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Minimizing LCMS Artifacts Using Arium® Ultrapure Water Systems

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Abstract

In Liquid Chromatography coupled with Mass Spectrometry (LCMS) applications, the quality of water is crucial. Ultrapure water, adhering to ASTM Type I specifications, is essential for mobile phase preparation, sample dilution, reagent preparation, and instrument cleaning. Water impurities such as particulates, bacteria, and ions can compromise chromatographic performance, leading to poor data quality, overlapping peaks, ghost peaks, and column damage. The freshly produced ultrapure water from our Arium® Mini UV (Arium®-water) systems can help scientists overcome water impurities and unlock the full potential of LCMS analysis.

Introduction

LCMS is a powerful analytical technique that combines the separating capabilities of liquid chromatography with the identification and quantification capabilities of mass spectrometry. LCMS has become a cornerstone in modern analytical chemistry and is widely used in various scientific fields, including pharmaceuticals, environmental analysis, food safety, metabolomics, proteomics, and forensic science.

In the pursuit of analytical excellence, ultrapure water plays a crucial role in LCMS applications. Ultrapure water, meeting ASTM Type I specifications, serves as the backbone of the entire analytical workflow, ensuring precision, reliability, and accuracy in results. Addressing the challenges posed by water impurities, particularly organic compounds, bacteria, and ions, becomes paramount to achieving an optimal performance in chromatography and mass spectrometry. By using Arium®-water purification systems, such as Arium® Mini UV, scientists and lab technicians can access ultrapure water of exceptional quality, elevating the sensitivity of analytical results. This article explores the vital role of ultrapure water in LCMS, the impact of water impurities on chromatographic performance, and the advantages of using cutting-edge water purification systems to ensure consistent, reliable, and accurate chromatographic data.

Effects of Impurities[1,2]

Organic Compounds

Organic compounds in the mobile phase are among the most critical contaminants in liquid chromatography applications. When present in the mobile phase, organic compounds may compete with the analyte to bind to the active sites of the stationary phase. This competition reduces the amount of analyte retained on the column, leading to reduced method sensitivity.

Accumulation of organic compounds on the column surface can restrict analyte and solvent access to active sites, resulting in mass transfer issues and loss of resolution. Furthermore, if organic contaminants accumulate at the head of the column, they can cause ghost peaks and interfere with the separation process.

In high-contamination scenarios, organic compounds can accumulate over time and act as a new stationary phase, leading to peak tailing and retention time shifts, ultimately affecting the accuracy and reliability of the analysis.

Organic Contamination

Bacteria and biofilm residue in the water can create blockages in columns and frits, leading to flow issues and degraded chromatographic performance. Additionally, organic by-products produced by bacteria, such as pyrogens, nucleases, or alkaline phosphatase, may result in chromatographic disturbances. These disturbances can affect the accuracy, precision, and reproducibility of the analysis, as well as the stability and lifespan of the column.

Inorganic Contamination

Contaminants, including excessive alkali ions, have the potential to skew results and introduce noise in analyses. To mitigate this risk, it is advisable to replace mobile phases instead of simply topping them off. Regularly replacing the mobile phases helps prevent the accumulation and concentration of contaminants in supply bottles, ensuring the integrity of the analytical process. Additionally, prolonged exposure to air can lead to phthalate contamination in HVAC systems, underscoring the importance of maintaining a clean and controlled environment for accurate and reliable measurements.

Ionic contaminants present in the water have the potential to alter the ionic strength of a solution, thereby exerting an influence on specific chromatographic separations. This adjustment in ionic strength can induce changes in diverse chemical and physical properties of the solution, encompassing alterations in conductivity, osmotic pressure, and the behavior of charged species within the solution.

Table 1: *Selected Product Water Quality*

	Conductivity [µS × cm^{-1}]	Resistivity [MΩ × cm]	TOC [ppb]	Endotoxin [EU × ml^{-1}]	Bacteria [CFU × mL^{-1}]
Arium® Mini UV	0.055	18.2	≤5	<0.001	<0.001

Advantages of Water Purification Systems
Commercially available LCMS grade water in bottles may provide inferior results due to inadequate bottle handling, or contamination from the presence of organic solvents in the production facility. For sensitive LCMS applications, it is crucial to use high-quality ultrapure water that meets the specific requirements of LCMS analyses to ensure accurate and reliable results.

To ensure optimal chromatographic performance, it is highly recommended to use freshly produced water from water purification systems. Sartorius' Arium® Mini UV systems are designed to deliver Type I ultrapure water with exceptional characteristics, such as high resistivity, low Total Organic Carbon (TOC) levels, and minimal bacterial content (see selected data in Table 1). These water purification systems ensure that the water used in LCMS applications meets the highest purity standards, resulting in reliable and accurate chromatographic data.

Total Organic Carbon
Measuring specific organic compounds in purified water can be impractical due to their diverse nature and complexity. Consequently, TOC serves as a practical indicator of overall organic contamination. In this method, organic substances in the water are oxidized, and the resultant products are measured to determine the level of organic contamination.[3]

Ultraviolet Radiation
UV radiation is employed to break down and oxidize organic compounds present in the water. A wavelength of 185 nm is effective in breaking down and oxidizing carbon-containing molecules, producing ionized fragments that can be subsequently removed by ion exchange.[3] UV light inactivates microorganisms by inducing the formation of dimers in RNA and DNA, resulting in damage that disrupts transcription, replication, and leads to bacterial demise.[4]

Arium® Mini UV systems utilize full-spectrum UV lamps to maximize the breakdown of organic molecules, ensuring that the water used in liquid chromatography applications is of the highest quality.

Materials and Methods

The following data are collected from feasibility studies conducted in the upstream laboratory at Sartorius Bohemia NY USA. A DG44 CHO-Cell mAb production process was carried out using the Sartorius Ambr® 15 platform. Low molecular weight fragments and intact glycan profiles were evaluated for clarified cell culture samples obtained during the production process. Samples were not purified via protein A, only clarified by syringe filtration and loaded onto the Waters BioAccord™ LCMS System equipped with a BioResolve™ RP mAb Polyphenyl Column. Any solvent and reagents not explicitly described are LCMS grade. The BioAccord™ system was controlled using waters_connect™ informatics software.

After being removed from Ambr® 15, samples were centrifuged at 380 RCF for 2 min before being filtered through Sartorius Minisart® PES15 0.2 µm syringe filters ensuring the absence of any cell particulate. Due to high titer concentrations, all samples were diluted to a standard 0.2 mg/mL using water from the conditions listed below. The LCMS system was purged of all prior water in the lines from the mobile phase A (aqueous) bottle between changing source conditions before the following sample was run.

Arium® Mini UV Ultrapure Water Systems

Compact and Reliable
Designed for smaller spaces, Arium® Mini ensures reliable results. With user-friendly displays and innovative bagtank technology, it's ideal for routine analyses even in limited laboratory spaces.

Arium® Mini UV Water Purification Systems are designed for Type I water requirements of 10 L per day from either tap water or pre-treated water. They meet the needs of smaller labs or research spaces and are compact yet robust solutions. Their slim design of only 28 cm in width allows them to fit easily into limited lab spaces. Arium® Mini systems are ideal for use in critical and non-critical life science applications and in analytical lab procedures.

Waters BioAccord LCMS

LSMS Method Condition

Table 2: *Water Samples for Comparison Study*

Water Conditions	Water Source	Stated Resistivity
1	Arium® Mini	> 18 MΩ
2	Utilities USP Water	> 18 MΩ
3	Commercial LCMS Bottled Water	Not Stated

Table 3: *LC and MS Condition for the Initial Study*

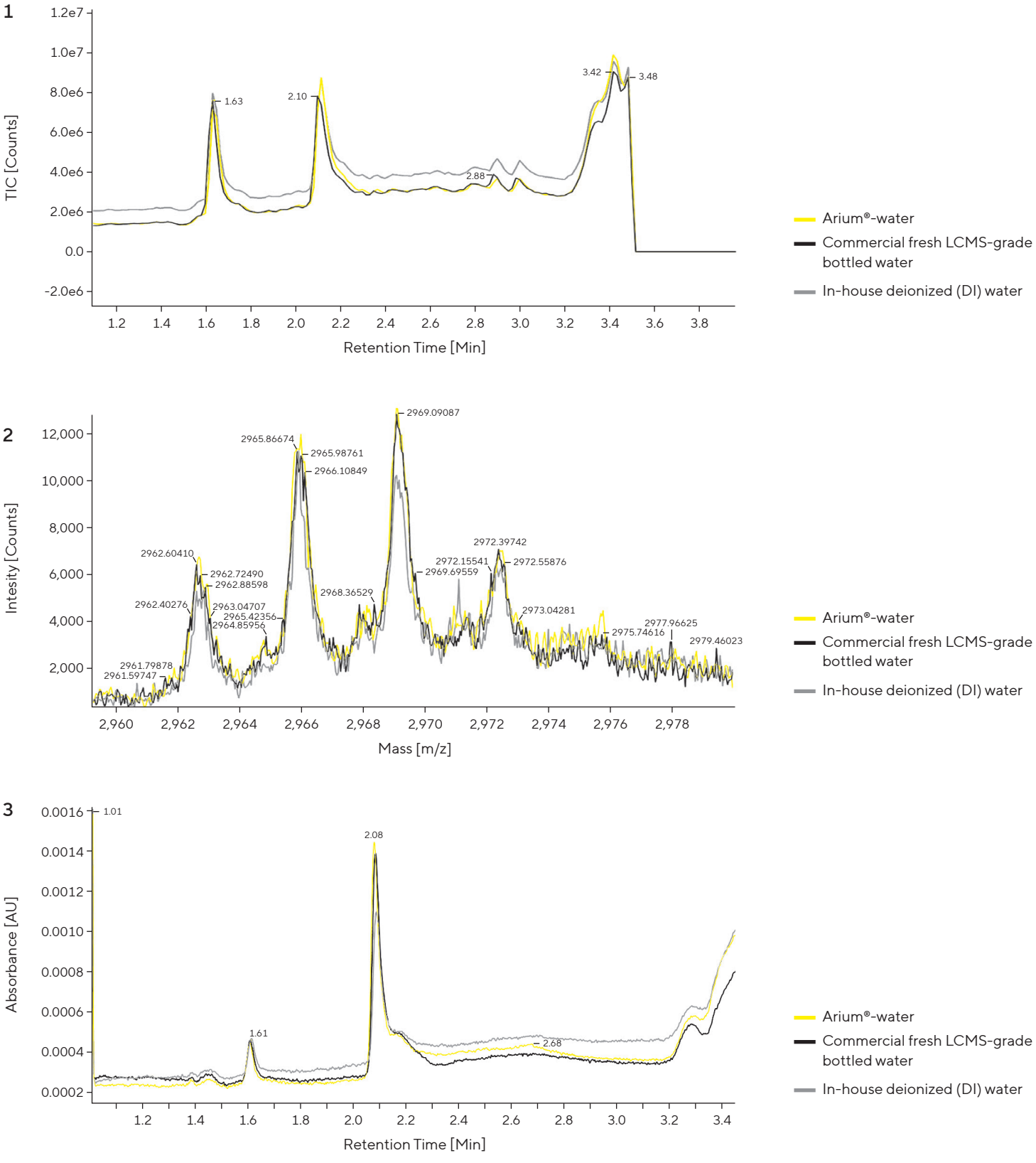
LC Conditions	
LC System	Waters ACQUITY™ Premier System
Detection	TUV
Column	2.1 × 100 mm BioResolve™ RP Polyphenyl
Column Temp.	60 °C
Sample Temp.	8 °C
Injection Volume	5 µL
Flow Rate	0.4 mL/min
Mobile Phase A	H_2O, 0.1% Formic Acid (Range of conditions)
Mobile Phase B	Acetonitrile, 0.1% Formic acid

MS Conditions	
MS System	Waters ACQUITY RDa™
Ionization Mode	Positive
Acquisition Range	500 – 7,000 m/z
Capillary Voltage	1.5 kV
Collision Energy	NA
Cone Voltage	95 V

Results

Comparison Study

Figure 1: *Total Ion Chromatogram (TIC; 1; TOF MS (400 – 7000), 95 V ESI+), Mass Spectral Data (2; Average Time 2.1751 min, TOF MS (400 – 7000), 95 V ESI+: Combined) From a Monoclonal Antibody Sample And UV Traces (3; Channel Name: TUV 280)*



The water sources included freshly opened LCMS-grade bottled water, known for its high purity, and Arium®-water, which also sticks to stringent quality standards. Additionally, the performance of in-house utilities deionized (DI) water (USP) was examined, a commonly used water source in laboratory settings due to its availability and cost-effectiveness.

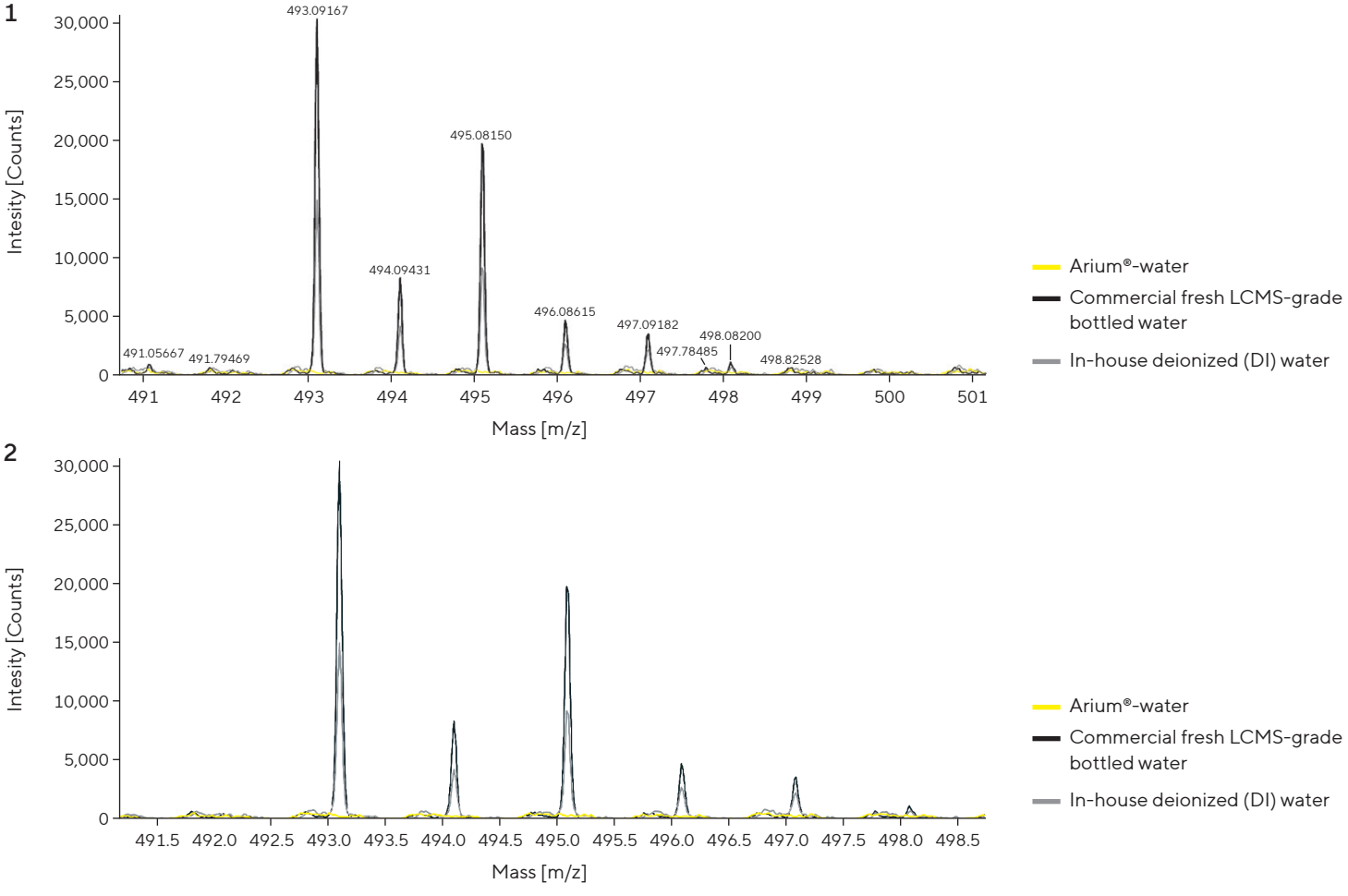
Upon analyzing the obtained data, interesting trends emerged. Both LCMS-grade bottled water and Arium®-water displayed similar chromatographic profiles, showcasing comparable peak shapes and baseline stability. These results indicate that the two high-purity water sources perform consistently and effectively in LCMS analyses.

However, contrasting results were observed when using in-house utilities DI water (USP). The chromatographic profiles exhibited a proportionately elevated background level, suggesting the presence of impurities or contaminants (TOC, inorganic and bacterial contamination) in the water. Additionally, a slight difference in peak area was noted, further emphasizing the potential impact of water quality on the accuracy and reliability of chromatographic measurements.

The peak visible at ~1.61 min corresponds to free Light Chain in the sample and is visible in all water conditions. This is not uncommon for unpurified cell-culture samples. Due to the exclusion of the Protein A purification step, it is possible to see other proteins in solution at the time of sample. The larger peak at ~2.08 min corresponds to the complete mAb. This peak is easily differentiated in all water conditions (replicate sample). While the Arium® and bottled water express very similar curves, the USP water causes a lower peak intensity and higher background absorbance
While investigating the mass spectra of the free light chain in solution, it was seen that a potential contaminant was visible in the lower section of the m/z range for some samples. On closer investigation and data analysis, the contaminant was observed to only be present in the USP and bottled water mobile phase samples (Figure 2).

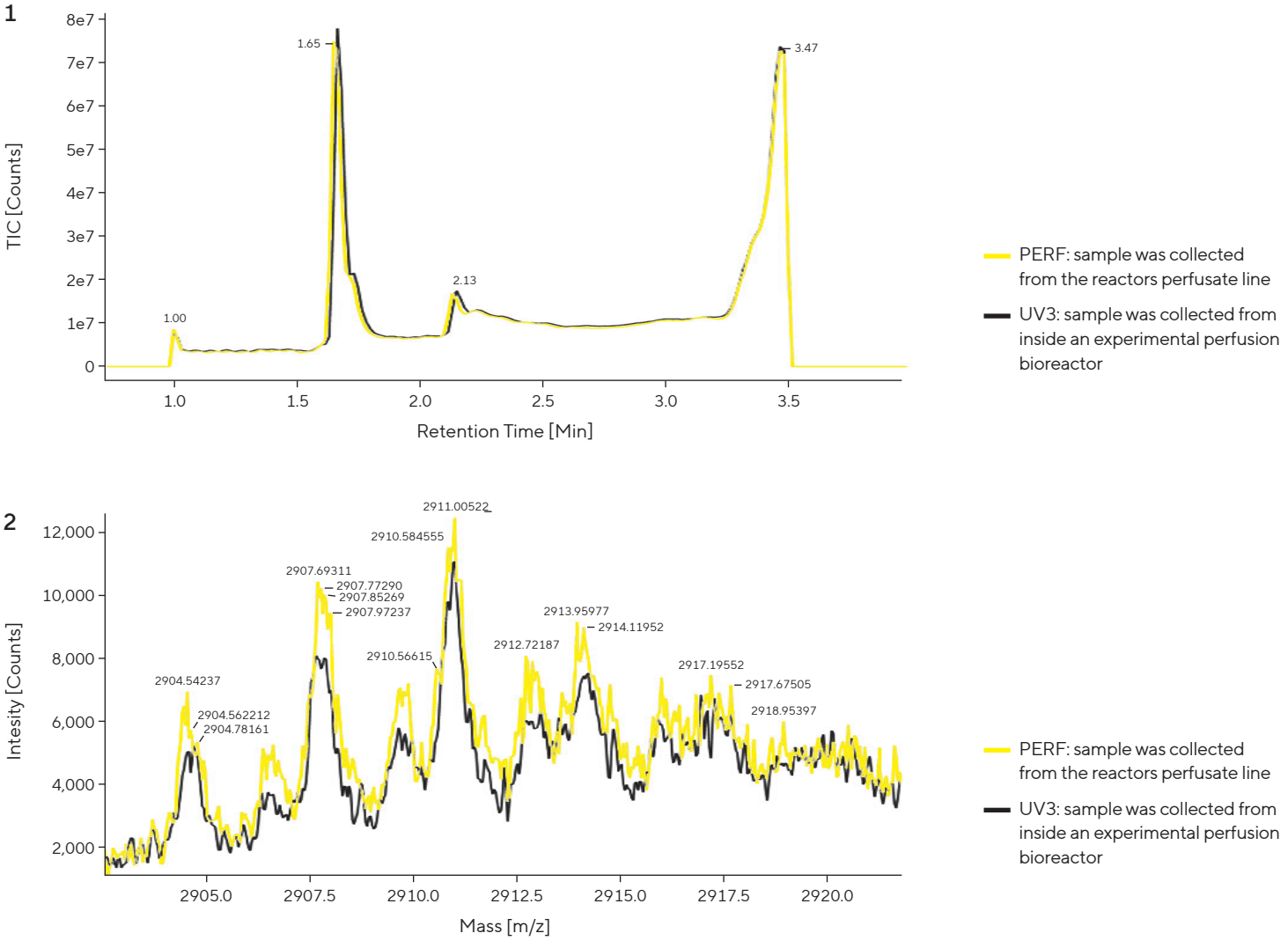
This phenomenon was observed for triplicate samples (data not shown). The isotopic abundances of the ion are consistent with a contaminant molecular formula of $C_{24}H_{19}N_6O_2Cl_2$. The exact structure was not elucidated but the clear difference in the abundance can be seen.

Figure 2: *Extracted Mass Chromatogram of Retention Time ~1.6 – 1.7 Min (Light Chain Peak, 1) And Zoomed in Spectra (2); Average Time 2.1168 min, TOF MS (400 – 7000), 95 V ESI+: Combined.*



Initial Experiment with Arium®-water

Figure 3: *Total Ion Chromatogram (TIC; 1, TOF MS (400 – 7000), 95 V ESI+) And Mass Spectrum From a Monoclonal Antibody Sample Prepared With Arium®-water (Average Time 2.1418 min (UV3) and 2.1668 min (PERF), 2, TOF MS (400 – 7000), 95 V ESI+: Combined).*

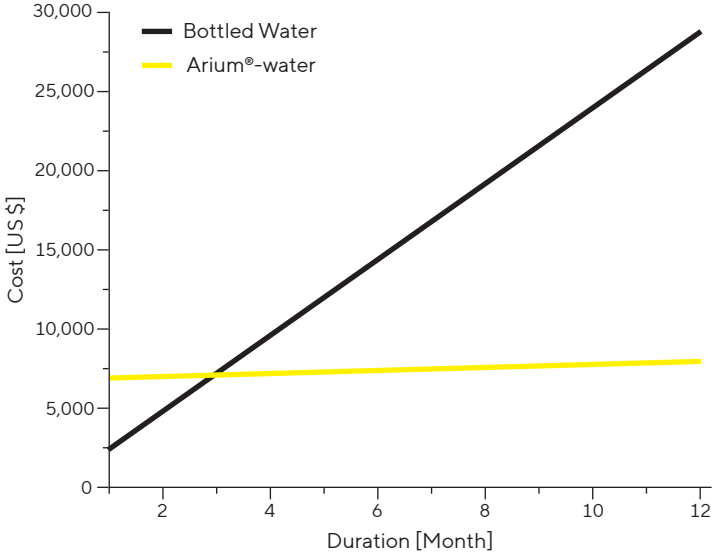


Given the success in performance and reliability of Arium®-water, it was fully adopted in the Sartorius upstream laboratory for LCMS applications. Figure 3 displays intact mass results from a perfusion CHO cell culture with samples from both inside the bioreactor and within the perfusate path (PERF).

Small changes in relative glycoform abundance between the two samples can be seen clearly with relatively low background making results easy to elucidate.

Return of Investment

Figure 4: *Comparison of Expenses Between In-House Produced Arium®-water Type I (Ultrapure Water) and Bottled Water (LCMS Grade, With PFAS Content Below Detection Limit)*



Note: Calculation based on following assumptions: 2 L water consumption per working day, 20 working days per month, Arium® Comfort I UV system plus consumables list price = $9,590, annual Arium® Comfort I UV consumables list price = $2,400 and costs bottled water LCMS grade = $60/L*.

Conclusion

The study underscores the necessity of high-quality water sources for dependable LCMS outcomes. The use of in-house DI water or even bottled LCMS water in some cases may introduce contaminants that impact the accuracy of the data and robustness of the system. Premium purified water sources like Arium®-water are imperative for accurate results. Water purification systems, especially Arium® Mini UV, ensure essential water quality and enhance chromatographic system efficiency, bolstering data quality. An illustrative cost analysis spanning a year demonstrates the benefits of adopting in-house treated water (Figure 4). The acquisition of the device becomes financially advantageous after approximately three months, especially when there is a daily demand of 2 L or more, resulting in significant cost savings within a year.

Overall, water quality has a significant impact on UPLC performance and systems require ASTM Type I ultrapure water to achieve the most accurate results. Water purification systems such as the Arium® Mini UV are essential for optimal chromatographic performance, accurate analysis, and improved laboratory efficiency.

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