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REVIEW ARTICLE

Overview on High Performance Liquid Chromatography (HPLC)

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ABSTRACT

A group of techniques known as chromatographic are used to isolate the elements of a mixture. Two phases make up this strategy: a fixed phase and a mobility phase. Among the several chromatographic techniques are Ion exchange chromatography, Paper chromatography, Thin layer chromatography, Gas chromatography and High performance liquid chromatography. HPLC is a most adaptable, trustworthy, and secure analytical technique for evaluating the quality of medicinal components. This important qualitative and quantitative methods is frequently utilized a estimate biological and medical products. This purpose of this article is to discuss several features of HPLC, including its principle, types, characteristics, manner of separation, instrumentation, key parameters and diverse applications.

Keywords:*HPLC,Normal Phase , Reverse Phase , Method Development , chromatographic conditions*

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INTRODUCTION

When separating, identifying, and quantifying the active compounds used for high-performance liquid chromatography (HPLC)[1].The preferred technique figuring out novel chemical entities' peak purity, monitoring reaction changes during scale-up or synthesis processes, evaluating new formulations, and performing quality control and quality assurance on final pharmaceutical products[2,3]. Both quantitative and qualitative drug analysis typically makes use of the most exact analytical methods, such as HPLC. The preferred method is HPLC, which may be used to evaluate new product formulations, analyse reaction changes during scale-up or manufacturing processes, and examine the peak purity of novel chemical entities[4,5].

TYPES OF HPLC

Normal Phase HPLC:

In the HPLC sample stage, the stationary phase is polar and the mobile phase is non-polar.Thus, the polar analysis continues to be in the stationary phase. The adsorption capacity and elution time both increase as the polarity of the solute molecules increases. In this chromatography, silica that has been chemically treated serves as the stationary phase[6]. When a combination is run through a column, polar silica will retain polar chemicals longer than non-polar ones. Therefore, the non-polar ones will move through the column more rapidly. [7].

Reverse Phase HPLC:

Primitive phase is non-polar, whereas the mobile phase in hplc is either polar or slightly polar .The hydrophobic interaction hypothesis serves as the cornerstone of rp-hplc [8]. In a mixture of components, the non-polar stationary phase will retain correspondingly fewer polar analyses for a longer amount of time than comparably more polar analyses. Since it is more polar, it will elute first. [9]

Size Exclusive HPLC:

In HPLC, sometimes referred to as gel-permeation chromatography, the primary criteria for particle separation are particle size. The material with precisely specified pore diameters is simply screened or filtered from the sample and added to the column. Larger molecules quickly pass through the column, but smaller molecules quickly elute after penetrating the packing particles' pores [10]

Ion Exchange HPLC:

Retaining by ion-exchange HPLC is based on the attraction between solute ions and charged sites attached to the solid surface. Ionic samples are almost always used in this procedure. The charge of the sample will affect how vigorously and how slowly it will elute from the ionized surface[10,11].

INSTRUEMENTATION:

A pump, injector, column, detector, integrator, and display system are components of the HPLC instrumentation. The separation takes place in the column. These are its components.

Solvent Reservior:

The portable phase's components are kept in a glass container. In HPLC, the organic phase, sometimes referred to as the fluid, is made up of non polar and polar fluids. Many different non-polar and polar solvents must be utilised, depending on the sample's makeup.

Pump :

After being extracted from the solvent reservoir and pushed into the column, the aqueous layer is then transported to the detector by the pump. The input power of the pump is 42000 KPa. The characteristics of the dissolution medium, mass flow, and column diameters all have an impact on this operating pressure**.**

Sample Injector :

The syringe could be a computerised infusion machine. An injector for HPLC should be capable of injecting fluid samples at high pressures and with excellent reproducibility in quantities ranging from 0.1 to 100 mL. (4000 psi maximum)

Columns :

Columns typically have an internal diameter of 2 to 5 mm and are made of cleaned stainless steel. Their typical length is 50 to 300mm. Typically, they have a stationary phase made up of molecules with sizes ranging from 3 to 10m. Optimal circumstances call for the column temperature and reversed - phase temperature to remain constant during the experiment.

Detector :

The chemical blog's sensors at the bottom makes it possible for theseparation of the analyses as they elute from the column. Detector types that are often employed include electrochemical, fluorescence, mass spectrometric, and UV spectroscopy.

Integrator :

Plot monitors that can examine, maintain, and recycle chemical data on a variety of high-quality devices can be used to capture indications out from sensor. It's not particularly difficult to comprehend the chromatograph that the PC uses to coordinate the indicator's reaction to every component[12–19].

METHOD DEVELOPMENT OF HPLC :

The preceding is a step in the creation of an HPLC method:

1. Being aware of the physio-chemical characteristic of drug molecule.
2. Picking chromatographic configuration
3. Creating the analytical strategy
4. Creating the specimens
5. Approach improvement.
6. Validating the method.

1)Being aware of the physio -chemical characteristic of drug molecule:

The creation of a technique depends on the physicochemical characteristics of a drug. Establishing a procedure requires research on the surface morphology of the active ingredient, such as its mobility, neutrality, basicity, and substrate concentration . Magnetic force is a material fact of a compound. It helps the observer choose the solvent and chemical composition of the mobile phase. The chemicals' potential can be utilised to account for how soluble they are depending on how soluble the analysis is, one can choose a excipients. The analytical must not interact in any way with its component parts and be soluble

in diluents. pH and pKa are significant factors when developing HPLC methods. The pH scale is based on the proportional base-10 negative of the ion concentration of hydrogen. [20,21]

pH = - log10[H_3O+].

2) Picking chromatographic configuration :

Selection of column :

In building a method, choosing a solid surface is a common crucial process from particularly purified, less acidic silica and created expressly for the. It is typically acceptable for all samples and highly encouraged toUse of a C8 or C18 matrix made from very pure, less acidic silica and developed specifically for the detachment of basic chemicals is normally appropriate for all samples and strongly encouraged [22].The most importantsare the characteristics of the bonded stationary phase, the silica substrate properties, and the column diameters. The majority of modern HPLC columns choose silica-based packing due to a variety of physical features [23].

Buffer selection:

The preferred pH determines the buffer to use. Reversed phase on silica typically operates in a pH range of 2 - 8.Seeing as barriers regulate acidity most near a basicity, the barrier must have a basicity near to the desired pH. As a general guideline, use a buffer with a pKa value lower than two components of the designing movable stage [24].

Buffer concentration:

Tiny compounds 10-50 mM saline values are adequate. Generally speaking, a saline shouldn't be employed with more organic material than 50%. This is dependent on the particular buffer and its strength. For reversed-phase HPLC, acetic acid and potassium based salt is a common often employed ion channels systems. [25,26].

Isocratic separation:

Variable eluent composition is part of the gradient mode of separation. This method dramatically boosts an software's moving element, mostly due to an increase in perceived efficiency. Maximal breadth is directly correlated with the rate of eluent composition change [27].

Internal Diameter:

An essential factor that affects the gradient elution's detection sensitivity and separation selectivity is an HPLC column's internal diameter (ID). Additionally, it establishes how much analyte can be placed onto a column. [28].

Particle size:

In contemporary HPLC, the outside of small, spherical silica particles frequently receives the stationary phase treatment. The pressure needed to generate the optimum linear velocity is anegatively related with the granular diameter's numerator, hence smaller particles frequently offer greater surface area and better separations. With column diameters ranging from 5 cm to >30 cm., bigger particles are employed in non-HPLC applications such solid-phase extraction and preparative HPLC.[29,30].

Pore size:

The size of the column's pores dictates how well molecules used for analysis can interact with a particle's inner surface [31].

Selection of Mobile phase:

In RP-HPLC separation, the mobile phase composition—also known as solvent strength—is crucial.The low UV cut-off wavelengths for RP-HPLC are 190, 205, and 212 nm for tetrahydrofuran (THF), acetonitrile (CAN), and methanol (MeOH), respectively. These solvents combine well with water[32**].**

Selection of Detectors :

The device is a crucial element of High - performance liquid chromatography. Choice of this analyzeris influenced by a number of variables, including the investigation's chemical makeup, inherent distraction, required detection range, accessibility, and detector price. The dual wavelength absorbance detector of the HPLC is UV-visible. The detector provides superior optical detection for solutions when used with operational HPLC, titrations HPLC, or Liquid chromatographic systems from Waters. Thanks to software architecture and improvements to the photonics, it provides excellent chromatographic and spectral sensitivity. Due to its absorptivity chromatographic and optical reactivity, reliability, and predictability, this detector is the best option for analysing substances with little to no Maximum absorbance. The inter fluorescence detector's precision and discrimination allow it to analyse target molecules even at low concentrations [33,34]

3.Creating the analytical strategy :

The selection of a variety of chromatographic parameters, such as the organic phase, barrier, mass flow, and mobile phase's pH, constitutes the initial step in creating an statistical approach for RP-HPLC. The system appropriateness parameters are then taken into consideration after all of these parameters have

been chosen through testing. The detection wavelength is frequently near its isobestic point when two components are estimated at the same time. To determine the concentration levels that a linear pattern can be observed, the medication's uniformity is then investigated. To assess the viability of the suggested technique for quantitative determination, evaluation of the experiment combination n is also conducted[35–40].

4.Creating the specimens :

Intended to create a consistent, homogenous solution suitable for insertion into the column in HPLC analysis.The goal of specimen preparation is to create an allocation of the test that is generally unaltered, won't damage the matrix, and works with the intended HPLC method. To do this, the specimen agent must disperse in the polar solvent not impairing collection stability. Sample preparation continues through sample processing into the Hplc system after sample collection. [41]

5.Approach improvement :

Identifies weakness of the approach as uses experimental design to improve the process. Recognize how the approach performs under various circumstances, instrument configurations, and sample types. [42].

6.Validating the method:

The practise of evaluating something and offering unbiased evidence that it fits the requirements for a specific intended usage is known as validation. A procedure for assessing highly accurate results and proving that it satisfies a specific requirement[43].

APPLICATIONS

Pharmaceutical applications:

- Tablet dissolution research of real world dosage forms.
- Identification of the dosage forms active components[44].

Environmental applications:

- Diphenhydramine can be detected in sediment samples.
- Phenolic chemicals can be found in drinking water [45]

CONCLUSION

HPLC is the analytical method that is utilised the most. It has various benefits. Using HPLC, one can produce substances that are incredibly pure. It can be applied to industrial and clinical science as well. HPLC can increase accuracy, specificity, and all three. The only problem with HPLC is the price. A statistic is measured as planned and the performance criteria of the measurement are identified by continuous collaboration across the phases of method development and validation. The separation selectivity is significantly influenced by the choice of column, buffer, detector, wavelength, and other factors (organic and pH) composition. Good selectivity, responsivity, economy, shorter time commitment, and minimal detection limitations were all benefits of HPLC technology.

REFERENCES

1. Martin, M.; Guiochon, G. (2005). Effects of high pressure in liquid chromatography. J Chromatography A ;1090(1):16-38.
2. Dwivedia, S.K.; Agarwala, D.D. (2015). A Review: HPLC Method Development and Validation. Int J Analytical Bioanalytical Chem;5(4):76-81.
3. Ahuja, S.; Rasmussen, H. Development for Pharmaceuticals, Separation Science and Technology, Elsevier: New York, 2007;8.
4. Rao, B.V.; Sowjanya, G.N.; Ajitha, A.; Rao, V.U. (2015). A review on stability indicating HPLC method development. World Journal of Pharmacy and Pharmaceutical Sciences. 4(8):405-23.
5. Ahuja, S.; W dong, M. (2005). Handbook of Pharmaceutical analysis by HLPC, Elsevier: New York;6
6. Snyder LR, Kirkland JJ, Glach JL. (1997). Practical HPLC Method Development, John Wiley and Sons, New York, 158-192.
7. HPLC – Chemiguide. May 2, 2007chemguide.co.in.
8. Rao G, Goyal A. (2016). An Overview on Analytical Method Development and Validation by Using HPLC. The Pharmaceutical and Chemical Journal, 3(2): 280-289.
9. McpolinOona.an Introduction to HPLC for Pharmaceutical Analysis. Mourne Training Service. 11-12.
10. Types of HPLC.http://hplc.chem.shu.edu/NEW/HPLC_Book/Introduction/int_t yps.html. (Accessed Feb 12, 2017.
11. Hearn, M.T. (1980). Ion-pair chromatography on normal and reversedphase systems. Advances in Chromatography.18:59-100.
12. Reinhardt TA, et al. (1983). A Microassay for 1,25-Dihydroxyvitamin D not requiring high performance liquid chromatography: application to clinical studies. JCEM. 58.

13. Parker JMR, et al. (1986). New hydrophilicity scale derived from high-performance liquid chromatography peptide retention data: correlation of predicted surface residues with antigenicity and x-ray-derived accessible sites. Biochemistry;25:5425-5432.
14. Shephard GS, et al. (2006). Quantitative determination of fumonisins b1and b2 by high-performance liquid chromatography with fluorescence detection. J Liquid Chromatogr.:13.
15. Hamscher G, et al. (2002). Determination of persistent tetracycline residues in soil fertilized with liquid manure by highperformance liquid chromatography with electrospray ionization tandem mass spectrometry. Anal Chem. ;74:1509-1518.
16. Mesbah M, et al. (1989). Precise measurement of the g+c content of deoxyribonucleic acid by high-performance liquid chromatography. Int J Syst Evol Microbiol.39:159-167.
17. Tamaoka J and Komagata K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. FEMS Microb let. 234-238
18. Svec F and Frechet MJJ. (1992). Continuous rods of macroporous polymer as high-performance liquid chromatography separation media. Anal Chem. 64:820-822.
19. Shintani H. (2013). Validation Study in membrane chromatography adsorber and phenyl hydrophobic membrane chromatography adsorber for virus clearance and removal of many other components. Pharm Anal Acta.S2:005.
20. http://www.scribd.com/doc/9508765/physical-properties-of -drug.
21. Buffers and pH Buffers: available from: www.xtremepapers.com.
22. Charde MS, Welankiwar AS and Kumar J. (2014). Method development by liquid chromatography with validation. International Journal of Pharmaceutical Chemistry. 4(2):57-61.
23. Ranjit Singh. (2013). HPLC method development and validation. J Pharm Educ Res; 4(1): 26-33.
24. Sabir AM, Molony M,Parminder SB. (2013). HPLC Method Development and validation: A Review. International research Journal of pharmacy. 4(4):39-46.
25. Noman A, Bukhaiti ALWedad Q, Alfarga A,AbedSherif M, Mahdi AA. And Waleed AA. (2016). HPLC technique used in food analysis-Review. International Journal of Agriculture Innovations and Research. 5(2):181-188.
26. Azim, MD. Sabir; Mitra, Moloy; Bhasin, Parminder S. (2013). HPLC Method Development and Validation: A Review. International research Journal of Pharmacy.4(4);39-43.
27. Schellinger, A.P.; Carr, P.W. (2006). Isocratic and gradient elution chromatography: a comparison in terms of speed, retention reproducibility and quantitation. J Chromatography A;1109(2):253-66.
28. Snyder LR, Kirkland JJ, Dolan JW. (2011). Introduction to modern liquid chromatography. John Wiley & Sons. New York.
29. Xiang Y, Liu Y, Lee ML. (2006). Ultrahigh pressure liquid chromatography using elevated temperature. Journal of Chromatography. 1104(1): 198-202.
30. Horvath CG, Preiss BA, Lipsky SR. (1967). Fast liquid chromatography. Investigation of operating parameters and the separation of nucleotides on pellicular ion exchangers. Analytical chemistry, 39(12): 1422-1428.
31. Malviya R, Bansal V, Palo P, and Sharma PK. (2010). High Performance Liquid Chromatography: A Short Review. Journal of Global Pharma Technology. 2(5):22-23.
32. Pratap B. et al. (2013). Importance of RP-HPLC in Analytical method development: A review. International journal of novel trends in pharmaceutical sciences; 3(1): 15-23.
33. Lindholm J. (2004). Development and Validation of HPLC method for Analytical and Preparative Purpose. Acta Universities Upsaliensis Uppsala. 13-14.
34. Snyder LR, Kirkland JJ, Glach JL. (1997). Practical HPLC Method Development, 2nd edition. New York. John Wiley &Sons. 233-291.
35. Sethi PD. (2001). Introduction – High Performance Liquid Chromatography, 1st edn, CBS Publishers, New Delhi. 1-28.
36. Belal F et.al. (2013). Stability-indicating HPLC Method for the Determination of Atenolol in Pharmaceutical Preparations. J Chromat Separation Techniq. 4(1): 1-7.
37. Chetta N. et.al. (2013). Development and validation of a stability indicating high performance liquid chromatographic (HPLC) method for Atenolol and hydrochlorothiazide in bulk drug and tablet formulation. Int J Chem tech res. 1(3): 654-662.
38. Kumar GS. et.al. (2013). Development and validation of RP-HPLC method for simultaneous estimation of Atenolol and Chlorthalidone in Bulk and dosage form. Int Res J Pharma; 3(10): 215-19.
39. FDA Guidance for Industry (2000)-Analytical Procedures and Method Validation, Chemistry, Manufacturing, and Controls Documentation, Center for Drug Evaluation and Research (CDER) and Center for Biologics Evaluation and Research (CBER).
40. Julia T, Mena AJ, Aucoin MG, Kamen AA. (2011). Development and validation of a HPLC methodfor the quantification of baculovirus particles. J Chromatogr B. 879: 61-68.
41. Santhosh G, Nagasowjanya G, Ajitha A, Uma Maheswara Rao Y. (2014). HPLC method development and validation: an overview. International Journal of Pharmaceutical Research & Analysis. 4(2): 274-280.
42. Kayode J, Adebayo. (2015). Effective HPLC method development. Journal of Health, Medicine and Nursing. 12: 123-133.
43. Gad S. (2006). Pharmaceutical manufacturing handbook of regulations and quality. John wiley and sons.

44. Stubbs C., Kanfer, I. (1990).Stability-indicating High-performance Liquidchromato- graphic Assay of Erythromycin Estolate in Pharmaceutical Dosage Forms. Int. J. Pharm. 3(2): 113-119.
45. Kwok Y. C., Hsieh D. P. H., Wong P. K. (2005). Toxicity identification evaluation (TIE) of pore water of contaminated marine sediments collected from Hong Kong waters. Marine Pollution Bulletin. 51(8-12): 1085-1091.