APPLICATION NOTE

Type I ultrapure water crucial for HPLC and UHPLC

Abstract

High performance liquid chromatography (HPLC) is a powerful analytical technique used to separate, identify and quantify compounds with a wide range of differing properties. The need to ensure that standards, organic solvents and HPLC mobile phases are of the highest purity is widely recognised, yet water quality, which may have a major impact on chromatographic performance, is frequently taken for granted. For ultra high performance liquid chromatography (UHPLC), a technique using sub-2 µm particles and high pressures to enhance analytical speed and sensitivity, water quality is very crucial.

Introduction

HPLC is a commonly used and extremely powerful chromatographic technique, with applications in areas such as pharmaceutical, bioanalytical, food and beverage, clinical, forensic, environmental and drug development laboratories. System pressure limits, generally around 6,000 psi, effectively restrict column particle sizes to 3 µm or larger, limiting the speed and efficiency that can be achieved. UHPLC is a recent extension of HPLC which can operate at pressures up to 15,000 psi and take advantage of sub-2 µm particle sizes, significantly increasing speed and efficiency without affecting the quality of separation.

Good chromatographic performance is dependent on the purity of the water used[1]. Water can contain a variety of impurities such as particulates, bacteria, nucleases, ions and trace organics. Poor quality water can degrade chromatographic performance by impacting on resolution, integration and baselines, introducing ghost peaks and affecting the selectivity of the stationary phase. Particulates found in poorly purified water may also cause damage to the LC system and create column blockages – UHPLC is particularly susceptible due to the very small particle sizes used – resulting in increased downtime.

Organic compounds

Organic compounds present in water are likely to be the most critical contaminants. When present in the mobile phase, organic compounds may compete with the analyte to bind to the active sites of the stationary phase. This reduces the amount of analyte retained on the column and subsequently eluted, with a corresponding reduction in method sensitivity. If organic compounds accumulate on the column surface they can restrict analyte and solvent access to active sites, resulting in mass transfer issues and loss of resolution, while any accumulation at the head of the column can cause ghost peaks. Additionally, the accumulation of organic contaminants on the column may result in increased back pressure and, ultimately, a shorter column lifetime. In situations where contamination levels are very high, it is possible for the organic compounds to accumulate with time and act as a new stationary phase, causing peak tailing and retention time shifts.

Bacteria

Bacteria and biofilm debris can lead to column and frit blockages and organic by-products – such as pyrogens, nucleases or alkaline phosphatase – may also result in chromatographic issues, such as those described above.

Ions

Ionic contaminants may modify the ionic strength of a solution, which can affect some chromatographic separations, and, if contaminants absorb UV light, additional peaks will be generated which may interfere with the analysis.

Particulates and colloids

Particulates may cause damage to the HPLC pump and can also cause columns and frits to block. This effect is much more significant for UHPLC users, as the very small particle sizes and decreased diameters of these columns and other components make them more susceptible to premature blocking than their HPLC counterparts. Colloids can be irreversibly adsorbed onto the stationery phase, resulting in a change to the separation efficiency of the column.

Advantages of water purification systems

Laboratories may use commercially available HPLC grade water, usually supplied in bottles of 1L or more, which, when used as an eluant, can give inferior results. An alternative to bottled water is to use freshly prepared water from water purification systems. The two types of water have often been compared[1] (Figure 1). The poorer performance of bottled water may be a due to limitations in the pure water system used by the manufacturers, inadequate cleaning of bottles, or the preparation and bottling of water in a facility that also prepares organic solvents. Even if these issues are avoided, there remains the risk of contamination on storage, due to leachates and bacterial growth.

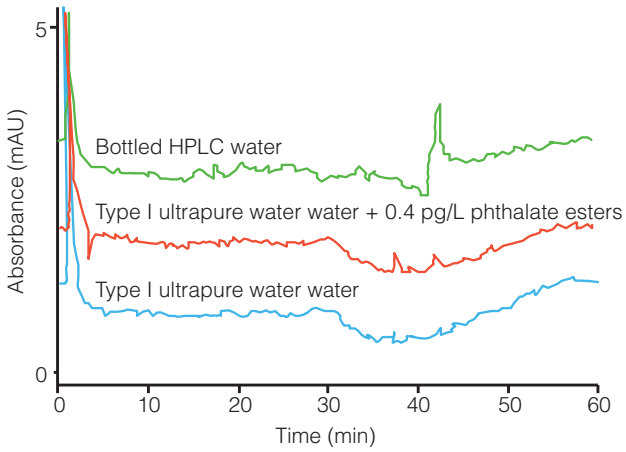

Figure 1: 50 ml water concentrated on a C18 column and eluted with a water:acetonitrile gradient, 0–100 % at 5 %/min, flow rate 2 ml/min, with UV detection at 254 nm.

Purifying water for HPLC

HPLC/UHPLC analysis requires the use of type I ultrapure water for the preparation of reagents, buffers, mobile phases and for any sample pre-treatments[2]. Combining HPLC/UHPLC with mass spectrometry for ultra-sensitive liquid chromatography-mass spectrometry[3] applications requires water with very low levels of Total Organic Carbon (TOC). ELGA's PURELAB® Ultra Analytic delivers type I ultrapure water with, typically, a resistivity of 18.2 MΩ.cm, a very low TOC value of less than 2 ppb and bacteria levels below 0.1 CFU/ml, which is highly recommended. ELGA's award winning PURELAB flex, with a TOC of less than 5 ppb, is suitable for all other HPLC applications.

Ultraviolet (UV) radiation

Passing water through a beam of ultraviolet light breaks down organic compounds. A wavelength of 185 nm effectively breaks down and oxidises carbon-containing molecules, yielding ionised fragments for subsequent removal by ion exchange, whereas longer wavelength UV radiation (254 nm) disrupts the activity of bacterial enzymes, preventing replication. To maximise breakdown of organic molecules, both the PURELAB Ultra Analytic and PURELAB flex use a full spectrum UV lamp.

Total Organic Carbon (TOC)

Routine measurement of organic compounds in purified water is impractical due to their potential variety and complexity. Consequently, an indicator of overall organic contamination is used. TOC, in which organic substances in water are oxidised and then the effects of the resultant products are measured, has proved the most practical method. TOC is mainly used for monitoring and trending purposes; in most water samples TOC cannot be related directly to the concentration of organic molecules, as different molecules contain differing amounts of carbon.

Media

The media cartridges in both the PURELAB Ultra Analytic and PURELAB flex contain synthetic, activated carbon beads, which adsorb a wide variety of organic compounds, and high purity ion exchange resins to minimise the release of impurities.

Conclusion

Type I ultrapure water with high resistivity (>18.0 MΩ.cm), free from particulates, bacteria, organic and ionic compounds, should be used for all HPLC and UHPLC applications to ensure good chromatographic performance.

To find out more about ELGA LabWater's water treatment technologies and solutions for analytical applications, visit **www.elgalabwater.com**

References

1. Whitehead, P. Ultra-pure water for HPLC. Why is it needed and how is it produced? Laboratory Solutions, December 1998
2. ASTM Standard Guide for Bio-applications Grade Water D 5196-06
3. ELGA Application Note: Ultrapure water enables excellent chromatographic performance for LC-MS analysis

About ELGA LabWater

ELGA LabWater manufactures supplies and services laboratory, healthcare and clinical water purification systems. ELGA offices and distributors are located in more than 60 countries worldwide. ELGA is the global laboratory water brand name of Veolia Water Solutions & Technologies.

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