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A brief primer for scientists in life science, pharmaceutical and clinical labs:

How to Get the Most Accurate and Reliable Data from HPLC using Ultrapure Water

Summary

As a scientist, getting reliable, accurate, and consistent data from your HPLC instrumentation is critical. Unreliable data means wasted time and effort on your part.

It has been estimated that most difficulties with HPLC are a result of column problems due to poor quality lab water. Water that's not purified to the highest standards can lead to ghost peaks, blocked chromatography columns, poor separations, and more.

This means you need to adopt the highest standards when it comes to your lab reagents – high purity is essential. This article will illustrate the problems with water contamination and how these contaminants affect HPLC performance. You'll also learn why using "ultrapure" water is crucial for getting the best data.

You are responsible for maintaining your HPLC instrumentation, and making sure your data is reliable. Using ultrapure water is one of the most important decisions you'll make. The right reagents will support you and your work for years to come, allowing you to produce quality data. On the other hand, poor quality water will hinder your lab efforts, and undermine your reputation.

1. Water: A Critical Laboratory Reagent for HPLC

"90% of all difficulties with high performance liquid chromatography are caused by column problems, most of which come from contaminated water"

The above quote comes from LC/MS: A Practical User's Guide, by M.C McMaster [1], and reflects the importance of using water of the highest quality for HPLC analysis.

HPLC is an extremely powerful technique, combining wide range of applications with high sensitivity. But as you'll see, these benefits aren't possible without the highest quality lab water. This article will focus on HPLC, and how impure water can severely degrade performance [2]. It'll also highlight the importance of using what's known as "ultrapure" water to ensure the highest standards in HPLC data.

2. The Risks of Poor Water Quality

Virtually all trace HPLC analyses require the use of significant quantities of pure water. It is needed throughout the process from sample preparation and pre-treatment, rinse water for solid-phase extraction, reagent water for preparing standards and blanks to eluent preparation. In these and other applications the purity of the water can be a critical factor in the sensitivity, reproducibility and robustness of the analytical results obtained. These applications need high quality water with very stringent levels of purity [2, 3, 4].

Water needs to be of high purity for accurate, cost effective, and reliable lab analysis. The problem is that water contains a very wide range of impurities that need to be removed and the resultant pure water is easily contaminated; even trace levels of impurities can affect one of the stages in HPLC and degrade your analyses. You need a system that provides you with a source of highly pure water for your experiments [5].

2.1 Water contamination and HPLC

Clearly, the most obvious impurities are those organic compounds which interfere directly with the chromatographic peaks being measured. These must be eliminated or, at the very least, minimized. However, there are also many contaminants which can interfere indirectly, but equally seriously; for example, by reaction with reagents or analytes during sample pre-treatment, by contamination of columns or detectors, or by effects on the lifetime of standards. To minimize the risks from these potential problems you need to use ultrapure water not just to reduce the levels of specific impurities but also to reduce the overall contamination levels as much as possible.

Total Organic Carbon (TOC) is used to give a measure of the overall organic purity of water. It is determined by oxidizing the organic contaminants and measuring the level of oxidized species produced by a variety of methods. This can be carried out on-line or off-line. Typical TOC levels range from 1 to 10 ppm for rivers and drinking water. For trace HPLC work TOC values need to be 10 ppb or less and, preferably, nearer to ultrapure water levels of 1 ppb. To reach these levels involves removing more than 99.9% of the organics in the feed-water.

The potential effects of various types of contaminants in water for HPLC are summarized in Table 1.

Organic contaminants are likely to be the biggest issue. Organics can contaminate the sample during preparation and pre-treatment. When present in the mobile phase, organic compounds may compete with the analytes to bind to the active sites of the stationary phase, reducing sensitivity.

Accumulation of contaminants can also restrict analyte and solvent access to these sites, resulting in mass transfer issues and loss of resolution. Any accumulation at the head of the column can cause ghost peaks causing confusion when they are near peaks of interest, and cause quantitative errors when they overlap. Long-term build-up of impurities can lead to increased back-pressure and, ultimately, shorter column life.

Bacteria and biofilm debris can lead to column and frit blockages and organic by-products – such as pyrogens, nucleases or alkaline phosphatase - may cause the problems outlined above. Ionic contaminants can interfere directly with ionic separations, absorb UV light and also modify the ionic strength of a solution and affect chromatographic separations. Particulates and colloids can damage the HPLC pump and block frits and columns. Column blockages can increase column back-pressure and reduce the overall lifetime of the column, which increases cost. Colloids can also adsorb irreversibly onto the stationary phase reducing separation efficiency.

These contaminants need to be removed to ensure high purity water. The standard JIS K 0124: 2011 "General Rules For High Performance Liquid Chromatography", specifies the following [6]:

"Water used for this standard shall be purified by a combination of reverse osmosis, distillation, ion-exchange, ultraviolet irradiation, filtering, or other methods, and shall have a water quality level that does not interfere with analysis. Water quality shall be evaluated based on index values such as specific resistance, total organic matter (TOC), or absorbance values."

Type of Contamination	Detrimental effect on HPLC
Dissolved organic compounds	Ghost peaks, reduction in sensitivity, reduction in the amount of analyte retained by the column, loss of resolution
Microorganisms	Blockages in the filters and column, growth of microbes and algae and, release of organic impurities
Colloids and suspended particles	Can damage the pump. Blockages in the chromatography column can affect performance (e.g. split peaks) and reduce column lifetime.
Ionic species	Can affect the sequence of separation of polar molecules, ghost peaks due to UV-absorbing ions, ionisation effects in detector
Dissolved nitrogen and oxygen	Bubble formation in column and detector. Voids in the column, can impede flow. Gas in detector can affect response.

Table 1 – The type of contaminants found in water and how these can affect HPLC

In modern laboratory water purification systems, the ions are removed by reverse osmosis followed by ion-exchange; the organic impurities are reduced by reverse osmosis, adsorption on activated carbon and residual levels are broken down by exposure to high energy UV light; bacteria and bio-active species are removed by reverse osmosis, ion-exchange, filtration and exposure to UV light and colloids and particulates are removed by reverse osmosis and further filtration.

The next section describes ultrapure water, and how it helps you avoid these contamination problems.

3. How Ultrapure Water Helps You Maximise HPLC Results

The contamination problems outlined above mean that ultrapure water is essential for HPLC analysis.

Making sure that you use water that is pure enough is a key step towards producing consistent, accurate results. Deionized or distilled water aren't good enough, since this water still contains organic substances and other impurities.

For general pharma lab work, it's recommended that you use Clinical Laboratory Reagent Water (CLRW), which meets the guidelines set forth by the Clinical Laboratory Standards Institute (CLSI) [7] as a minimum requirement. CLRW water is often not pure enough for HPLC. To be absolutely sure you're using water of the highest purity for sensitive analytical techniques you need Type I (ultrapure) water. This water reaches extremely high levels of purity, with a resistivity of 18.2 MΩ.cm, total organic carbon (TOC) of <10 ppb, and bacterial count of <1 CFU/ml [8].

For clarification, Table 2 below (adapted from Reference 5) lists the types of water based on the limits for the major types of impurities, along with CLRW.

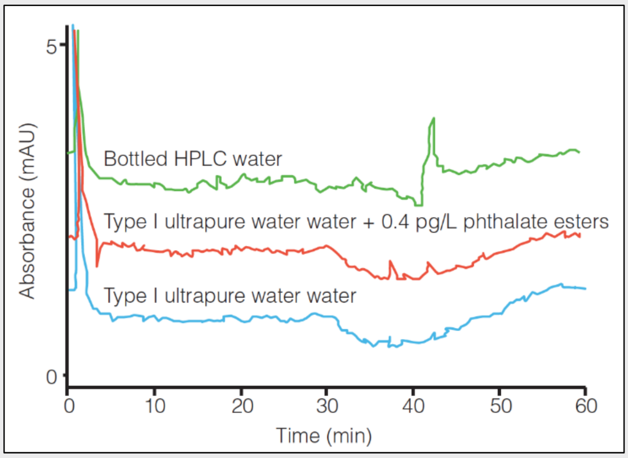

Figure 1 - 50 ml water concentrated on a C18 column and eluted with a water:acetonitrile gradient, 0–100 % at 5 %/min, flow rate 2 ml/min, with UV detection at 254 nm. Adapted from Reference 10.

3.1 Bottled HPLC water or in-house purification system?

You have two options for sourcing water for HPLC in your lab:

- **You can use bottled HPLC grade water, or**
- **You can use an in-house purification system**

Commercially available bottled HPLC grade water can be used as an HPLC eluent and for preparing standards and blanks. But, in general, relying on bottled water is inferior to using an in-house purification system [9,10].

Bottled water is more expensive when significant volumes are used and the user is dependent on the analyses provided with the bottle which are usually on bulk sampling. Suitability for any particular analysis needs to be confirmed and, once the bottle is opened, there are real risks of contamination.

	Resistivity (MΩ-cm)	TOC (ppb)	Bacteria (CFU/ml)	Endotoxins (EU/ml)
Type I+	18.2	<5	<1	<0.03
Type I	>18.0	<10	<1	<0.03
Type II+	>10	<50	<10	N/A
Type II	>1.0	<50	<100	N/A
Type III	>0.05	<200	<1000	N/A
CLRW (7)	>10	<500	<10	N/A

Table 2 – Types of water classified by ELGA LabWater, as defined by their measurable physical and chemical properties. Adapted from Reference 5

Another study showed that HPLC-grade bottled water resulted in several large peaks, when pre-concentrated on a chromatography column, followed by elution [9]. These peaks were absent when freshly produced ultrapure water was used. When used to separate a drug mixture, bottled water caused large shifts in baselines, as well as ghost peaks.

Again, these problems were not encountered with freshly produced ultrapure water. The authors attributed this to the much higher levels of organic contaminants in bottled HPLC-grade water, when compared to fresh ultrapure water

The important thing to remember is a well designed in-house purification system ensures your ultrapure water source is consistent, and the water used for your HPLC tests is freshly purified and of the highest quality.

Our PURELAB® Chorus 1 delivers type 1 ultrapure water with TOC levels as low as 1ppb that is highly suitable for HPLC experiments.

Conclusion and Next Steps…

You now know that choosing ultrapure water for your HPLC tests will help you get consistent, accurate results. And you need a reliable method for providing this ultrapure water for your lab. You also know that installing an in-house purification system is the best way to do this, saving you time and money in the long term, and reducing your impact on the environment. The risk of doing otherwise is too great.

So for more information on ELGA LabWater purification systems for ensuring the highest standards in ultrapure water for HPLC, or to request a formal quotation, visit https://www.elgalabwater.com/products.

Contact Us

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