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Review: common trouble shooting problems in RP-HPLC

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Abstract

High performance liquid chromatography is one the powerful analytical tool regularly employed for the analysis of the drugs in the pharmaceutical formulations. Day by day advancement in instrumentation is increasing but still the problems have been encountered while performing analysis. Here in this review article different troubleshooting has been described with their causative and preventive parameters during performing the method development for separation and identification by RP-HPLC.

Keywords: Problem, Causes, Prevention, Trouble shooting, Instrumentation

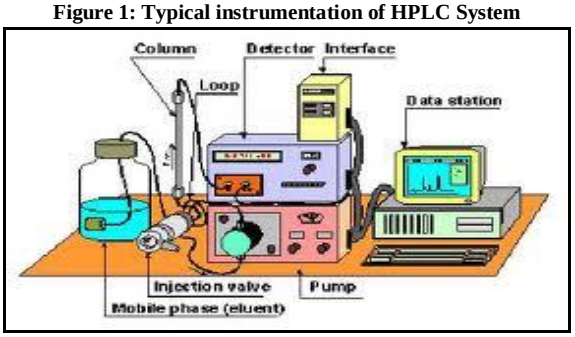
1. Introduction

The techniques high performance liquid chromatography is so called because of its improved performance over classical column chromatography and it is the most important tools of analytical chemistry today. Principle is the solution of sample is injected into a column of porous material (stationary phase) and liquid phase (mobile phase) is pumped at higher pressure through the column. The principle of separation followed is the adsorption of solute on stationary phase based on its affinity towards stationary phase. The technique of HPLC has following features.

- High resolution
- Small diameter, Stainless steel, Glass column
- Rapid analysis
- Relatively higher mobile phase pressure
- Controlled flow rate of mobile phase[2]

Typical HPLC system consists of the following main components and schematic diagram shown in Figure 1.

Figure 1: Typical instrumentation of HPLC System



1.1. Solvent Reservoirs & Degasser

Solvent Reservoirs are used for storage of sufficient amount of HPLC solvents for continuous operation of the system and equipped with an online degassing system and special filters to isolate the solvent from the influence of the environment. Dissolved gas in the mobile phase can cause pump malfunctions leading to blending errors and shifting retention times. Degassing by stirring or ultrasonication under vacuum is inadequate while helium sparging is inconvenient and expensive. Online vacuum degassers are now built-in accessories in many integration HPLC systems. In these vacuum degassers, solvents passing through tubes of semi porous polymer membranes inside a chamber, which is evacuated to eliminate dissolved gaseous molecules.

1.2. Column Oven

In reversed-phase HPLC, column temperature is a strong determinant of retention time and also affects column selectivity. A column oven is therefore required for most automated pharmaceutical assays to improve retention time precision, typically at temperatures of 30-50°C. Temperatures >60° are atypical due to concerns about thermal degradation of the analytes and column lifetimes. Column ovens operate either by circulating heated air or by direct contact (clam-shell type). Solvent pre-heating is achieved by passing the mobile phase through a long coiled tube embedded onto the heating element before the column. Trends in column oven design are moving towards wider temperature ranges (e.g., 4-80°C with the use of a peltier cooling device.

1.3. Pump

This provides the constant and continuous flow of mobile phase through the system; most modern pumps allow controlled mixing of different solvents from different reservoirs. Pumps are operated in a following pressure range-

1) 0-40 MPa (0-400 bar, 0-5880 psi) up to 5 ml/min
2) 0-20 MPa (0-200 bar, 0-2950 psi) up to 10 ml/min

HPLC pumps can be categorized in several ways:

a. **Flow range:** A typical analytical pump has flow range of 0.001-10 ml/min, which handles comfortably the flow rates required by most pharmaceutical assays (e.g. 0.5-3 ml/min). Preparative pumps with flow ranges of 30 ml/min up to liters/min.
b. **Driving mechanism:** Most pumps use a reciprocating driving mechanism. The exceptions are screw driven syringe pumps for capillary LC applications.

Low pressure mixing system uses a single pump to deliver mobile phases generated by an upstream proportioning valve. High pressure mixing system

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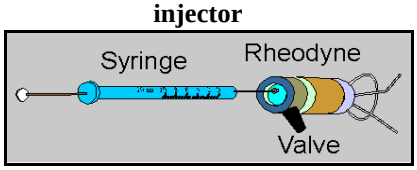
uses two or more pumps to proportion solvent downstream at high pressure and it is more preferred.

1.4. Injector

HPLC injector allows an introduction (injection) of the analyte mixture into the stream of the mobile phase before it enters the column under high pressure. A common injector is the Rheodyne model 7125 or 7725 injector which consists of a six-port valve with a rotor, a sample loop, and a needle port. For manual injection, a syringe with a 22-gauge blunt-tip needle is used to introduce a precise sample aliquot into the sample loop at the LOAD position. The sample is delivered into the column by switching the valve to the INJECT position.

Figure 2: Schematic representation of rheodyne injector



1.5. Column

This is the heart of HPLC system; it actually produces a separation of the analyte in the mixture. A column is the place where the mobile phase is in contact with the stationary phase, forming an interface with enormous surface. Most of the chromatography development in recent years went toward the design of many different ways to enhance this interfacial contact. Generally column operating pressure ranges are 500–3,000 psi having life time of 500–2,000 injections or 3 -24 months.

Figure 3: HPLC column

1.6. Detector

This is a device for continuous recording of specific physical (sometimes chemical) properties of the column effluent. The most common detector used in pharmaceutical analysis is UV (ultraviolet), which allows monitoring and continuous recording of the UV absorbance at a selected wavelength or over a span of wavelengths (diode array detection). Appearance of the analyte in the detector flow-cell causes the change of the absorbance. Many modern detectors have dual- or multiple-wavelength detection capability. A photodiode array detector (PDA), also known as a diode array detector (DAD), provides UV spectra of eluting peaks while functioning as a multiwavelength UV/Vis. detector. It facilitates peak identification or peak purity evaluation, by comparing the upslope, apex and down slope spectra, assessment of UV spectral peak purity can give some limited assurance that there is not an impurity with different characteristics co-eluting with the main peak.

1.7. Data Acquisition and Control System

Computer-based system that controls all parameters of HPLC instrument [eluent composition (mixing of different solvents); temperature, injection sequence, etc.] and acquires data from the detector and monitors system performance (continuous monitoring of the mobile-phase composition, temperature, backpressure, etc.)[5].

2. HPLC Trouble Shooting[1,6-12]: A systematic approach is best to identify when trouble shooting occurs in HPLC. The trouble shooting problems in HPLC are categorised in following categories.

1. Problem with peaks.
2. Problem with baseline.
3. Problem with retention time.
4. Problem with pressure.
5. Miscellaneous problems.

2.1. Problem with peaks: Broad peaks

Cause	Prevention
Injection volume too large.	Inject smaller volumes or reduce solvent strength for injection to focus the sample components.
Poor column efficiency.	Use mobile phases of lower viscosity, elevated column temperature, lower flow rate or a packing with smaller particle size
Extra column volume of LC system is too large	Use zero dead volume fitting and connectors; use smallest possible size tube diameter.
Volume of detector cell too large.	Use smallest possible cell volume for the sensitivity required; use a detector without heat exchanger in the system.
Detector time constant is too slow.	Adjust the time constant to the peak width.
Sampling rate of the data system is too low.	Increase the sampling rate.
Retention time is too long	Use gradient elution or stronger mobile phase composition.

Fronting:

Cause	Prevention
Column overload	Decrease sample amount.
Formation of channels in the column.	Buy a new column or have the column repacked.

Tailing:

Cause	Prevention
Basic Analytes.	Use Silica base de-activated RP phases.
Sample components which can form chelates: metal traces in the packing.	Only use high-purity silica-based packings with their very low metal contamination; add EDTA or another chelating compound to the mobile phase; switch to polymer-based columns
System dead volume	Minimize the number of connections; use zero-dead-volume connectors; use new capillaries to column/ to detector; check whether all fittings are tight.
Degradation at higher temperatures	Use column oven temperature below 50^{o}c

Double Peaks:

Cause	Prevention
Injection solvent too strong.	Use a weaker solvent for the sample or a stronger mobile phase.
Dead volume or formation of channels in the column.	Replace the column or, if possible, open the upper end fitting and fill the void with the same packing; have the column repacked.
Simultaneous late elution of a substance from a previous run.	Flush the column with a strong eluent after each run, or end gradient at a higher concentration.

Negative Peaks:

Cause	Prevention
RI of the analyte is lower than mobile phase.	Reverse detector to polarity to obtain positive peaks.
UV absorption of the analyte is lower than the mobile phase.	Use mobile phase with lower UV absorption.

Ghost Peaks:

Cause	Prevention
Contamination	Use only HPLC grade solvents as mobile phase.
Late elution of analyte from the previous run	Flush the solvent with stronger eluent after each run.
Unknown interfering substance in sample	Use fractioning prior to sample run.
Contaminated water	Check suitability of water for the column

Spikes:

Cause	Prevention
Air bubble in the mobile phase.	Degas the mobile phase
Column was stored without end caps.	Always stored column tightly cap.

2.2. Problems with baseline: Baseline noise

Causes	Prevention
Air in mobile phase, detector, pump	Degas the mobile phase.
Pressure close to maximum	Minimize the backpressure by reducing the flow rate.
Pump Pulsation	Clean or exchange the valves of pulse heads.
Weak detector lamp	Replace the lamp.

Baseline drifting to higher and lower absorptions:

Cause	Preventions
Retention time is too long.	Use gradient elution or a stronger mobile phase for isocratic elution.
Accumulation and elution of impurities.	Use only HPLC grade solvents; clean the contaminated column with a strong solvent.
Poor column efficiency	Use mobile phase of low viscosity and higher column oven temperatures, flow rate.
Strong UV absorption of mobile phase.	Non UV absorbing mobile phases
Viscosity of mobile phase is too high	Increase the column oven temperature

2.3. Problem with Retention time: Decreasing and increasing retention time

Cause	Prevention
Loss of bonded stationary phase.	Replace column; for silica adsorbents use mobile phases between pH 2 and pH 8 or switch to phases with higher pH stability
Decreasing flow rate.	Check and – if necessary – adjust the pump flow rate; check for pump cavitations; check for leaking pump seals and other leaks in the system.
Increasing flow rate	Adjust the flow rate.
Column overload with sample	Decrease the sample amounts.
Changing the mobile phase composition	Cover the solvent reservoir to avoid the evaporation of solvents.

Fluctuating Retention times:

Cause	Prevention
leaks	see at "leaks"
Insufficient buffer capacity.	use buffer concentrations above20 mmol/L.
Insufficient equilibration at isocratic separation.	Pass 10 to 15 column volumes of mobile phase through the column for equilibration
Insufficient equilibration at reversed phase ion-pairing chromatography.	Increase the equilibration time; in ion-pairing chromatography sometimes 50 column volumes may be required for equilibration

2.4. Problem with Pressure:

Increasing or decreasing Pressure:

Cause	Prevention
Contamination at the column inlet	Use an 0.5 µm in-line filter; use guard columns; back flush column with a strong solvent in order to dissolve the impurity; replace plugged inlet frits /guard column.
Viscosity of mobile phase too high.	Use a solvent of lower viscosity or increase the temperature.
For polymer-based columns: swelling of the adsorbent caused by eluent changes.	Use only solvents compatible with the column; check proper eluent composition; consult instructions for use for solvent compatibility; use a column with a higher degree of cross-linking.
When the injector is disconnected from the column: plugged injector.	Clean the injector or replace the rotor.
Precipitation of buffer components	Proper clean up of column
Air bubble in the pump	Proper degas the column
Leaks the tube line between column and detector	Tighten all fitting replace the defective ones.

2.5. Miscellaneous Problems:

Lack of selectivity:

Cause	Prevention
Not enough sample injected.	Increase amount of sample for injection.
Peaks outside the linear range of the detector.	Dilute or enrich the sample until the concentration is in the linear range of the detector.
Auto sample line is blocked	Check the flow and clear any blockages.
Detector attenuation set is too high	Reduce the detector attenuation.

Poor sample recovery:

Cause	Prevention
Hydrophobic interactions between stationary phase and biomolecules.	Use short-chain reversed phase packings; as an alternative you may use hydrophilic stationary phases or ion exchangers.
Adsorption on tubing and other hardware components.	Use inert tubing and fittings made from, e.g., PEEK or titanium.
Adsorption on to stationary phase	Increase the mobile phase strength

Leaks:

Cause	Prevention
Serious leaks at column or fittings.	Tighten loose fittings or use new fittings.
Serious leak at the injector.	Replace worn or scratched valve rotors.
Serious leak at the pump	Replace the defective pump seals

3. Conclusion

High performance liquid chromatography is the widely encountered technique for the routine analysis of the drugs in pharmaceutical formulations. The various problems has been get encountered while performing the method development by RP-HPLC. Here in this review article the trouble shooting parameters are well described. These trouble shooting guidelines will help the analyst to maintain the HPLC system free of problems and also allows the smooth running of the system. It reduces the cost of the operation too.

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