump		
	Capacity	Low	High
	Flow rate	1-10 ml/min	10-100 ml/min
	Injector capacity	Upto 100µl	0.1-100 ml
	Column:		
	Internal diameter	1-5mm	1-10cm
	Particle size	5µm or smaller	7µm or larger
	Sensitivity of detector	High	Low
	Fraction collector	Absent	Present
	Diverter valve	Absent	Present
Applications	Analytical use	For quantitation and/or identification of compound	For isolation and/or purification of compound

A. Solvent reservoir:

Large capacity (Upto several gallons) reservoirs made up of glass or stainless steel are used in prep. HPLC. Material of construction varies with type of material. For biologically sensitive material, biocompatible material is used.[6]

B. Pump:

Prep. HPLC requires high eluent flow rate and internal diameter of column used in prep. HPLC is usually larger. The required flow rate for prep. HPLC is usually between 10 to 100 ml/min (For larger columns, flow rate of 500 ml to 1 litre/min. may be required). For prep. HPLC, analytical pumps are modified with respect to high pumping rates and large volume. The main modification that is required to work at flow rate of 10-100ml/min is a larger piston head with higher volume liquid filled chamber.[6]

C. Preparative injector:

Rheodyne injector should be able to inject the sample within the range of 0.1 to 100 ml of volume under high pressure (up to the 4000 psi). It is also referred as rotary sampling valve. It involves four-steps of operation. [7]

A preparative rheodyne injector and positions of rheodyne injector are shown in figures 2 and 3.

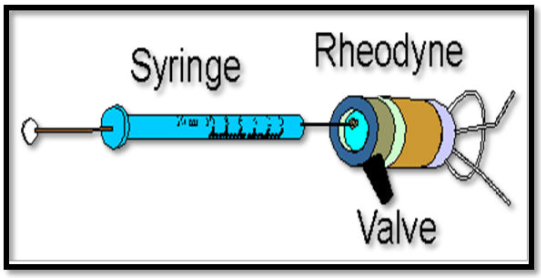

Figure 2: Rheodyne injector

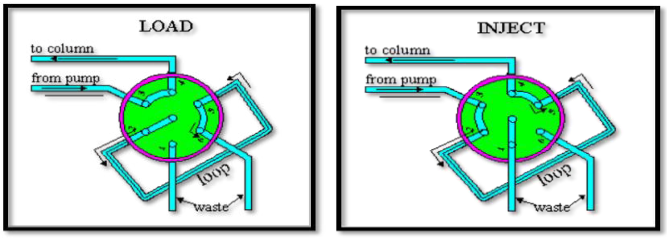

Figure 3: Positions of rheodyne injector.

D. Preparative column:

The role of the column is very important in developing a reproducible prep. HPLC method. Preparative columns should be able to withstand the high inlet pressure, necessary to obtain the required flow rate. Particle size of a packing material used for column defines the performance of prep. column. As the preparative columns are wide sample distribution plate is placed for the distribution of the sample across the column. The distribution plate consists of a disc with a radial slots.[6]

Typical preparative column is shown in figure 4.

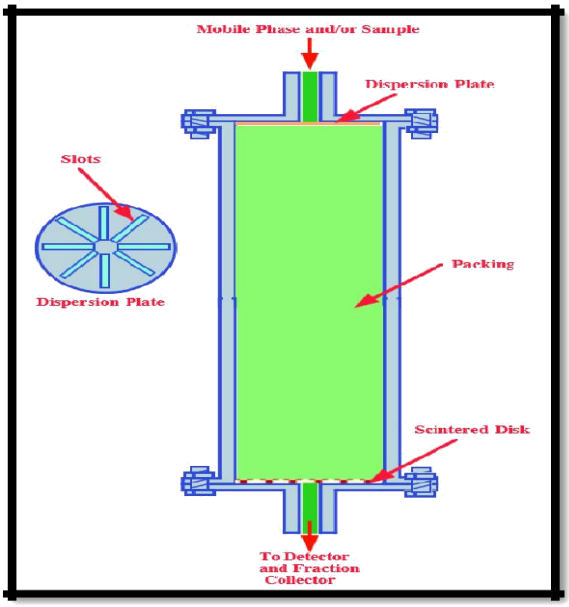

Figure 4: A typical preparative column [6]

i) Packing of preparative column:

Choice for the type of technique used for packing of preparative columns depends on the particle size of packing material, scale of the preparation and nature of the material to be separated. Dry packing and/or slurry packing is used for packing of preparative columns. The packing pressure should be at least twice the working pressure in order to prevent resettling of the packed bed. To pack the prep. HPLC columns efficiently, the pressure upto 650 bar or 9500 psi is necessary.

a. Dry packing:

Dry packing is used when the particle size of the packing material is more than 20 µm. It can be packed with tapping or with sonic vibrations.

b. Slurry packing:

Liquid chromatographic columns are packed with slurry when the particle size of packing material is less than 20 µm. Slurry is prepared by adding silica (2.2 g) in suitable solvents like dibromomethane, tetrachloroethylene, carbon tetrachloride, diiodomethane etc. Prep. HPLC columns packed with slurry are not operated at pressure greater than the bursting strength of the tube used for the column. After preparation slurry is placed in the packing reservoir. After connecting the slurry packing reservoir to pump, pump parameters such as flow rate and pressure are adjusted as per column diameter and column length. The column is disconnected when it is tightly packed with the slurry and packing is secured with proper sealing. Apparatus for packing of prep. HPLC column is shown in figure 5.

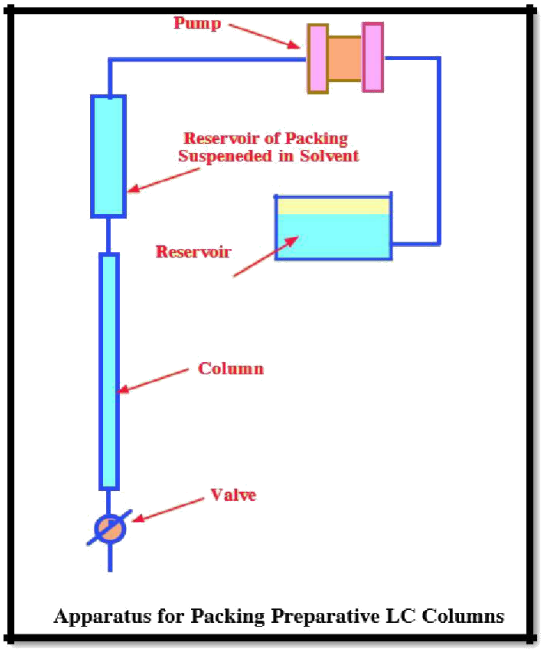

Figure 5: Apparatus for slurry packing of preparative LC columns.

ii) Column packing techniques:

a. Radial compression packing:

In this technique walls of the column should be flexible and columns are packed into a tubular polymer cartridge. This cartridge is placed inside stainless steel cylindrical column. The column is filled with dense slurry of the packing material and seal with suitable material. The bed is then compressed radially by applying pressure (pneumatic or hydraulic) to the outside walls of the polymer cartridge. The radial compression technique is generally applied in columns of up to 20 cm in diameter.

Radial compression packing is shown in figure 6.

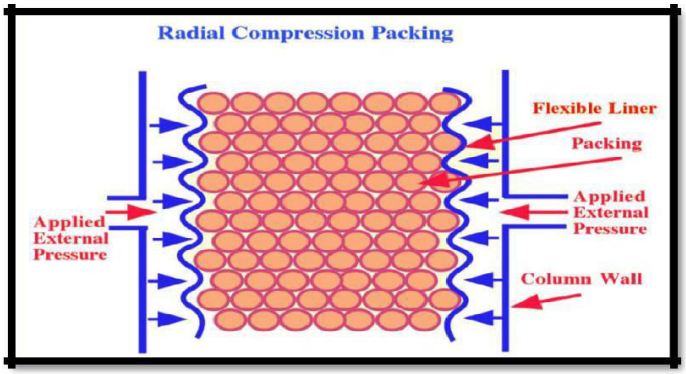

Figure 6: Radial compression packing

b. Longitudinal/axial compression packing:

Axial compression packing is an alternative column packing technique. This technique can be used to the Columns with larger diameter. This is highly efficient technique of column packing.

Column contains piston which can be moved throughout the entire length. Mobile phase is passed through the channels present in center. The channel is connected to the detector and then to the fraction collector. After packing, the piston is withdrawn from bottom of the column.

The length of the packing is decided by the amount of packing material used for packing. The use of this techniques for prep. chromatography is increase in greater extent and it can also used for expensive materials (antibiotics and other biotechnology products) to be processed in kilogram quantities and more.[8-12]

Longitudinal compression packing is shown is figure 7.

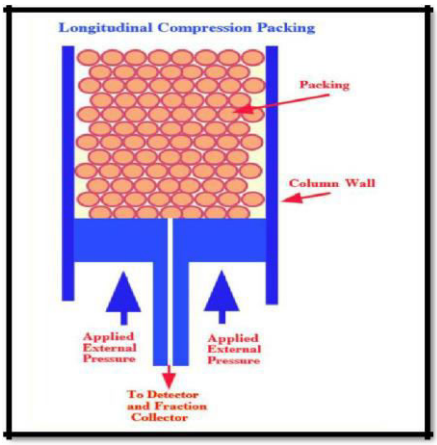

Figure 7: Longitudinal Compression Packing

F. Detector:

Prep. chromatographic detectors can have very limited specifications. They need not be sensitive as the sample size and the eluent solute concentrations are very large. Analytical detectors can be used for preparative purposes. In prep. HPLC sample should be diluted with large volume of mobile phase and then passed through the detector. [6]

G. Fraction collector:

In prep. HPLC the diverter valve is used to divert the flow of sample either to waste or the desired part of the injected sample into a fraction container via the fraction collection needle. The analytical scale fraction collector required flow rate below 10 ml/min and the preparative scale fraction collector requied flow rate up to 100 ml/min.

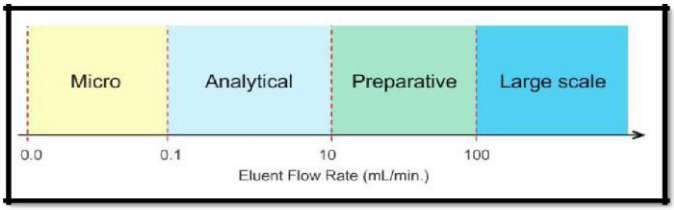

Figure 8: Eluent flow rate[1]

Some commercially available instruments provides the combination of auto-sampler and the fraction collector on a single platform. Sample fraction collector system is as shown in figure 9.

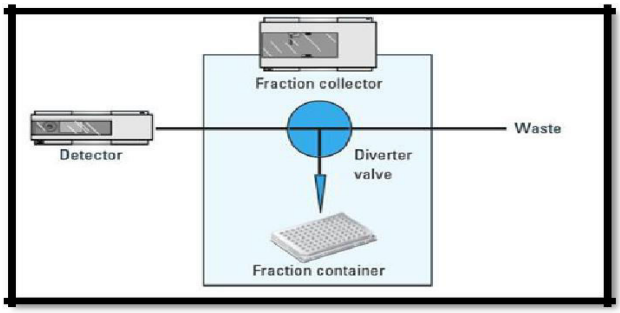

Figure 9: Fraction collector system

Different fraction collection methods[1-4]

Sample fractions can be collected into vials or test tube with suitable method.

i. Manual fraction collection:

In this the switching of the diverter valve is triggered manually by pressing a button on the instrument.

ii. Time-based fraction collection:

It involves the collection of analyte of interest in certain time intervals from a purification run.

iii. Peak-based fraction collection:

It involves the collection of fraction based on a detector signal.

iv. Mass-based fraction collection:

It involves collection of fraction depends on user-selected target mass which is found by mass detector.

3. HYPHENATION OF PREPARATIVE HPLC WITH OTHER ANALYTICAL TECHNIQUES:

Prep. HPLC when coupled/ hyphenated with other analytical techniques, it offers several advantages in many ways. Prep. HPLC can be hyphenated with mass spectroscopy and other

chromatographic techniques such as counter current and flash.

Literature survey reveals that it is effectively used for purification and separation by combining it with mass spectroscopy, counter current chromatography as well as with flash chromatography.

Reported studies of analysis making use of hyphanation of prep. HPLC are reviewed as follows,

3.1 Preparative high-performance liquid chromatography–mass spectrometry (MS) for the high-throughput purification of combinatorial libraries.

Prep. HPLC has been used for peptide and combinatorial chemistry. Fraction collection is achieved after UV detection. Every UV- active compound can be collected above a certain threshold. The main advantage of prep. HPLC–MS is that, only the target molecule is selected by detecting the ion current of the desired target molecule. [13]

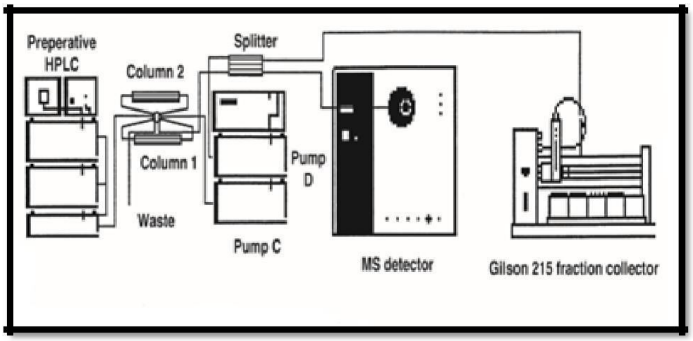

Figure 10: Set up of preparative HPLC-MS system

Advantages:

1. Online identification.
2. Ease of operation.
3. Non-critical fraction collector capacity.
4. There is one-injection, one-fraction purification procedure which solves many problems, normally encountered with large libraries.

Disadvantages:

If for any reason an existing product is not detected by MS, the sample is lost.

3.2 Combination of counter-current chromatography and preparative high-performance liquid chromatography to separate galactolipids from pumpkin.

Galactolipids in the fruits of *Cucurbita moschata* (pumpkin) could not be completely separated by HPLC. In the reported study, a combination of high-speed counter-current chromatography (HSCCC) and prep. HPLC was used for separating the galactolipids. A fraction containing galactolipids from the purification of the *n*-butanol extract of pumpkin by macro-porous resin column chromatography was first separated by HSCCC to result in three sub-fractions of each containing two galactolipids monomers. The three sub-fractions were further separated by prep. HPLC respectively to yield six galactolipids monomers with purity more than 96%. It can provide excellent solution to separate galactolipid with different sugar group and alkyl chain.[14]

3.3 Preparative high pressure liquid chromatography-flash chromatography (PuriFlash).

The commercially available instrument puriFlash (Interchim laboratory suppliers) combines the simplicity of Flash purification with the power to run Prep. HPLC columns into one instrument. With this instrument one can switch from normal to reverse phase in less than 10 seconds. The puriFlash software allows user to alter run conditions such as gradient slope, flow rate, tube collection volume at anytime during run.

4. APPLICATIONS OF PREPARATIVE HPLC:

Preparative HPLC is preffered choice for numerous applications. Some of the common applications of prep. HPLC are as follows:

4.1. Purification in medicinal or high-throughput chemistry:

Most of the compounds are specifically synthesized by high-throughput chemistry. All newly synthesized compounds must be purified before they released for activity testing. Prep. HPLC is widely used for this purpose. In this case mass-based fraction collection is method of choice for purification because the structures of the synthesized compounds are known.

4.2. Purification of compounds in natural product chemistry:

In natural product chemistry, crude extract is a very complex mixture contaning active components along with other

ingredients. The prep. HPLC process is used to isolate and purify individual component from mixture of crude extract. The natural product chemistry involves consecutive purification and activity testing steps until the active component is extracted in the pure form for structural elucidation.

4.3. Purification of by-products, formed during impurity analysis:

In the drug discovery and development process it is not only important to isolate the compound of interest in pure form but also with traces of impurities. These isolated impurities are treated as reference standards for the later production process. When an unexpected compound (by-product) is observed during the production cycle, it can be compared to the previously isolated impurity reference standards. In prep. HPLC, starting material is required in larger amount therefore purification run can be optimized to make sure the impurities are well separated from the main peak of interest.

4.4. Recovery collection:

It is necessary to collect all fractions of a sample at specific location during the prep. HPLC analysis which is referred as recovery collection. In prep. HPLC, diverter valve is used to isolate fraction of interest from waste material. These collected fractions are used as a safety feature in case of failure of analytical run. Thus recovery collection is very valuable for reanalysis.

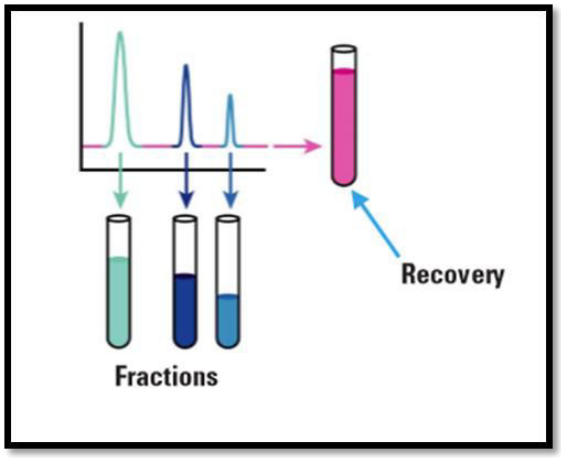

Figure 11: Recovery Collection

4.5. Automated fraction re-analysis:

Automated fraction re-analysis consist of three steps:

a. Pre-preparative analysis.

b. Purification.

c. Post preparative analysis.

In first step identity, purity and amount of target compound is measured prior to the purification run to decide whether it is worthwhile to purify the sample or not. After the completion of purification run, fraction containing compound of interest must be analysed at certain point to assure the purity and activity of right compound.[1]

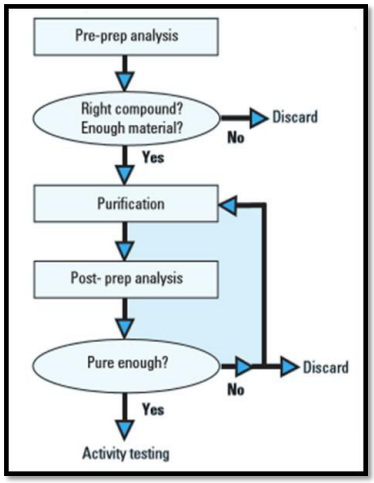

Figure 12: Automated Fraction Reanalysis.

5. REPORTED STUDIES:

Literature survey revealed that prep. HPLC is widely used for isolation and purification, some of the reported studies are tabulated in table 2.

Table 2: Reported studies using preparative HPLC

Sr. No.	REPORTED STUDIES	CHROMATOGRAPHIC PARAMETERS	RESULTS	Reference No.
1.	Separation of punicalin from waste water of hydrolysed pomegranate husk	**1. Mobile phase**: 10 % (V/V) of methanol and 0.1 % TFA in water **2. Column**: C18 (19 x 300 mm, 7 µm) **3. Flow rate**: 8 ml/min. **4. Detector:** 2487 duel wavelength absorbance detector	About 120 ml of waste water which contains 480 mg of punicalin was purified by using preparative HPLC. After purification it was found that 349mg of 97% punicalin separated from the waste water	15
2.	Isolation of recombinant hirudin by preparative high-performance liquid chromatography	**1. Mobile phase:** water and 2-propanol (70:30) **2. Column:** C8 (50 cm x 4 in, 50 µm) **3. Flow rate:** 300 ml/min. **4. Detector:** Photodiode-array detector	Recombinant hirudin varaint 2-Lys47 (rHV2-Lys47) which is produced by a genetically engineered yeast strain was purified by successive preparative reversed-phase HPLC	16
3.	Isolation of six isoflavones from *Semen sojae praeparatum* by preparative HPLC	**1. Mobile phase:** MeCN- water- AcOH (25:75:2and 35:65:2 v/v/v) **2. Column:** C18 (20 x 250 mm, internal diameter [I.D.] 10µm) **3. Flow rate:** 3.5 and 4.5 ml/min. **4. Detector:** Photodiode-array detector	Successful isolation of six isoflavones (genistein, genistin, daidzein, daidzin, glycitein and glycitin) with the high purity	17
4.	Preparative high-performance liquid chromatographic purification and structural determination of 1-O-α-d-glucopyranosyl-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	18
5.	Preparative high-performance liquid chromatographic purification of saffron secondary metabolites	**1. Mobile phase:** methanol 45 % and acetonitrile 90% **2. Column:** C18 (25 cm x 0.46 cm I.D. and 25 cm x 2.12 cm I.D.) **3. Flow rate:** 42 ml/min. **4. Detector:** UV-Vis spectrophotometric detector (SPDdAV)	The highest yield of picrocrocin with the 80% purity, obtained by preparative HPLC method development.	19
6.	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%	20
7.	Preparative separation of lithospermic acid B from Salvia miltiorrhiza by polyamide resin and preparative high-performance liquid chromatography	**1. Mobile phase:** Aqueous acetic acid 1% v/v and acetonitrile: methanol 3:2 v/v **2. Column:** C8 (20mm×250mm,15m) **3. Flow rate:** 8 ml/min. **4. Detector:** G1314A UV–Vis detector	After preparative HPLC, the maximum HPLC purity obtained was 99.28% with a recovery of 75.2%	21
8.	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	22
9.	Improved gram-quantity isolation of malto-oligosaccharides by preparative HPLC	**1. Mobile phase:** Acetonitrilc- H_2O (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%	23
10.	Preparative high-performance liquid chromatographic separation and analysis of Maltacine complex – a family of cyclic peptide antibiotic from Bacilus subtilis	**1. Mobile phase:** Water- 0.1 % trifluroacetic acid or ACN – 0.1% trifluroacetic acid **2. Column:** Wydac C18 (25x 250mm) **3. Flow rate:** 10 ml/min. **4. Detector:** UV detector	Isolation of family 18 closely related to cyclic peptides within 110 min with minimal loss of activity	24

Sr. No.	REPORTED STUDIES	CHROMATOGRAPHIC PARAMETERS	RESULTS	Reference No.
11.	Purification and identification of several sulphonated azo dyes using reversed-phase preparative high-performance liquid chromatography	**1. Mobile phase:** Methanol and ammonium acetate **2. Column:** C18 column 250321.2 mm) **3. Flow rate:** 13.5 ml/min. **4. Detector:** Tunable absorbance detector.	Reversed-phase preparative HPLC has been successfully used to isolate several sulphonated azo dyes (Acid Red 1, Acid Red 8, Acid Red 106, Acid Violet 5, Chromotrope 2R, Reactive Orange 16 and Cibacron Brilliant Red 3B-A) from their impurities	25
12.	Preparative hplc purification of prostaglandin endoperoxides and isolation of novel cyclooxygenase-derived arachidonic acid metabolites	**1. Mobile phase:** Hexane: isopropanol: acetonitrile: acetic acid (95 : 5 : 0.05: 0.05 v/v) **2. Column:** Straight-phase preparative HPLC column, (10 mm (I.D.) X 250 mm, 7 µm) **3. Flow rate:** 4ml/min. **4. Detector:** UV detector	Milligram quantities of prostaglandin G_2 (PGG2) and prostaglandin H_2 (PGH2) were obtained in ~95% purity within shortest time	26
13	Preparative HPLC. Part 1: A comparison of three equipment for the purification of steroid esters	**1. Mobile phase:** CH_2Cl_2: MeCN (95:5 V/V) **2. Column:** C18, 8 cm I.D. **3. Flow rate:** 26 ml/min **4. Detector:** UV detector	Economic method, gram quantities of steroid esters are purified in efficient way	27
14	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	28
15	Isolation and characterisation of a potential process related impurity of phenazopyridine HCl by preparative HPLC followed by MS-MS and 2D-NMR spectroscopy	**1. Mobile phase:** Water: ACN (30:70 v/v) **2. Column:** C18(10 mm x 250 mm) **3. Flow rate:** 5 ml/min **4. Detector:** Photodiode-array detector	Separation of trace level impurity of 3-phenyl-5-phenylazo-pyridine-2,6-diamine	29
16	Preparative HPLC separation of methoxytetralins, ligands for melatonin receptors, contaning two chiral centers with polysaccharide chiral stationary phases. Determination of enantiomeric purity	**1. Mobile phase:** Hexane: ethanol (90:10 v/v) **2. Column:** Chiral 250x 50 mm I.D. **3. Flow rate:** 120 ml/min **4. Detector:** UV detector	Each enantiomers obtained rapidly and quantitatively.	30

6. CONCLUSION

In preparative HPLC we can reuse the sample with the help of recovery fraction collection till satisfactory results are obtained. If we optimise all the analytical process parameters like column loadability, selectivity, flow rate, particle size, we can scale up the technique from analytical to preparative scale. Since preparative HPLC is expensive technique, compared to traditional purification methods such as distillation, crystallization or extraction, it has been used only for rare or expensive products. On an industrial scale prep. HPLC works in continuous manner to produce tons of pure product by applying the principle of simulated moving bed (SMB) chromatography. Prep. HPLC is widely used as method of choice in the field of high throughput chemistry and natural product chemistry for the purpose of isolation, purification of purest form of analyte of interest from synthesized product or from crude extract respectively.

REFERENCES

1. Huber U, Majors R, Principles in preparative HPLC, Agilent Technologies Inc., Germany, 2007, (2): 60-71.
2. Lindholm J, Development and validation of HPLC methods for analytical and preparative purposes, Acta Universitatis Upsaliensis, 2004, 41-42.
3. Katz E, High performance liquid chromatography: Principles and methods in biotechnology, 1995, 596-606.
4. Marvin C, HPLC: A practical user guide, 2nd edition, John wiley and sons, 2007, 137-142.
5. Snyder L, Kirkland J, Practical HPLC method development, 2nd edition, Wiley- interscience, 1997, 616-618.
6. Scott P, Preparative chromatography, Chrom-Ed Book Series, 2003, 22-36.

7. Mahajan S, Instrumental methods of analysis, 1st edition, Popular prakashan, Mumbai, 2010, 250-251.
8. Meyer V, Practical high performance liquid chromatography, 5th edition, Wiley blackwell, 2010, 387-395.
9. Kaushal R, Kaur N, et al, International research journal of pharmacy, 2011, 2(5): 2.
10. Yuri K, HPLC for pharmaceutical scientist, 10.1002, Wiley blackwell, 2007, 954-955.
11. Vank N, Bal L, Journal of separation science, 1979, (2): 667.
12. Wellings D, A Practical handbook of preparative HPLC, Elsevier, Netherland, 2006, 59-65.
13. Bauser M, Journal of chromatographic science, 2002, (40): 292-295.
14. Qizhen D, Zhiguo J, et al, Journal of chromatography A, 2009, (1216): 4176–4180.
15. Yuan Q, Zhou H, et al, Food chemistry, 2011, (126): 1361-1365.
16. Bischoff R, Clesse D, et.al, Journal of chromatography, 1989, (416): 245-255.
17. Fan G, Mi H, Fitoterapia, 2007, (78): 200–204.
18. Cookson D, Cheetham P, et al, Journal of chromatography, 1987, (402): 265-272.
19. Castellar M, Montijano H, et al, Journal of chromatography, 1993, (648): 187-190.
20. Guo Z, Zheng H, et al, Forensic science international, 2012, (221): 120-124.
21. Guo Y, Zhou L, et al, Journal of chromatography A, 2011, (1218): 4606– 4611.
22. Ramulu K, Rao B, et al, Journal of chemistry, 2013, (6): 274 – 283.
23. Hotchkiss A, Haines R, et al, Carbohydrate research, 1993, (242): l-9.
24. Hagelin G, Oulie I, et al, Journal of chromatography B, 2004, (811): 243-251.
25. Chen M, Moir D, et al, Journal of chromatography A, 1998, (825): 37–44.
26. Hecker M, Hatzelmann A, et al, Biochemical pharmacology, 1987, (36): 851-855.
27. Matlin S, Chan L, Journal of separation science, 1984, (7): 570-576.
28. Matlin S, Belenguer A, et al, Journal of separation science, 1987, (10): 86-91.
29. Rao R, Maurya P, et al, Journal of pharmaceutical and biomedical analysis, 2009, (49): 1287-1291.
30. Lipka E, Guelzim A, et al, Journal of biochemical and biophysical methods, 2005, (64): 46-58.