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Review Article

Review on Common Observed HPLC Troubleshooting Problems

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ABSTRACT

HPLC provide much higher resolution, more accurate quantitative results, as well as shorter analysis times in comparison to the earlier techniques, HPLC has evolved into an indispensable tool in many analytical laboratories. Troubleshooting is a form of problem solving, often applied to repair failed products or processes. Actually, HPLC refers to a number of separation techniques that use a liquid mobile phase, or eluent. Troubleshooting HPLC instrumentation and separations require a fundamental understanding of how the instrument functions and how the separation works. Common HPLC problems are caused by component malfunctions (pump, degasser, injector, detector, data system, column), and faulty preparation of the mobile phase or sample preparation. Always use well cleaned glassware for preparation for better results. Main key to resolve HPLC problem is Cleaning of HPLC with appropriate solvent. If any problem occurs, it is advisable to perform a quick visual check of the instrument and column. If Monograph or any protocol is provided then try to follow same process. Troubleshooting is a form of problem solving, often applied to repair failed products or processes. Otherwise, we suggest that you read the entire article so as to pick up some ideas that will help you avoid problems in the future. We hope that this article useful to diagnose problems and to gain understanding of underlying causes so that you can prevent or minimize future occurrence. Colum and HPLC care is most important part of troubleshooting. In This article, we tried to cover all major troubleshooting problems.

Key words: HPLC troubleshooting, Column Storage & Cleaning, Basic of HPLC.

1. INTRODUCTION

Before starting any troubleshooting, whether it is related to instruments or columns, it is essential that safe laboratory practices be observed. The chemical and physical properties of any solvents used should be known and the material safety data sheet (MSDS) for theses solvents should be readily available. Troubleshooting is a form of problem

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solving, often applied to repair failed products or processes. It is a logical, systematic search for the source of a problem so that it can be solved, and so the product or process can be made operational again. Troubleshooting is needed to develop and maintain complex systems where the symptoms of a problem can have many possible causes.

Troubleshooting is used in many fields such as engineering, system administration, electronics, automotive repair, and diagnostic medicine. Troubleshooting requires identification of the malfunction(s) or symptoms within a system. Then, experience is commonly used to generate possible causes of the symptoms [1-2].

Having troubles on HPLCs can be incredibly frustrating. For example, You're trying to get some analysis done, and your research can't move forward until you figure out why your tools aren't working. There are some common problems of HPLC columns that pop up from time to time. Knowing what these are and how to fix them can save you hours of frustration. Read on to discover some of these problems and how to address them. We limited this article in basic level. We would recommend receiving consultation with the manufacturer of HPLC system. Main key to resolve HPLC problem is Cleaning of HPLC with appropriate solvent [10].

1.1 Types of HPLC

Depending on the substrate used i.e. stationary phase used, the HPLC is divided into following types [11]

- Normal Phase HPLC: In this method the separation is based on polarity. The stationary phase is polar, mostly silica is used and the non-polar phase used is hexane, chloroform and diethyl ether. The polar samples are retained on column.
- Reverse Phase HPLC: It is reverse to normal phase HPLC. The mobile phase is polar and the stationary phase is non polar or hydrophobic. The more is the non-polar nature the more it will be retained.
- Size-exclusion HPLC: The column will be incorporating with precisely controlled substrate molecules. Based on the difference in molecular sizes the separation of constituents will occur.
- Ion-exchange HPLC: The stationary phase is having ionically charged surface opposite to the sample charge. The mobile phase used is aqueous buffer which will control pH and ionic strength [11]

HPLC is an advanced form of liquid chromatography used in separating the complex mixture of molecules encountered in chemical and biological systems, in order to recognize better the role of individual molecules. It was in the year 1980, HPLC methods appeared for the first time for the assay of bulk drug materials (United States Pharmacopoeia, 1980). [1, 29]

Before the discovery of chromatography, techniques like gravimetric analysis, photometry, colorimetry (UV, visible detection), titrimetric (acid-base detection), etc. were sole methods available for analysis. Even the requirements of analysis for research were simple, i.e., there was no necessity for analysis of complex molecules, similar molecules (i.e., molecules with the same chemical and physical properties). But as research advanced there was the requirement to analyze all the molecules in a given sample for better detection of the problem (in the clinic), impurities and also deficiencies in industry and research. This was not possible with a single technique like photometric, titrimetric, etc. due to the greater physical and chemical similarity in molecules of a sample like phytoconstituents, amino acids and neurotransmitters etc [15, 16]. The components in the sample are separated based on their affinity to the molecules in the column. After the compounds in the sample are separated, they pass the detectors. User software is utilized in the HPLC technique for the data analysis [12].

Actually, HPLC refers to a number of separation techniques that use a liquid mobile phase, or eluent. Troubleshooting HPLC instrumentation and separations require a fundamental understanding of how the instrument functions and how the separation works. The practical approach presented here is meant to serve as both a troubleshooting guide and an HPLC learning tool [3].

1.2 Visual Inspection:

When a problem occurs, it is advisable to perform a quick visual check of the instrument and column. This will pick up leaks, lose or disconnected tubing, changes in instrument settings, etc [4, 9].

1.3 No Peaks:

Single or multiple missing peaks are usually due to the wrong sample being injected of the sample degrading. Equally likely though is a loss of resolution due to column/solvent inconsistencies. There can be several factors that cause no peaks or very small peaks to show up on HPLC outputs. Normal readings should have large, thin peaks that may vary somewhat in height. Small peaks or no peaks at all may mean your detector lamp is turned off, you have no mobile phase flow, your sample is missing or deteriorated, or there's a problem with your

Detector, integrator, injector valve or Start by making sure your detector is turned on, and then check all the electrical connections and cables. Make sure your auto sampler vials have enough liquid and that there are no air bubbles in the sample, and recheck the system with a new standard solution. If that doesn't work, check the attenuation or gain settings status, and auto zero. Also check for Loose/broken wire between detector and integrator or recorder, no mobile phase flow, no sample/deteriorated sample/wrong sample. Settings too high on detector or recorder [5].

1.4 No Flow

If it is getting absolutely no peaks on your output, you may have no flow in your HPLC column. This may mean your pump is off or the flow is interrupted or obstructed somehow. It may also have a leak or air trapped in the pump head. Start the pump if it's off, and check the mobile phase levels in the reservoir and flow throughout the system. Check the sample loop for any obstructions or air locks, and

make sure the mobile phase components are miscible and the mobile phase is properly degassed.

Also check for Pump off., Flow interrupted/obstructed, Any Leak or any Air trapped in pump head. From there, move on to checking the system for loose fitting and the pump for leaks or other issues. Disconnect the tubing at the guard column if you have one, and check for flow. If you're still having problems, purge the pump at a high flow rate, prime the system, loosen any check valves your system may have, and, if all else fails, flush the system with 100percent methanol or isopropanol [6].

1.5 Detector leaks

It is always recommend to check parameters like possible cause Solution, Cell gasket failure, prevent excessive backpressure or replace gasket, cracked cell window(s) Replace cell window(s), Leaky fittings Tighten or replace fittings, Blocked waste line Replace waste line, Blocked flow cell [4-9]. It is also recommended to choose the proper HPLC detector for a given application [13].

1.6 Pressure

If HPLC pressure is lower than usual or you have no pressure, you may have a leak or air rapped somewhere in the system. You might also have a faulty check valve or an obstructed or interrupted mobile phase flow. If your system pressure is too high, there's likely a problem in the pump, injector, in-line filter, or tubing, or you might have obstructed guard or analytical columns.

For high pressure, start by removing the guard and analytical columns from the system and replacing them with unions to reconnect the injector to the detector. Run the pump at 2-5 mL/min, and work on isolating the cause, starting with the detector, then the in-line filter and working back to the pump. If the problem seems to be with the analytical column, reverse and flush the column while it's disconnected from the detector, and change the inlet frit or replace the column if necessary.

For low pressure, start by checking the whole system for leaks, loose fittings, faulty pump seals, or bad valves. If everything looks okay, run the same mobile phase check we discussed earlier and check for flow at the analytical and guard columns. If that doesn't work, reconnect everything and try pumping solvent at double the flow rate. Also check for any Leak, Mobile phase flow interrupted/obstructed. Air trapped in pump head or any Leak at column inlet end fitting.

System pressure is affected by a number of variables including the viscosity of the solvent used, column variables, flow rate and temperature. It is important to have a reference point when high or low pressures to the norm. This reference point should be the pressure generated in the system when everything if functioning correctly.

Pressure problems fall into one of three categories: high, low or fluctuating pressure. They can occur suddenly or be a gradual process. Sudden pressure rise stand to be due to particles from the sample, blocked or damaged tubing or column packed bed collapse. Gradual pressure rises can also be 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. You can also check Problem in pump, injector, in-line filter, or tubing. Obstructed guard column or analytical column [9].

2. PEAK AREA PRECISION SYMPTOM

2.1 Inappropriate detector settings

1) Possible Cause: Wrong detection wavelength(s) Solution: Measuring in a UV/fluorescence spectrum flank can compromise the precision. Choose a detection wavelength or an excitation/emission wavelength pair near the apex of the spectrum/spectra. If spectra of analytes are very different, a wavelength switch might be required.

2) Possible Cause: Response time too short, high noise, imprecise integration at trace level

Solution: Make sure to use suitable response time (or time constant) settings. Typically set a response time that is about 1/4 of the peak width at half-height of the narrowest peak. Follow the operating instructions for details.

3) Possible Cause: Incorrect nebulizer temperature (Thermo Scientific Dionex Corona Detectors ultra (RS)

Solution: Check nebulizer temperature setting. If using large amounts of THF or halogenated solvents in mobile Phase, set temperature to 30 °C. If analyte is semi volatile, it may be necessary to turn off the nebulizer heater in order to recover response.

4) Possible Cause: Not enough data points

Solution: Set the data collection rate at least to 20-30 data points for reproducible peak integration [10].

2.2 Injection volume variation

1) Possible Cause: Auto sampler draws air from the vial

Solution: Check the sample filling height and the sampling height of the injector needle.

2) Possible Cause: Sample degradation

Solution: Use appropriate storage conditions, e.g., thermostatted auto sampler.

3) Possible Cause: Air in the auto sampler fluidics

Solution: Flush out auto sampler fluidics following the steps laid out in the respective operating instructions.

2.3 Peak Integration Settings

1) Possible Cause: Positions of integration delimiters vary

Solution: Check the software integration settings. For instance, with the support of the Thermo Scientific Dionex Chromeleon help. Best, facilitate the Chromeleon 7 Cobra algorithm and let it identify the ideal settings foryou. Avoid automatic data rate settings and use a fixed data rate [10].

2.4 Variable Retention Times:

Increase RT: Changing mobile Phase composition, Decrease flow rate, Bubble in mobile phase

Decrease RT: Increase flow rate, Column overloaded, Active group on stationary phase

Temperature: Reduction of Retention with Increasing

Temperature: 1% to 2% Change / per 1° Celsius

Pump Flow Rate Problem (check actual volume/time being delivered)

• Wrong Column Type (C8 – less retention, vs C18 – more retention)

• Temperature Problem (warmer – less retention, colder – more retention)

• % organic in Mobile Phase (more organic – less retention, less organic –more retention)

Other factors which can be effect Retention time:

Solvent Composition, Temperature, pH-Control, Ion Pairing

Drifting Retention: Equilibration, Stationary Phase, Stability, Column Contamination and any Leakage. Change in mobile phase composition. (Small changes can lead to large changes in retention times.) Air trapped in pump. (Retention times increase and decrease at random times. Column temperature fluctuations, Column overloading. (Retention times usually decrease as mass of solute injected on column exceeds column capacity.) Sample solvent incompatible with mobile phase. Column problem. (Not a common cause of erratic retention. As a column ages, retention times gradually decrease) Mobile Phase composition change, Column chemistry change, Flow problem, Valve failure [3-9]

2.5 Column Back Pressure

It may be depend on but not limited to, Locate Pressure problem, in line filter, Guard Cartridge, and Buffer precipitation

2.6 Baseline problems:

Baseline irregularities can be non-cyclic or cyclic. They can originate from electrical interferences, detector faults, solvent impurities, column contamination, etc. To isolate the source of a baseline irregularity, it is important to determine whether the problem lies with the fluid path, detector or electrical connections [14].

2.7 Non-cyclic noise – Detector electronics problems

The most common cause of problems related to electronic baseline noise is the detector. Usually, if the detector is allowed insufficient time to equilibrate before an injection is performed, then the resultant chromatogram will contain spurious peaks and there will also be some evidence of baseline drift.

2.8 Loss of Resolution

Mobile phase contaminated/deteriorated (causing retention times and/or selectivity to change). Obstructed guard or analytical column. Resolution can be failed if any expired standard or sample used. Resolution also depend on correct column and correct temperature.

2.9 Abnormal peak shape:

Abnormal peak shape encompasses a range of possible peak shape problems:Fronting or tailing peaks, no peaks, smaller than expected peaks, broad peaks – early eluting analytes or all analytes, double peaks/shouldering peaks, flat topped peaks, negative peaks

2.10 Broad Peak:

Broad peaks are most often due to errors in instrumentation or column. It is worth while investigating the column and guards first as they often are the critical part of the system.

2.11 Split Peak:

If you start seeing peaks show up that dip down in the middle, making an M shape, you may have some contamination on your guard or analytical column inlet. You may also have a partially blocked frit or an uneven void at the column inlet. It's also possible that your sample solvent is incompatible with the mobile phase [4]

If you think you have contamination at an inlet, reverse and flush the column. You can also repack the top of the column with pellicle particles of the same bonded phase functionality and continue using the column in reverse flow direction.

If you think the problem is with the sample, adjust the sample in the mobile phase. Difference of pH in the sample and mobile phase causes split peaks. Contamination on guard or analytical column inlet. Partially blocked frit. Small (uneven) void at column inlet. Sample solvent incompatible with mobile phase.

2.12 Peaks Tail on Initial and Later Injections

It can be depend on several factor like, Sample reacting with active sites, Wrong mobile phase pH, Wrong column type, Small (uneven) void at column inlet, Wrong injection solvent, Column Destroyed, Incorrect Sample Solvent, Secondary Interactions ,Column Overload, Mass Overload, Volume Overload, Other Extra-Column Effects like Sampling Rate & Time Constant

2.13 Tailing Peaks:

We can be resolving this problem by washing Column with Isopropyl alcohol. Tailing peaks are typically caused by column degradation or inlet contamination. Carefully maintained columns and guards will considerably reduce the incidence of tailing peaks. The other reason for tailing factor can be operate at a lower pH, use a highly deactivated column, consider the possibility of mass overload, consider the possibility of column bed deformation, work at high pH when analyzing basic compounds, use a sample clean-up procedure

You may notice that rather than coming up and down in straight lines, your peaks start to develop a slight slant at the front or back, called fronting or tailing. This usually means guard or analytical column may be worn out or column may be overloaded. Tailing maybe caused by a contaminated or deteriorated mobile phase or interfering mobile components in the sample, while fronting may result from problems with the sample solvent.

If you have tailing peaks, start by removing the guard column and attempting analysis, replacing it if necessary. You may also need to restore or replace the analytic column. Be sure to check on the make-up of the mobile phase and the column performance. Guard Or Analytical Column Contaminated/Worn Out. Mobile Phase

Contaminated/Deteriorated. Interfering Components In Sample Partially Plugged Column Inlet Filter
Remove end-fitting, Contaminated In-Line Filter, Contaminated Guard Column, Replace guard column/insert, Fronting Peaks [1-9]
Column: Connection, Replace frit/ Guard Column, Regenerate or replace column, Column overloaded. Sample solvent incompatible with mobile phase. Shoulder or gradual baseline rise before a main peak may be another sample component.

2.14 Negative peaks:

Negative peaks are most often caused by difference in refractive index between the sample solvent, sample and mobile phase. They are also caused after routine maintenance when the system has not been reconfigured correctly.

2.15 Rounded Peaks:

Detector operating outside linear dynamic range. Recorder gain set too low. Column overloaded.
Sample-column interaction. Detector and/or recorder time constants are set too high.

2.16 Baseline Drift:

Column temperature fluctuation. (Even small changes cause cyclic baseline rise and fall. Most often affects refractive index and conductivity detectors, UV detectors at high sensitivity or in indirect photometric mode.) Nonhomogeneous mobile phase (Drift usually to higher absorbance, rather than cyclic pattern from temperature fluctuation.) Contaminant or air buildup in detector cell. Plugged outlet line after detector. (High pressure cracks cell window, producing noisy baseline.) Mobile phase mixing problem or change in flow rate. Slow column equilibration, especially when changing mobile phase. Mobile phase contaminated, deteriorated, or not prepared from high quality chemicals. Strongly retained materials in sample can elute as very broad peaks and appear to be a rising baseline. (Gradient analyses can aggravate problem.) Detector (UV) not set at absorbance maximum but at slope of curve.
The following steps may be tried.
1. Turn off the instrument pump - fluid flow must be zero
2. Monitor the baseline for 5 to 10 min. note if there is any improvement in the baseline's appearance. If yes, then the problem lies within the instrument fluid path. If no, the problem is either electrical or detector related.
3. Disconnect the detector electrical cables from the A/D interface with PC, integrator and chart recorder, *i.e.* the data handling devices. Attach a jump source to the input terminals on the data-handling device (a crocodile clip, paper clip). If the noise continues, then the problem is within the data handling device [9].

2.17 Too low signal-to-noise:

1) Possible Cause: Non-ideal fluorescence detector settings
Solution: Scan for best excitation and emission wavelengths, optimize the gain of the photomultiplier, use high-quality mobile phase only, and set suitable response time.
2) Possible Cause: Non-ideal UV detector settings Solution: Scan for best absorption wavelength(s), set suitable response time and optimize slit widths and bandwidths according to the operating manual [10].

2.18 Bubbles Problem in Solvent line:

The Best Solution is Consider an in-line degassing accessory unit for your HPLC system. Some modern HPLC systems contain a built-in degasser.
Alternatives: Helium sparging – be careful not to alter mobile phase composition by evaporation of volatile components. Offline vacuum degassing in an ultrasonic bath –incomplete method which provides short-lived degassing

2.19 Degas:

It improves unstable and noisy baselines. Commonly used degassing practices for HPLC mobile phase are Helium purging, vacuum degassing, sonication [17].
Boiling is the most effective technique to get rid of dissolved air completely but it is never advised because of loss of volatile components along with the gases and also it takes a long time to equilibrate the mobile phase to the required ambient temperature conditions.
Helium purging removes up to 80% of dissolved air. For organic – aqueous mobile phase an equal volume of helium for purging is adequate. The rate of supply of helium can be reduced after some time as excessive purging can lead to loss of more volatile mobile phase components.
Vacuum degassing removes more than 60% of dissolved air. One option is to apply vacuum during filtration of mobile phase through 0.45 or 0.22 m porosity membrane filter. On – line vacuum degassing is available on most commercial available systems. Mobile phase is passed through porous polymer tubing placed in a vacuum chamber inside the HPLC. The porosity of the tubing allows expulsion of gases through the walls but the liquid is retained in the tubing.
Sonication using ultrasonic baths is common in most laboratories but as a stand-alone technique it removes only up to 30% dissolved air so sonication in combination with any other technique is recommended.
For practical purposes it is advisable to degas mobile phase in both low and high pressure mixing systems and combination of different techniques can eliminate most of the problems associated with bubble formation. As on-line vaccum degassing is offered on most commercially available systems sonication in combination with on-line degassing gives satisfactory results.

2.20 Improper Prime of System : Failure to flush all of the lines with freshly degassed mobile phase before use (every day) will often result in all kinds of instabilities until all of the old gas filled mobile phase has been purged from the system. This could take many column volumes of liquid [22-24].

3. HPLC TROUBLESHOOTING FILTRATION

Even when you have carefully chosen a suitable method and equipment for HPLC, problems can still occur, and troubleshooting becomes an unfortunate use of valuable time. When troubleshooting, sample preparation is often a key consideration. So, what are some of the possibilities you might consider regarding your sample preparation filtration!

Why is filtration recommended: Although some high throughput approaches may not allow for filtration, filtering your sample as a minimum preparation step is highly recommend. Unfiltered particulates in samples can often clog instrument interfaces and HPLC columns, causing increased backpressure or blocking the device altogether. Filtering samples is a simple, cost-effective way of removing particulate material from sample, thereby increasing instrument and column longevity, right-first-time rate of analysis, and potentially providing better chromatographic results. Filtration is usually a way to physically remove particulate species out of solution. Generally, there is no chemical separation exerted by the filtration and, if sample clean-up is required, you may need to consider other, more selective, sample preparation methods One notable exception is ultra filtration, where pressure or concentration gradients are used to force separation through a semi-permeable membrane. Fundamentally, filtration is a simple and actionable method to improve your analytical outcomes. So how do you go about selecting the correct syringe filter like Non-specific binding, Filter clogging, choosing the right size filter, Extractable and Evaluating Filtration Efficiency [25, 9].

3.1 Purge HPLC:

When setting up an HPLC system, the aim of the purge is simply to flush through all the lines so that any remaining solvent in them from a previous analysis or wash is replaced with the new mobile phase [18]. As you would expect, the amount of new solvent required depends on the volume of the tubing in the system. The major contribution to the volume of tubing is usually the on-line vacuum degasser, if installed. The design of these modules is such that they require a relatively large volume to perform well. For example, a typical Agilent 1200 degasser has a volume of ~12 mL for each line. Agilent recommends a purge of at least 30mL in total for the 1200 systems. Therefore, to estimate how much volume is required for a purge, check the volume specification of the inlet tubing, the degasser and the pump that you are using, and then double it to give an approximate purge volume. You may find that the manufacturer of your particular instrument has a recommended amount to use. A flow rate of 5mL/min is commonly used for standard HPLC systems.

3.2 Vial Related Issue:

Use Self Sealing Septum to minimize evaporative loss of volatiles. Wrong choice of Septum may result in:

- Evaporative loss of sample
- Lack of reproducibility for repetitive injections
- Septum coring
- Needle damage
- Septum dislodging

Peak area increases after first injection from thesame vial (first injection – low, latter injections OK). Possible cause: Inadequate venting upon needle piercing the septum/cap for the FIRST Time (Vacuum formation) caused by the septum/cap sealing around the injection needle. Vacuum draws some sample back out of the needle situation can be aggravated by over filling the vial (Never fill the vial all the way to the top)

Test: remove cap and septum from vial, perform multiple injections, measure peak areato determine if the septum/cap is the cause.

Symptom: Peak area varies (increases /decreases) from injection to injection from the same vial

Possible cause: Coring of Septum by Needle If using a bottom draw port needle, the draw port could be plugged with septum material.

Check needle draw port for septum material, remove / replace.

Solution: if using self-sealing PTFE/silicone septum:

Switch to preslit PTFE/silicone septum. Preslit septum will eliminate coring and deliver good Resealing capability Or, Switch to PTFE septum. PTFE will eliminate coring. However, it will not reseal [1-9]

3.3 HPLC columns require relatively little care:

However, they can be damaged if:

1. Dropped on the ground
2. Banged around (drawer)
3. Stored in the freezer/ refrigerator (depends on type of solvent)

3.4 Column Regeneration:

It may be regenerate column with 0.1N HCL.

Always follow column vendor's guideline for regeneration. Regeneration can bring back a column's performance if problem relates to compounds, which are retained under method conditions, causing changes in chromatography. Washing them off with more aggressive solvents can return performance. If surface has been chemically altered, i.e. hydrolysis of ligands and end capping, then performance may not be restored

3.5 Column Storage:

Store in Mobile Phase for Short Periods of Time (<72hrs.). Store in Shipping Solvent for Longer

Periods of Time. Column should be stored in solvent which manufacturer recommends. For bonded phases, use organic solvent (eg. MeOH or ACN) -- Using non-aqueous solvents minimizes hydrolysis. Some bonded phases (CN) become unstable in polar organic mobile phases. Storage in water or buffer is then okay. Columns which may be stored in Water or Buffered Solvents like Ion exchangers or Aqueous SEC packings.

However you can Prevent microbial growth by using0.05% sodium azide in mobile phase or Small quantity of organic solvent (Acetonitrile 5% or methanol 10%)

3.6 Aggressive buffers that should be avoided:

Phosphate has been found more aggressive in the alkaline pH range other buffers. So, I do not recommend to use alkaline phosphate buffers with silica based or related packaging based on inorganic-organic hybrid. Phosphate has a limited pH range anyway. Ammonia is also fairly aggressive, but it can be substituted without difficulty with organic amine.

3.7 Colum damage by Air:

If the reservoir runs dry in your HPLC pump you will draw air into the pump, but the pump will not pump air through the system. HPLC pumps are designed to pump liquid, not air. When they fill with air, they lose their prime and stop pumping [26]. You may pump a few bubbles of air into the column before the pump quits entirely, but the pump will not have pumped air through the column continually if it was left running all night. Even if it had, it is unlikely to have caused problems. To correct the problem, you will need to first re-prime the pump by purging the air from all the lines. I would remove the column before starting. Start by degassing the solvent thoroughly. If you use an in-line degasser, this may be sufficient, otherwise I recommend helium sparging. Then open the purge valve at the pump outlet and prime the pump in the normal manner. With some pumps, this will mean filling the tubing between the reservoir(s) and pump with the aid of a syringe [30].

3.8 Gradient Dwell volume:

It is volume between the point where the gradient is mixed and the column inlet. This volume delays the onset of the gradient & is so, also called gradient delay volume.

4. MOBILE PHASE pH and pH BUFFERS

Choosing the mobile phase you use is one of the most important decisions you make in determining the outcome of your HPLC analysis. Second only to the column, the mobile phase type and its polarity and quality greatly affect the efficiency and accuracy of your results. For something this important, anyone using an HPLC, whether directly or as part of a manufacturing/release process, should understand what mobile phase is and how it works [20, 21].

The purpose of mobile phase is to move the sample through the column and separate the components of the sample by slowing them down. These components are separated by size, shape, charge, polarity, hydrophobic state, and binding capacity and are captured in the column. The separation occurs when the sample "slows down" compared to the mobile phase and the individual components that begin to separate from the mobile phase are detected and quantified.

pH plays a crucial role in determining retention and selectivity, as well as in controlling the accuracy and precision of a method [27, 28]. There is no universal "best" pH for mobile phase, since the sample characteristics and the desired analytes play a factor in determining the ideal pH for a run. What is universal is the necessity of having a consistent and accurate pH that will remain at the required value for the usable life of the mobile phase. This can be minutes or hours (the time to run a sample set through the HPLC), all the way up to 6 months or longer in certain closed systems. In cases where the sample could affect the pH during a typical run, buffer should be included in the make-up of the mobile phase [29]

pH Effects Ionization

– Silica Surface of Column

– Sample Components of Interest

• Buffers

– Resist Changes in pH and Maintain Retention

– Improve Peak Shape for Ionizable Compounds

• Effects Column Life

– Low pH strips Bonded Phase

– High pH Dissolves Silica

Column and HPLC Cleaning:

Frequency: [19]

- General cleaning Daily
- Syringe, loop & injector plunger seal: After completion of each analysis.
- Flow cell, pipeline, suction filter: When Problem Arise
- Keep the record of cleaning.

Flush with stronger solvent then your mobile phase.

A. Water Soluble Samples Flush with the following:

1. Flush with warm (50 °C) distilled water
2. Acetonitrile
3. Methanol

B. Samples Not Soluble in Water Flush with the following:

1. Methylene chloride
3. Hexane
4. Isopropyl Alcohol (Also Improve Tailing Factor)

When Complete your run always make habit to wash your system with your column with Acetonitrile: Water (50%:50%) or Methanol: Water (50%:50%)

Also clean with same solvent by removing column and by fixing union in place of column. [30, 31]

5. CONCLUSION

It states, prevention always better than the cure in case of HPLC Problem. By the way often these problems are not associated with the column and may be caused by instrument and chemistry issues like pH of mobile Phase, Instrument Connections, Detector Settings, and Metal Contamination. The thing is start with the correct questions and finds the answers & the answers will lead to solutions. HPLC has a place in research, product assessment, and environmental monitoring. The best solution to avoid HPLC problem is the Maintenance of HPLC system.

6. REFERENCES

1. Troubleshooting in high performance liquid chromatography. https://www.pharmatutor.org/articles/troubleshooting-in-high-performance-liquid-chromatography-hplc (accessed 4th June 2020).

2. Only a world-class drug development process can have a worldwide effect. https://www.waters.com/nextgen/us/en.html (Accessed: 6th June 2020).
3. The Common Problems of HPLC Columns and How to Deal With It. https://develosil.us/common-problems-of-hplc-columns-and-how-to-deal-with-it/ (Accessed 16th June 2020).
4. HPLC Trouble shooting guide. https://www.sigmaaldrich.com/technical-documents/articles/analytical/hplc-troubleshooting-guide.html (Accessed 20th June 2020).
5. HPLC Troubleshooting hints and tips. https://www.chromservis.eu/c/hplc-troubleshooting-Hints and tips - Chromatography (Accessed18th June 2020).
6. Johan Dolan, HPLC Troubleshooting Guide. www.ace-hplc.com (Accessed on18 June 2020).
7. Tips and Tricks of HPLC System Troubleshooting. https://www.agilent.com/cs/library/slidepresentation/Public/Tips_and_Tricks_HPLC_Troubleshooting.pdf (Accessed on14th June 2020).
8. Chromatography Blog. https://www.crawfordscientific.com (Accessed on 17th June 2020).
9. Armelle Vallat, Troubleshooting Guide HPLC, www.pbf.unizg.hr (Accessed on16th June 2020).
10. Akshaykumar VB, Jitendra SS, Hitendra BP et al, A Review on HPLC-Trouble Shooting Guide. Int J Pharm Sci Rev Res 2014; 27: 200-9.
11. Mukthi T. A Review on High Performance Liquid Chromatography (HPLC). Research & Reviews: J Pharm Anal 2016; 5: 22-8.
12. Divya TN. High Performance Liquid Chromatography in analysis. Research & Reviews in Pharmacy and Pharmaceutical Sciences 2014; 3: 73-5.
13. Michael S. HPLC detectors: A brief review, J Liq Chromatogr R T 2010; 9: 1130-50.
14. Pankti PD. A Guide to Problem-Solving in High Performance Liquid Chromatography. J Pharm Res 2012; 5; 4411-20.
15. HPLC Chromatography | Its Principle and Working Methodology. https://www.studyread.com/hplc-chromatography-principle/(Accessed on 7th July 2020).
16. Quantitative errors in HPLC. http://www.interchromforum.com/html/qnt_err_hplc.html (Accessed on7th July 2020).
17. Why degassing of HPLC mobile phase necessary. https://lab-training.com/2013/11/18/why-is-degassing-of-hplc-mobile-phase-necessary/(Accessed on 7th July 2020).
18. Help on: How long should it take to purge the lines on my HPLC system? http://blog.mournetrainingservices.co.uk/2014/01/help-on-how-long-should-it-take-to.html (Accessed on 7th July 2020).
19. SOP for cleaning the high performance liquid chromatography. https://www.pharmaguideline.com/2011/08/sop-for-cleaning-of-high-performance.html#gsc.tab=0 (Accessed on 7th July 2020).
20. Mobile Phase: Know what you are putting into your HPLC. https://www.lifecyclebio.com/2018/05/25/mobile-phase-know-what-you-are-putting-into-your-hplc/ (Accessed on 6th July 2020).
21. Ozlem C. Separation techniques: Chromatography, North Clin Istanbul 2016;3:156–60.
22. HPLC Chromatography Hints and Tips For Chromatographers. https://hplctips.blogspot.com/2014/01/diagnosing-troubleshooting-hplc.html (Accessed on 20th July 2020).
23. Michael ES. UPLC: An Introduction and Review. J Liq Chromatogr R T 2005; 28: 1253-63.
24. A Review on Analytical Method Development and Validation of Pharmaceutical Technology. https://www.pharmatutor.org/articles/review-on-analytical-method-development-and-validation-of-pharmaceutical-technology (Accessed on 6th July 2020).
25. Stavros Kromidas. More Practical Problem Solving in HPLC, Wiley: 2004.
26. HPLC. https://lab-training.com/hplc/ (Accessed on 10th July 2020).
27. Patil MP. HPLC Method Development–A Review. J Pharm Edu Res 2017;1:243-60.
28. Mobile phase degasing. https://www.chromatographytoday.com/news/hplc-uhplc/31/breaking-news/what-is-mobile-phase-degassing/31347 (Accessed on 20th July 2020).
29. Siddiqui MR, AlOthman ZA, Rahman N. Analytical techniques in pharmaceutical analysis: A review. Arab J Chem 2017;10:S1409-21.
30. Does Air Damage my Column? https://blog.sepscience.com/liquidchromatography/hplc-solutions-8-does-air-damage-my-column (Accessed on 20th July 2020).
31. Anagha SP. A Review on Ultra Performance Liquid Chromatography (UPLC). Asian J Pharm Tech Innovation 2015; 3(10); 86-96.

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