International Journal for Pharmaceutical Research Scholars (IJPRS)

V-5, I-3, 2016 ISSN No: 2277 - 7873

REVIEW ARTICLE

A Concise Review on HPLC Method Development and Validation

Divya Gupta*, B. K. Singh

Department of Pharmaceutical Sciences, Kumaun University, Bhimtal Campus, Nainital-263136, Uttarakhand, India.

Manuscript No: IJPRS/V5/I3/00138, Received On: 28/09/2016, Accepted On: 17/10/2016

ABSTRACT

HPLC is an analytical tool which is able to detect, separate and quantify the drug, its various impurities and drug related degradants that can form on synthesis or storage. HPLC method development and validation play important role in new discovery, development and manufacture of pharmaceutical drugs. An analytical procedure is developed to test a defined characteristic of the drug substance or drug product against established acceptance criteria for that characteristic. A number of chromatographic parameters were evaluated in order to optimize method. This review gives information regarding various stages involved in development and validation of HPLC method. Validation of HPLC method as per ICH guidelines covers all the performance characteristics of validation.

KEYWORDS

HPLC, Validation, Method development, Degradants

INTRODUCTION

Chromatography is "The technique through which the chemical components present in complex mixtures are separated, identified and determined quantitatively and qualitatively". This technique is a powerful tool not only for analytical methods but also for preparative methods. Compounds of high grade purity can be obtained by this method.

The origins of Liquid Chromatography began in the early 1900's with the work of the Russian botanist, Mikhail S. Tswett. His famous studies focused on separating compounds (leaf pigments), which were extracted from plants using a solvent. He named his process "chromatography" [from the Greek words "chroma", meaning "color", and "graphy", meaning "writing" (literally "color writing")] to describe his "colorful" experiment.

***Address for Correspondence:**
Divya Gupta,
Department of Pharmaceutical Sciences, Kumaun University, Bhimtal Campus, Nainital-263136, Uttarakhand, India.
E-Mail Id: divya.varshney126@gmail.com

Today, Liquid Chromatography, in its various forms, has become one of the most powerful techniques in Analytical Chemistry.

Prior to the 1970's, few reliable chromatographic methods were commercially available to the laboratory scientist. During 1970's, most chemical separations were carried out using a variety of techniques including open-column chromatography, paper chromatography, and thin-layer chromatography. However, these chromatographic techniques were inadequate for quantification of compounds and resolution between similar compounds. During this time, pressure liquid chromatography began to be used to decrease flow through time, thus reducing purification times of compounds being isolated by column chromatography. Liquid chromatography can be performed in three primary approaches i.e. thin layer chromatography, Paper Chromatography and HPLC. In all cases, the "sample" must be dissolved into a liquid that is then transported by

the solvent onto or into, the chromatographic device.

High pressure liquid chromatography was developed in the mid-1970's and quickly improved with the development of column packing materials and the additional convenience of on-line detectors. In the late 1970's, new methods including reverse phase liquid chromatography allowed for improved separation between very similar compounds was evolved.

By the 1980's HPLC was commonly used for the separation of chemical compounds. New techniques improved separation, identification, purification and quantification for above the previous techniques. Computers and automation added to the convenience of HPLC. Improvements in type of columns and thus reproducibility were made as such terms as micro-column, affinity columns, and Fast HPLC began to immerge. Currently HPLC is used by a variety of fields including cosmetics, food, and environmental industries.

The early 1970's saw a tremendous leap in technology. These new "HPLC" instruments could develop up to 6,000psi (400 bars) of pressure, and included improved detectors and columns. HPLC really began to take hold in the mid to late 1970's. With continued advances in performance, the name was changed to High Performance Liquid Chromatography (HPLC).

Now-a-days, HPLC instruments of different makers are used in industries. Out of them Agilent technologies & Waters are popular[1,2 3].

Scope of HPLC

High Performance Liquid Chromatography (HPLC) is most widely used of all of the analytical separation techniques, with annual sales of HPLC equipment approaching the billion dollar mark. The reason for the popularity of the method is its

1. Sensitivity
2. Ready adaptability to accurate quantitative determination
3. Suitability of separating non-volatile species or thermally fragile ones and above all, its widespread applicability to substances that are of prime interest to industry, too many field of science & to the public. Today, trace concentrations of compounds, as low as "parts per trillion" (ppt), can be easily obtained. HPLC can be applied to just about any sample, such as pharmaceuticals, food, nutraceuticals, cosmetics, environmental matrices, forensic samples, and industrial chemicals[3,4,5].

Characteristic Features of HPLC

1. High resolving power.
2. Speed of separation.
3. Accurate quantitative measurement.
4. Continuous monitoring of column effluent.
5. Repetitive & reproducible analysis using the same column.
6. HPLC has promoted the discovery of ion chromatography.
7. HPLC is provided with automatic analytical procedure and data handling.
8. It can be applied to the separation and analysis of very complex mixtures.
9. It can determine multiple components in a single analysis.
10. A variety of solvents and column packing are available in HPLC, providing a high degree of selectivity for specific analysis.
11. Analyses are completed in few minutes with excellent precision and accuracy[5,6].

Instrumentation for HPLC

From the above discussion we can say that a typical modern liquid instrument consists of following components:

1. Solvent delivery system which includes a pump.
2. Sample injection system.
3. Column.
4. Solvent detector.
5. Recorder.

6. Data handling device and microprocessor control[6].

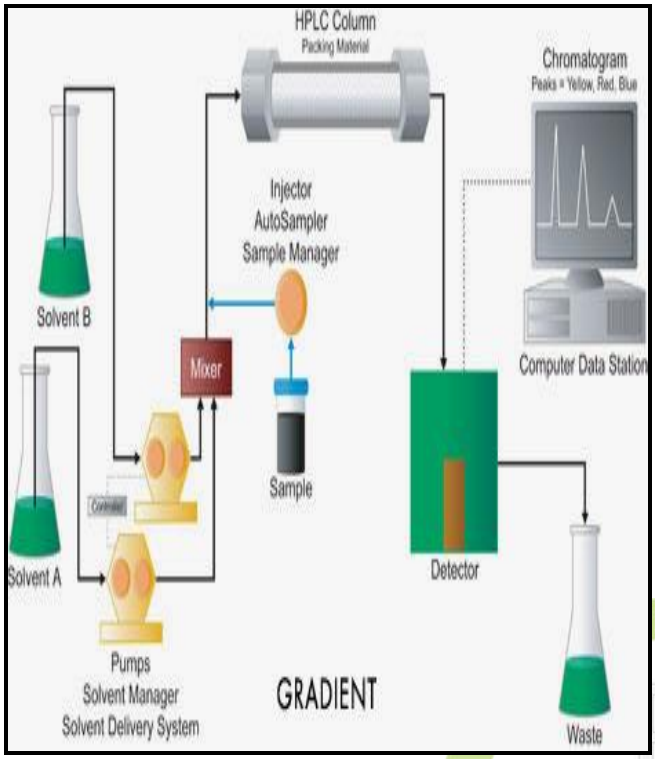

Figure 1: Simple diagram of a HPLC system

Solvent Delivery System

High Pressure Pumps

The pumps are one of the most important components of HPLC, since its performance directly affect the retention time, reproducibility & detector sensitivity. It delivers a steady stream of solvent from the reservoir to the detector through the column. The pumps can deliver solvent at a pressure up to 10,000 psi with a flow rate over 50ml/ min.

Types of Pumps

1. ***Gas Displacement Pump:*** These pumps offer non pulsating flow but have limited solvent capacity.
2. ***Reciprocating Pump:*** This pump consists of a small chamber in which the solvent is pumped by a motor driven system. These pumps facilitate replacement of solvents but provide a pulsating flow.
3. ***Pneumatic Pump:*** These pumps contain a mobile phase which is contained in a collapsible container and placed in a vessel. These pumps are inexpensive and pulse free but depend on solvent viscosity and column back pressure.
4. ***Syringe Pump:*** These pumps work on the principle of solvent displacement by a piston mechanically driven at a constant rate in a piston chamber of 500ml capacity[7,8,9].

Mobile Phase

The choice of mobile phase is vital in HPLC. The eluting power of the mobile phase is determined by its overall polarity, the polarity of stationary phase and nature of the sample components. The mobile phase in HPLC refers to the solvent being continuously applied to the column, or stationary phase. The mobile phase acts as a carrier for the sample solution. There are several types of mobile phases, these include:

Isocratic Elution: Compounds are eluted using constant mobile phase composition.

Gradient Elution: Different compounds are eluted by increasing the strength of the organic solvent. The strength of the mobile phase is later increased in a linear fashion by raising the organic solvent fraction, which subsequently results in elution of retained components.

Gradient elution is frequently used where solvents vary widely in polarity. For gradient elution using a low pressure mixing system, the solvents from separate reservoirs are fed to a mixing chamber & the mixed solvent is pumped to the system. In modern instruments delivery of solvent to the pump is controlled by GPV (Gradient proportionating valves) regulated by microprocessor. Other properties of the solvent which need to be considered are detector compatibility, viscosity, flammability & toxicity. Solvents used for HPLC can be purified to remove UV- absorbing impurities and particulates. Strongly UV- absorbing impurities affect the detector. If the impurity is of higher polarity than the solvent, it influences the separation. Suspended bubbles or dissolved air affect the operation of the pump, detector and spread the bands. So degassing of the mobile phase is accompanied by placing the mobile phase under vacuum or by ultrasonic stirring[9,10].

Sample Injection System

The sample (2 to 200 µl) is introduced into the flowing stream of solvent with an injector.

Injection method:

1. ***Syringe Injection:*** Septum injectors allow sample introduction by a high pressure syringe through a self-sealing elastomer septum. However, the problem associated with septum injectors is the leach effect of the mobile phase in contact with the septum, which may lead ghost peaks. This problem can be eliminated by using stop flow septum less injection.
2. ***Stop Flow Injections:*** The flow of solvent is momentarily stopped and the sample is directly injected on the head of the column.
3. ***Micro Volume Sampling Valves:*** These valves enable samples to be introduced reproducibly into pressurized columns without interruption of the mobile phase flow. The sample is loaded at atmospheric pressure into an external loop in the valve and introduced into the mobile phase by an appropriate rotation of the valve. Automatic sample injectors allow continuous operation of the instrument. Valve injection is preferred for work because of its higher precision compared to syringe[11,12,13].

Columns

The packing used in modern HPLC consist of small, rigid particles having a narrow particle-size distribution.

Types of Packing

The types of packing may be conveniently being divided into following categories:

1. ***Porous, Polymeric Beads:*** These are based on styrene divinyl benzene copolymers. These are used for ion exchange and size exclusion chromatography, but have been replaced by silica based packing which is more efficient and mechanically stable.
2. ***Porous-layer Beads:*** These beads (diameter 30 to 55µm) are consisting of thin shell (1 to 3µm) of silica on an inert spherical core (e.g., glass beads). These pellicular types packing are still used for some ion exchangeapplications, but there use in HPLC has declined with the development of totally porous micro particulate packing.
3. ***Total Porous Silica Particles:*** These are now the basis of most commercially important column packing. Porous silica particles improve considerably column efficiency, sample capacity and speed of analysis. Packing materials are based on the principle of separation by size, absorption, solubility and ion-exchange[14,15,16].

Procedure for Column Packing

The procedure chosen for column packing depends mainly on the mechanical strength of the packing and its particle size. Particles of diameter >20 µm can usually be dry-packed, whereas for particles with diameter <20 µm slurry packing techniques are used in which the particles are suspended in particles in a suitable solvent and the suspension driven into the column under pressure.

Guard column is placed between the injector and HPLC column to protect the later from damage caused by particulates or strongly adsorbed substances in samples or solvents. It can also be used to saturate the eluting solvent with soluble stationary phase guard columns may be packed with micro particulate stationary phases or with porous layer beads[17].

Mobile Phase

Selection of mobile phase is most important in method development via HPLC. In general, polar materials are separated using partition chromatography while non-polar substances are separated using adsorption chromatography. Better sample separation is achieved by matching the polarities of the sample and packing and by using a solvent that has different polarity. Beside polarity, hydrogen bonding and London dispersive forces also affect the solvent strength[17].

Detectors

The detector for an HPLC is the component that

emits a response due to the eluting sample compound and subsequently signals a peak on the chromatogram. The detector is connected electrically to the computer data station that records the electrical information needed to generate the Chromatogram that is used to identify and quantitate the concentration of the compounds. Several different types of detectors are available. For example, if a compound can absorb ultraviolet light, then a UV Absorbance Detector is used. If the compound can fluoresce, then a Fluorescence Detector is used. If the compound does not have either of these characteristics then a more universal type of detector is used such as Evaporative Light Scattering Detector (ELSD). Today, the most powerful approach to gain as much information as possible from a run is to use multiple detectors in series. Typically, a UV and ELSD detector are used in combination with a Mass Spectrometer (MS) to analyze the results of the chromatographic separation.

Identification of compounds by HPLC is a crucial part of any HPLC assay. In order to identify any compound by HPLC, a detector must first be selected. Once the detector is selected and is set to optimal detection settings, a separation assay must be developed. The parameters of this assay should be such that a clean peak of the known sample is observed from the chromatograph. The identifying peak should have a reasonable retention time and should be well separated from extraneous peaks at the detection levels which the assay will be performed.

However, detectors can be divided into two categories:

1. ***Bulk Property Detectors:*** These detectors measure the difference in some physical property of the solute in the mobile phase compared to the mobile phase alone e.g., refractive index and conductivity detectors. The latter detector is a universal detector for ionic species and is widely used in ionic chromatography and HPLC but they tend to have poor sensitivity and limited range. Such detectors are usually affected by even small changes in the mobile phase composition which precludes the use of gradient elution technique.
2. ***Solute Property Detectors:*** these are like electrochemical, fluorescence detectors. These respond to a particular physical or chemical property of the solute, being independent of the mobile phase. They generally provide high sensitivity and a wide linear response range.

Recorder

The signals from the detectors are recorded as deviation from the base line. With proper calibration, the height or area of peak is a measure of the amount of the component present in the sample.[18]

Data Handling Device

In high performance liquid chromatography the "Empower pro" Software is used in 'Waters' HPLC system for data handling during analysis.

Basic Terminology Used in HPLC

Performance

Column efficiency is defined in terms of number of theoretical plates per meter (N) Theoretical Plate Model of Chromatography.

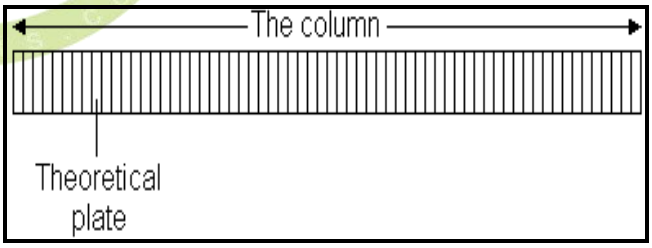

Figure 2: Theoretical plate model

The plate model supposes that the chromatographic column contains a large number of separate layers, called theoretical plates. Separate equilibrations of the sample between the stationary and mobile phase occur in these plate.

$$N = 5.54\ V_R/L.W_h^2$$

Where, V_R is the distance between the point of injection and a perpendicular dropped from the maximum of peak, L is the length of the column in meters and W_h is the Width of the peak at half peak height.

Retention Time

The time between sample injection and an analyte peak reaching a detector at the end of the column is termed the retention time (t_R). Each analyte in a sample will have a different retention time. The time taken for the mobile phase to pass through the column is called t_M.

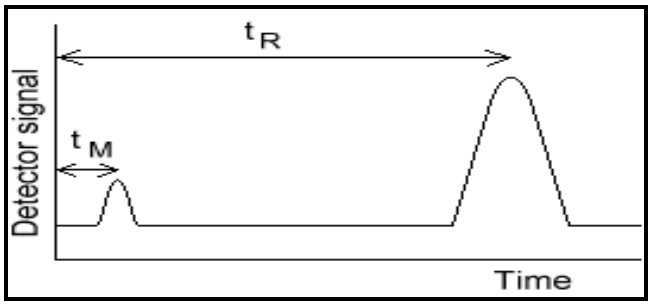

Figure 3: Model diagram of Retention Time

A term called the retention factor, k', is often used to describe the migration rate of an analyte on a Column. You may also find it called the capacity factor. The retention factor for analyte A is defined as;

$$k'_A = t_R - t_M / t_M$$

t_R and t_M are easily obtained from a chromatogram. When an analytes retention factor is less than one, elution is so fast that accurate determination of the retention time is very difficult. High retention factors (Greater than 20) mean that elution takes a very long time. Ideally, the retention factor for an analyte is between one and five[19,20].

Selectivity Factor

We define a quantity called the selectivity factor, a, which describes the separation of two species (A and B) on the column;

$$a = k'_B \ / k'_A$$

When calculating the selectivity factor, species A elutes faster than species B. The selectivity factor is always greater than one[19,20].

CONCLUSION

This review describes the general technique of HPLC method development and validation of optimize method. The general approach for the method development for the separation of pharmaceutical compounds was discussed. The selection of buffer and mobile phase composition plays an important role on a separation selectivity. Final optimization can be performed by changing the gradient slope, temperature and flow rate as well as the type and concentration of mobile phase. Optimized method is validated as per ICH guidelines.

REFERENCES

1. Lunn, G. (2006). HPLC Method for Pharmaceutical Analysis, Vol-2, A, Wiley-Inter-science Publication, New-York, 864.
2. Sharma B. K. (1999). Instrumental Methods of Chemical Analysis, 18th edition, Goel Publishing House, 1-6.
3. Skoog F. A., Holler F. J. (2009). Introduction to UV Spectroscopy, Principle of instrumental analysis, 5th edition, Brooks/Cole publication, 301.
4. Beckett, H., Stenlake, J. B. (2001). UV-visible Spectrophotometry: Practical Pharmaceutical Chemistry, 4th edition, Part-II, C.B.S. Publishers, New Delhi, 285-97.
5. Jeffrey (1997). Introduction: Vogel Textbook of Quantitative Chemical Analysis, 5th edition, ELBS, Longman, 3-8.
6. Sharma, Y. R. (2000). Elementry Organic Spectroscopy, 2nd edition, S.Chand Publishers & Distributors.
7. Standard Base techniques "High Performance Liquid Chromatography" Drenthe Collage, the Netherlands, Vol 2, 1-8.
8. Braun R. D. (2005). Introduction to Instrument Analysis, Pharma Book Syndicate, Hyderabad, 261.
9. Silverstein, R. M., Webster, F. X. (2008). Spectrometric Identification of organic compounds, 6th Edition. Wiley India Pvt. Ltd
10. Willard, H. H. , Merrit, L. L., Dean, J. A., Settle, F. A. (1986). HPLC Methods and applications in: Instrumental Methods of analysis, 7th Edition, C. B. S. Publishers, New Delhi, 626-627.

11. Snyder, L. R., Kirkland, J. J., Glajch, J. L. (2000). Practical HPLC method development, 2nd edition, A Wiley-Interscience Publication, 697- 709.

12. Chatwal, G. R., Anand, S. K. (2007). Instrumental methods of Chemical analysis, 5th edition, chapter 31, Himalyan Publication House; page number-2.272, 2.273, 2.565-2.585, 2.625-2.638.

13. Sethi, P. D. (2001). Introduction in HPLC: Quantitative Analysis for Pharmaceutical Formulation, 1st edition, CBS Publishers and Distributors, New Delhi, 51-66.

14. Fronk S. (2004). Introduction: Handbook of Instrumental Techniques for Analytical Chemistry, 1st edition, Pearson Education, 7.

15. Stout T. H. , Dorsey J. G. (2002). High Performance Liquid Chromatography. In, L. Ohannesian, A. J. Streeter editors. Handbook of Pharmaceutical Analysis, NY: Marcel Dekker Inc., 127-40.

16. Nadig D. E. (2002). Preparation of Drug sample for analysis. In, L. Ohannesian, A. J. Streeter editors. Handbook of Pharmaceutical Analysis, NY: Marcel Dekker Inc., 80-83.

17. Nash R. A., Watcher A. H. (2003). Pharmaceutical Process Validation, Marcel Dekker Inc.; New York, 7th edition, 159-190.

18. Singh, R. B. (2011). Validation and process development: a review, *International Research Journal of Pharmacy*, 2, 32-39.

19. Lindsay, S. High Performance Liquid Chromatography, 2nd Edition, 1-10.

20. Hung, L. B., Parcher, J. F., Shores, J. C., & Ward, E. H. (1988). Theoretical and experimental foundation for surface-coverage programming in gas-solid chromatography with an adsorbable carrier gas. *Analytical Chemistry*, 60(11), 1090-1096.

21. Horvath, C. G., Preiss, B. A., & Lipsky, S. R. (1967). Fast liquid chromatography. Investigation of operating parameters and the separation of nucleotides on pellicular ion exchangers. *Analytical Chemistry*, 39(12), 1422-1428.

