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Review Article

Troubleshooting and maintenance of high-performance liquid chromatography- A Review

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ABSTRACT

HPLC (High Performance Liquid Chromatography) has evolved into an indispensable tool in many analytical laboratories and is applied to diverse analytical problems. Troubleshooting HPLC instrumentation and separations require a fundamental understanding of how the instrument functions and how the separation works. This review gives an overview of HPLC system maintenance and summarizes strategies and guidelines for HPLC troubleshooting. Every HPLC system consists of the same basic components, no matter if it is a modular system. Problems can take place in each component, can change the overall performance, and will consume more cost to recover the problems. This review paper provides common maintenance procedures that can be performed by the user and simple guidelines for maintenance and troubleshooting of HPLC and solving characteristic and common problems in HPLC. An easy-to-use table describes probable cause and solutions. The objective behind this article to help the Analyst in troubleshooting and maintenance of High Pressure Liquid Chromatography, and to overcome the shortcomings that may arise during their projects.

KEYWORDS: HPLC, HPLC Troubleshooting, Maintenance, HPLC guide

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INTRODUCTION

HPLC is a potent analytical tool, allowing for the separation, identification and quantification of drug substances. Every HPLC system consists of the same important components, no matter if it is a modular system or a specialized all-in-one unit. Various parts of a typical HPLC instrument are depicted in Fig.1.Some of the most common major problems observed in HPLC separations are Pressure abnormalities, Leaks, Problems with the chromatogram, Injector problems, other problems detected by smell, sight, and sound.

Troubleshooting helps us to quickly identify problem causes and solutions. Before starting any troubleshooting, whether it is related to instruments or columns, it is essential that safe laboratory practices be observed [10].

A typical troubleshooting process involves the following steps:

1) Verify that a problem actually exists.
2) Isolate the cause of the problem.
3) Correct the cause of the problem.
4) Verify that the problem has been corrected.
5) Follow up to prevent future problems

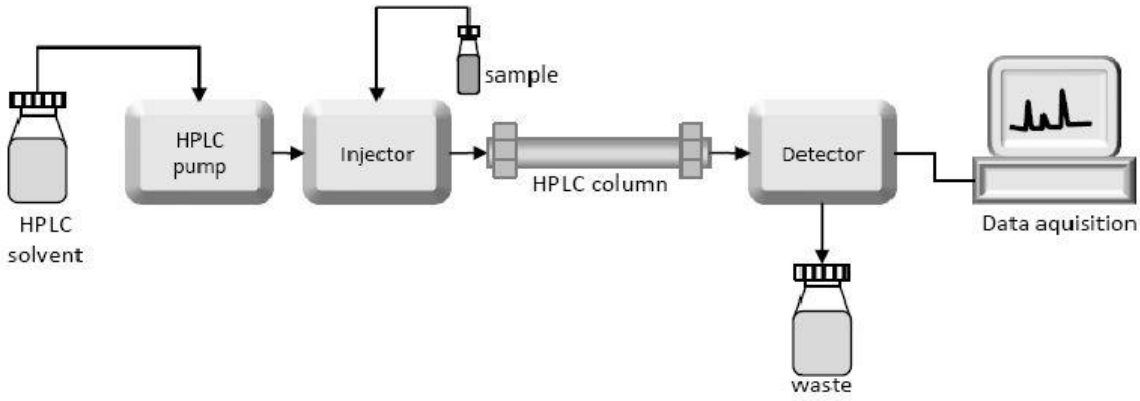

Figure 1: Schematic diagram of HPLC

TROUBLE SHOOTING PROBLEMS:

It is important to remember that once the problem is defined and possible corrective action is identified, only one change at a time should be made; after each change, the whole system should be checked again to determine whether the problem still exists or whether the change corrected the problem [2].

Mobile phase problems: The reagents and solvents should be of the highest prescribed quality. Deionized water often contains trace levels of organic compounds and so therefore is not recommended for HPLC use [2, 4, 8]. Ensure that any water used in buffer preparation is of the highest purity. HPLC grade reagents contain no impurities to produce spurious peaks in a chromatogram baseline, whereas AR grade reagents do contain trace levels of impurity, which may produce spurious baseline peaks. Ultra-pure HPLC water (18MΩ resistivity) is generated by passing deionized water through an ion exchange bed [2, 4-5, 8]. Alternately, HPLC grade water can be purchased from solvent suppliers.

Buffers: All buffers should be prepared freshly on the day required. This practice ensures that the buffer pH is unaffected by prolonged storage and that there is no microbial growth present. Changes in pH and microbial growth will affect chromatography. Buffer reagents can contain a stabilizing agent, for example, sodium metabisulphite [6].

Filtration: Ideally, all HPLC solvents should be filtered through a 0.45 µm filter before use [2]. This removes any particulate matter that may cause blockages. After filtration, the solvents should be stored in a covered reservoir to prevent contamination with dust etc. Filtering HPLC solvents will be benefit both chromatography and the wear and tear of the HPLC system. Pump plungers, seals and check valves will perform better and lifetimes will be maximized [2, 8].

Degassing: Before the freshly prepared mobile phase is pumped around the HPLC system, it should be thoroughly degassed to remove all dissolved gasses [2, 6]. Dissolved gas can be removed from solution by Bubbling with helium, Sonication, Vacuum filtration. If the mobile phase is not degassed, air bubbles can form in the high-pressure system resulting in problems with system instability, spurious baseline peaks etc. [2, 6].

Pump problems: The pump must deliver a constant flow of solvent to the column over a wide

range of conditions. Modern HPLC pumps incorporate single or dual piston, syringe, or diaphragm pump designs [1, 2, 4, 8]. Pumping system problems are usually easy to spot and correct. Some of the most common symptoms are erratic retention times, noisy baselines, or spikes in the chromatogram. Leaks at pump fittings or seals will result in poor chromatography. A sure sign of a leak is a buildup of salts at a pump connection. Buffer salts should be flushed from the system daily with fresh deionized water. Pump seals require periodic replacement [2, 4]. Regular maintenance should be performed rather than waiting for a problem to occur. HPLC system should be run constantly at low flow rates (e.g. 0.1ml/min) to avoid crystallization effects. Other locations where problems can occur are the check valves in the pump head. For e.g. when the pump is not able to produce a constant flow/pressure, clean the check valves with isopropanol or dismantle the check valves and clean them in an ultrasonic bath using isopropanol. Be sure while refitting the check valves in the pump head, that the valves are inserted in the right direction. If this procedure is not successful, replace check valves [2, 8]. In some cases, prolonged use of ion pair reagents has a lubricating effect on the pump pistons that produce small leaks at the seal [2, 6].

Pressure Problems: The pressure problem can occur suddenly or be a gradual process. Sudden pressure rises tend to be due to particles from the sample, blocked or damaged tubing or column packed bed collapse. Gradual pressure rises due to particles in the sample, but they can also arise from particles generated in the instrument, for example, debris from vial septa or degrading seals. Pressure problems fall into one of three categories: low pressure, fluctuating pressure, high pressure [6, 10]. The simplest way to troubleshoot pressure problems is using a systematic approach, as highlighted in following tables for high, low or fluctuating pressure [6, 10].

Injector/injection problems: The injector rapidly introduces the sample into the system with minimal disruption of the solvent flow. HPLC systems currently use variable loop, fixed loop, and syringe-type injectors [2,]. Mechanical problems involving the injector (e.g., leaks, plugged capillary tubing, worn seals) are easy to spot and correct [4]. Variable peak heights, split peaks and broad peaks can be caused by incompletely filled sample loops, incompatibility of the injection solvent with the mobile phase, or poor sample solubility. Whenever possible, dissolve and inject samples in the mobile phase [2]. Be aware that some auto samplers use separate syringe wash solutions. Make sure that the wash solution is compatible with and weaker than the mobile phase. This is especially important when switching between reversed phase and normal phase analyses [4, 8].

Column Protection: Protect your analytical column from sample and system debris and contaminants to maintain the column performance and efficiency. Guard columns or cartridges are one of the most cost effective and efficient ways of trapping these unwanted system components [2]. A 10 mm length guard for moderate to heavy contamination. Although not an integral part of most equipment, mobile phase inlet filters, pre-injector and pre-column filters, and guard columns greatly reduce problems associated with complex separations [1-2]. All samples are filtered through 0.45µm or 0.2µm syringe filters [2, 8]. The useful life of these disposable products depends on mobile phase composition, sample purity, pH, etc. As these devices become contaminated or plugged with particles,

Detector problems: Detector-related problems include leaks, air bubbles, and cell contamination. These usually produce spikes, baseline noise or drift in the chromatograms or low sensitivity [3]. The sensitivity of universal detector for HPLC has not been devised yet. A number of different detectors are available for HPLC systems [2-3]. The most common are fixed and variable wavelength ultraviolet spectrophotometers, refractive index, and conductivity detectors[3]. Detector problems fall into two categories – electrical and mechanical/optical. For electrical problems, we should contact the instrument manufacturer. Mechanical or optical problems can usually be traced to the flow cell. Some flow cells – especially those used in refractive index detectors – are sensitive to pressure [2, 7]. Flow rates or back pressures that exceed the manufacturer's recommendation will break the cell window. Faulty or reversed cable connections can also be the source of problems [2, 3, 7].

PROBLEMS AND SOLUTIONS

Abnormal Pressure: A change in the operating pressure is a sign that there may be a problem. Choose the category below that best fits the symptoms and follow the suggestions to correct the problem [1, 5, 8, 9].

Leaks: Leaks are usually stopped by tightening or replacing a fitting. Over tightened metal compression fittings can leak and plastic finger tights can wear out and damaged fittings should be discarded and replaced [1, 2, 5, 8, 9].

Problems with the Chromatogram: Many problems in an HPLC system show up as changes in the chromatogram. Selecting the proper column type and mobile phase are keys to "good chromatography" [1, 5, 8, 9].

Problems with the Injector: The problems are usually detected while using the injection valve. Leaky injection valves are discussed in leak section [1-3, 5, 8- 9].

Problems Detected by Smell, Sight, or Sound: All senses must be used to identify HPLC problems. This will help to locate problems quickly. The majority of problems are identified by sight; most of these are included in the proceeding section [1, 5, 8, 9].

Table 1:

Problem	Possible Cause	Solution
No pressure reading, no flow	1. Power off 2. Fuse blown 3. Broken piston 4. Air trapped in pump head 5. Insufficient mobile phase 6. Faulty check valve(s)	1. Turn on power 2. Replace fuse 3. Replace piston 4. Degas solvents: bleed air from pump, prime pump 5. Replenish reservoir or Replace inlet frit if blocked 6. Replace check valve(s)
No pressure reading, flow is normal	1. Faulty meter 2. Faulty pressure transducer	1. Replace meter 2. Replace transducer

Table 2:

Problem	Possible Cause	Solution
Steady high pressure or pressure climbing	1. Flow rate set too high 2. Blocked column frit 3. Improper mobile phase; precipitated buffer 4. Injector blockage 5. Column temperature too low 6. Blocked guard column	1. Adjust setting 2. Back flush column or Replace column 3. Use correct mobile phase and Wash column 4. Clear blockage or replace injector 5. Raise temperature 6. Remove/replace guard column
Steady low pressure or pressure dropping, but not to zero	1. Flow set too low 2. Leak in system 3. Column temperature too high	1. Adjust flow rate 2. Locate leak and correct 3. Lower temperature
Pressure cycling	1. Air in pump 2. Pump seal failure 3. Insufficient degassing	1. Bleed air from pump 2. Replace pump seal 3. Degas solvent

Table 3:

Problem	Possible Cause	Solution
Leaky fittings	1. Loose fitting 2. Stripped fitting 3. Over tightened fitting	1. Tighten 2. Replace 3. Loosen and retighten or replace
Leaks at pump	1. Loose check valves 2. Loose fittings 3. Mixer seal failure 4. Pump seal failure 5. Pressure transducer failure 6. Pulse damper failure	1. Tighten check valve 2. Tighten fittings 3. a. Replace mixer seal b. Replace mixer 4. Repair or replace 5. Repair or replace 6. Replace pulse damper

Table 4:

Problem	Possible Cause	Solution
Injector leaks	1. Rotor seal failure 2. Loose injection-port seal 3. Improper syringe-needle diameter 4. Waste-line blockage	1. Rebuild or replace injector 2. Adjust 3. Use correct syringe 4. Replace waste line
Column leaks	1. Loose end fitting 2. Column packing in ferrule	1. Tighten end fitting 2. Disassemble, rinse ferrule, reassemble
Detector leaks	1. Cell gasket failure 2. Cracked cell window(s) 3. Leaky fittings 4. Blocked waste line	1. Prevent excessive back pressure or Replace gasket 2. Replace window(s) 3. Tighten or replace 4. Replace waste line

Table 5:

Problem	Possible Cause	Solution
Peak tailing	1. Blocked frit 2. Interfering peak 3. Wrong mobile-phase pH 4. Sample reacting with active sites	1. a. Reverse flush column or Replace column 2. Change mobile-phase and/or column 3. Adjust pH 4. Add ion pair reagent or volatile basic Modifier or change column
Peak fronting	1. Low temperature 2. Sample overload	1. Increase column temperature 2. Decrease sample concentration
Split peaks	1. Contamination on guard or analytical column inlet 2. Sample solvent incompatible with mobile phase	1. a. Remove guard column and attempt analysis b. Replace guard if necessary c. If analytical column is obstructed, reverse and flush 2. Change solvent; whenever possible, inject samples in mobile phase

Table 6:

Problem	Possible Cause	Solution
Distortion of larger peaks	1. Sample overload	1. Reduce sample size
Distortion of early peaks	1. Wrong injection solvent	1. Reduce injection volume or Use weaker injection solvent

Table 7:

Problem	Possible Cause	Solution
Increased tailing as k' increases	1. Secondary retention effects, reversed-phase mode 2. Secondary retention effects, ion-pair	1. a. Add triethylamine (basic samples) b. Add acetate (acidic samples) c. Add salt or buffer (ionic samples) 2. Add triethylamine (basic samples
Acidic or basic peaks tail	1. Inadequate buffering	1. a. Use 50–100mm buffer concentration b. Use buffer with pKa equal to pH ofmobile phase

Extra peaks	1. Other components in sample 2. Late-eluting peak from previous injection 3. Vacancy or ghost peaks	1. Normal 2. Increase run time or gradient slope or Increase flow rate 3. a. Check purity of mobile phase b. Use mobile phase as injection solvent

Table 8:

Problem	Possible Cause	Solution
Retention time drifts	1. Poor temperature control 2. Poor column equilibration	1. Thermostat column 2. Allow more time for column equilibration between runs
Abrupt retention time changes	1. Flow rate change 2. Air bubble in pump 3. Improper mobile phase	1. Reset flow rate 2. Bleed air from pump 3. Replace with proper mobile phase

Table 9:

Problem	Possible Cause	Solution
Baseline drift	1. Column temperature fluctuation 2. Nonhomogeneous mobile phase 3. Contaminant or air buildup in detector cell 4. Slow column equilibration, especially when changing mobile phase 5. Strongly retained materials in sample can elute a very broad peaks and appear to be a problem 6. Detector (UV) not set at absorbance maximum but at slope of curve	1. control column and mobile phase temperature 2. a. Use HPLC-grade solvents, high-purity salts, additives and Degas mobile phase 3. Flush cell with methanol or other strong solvent 4. a. Flush with intermediate strength solvent b. Run 10–20 column volumes of new mobile phase before analysis 5. a. Use guard column b. If necessary, flush column with strong solvent between injections or periodically during analysis 6. Change wavelength to UV absorbance maximum
Baseline noise (regular)	1. Air in mobile phase, detector cell, or pump 2. Incomplete mobile phase mixing 3. Pump pulsations	1. a. Degas mobile phase b. Flush system to remove air from detector cell or pump 2. Mix mobile phase by hand or use less viscous solvent 3. Incorporate pulse dampener into system

Table 10:

Problem	Possible Cause	Solution
Baseline noise (irregular)	1. Mobile phase solvents immiscible 2. Air trapped in system 3. Air bubbles in detector 4. Detector cell contaminated 5. Column leaking silica or packing material	1. Select and use only miscible solvents 2. Flush system with strong solvent 3. Purge detector or Install back-pressure device after detector 4. Clean cell by flushing with 1 n HNO_3 5. Replace column

Broad peaks	1. Mobile-phase composition changed 2. Mobile-phase flow rate too low 3. Leaks (especially between column and detector) 4. Detector settings incorrect 5. Buffer concentration too low 6. Column contaminated 7. low plate number 8. poorly resolved compounds Column temperature too low 9. Detector time constant too large	1. Prepare new mobile phase 2. Adjust flow rate 3. Check for loose fittings Check pump for leaks, salt build-up, and unusual noises 4. Adjust setting 5. Increase concentration 6. Replace column with new one of same type. If new column provides symmetrical peaks, flush old column with strong solvent 7. Open inlet end and fill void or replace column 8. Increase temperature; do not exceed 75°C unless higher temperatures are acceptable to column manufacturer 9. Use smaller time constant

Table 11:

Problem	Possible Cause	Solution
Loss of resolution	1. Mobile phase contaminated/deteriorated 2. Obstructed guard or analytical column	1. Prepare new mobile phase 2. a. Remove guard column and attempt analysis or Replace guard if necessary b. If analytical column is obstructed, reverse an flush
All peaks too small	1. Detector attenuation too high 2. Injection size too small	1. Reduce attenuation 2. Use larger sample loop
All peaks too large	1. Detector attenuation too low 2. Injection size too large 3. Improper recorder connection	1. Use larger attenuation 2. Use smaller sample loop 3. Use correct connection

Table 12:

Problem	Possible Cause	Solution
Manual injector, hard to turn	1. Damaged rotor seal 2. Rotor too tight	1. Rebuild or replace valve 2. Adjust rotor tension
Manual injector, hard to load	1. Valve misaligned 2. Blocked loop 3. Dirty syringe	1. Adjust alignment 2. Replace loop 3. Clean or replace syringe

Table 13:

Problem	Possible Cause	Solution
Auto injector will not turn	1. No air pressure (or power) 2. Rotor too tight 3. Valve misaligned	1. Supply proper pressure (power) 2. Adjust 3. Adjust alignment
Auto injector, other problems	1. Blockage 2. Jammed mechanism 3. Faulty controller	1. Clear or replace blocked portion 2. See service manual 3. Repair or replace controller

Table 14:

Problem	Possible Cause	Solution
Solvent smell	1. Leak 2. Spill	1. See leak section 2. a. Check for overflowing waste container b. Locate spill and clean up
"Hot" smell	1. Overheating module	1. a. Check for proper ventilation, temperature setting adjust b. Shut module off, see service manual
Abnormal meter readings	1. Pressure abnormality 2. Column oven problem 3. Detector lamp failing	1. See abnormal section 2. a. Check settings, adjust b. See service manual 3. Replace lamp

Table 15:

Problem	Possible Cause	Solution
Warning lamps	1. Pressure limit exceeded 2. Other warning lamps	1. Check for blockage or Check limit setting, adjust 2. See service manual
Warning buzzers	1. Solvent leak/spill 2. Other warning buzzers	1. Locate and correct 2. See service manual
Squeaks and squeals	1. Bearing failure 2. Poor lubrication 3. Mechanical wear	1. See service manual 2. Lubricate as necessary 3. See service manual

Conclusion

High Performance Liquid Chromatography has wide variety of applications in many fields such as analysis & separations of pharmaceuticals, biochemistry, analyzing the air and water pollutants, monitoring the pesticide levels in the environment. HPLC is made of several critical components. This chapter gives an overview of HPLC system maintenance and summarizes strategies and guidelines for HPLC troubleshooting.

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