Good Laboratory Practices for High Performance Liquid Chromatography (HPLC): A Overview

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Summary: High performance liquid chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantitate the compounds that are present in any sample that can be dissolved in a liquid. Today, compounds in trace concentrations as low as parts per trillion [ppt] may easily be identified. HPLC can be, and has been, applied to just about any sample, such as pharmaceuticals, food, nutraceuticals, cosmetics, environmental matrices, forensic samples, and industrial chemicals. Nowadays, GLPs are solid standards for government registration and regulatory research facilities. Outside the U.S., OECD GLP is more familiar and has been applied in some other countries [1-2]. It is important that not only proper science must be used in the scientific research but also quality assurance systems should be implemented while we attempt to produce reliable and reproducible data in regulatory research because all of these activities have great impacts on toxicology and the environment. This article discussed the Good Laboratory practices for High performance Liquid Chromatography for precise results and long life of the instruments and its consumable accessories.
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Preparation of solvents.

Correct solvent preparation is very important. It can save vast amounts of time Spent roubleshooting spurious peaks, baseline noise, etc.

Quality

All reagents and solvents should be of the highest quality. HPLC grade reagents may cost slightly more than lower grade reagents, but the difference in purity is marked. HPLC grade reagents contain no impurities to produce spurious peaks in a chromatogram baseline. Ensure that any water used in buffer preparation is of highest purity. Deionized water often contains trace levels of organic compounds and so therefore is not recommended for HPLC use. Ultra pure HPLC water (18M_ resistivity) is generated by passing deionized water through an ion exchange bed. Modern water purification instruments use this mechanism to produce water of suitable quality in high volumes. 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. These stabilizing agents often affect the optical and chromatographic behavior of buffer solutions, so it is often worth buying reagents that contain no stabilizer. Containers of solid reagent are easily contaminated by repeated use. For this reason, we recommend that reagents be purchased in low container weights.

Filtration

All HPLC solvents should be filtered through a 0.45 µm filter before use. 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 benefit both your chromatography and HPLC system. Pump plungers, seals and check valves will perform better and lifetimes will be maximized.

Degassing

Before the freshly prepare mobile phase is pumped around the HPLC system, it should be thoroughly degassed to remove all dissolved gasses. 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. The most efficient form of degassing is bubbling with

helium or another low solubility gas. If this method is available, we recommend that the mobile phase is continually degassed at very low levels throughout the analysis. This will inhibit the re-adsorption of gases over the analysis time.

Solvent use

Each solvent line should be fitted with an inlet filter. This is the first line of system defense against particulate contamination from solvents. The filters should be kept clean to prevent cross contamination. When they are not being used, it is recommended that they stored in a solution of 50% acetonitrile / 50% water. This will inhibit microbial growth and stop dust and dirt from embedding in the filter pores the solvent lines should be clean, growth-free and should have no sharp bends or creases in them. Solvent reservoirs should be placed as high as possible on or in the instrument – always higher than the pump inlet manifold.

Mobile phase properties

Do not use highly acidic or basic solvents unless your HPLC system and Column have been engineered to accommodate them. Seals, plungers, etc… can be damaged by extreme pH conditions. The use of highly aqueous mobile phase is becoming more popular as safety guidelines demand less exposure to organic solvents. Care should again be taken that the HPLC column has been engineered to accommodate highly aqueous solvents – traditional alkyl chain media can be prone to phase collapse in low organic composition solvents mixes, for example at less than 5% organic solvent. Highly aqueous mobile phases are ideal breeding grounds for microbes. Ensure that an organic solvent is flushed through the HPLC system and column at least once every 48 hours to kill unwanted microbial growth. Alternatively add a small amount of sodium azide to the aqueous solvent to inhibit growth**.**

Changing solvents

Buffered phase to wash or storage phase

Ensure that the buffer is soluble in the proposed wash or storage phase. Is it is not, first flush the system with a solvent mix that is highly aqueous to remove the buffer from the system and column, then change to the proposed wash or storage solvent mix. Few columns like Hypercarb TM can be used in both normal and reversed phase, then with both solvent types. To convert a normal phase system/column to a reversed phase system/column, flush with a solvent that is miscible with both the current normal phase solvents and ideally, the proposed reversed phase solvents. If the final reversed phase solvents include a buffer, then it is advisable to move from the 100% methanol flush to a 50% aqueous methanol flush. For example: Normal phase Hexane/Ethyl acetate Flush IPA (isopropenyl acetate) then Methanol finally (50:50) Methanol/Water Reversed phase Buffered aqueous methanol To convert a reversed phase system/column to a normal phase system/column, follow a similar path to the one listed previously, but in reverse For example: Reversed phase Buffered aqueous methanol Flush (50:50) Methanol/Water Methanol then IPA Normal phase Hexane/Ethyl acetate HPLC solvents and their most pertinent physical properties, including viscosity and miscibility number. The miscibility numbers can be used to predict the miscibility of solvents. If the smaller miscibility number is subtracted from the larger and the difference is 15 units or less, then the 2 liquids are soluble in all proportions at 15°C. If the smaller miscibility number is subtracted from the larger and the difference is 16 units, then the 2 liquids have a critical solution temperature between 25 and 75°C with 50°C as the optimum temperature. If the smaller miscibility number is subtracted from the larger and the difference is 17 or greater, then the 2 liquids are immiscible, or their critical temperature is greater than 75°C.

Buffer properties

The following table list a series of commonly used HPLC buffers, their alternative name, where applicable, and their pKa values at 20°C. Buffer transparency is a variable that should be measured prior to buffer use, as it will vary with salt concentration. Buffer choice will be very dependent on the analyte and the instrumentation used. Ideally, LC/MS applications should use a volatile buffer as this will not form a containing deposit on the cone and source. Inorganic acids, in volatile buffers and ion pair reagents should all be avoided.

Porous graphitic carbon

There are four wash or regeneration procedures associated with porous graphitic carbon. The one(s) used will depend on the analytes and solvents that have been used with the column.

Acid/Base regeneration

Suitable for ionized species analyzed in strongly aqueous mobile phases.

1. invert the column
2. flush at 1 mL/min with 50 mL tetrahydrofuran/water (1:1) containing 0.1% trifluoroacetic acid
3. flush at 1 mL/min with 50 mL tetrahydrofuran/water (1:1) containing 0.1% triethylamine or sodium hydroxide
4. flush at 1 mL/min with 50 mL tetrahydrofuran/water (1:1) containing 0.1% trifluoroacetic acid
5. Flush with methanol/water (95:5) to re-equilibrate
6. Re-invert the column

Strong Organic regeneration

Suitable for applications involving polar and/or ionized species analyzed in aqueous mobile phases.

1. flush at 1 mL/min with 50 mL acetone
2. flush at 1 mL/min with 120 mL dibutylether
3. flush at 1 mL/min with 50 mL acetone
4. flush with aqueous mobile phase until equilibrated

Normal Phase regeneration

Suitable for applications running predominantly in normal phase mobile phases.

1. flush at 1 mL/min with 50 mL dichloromethane
2. flush at 1 mL/min with 50 mL methanol
3. flush at 1 mL/min with 50 mL water
4. flush at 1 mL/min with 50 mL 0.1 M hydrochloric acid
5. flush at 1 mL/min with 50 mL water
6. flush at 1 mL/min with 50 mL methanol
7. flush at 1 mL/min with 50 mL dichloromethane
8. flush with mobile phase until equilibrated

Removal of Trifluoroacetic acid

Suitable for applications running mobile phases containing trifluoroacetic acid. Flush the column with acetonitrile that has been heated to 75°C. The column should also be maintained at this temperature.

System plumbing and fittings

The purpose of a well-plumbed HPLC system is to minimize dead volume between it's components and to eliminate leak System tubing errors show themselves in many ways, for example as band broadening, baseline noise, etc detection of incorrect diameter tubing is often very difficult once it is in-situ. The internal diameter of tubing used in a HPLC system varies with the position in the instrument; refer to your system maintenance manuals. The type of tubing used is determined by the application that is being performed. When changing tubing, make sure that the replacement is manufactured from a material that is compatible with any solvents that may be flushed through it. The Sample preparation is about more than just the dissolution of s solid in a liquid. Samples may require other techniques such as filtration, extraction or derivatization as well as accurate weighing and /or dissolution. Samples require filtration if they contain suspended solids. This can be performed on-line using a pre-column filter or as the sample is introduced to the vial.

Conclusion

Although liquid chromatography is barely 100 years old, an extraordinary variety of instrumental and ancillary equipment is available, notably in the domain of high-performance liquid chromatography. Systematic approach is best to identify any problems when troubleshooting the high-performance liquid chromatography (HPLC) system. Commonly five major categories of symptoms to help quickly identify the source of the problem if not properly fallow good laboratory practices for HPLC such as Pressure abnormalities Leaks Problems with the chromatogram Injector problems other problems detected by smell, sight, and sound when the problem has been corrected, record the incident in the HPLC system record book to help with future problems. Many liquid chromatography (LC) problems can be prevented with routine maintenance. For example, replacing pump seals at regular intervals should eliminate pump-seal

failure and its associated problems common problem areas for each HPLC module, as well as preventive maintenance practices to reduce their frequency. Articles Recommended if every user scientist adapt guidelines of GLP on regular basis for laboratory routine analysis work so that minimized accuracy and authenticity of results and life of instruments.

Figurre-1 Typical High Performance Liquid Chromatography

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