International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 1635-1646 www.ijprajournal.com ISSN: 2456-4494

An Overview on Identifying and Solving Common Problems in HPLC Troubleshooting.

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Date of Submission: 28-05-2024 Date of Acceptance: 05-06-2024

ABSTRACT –
In many industries, such as pharmaceuticals, food science, and environmental analysis, High-performance liquid chromatography (HPLC), is useful analytical method. The accuracy and dependability of the results can be jeopardized by a number of issues, just like with any analytical technique, including HPLC. The causes, remedies, and common issues with HPLC analysis are covered here. In HPLC analysis, column leakage and blockage are among the most frequent issues. Reduced resolution and peak shape can result from column leakage, which can be caused by a broken or badly installed column. optimum calibration curve establishment, improving the signal to noise ratio, enhancing specificity, checking pump performance, etc. However, a build-up of particulate matter or sample residue in the column can cause column blockage, which lowers flow rate and delays retention time. Baseline noise and drift in HPLC analysis are another issue that can arise from impurities in the sample or mobile phase, as well as from insufficient degassing of the mobile phase. So based on such common issues of HPLC here are some beneficial hints for developing methods, such as selecting gradient or isocratic conditions, and advice on maintaining the HPLC system and columns. Users of HPLC may also find this review paper to be a helpful guide. This information covers HPLC system maintenance and troubleshooting strategies. HPLC is widely utilized in research, product evaluation, and environmental monitoring. Regular maintenance is crucial to prevent issues. HPLC is effective for separating higher molecular weight compounds and offers both qualitative and quantitative information.

KEYWORDS –
Maintain HPLC system, HPLC Troubleshooting, Troubleshoot guide, Analytical Errors, HPLC related issues.

I. INTRODUCTION –

Troubleshooting is a methodical approach to problem-solving that is frequently used to fix problems with broken processes or products[1]. Each component of HPLC has the potential to develop issues that could alter overall performance and increase recovery cost Issues may emerge in every part and impact the overall functionality of the system[2]. In order to solve an issue and bring back the operational functionality of a process or product, it entails a logical and systematic investigation to determine the underlying cause of the issue[4]. This methodical approach is especially important and annoying to run into problems with High-Performance Liquid Chromatography (HPLC)[8]. Understanding these research analysis issues and their fixes can help you save a lot of time and frustration. The purpose of this article is to give a basic understanding, primarily for those with a basic level of expertise[15].

One of the most important steps in fixing HPLC problems is to carefully clean the system using the right solvent. Based on the stationary phase that is used HPLC can be of several types such as Normal, Reverse, Size-exclusion, Ion-exchange HPLC. High-Performance Liquid Chromatography (HPLC) is a sophisticated version belonging to chromatography intended to efficiently separate intricate mixtures of molecules found in biological and chemical systems. Before the development of chromatography, the only methods available for analysis were photometric techniques, colorimetric analysis (UV and visible light detection), gravimetric analysis, and titrimetry (acid-base titration methods). [1]

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Volume 9, Issue 3 May-June 2024, pp: 1635-1646 www.ijprajournal.com ISSN: 2456-4494

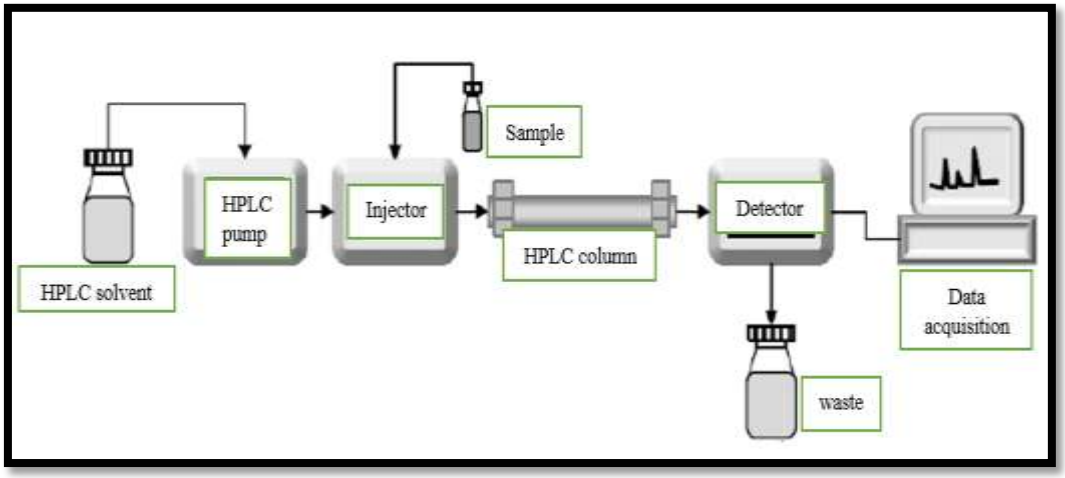

Fig no 01- The diagrammatic representation of HPLC System

As research developed, it became necessary to thoroughly examine every molecule in a sample in order to identify contaminants, identify shortcomings in industry and research, and improve problem detection in clinical settings. The need for such a comprehensive analysis was too great for a single method like titrimetric or photometric analysis. This limitation was especially noticeable because molecules in a sample, such as phyto-constituents, amino acids, neurotransmitters, and others, shared a great deal of physical and chemical similarities. The sample's constituents are categorized based on how well they attach to the molecules within the column. The compounds go through detectors after separation. High-Performance Liquid Chromatography (HPLC) data analysis necessitates the utilization of dedicated software[1].

HPLC includes several separation methods that make use of a liquid mobile phase, or eluent. A basic grasp of the operation of the instrument and the rules guiding the separation process is necessary to troubleshoot HPLC instrumentation and separations in an efficient manner. This context's practical approach is meant to serve as an HPLC learning tool as well as a troubleshooting guide.

1. MOBILE PHASE RELATED PROBLEM IDENTIFICATION WITH ITS SOLUTIONS-

Issues related to the mobile phase can lead to challenges such as reduced sensitivity, fluctuations, and the presence of interference or peaks in the chromatogram. Impurities present in the mobile phase pose significant challenges, particularly in gradient elution[1]. The baseline may exhibit an increase, and false peaks might emerge as the concentration of the contaminated component rises. Nonhomogeneous Mobile Phase Troubleshooting where there may be a drift, usually in the direction of greater absorbance instead of a recurring pattern caused by variations in temperature. Contaminants or accumulation of air in the detector cell, a blocked outlet line downstream of the detector causing cracks under high pressure in the cell window and a noisy baseline, problems with mobile phase mixing, flow rate variations, reversed phase analyses are particularly susceptible to water-based contaminants.

It is imperative to utilize solely high-grade deionized (DI) water when preparing the mobile phases. Nevertheless, certain deionization methods can introduce organic impurities into the water [2]. Ion pair reagents may precipitate in mobile phase when acetonitrile and some other organic solvents are present in high concentrations (>50%). Furthermore, precipitation results from certain salts made from ion-pair reagents having low water solubility. This precipitation problem can be avoided by substituting sodium-containing buffers for potassium-containing buffers when used with long-chain sulfonic acids (such as sodium dodecyl sulfate). Volatile acidic and basic modifiers, like triethylamine (TEA) and trifluoroacetic acid (TFA), are helpful for the recovery of compounds meant for additional analysis. To fix this problem, you can also with concentrations ranging from 0.1% to 1.0% TEA and 0.05% to 0.15% TFA[14].

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1.1 Isocratic or gradient mobile phase-

A resolution serves as a helpful visual tool for optimizing spacing between peaks, achievable through the graphical representation of resolution against the percentage of organic solvent. While comparing both isocratic and gradient mobile phase, when it comes to isocratic its easier and more excellent for method transfer in system and column of different dimensions and baseline noise. But the gradient is easier and more excellent, when it comes to peak shape, limit of detection, with ability to alter selectivity. Increasing the Speed of an Isocratic Method for some variables affect column efficiency without changing selectivity. These variables include particle size, length of column, and flow rate. Conversely, temperature is a non-column parameter that can affect efficiency and selectivity. Increasing the flow rate can shorten the analysis time, but going over the ideal flow rate as the Van Deemter plot shows that it reduces efficiency, especially when dealing with larger particles. Reducing the length of the column presents an additional method to accelerate analysis.

The length adjustment of the column directly affects the pressure, analysis time, and column efficiency. A third strategy is to change the particle sizeto shorten the runtime. But unlike with column length adjustments, the corresponding Altering column efficiency, pressure, and resolution is a more complex process[14]. Pressure varies with the particle size squared adjustment, resolution varies using the square root of the particle size, and column efficiency varies proportionately with changes in particle size.

It is essential to understand the dwell volume of the systemto account for gradient dwell time, mixer purging, and gradient deformation when executing fast gradients. When working with smaller chromatography columns or shorter gradient times, this concern becomes more apparent. The gradient time should ideally be at least twice as long as the dwell time. high pressure tolerance, and compact mixer volume. Longer gradients are less difficult to work with and less likely to distort. To verify to assess the precision of the mixing, a gradient step test is performed. To provide adequate back pressure, a capillary tubing is used in place of the column[15].

Tablet no 01 - Techniques for HPLC Mobile Phases

TECHNIQUES	ACTION TAKEN FOR TECHNIQUES
Helium Purging	Eliminate dissolved air to 80% To prevent losing more volatile components, use an equal volume of helium for purging in organic-aqueous mobile phases and gradually decrease the helium supply. Using Undergoing vacuum degassing removes over 60% of the dissolved air. Negative pressure can be applied through a specific filter during mobile phase filtration. Porous polymer tubing is used in numerous commercial systems to provide online vacuum degassing[15.]
Sonication	Eliminates 30% of the dissolved air when used alone. For optimal effects, it is usually advised to combine sonication with other techniques. Degassing the mobile phase makes sense from a practical standpoint in both high-pressure and low-pressure mixing systems. [15]

2. PERFORMING A VISUAL EXAMINATION-

To prevent any noticeable abnormalities such as leaks, loose tubing, disconnected components, or changes in instrument parameters, conduct a brief visual examination of both the column and the instrument[1].

3. INSUFFICIENT OR INTERRUPTION OF FLOW TO COLUMN OR TO A PUMP-

The lack or absence of peaks caused by a malfunctioning detector lamp, absence of mobile phase flow, missing or deteriorated samples, alternatively issues with the integrator, injector valve, or detector could all be indicated by small peaks, or by their complete absence. First ensure

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your detector is activated, then check all of the wires and connections before beginning any troubleshooting. Make sure there are enough liquid bubble-free vials in your auto-sampler, and use a fresh standard solution to reevaluate the system. Inspect the status of attenuation or gain settings and initiate an auto-zero procedure if the problem continues[2]. Next, verify the levels of the mobile phase in the reservoir and evaluate the systems overall flow to look for any possible disruptions or blockages. Check for any air locks or obstructions in the sample loop. Make sure the constituents of the mobile phase are miscible and sufficiently degassed. Look for possible issues like air trapped in the pump head, leaks, flow obstructions or interruptions, or the pump being turned off. Examine any loose fittings in the system and check the pump for any anomalies or leaks.

If required, flush the system by using 100% methanol or isopropanol. If the issue Continues, clean the valves using isopropanol. If this proves ineffective, resort to an ultrasonic bath with isopropanol. The efficiency of pump seals can be compromised by highly concentrated salts and caustic mobile phases[2]. Potential problems could be trapped air or leaks in the system, a malfunctioning check valve, Inspect the system for leaks or trapped air, and make sure the check valve is operating[1]. Controlling the pressure of analytical columns, pump, injector, in-line filter, and tubing.

4. ADJUSTING PEAK SPACING-

A resolution map serves as a valuable visual tool for fine-tuning spacing between peaks and can be generated by graphing resolution against the percentage of organic components. This information can be derived utilizing the general resolution formula and the correlation with solvent potency. The optimization of the retention factor for effective separation for following.

Table no 02-PEAK SPACING

FACTORS	SOLUTION
Peak spacing	samples comprising analytes with diverse functional groups, altering the percentage of organic components can significantly alter peak spacing To enhance resolution to its maximum potential
Selectivity(Column-type selectivity)	Adjustments through modifications to the composition of the mobile phase The selectivity, as well as the creation of peak spacing, is notably impacted by the chemical composition of the stationary phase. Column bonded phase type, mobile phase composition, and analyte chemistry all affect selectivity. Selectivity for non-ionic compounds cannot be changed by adjusting the chemistry of the analyte Selectivity changes dramatically when one switches from a C18 group column to a cyano or polar-embedded group column Type of organic solvent simultaneously with the chromatography column may result in a more significant shift in selectivity. Mobile phase pH is a powerful tool that provides an additional means of adjusting selectivity, especially for ionizable compounds (such as bases and acids).
Distortions (fronting, tailing, or split peaks)	Introduced by large injection volumes, strong sample solvents, or a combination of both The injection solvent's volume and strength must be carefully considered in order to reduce peak distortion. It is advised to use the least concentrated solvent feasible to dissolve the sample and The injected volume must not exceed 15% of the volume of the initial peak of interest. Either aexcessive mass or volume loading on the column can lead to peak distortion. If you inject ten times less mass into the column and observe changes in the shape of peaks and their retention times, you can determine if mass overload is the cause

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Changes in peak shape	split peak issue - indicate overloading, A singular instance reported in demonstrated a unconnected to overload, column frit obstruction, problems with the inline filter, or secondary interactions with the column. Peak tailing - unwanted secondary interactions, especially when basic or acidic compounds interact with metal contamination in the silica or participate in ion exchange interactions. Peak distortion - significant increase in extra-column volume or by column degradation in extremely acidic environments

5. VALUABLE INSIGHTS AND STRATEGIES, TIME-SAVING APPROACHES, AND KEY INFORMATION:

Tablet no 03- Guideline & Guiding Principles Rule

FACTORS	HOW TO ADDRESS ISSUE
Temperature and retention adjustment (isocratic separation)	(2% per °c) 1 °C rise results in 2% shift in retention time
Rule of singular adjustment and rule of dual validation	For that addressing an issue, modify a single variable at a time and also to ensure the issue is consistently reproducible
Isocratic separation	Expect the retention factor to undergo a roughly threefold change with 10% variation in the concentration of the potent solvent.

6. FINDING AND FIXING LEAKS IN DETECTORS-

The prevalent detectors found in HPLC systems include UV spectrophotometers with fixed and variable wavelengths, refractive index sensing devices, and conductivity sensors[2]. If a flow cell gasket failure is suspected, promptly replace the gasket or take preventive measures to prevent excessive backpressure. Address any damaged cell windows immediately, ensuring proper fittings and addressing clogs in the waste line to restore normal flow. Operate the pump at a flow rate of 2 to 5 ml/min[1]. Check for leaks at the inlet end fitting of the column, air entrapment in the pump head and interruptions or blockages in the flow of the mobile phase.

Understanding the number of factors, such as solvent viscosity, column specifications, flow rate, and temperature, affect system pressure. One of the reasons for the slow rise in pressure is when the column packed bed collapsing. This phenomenon could be caused by particles created inside the instrument, like broken seals or debris from vial septa, in addition to particles in the sample itself. Furthermore, problems with the pump, injector, in-line filter, or tubing may be connected to pressure problems[1]. Re-prime the pump accordingly. Remove the column before initiating. Ensure thorough degassing of the solvent. When employing an in-line degasser, this might suffice; alternatively, consider helium sparging if needed. Open the purge valve at the pump outlet and prime the pump as usual. Some pumps may require filling the tubing between the reservoirs and the pump using a syringe[14].

7. PEAK SHAPE ABNORMALITIES AND SOLUTIONS:

These include no peaks, regular/problematic peaks, fronting or tailing peaks, missing peaks, surprisingly small peaks, broad peaks impacting all or early-eluting analytes, double peaks or shouldering peaks, flat-topped peaks, and negative peaks due to Instrumentation or column errors.

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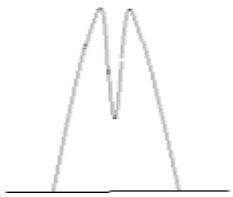
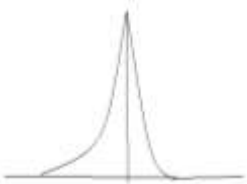
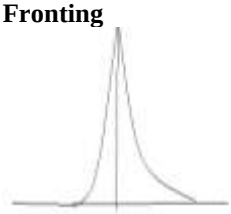
Table no 04- PEAK SHAPE ABNORMALITIES

FACTORS AFFECTING PEAK SHAPE	CAUSES	DIAGRAMMATIC REPRESENTATION
Negative Peaks	**Causes-** Variations in the refractive indices of the sample, sample solvent and mobile phase **How to address-** It mayarise from lower analyte absorbance than the mobile phase or disruptions in the mobile phase equilibrium post-injection through the column. **To deal with Negative peaks –** Modify the sample solvent as needed, and consider diluting the sample in the mobile phase whenever feasible However, for PDA or UVD, consider employing a solvent with absorbance similar to or slightly lower than the analyte. Inspect the column inlet ferrule for tightness, as loose connections may introduce air and result in negative peaks. Ensure thorough filtration and sonication of solvents before usage.	**Negative** 
Split Peak	**Cause-** In the mobile phase, modify the sample if it is thought to be the cause of the problem. **How to address split-** The emergence of M-shaped peaks with a midpoint dip indicates possible contamination A small (uneven) void at inlet of on or variations in the pH of the mobile phase and the sample the guard or analytical column **To deal with-** Peak splitting issues can often be resolved by employing reverse flushing, which effectively eliminates contaminants from the column. This method may also dissolve absorbed impurities if they are soluble in the mobile phase.	**Splits**  
Tailing Peaks or peaks	**Causes-** Column degradation or inlet contamination **How to address fronting-** A tainted or degraded mobile phase or mobile components that interfere with the sample could cause tailing, and problems with the sample solvent Replace mobile phase Cleaning the column with isopropyl alcohol **To deal with fronting peaks-** take off the end fittings and check for contamination. **Strategies To Address Tailing Factors-** operating at a lower pH Considering the potential for mass overload	 **Fronting** 

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	or distortion of the column bed, operate at an elevated pH when examining basic compounds.	**Tailing**

8. CHANGES IN INJECTION VOLUME-

The air in the vial is drawn out by the auto-sampler, Checking the height of the injector needle sampling and sample filling is the solution. Degradation of the sample potential root cause might be using a thermostatted auto-sampler or other appropriate storage measures to ensure the right conditions. Air in the auto-sampler fluidics could be the cause. Use the operating instructions that come with the auto-sampler to flush the fluidics will resolve this issue.

9. PROBLEMS WITH THE GUARD OR ANALYTICAL COLUMN :

1. A number of chromatographic issues can arise from wear and tear on column, mobile phase contamination or degradation, the presence of components in the sample that interfere with the process, or a partially plugged column inlet filter.
2. Inspect and swap out an infected in-line filter, Clean or replace a guard column that is contaminated. If necessary, think about changing the guard column or insert.
3. If column problems are the cause of fronting peaks, check the connection, regenerate, replace the column, or replace the guard or frit column. Fronting can also result from sample solvent incompatibility with the mobile phase or overloading the column.
4. Precipitation occurs when a significant amount of the target protein that was first loaded experiences depletion and is unable to elute from the chromatography column, precipitation phenomena become apparent.
5. A common explanation for this phenomenon is the presence of incredibly tiny precipitated or precipitating protein particles that become trapped at the upper segment of the column.
6. It is important to understand that this happens because the protein is physically filtered rather than adhering to the column. Precipitation is likely to occur if the protein is added to the column in a buffer that is noticeably different from the column buffer[14].

7. ELEMENTS AFFECTING RETENTION DURATION:

Table no 05- Factors Affecting Retention Time

Factors Affecting Retention Time(RT)	Implementing Remedy	Effects
Flow Rate	Adjust by decreasing the flow rate and vice versa To address bubbles in the mobile phase composition, ensure the column is not overloaded Inspect for active groups on the stationary phase.	To see increase in retention time (RT)
Temperature	Increase the temperature lower temperatures	1-2% decrease in retention time per 1°Cincrease in retention time
Column Issues	choosing the incorrect kind of column Column back pressure can be caused by such as buffer precipitation,in-line filter problems, pressure problems, and guard cartridge inefficiencies, age-related changes	Different retention times Retention is generally lower in C8 than in C18
% of Organic Phase in Mobile phases	Higher organic contentoverloading, or incompatibility among the sample solvent and the mobile phase.	lower retention time Retention periods may differed

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Additional Elements	Drifting retention pH regulation, composition of the solvent, and ion pairing	column contamination equilibration, stationary phase stability, leakage, and modifications in mobile phase composition, air trapped in the pump[30].

8. BASELINE ISSUES-

Non-cyclic or cyclic baseline problems can be caused by a variety of things, including electrical interferences, issues with the detector, impurities in the solvent and contamination of the column finding out if the issue is with the detector, the fluid path, or the electrical connections is essential to identifying the source of a baseline irregularity.

8.1 Detector Electronics Problems with Non-cyclic Noise-

The resulting chromatogram may show spurious peaks along with evidence of baseline drift if the detector is not given enough time to equilibrate before performing an injection.

8.2 Reduction in Resolution-

Resolution loss can be worsened by changes in retention times and/or selectivity brought on by contamination or deterioration of the mobile phase. An obstructed guard or analytical column may also jeopardize resolution. The use of non-expired standards or samples, proper column pairing, and temperature control are all necessary for maintaining resolution[31].

8.3 How to Handle the Problem-

To achieve zero fluid flow, turn off the instrument pump. For five to ten minutes, keep an eye on the baseline and note any improvements. If the problem continues, it might be related to detector or electrical issues. Cut the electrical cables connecting the detector to the PC, integrator, and chart recorder in the A/D interface. Connect a jump source, such as a crocodile clip, to the data-handling device's input terminals. Should the noise continue, the data-handling device is the source of the issue.

8.4 Insufficient Signal-to-Noise Ratio:

Conduct a scan to determine the optimal excitation and emission wavelengths, fine-tune the photomultiplier gain, utilize only high-quality mobile phase, and establish an appropriate response time for Suboptimal fluorescence detector settings

8.5 How to Handle Solvent Line Bubbles:

An in-line degassing accessory is a device that you may want to use in order to solve the bubble problem in your HPLC system. Even some of the more recent HPLC systems have a built-in degasser, which is advantageous.

8.6 Degassing Methodology:

Degassing is a procedure that aids in baseline stabilization and noise reduction.

8.7 Alternative Methods:

Proceed with caution if you plan to remove bubbles with helium. Through the evaporation of volatile components, helium sparging can alter the composition of your mobile phase. Using an ultrasonic bath, offline vacuum degassing is an additional technique. Although it only offers short-term relief, this strategy is reasonably effective[15].

9. COMMON PROBLEMS RELATED TO FILTRATION

9.1 Filtration of Sample Preparation:

Issues can arise even with a well-planned HPLC method and apparatus, which makes troubleshooting an important but time-consuming task. It is important to take sample preparation especially filtration into account when troubleshooting.

9.2 Reasons for Suggestion of Filtration:

The very least amount of sample preparation that is strongly advised is filtration. Although some high-throughput techniques might omit this stage, filtering your sample can stop unfiltered particles from clogging HPLC columns and instrument interfaces. Increased backpressure or total device blockage may result from this. Particulate material can be removed at a reasonable cost through filtration, which also increases the longevity of columns and instruments, increases the success rate of right-first-time analyses, and may improve chromatographic results[14].

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9.3 Types of Filtrations:

The main physical technique for removing particulate matter from a solution is filtration. One exception is ultrafiltration, which forces separation through a semipermeable membrane by applying pressure or concentration gradients.

9.4 Choosing the SuitableSyringe Filter:

Non-specific binding, filter blockage, appropriate filter size, extractables, and filtration efficiency when choosing a syringe filter. To put it simply, filtration represents a straightforward and effective approach to enhancing your analytical outcomes. The criteria for selection include compatibility with solvents and porosity.

14 HPLC PURGING:

The purge configuration serves to flush all the lines and replace any residual solvent from previous analyses or washes with a fresh mobile phase. The quantity of fresh solvent needed is determined by the tubing volume within the system. The on-line vacuum degasser, known for demanding a substantial volume for optimal performance, notably impacts this factor.[8] To determine the necessary purge volume, assess the volume specifications of the currently utilized pump, degasser, and inlet tubing. It is standard practice to double this volume for an appropriate purge. selection of the incorrect septum leads to Sample loss by evaporation which leads to issues with reproducibility during repeated injections

15 THE NEEDLE'S CORING OF THE SEPTUM LEADS TO DAMAGE:

Consider a self-sealing septum when working with vials in your HPLC analysis to avoid losing volatile materials to evaporation. Insufficient venting producing a vacuum when the needle punctures the septum or cap for the first time, causing the septum or cap to seal around the injection needle. Some of the sample is drawn back out of the needle by this vacuum. Overfilling the vial may aggravate this problem. To find out if the septum or cap is the problem, remove the cap and septum from the vial, give several injections, and measure the peak area. Changes in peak area (either increases or decreases) observed within the identical vial from one injection to another. A septum plug could be placed inside the draw port if a bottom draw port needle is being used. When utilizing a silicone self-sealing septum change to a preslit silicone septum for better resealing capability and to avoid coring. As an alternative, use septum. It does not reseal, but it does remove coring.

10. HPLC COLUMN

10.1 Maintenance:

If necessaryuse 0.1N HCL to regenerate a column. Always adhere to the regeneration instructions provided by the column vendor. If the problem stems from compounds retained in the column under method conditions that alter the chromatography, this procedure can recover the column's performance. If the issue is surface contamination, washing with stronger solvents may be beneficial. Performance might not entirely return, though, if the surface has undergone chemical modification (ligand hydrolysis, for example, or end capping).

10.2 Storage:

Store the column in the mobile phase for brief intervals (less than 72 hours). Use the shipping solvent recommended for longer periods of time. Organic solvents such as MeOH or ACN help bonding phases by reducing hydrolysis. In polar organic mobile phases, certain phases, such as CN, may be unstable; in these situations, storage in buffer or water is acceptable. Aqueous SEC packings or ion exchangers are examples of columns that can be stored in water or buffered solvents. Incorporate 0.05% sodium azide or a small amount of organic solvent (such as 10% methanol or 5% acetonitrile) into the mobile phase to stop microbiological growth.

10.3 Air Damage to Column:

Your HPLC pump may draw in air if its reservoir becomes empty. But the pump's intended purpose is to pump liquid, not air. It loses its prime and stops pumping when filled with air.

10.4 Correcting Air in the Column:

If the HPLC pump draws a few bubbles of air into the column before quitting, it is unlikely to continuously pump allowing air to pass through the column if left in operation overnight. Even if it did, it is unlikely to cause significant problems. To address the issue, efficiency and accuracy of the results are greatly influenced by the type, polarity and quality of the mobile phase. Keeping the pH constant and precise during the mobile phases whole life is universally vital. It is advisable to add a buffer to the mobile phases composition if there is a chance the sample could affect the pH during a normal run, while pH impacts ionization on the

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silica surface of the column and the sample components of interest.

11. HPLC COLUMN CLEANING:

Clean the syringe, loop, and injector plunger seal daily after concluding each analysis. Ensure the flow cell, pipeline, and suction filter are clear in case of issues, and maintain a record of the cleaning process. Use a stronger solvent to flush your mobile phase for samples that are water soluble run a warm, distilled water flush (50 °C). for materials that are not water-soluble use methylene chloride, hexane, isopropyl alcohol which boosts tailing factor as well.

11.1 After Finishing Your Run:

Make it a habit to use your column to wash your system with either 50% methanol and 50% acetonitrile. Also additionally, clean using the same solvent by removing the column and replacing it with a union.

II. CONCLUSION:

It highlights the fact that, in cases of HPLC issues, prevention is always preferable to treatment. These problems often have nothing to do with columns; instead, they can be caused by chemistry and instrument issues such as metal contamination, detector settings, instrument connections, and mobile phase pH. An overview of HPLC system maintenance is given in this chapter, along with tips and techniques for troubleshooting information to lead you toward solutions. Research, product evaluation, and environmental monitoring all heavily rely on HPLC. Regular maintenance of the HPLC system is the best way to prevent HPLC issues. HPLC effectively separates higher molecular weight compounds, offering both qualitative and quantitative insights. Comprising various essential components, these guidelines assist in the maintenance of the HPLC system, addressing common issues for improved performance and cost-effectiveness across different manufacturers.

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