Using Type 1 Water (18.2 MΩ-cm) for High Performance Liquid Chromatography in the Food & Beverage Industry

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High Performance Liquid Chromatography (HPLC) is widely used in the food and beverage industry at all stages, from new product development to quality assurance. HPLC can be used to determine the components of a given product in its raw, intermediate, and final states, and is commonly used to test these products for additives and contaminants in order to comply with regulatory standards. Additionally, important product information such as vitamin, lipid, and protein content can be determined (Nollet and Toldra, ix).

The process of HPLC involves the injection of a solvent (eluent) containing the sample (mobile phase), under high pressure, into a column with specific adsorbent material (stationary phase) that separates the sample components so they can then be identified and/or quantified (Nollet and Toldra, ix). One of the most common eluents is water mixed with a varying ratio of organic solvent, such as methanol. Because impurities in the water can interfere with accurate and reproducible results, it's imperative to pay close attention to using ultrapure water with a total organic carbon (TOC) content below 5ppb.

There are two different types of water that are acceptable for HPLC eluents: bottled HPLC grade water or Type 1 ultrapure laboratory water. Bottled water has a number of advantages. It's guaranteed to be the right quality when it is first opened, before there is any chance of environmental contamination, and is a good choice for users needing small volumes. However, bottled water also has several disadvantages. The largest drawback is that bottled water can easily be contaminated if good laboratory practices (GLP) are not followed. Also, TOC levels are not always available for bottled water, and organics from the atmosphere can contaminate the water once the bottle is opened. An example would be CO_2, because it easily dissolves in pure water. Finally, bottled water can quickly become very expensive for high-volume users.

Ultrapure water produced from Type 1 water purification systems offers many benefits. The water is always fresh, so concerns about passive environmental contamination are removed. Additionally, ultrapure water purification systems fitted with a UV lamp can control the amount of organic carbons in the product water. Water systems that display the amount of TOCs in the product water are also available. The disadvantages of a water purification system include the cost to purchase the system, which may or may not include the first set of consumables required to purify water. Additionally, these consumables must be replaced on a regular basis to prevent contamination and ensure the water is consistently pure.

How Contaminants Can Impact HPLC

Organic compounds, bacteria, ions, colloids, pyrogens, and particles can all potentially be present in water and can interfere with HPLC in a variety of ways. Bacteria, colloids, and particles can block pumps, injectors, and columns, and contaminating organics can create ghost peaks that can interfere with results. In addition, as the amount of organics increases, or if other ions are present, the reproducibility of the HPLC trace is more challenging. **Figure 1** shows an example of how organic contaminants can adversely affect an HPLC chromatograph. The contaminants in the deionized water produce several peaks that could be mistaken for sample peaks or even hide the peaks from the sample, thus negatively impacting the ability to obtain accurate, reproducible data. The "HPLC grade bottled water" trace shows a potential ghost peak caused a contaminant organic(s) as well. The "Ultrapure Water System with UV Lamp" trace shows it has no background peaks, making it an ideal eluent source; the only peaks observed will belong to the sample, not the eluent.

Water purification systems directly monitor two important characteristics of the water being produced. One measure of the purity of Type 1 water is its resistivity. This is the ability of the water to resist conducting electricity, and is the mathematical inverse of conductivity. Resistivity is measured in megohm-centimeters (MΩ-cm). The theoretical maximum resistivity, indicating maximal ion removal, is 18.2 MΩ-cm. In addition to this high resistivity, water systems can also display the amount of TOCs in the product water. Ideally, ultrapure water with a UV lamp will have a TOC of 1-5 ppb.

How a Type 1 Water Purification System Produces 18.2 MΩ-cm Quality Water with Low TOCs

Water purification systems are able to provide reproducible quality water due to the group of purification technologies enclosed in the system. Incoming water in a typical Type 1 system has already been pretreated by deionization, distillation, or reverse osmosis (RO). Once the water is

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inside the water system, it passes through a UV lamp assembly which oxidizes organics to 1-5 parts per billion (ppb), and inactivates bacteria. This is achieved by using a dual wavelength UV lamp, 254 and 185nm. The 254nm wavelength inactivates bacterial DNA and the two wavelengths combined effectively oxidize organic compounds. The water then moves to a deionization cartridge containing a high grade resin to remove ions (including any leftover cell walls carrying a slight charge after the UV inactivation) to the theoretical maximum of 18.2 MΩ-cm. This resin also captures dissolved gasses that can cause air bubbles in columns. The last pass is through a 0.2µm filter to capture any remaining particles or bacteria that could cause clogging in the columns. At the end of the process the TOC levels are 1-5 ppb and the resistance is 18.2 MΩ-cm, which is ideal for HPLC working conditions.

In closing, HPLC is an important analytical tool in the food and beverage industry. Its proper use not only helps to ensure food safety, it ensures that we can all continue to enjoy tasty products in the future. But the process itself is highly sensitive to the components being introduced into the analysis, including any impurities that are potentially present in the water used in the analysis. One of the key components is the water used in the eluent. It is important to consider using ultrapure, Type 1, 18.2 MΩ-cm water, with 1-5 ppb TOCs to prevent potential physical interference such as clogging of columns. And more importantly, its use prevents interferences that could result in misread results.

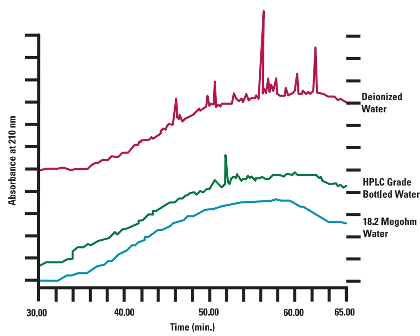
Ultrapure Water Advantages and Disadvantages

Advantages	Disadvantages
High quality every time water is dispensed (removes risk of environmental contamination)	Higher cost to purchase and maintain system for low volume usage
TOCs are tightly controlled and end-user is alerted when unacceptable levels are present when system with TOC monitoring option is purchased	System must be properly maintained to ensure high quality water
Good option for customers that are using a high volume of water	Lab space required for system

Bottled Water Advantages and Disadvantages

Advantages	Disadvantages
Guaranteed quality first time opened but then end-user must exercise GLP to ensure quality	TOCs cannot be controlled and are in some cases not reported
Convenient to simply open and use without worry about maintenance	Can become quite expensive if demand becomes high, and expensive to ship and store
Good option for customers using low to mid-range water volumes	Must store the water so it is always on hand, requiring space in lab for bottles

Figure 1



Determined with a Beckman HPLC system; 165 detector at 210 nm, 4.6 x 250 nm C-18 column; linear gradient mobile phase from 100% water to 100% acetonitrile at 2 mls/minute in 30 minutes; 60 ml sample enrichment at 2 mls/minute. Direct-Connecte™ Guard column.

Ultrapure Water System with UV Lamp

References

Leo M.L. Nollet and Fidel Toldra, Ed. Food Analysis by HPLC 3rd Ed., Boca Raton, FL: CRC Press, 2013.

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