EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 8
159-161
Year: 2019

A REVIEW ON CRITICAL CONSIDERATIONS IN HPLC SOLVENT SELECTION

Deelip Derle* and V. V. Shelke

Department of Quality Assurance M.V.P. Samaj's College of Pharmacy, Nashik-422002, Maharashtra, India.

***Corresponding Author: Deelip Derle**
Department of Quality Assurance M.V.P. Samaj's College of Pharmacy, Nashik-422002, Maharashtra, India.

Article Received on 27/05/2019 **Article Revised on 18/06/2019** **Article Accepted on 09/07/2019**

ABSTRACT
The main objective of this paper is to understand the critical considerations in selection of solvent for HPLC. As the mobile phase is the one of the most important parameters in HPLC especially in method development and its validation. The mobile phase (solvent) used may have a big effect on retention. It may directly effects on ionization analyte molecules or in some cases, may suppress ionization. This review discusses the some of the important considerations which plays an important role in selection of solvent and helps in deciding the suitability of solvent as mobile phase.

KEYWORDS: HPLC, Solvent Selection, Normal Phase Chromatography, Reverse Phase Chromatography.

INTRODUCTION

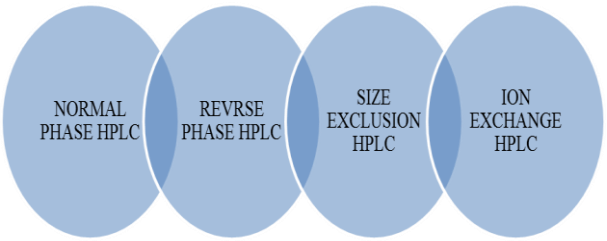
Basics of Hplc

HPLC is a one of the analytical techniques use to separate, identify, or quantify each component in a mixture. It works on following basic principles of TLC or column chromatography, were it has two phases.

1) Stationary phase (solid like silica gel)
2) Mobile phase (liquids)

Types of HPLC

In HPLC following are the variants of HPLC, depending upon the phase system in the process.



Normal Phase HPLC

In normal phase HPLC the separation of analyte is based on polarity. It consists of polar stationary phase and non-polar mobile phase. Hence stationary phase is silica and mobile phases are chloroform, diethyl ether, hexane etc.

Reverse Phase HPLC

In reverse phase HPLC the separation of analyte is based on polarity. It consists of non-polar (hydrophobic) stationary phase and polar mobile phase. It works on principle of hydrophobic interaction hence the more non-polar material it will retained longer.

Size Exclusion HPLC

In size exclusion HPLC, column is filled with material having uniform pore size, and particles are separated according to its molecular size. In case of larger molecules, they washed rapidly through the column and smaller molecules penetrate inside the porous of the packing particles and elute later.

Ion Exchange HPLC

In ion exchange HPLC stationary phase has an ionically charged surface of opposite charge to the sample ions. This technique is widely used with ionic samples. If the charge is stronger it will be attracted to the ionic surface and thus it takes more time to elute.

Selection of Mobile Phase

Mobile phase selection is the important parameter in HPLC. Type of mobile phase used may have a big effect on the retention. It can promote or suppress an ionization of the analyte molecules and it also can shield an accessible residual silanol or any other active adsorption centers on the adsorption surface.

Proper selection of the mobile phase is the second most important step in the development of the separation method (the first one is the selection of adsorbent type). The main requirement for the mobile phase is that it has to dissolve the analyte up to the concentration suitable for the detection. The selection/preparation of the sample crucial to the effectiveness of the HPLC technique, as the sample must interact with both stationary and mobile phases. Additionally, selection of a correct mobile phase is important.

The sample itself must satisfy a number of criteria before it can be analyzed. The main points to remember are the following.

- The mobile and stationary phases should be selected to provide optimum separation of the analyte in the sample.
- The sample should be dissolved in a buffer that is compatible with mobile phase.
- The sample should not contain any particulate matter.
- The sample should not contain any material that will poison (that is, irreversibly bind) the column.
- The sample should be concentrated in a small volume (injection volume is small).

The mobile phase its self is of equal importance to the stationary phase and is chosen to elicit separation of the analytes of interest during the HPLC run. A good mobile phase has the following characteristics.

- Compatible with the column packing/ stationary phase and the sample.
- Gives good separation of the analytes of interest.
- Is of the highest purity.
- Must not contain particulate matter.
- Must be degassed immediately prior to use.

Mobile phase and their properties: The mobile phase is important in the separation process, and several other of its properties are important in the HPLC analysis. One such property is the mobile phase viscosity, and which affects the backpressure in the HPLC system. Other properties are related to the detection process. The mobile phase must differ from eluted molecules by certain physicochemical properties in order to be detected. These properties can be UV-absorption, fluorescence, molecular fragmentation in a mass spectrometer, refractive index or others that makes the analytes detectable. For this reason, some physicochemical properties of the mobile phase must be known, and the detection procedure must be selected such that the solvent (mobile phase) should not interfere with the detection. Other properties of the solvent may also be of interest, such as boiling point. The values for the boiling point (at atmospheric pressure) of several common solvents.

Solvents for HPLC: There is a wide range of solvents for HPLC. It depends upon sample and what kind of solvents should be used. First criteria are to check miscibility of solvents with samples.

List of solvents is as below

1. Acetone
2. Methylene chloride
3. Chloroform
4. Pentane
5. 2-Propanol
6. Cyclohexane
7. Toluene
8. Ether
9. Water
10. Methanol
11. Isobutyl alcohol
12. Methyl ethyl ketone
13. 1,2,4-Trichlorobenzene
14. 2,2,4-Trimethylpentane
15. n-Heptane
16. Ethyl acetate
17. Methyl tert-butyl ether

These are milli Q water (HPLC grade) solvents, which are widely used solvents in HPLC for development and method validation in various

The article discusses some of the essential considerations that play an important role in deciding suitability of solvents as mobile phase. Some critical parameters are as follow

- Cost
- Solubility
- Absorbance
- Volatility
- Viscosity
- Inertness
- Water

1. Cost

Cost is an important consideration as HPLC requires superior purity grade solvents and it is common to see dozens of HPLC systems operating round the clock in large laboratories. This means consumption of high grade solvents in bulk quantities and therefore cost considerations play a vital role.

2. Solubility

The sample should be completely soluble in the mobile phase. Slightest insolubility will result in phase separations or suspensions which will contribute to operational problems.

3. Absorbance

Generally, the detectors used in HPLC are based on absorbance of light by sample constituents. The intrinsic absorbance of the mobile phase components in the selected wavelength range should not interfere with the absorbance of the sample. The mobile phase solvent should ideally have no absorbance at the wavelength of interest.

4. Volatility

The mobile phase solvents should have low volatility especially for use with light scattering detectors. Highly volatile solvents can lead to compositional changes in mobile phase composition over use and storage. This can lead to poor reproducibility of chromatograms.

5. Viscosity

The selected solvents should have low viscosity so that flow through the column does not lead to development of high back pressures. The viscosity of mobile phase plays a crucial role. Clearly, the mobile phase with lower viscosity shows lower instrumental pressure. Thus,

higher flow rates of the mobile phase can be used and leads to the shorter analysis time.

6. Inertness

The selected solvents should be inert to sample components, column packing and column material. Any reactivity with any of these components can lead to formation of precipitates, gases or other reaction products which can upset the system performance. The solvents should not form separate phase on coming in contact with the sample. In other words, there should be complete miscibility of solvents.

7. Water

Water is also an important component of reverse phase mobile phase composition either in combination with other solvents or as a buffer media. Water purification systems are commercially available. The water generated by such systems should be used at the earliest possible so that its quality does not degrade over the storage period.

HPLC technique is highly sensitive to changes in the composition of the mobile phase reaching the detector so it is essential to filter the solvents through 0.45um filter to remove any solid suspensions. Degassing is also an essential requirement to remove any trace of dissolved air which can lead to flow restriction or spurious peaks.

CONCLUSION

Mobile phase is the most important parameter in reversed phase HPLC. Type of mobile phase used may have a big effect on the retention. It can promote or suppress an ionization of the analyte molecules, and it also can shield an accessible residual silanol or any other active adsorption centers on the adsorbent surface. So, the above various critical considerations for the selection of mobile phase in HPLC.

REFERENCES

1. Hugo A, Billiet L., Selection of mobile phase parameters and their optimization in reversed phaseliquid chromatography Journal of chromatography A., 1989; 285: 27-5.
2. Dr. Bhanot D. Critical consideration in HPLC solvent selection. Lab training.com, 2017: 1-7.
3. Patil P. HPLC Method Development – A Review Journal of pharmaceutical Research and calculation, 2017; 1(2): 243-60.
4. Serban C. Mobile phase and their properties essentials in Modern HPLC seperatitons, 2013: 1-11.
5. Sufgiuama T, Matsuyama R., Usui S., Katagiri Y, Hiraano K. Selection of Mobile phase in High-performance liquid chromatographic separation for medicines, Biolo Ohara Bull, 2000; 23(3): 274-78.
6. Vamber P., Schoennmakers P., Selection of adequate optimization criteris in chromatographic separations, Analytical and bionaanlytical chemistry, 2009; 394(5): 1283-89.
7. Sanjay Kumar D & D.R, Kumar. Importance of RP-HPLC in analytical method development : A review International Journal of Oharmaceutical science and research, 2012; 4626-33.
8. Jandera P. Stationary And Mobile phases in hydrophilic interaction chromatography : a review Analytical chimica acta, 2011; 692(1-2): 1-125.
9. G.Ranis-Ramos, M.C. Garcia, Solvent selection in liquid chromatography. Liquid chromatography fundamentals and instrumentation, 2013; 225-49.
10. Valka K, Snyder L. Retention in Reversed phase liquid chromatography as a functional of mobile phase composition, Journal of Chromatography A., 1993; 656(1-2): 501-20.
11. Snyder L., Quarer M. Grlajch T. Solvent strength selectivity in reversed phase HPLC chriomatographia, 1987; 24(1): 33-44.