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DEVELOPMENT AND THE VALIDATION OF HPLC METHOD

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ABSTRACT:

The High performances liquid chromatography (HPLC) is the type of column chromatography that is an essential analytical tool for drug product assessment. HPLC is the separating technique characterized by detection, separation, analysis and quantifies the drug. In the development and manufacturing of new pharmaceutical product the analytical method play an important role. The word validation means simply Validity or action of providing effectiveness. Validation is an analytical method that provides information about various parameters such as accuracy, precision, linearity, Limit if Detection, Limit of Quantification, specificity, robustness, range. As per ICH guidelines, the validation should be done. This article was prepared with the aim to review the method development and validation of HPLC.

Keywords: Chromatography, HPLC, Development method, Validation, impurity, Detector, Instrumentation.

INTRODUCTION:

Analytical chemistry is the branch of chemistry to study separation, identification and quantification of the chemical composition of artificial and natural product/ material. In the research laboratory and process industry, Analytical chemistry plays an important role. The main two techniques of analytical chemistry are qualitative and quantitative techniques. Analytical data is not only used in only a pharmaceutical product is also used for other things such as biology clinical diagnosis, art etc. The analytical method developed by using instruments such as HPLC, TLC, HPTLC, Spectrophotometer GC, have wide applications in assessing the quantity and quality of the finished product or raw mater.

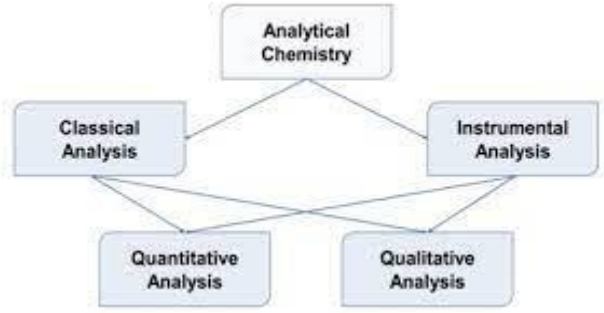

Figure1: Classification of Analytical chemistry.

Chromatography

The term Chromatography (Greek word kromatos meaning Colour and Graphos meaning-writing) means colour writing. Tswett (1906) define the chromatography method in which the component of a mixture is separated on an adsorbent column. In all types of chromatography separation of substances or components is based on mainly adsorption and partition. Adsorption means the binding of a substance to the surface of the solid phase while partition means the substance get

distributed into two liquid phases. Basically, the chromatography is based on two phases mainly the solid phase and liquid phase. Depending upon the two phases the chromatographic method is classified as:

I. Partition chromatography: In the partition chromatography involved the gas or liquid as a mobile phase and another liquid and solid as a stationary phase.

1. Thin layer chromatography.
2. Paper chromatography.
3. Gas – Liquid chromatography.
4. High performances liquid chromatography.
5. Partition column chromatography.

II. Adsorption chromatography: This involved the Gas as a mobile phase and adsorbent solid as a stationary phase. Category under these types includes:

1. Adsorption column chromatography
2. Thin layer chromatography
3. Gas – Solid chromatography.
4. Ion exchange:

III Molecular sieve:

1. **Gel chromatography.**

HPLC: HPLC stand for the High-Performance Liquid Chromatography or High-Pressure Liquid Chromatography. The compound present in the sample can be separated, identify, quantify by dissolving in the liquid (Chawla G Chaudhary KK, 2019). Adsorption is the main principle of the HPLC. HPLC is the chromatographic technique in which liquid is the mobile phase and solid is the stationary phase. With the high pressure delivered by a pump, the sample moves through the column with the mobile phase. The sample component travels according to their affinity towards the stationary phase. The component travelled faster when the component has a higher affinity towards the stationary phase, while the component shows less travelling, that time the component has a lower affinity towards the stationary phase. N-hexane, methylene, methylene chloride, chloroform, methyl-t-butyl-ether, Tetrahydrofuran (THF), Acetonitrile (MeCN or CAN) Methanol (MeOH), Isopropanol (IPA), Water (McPolin O, 2009) are the common solvent used for running HPLC. Application of HPLC involved separation, identification and purification. Other applications include forensic applications pharmaceutical industrial applications, environmental applications, clinical applications, food and flavour.

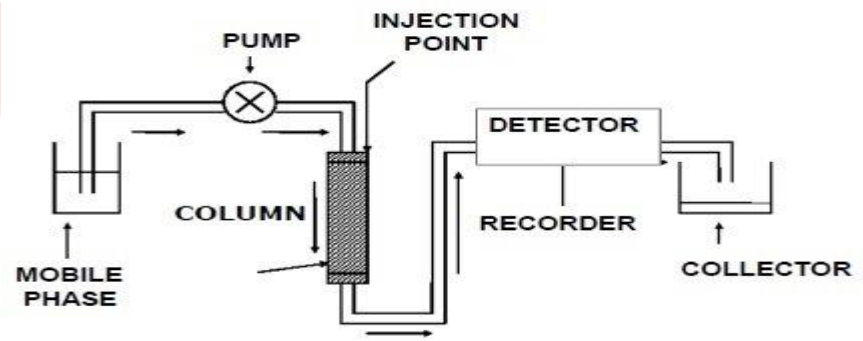

Figure2: HPLC system

Instrumentation of HPLC

Component of the HPLC system:

A. Solvent reservoir, degassing system and mixing system
B. Sample injector
C. High Pressure pump
D. Column
E. Detector
F. Data Recording System

1. Solvent reservoir, degassing system and mixing system: A modern HPLC apparatus involved one or more glass or stainless steel reservoirs, containing 500ml or more solvent. The solvent reservoir store the mobile phase. The reservoir is often equipped with a means of removing dissolved gases usually O2 and N2 that interfere by bubbles forming in the columns and detector system. This bubble causes the band to spread; in addition, interfere with the detector function.

Degasser includes: (A) The vacuum pump (b) Distillation system (c) Solvent heating and stirring equipment.

2. Sample Injector: The sample is introduced into the mobile phase by the sample injector. The sample valves come between the column and pump. Auto sampler is able to inject the sample into the mobile phase (continuous flowing) that carries the sample in to the HPLC column.

Sample injector type: (1) Syringe injector (2) Stop flow injector (3) Solvent flowing injector. These are the above three types of sample injector.

3. High Pressure Pump: The role of the pump is to force the liquid (mobile phase) into the column and give the specific flow rate. The normal flow rate is around 1-3ml/min. The high pressure used in the HPLC ranges from 6000 - 9000 psi (400 - 600 bar). Commonly used pump types in the HPLC are constant pressure pump, reciprocating piston pump, syringe pump.

4. Column: The actual separation of components takes place in a column. Columns are made up of glass or stainless steel, sizes ranging from 5 -20 cm long and 2 -4.6cm internal diameter (Chawla G and Chaudhary KK, 2019).

5. Detector: The detector is the brain of the HPLC system. Detector required to sense the presence, and amount of components in the column effluent. A detector that measures properties possessed by both solute and mobile phase is called a **bulk property detector**. If the solute possesses the property e.g. absorption of UV/visible light of electrochemical property, the detector are called a **solute property detector**.

6. Data recording system: A series of peaks and areas under the peak can be calculated automatically by the computer link to the display.

METHOD OF DEVELOPMENT OF HPLC:

A series of steps involved in the development of HPLC.

1. Understanding of physicochemical properties of drug molecules.
2. Selection of chromatographic condition
3. Development of approach of analysis
4. Sample preparation
5. Method optimization
6. Method validation.

1. **Understanding of physicochemical properties of drug molecules:**
   Physicochemical properties of drug molecules are important for method development. The physical properties of drug molecules like solubility, pKa, pH, the polarity of drug molecules. Polarity helps to analyse the selection of the solvent composition of the mobile phase. The solubility of molecules basically depends upon the polarity of the molecules. In the development of HPLC PH and pKa play an important role. The PH values define as the negative of the logarithm to base 10 of hydrogen ion concentration.

$$pH = -\log_{10} [H3O+].$$

2. Selection of chromatographic condition:

(1) Selection of column: The most important step in the development method of HPLC is the selection of columns. C8 or C18 columns are specially purified, less acidic silica specially designed for the separation of a basic compound.

(2)Buffer selection: pH range for the reversed-phase on silica-based packing is pH 2 to 8. It is important that buffer has a pKa close to the desired pH that buffers control pH at their specific pH. A rule for choosing a buffer with a pKa value < 2 to unite the mobile phase pH.

General consideration for buffer selection:

1. Phosphate is soluble in methanol/water than in acetonitrile/water or THF/water.
2. Some salt buffers are hygroscopic in nature and this may lead to changes in the chromatography like increased tailing of basic compounds and possibly selectivity differences.
3. Ammonium salts are more soluble in organic/water mobile phases.
4. Trifluoroacetic acid can degrade with time. It is volatile and absorbs at low UV wavelengths.
5. Microbial growth can quickly occur in buffered mobile phases that contain little or no organic modifier. The growth accumulates on column inlets and can damage chromatographic performance.
6. At pH greater than 7, phosphate buffer accelerates the dissolution of silica and severely shortens the lifetime of silica-based HPLC columns. If possible, organic buffers should be used at pH greater than 7.
7. Ammonium bicarbonate buffers are usually prone to pH changes and are usually stable for only 24 - 48 hrs. The pH of this mobile phase becomes more basic due to the release of carbon dioxide (CO2).
8. After preparation of buffer, they should be filtered through a 0.2-µm filter.
9. Mobile phases should be degassed.

(3) Buffer Concentration

Generally, a buffer concentration of 10-50 mm is for small molecules. No more than 50% organic should be used with a buffer. This will depend on the specific buffer as well as their concentration. The most common buffer systems for reversed-phase HPLC are phosphoric acid and its salts (sodium and potassium). Sulfonate buffers can replace with phosphonate buffers when analysing organophosphate compounds.

(4) Isocratic and Gradient Separations:

Isocratic separation means equilibrium conditions in the column and the actual velocity of compounds moving through the column are constant. The peak capacity is low and the longer the component is retained on the column the wider is the resultant peak. Gradient mode of separation includes increases in the separation power of a system due to an increase of the apparent efficiency (decrease of the peak width). The peak of width depends on the rate of the eluent composition variation. The calculated ratio is <0.25 isocratic is adequate. When the ratio is >0.25 gradient would be adequate.

(5) Internal Diameter

The internal diameter (ID) of an HPLC column is an important parameter that affects the detection sensitivity and separation selectivity in gradient elution.

(6) Particle size

Most traditional HPLC is performed with the stationary phase attached to the small spherical silica particles. These silica particles are available in many sizes with 5 µm beads most commonly used. The smaller particle size provides larger surface area and better separations but the pressure required for the optimum velocity increases by the inverse of the particle diameter squared.

(7) Selection of Mobile Phase:

The mobile phase directly affects resolution, selectivity and efficiency. The composition of the mobile phase (or solvent strength) plays an important role in RP-HPLC separation. Acetonitrile (ACN), methanol (MeOH) and tetrahydrofuran (THF) are commonly used solvents in RP-HPLC. These solvents are miscible with water. A mixture of acetonitrile and water is the best initial choice for the mobile phase selection.

(8) Selection of detectors:

Detector is an essential part of HPLC. Selection of detector depends on the chemical nature of analyses, limit of detection, availability and/or cost of detector. UV visible detector is a dual-wavelength absorbance detector for HPLC. This detector gives the high sensitivity required for routine UV-based applications to low-level impurity identification and quantitative analysis. The detector offers advanced optical detection for Waters analytical HPLC, preparative HPLC, or LC/MS system solutions. Its integrated software and optics innovations deliver high chromatographic and spectral sensitivity. Multi-wavelength Fluorescence Detector offers high sensitivity and selectivity fluorescence detection for quantitating low concentrations of compounds.

Detector	Type of compound can be detected
UV-Visible and photodiode array	Compounds with chromospheres, such as aromatic rings or multiple alternating double bonds
Conductivity detector	Charged compounds, such as inorganic ions and organic acid.
Electrochemical detector	For easily oxidized compounds like quinines or amines
Refractive Index detector & Evaporative light scattering detector	Compounds that do not show characteristics usable by the other detectors, eg.polymers, saccharides.

3. Developing the approach for analysis:

While developing the analytical method on Reverse phase-HPLC the first step is the selection of various chromatographic parameters like the selection of mobile phase, selection of column, selection of flow rate of mobile phase, selection of pH of the mobile phase. These all parameters are selected on the basis of trials and considering the suitability parameters. Retention time ≥ 5 min, number of theoretical plates ≥ 2000, tailing factor ≤ 2, resolution of two peaks ≥ 5, the area of the target peak analyzed within the standard %RSD chromatogram must not exceed 2.0%.

4. Sample preparation:

Sample preparation is an essential part of HPLC analysis, intended to provide a reproducible and homogenous solution that is suitable for injection onto the column. The purpose of sample pre-treatment is to form sample aliquots with relatively low interference, which will not damage the column, which are compatible with the specified HPLC method. That is, the sample solvent dissolves within the mobile phase without affecting sample retention or resolution.

5. Method optimization:

Identify the "weaknesses" of the method and optimize the method through experimental design. To understand the method performance with different conditions, different instrument setups and different samples.

6. Method Validation:

The concept of validation was pioneered by two FDC officials Ted Byers and Bud Loftus in the mid-1970s. Validation in general required meticulous preparation and careful planning of the various steps in the process.

The component of validation:

a) Accuracy
b) Precision
c) Range
d) Ruggedness
e) Robustness
f) Limit of detection and limit of quantification
g) Linearity

a) Accuracy: The accuracy of an analytical method is the extent to which the test results generated by the method and the true value agree. The true value of accuracy assessments can be obtained in several ways.

b) Precision: Precision refers to the variability between repeated measurements of the identical number. It is defined as **the degree of the reproducibility of a series of measurements of the same properties** i.e. a result is said to be precise if a number of parallel determinations of the same properties agree well.

c) **Range:** The range of an analytical method is the interval between the upper and lower level that has been demonstrated to be determined with precision, accuracy, and linearity using this method. The range is normally expressed in the percentage, parts per million. The method is validated by verifying that the analytical method provides acceptable accuracy, precision, and linearity when applied to a sample containing the test item.

d) **Ruggedness:** Ruggedness could be a measure of the repeatability of test results from the laboratory to laboratory, analyst to chemical, and person to person under normally expected changes in conditions. The ruggedness of an analytical method is a degree of reproducibility of the test result by the analysis of the same sample under a variety of conditions, such as different laboratories analysts, instruments, reagents, temperature time, area etc.

e) **Robustness:** It is defined as the measurements ability of analytical methods that remain unaffected by the small variation in the method parameter such as temperature, the composition of mobile phase instrument setting, and providing an indication of its reliability during normal phase robustness determination is a systematic process of varying parameter and measuring effects on the method that monitoring by the system suitability and the analysis of the sample.

f) **Limit of Detection and Quantification:** The limit of detection (LOD) is defined as it is the lowest concentration of an analyte in an exceedingly sample that may be detected. The LOD is expressed as a concentration at a specified signal/noise ratio (typically 3:1). The limit of quantification (LOQ) is defined as the lowest concentration of an

analyte in a sample that can be determined with acceptable precision and accuracy under the stated operating conditions of the method. ICH has recommended a signal: noise ratio of 10:1 for LOQ. LOD and LOQ were calculated based on the standard deviation of the response (SD) and the slope of the calibration curve(s) at levels approximating the LOD according to the given below formulae.

$$LOD = 3.3 \times S / SD$$

$$LOQ = 10 \times S / SD$$

g) **Linearity:** Linearity is the ability of the analytical method is its ability to elicit test results that are directly proportional to the concentration (amount) of analyte in the sample. Linearity is determined by a series of three to six injections of five or more standards whose concentration span 80-120 per cent of the expected concentration range. The response should be proportional to the concentration of an analyte.

CONCLUSION:

This review describes the general HPLC method development and validation. The knowledge of pKa and PH, solubility, polarity, partial size was discussed. The selection of buffer, column, detector, and pH play a dramatic role in separation selectivity. The advantages of HPLC were high selectivity, sensitivity, less time-consuming. Optimized the method is validated with various parameters such as specificity, precision, accuracy, limit detection, linearity as per ICH guidelines.

REFERENCE

1. Rao BV, Sowjanya GN, Ajitha A, Rao Uma MV. A review on stability indicating HPLC method development, World journal of pharmacy and pharmaceutical sciences.2015; 4(8):405-423.
2. Rajan HV. Development and validation of HPLC method - A Review.International Journal of current research in pharmacy. 2015; 1 (2):55-68.
3. Kumar V, Bharadwaj R, Gupta G, Kumar S. An Overview on HPLC Method Development, Optimization and Validation process for drug analysis. The Pharmaceutical and Chemical Journal. 2015; 2(2):30-40.
4. Gupta V, Jain AD, Gill NS, Gupta K. Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences. 2012; 2(4):17-25.
5. Sonia K, Nappinnai M. Development and validation of HPLC and UV-visible spectrophotometric method for the pharmaceutical dosage form and biological fluid –review. European Journal of Biomedical and Pharmaceutical sciences. 2016; 3(3): 382-391.
6. Sánchez MLF. Chromatographic techniques, European RTN Project, GLADNET, retrieved on 05-09-2013.
7. Snyder LR, Kirkland JJ, Glach JL. Practical HPLC Method Development, John Wiley and Sons, New York, 1997; 158-192.
8. HPLC – Chemiguide. May 2, 2007. www.chemguide.co.uk
9. Rao G, Goyal A. An Overview on Analytical Method Development and Validation by Using HPLC. The Pharmaceutical and Chemical Journal, 2016; 3(2): 280-289.
10. McpolinOona.an Introduction to HPLC for Pharmaceutical Analysis. Mourne Training Service. 11-12.
11. http://www.scribd.com/doc/9508765/Physical-Properties-of-Drug.
12. Buffers and pH Buffers: available from: www.xtremepapers.com.
13. Charde MS, Welankiwar AS and Kumar J. Method development by liquid chromatography with validation. International Journal of Pharmaceutical Chemistry.2014; 4(2):57-61.
14. Ranjit singh. HPLC method development and validation. J Pharm Educ Res2013; 4(1): 26-33.
15. Sabir AM, Molony M, Parminder SB. HPLC Method Development and validation: A Review. International research Journal of pharmacy. 2013; 4(4):39-46.
16. Noman A, Bukhaiti ALWedad Q, Alfarga A,AbedSherif M, Mahdi AA. And Waleed AA. HPLC technique used in food analysis-Review. International Journal of Agriculture Innovations and Research. 2016; 5(2):181-188.
17. Snyder LR, Kirkland JJ, Dolan JW. Introduction to modern liquid chromatography. John Wiley & Sons. New York. 2011.
18. Xiang Y, Liu Y, Lee ML. Ultrahigh pressure liquid chromatography using elevated temperature. Journal of Chromatography. 2006; 1104(1): 198-202.
19. Horvath CG, Preiss BA, Lipsky SR. Fast liquid chromatography. Investigation of operating parameters and the separation of nucleotides on pellicular ion exchangers. Analytical chemistry, 1967; 39(12): 1422-1428.
20. Malviya R, Bansal V, Palo P, and Sharma PK. High Performance Liquid Chromatography: A Short Review. Journal of Global Parma Technology. 2010; 2(5):22-23.
21. Pratap B. et al. Importance of RP-HPLC in Analytical method development: A review. International journal of novel trends in pharmaceutical sciences 2013; 3(1): 15-23.
22. Lindholm J. Development and Validation of HPLC method for Analytical and Preparative Purpose. Acta Universities Upsaliensis Uppsala. 2004; 13-14.
23. Snyder LR, Kirkland JJ, Glach JL. Practical HPLC Method Development, 2nd edition. New York. John Wiley &Sons. 1997; 233-291.
24. Sethi PD. Introduction – High Performance Liquid Chromatography, 1st edn, CBS Publishers, New Delhi. 2001; 1-28.
25. Belal F et.al. Stability-indicating HPLC Method for the Determination of Atenolol in Pharmaceutical Preparations. J Chromate Separation Techniq. 2013; 4(1): 1-7.
26. Chetta N. et.al. 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. 2013; 1(3): 654-662.

27. Kumar GS. et.al. Development and validation of RP-HPLC method for simultaneous estimation of Atenolol and Chlorthalidone in Bulk and dosage form. Int Res J Pharma 2013; 3(10): 215-19.
28. FDA Guidance for Industry (2000)-Analytical Procedures and Method Validation, Chemistry, Manufacturing, and Controls Documentation, Centre for Drug Evaluation and Research (CDER) and Centre for Biologics Evaluation and Research (CBER).
29. Julia T, Mena AJ, Au coin MG, Kaman AA. Development and validation of a HPLC method for the quantification of baculovirus particles. J Chromatogram B. 2011; 879: 61-68.