Troubleshooting in HPLC: A Review

[1]S. A. Bhor, [2]P. P. Atnure, [3]M. S. Charde, [4]R. D. Chakole,[5]S.T. Hajare

[1,2,5]Research Scholar, [3]Assistant Professor, [4]Associate Professor
Department of Pharmaceutical Chemistry,
Government College of Pharmacy, Vidyanagar Karad, Dist.: Satara,
Pin: 415124, Maharashtra, India,
For correspondence**: Ms. Sayali Adinath Bhor**

***Abstract*- The most effective method for many analytical procedures is chromatography. Compared to other chromatographic procedures, HPLC yields results with higher resolution and accuracy in less time. There are different types of HPLC from which reverse phase HPLC is mostly used because various components are polar in nature and easily separated. The approach to problem-solving used to identify and resolve HPLC system-related issues is known as HPLC troubleshooting. Problems with the HPLC System can come from a variety of causes. Define the issue first, then narrow down the source to identify the potential troublemaker. Troubleshooting HPLC instrumentation and separations require a fundamental understanding of how the instrument functions and how the separation works. This review is an attempt to describe overview of HPLC with its types and applications. Along with this, this article includes troubleshooting strategies and the process to overcome problems related to mobile phase, injector, detector, column and pumps. All these problems are due to pressure difference, leaks, chromatogram, baseline irregularities and smell, sight or sound related issues.**

Key words: **Troubleshooting, HPLC, etc.**

INTRODUCTION:

Chromatography the term used to describe the process which is used to separate, identify and determine the chemical components present in the complex mixture. Similar to spectroscopy, this method is frequently used and very potent instrument not just for preparative methods but also for analytical approaches. By using this technique, high-grade pure compounds can be produced.A simple definition of chromatography is as follows" It is a method in which the components of a mixture are separated based on rate at which they are moved through stationary phase by the gaseous or liquid mobile phase."[1]

In biochemistry and analysis, active components are regularly separated, identified, and quantified using a special type of column chromatography called high-performance liquid chromatography, sometimes known as high-pressure liquid chromatography.[2]A little amount of the sample is added to the mobile phase stream, which is then slowed down by a specific chemical or physical interaction with the stationary phase.The kind of analyte and the packing of stationary and mobile phases have an impact on the amount of retardation. The retention time measures how long it takes for a specific analyte to elute, or leave the column.[3]

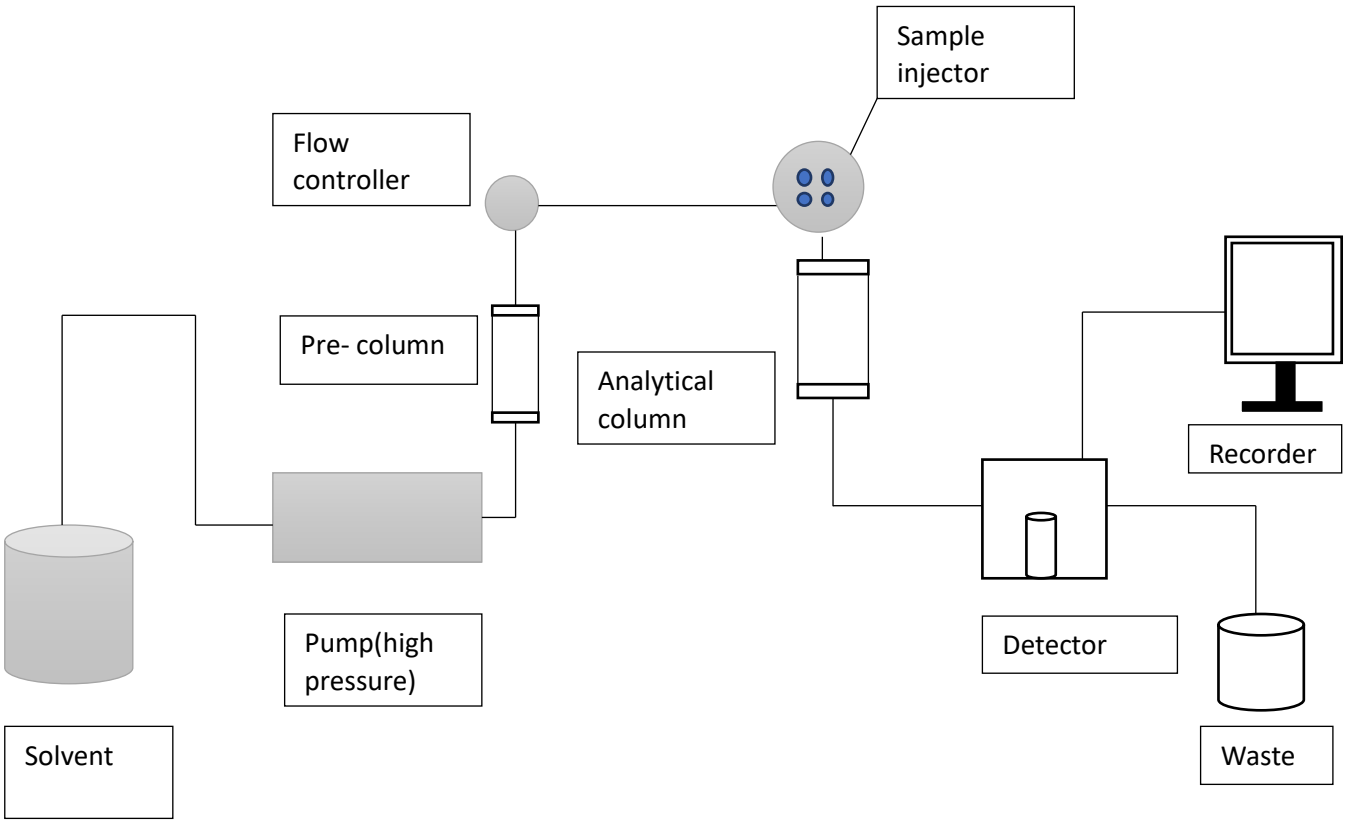

Figure 1: HPLC Instrument

Types of HPLC:

Based on the stationary phase used. HPLC is divided into the following types:

1. Normal Phase: For the HPLC separation, polarity is a keycomponent. In this type stationary phase is polar in which silica is used. Hexane, chloroform & diethyl ether are employed as non-polar mobile phase. Polar Sample kept on the column.

2. Reverse Phase: HPLC of reverse phase is opposite of the normal phase HPLC. The stationary phase is hydrophobic or non-polar while the mobile phase is Polar. If the compound is non-polar it will be retained on Stationary phase.

3.Size exclusion: The column will induce precisely regulated substrate molecules during separation in HPLC. Because of the variance in molecular sizes,components will separate.

4. Ion exchange: The stationary phase has an ionized surface that is opposite the charge of sample. Aqueous buffer is utilised as the mobile phase and it will regulate the pH and ionic strength.[4][5][6]

HPLC trouble shooting:

The problem-solving techniques like troubleshooting are frequently used to fix broken items or ineffective Procedures. Searching for a problem's root cause is rational and methodical therefore that a solution exists. Enabling the process of the product to reactivate and made to work. The process of trouble shooting creates and keeps it sophisticated systems where there are numerous potential Sources of troublesome symptoms. There are several industries that require troubleshooting strategies including technology, electronics and engineering, medical diagnostics and auto repair. Finding the issue is necessary for troubleshooting. After then experience is frequently used to come up with potential causes for the symptoms.[7]

HPLC Trouble shooting strategy following stages are required for every troubleshooting method

1) Recognition of the issue.
2) Understanding the issue root of problem cause.
3) Determining the precise root of issue.
4) Correcting the issue, if possible.
5) Returning the device to regular usage or informing your maintenance manager of the issue.

Process for HPLC trouble shooting:

i. Troubleshooting technique is a methodical strategy that will solve any difficulty is needed to carry out the plan. So that the precise root of the issue may be identified, the systematic method should proceed in a logical order.
ii. Collect facts rather than theories.
iii. Start by checking the simplest items because it is easy.
iv. Evaluate the performance against what was anticipated.
v. Potential reasons should be listed.
vi. Go over each potential reason in detail and examining the results of any adjustments made.
vii. As a last resort, seek assistance elsewhere, such as at the help desk of supplier or your neighbourhood team, your instrument technical support. [8]

Trouble shooting problems: These problems are related to
1) Abnormal Pressure
2) Leaks
3) Problem with a chromatogram.
4)Problem with injector
5)Baseline irregularities
6)Problem detected by smell, sight, or sound.
7) Key problem areas & preventive measures.

1) Abnormal pressure [9]

i) Low Pressure

Causes	Solution
Insufficient flow	Modify flow rate
A system leak	Find a fix leaks
Incorrect column	Use the correct column
A high column temperature	Lower the temperature
A broken controller	Upgrade or change the controller

ii) Fluctuating pressure[10]

Causes	Solution
Bubbles present in pump	Purge the solvent with helium after degassing it
Leak check valves or seals on pumps	Replace seals and clean or replace check valves

iii) High Pressure

Causes	Solution
An excessive flow rate	Modify settings and allow back flush column
Column frit that is blocked	Replace the frit or substitute column
Precipitated buffer or incorrect mobile phase	Use the proper mobile phase and wash the column
Incorrect column	Use the correct column
Injector obstruction	Remove obstruction or replace injector
Insufficient column temperature	Increase the temperature
A controller issue	Fix or change the controller
Blocking the guard column	Replace or remove the guard column
An in-line blocked filter	Discard or replace the in- line filter

iv) No Pressure Reading

Causes	Solution
A faulty meter	Replace the meter
Inefficient pressure transducer	Change the transducer

2) Leaks

i) Column end leaks

Causes	Solution
Simple and fitting	Firm up the end fitting
Column packing within the ferrule	Separate, clean and attach the ferrule
Insufficient frit thickness	Use the right frit

ii) Leak at pump

Causes	Solution

Failed pump seal	Replace the pump seal, look for the scratches on the piston and replace if it necessary

iii) Injector Leak[11]

Causes	Solution
Rotor seal breakdown	Replace or rebuild the injector
Unbroken loop	Swap out loop
Injection- port seal that is loose	Adjust
Incorrect syringe- needle size	Use the proper syringe
Sewage syphoning	Maintain the waste line above the surface waste
Waste-line obstruction	Change the waste line

iv) Detector leak[12]

Causes	Solution
Failing the Celt gasket	Avoid high back pressure or replace the gasket
Cell window cracks	Change the window
A blocked drain	Change the waste line
Faulty fixtures	Adjust or Substitute

v) Leaky Fitting

Causes	Solution
Unsecure fitting	Tighten
Worn-out Fitting	Substitute
Excessive tight fitting	Replace or loosen then retighten

3) Problem with chromatogram

i) Increased peak Retention time[13]

Causes	Solution
Decrease in column temperature	Set the oven's temperature
A column that is unstable	If the problem was caused by the preceding solvent wash the column thoroughly. If the problem was caused by the stationary phase, wash the column thoroughly
Speed of chart	Repair Recorder

ii) Decrease peak Retention time

Causes	Solution
An older Stationary phase	Replace the column or Stationary phase if it is soluble switch the chromatographic system
Remains of earlier elution, particularly gradient elution	Adequate column cleaning
Rising column temperature	Set the oven temperature

ii) Split peak

Causes	Solution
A guard or analytical column inlet contamination	Remove the guard column and try your analysis. If necessary, swap out the guard column. Reverse and flush if the analytical

	column is clogged. If the issue persists, the column can be plugged. Use the proper restoration technique. If the issue still exists then inlet frit may be partially clogged. Replace the frit or the column.
A little bit blocked frit	The column's top frit functionality should be changed.
A tiny, amorphous voidnearthe column inlet	Put another batch of pellicular particles into the column using the same bonded phase and the reverse flow direction.
Incompatibility of the sample solvent with the mobile phase	Modify the solvent and inject samples while they are still in the mobile phase.

v) Fronting peak[14]

Causes	**Solution**
An overloaded column	Use a smaller volume while injecting. If there is a mass overflow.
Mobile phase and stationary phase areincompatible	Change the solvent. Samples should always be injectedwhen they are still in mobile phase. Flush the polar bonded phase column with 50 column volumes of HPLC grade ethyl acetate and an intermediate polarity solvent at a rate that is 2- 3 times the typical flow rate before analysis.
Slow rise in the baseline before to the major peak could also be included in the sample	Improve the system efficiency or selectivity to boost resolution, alter column types changing from non polar C18 phase to polar cyano phase of necessary.

vi) Rounded peak

Causes	**Solution**
A detector with nonlinear dynamic range	Decrease sample size and composition
Insufficient recorder gain	Modify gain
A crowded column	Inject a lesser volume of the sample
Interaction between samples and columns	Alter the pH, mobile phase composition or buffer strength. Change the type of column or boost the temperature if necessary
The recorder or detector time there are too many constants	Lower the parameters to the point where no more benefits are seen

vii) Broad peak

Causes	**Solution**
The makeup of the mobile phase changed	Set up a fresh mobile phase
Insufficient mobile phase flow rate	Modify the flow rate

Leak between detector and column	Inspect the system for any loose parts. Look for leaks, salt accumulation and odd noises in the pump. Pump seals may need to be changed.
Incorrect detector settings	Change the parameter
Insufficient buffer concentration	Sharpen your focus
Worn- out or polluted guard column	Change the guard

viii) Negative peak

Causes	**Solution**
Reversed recorder leads	Verify the polarity
The solute refractive index is lower than mobile phase	Reverse recorder leads or mobile phase with a lower index of refraction
The composition of the sample mobile phase and solvent differs significantly	Alter or modify the sample solvent and sample should always be diluted in the mobile
Mobile phase is more UV- absorbing than sample components	When employing indirect UV detection either change the polarity or modify UV wavelength.

ix) Ghost peak

Causes	**Solution**
Injector or column contamination solution	Flushing the injector between analysis is wise routine. Run a strong solvent through the column if necessary including last rinse.

x) Change in peak height

Causes	**Solution**
The column activity changed or one or more sample components worsened	To confirm that a sample is the problem cause, use a new sample or a standard. Replace column if any or all peaks remain smaller than anticipated.If the new column makes the analysis better, try restoring the old column using proper process.
Leak between the column inlet and the injection port	Inspect the system for faulty fitting, look for leaks, salt accumulation and odd noses in the pump. Pump seal may need to be changed.
Variable sample volume	Verify that samples are uniform. Use two to three times the loop volume for fixed volume sample to make sure the loop is entirely filled.

4) Problem with injector

i)Injector volume variation[15]

Causes	**Solution**

Air is drawn from the Vail by the auto sampler	Verify the injector needle sampling height and sample filling height
Degrading samples	Use appropriate storage condition
Air in the fluidics auto sampler	As outlined in the relevant operating instructions, flush out the auto sampler fluidics

5) Baseline irregularities

i)Baseline drift[16]

Causes	**Solution**
Variations in column temperature	Temperature of the control column and mobile phase
A nonhomogeneous mobile phase	Employ mobile phase degassing, high purity salts and solvents of HPLC column
Air or contamination accumulation in the detector cell	Use methanol or another powerful solvent to flush the cell
Slow column equilibration, especially when switching mobile phases	Use a medium strength solvent to flush.Before analysis, run 10-20 column volumes of fresh mobile phase
Strongly retaining components in the sample can elite in the form of very broad peaks and appear to be an issue	Employ a guard column if necessary, frequently during analysis or between injections, flush the column with a potent solvent
The detector (UV) is not placed at the greatest absorbance, but rather than the slope of the curve	Adjust wavelength for the highest UV absorption.

ii) Baseline noise (regular)[17]

Causes	**Solution**
Air in a detector cell, mobile phase or pump.	Mobile phase of Degas. Remove air from the pump or detector cell by flushing the system.
Pulses from the pump	Add a pulse damper to the system
Incomplete mixing of the mobile phase	Manually stir the mobile phase or use less fluid with viscosity
Effect of temperature on the same line, other electronic devices	Eliminate difference or include a heat exchanger. To ascertain if the problem has an external cause, isolate the LC, detector, and recorder
Leak	Look for any loose fitting in the system

iii)Baseline noise (irregular)

Causes	**Solution**

Contaminated, degraded or made of sub-par material is the mobile phase	Ensure the mobile phase composition. Separate the recorder and detector electronically. To fix issue, consult the instruction manual. Use a powerful solvent to flush the system. Afterwards install the back pressure regulator
Electronics for detector/ recorders system airlock, air bubbles in detector infected detector cell. Inadequate detector lamp leak	For information on RI detectors, consult the instrumental manual.A tidy cubicle alter the lamp

6) Problem detected by smell, sight or sound

i) Solvent smell

Causes	**Solution**
Leak	See section on leak
Spill	Look for overflowing trash cans, find spills and clean them up
Module that is too hot	Examine the ventilation, adjust the temperature or turn off the module and consult the service manual

ii) Abnormal meter reading

Causes	**Solution**
An abnormal pressure reading	Review the section on anomalies
Issue with the Column oven	Review the service manual and check settings
Detector lamp failure	Switch the lamp

iii)Warning lamps

Causes	**Solution**
Pressure ceiling breached	Examine the flow and limit settings then make necessary adjustments
Additional alert lamps	Consult the service manual

iv) Warning buzzers

Causes	**Solution**
Solvent spill or leaks	Identify and fix
Additional alarm buzzers	Consult the service manual

7) Key problem area and preventive measures

i) Reservoir

Causes	**Solution**
Blocked inlet sand	Change the mobile phase filter every 3-6 months
Bubbles of gas	Mobile phase degas

ii) Pump

Causes	**Solution**
Bubbles of air	The mobile phase firstly degas
A failed pump soal	Change after 3 months

Check valve malfunction	Filter the mobile phase, preserve a spare inlet- line frit.

iii) Injector

Causes	Solution
Rotor seal ageing	Avoid overtightening and sample filtering

iv) Column

Causes	Solution
Blocking frit	Filter the mobile phase, the samples, and utilise an in-line filter or guard column
Vacuum at top of column	Precolumn, guard column and avoid mobile phase pH>8

v) Detector

Causes	Solution
Failure, reduced response and enhanced noise detector	Replace after six months or save spare lamp
Cellular bubbles	Maintain cell cleanliness, use a restrictor after cell and degas the mobile phase

vi) General

Causes	Solution
Corrosion and abrasion	When not in use, clean and flush the buffer from LC

Applications of HPLC:

The Forensic, environmental, pharmacology and clinical domains are just a few where HPLC is used. Additionally, it is used in compound separation and purification.

1. **Pharmaceutical Application:** These applications include quality control, dissolution studies medication storage stability
2. **Environmental Applications:** To identifying elements in drinking water and monitoring pollution.
3. **Forensic Application:** In forensic include the examination of textile dyes & the measurement of narcotics and steroids in biological samples.
4. **Food and Flavour Applications:** Polycyclic chemical detection and analysis in fruit juices. Preservative analysis,vegetables, sugar.
5. **Clinical Applications:** Blood urine at analyses, detection of endogenous neuropeptides.[18]

CONCLUSION:

High Performance Liquid Chromatography is used in a wide range of disciplines, includingbiochemistry, air and water pollution studies, pharmaceutical analysis and separation, andenvironmental pesticide level monitoring. Federal and state regulatory organisations employ HPLC to examine food and drug goods for thepresence of narcotics or to verify that the label claim is being followed, as well as for neutraceuticals, forensic purposes, and clinical diagnostics. Higher molecular weight substances can be separated using HPLC to get qualitative and quantitative data. HPLC is made up of numerous essential parts.The HPLC system can be maintained and frequent problems can be resolved more easily. It results in cost reduction and system performance improvement.

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