HPLC applications

Laboratory Reagent Grade Water for HPLC applications

Laboratory reagent grade water is used extensively in High Performance Liquid Chromatography (HPLC) applications. This method may also be called High Pressure Liquid Chromatography and is used to separate, identify and quantify organic compounds in solutions. For HPLC applications, laboratory grade water must have a low organic carbon content and can be referred to as chromatography grade water.

It has been estimated that well over one million chromatography methods are performed every day around the world. Of these, over eight hundred thousand methods are reverse phase gradient HPLC. These procedures will include applications in the pharmaceutical, food and beverage, forensic and environmental industries. This indicates the importance of the method as well as the purity of the mobile phase solvents.

The largest consumption of laboratory grade water for HPLC applications is as a mobile phase solvent for reverse phase HPLC methods. A mobile phase solvent is the carrier solvent that is used to transport a sample from the injection point to the separation column and detector of the chromatograph. Reverse phase HPLC, as opposed to normal phase, is a method that defines the mobile phase solvent as being more polar than the packing material used in the chromatography column. This type of method is generally applied to analyses of organic compounds that are soluble in water.

More than one mobile phase solvent may be used during a method at varying concentrations to enhance sample separation. I.E.: A method may start with 90% water and 10% THF and finish with 10% water and 90% THF. If variable concentrations of mobile phase solvents are used during the method, the method may be further defined as a gradient technique. (Reverse phase gradient HPLC.) Purified water is used quite often for this method due to the polar nature of the water molecule. Other polar solvents, such as alcohol, acetonitrile or tetrahydrofuran (THF) may be used with the water as gradient mobile phase solvents. (Chromatography instrument manufacturers supply application methods and miscibility charts for most solvents).

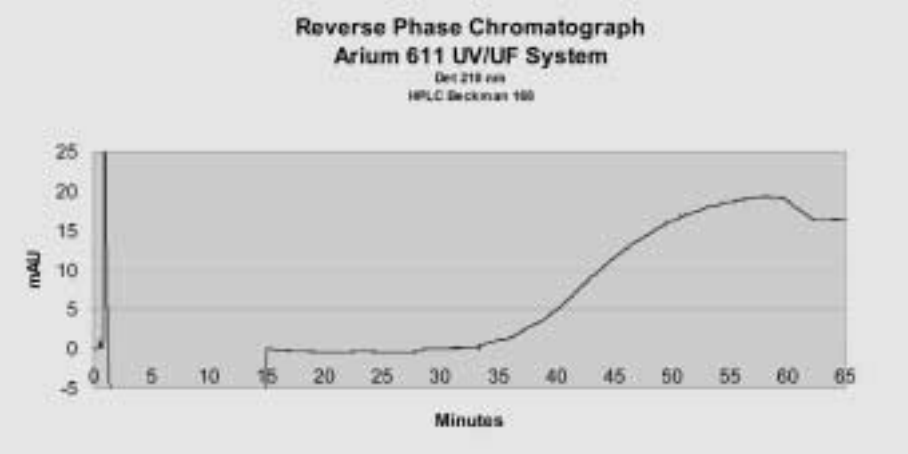
The importance of the solvent purity increases inversely with the detector wavelength being used with the HPLC method. The lower the wavelength, the higher the water purity must be to avoid interference. At wavelengths above 250 nm, a laboratory may get by with bottled water or deionized water. However, when working with wavelengths below 250 nm, specially treated deionized water is required. Even at the higher wavelengths, it is possible to detect unwanted peaks due to concentration effects if too much organic carbon is present in the water.

Therefore, it is necessary to produce reagent water on demand as opposed to using bottled water or low quality DI water for this application. Bottled water will absorb contaminants from the atmosphere every time the bottle is opened. This water may already contain interfering organic material that leached from the bottle or cap. In any case, it is difficult to control this type of contamination. Furthermore, bottled water is most likely more expensive than the water taken from a point of use system designed to produce chromatography grade water. Another advantage is that the water produced from this type of system may be used for most, if not all, of the other methods performed in the lab.

Deionized water produced from a system that is not designed for chromatography work can also present problems. These types of system normally do not contain the pretreatment materials needed to remove the broadest range of organic contaminants from feed water sources. These systems may also not contain an ultraviolet (UV) oxidation chamber to further reduce organic contaminants. Proper pretreatment coupled with UV radiation provides the most consistent purified water with the lowest background organic carbon levels. Many HPLC procedures utilize UV detection for sample analysis. By providing UV radiation/oxidation at 185 nm wavelength in the water treatment system, most if not all potentially interfering organic compounds are eliminated.

There are several other important factors that must be taken into account when using reagent grade water and other solvents for chromatography methods. They are; rinse all filters with a small amount of the solvent to be used prior to collecting the solvent. Waste the rinse water or solvent. This eliminates wetting agents and other surface contaminants from the filter that could interfere with the test. This is true of final filters on water systems and filters used for other mobile phase solvents during degassing or sample preparation.

Glassware used to contain mobile phase solvents should be cleaned, meticulously rinsed and baked in an oven or furnace at 425°C for at least two hours covered. Limit exposure of any volatile solvent in the lab area. Organic solvents will easily absorb into exposed reagent water very quickly.



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