DOI: 10.21276/IJIPSR.2017.05.05.193

International Journal of Innovative Pharmaceutical Sciences and Research
www.ijipsr.com

A REVIEW ON HIGH PERFORMANCE CENTRIFUGAL PARTITION CHROMATOGRAPHY

Sai Sruthi Arige, Sai Datri Arige* and Lakshmana Rao A

Department of Pharmaceutical Analysis, V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, A.P., ***INDIA***

Abstract

High Performance Centrifugal Partition Chromatography (HPCPC) is a new technique in liquid chromatography which is support free, it effectively separates, isolates and purifies wide range of samples from milli grams to multigrams. The Centrifugal Partitioning Chromatography thus functions based on the principles of liquid/liquid partitioning chromatography: two immiscible liquid phases are mixed together to form a two-phase system, and are then separated multiple times. The individual solutes are isolated based on the different partitioning coefficients of each compound of the solute in this two phase system of the chromatograph.

Keywords: HPCPC, Partition, Immiscible liquids and Chromatography.

Corresponding Author:

Sai Datri Arige

Department of Pharmaceutical Analysis,

V. V. Institute of Pharmaceutical Sciences,

*Gudlavalleru, A.P., **INDIA***

E-mail: *sai.dhatri_arige@yahoo.co.in*

Phone: *+91-8500542419*

DOI: 10.21276/IJIPSR.2017.05.05.193

INTRODUCTION

HPCPC (high performance centrifugal partition chromatography) is a one analytical technique to separation of components from mixture of sample. By application of centrifugal force and separation occurs due to difference in partition co-efficient of the samples. It is advanced technique of liquid chromatography. It is also called as counter current chromatography (CCC). Without a solid stationary phase support, effectively separate, isolate and purify Milligrams to Multi grams [1-5].

- Bio Polymers
- Fermentation Products
- Foods &Additives
- Fine Chemicals
- Genetically Engineered Substances
- Natural Organic Compounds
- Physiological Activated Substances
- Pharmaceuticals
- Petrochemicals
- Rare Metal &Earth
- And much more

WITH

- High efficiency
- Ultra selectivity
- No column
- High recovery
- High throughput
- Volume ratio of stationary/ mobile phase is very high (better resolution)
- Short run times
- Retention of virtually all biological activity and molecular integrity

HISTORY

Around fifty years ago, the concept of partitioning solutes between two liquids gave birth to two cognate methods, one was the counter-current distribution, and another was the liquid-liquid partition chromatography. Thirty years ago, Sanki Engineering Ltd. opened the way to high

DOI: 10.21276/IJIPSR.2017.05.05.193

performance centrifugal partition chromatography (HPCPC), which was taking the better of the two first techniques, namely the versatility of a true liquid-liquid process combined with the quickness and advanced technology of chromatography. The HPCPC is gaining more and more interest as a semi-prep and preparative scale chromatographic method for better separation, isolation and purification of components of solute [6-8].

CLASSIFICATION OF LIQUID CHROMATOGRAPHY

Liquid chromatography can be differentiate based upon packing material of stationary phase such as packed column chromatography and non –packed column chromatography. And packed column chromatography is again divided into liquid chromatography (LC), High performance liquid chromatography (HPLC), Gas liquid chromatography (GLC), paper chromatography, column chromatography. And non- packed column chromatography is again divided into centrifugal partition chromatography and Gravitational partition chromatography [9].

Centrifugal partition chromatography further classified into single axis Hydrodynamic partition chromatography such as high performance partition chromatography (HPCPC) and double axis Hydrostatic centrifugal partition chromatography (HSCCC). And gravitational partition chromatography is classified as droplet counter current chromatography (DCCC) [10].

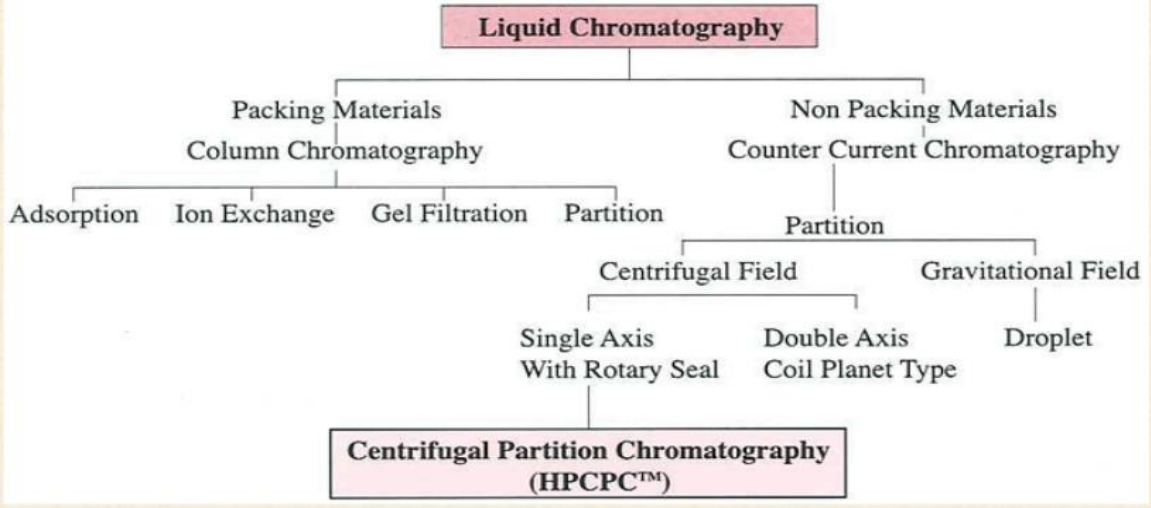

Fig. 1: Classification of High Performance Centrifugal Partition Chromatography

PRINCIPLE

"Two Immiscible liquid phase come in contact with each other as at least one phase is pumped through a column, a Helical coil or micro droplet cell or partition cell connected to that channels, which contain both resulting dynamic mixing and settling action allows the components to be separated by their respective solubility in the two phase in the presence of centrifugal force on

DOI: 10.21276/IJIPSR.2017.05.05.193

it.". The affinity of the solute for each phase can be measured by their partition co-efficient that turn dictates the order of elution for each compound [11-16].

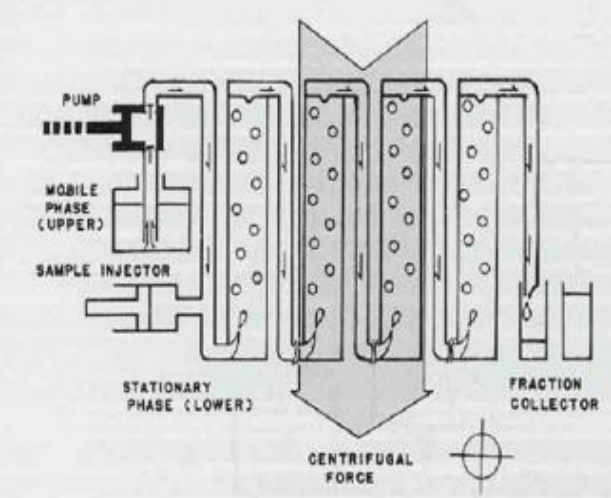

Fig. 2: Working principle of HPCPC

THEORY

High Performance Centrifugal Partition Chromatography (HPCPC) is a practical and suitable method, particularly on the preparative scale, for the separation of bio molecules such as proteins, enzymes, etc. it is also known as "counter current chromatography" HPCPC is an analytical chemistry technique that is used to separate, identify, and quantify the chemical components of a mixture. In HPCPC solid stationary phase is not used. Instead of stationary-phase liquid is retained by centrifugal force in discrete partition channels within a unique patented circular Partition Disk Pack. A packed column generally contains only 2 to 7 percent of stationary phase, severely limits its capacity. In an HPCPC system, the column contains between 50 and 80 percent stationary phase. The stationary phase is held in numerous discrete partition cells. Micro droplets of mobile phase liquid pass continuously through the stationary phase liquid. Any two-phase solvent mixture can be used, at any pH, to perform normal and reversed phase chromatographic separations In HPCPC is based on the principle of counter current chromatography (CPC). The stationary phase of a two –phase system is maintained in the instrument under a centrifugal force while the mobile phase is pumped through a series of chambers or cells. The stationary phase is retained inside the rotor by the centrifugal force generated by rotation around a single axis. Discrete chambers connected by a channels are the sites of mixing and settling action that allows the compounds being separated to distributed between the two phase as the mobile phase exits the columns it is collected in test tubes for further Analysis . Chemical compounds subjected to CPC

DOI: 10.21276/IJIPSR.2017.05.05.193

are separated based on the partition co-efficient of each compound in the two – phase system [17-23].

MODES OF OPERATION

NORMAL PHASE

A polar solvent used as stationary phase and mobile phase as non-aqueous or biphasic solvent system used as mobile phase [24].

REVERSE PHASE

A non polar or less polar solvent used as stationary phase and mobile phase aqueous phase.

GRADIENT

By changing the polarity of mobile phase either increase or decrease elution take place.

Ex: methanol-water as mobile phase heptanes as stationary phase [25].

DUAL MODE

Mobile phase and stationary phase Direction of flow change by switching such as

ASCENDING MODE

DESCENDING MODE

In Ascending Mode, A four way valve allows a change in the direction of the elution and therefore will work either in ascending mode (figure 1) when the lightest phase is the mobile phase.

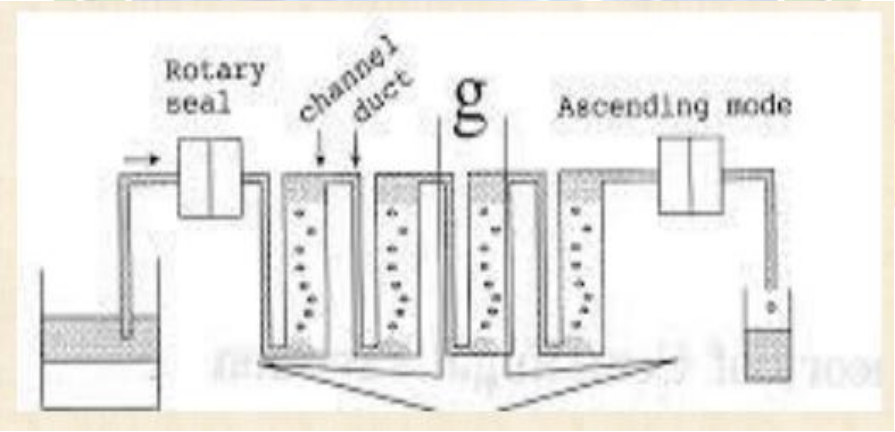

Fig. 3: Ascending mode of operation

In **Descending Mode,** When the heaviest phase is the mobile phase. By working this way, it is possible to work both in normal and reversed mode without replacing the column.

DOI: 10.21276/IJIPSR.2017.05.05.193

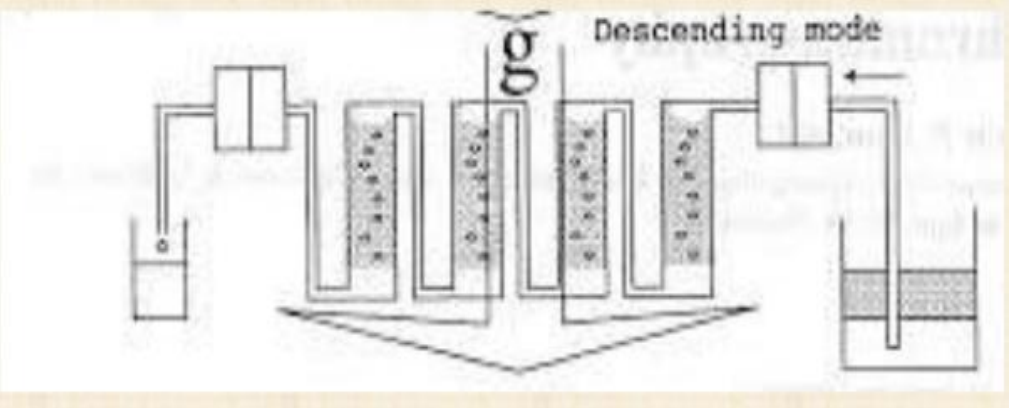

Fig. 4: Descending mode of operation

INSTRUMENTATION

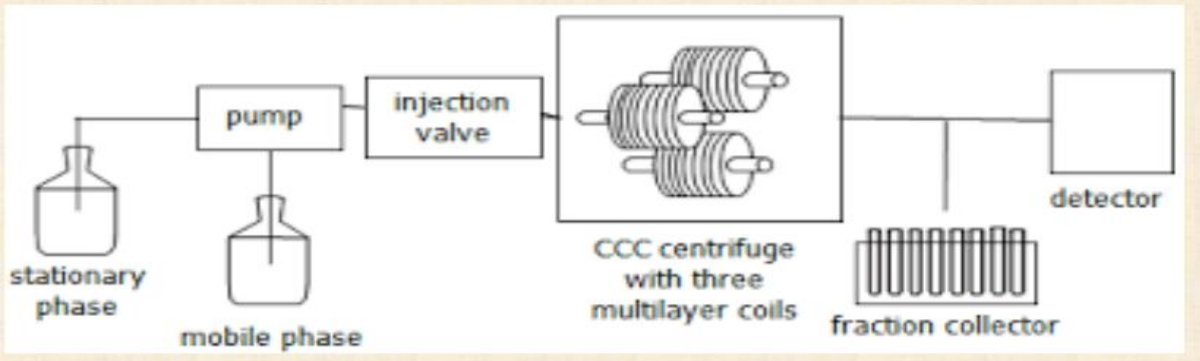

Fig. 5: Instrumentation of HPCPC

PARTS

- Solvent system
- pump
- Sample Injector
- Elution mode switching valve
- Rotary Joint
- Partition Channel
- Rotary
- UV Detector
- Fraction Collector

SOLVENT SYSTEM

Three Criteria

Numerous two-phase solvent systems with a broad spectrum of polarity or containing extracting reagents can be applied to separate organic, bioorganic, and inorganic substances.

DOI: 10.21276/IJIPSR.2017.05.05.193

There are three important criteria for choosing a two-phase liquid system.

- First and obviously, it should form two immiscible phases.
- Second, the phase selected to be the stationary phase should be retained by the column.
- Third, the sample should be separated by the twophase liquid system selected.

Two Immiscible Phases

Two-phase solvent system is used as separation medium in Centrifugal Partition Chromatography. One serves as the stationary phase; the other serves as the mobile phase. The solvents may be selected from an infinite variety of possible combinations. There are countless solvent mixtures that form two immiscible phases. When the nature of the organic substances to be separated is known, one may find a suitable solvent system by searching the literature for solvent systems that have been successfully applied to similar compounds. In case of organic-aqueous two-phase systems, the organic phase consists of one solvent or of a mixture of different solvents. Various non-aqueous/nonaqueous solvent systems (e.g., heptane/acetonitrile) have been used for separation of non polar compounds and/or compounds that are unstable in aqueous solutions. Separation of macromolecules and cell particles can be performed with a variety of aqueous/aqueous polymer phase systems.

Followings are some frequently used solvent combinations

If the sample is water soluble compound with molecular weight more than 2000 A^{O},

- For Bio high polymers (proteins), denaturalization/sluggish is occurred in organic solvents. So, Dextran/PEG is used as solvent system (or) in high temperature, PEG/Aqueous salt solution (or) EtOH/Aqueous salt solution (or) Acetonitrile/Aqueous salt solution.
- For Bio high polymers (nucleic acid, sugars), PEG/Aqueous salt solution (or) EtOH/Aqueous salt solution (or) Acetonitrile/Aqueous salt solution is used as solvent system.
- For Oligomers (Peptides, sugars), Butanol/acetic acid/water (or) Butanol/Ethanol/water (or) Butanol/Methanol/water is used as solvent system.
- For Ionic Substances, Butanol/acetic acid/water (or) Butanol/Ethanol/Buffer (or) Butanol/Methanol/ Buffer (or) Ethyl acetate/ buffer are used as solvent system.
- For Non-ionic Substances, Butanol/acetic acid/water (or) Butanol/Ethanol/water (or) Butanol/Methanol/water (or) Butanol/Ethyl acetate/water are used as solvent system.

DOI: 10.21276/IJIPSR.2017.05.05.193

If the sample is water insoluble compound,

- For High polar (soluble in Methanol, Acetone), Butanol/acetic acid/water (or) Butanol/Ethanol/water (or) Butanol/Methanol/water (or) Butanol/Ethyl acetate/water are used as solvent system.
- For Normal Polar (soluble in Chloroform), Chloroform/Methanol/water (or) Methyl-tert. butyl ether/Acetonitrile/water (or) Methyl-isobutyl ketone/Acetone/water (or) Heptane/Ethyl acetate/Methanol/water.
- For Low Polar (Soluble in Hexane), Hexane/Acetonitrile (or) Hexane/Methanol/water (or) Hexane/Ethanol/water (or) Heptane/Ethyl acetate/Methanol/water.
- For pre concentration and separation of inorganic species, a stationary phase containing extracting reagents of different types (cation-exchange, anion-exchange, and neutral) in an organic solvent is usually applied. The mobile-phase components should not interfere with the subsequent analysis. Solutions of inorganic acids and their salts are most often used. The mobile phase may also contain specific complexing agents, which can bind one or several elements under separation

Stationary Phase

In normal phase polar solvents used as solvent and reversed phase non-polar solvent used as stationary phase. Generally heptane used as stationary phase in gradient mode.

Mobile Phase

In normal phase non-aqueous or biphasic solvent like Butanol and water system used as mobile phase.

In case of reverse phase aqueous phase used as mobile phase system.

PUMP

Pumps are used to pump the solvent system into the columns. The performance of pumps directly influences the retention time, reproducibility and detector sensitivity.

Criteria for the Selection of Pumps

Selection is defined by a few key specifications, including flow rate, head, power, and efficiency.

- Flow rate describes the rate at which the pump can move fluid through the system, typically expressed in gallons per minute (gpm). The rated capacity of a pump must be matched to the flow rate required by the application or system.

DOI: 10.21276/IJIPSR.2017.05.05.193

- Pressure is a measure of the force per unit area of resistance the pump can handle or overcome, expressed in bar or psi (pounds per square inch). As in all centrifugal pumps, the pressure in axial flow pumps varies based on the pumped fluid's specific gravity. For this reason, head is more commonly used to define pump energy in this way.
- Head is the height above the suction inlet that a pump can lift a fluid. It is a shortcut measurement of system resistance (pressure) which is independent of the fluid's specific gravity, expressed as a column height of water given in feet (ft) or meters (m).
- Net positive suction head (NPSH) is the difference between the pump's inlet stagnation pressure head and the vapour pressure head. The required NPSH is an important parameter in preventing pump cavitation.
- Output power, also called water horsepower, is the power actually delivered to the fluid by the pump, measured in horsepower (hp).
- Input power, also called brake horsepower, is the power that must be supplied to the pump, measured in horsepower (hp).
- Efficiency is the ratio between the input power and output power. It accounts for energy losses in the pump (friction and slip) to describe how much of the input power does useful work.

Types of pumps

Centrifugal pumps are dynamic pumps which move fluids through a system using one or more impellers. Centrifugal pumps generate flow by using one of three actions: radial flow, mixed flow, or axial flow.

- **Axial** flow pumps are characterized by high flow and low pressure. They lift liquid in a direction parallel to the impeller shaft, operating essentially the same as a boat propeller. Pressure is developed wholly by the propelling action of the impeller vanes.

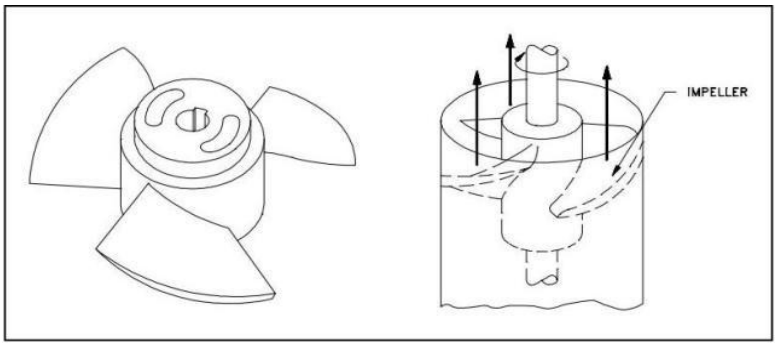

Fig. 6: Axial flow impeller

DOI: 10.21276/IJIPSR.2017.05.05.193

- **Radial** flow pumps are characterized by high pressure and low flow. They accelerate liquid through the center of the impeller and out along the impeller blades at right angles (radially) to the pump shaft. Pressure is developed wholly by centrifugal force.

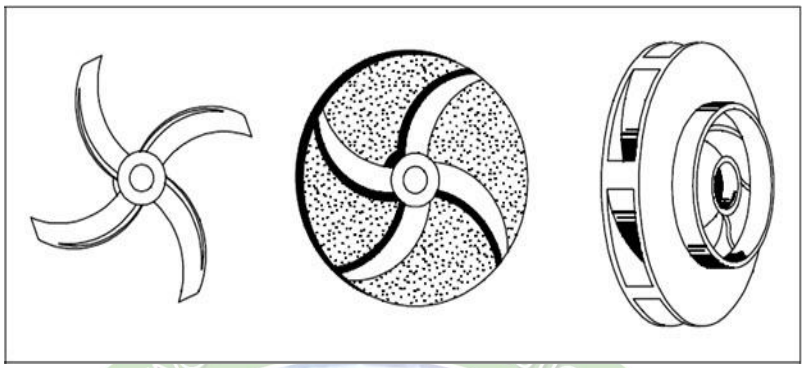

Fig. 7: Radial flow impeller

- **Mixed** flow pumps incorporate characteristics from both axial and radial flow pumps, with typically medium flow and medium pressure. They push liquid out away from the pump shaft at an angle greater than 90°. Pressure is developed partly by centrifugal force and partly by the lifting action of the impeller.

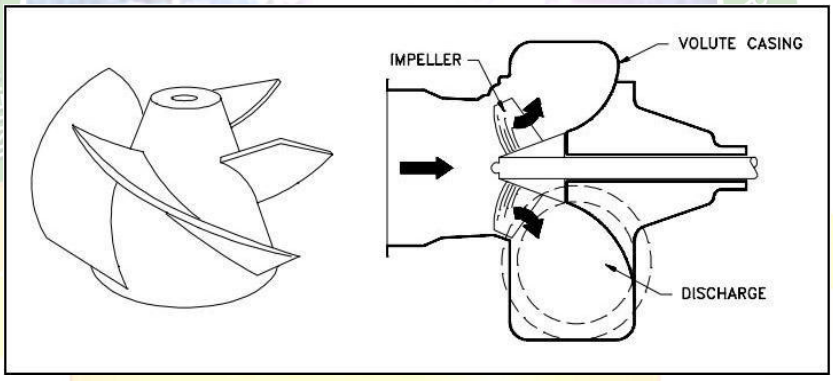

Fig. 8: Mixed flow impeller

Materials

Pumps and their various components are made up of a number of different materials. Media type, system requirements, and the surrounding environment all are important factors in material selection. Some of the most common materials used in pumps are described below:

- Cast iron provides high tensile strength, durability, and abrasion resistance corresponding to high pressure ratings.
- Plastics are inexpensive and provide extensive resistance to corrosion and chemical attack.
- Steel and stainless steel alloys provide protection against chemical and rust corrosion and have higher tensile strengths than plastics, corresponding to higher pressure ratings.

DOI: 10.21276/IJIPSR.2017.05.05.193

- Other materials used in pump construction include:
- Aluminum, Brass, Bronze, Ceramics and Nickel-alloy.

Pump Operation

Centrifugal pumps operate using kinetic energy to move fluid, utilizing an impeller and a circular pump casing. A vacuum is created in the pump which draws fluid to the impeller by suction.The impeller produces liquid velocity and the casing forces the liquid to discharge from the pump, converting velocity to pressure. This is accomplished by offsetting the impeller in the casing and by maintaining a close clearance between the impeller and the casing at the cutwater. By forcing fluid through without cupping it, centrifugal pumps can achieve very high flow rates.

SAMPLE INJECTOR

A centrifugal chromatographic system comprises a controller arranged automatically to trigger the introduction of the sample fluid into the chromatographic enclosure. A centrifugal chromatographic system comprises a chromatographic enclosure, the rotor is configured to rotate the chromatographic enclosure wherein sample introduction mechanism is in fluid communication with the chromatographic enclosure, said sample introduction mechanism being arranged to introduce a sample fluid to the chromatographic enclosure while the rotor is rotating, wherein the sample introduction mechanism is configured to receive a sample introduction signal and to trigger the introduction of the sample fluid in response to receiving the sample introduction signal [26-29].

Generally, automated loop injector is used in HPCPC for sample introduction.

Fig. 9: Sample injector

DOI: 10.21276/IJIPSR.2017.05.05.193

ROTORS

In HPCPC, Rotor is nothing but the column for the chromatography which facilitates the separation of the sample into components. Rotor is a series of channels linked in cascade by ducts and aligned in cartridges or disks in a circle around a rotor; setting the rotor in motion submits this assembly to a constant centrifugal field. Rotor is generally made up of Stainless Steel. Chromatographic system comprises a rotor configured to rotate about an axis. A chromatographic enclosure carried on the rotor being arranged to contain an associated chromatographic stationary phase and to facilitate transmission of a fluid through the chromatographic stationary phase contained within the chromatographic enclosure.

Fig. 10: Rotors

The fluid is driven through the chromatographic stationary phase via centrifugal force generated from the rotation of the rotor and a flow cell in fluid communication with the chromatographic enclosure, the flow cell including a flow window, wherein an inner cross sectional area of a flow path through the chromatographic enclosure and the flow cell is substantially constant starting at a location in the chromatographic stationary phase of the chromatographic enclosure and progressing past the flow window. Each chromatographic column enclosure having an associated flow cell, wherein the flow cells associated with each of the chromatographic columns are positioned at substantially the same radial distance from the rotor's axis of rotation so that the light detection mechanism is able to detect characteristics of the fluids passing through each of the flow cells once per revolution of the rotor. Via centrifugal forces, a mobile phase fluid including a sample can be driven through a stationary phase within the chromatographic enclosure to perform a chromatographic separation process on components of the sample [30].

DOI: 10.21276/IJIPSR.2017.05.05.193

Rotary Joint

Which connect the two rotor rods which are directly connected to discrete channel.

Partition Channel

It is cylindrical or rectangular cell. Which made up of stainless steel and metallic joints contain holes to passage of stationary phase.

Centrifuge

Which used produce the centrifugal force by using rotor. And it is retain the stationary phase in column or discreate partition cell by the two forces such as

- Hydro dynamic force
- Hydrostatic force

FRACTION COLLECTORS

Fraction Collectors are automatically arranged in the chromatographic system which efficiently collects the eluted components of the sample into the fraction collector. It allows the collection of fractions in large volume containers which dispenses sample fractions (via a tube connected to the dropper assembly) into multiple containers over a wide horizontal plane. Because of the distance from the UV detector and other instruments to the fraction collector dispense nozzle, there is a delay from the point of graphing until the sample is collected in the tube. By inputting the lag time, the collected sample can be set to accurately conform to the recorder.

DETECTOR

The detector monitors the samples that gets eluted from the column in High Performance Centrifugal Partition Chromatography, the detector used is

- UV-Visible diode array detector(DAD) detector
- Mass detector

Diode Array Detector

Photo Diode arrays (semiconductor devices) are used in the detection unit. A DAD detects the absorption in UV to VIS region. While a UV-VIS detector has only one sample-side light-receiving section, a DAD has multiple photodiode arrays to obtain information over a wide range of wavelengths at one time, which is a merit of the DAD. The idea is that spectra are measured at intervals of 1 second or less during separation b with continuous eluate delivery. If the measurement is performed at a fixed wavelength, components are identified from only their retention time; thus, a minor deviation in retention time can make identification of components difficult. In such a case, the DAD can be used to identify components by a comparison of the

DOI: 10.21276/IJIPSR.2017.05.05.193

spectrum. In DADs light from the lamps is shone directly onto the flow cell, light that passes through the flow cell is dispersed by the diffraction grating, and the amount of the dispersed light is estimated for each wavelength in the photodiode arrays.

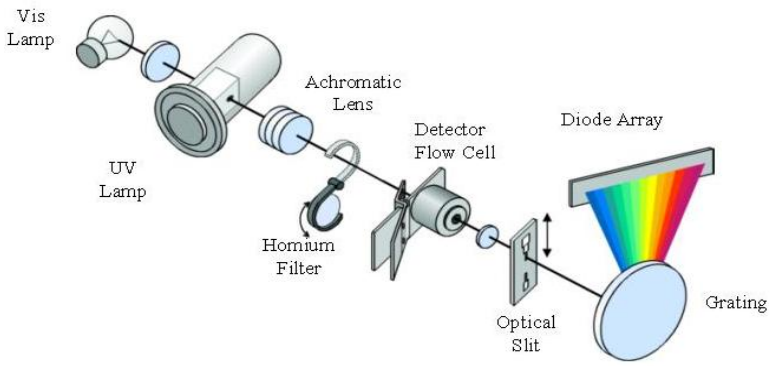

Fig. 11: UV-Visible diode array detector (DAD) detector

Mass Detector

The combination of Liquid Chromatography and Mass Spectroscopy helps in chemical separation of an analyte and the conformation of its molecular identity. The data about the fragmentation pattern of the analyte molecule obtained from MS analysis helps in conforming the identities of compounds [31].

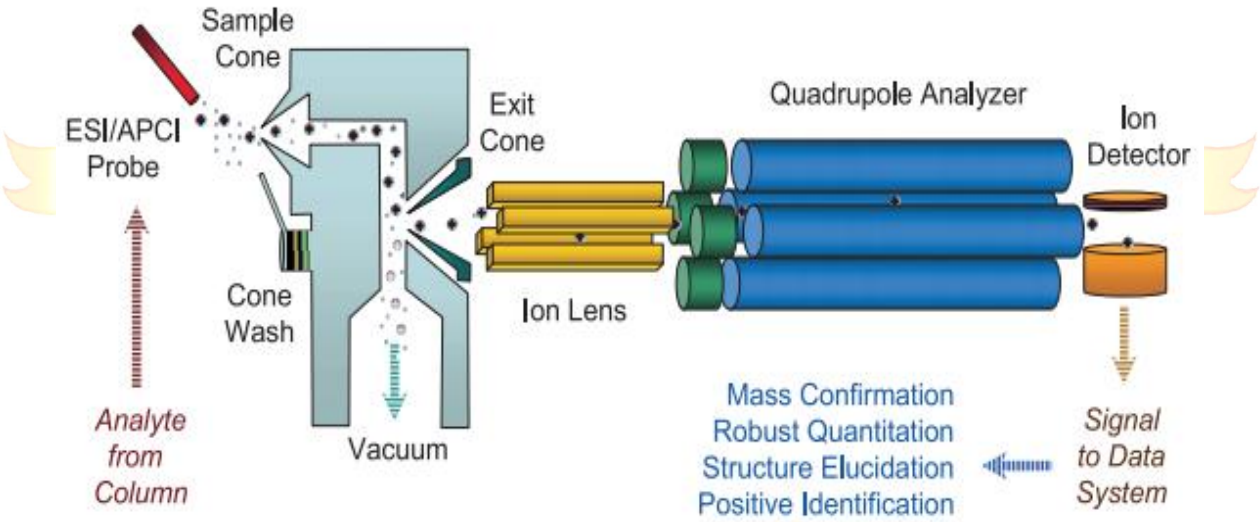

Fig. 12: Mass Detector

ADVANTAGES

Cost Effective

- No solid packing material that is costly to buy, replace or reprocess
- Low solvent consumption (10 to 25% less)

DOI: 10.21276/IJIPSR.2017.05.05.193

- One single rotor adapted to all types of separations

A Soft Technique

- No irreversible adsorption or sample loss -> 100% recovery
- No denaturation of fragile molecules due to interaction with silica -> adapted to fragile molecules

Efficient

- High purification levels up to 99.9%

Faster

- 3 to 5 times faster than other current techniques

Flexible

- Adapted to all types of products, natural or synthetic
- Well-adapted to a very wide range of polarities
- Ideal for easy Scale-up
- One single separation/purification technique adapted to samples ranging from mg to several kg

APPLICATIONS OF HPCPC

- Separation of Scandium, yttrium and lanthanum in HPCPC with S-Octyl phenyl oxy acetic acid
- Isolation of liquiritigenin-4'-Apiosyl glucoside by HPCPC
- Xanthones separation from mangosteen pericarp by using HPCPC
- Bio Polymers
- Fermentation Products
- Foods &Additives
- Fine Chemicals
- Genetically Engineered Substances
- Natural Organic Compounds
- Physiological Activated Substances
- Pharmaceuticals
- Petrochemicals
- Rare Metal &Earth

DOI: 10.21276/IJIPSR.2017.05.05.193

CONCLUSION

It can be concluded from the entire review that HPCPC is a versatile, reproducible chromatographic technique for the estimation of drug products. It has wide applications in different fields in term of quantitative and qualitative estimation of active molecules. HPCPC is the modern and most convenient approaches for high-throughput, efficient, economic and ultra-fast analysis. Also, HPCPC is the best method of analysis because there is no loss of sample; the theoretical plates required are less compared to other liquid chromatographic techniques. It can be applicable for any ph range which gives better resolution and separation.

REFERENCE

1. Yoichiro Ito, Walter D.Conway. Highspeed countercurrent chromatography. Vol.182. J.Wiley: New York; 1996.
2. Liu Yang, Friesen J.Brent, McApline James B, Pauli Guido F. Solvent System Selection Strategies in Countercurrent Separation. Planta Medica. 2015; 81:1582–1591.
3. Mekaoui Nazim, Faure Karine, Berthod Alain. Advances in Countercurrent Chromatography for Protein Separations. Bioanalysis. 2012; 4:833–844.
4. Kendall D, Booth A.J, Levy M.S, Lye G.J. Separation of supercoiled and opencircular plasmid DNA by liquidliquid countercurrentchromatography. Biotechnology Letters. 2011; 23:613.
5. Satyajit D.Sarker, Lutfun Nahar (eds.). Natural Products Isolation. Vol.864. Humana Press: Totowa, NJ. 2012. 221–254.
6. Ian A.Sutherland. Recent progress on the industrial scale pof countercurrent chromatography. Journal of Chromatography. A. 2007; 1151: 6–13.
7. Sumner Neil. Developing counter current chromatography to meet the needs of pharmaceutical discovery. Journal of Chromatography. A. 2011; 1218(36): 6107–6113.
8. Kurumaya Katsuyuki, Sakamoto Tetsuto, Okada Yoshihito, Kajiwara Masahiro. Application of droplet countercurrent chromatography to the isolation of vitamin B12. Journal of Chromatography. A. 1988; 435: 235–240.
9. Friesen J.Brent, McAlpine James B, Chen ShaoNong, Pauli Guido F. Countercurrent Separation of Natural Products: An Update. Journal of Natural Products. 2015; 78(7): 1765–1796.

DOI: 10.21276/IJIPSR.2017.05.05.193

10. Friesen J.B, Pauli GF.J. Phytoconstituents from Vitex agnus-castus fruits. Journal of Agricultural and Food Chemistry. 2011; 56: 19.

11. Ikehata J.I, Shinomiya K, Kobayashi K, Ohshima H, Kitanaka S, Ito Y. Effect of Coriolis force on counter-current chromatographic separation by centrifugal partition chromatography. Journal of Chromatography A. 2011; 1025: 169-175.

12. Shin Omiya.K, Kabasawa Y, Yanagidaira K, Sasaki H, Muto M, Okada T, Ito Y. Protein separation by nonsynchronous coil planet centrifuge with aqueous-aqueous polymer phase systems. Journal of Chromatography A. 2010; 1005: 103-112.

13. Zhang T.Y. Separation and purification of natural products (medicine herbs) by HSCCC. Counter current Chromatography. Berthod, 2009; 201-260.

14. Jin Y.R, Zhang L.X, Zhang L.Z, Han S.J. Separation of inorganic compounds: theory, methodology and applications. Counter current Chromatography. Berthod, 2008; 261-300.

15. Marchal L, Foucault A, Patissier G, Rosant J.M, Legrand J. Influence of flow patterns on chromatographic efficiency in centrifugal partition chromatography. Journal of Chromatography A. 2006; 869: 339-352.

16. Menet J.M, Thiebault D. Counter current Chromatography. Chromatographic Science++ Series: Marcel Dekker, New York. 2005; 85.

17. Foucault A.P, Chevolot L. Counter-current chromatography: instrumentation, solvent selection and some recent applications to natural product purification. Journal of Chromatography A. 2003; 808: 3-22.

18. Oka H. High-Speed Countercurrent Chromatography. JohnWiley: Chichester, 2002; 73.

19. Conway W.D, Petroski R.J. Modern Counter current Chromatography. American Chemical Society: Washington, DC, 2001; 593.

20. Conway W.D. Counter current Chromatography, Apparatus, Theory and Applications. VCH: Weinheim, 2000.

21. Mandava N.B, Ito Y. Counter current Chromatography. Marcel Dekker: New York. 2000; 44.

22. Oka H. High-Speed Countercurrent Chromatography. John Wiley: Chichester. 1996; 73.

23. Oka H, Ito. Y. In Encyclopedia of Chromatography, J. Cazes (Ed.), p. 208, Marcel Dekker, New York (2001).

24. Oka H, Ito Y. Abstracts of Papers of the 4th International Conference on Countercurrent Chromatography. 2006; 41.

DOI: 10.21276/IJIPSR.2017.05.05.193

25. Kong Z, Rinehart K.L, Milberg R.M, Conway W.D. Journal of Liquid Chromatography & Related Technologies. 1998; 21: 65.

26. Kidwell H, Jones J.J, Games D.E. Rapid Communication in Mass Spectrometer. 2001; 15: 181.

27. Chen L.J, Song Y, Games D.E, Sutherland I.A. Journal of Liquid Chromatography & Related Technologies. 2003; 28: 1993.

28. Winterhalter P. Abstracts of Papers of the 4th International Conference on Countercurrent Chromatography. 2006; 40.

29. Yue Yu, Qing-Wen Zhang, Shao-Ping Li. Preparative purification of coniferyl ferulate from Angelica sinensis oil by high performance centrifugal partition chromatography. Journal of Medicinal Plants Research. January, 2011; 5(1): 104-108.

30. Dr. Foucault Alain.P. Centrifugal Partition Chromatography. Chromatographic science series. 1995; 431.

31. Hostettmann K, Marston A, Hostettmann M. Preparative Chromatography Techniques: Applications in Natural Product Isolation. Springer: Germany; 1998; 260.