Milli-Q®
Lab Water Solutions

**Application Note**

# Four Ways to Better Water Quality in LC-MS

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High purity water is key to the success of analyses performed using (ultra) high-performance liquid-chromatography-mass spectrometry, (U)HPLC-MS — or as it is more commonly known, LC-MS. Even so, chromatographers may consciously select top-quality salts and organic solvents for mobile phase preparation, but not apply the same level of care when choosing their source of high purity water.

This article will discuss several aspects of working with high purity water in LC-MS analyses including quality and handling. Solutions will be proposed for optimal water purification based on the use of complementary purification technologies combined with application-specific final filtering, and finally, the importance of water purification system maintenance will be discussed.

## 1) Start with the best-quality water

Natural water contains several major classes of contaminants, including inorganic ions; organic molecules; particulates and colloids; and bacteria and their byproducts. As these contaminants are also present in tap water, water must be carefully purified before it can be used in highly sensitive analytical techniques such as LC-MS in order to avoid impacting analyses.

Laboratory water purification system manufacturers have defined laboratory water types as Type 1, 2, or 3 according to the level of contaminants contained in the water. Table I shows the specifications for the different types of water delivered by Merck Millipore water purification systems. Type 1 is the purest, and is referred to as "ultrapure," "high purity," or "Milli-Q® water." Type 2 water refers to pure water, or "Elix® water." Type 3 water is the lowest grade of laboratory water.

**Table I.**

| Contaminant | Parameter & unit | Type 1 | Type 2 |
|---|---|---|---|
| **Ions** | Resistivity [MΩ˙cm] - 25 °C | 18.2 | ≤5 |
| **Organics** | TOC [ppb] | ≤5 | <30 |
| **Particulates** | Particulates > 0.2 µm [units / mL]* | <1 | <1 |
| **Bacteria** | Bacteria [CFU / 100 mL]* | <10 | <10 |

*With Millipak® polisher

The table above outlines the different Merck Millipore water specifications for Types 1 and 2 water. (At Merck Millipore, the quality of Type 3 water is assessed through successful ionic, o
rganic and particulate removal with respect to feed water quality.)

To avoid interferences with measured analytes in (U)HPLC and LC-MS applications, and to maintain the (U)HPLC system in proper working condition, Type 1 ultrapure water is generally used — and for a number of purposes. Water is used extensively in analyses in the preparation of standards, blanks, and samples, and as a component of mobile phase. Moreover, as water is present in mixtures at a high percentage, the contribution of water contamination will be relatively more important compared to the other mixture components. Water is also needed to wash and rinse containers: for LC-MS applications, this water should also be ultrapure, in order to avoid introducing a contaminant to an otherwise uncontaminated sample.

Table II shows the different classes of contaminants present in both natural and tap water, as well as their effects on analyses and instrumentation. Contamination can seriously undermine analyses, leading to difficulties in data interpretation, wrong conclusions, and time wasted troubleshooting instruments and having to repeat analyses.

**Table II. Effect of water contaminants in LC-MS.**

| Contaminant | Components | Possible Effects |
|---|---|---|
| **Inorganic Ions** | • Cations: sodium, calcium, magnesium, iron<br>• Anions: bicarbonate, chloride, sulfate | • Create complicated mass spectra, due to adduct formation<br>• Contribute to ion suppression |
| **Organics** | • Dissolved organic molecules in tap water are mainly of biological origin; plant decay releases humic/fluvic acids, tannins and lignin.<br>• Man-made contaminants, such as phthalate esters, can leach into water from PVC pipes. | • Create ghost peaks and interferences<br>• Cause baseline drifting<br>• Decrease sensitivity and resolution<br>• Cause poor repeatability<br>• Contribute to reduced column lifetime |
| **Particulates and colloids** | **Soft Particulates:** vegetal debris<br>**Hard Particulates:** sand, rock<br>**Colloids** | • Can interfere with instrument operation<br>• Damage the (U)HPLC pump and injector<br>• Plug the column and frits<br>• Increase backpressure |
| **Bacteria and their byproducts** (pyrogens, nucleases, alkaline phosphatase) | **Live microorganisms in tap water** (Harmful bacteria are removed by the chlorination process). | • Create ghost peaks<br>• Plug the column and frits<br>• Increase backpressure |

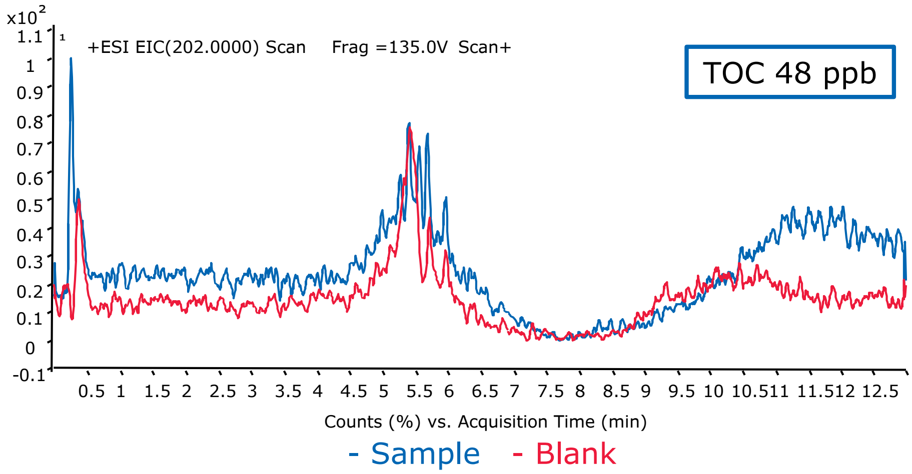
An important way to keep control over contaminants is to make sure that the water purification system used for LC-MS work is capable of advanced water quality monitoring. In order to obtain information about both ionic and organic contaminants, two types of monitoring capabilities are recommended: resistivity monitoring and Total Oxidizable Carbon (TOC) monitoring.

High-precision resistivity monitors provide information on the ionic purity of the water dispensed from the water purification system. This information is of particular value to scientists analyzing ions or metals. A resistivity value of 18.2 MΩ·cm means that the number of ions contained in the ultrapure water is not significant (total ionic concentration is < 1 ppb).

The presence of ions is particularly important in mass spectrometry detection. Ions, indeed, can combine with some organic molecules to generate adducts, making the MS data analysis much more complicated. In addition, the presence of ions may lead to ion suppression, reducing the intensity of the signals.

TOC monitors measure organic purity in general, and show levels of organic contamination in terms of parts per billion (ppb). Traditionally, a low TOC level is achieved through UV photooxidation technology that reduces TOC in ultrapure water to less than 5 ppb.

The chromatogram in Figure 1 shows high TOC levels in ultrapure water, with resulting ghost peaks, interferences with measured analytes, and a noisy baseline. When TOC levels are high, there is a greater probability that interferences with analytes will occur, or that experiments will fail. This, of course, leads to time lost trying to understand the reason for failure, and/or to repeat the experiment.




Hibar® HR Purosphere® STAR RP-18
Agilent 1290 Infinity® HPLC coupled to an Agilent® 6420 Triple Quad LC-MS
Ultrapure water and ACN
Sample: Simazine in drinking water

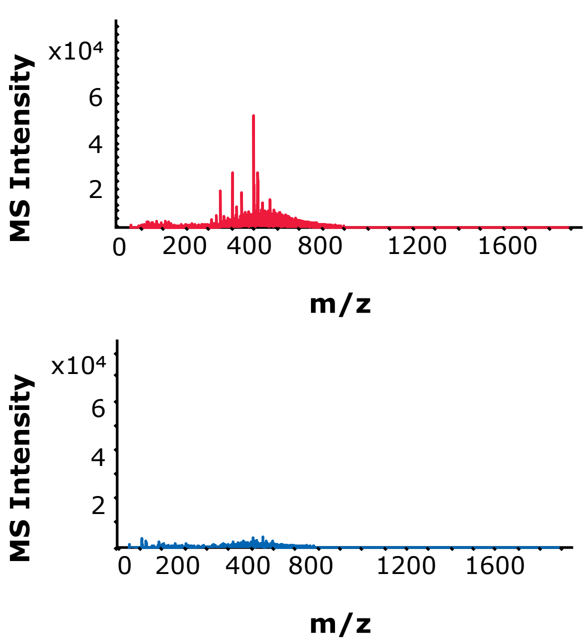
**Figure 1.**

High TOC levels: ghost peaks, interferences, and noisy baseline in the water used for the blank.

Overall, dual resistivity and TOC monitoring provides important information that allows the water purification system user to keep control over water purity at all times, leading to confidence that any problems in experiments are not water-related.

## 2) Handle with care

Making sure you have the best-quality water possible is the best way to begin your LC-MS analyses. Ultrapure water, however, is an extremely good solvent. Once dispensed from the water purification system, ultrapure water tends to be contaminated very quickly from a variety of sources including the lab environment and any storage containers that are used. , Water quality can also degrade if the water purification system does not work properly, but this aspect will be discussed separately in Section 4.




bottled ultrapure water from competitor B stored for four weeks

freshly produced Milli-Q® water

**Figure 2.**

Comparison of the purity of LC-MS grade water from competitor B (top) stored for four weeks to the purity of freshly produced Milli-Q® water (bottom). Milli-Q® water, with Total Organic Carbon (TOC) below 5 ppb, and resistivity of 18.2 MΩ·cm, was produced from a Milli-Q® water purification system fed by an Elix® system with a 0.22 µm membrane final point-of-delivery purifier (Millipak®). The analyses were performed via direct injection of the solvents into the MS operated in ESI positive mode.

In addition, it is recommended that ultrapure water not be stored: ideally water should be dispensed from the water purification system just before use in LC-MS analyses.

In real-life situations, however, everyone may not have this option: if it is necessary to store ultrapure water for a short time, there are several factors to take into consideration. First and foremost, it is important to choose a container that is appropriate for the application — and to select the best quality possible.

In addition to absorbing contaminants from the laboratory atmosphere, ultrapure water that is in contact with a storage container will also absorb ions and organics. Glass containers will release ions, and over time, ultrapure water stored in a glass bottle will pick up these ions. For LC-MS users, ionic contamination can be an issue when ions form adducts, creating additional peaks in mass spectra, complicating data analysis, and creating interferences with measured analytes.(2)

Glass containers can also release organics into water, but in smaller quantities than plastic containers, (such as polyethylene carboys), which can leach plasticizers, as shown below (Figure 3). If you absolutely must store ultrapure water for LC-MS, then it is best to do so in glass (preferably borosilicate bottles) rather than plastic containers, and for the shortest time possible.

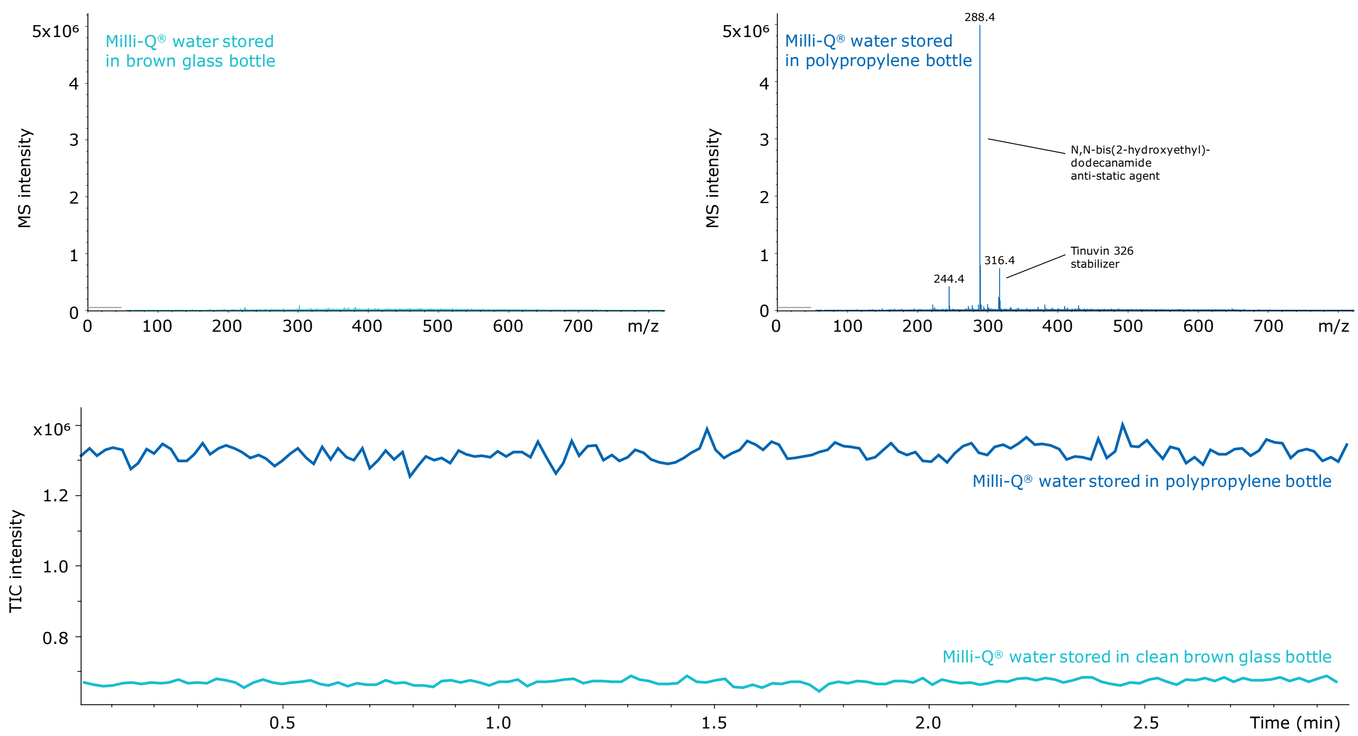



**Figure 3.**

The figure shows mass spectra of two Milli-Q® water samples stored in brown glass and polypropylene bottles, respectively (top), and TICs of the same samples (bottom). The analyses were performed via direct injection of the solvents into the MS operated in ESI positive mode.

LC-MS users of ultrapure water should also be aware that any containers used for water collection (including caps) should always be cleaned thoroughly, and then rinsed several times with ultrapure water to remove any traces of contaminants or detergents before water collection.

## 3) Combine purification technologies

No single water purification technology is capable of fully removing all of the classes of water contaminants. Therefore, to remove and control the level of contaminants in water, it is recommended to select an ultrapure water purification system that uses a combination of several purification technologies.

In addition to complementary technologies, LC-MS scientists will also want to select their system to meet additional criteria, including the sensitivity of their analyses, monitoring needs, and system size — both in terms of actual physical footprint, as well as volumes of water required daily, which can range from several liters a day up to several thousand liters a day.

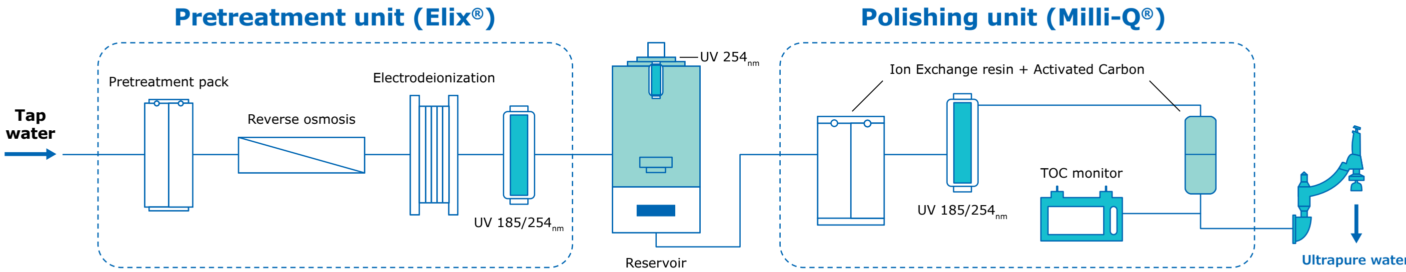



**Figure 4.**

Schematic of a water purification chain that delivers ultrapure water suitable for LC-MS use

Figure 4 shows the combination of water purification technologies that is recommended for (U)HPLC and LC-MS analyses. The water purification chain starts with a pretreatment pack following by a reversed osmosis (RO) membrane. Together these technologies will decrease the level of organics from 1000 ppb to 50-100 ppb and provide Type 3 water in which > 95% of all ions have been removed.

RO treatment is followed by electrodeionization in the Elix® module to further remove ions, as well as treatment with a germicidal UV lamp to prevent bacterial growth. This Type 2 water has a low TOC level and resistivity at or above 5 MΩ.cm. However, the water must be further treated to obtain water of a quality suitable for (U)HPLC analyses. This last stage consists of final purification (or polishing) steps, using ion-exchange resins, synthetic activated carbon, and UV photooxidation, in order to produce 18.2 MΩ·cm ultrapure water with a TOC level at or below 5 ppb.

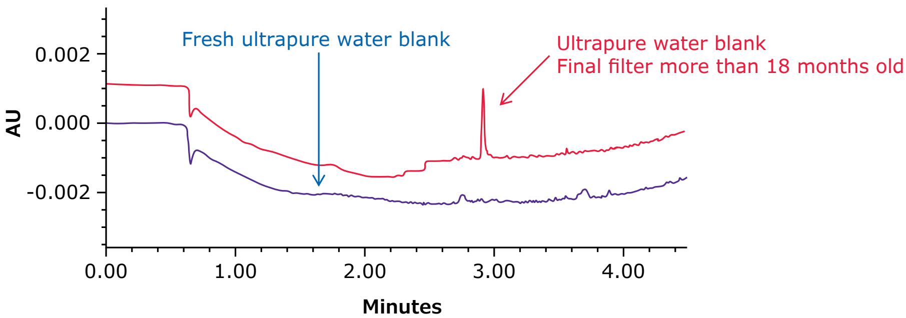
Finally, a point-of-use filter can be placed at the end of the chain, with the dual purpose of adding a purification step immediately before water is delivered and used, and also preventing retro-contamination of the purification chain from airborne sources. For LC-MS, the focus of this final purification step can be either to prevent particles and bacteria in ultrapure water, and/or to remove traces of organics that very rarely are still present in the water. In the first instance, a 0.22 µm screen filter is used, and in the second, a C18-based polisher. Selection of the final filter will depend on the sensitivity of the analyses, and the type of instrument and detection method used.


**Figure 5.**

Water purification system producing water suitable for LC-MS use; the Q-POD® ultrapure water dispenser is fitted with a 0.22 µm point-of-use final filter.

## 4) Don't neglect maintenance

Last, but certainly not least — proper and regular maintenance of the water purification system will extend the system's lifetime and also help ensure optimum ultrapure water quality. It is important to follow the manufacturer's recommendations concerning replacement dates for purification cartridges, UV lamps, and point-of-use final filters. Water purification systems will generally display warning messages when replacement of any of these items is needed; these warnings should not be ignored.




**System:** Acquity UPLC®

**Column:** RP18

(1.7µm, 2.1 x 50 mm) at 35 °C

**Gradient** from

0% ACN to 100% ACN in 5 min

**Flow rate** 0.6mL/min

**Detection** at 254 nm

**Figure 6.**

The peak in the red chromatogram is potentially due to contamination of ultrapure water when the point-of-use filter was used beyond its recommended lifetime. The blue chromatogram shows an ultrapure water blank following replacement of the filter.

The UHPLC chromatograms above (Figure 6) demonstrate the effect on fresh ultrapure water of using a 0.22 µm final filter beyond its recommended replacement date. The red chromatogram shows a peak that potentially is the result of bacterial contamination that can release organics; the blue chromatogram shows the water blank when the final filter was replaced.

Another aspect to take into account when using point-of-use final filters, is that contaminants in the lab can adhere to the final polisher membrane and come out as ghost peaks in chromatograms. This can be avoided easily by discarding the first few liters of ultrapure water in the morning before collecting water for use during the day.

Ghost peaks may also be caused by failing to replace the pretreatment cartridges upstream from the RO membrane in a timely manner. It is thus critical to properly maintain the water purification system and to replace consumables in time.

Proper maintenance also includes regular calibration of any monitoring equipment present in the water purification system, such as the resistivity and TOC monitors. Well-maintained monitors help water purification system users keep control over the resistivity and TOC values of water produced by the system — and take corrective action when needed.

## Summary


By taking the above recommendations into account, LC-MS users will be able to eliminate from the start many of the water-related issues that could damage water purification and LC-MS systems, and also impact and possibly compromise LC-MS experiments and analyses.

## Acknowledgments

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