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WATER PURIFICATION IN THE CHROMATOGRAPHY LAB

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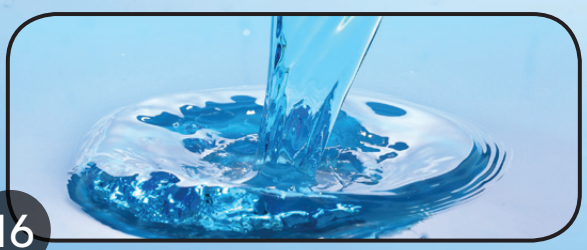

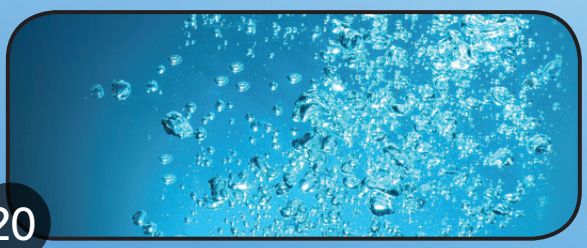

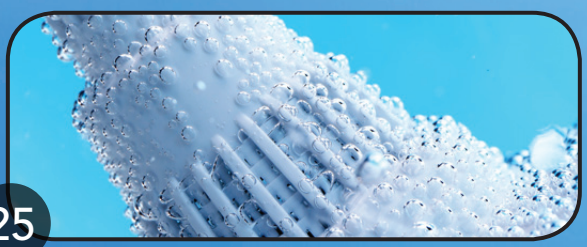

LCGC UBM

WHY AND HOW TO AVOID IONIC CONTAMINATION IN WATER USED FOR LC–MS ANALYSES

Anastasia Khvataeva-Domanov and Stéphane Mabic

Ionic contaminants in the water used in ultrahigh-pressure liquid chromatography (UHPLC) analyses with mass spectrometry (MS) detection lead to adduct formation and reduced analytical signals because of ion suppression. In MS, the preferred ion type is the protonated molecular ion, especially in peptide analysis, since the partially mobile proton charge enables more meaningful fragmentation analysis, as compared to a sodiated peptide ion. Moreover, the occurrence of protonated analyte signals demonstrates that solvents and reagents, as well as the MS instrument used in analyses, were clean and did not contribute any contaminating cationic components to the analytical process. In the experiments presented here, it was observed that the signal intensities of the protonated species decreased as the sodium ion concentration in the water increased. This was accompanied by an increase in the intensity of sodiated adducts..

Water plays an essential role in liquid chromatography–mass spectrometry (LC–

Table I: Direct infusion experiments

	First set of experiments	Second set of experiments
Sample	Bradykinin (fragment 1-7) Monoisotopic Mass 756.40 Da [M+H] ion (*m/z* 757) [M-115+Na] ion (*m/z* 664) [M-202] ion (*m/z* 554)	Glu1-fibrinopeptide B Monoisotopic Mass 1569.65 Da [M+H] ion (*m/z* 1570) [M+2H] ion (*m/z* 785) [M+Na+H] ion (*m/z* 796) [M+2Na] ion (*m/z* 807) [M+3Na-H] ion (*m/z* 818)
Instrument	Applied Biosystems API 2000	Waters Synapt HDMS
Ionization mode	ESI+	ESI+
Flow	10 µL/min	10 µL/min
Solvents	(a) 96:4 fresh ultrapure water–acetonitrile (b) 96:4 1 ppm Na+ in ultrapure water/ acetonitrile	(a) 50:50 fresh ultrapure water–acetonitrile (b) 50:50 1 ppb Na^+ in ultrapure water–acetonitrile (c) 50:50 100 ppb Na^+ in ultrapure water–acetonitrile (d) 50:50 1000 ppb Na^+ in ultrapure water–acetonitrile
Sample concentration	1 µM	500 pM

MS), where it is used extensively in the workflow. Contaminants in the water can affect the quality of data and instrument performance; therefore, it is recommended and prudent to use only the highest purity solvents. Organic contamination of the water used in high performance liquid chromatography (HPLC) is an important issue and has been addressed accordingly (1), but the ionic purity of the water should also be considered, especially when MS is used as a detection technique (2). Ionic contaminants lead to adduct formation and reduced analytical signals because of ion suppression.

Electrospray ionization (ESI) remains the most popular MS technique. In positive-ion analyses, it is ideal to have only protonated peaks of the parent ion or its fragments in the mass spectrum. The presence of metal adduct peaks, such as sodium adducts (M+Na), makes data analysis more challenging and complicated. Metal ions may come from several possible sources (3) such as solvent reservoirs, gloves, the analyst, and the solvents used in preparing the mobile phase. Therefore, using ultrapure water free of metal ions will contribute to the success of any LC–MS analysis.

Experimental

Experiments to Evaluate the Effect of Ionic Contamination in Water on MS Data

Two compounds, bradykinin fragment 1-7 and Glu1-fibrinopeptide B, were used to investigate the effect of ionic contamination on LC–MS analyses. Different samples of peptides were prepared and infused directly to a mass spectrometer. The experimental details for peptide analyses are described in Table I.

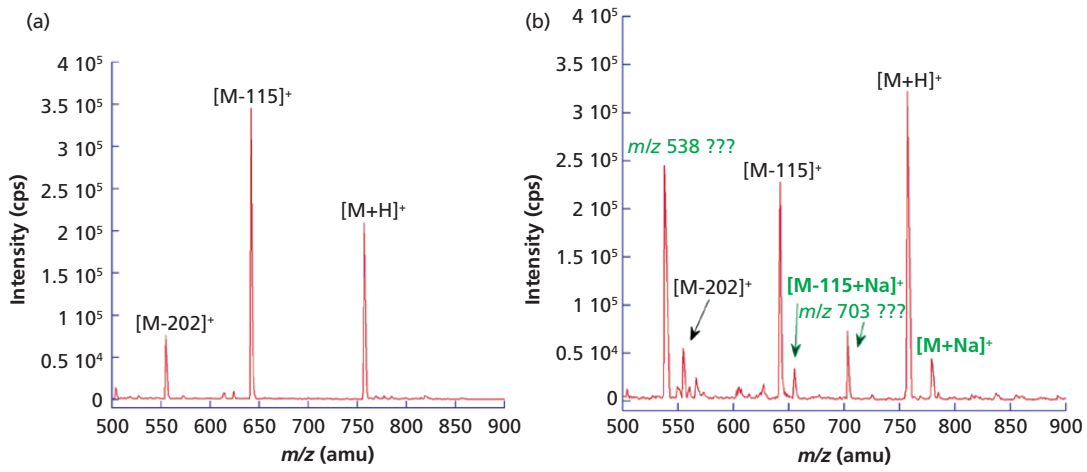

Figure 1: Mass spectra of bradykinin (fragments 1-7). Direct infusion, ESI+, using (a) 96% fresh ultrapure water, 4% acetonitrile and (b) 96% 1 ppm Na+, 4% acetonitrile.

Experiments to Identify the Levels of Ions in Ultrapure Water

To evaluate the level of sodium in ultrapure water, water samples from an EMD Millipore Milli-Q Advantage A10 water purification system were analyzed using an Agilent 7700s inductively coupled plasma–mass spectrometry (ICP-MS) instrument (4). The calibration standards used in experiments were a mixture of Agilent and Spex CertiPrep, and containers were all perfluoralkoxy (PFA) polymer precleaned with ultrapure water.

Water Purification Systems

All ultrapure water samples (resistivity of 18.2 MΩ·cm and total oxidizable carbon [TOC] below 5 ppb) from EMD Millipore water purification systems were analyzed immediately after water collection.

Results and Discussion

Effect of Ionic Contamination in Water on MS Data

The quality of LC–MS data is influenced by many factors, such as instrumentation, experimental parameters, sample preparation, the quality of reagents used, and the quality of solvents (5). Water is used extensively in reversed-phase LC–MS workflows; therefore, its purity plays a critical role in instrument performance and the quality of data generated.

The purity of water for LC–MS work is mainly assessed through organic contamination, which can be expressed by the level of TOC (6), or by certificates of analysis reporting results of suitability tests.

The second approach to evaluate water quality for LC–MS analyses is to assess its level of ionic purity. Thus, for bottled water, certificates of analysis usually detail maximum concentrations for certain ions because when stored in a glass bottle, the water will leach

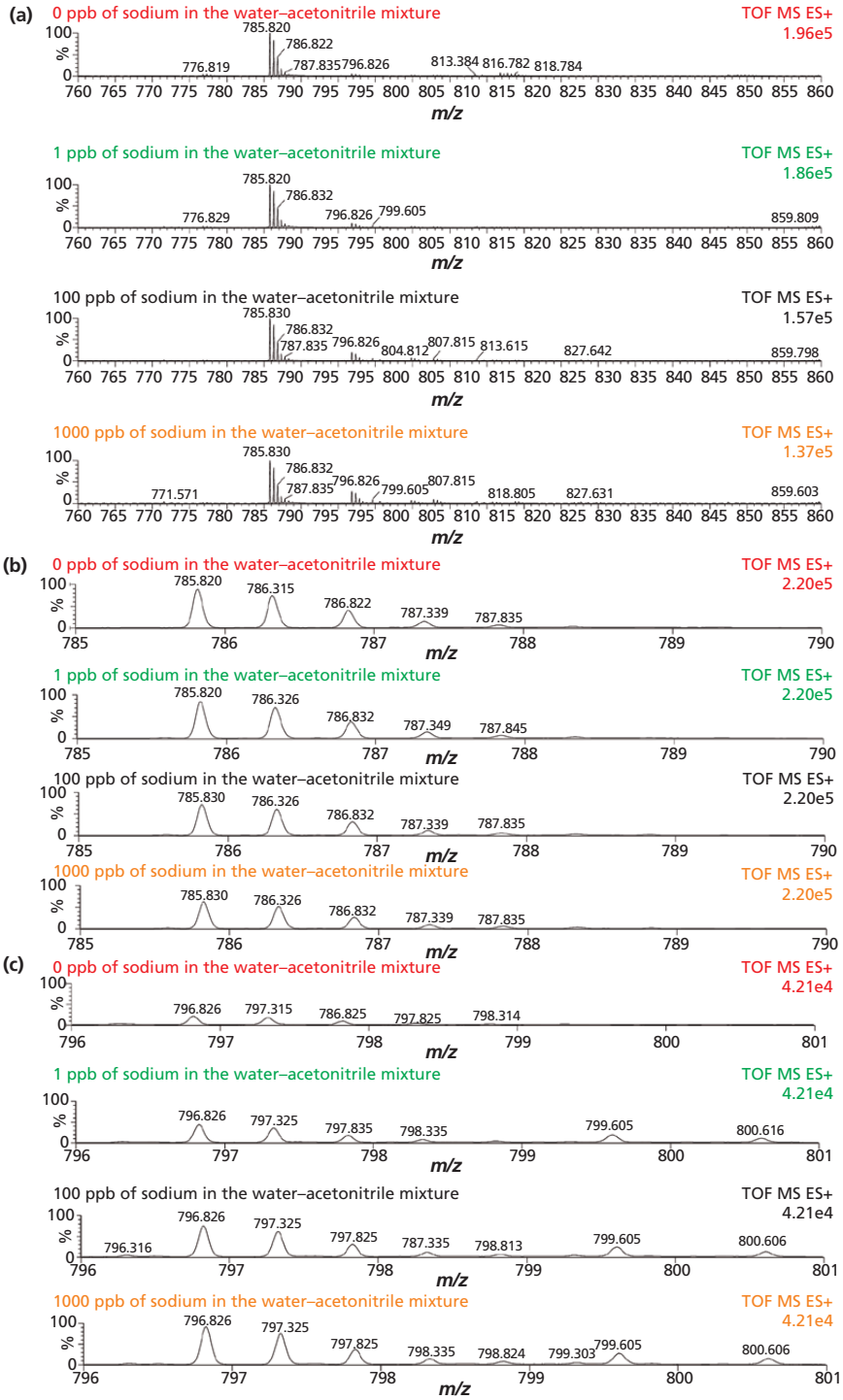

Figure 2: (a) Mass spectra of protonated molecular ion [M+2H] of Glu1-fibrinopeptide B sample, (b) and (c) extracted ion chromatograms of [M+Na+H] ion as examples of the effect of ionic concentration on MS detection.

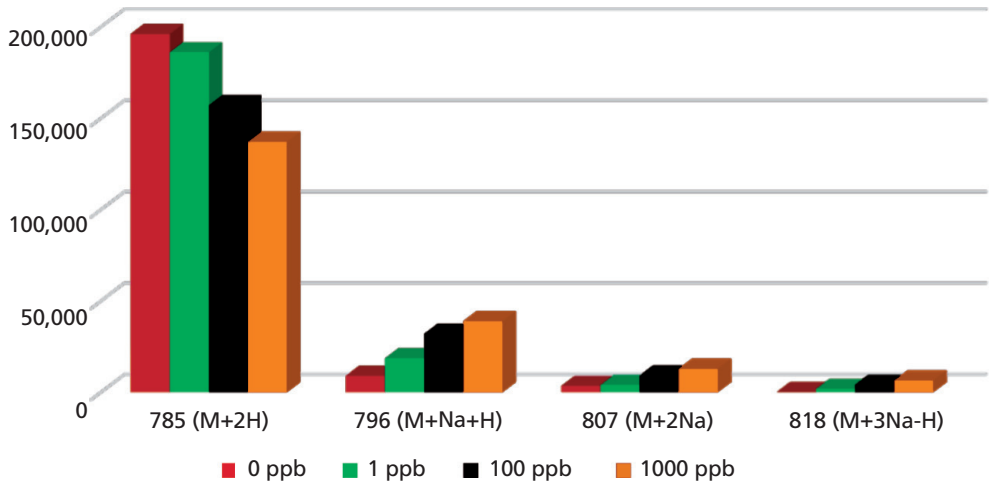

Figure 3: Signal intensities of 500 pmol Glu1-fibrinopeptide B in 50:50 (v/v) acetonitrile–water. The mixture was spiked with different amounts of sodium ions and injected directly into the mass spectrometer.

out contaminants from the container even though the water may have been of very high purity immediately after production. For example, standard glass bottles leach out alkali, contaminating ultrapure water and leading to a higher count of adducts. Since the quality of bottles can vary strongly from one to another, the nature of ions selected to report in the certificate of analysis depends on the quality of the bottle used to store the water. Table III compares the specifications for some metal ions in ultrahigh-pressure liquid chromatography (UHPLC)–MS-grade, LC–MS-grade bottled waters from three vendors, and in fresh ultrapure water.

Because ionic impurities present in water increase its conductivity, the conductivity parameter, or alternatively its inverse, resistivity, can be used to characterize the ionic purity of water.

Table II: Freshly produced ultrapure water sources

Experiment	Water purification system used
Bradykinin direct infusion and ICP-MS	Elix + Milli-Q Advantage A10 (Q-Gard and Quantum TEX cartridge, Millipak final filter)
Glu1-fibrinopeptide B direct infusion	Milli-Q Integral (Quantum TEX cartridge)

Thus, at 25 °C, conductivity of 0.055 µS/cm, or resistivity of 18.2 MΩ·cm, implies that the water is ultrapure, whereas at 1 ppb of Na^+ in ultrapure water, resistivity decreases to 17.6 MΩ·cm, and at 5 ppm, dramatically drops down to 0.093 MΩ·cm; these values can be calculated based on the concentration of sodium, its charge, and mobility (7). Therefore, when fresh ultrapure water of 18.2 MΩ·cm resistivity measured via an in-line monitor was used to dissolve a peptide sample (bradykinin fragment 1-7), and infused directly to a mass spectrometer, the resulting spectrum was clean (Figure 1a).

Table III: Specifications for some metal ions in UHPLC–MS-grade or LC–MS-grade water and fresh ultrapure water produced by a laboratory water purification system specifically for LC–MS practice*

	Maximum Concentration (ppb)			
Metal Ion	UHPLC–MS Grade Water Brand X	LC–MS Grade Water Brand Y	LC–MS Grade Water Brand Z	Fresh Ultrapure Water for LC–MS Analyses
Aluminum	20	500	10	1
Calcium	50	100	100	1
Iron	30	100	5	1
Potassium	50	100	10	1
Magnesium	20	100	20	1
Sodium	50	100	200	1

*Specifications of different sources of laboratory water dedicated for MS analyses were collected from official websites of manufacturers but their names are not provided to avoid direct comparison.

The parent and fragment peaks represented only protonated species. However, when the water used was contaminated with sodium ions, the spectrum was more complex with the presence of sodium adduct peaks (Figure 1b).

The presence of metal ions can suppress the signal of the protonated ion peak of interest. The effect of the sodium ion on the signal intensity of Glu1-fibrinopeptide B was analyzed by varying the ion concentration in the water–acetonitrile mixture that was used to dissolve the peptide. It was observed that signal intensity of the [M+2H] molecular ion decreased with the increase of sodium ion concentration in the water. In parallel, it was observed that signal intensities of the sodium adducts increased with the increase in Na^+ concentration (Figures 2a, 2b, and 2c).

Further signal intensities of Glu1-fibrinopeptide B such as the [M+2H], [M+Na+H], [M+2Na], and [M+3Na-H] ions were recorded for each analyzed sample and presented as a function of Na^+ concentration in water (Figure 3).

Specifically, the presence of 1 ppb of Na^+ decreased the [M+2H] signal intensity by 5%. But the decrease in signal was 20% with 100 ppb Na^+, and 30% when it was 1000 ppb. Altogether the presence of sodium results in more complex spectra, leading to difficulties in data characterization, analyte quantification, and subsequently more time spent on data analysis. Therefore, using water in LC–MS analyses that is free of ions is of high importance. In addition, there are a few key considerations to keep in mind when choosing the source of ultrapure water, and also when handling it to minimize contamination.

Five Tips to Avoid Ionic Contamination in LC–MS Practice

1. Choose the Best Source of Ultrapure Water

Common choices are freshly produced ultrapure water from a laboratory water purification system, and bottled water, such as UHPLC–MS grade or LC–MS grade. Fresh ultrapure water produced

Table IV: Concentration of sodium ions in fresh ultrapure water and in the same ultrapure water that was stored in a glass bottle for one day

Sample	Na^+ (ppb)
Freshly produced ultrapure water	0.020
Ultrapure water in a glass bottle after one day	0.212

using an optimal combination of purification technologies is usually of very high purity, with ionic levels below 1 ppb at 18.2 MΩ·cm of resistivity, which can be monitored online at the moment of water collection. In the case of bottled water, specifications of ionic purity for laboratory water are usually provided and can be easily consulted to assess the potential risk of ionic contamination on LC–MS analyses.

Because ultrapure water is a very aggressive solvent, it tends to absorb ions and organic compounds from the container. Thus, maximum concentrations of ions in final ultrapure product water depend significantly on the level of extractables released by the containers used to collect freshly produced ultrapure water, or to store it, in the case of bottled water. Table IV shows the concentration of Na^+ in freshly produced ultrapure water and ultrapure water after storage in a glass bottle for one day.

As shown in Table IV, the Na^+ concentration in freshly produced ultrapure water was 0.020 ppb. After storage in a glass bottle, the concentration increased to 0.212 ppb.

2. Choose Glassware of the Highest Quality That Leaches the Minimum Amount of Contamination

It is also recommendable to have dedicated glassware for LC–MS practice, and glassware should be cleaned thoroughly before use.

3. Keep Your Work Area Clean

Moreover, since laboratory air is characterized by a lot of contamination and as ultrapure water absorbs contamination from the air including volatile molecules (8), the working area should be kept clean (9). Appropriate covers and caps for mobile-phase reservoirs and glassware can help to avoid contact between ultrapure water and the laboratory air, as well. It is also a good idea to wear gloves, and choose ones that are powder-free and have the fewest metal or ion extractables (for example, polyethylene) because simply touching glassware with a bare hand can transfer enough salt to cause a significant appearance of metal adduct ions.

4. Select Reagents and Organic Solvents of the Highest Purity

Here, to evaluate the purity level necessary for sensitive MS applications, a certificate of analysis is a good source of information. Also, it is recommended to obtain details from the supplier beforehand concerning the level of leachables and extractables from solvent and reagent packaging.

5. Routine Maintenance

When using a laboratory water purification system, make sure that it undergoes routine maintenance to ensure the highest water quality.

Table V: Water purification technologies used in the production of ultrapure water from tap water	
Pretreatment (filter, activated carbon)	• Filtration • Removal of oxidizing agents
Reverse osmosis	• Removal of the bulk of contaminants (95–99%): organics, ions, microorganisms, particulates
Electrodeionization	• Further removal of ions, and charged organic species
UV (254 nm)	• Prevents bacterial contamination
Virgin ion-exchange resins	• Removal of trace amounts of ions
Synthetic activated carbon	• Removal of trace levels of organics
UV (185, 254 nm)	• Photo-oxidation to further remove organics
Point-of-use end filters	• Prevents particle and bacterial contamination • Allows removal of trace levels of organics

Ion Removal in Laboratory Water Purification Systems

The laboratory water purification systems used in this study combine the technologies shown in Table V to efficiently remove different types of contaminants from tap water feed. The removal of ions is carried out by using reverse osmosis, electrodeionization, and ion exchange resins.

To ensure the efficiency of the purification process and make sure that the ultrapure water contains the lowest possible ionic content, resistivity monitoring can be used. Ultrapure water of 18.2 MΩ·cm resistivity refers to ultrapure water free of a significant level of ions for LC–MS analyses.

Conclusion

The presence of ions in the water used for LC–MS analyses influences the quality of data by forming metal adducts and suppressing analyte signals. To avoid ionic contamination, it is recommended to use fresh ultrapure water when preparing mobile phases. It is also important to apply best laboratory practices.

Acknowledgment

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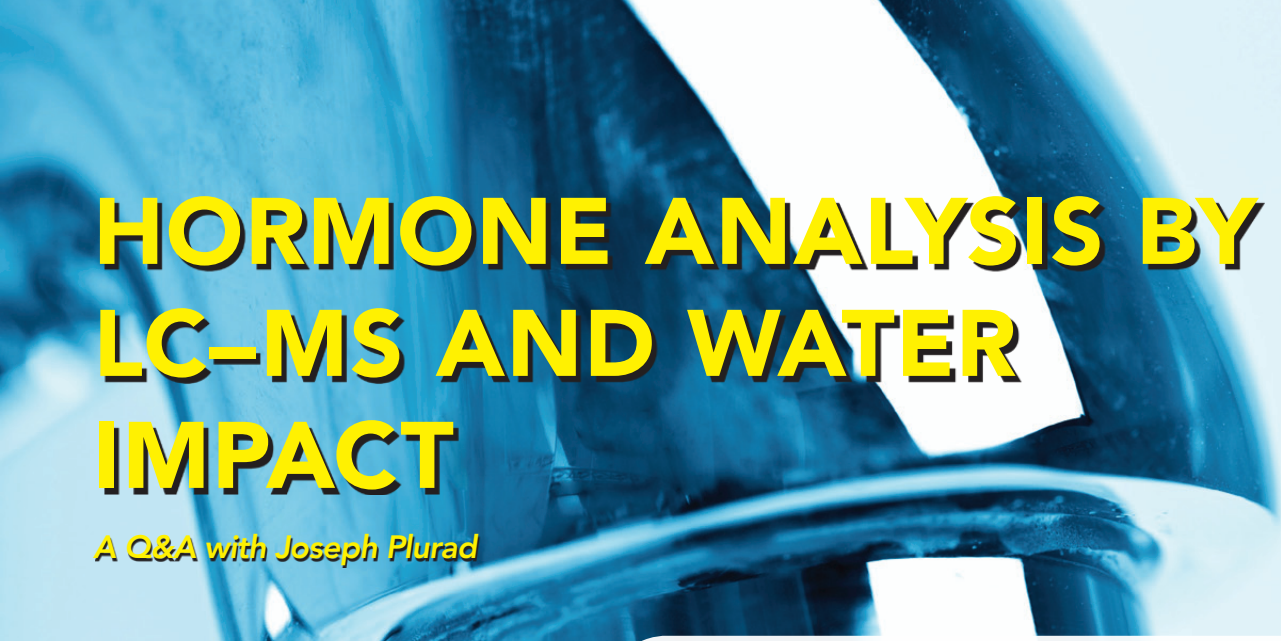
HORMONE ANALYSIS BY LC–MS AND WATER IMPACT

A Q&A with Joseph Plurad



The increased use of hormone-based therapies in health care throughout the world has resulted in hormones finding their way into municipal water supplies. The potential health risks of unintended consumption of hormones through drinking water have significantly increased the interest in identifying these compounds in our water supplies. The Milli-Q system incorporates a combination of purification processes that provides ultrapure, hormone-free lab water for the LC–MS techniques used for interference-free analysis of these contaminants.

LCGC: I know we've heard it before, but why is water quality important in liquid chromatography and mass spectrometry?

PLURAD: Water is probably the most used chemical in a laboratory. In liquid chromatography and mass spectrometry, water is used throughout the entire workflow. Any contaminants that remain in the purified water used in an analysis that have a direct impact on the separation or detection are of concern.

Also, if there are any traces of the molecules you're trying to analyze in the water you're using, you may have inaccurate results. Water quality is

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important to avoid interference with the analytes you're measuring or identifying as well as for optimizing instrument operation.

LCGC: Which specific contaminants can affect the LC–MS process?

PLURAD: The most obvious are organics, and in LC–MS, that's typically what you're looking for. Reduction to trace levels is key, particularly if the organics are similar to what you're analyzing.

Water that's heavy with organics can also cause issues with column efficiency by coating the separation media, resulting in poor peak resolution and shifting peaks. Ions can be a problem. Certain metals can create adducts resulting in noisy mass spectra.

Particle-free water is important to ensure proper flow through the system. With shrinking columns and tubing, as well as improvements and changes to separation media and higher pressures, the impact of particles clogging an LC–MS becomes even more magnified.

Bacterial contamination is a two-headed monster. Bacteria behave as particles, so you run the risk of blocking and clogging tubing or columns. But as bacteria die off, they leech out and reintroduce various organic and ionic contaminants into the previously clean water.

LCGC: Regarding the work MilliporeSigma recently completed, why is there so much interest in analyzing for hormones in water today? And how did you pick the water samples you chose to analyze?

PLURAD: In the last 20 years, a lot of attention has been given to "persistent organic pollutants" in drinking water sources. Much of the original focus in this area was on organic molecules and species that came from so-called chemical sources such as pesticides and solvents.

With advances in health care, however, pharmaceutical sources of these persistent organic pollutants have become more significant. As many of our listeners may remember, we were once told to dispose of our expired or unused pharmaceuticals by flushing them down the toilet, which in retrospect was not the best idea because this water, now carrying these drugs, eventually finds its way back into the municipal drinking water supply.

With the escalation of use of hormone-based therapies such as topical steroids, birth control, and hormone-replacement therapies, there may be long-term effects if these therapeutics exist in our drinking water, such as effects on human fertility and actual embryo development, as well as endocrine and other general health issues.

Consequently, there's extremely high interest in identifying what's in the water and at what levels to determine imminent or long-term health risks. Because our lab water systems rely on potable tap water as a feed source and are used throughout the world, we felt it was

important to understand what influence hormones could have on our ability to provide ultrapure water. We also wanted to demonstrate that our purification techniques can provide high-purity water for the detection of hormones in drinking water via LC–MS.

Because this is a global issue, we selected drinking water samples from various geographies including China, France, and Spain. We're not stating that these samples reflect the overall water qualities in these countries or the safety of the drinking water sources. These are single points of analysis chosen to show that the problem exists to some degree everywhere.

LCGC: What are the challenges in analyzing hormones at trace levels?

PLURAD: We found that these hormones are everywhere and that simple or single-stage purification techniques may not be effective in removing them. Consider deionization, for example. As a purification technique, it only works on contaminants that have an electrical charge. Most organics are neutral or very weakly charged. So deionization is not very effective at removing these contaminants.

Or, consider reverse osmosis. Although this is a workhorse in water purification, a reverse-osmosis system operating well removes only 95% to 99% of the contaminants in the water feeding that membrane. This means that in water systems that have relatively higher levels of these persistent contaminants you can expect to see some residual contaminants post purification.

Clearly, a combination of techniques is required to ensure full removal of these molecules and to have water free of hormone residues for your analytical work. We can consider purification that includes activating carbon, reverse osmosis, UV photo oxidation of organics, and ion exchange. And if that's still not enough, we can consider other purification media at the point of use such as additional activated carbon that targets specific contaminants.

LCGC: What were the results of your analyses?

PLURAD: We found hormones in all of the water sources we tested. It stands to reason that in highly industrialized and developed countries you would expect to see various hormones at various levels. Our R&D team found androsterone and estradiol in city water sources in France and Spain, and corticosterone was detected in China.

I'd like to reiterate it's understood that these municipal drinking water samples are safe and suitable and approved for human consumption. Agencies worldwide recognize the existence of this issue and are taking a hard look at the long-term effects of having these molecules in the drinking water.

The combination of purification processes embedded in a Milli-Q water

purification system allows us to provide hormone-free lab water for the LC–MS techniques used to analyze for these contaminants.

LCGC: The sensitivity of analyses is constantly improving. How is MilliporeSigma responding to this laboratory market demand?

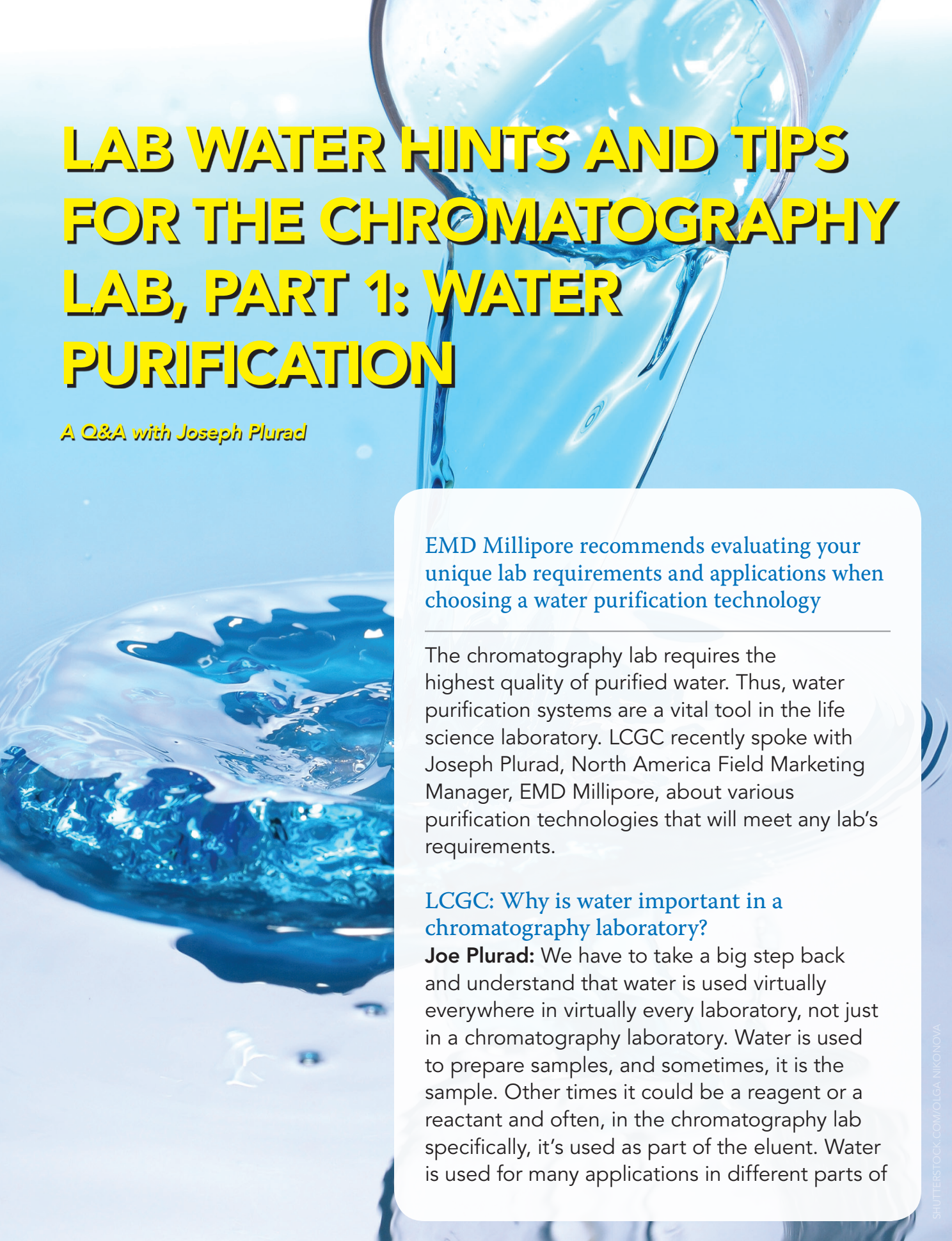
PLURAD: We now have scientists who are able to analyze and quantify trace levels of contaminants that are far below the detection limits provided by traditional quality measures for ultrapure water; in fact, by orders of magnitude at this point.

MilliporeSigma continues to develop more efficient means to remove general classes of contaminants and continues to develop purification packs that are adapted to remove very specific classes of molecules at the actual point of use. Being able to control the purification process 100% from tap water feeding the water purification unit gives users the best chance at managing the impact of persistent contaminants.

Joseph Plurad, North America Field Marketing Manager, MilliporeSigma

LAB WATER HINTS AND TIPS FOR THE CHROMATOGRAPHY LAB, PART 1: WATER PURIFICATION

A Q&A with Joseph Plurad



EMD Millipore recommends evaluating your unique lab requirements and applications when choosing a water purification technology

The chromatography lab requires the highest quality of purified water. Thus, water purification systems are a vital tool in the life science laboratory. LCGC recently spoke with Joseph Plurad, North America Field Marketing Manager, EMD Millipore, about various purification technologies that will meet any lab's requirements.

LCGC: Why is water important in a chromatography laboratory?

Joe Plurad: We have to take a big step back and understand that water is used virtually everywhere in virtually every laboratory, not just in a chromatography laboratory. Water is used to prepare samples, and sometimes, it is the sample. Other times it could be a reagent or a reactant and often, in the chromatography lab specifically, it's used as part of the eluent. Water is used for many applications in different parts of

the chromatography workflow.

And think about other places where water is used in terms of cleaning the equipment or cleaning the glassware and the lab ware, such as bottles that go on top of an HPLC or beakers used to prepare the sample.

In the biological world, water is used in the autoclaves. And often will condense or collect on what you are trying to decontaminate. It is used in humidity chambers as well. These are all touch points that could impact any experiment, but there are some specific contaminants and specific issues that impact chromatography. So the impact of water becomes much more important if it's not properly managed.

Water is a universal solvent, which means that it can, and will, pick up contaminants from the environment, water will dissolve gasses from the air and change the quality. When you think about all of these touch points and all of these possibilities where something can enter the water that is being used in your experiment, the gravity of its impact on chromatography specifically, becomes much more important.

LCGC: What is the impact of poor quality water in chromatography?

Joe Plurad: I don't think it's a coincidence that in the ultrapure water world, chromatography is the number one application demanding highly purified water. So any changes or deviations from water quality that is ideal for chromatography will negatively impact your work. And a lot of companies, both in the chromatography side as well as on the water side, have put a lot of work into understanding what actually is in the water and what it will actually do in chromatography. Water quality, in sample prep, separation or detection, can result in a lot of different problems. Certainly, contaminants in the water can react with the sample, which could change the nature or degrade it, or potentially enhance what you're trying to look for. These same contaminants could interact with the solutions that you're using. It could impact the quality of the eluent, especially as the solutions sit out because water and organics are good nutrient sources for biologics. Particles, and bacteria that behave as particles, can block columns, and foul the resin and the media. The impact of that is poor separations, but if you are successful in blocking up a column, that could result not only in column damage but instrument damage as well because pressure is being placed on the column and these pumps are designed to run at very high pressures.

Post separation, if these contaminants are allowed to flow through, you will continue to have issues with detection techniques and each detection technique will have a specific impact.

LCGC: What are the key contaminants in water that can affect an experiment? And what would happen?

Joe Plurad: In the chromatography world, there are generally three classes of contaminants. The first is particles, or bacteria as particles in this case. These particles can clog frits, fittings, pre-columns, and the columns themselves. This will result in column damage; damage will occur if you try to over-pressurize the system.

Another contaminant is organics. Trace organics in ultrapure water can foul the media of the columns. It will coat the columns, reducing efficiency. If you reduce the efficiencies of the columns, they won't be as good at separating your targets. This results in shifting baselines, or even tailing and masked peaks.

The last contaminant is ions. Within the world of ion chromatography, you don't want your target ions in the water that you're using. However, in LC and LCMS, alkali metals, and specifically sodium, can form adducts that will suppress signals in the mass spec. Sodium is a particular concern in water purification and any purification has to work extra hard to reduce sodium levels prior to dispensing ultrapure water.

LCGC: What technologies in water purification can address these contaminants?

Joe Plurad: At EMD Millipore, we want to educate people that the best way to manage water is to own 100% of the process at the point of use. Take tap water, and bundle or sequence the best series of purification techniques to achieve the water quality that you need for your application. From tap water we would recommend a pre-treatment to remove the larger particles.

Once you've gone through that prefiltration, we recommend reverse osmosis, which is really the workhorse in water purification. This removes 95% of all the contaminants in the incoming tap water and across all classes, whether inorganic, organic, or particulate.

The next step is EDI, or electrodeionization, which is similar to our Elix technology. EDI technology uses membranes and ion exchange media and electrical current to remove additional ions from the water purification stream. It builds on the work that reverse osmosis did and pulls out additional ions. This is going to provide additional benefit for sodium as well as difficult-to-remove ions like silica.

The next step is to pass the water through ion exchange media again to continue to remove more ions from that purified water, and hit the water with UV photo oxidation to remove organics. This will impart a charge to them that can be removed by downstream ion exchange media. The UV photo oxidation will also deactivate bacteria, and remove them from the equation in the ultrapure water.

After UV photo oxidation, we will chase it with additional ion exchange media to do its final polishing to get to that ultrapure water quality. And finally, at the point of use, where you're dispensing the water, we recommend a

final stage purification that is specific to your analytes and your application. At EMD Millipore, we have specific activated carbon cartridges that are designed to remove volatile organic carbons as well as a separate activated carbon filter that is designed for endocrine disruptors.

Other options include a C-18 reverse-phase silica cartridge, a .2 micron filter at the dispensing point an ultrafilter at the point of use. However, the downside to ultrafiltration, is that it generally does leach out trace organics, which are the nature of the membrane.

The best combination of techniques specific to what you're looking for and the specific chromatography technique that you're using will determine which are the best techniques for a specific application.

LCGC: What else can be done to optimize results?

Joe Plurad: Step one is again to use the best technologies to purify specifically for the analyte in your application. One thing that we can do is as simple as producing the right amount of water at the time you're about to use it.

Another practical technique includes trying to avoid plastic containers for sample preparation. Plastics typically use organics as release agents in their molding process and these release agents stick to the sides of the containers. So very fresh glassware, very clean glassware, is preferred.

Finally, as a point of practice, flush a little bit of water from your system before you actually collect it for use. Depending on how often the system is used, we recommend flushing a few liters from the system if it hasn't been used in a few days. If you are using a water purification system daily, or several times a day, as little as 250–500 mL of water can be flushed from the system to ensure the freshest, most recently purified water. The goal is to be prudent about what you're picking in terms of purifying water and immediately be using it as it's being made. In doing so, you will most likely get the best results in your chromatography.

EMD Millipore is the U.S. Life Science subsidiary of Merck KGaA, Darmstadt, Germany. Headquartered in Billerica, Massachusetts, the global business has around 10,000 employees, operations in 66 countries, and 2014 revenues of $2.92 billion.

Joseph Plurad, North America Field Marketing Manager, MilliporeSigma

LAB WATER HINTS AND TIPS, PART 2: OPTIMAL HANDLING OF PURIFIED WATER

A Q&A with Joseph Plurad

Following on our previous Dedicated Dialogue which discussed lab water contaminants and purification technologies, this Dedicated Dialogue will consider specific ways to handle and manage a lab water purification system and the product water from these systems in order to ensure consistent and reliable results. To learn more about the handling of purified water, LCGC talked with Joseph Plurad, North America Field Marketing Manager at EMD Millipore.

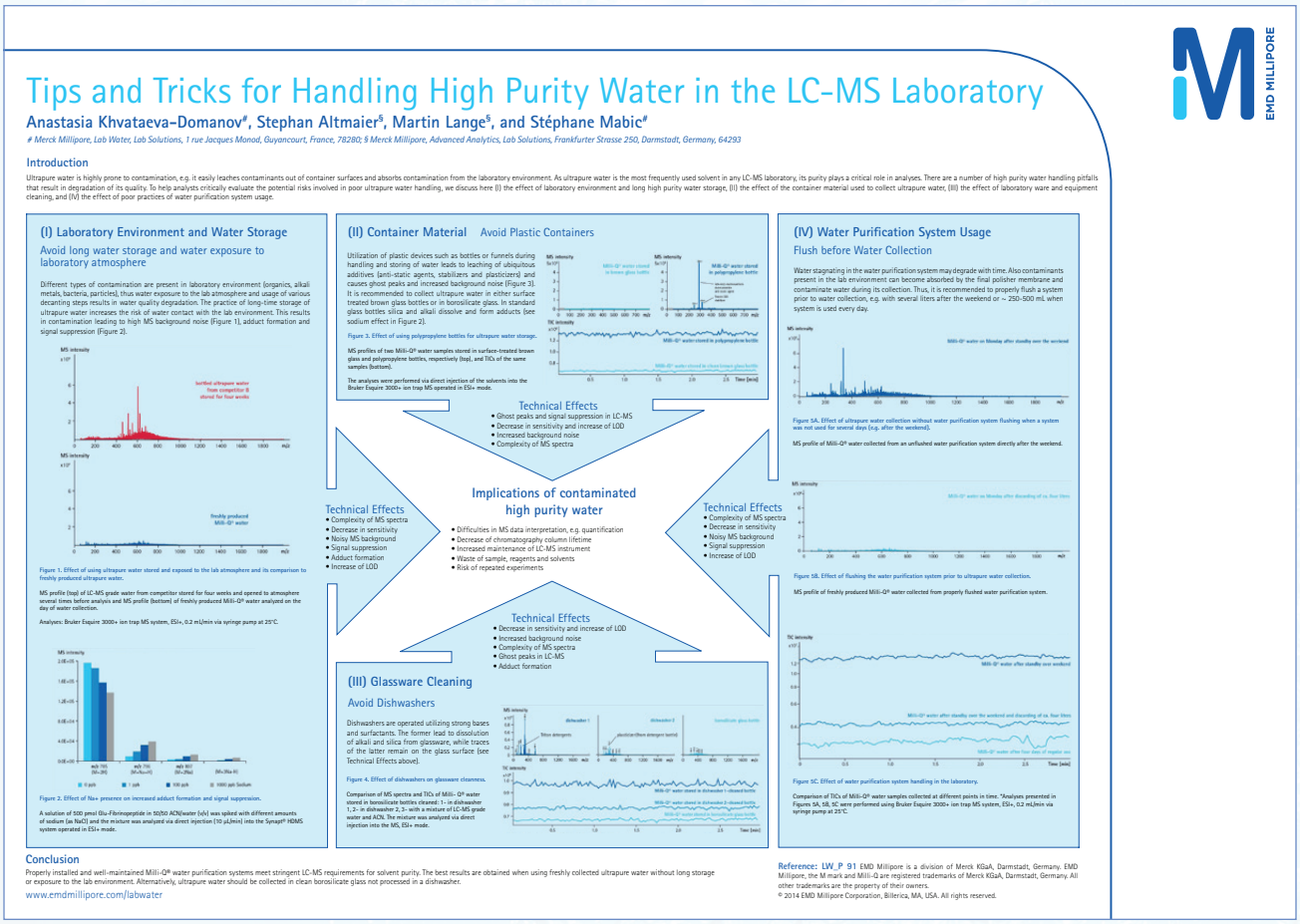
LCGC: Can you quickly review some key contaminants that could be in ultra-pure water and what they can do to an analysis?

Joseph Plurad: As discussed in our last podcast, the primary contaminants of focus are organics, ions and particles. Bacteria are also included in the particles category because of how they behave in a chromatography system. It should be pretty clear the issues that organics can cause since most chromatographic analyses are for organic compounds and molecules. Organic contaminants that will either compete or mask the organic compounds that you seek to purify or identify need to be minimized. Some organics can foul chromatography

media or resins which results in reduced column efficiency, unstable baselines, and poor peak resolution. Similarly, ions can cause competing reactions along with separation and resolutions issues, especially in ion chromatography. When chromatography is coupled with mass spectrometry (MS) some ions will create what are known as adducts, which suppress the MS signals and make molecule identification difficult. In addition, particles, including bacteria can clog frits, fittings, pre-columns and the columns themselves. In high pressure systems, while it's not likely that particulate contamination will cause the column themselves to rupture or burst—remember that these columns are designed to take lots of pressure—there are real risks of having the fittings and the pumps fail as a result. At a more basic level, pre-columns and fritted fittings will need to be replaced more frequently if contaminated by particulates, adding cost and time to your analysis. So clearly, a well-designed and well-managed water system can help reduce the impact of contaminants in everyday laboratory work.

LCGC: What are some good practices in using a water system to generate your chromatography-grade water?

Joseph Plurad: First and foremost, make sure that the water system you're using is optimized for the analysis and the experiments you plan on running. Today's high purity water systems employ multiple purification technologies to deliver water that meets technical requirements, regardless of whether using tap water or water from de-ionized, distilled, or reverse osmosis systems. In addition to the standard ion exchange and UV technologies, for example, companies like EMD Millipore have contaminant-specific products to remove specific classes of contaminants, like trace organics, volatile organics, persistent organic contaminants like endocrine disruptors, and/or bacteria and particles. There are also filters that can remove certain enzymes like nucleases and proteases. However it's important to remember that one size does not fit all. While a nuclease filter may be perfect for certain life science applications, it could actually complicate things for basic organic separation and identification. Once a system is chosen, operate it regularly and keep it well maintained. Purification elements like ion-exchange media and physical filters do need to be replaced regularly as they become fouled or exhausted. Cartridges and filters should be rinsed prior to collecting water to ensure that any preservatives that may remain from manufacturing are flushed out of the system. And of course, proactive cartridge and filter replacement ensure that you stay ahead of your contaminants. Remember that organic contaminants can reduce the ion removal efficiency of resin, and once all of the ion exchange active sites are consumed or blocked, the cartridge provides no



Tips and Tricks for Handling High Purity Water in the LC-MS Laboratory

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Introduction

Ultrapure water is highly prone to contamination, e.g. it easily leaches contaminants out of container surfaces and absorbs contamination from the laboratory environment. As ultrapure water is the most frequently used solvent in any LC-MS laboratory, its purity plays a critical role in analyses. There are a number of high purity water handling pitfalls that result in degradation of its quality. To help analysts critically evaluate the potential risks involved in poor ultrapure water handling, we discuss here (I) the effect of laboratory environment and long high purity water storage, (II) the effect of the container material used to collect ultrapure water, (III) the effect of laboratory ware and equipment cleaning, and (IV) the effect of poor practices of water purification system usage.

(I) Laboratory Environment and Water Storage

Avoid long water storage and water exposure to laboratory atmosphere

Different types of contamination are present in laboratory environment (organics, alkali metals, bacteria, particles), thus water exposure to the lab atmosphere and usage of various decanting steps results in water quality degradation. The practice of long-time storage of ultrapure water increases the risk of water contact with the lab environment. This results in contamination leading to high MS background noise (Figure 1), adduct formation and signal suppression (Figure 2).

Figure 1. Effect of using ultrapure water stored and exposed to the lab atmosphere and its comparison to freshly produced ultrapure water.

MS profile (top) of LC-MS grade water from competitor stored for four weeks and opened to atmosphere several time before analysis and MS profile (bottom) of freshly produced Milli-Q® water analyzed on the day of water collection.

Analyses: Bruker Esquire 3000+ ion trap MS system, ESI+, 0.2 mL/min via syringe pump at 25°C.

Figure 2. Effect of Na+ presence on increased adduct formation and signal suppression.

A solution of 500 pmol Glu-Fibrinopeptide in 50/50 ACN/water (v/v) was spiked with different amounts of sodium (as NaCl) and the mixture was analyzed via direct injection (10 µL/min) into the Synapt® HDMS system operated in ESI+ mode.

(II) Container Material

Avoid Plastic Containers

Utilization of plastic devices such as bottles or funnels during handling and storing of water leads to leaching of ubiquitous additives (anti-static agents, stabilizers and plasticizers) and causes ghost peaks and increased background noise (Figure 3). It is recommended to collect ultrapure water in either surface treated brown glass bottles or in borosilicate glass. In standard glass bottles silica and alkali dissolve and form adducts (see sodium effect in Figure 2).

Figure 3. Effect of using polypropylene bottles for ultrapure water storage.

MS profiles of two Milli-Q® water samples stored in surface-treated 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 Bruker Esquire 3000+ ion trap MS operated in ESI+ mode.

(III) Glassware Cleaning

Avoid Dishwashers

Dishwashers are operated utilizing strong bases and surfactants. The former lead to dissolution of alkali and silica from glassware, while traces of the latter remain on the glass surface (see Technical Effects above).

Figure 4. Effect of dishwashers on glassware cleanness.

Comparison of MS spectra and TICs of Milli- Q® water stored in borosilicate bottles cleaned: 1- in dishwasher 1, 2- in dishwasher 2, 3- with a mixture of LC-MS grade water and ACN. The mixture was analyzed via direct injection into the MS, ESI+ mode.

(IV) Water Purification System Usage

Flush before Water Collection

Water stagnating in the water purification system may degrade with time. Also contaminants present in the lab environment can become absorbed by the final polisher membrane and contaminate water during its collection. Thus, it is recommended to properly flush a system prior to water collection, e.g. with several liters after the weekend or ~ 250-500 mL when system is used every day.

Figure 5A. Effect of ultrapure water collection without water purification system flushing when a system was not used for several days (e.g. after the weekend).

MS profile of Milli-Q® water collected from an unflushed water purification system directly after the weekend.

Figure 5B. Effect of flushing the water purification system prior to ultrapure water collection.

MS profile of freshly produced Milli-Q® water collected from properly flushed water purification system.

Figure 5C. Effect of water purification system handling in the laboratory.

Comparison of TICs of Milli-Q® water samples collected at different points in time. *Analyses presented in Figures 5A, 5B, 5C were performed using Bruker Esquire 3000+ ion trap MS system, ESI+, 0.2 mL/min via syringe pump at 25°C.

Technical Effects

- Ghost peaks and signal suppression in LC-MS
- Decrease in sensitivity and increase of LOD
- Increased background noise
- Complexity of MS spectra

Technical Effects

- Complexity of MS spectra
- Decrease in sensitivity
- Noisy MS background
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- Ghost peaks in LC-MS
- Adduct formation

Implications of contaminated high purity water

- Difficulties in MS data interpretation, e.g. quantification
- Decrease of chromatography column lifetime
- Increased maintenance of LC-MS instrument
- Waste of sample, reagents and solvents
- Risk of repeated experiments

Conclusion

Properly installed and well-maintained Milli-Q® water purification systems meet stringent LC-MS requirements for solvent purity. The best results are obtained when using freshly collected ultrapure water without long storage or exposure to the lab environment. Alternatively, ultrapure water should be collected in clean borosilicate glass not processed in a dishwasher.

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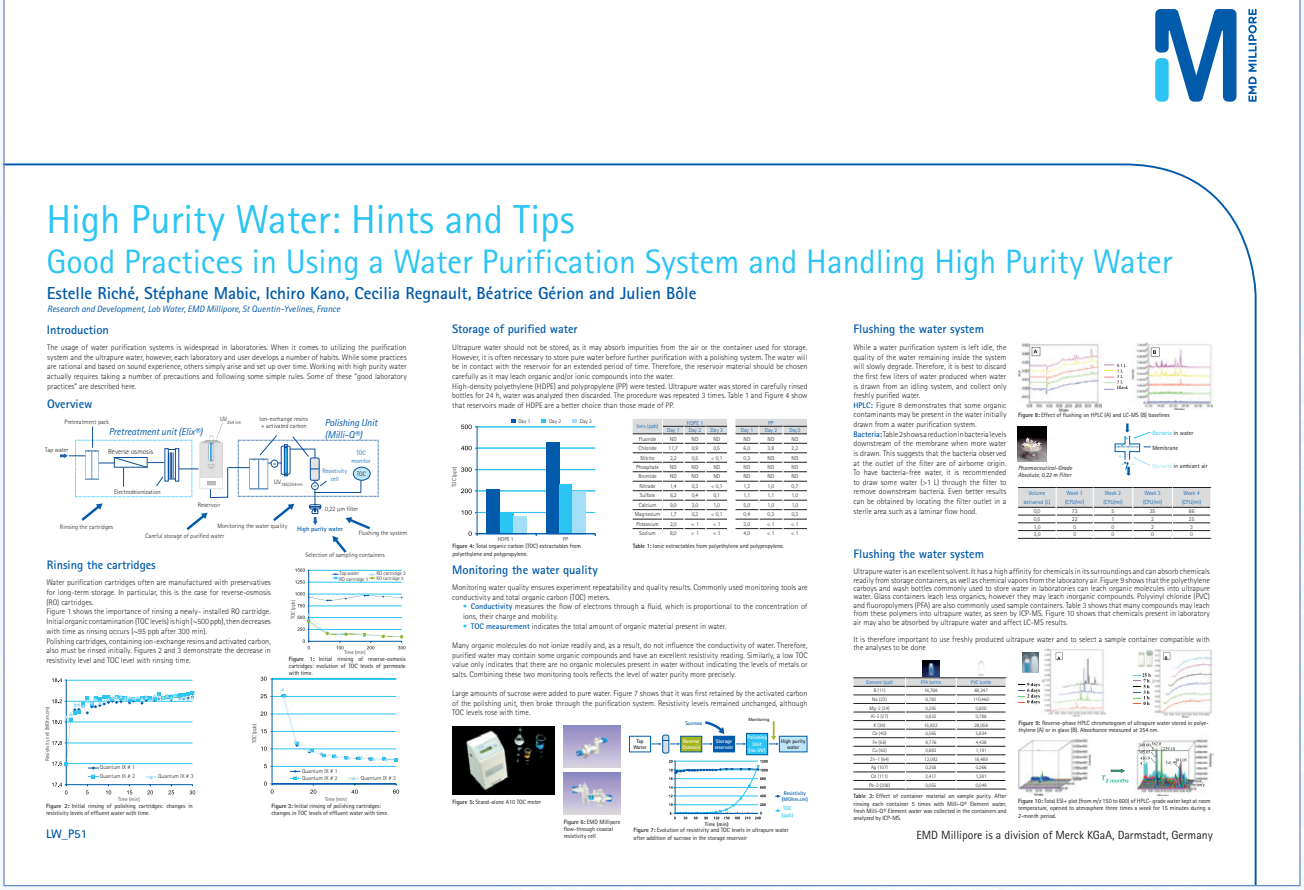
Reference: LW_P 91

purification power or benefit at all, which can happen relatively quickly. UV lamps should also be replaced when they're unable to oxidize organics or kill bacteria, and depending on the manufacturer, replacement could be required every two years. Finally, make sure you're regularly monitoring and reading the water quality prior to collecting the water for use; both resistivity, which gives you an idea of the ionic removal, and Total Organic Carbon (TOC), which measures trace organics in water, can put the system and subsequent experiments at risk. Generally, freshly produced water that is 18.2 Mega-ohm/centimeter resistivity with TOCs less than five parts per billion are fine for most chromatographic techniques.

LCGC: What are some best practices for handling the purified water once it's been dispensed from the system?

Joseph Plurad: One thing to remember is that water is a universal solvent so it will absorb contaminants from the air as well as the container you put it in. To minimize impact on experiments, a few things need to be considered. First, only dispense what is needed for a particular experiment or "run" to minimize the total



High Purity Water: Hints and Tips

Good Practices in Using a Water Purification System and Handling High Purity Water

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Introduction

The usage of water purification systems is widespread in laboratories. When it comes to utilizing the purification system and the ultrapure water, however, each laboratory and user develops a number of habits. While some practices are rational and based on sound experience, others simply arise and set up over time. Working with high purity water actually requires taking a number of precautions and following some simple rules. Some of these "good laboratory practices" are described here.

Overview

Pretreatment pack
Pretreatment unit (Elix®)
Tap water
Reverse osmosis
Electrodeionization
Reservoir
UV 254 nm
Ion-exchange resins + activated carbon
Polishing Unit (Milli-Q®)
UV 185/254 nm
TOC monitor
Resistivity cell
TOC
0.22 µm filter
Rinsing the cartridges
Careful storage of purified water
Monitoring the water quality
High purity water
Flushing the system
Selection of sampling containers

Rinsing the cartridges

Water purification cartridges often are manufactured with preservatives for long-term storage. In particular, this is the case for reverse-osmosis (RO) cartridges.
Figure 1 shows the importance of rinsing a newly- installed RO cartridge. Initial organic contamination (TOC levels) is high (~500 ppb), then decreases with time as rinsing occurs (~95 ppb after 300 min).
Polishing cartridges, containing ion-exchange resins and activated carbon, also must be rinsed initially. Figures 2 and 3 demonstrate the decrease in resistivity level and TOC level with rinsing time.

Figure 1: Initial rinsing of reverse-osmosis cartridges: evolution of TOC levels of permeate with time.

Figure 2: Initial rinsing of polishing cartridges: changes in resistivity levels of effluent water with time.

Figure 3: Initial rinsing of polishing cartridges: changes in TOC levels of effluent water with time.

LW_P51

Storage of purified water

Ultrapure water should not be stored, as it may absorb impurities from the air or the container used for storage. However, it is often necessary to store pure water before further purification with a polishing system. The water will be in contact with the reservoir for an extended period of time. Therefore, the reservoir material should be chosen carefully as it may leach organic and/or ionic compounds into the water.
High-density polyethylene (HDPE) and polypropylene (PP) were tested. Ultrapure water was stored in carefully rinsed bottles for 24 h, water was analyzed then discarded. The procedure was repeated 3 times. Table 1 and Figure 4 show that reservoirs made of HDPE are a better choice than those made of PP.

Figure 4: Total organic carbon (TOC) extractables from polyethylene and polypropylene.

Ions (ppb)	HDPE 1			PP		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Fluoride	ND	ND	ND	ND	ND	ND
Chloride	11.7	0.9	0.3	6.0	3.8	2.2
Nitrite	2.2	0.5	< 0.1	0.3	ND	ND
Phosphate	ND	ND	ND	ND	ND	ND
Bromide	ND	ND	ND	ND	ND	ND
Nitrate	1.4	0.3	< 0.1	1.3	1.0	0.7
Sulfate	6.2	0.4	0.1	1.1	1.1	1.0
Calcium	9.0	2.0	1.0	5.0	1.0	1.0
Magnesium	1.7	0.3	< 0.1	0.4	0.3	0.3
Potassium	2.0	< 1	< 1	3.0	< 1	< 1
Sodium	6.0	< 1	< 1	4.0	< 1	< 1

Table 1: Ionic extractables from polyethylene and polypropylene.

Monitoring the water quality

Monitoring water quality ensures experiment repeatability and quality results. Commonly used monitoring tools are conductivity and total organic carbon (TOC) meters.

- **Conductivity** measures the flow of electrons through a fluid, which is proportional to the concentration of ions, their charge and mobility.
- **TOC measurement** indicates the total amount of organic material present in water.

Many organic molecules do not ionize readily and, as a result, do not influence the conductivity of water. Therefore, purified water may contain some organic compounds and have an excellent resistivity reading. Similarly, a low TOC value only indicates that there are no organic molecules present in water without indicating the levels of metals or salts. Combining these two monitoring tools reflects the level of water purity more precisely.

Large amounts of sucrose were added to pure water. Figure 7 shows that it was first retained by the activated carbon of the polishing unit, then broke through the purification system. Resistivity levels remained unchanged, although TOC levels rose with time.

Figure 5: Stand-alone A10 TOC meter

Figure 6: EMD Millipore flow-through coaxial resistivity cell

Figure 7: Evolution of resistivity and TOC levels in ultrapure water after addition of sucrose in the storage reservoir

Flushing the water system

While a water purification system is left idle, the quality of the water remaining inside the system will slowly degrade. Therefore, it is best to discard the first few liters of water produced when water is drawn from an idling system, and collect only freshly purified water.
HPLC: Figure 8 demonstrates that some organic contaminants may be present in the water initially drawn from a water purification system.
Bacteria: Table 2 shows a reduction in bacteria levels downstream of the membrane when more water is drawn. This suggests that the bacteria observed at the outlet of the filter are of airborne origin. To have bacteria-free water, it is recommended to draw some water (>1 L) through the filter to remove downstream bacteria. Even better results can be obtained by locating the filter outlet in a sterile area such as a laminar flow hood.

Figure 8: Effect of flushing on HPLC (A) and LC-MS (B) baselines

Pharmaceutical-Grade Absolute, 0.22 m Filter

Volume delivered (L)	Week 1 (CFU/mL)	Week 2 (CFU/mL)	Week 3 (CFU/mL)	Week 4 (CFU/mL)
0.0	73	5	35	84
0.5	22	1	2	28
1.0	0	0	2	23
3.0	0	0	0	0

Flushing the water system

Ultrapure water is an excellent solvent. It has a high affinity for chemicals in its surroundings and can absorb chemicals readily from storage containers, as well as chemical vapors from the laboratory air. Figure 9 shows that the polyethylene carboys and wash bottles commonly used to store water in laboratories can leach organic molecules into ultrapure water. Glass containers leach less organics, however they may leach inorganic compounds. Polyvinyl chloride (PVC) and fluoropolymers (PFA) are also commonly used sample containers. Table 3 shows that many compounds may leach from these polymers into ultrapure water, as seen by ICP-MS. Figure 10 shows that chemicals present in laboratory air may also be absorbed by ultrapure water and affect LC-MS results.

It is therefore important to use freshly produced ultrapure water and to select a sample container compatible with the analyses to be done

Element (ppt)	PVC bottle	PVC bottle
B [11]	14,768	68,347
Na [23]	3,782	110,460
Mg [24]	1,206	3,830
Al [27]	1,830	12,780
K [39]	15,863	26,350
Ca [40]	1,546	5,834
Ti [56]	3,176	4,436
Cu [63]	1,889	1,191
Zn [64]	12,082	18,489
Ag [107]	1,238	1,196
Cd [111]	2,410	1,581
Pb [208]	1,592	1,046

Table 3: Effect of container material on sample purity. After rinsing each container 5 times with Milli-Q® Element water, fresh Milli-Q® Element water was collected in the containers and analyzed by ICP-MS.

Figure 9: Reverse-phase HPLC chromatogram of ultrapure water stored in polyethylene (A) or in glass (B). Absorbance measured at 254 nm.

Figure 10: Total ESI+ plot (from m/z 150 to 600) of HPLC- grade water kept at room temperature, opened to atmosphere three times a week for 15 minutes during a 2-month period.

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amount of exposure the water gets to the air and to the container. Stored high purity water will degrade with simple absorption of CO2 from the atmosphere, and if the container itself isn't well managed, organics and particles can enter the bottle. Letting water stand for days in between runs should be avoided since it introduces the risk of bacteria and other contamination. Also, be mindful of the container used. The last podcast discussed avoiding plastic containers. Plastic containers, depending on the material of construction, could include anti-static agents, plasticizers, stabilizers or even dust and plastic fines that result from the manufacturing package or storage processes. However, if using glass containers, surface-treated amber glass bottles or borosilicate glass containers are recommended. Regular glass bottles can leach silica and alkali metals which will cause problems. So basically, anything that can be done to minimize storage and exposure to contamination risks should become best practice.

LCGC: Is there anything else you can do to minimize the recontamination of ultra-pure water?

Joseph Plurad: Finding ways to reduce exposure is always the best practice. In addition to picking the right size and material of construction, you should really try to use the smallest container possible should be used to help minimize exposure and contamination and to keep everything clean. Another thing to consider is to minimize exposure and contamination from the tubing used to introduce ultra-pure water into the container, especially if transferring caps and moving from one container to the next. For example, bench top or atmospheric contaminants can easily be introduced if tubing or caps are left on the countertop. If experimental work is sensitive to CO2, bottled vent filters or organic traps should be considered. Container cleaning should also be avoided if possible, especially in automatic glassware washers. Most commercially available detergents utilize strong bases that can etch the glass and dissolve alkali and silica which will be absorbed into the water. Traces of surfactants and detergents, which are organic, could also remain on the surface of the glassware, only to be leached off with the ultra-pure water dispensed into the bottle. The same risks exist for bottled water. The certificate of analysis (CoA) lists the condition of the water when it was bottled, not necessarily when used. The CoA is no longer effective one the lid is removed, and the result is the same risk of contamination compared to water from any water purification system.

About EMD Millipore

EMD Millipore is the Life Science division of Merck KGaA of Darmstadt, Germany and offers a broad range of innovative, performance products, services and business relationships that enable our customers' success in research, development and production of biotech and pharmaceutical drug therapies. Through dedicated collaboration on new scientific and engineering insights, and as one of the top three R&D investors in the Life Science Tools industry, EMD Millipore serves as a strategic partner to customers and helps advance the promise of life science. EMD Millipore is known as Merck Millipore outside of the U.S. and Canada.

Joseph Plurad
North America Field Marketing Manager
EMD Millipore

SELECTING A LABORATORY WATER PURIFICATION SYSTEM

Jean-Francois Pilette

Identifying the most appropriate water purification system for your laboratory requires that you determine the types of contaminants you need to removed, the quality of the available feed water, the instant and daily volumes of Type 2 (pure) or Type 1 (ultrapure) water you need, and the type of water quality monitoring required. This article discusses these parameters and describes how they affect laboratory water use.

Primary Classes of Contaminants

Water contaminants can be divided into five categories: inorganic salts, dissolved organic substances, particulates and colloids, microorganisms, and gases. Certain types of contaminants are removed to some extent when natural water is processed into drinking water. The levels of these contaminants must however be reduced further and additional contaminants must be removed when tap water is further purified to become laboratory-grade water.

Inorganic salts are composed of cations (positively charged ions) and anions (negatively charged ions). The cations present in natural water are typically sodium, calcium, iron, manganese, lead, and aluminum. The most

common anions in natural water are chloride, nitrate, sulfate, phosphate, and carbonate. The presence of unknown or unwanted ions, or an excessive concentration of ions, in laboratory-grade water can adversely affect laboratory processes. For example, if lead ions are being analyzed and the water used to wash glassware or prepare standard solutions contains lead, the results will be inaccurate. Ion concentrations therefore have to be minimized in laboratory-grade water.

Silica, a negatively charged mineral, may also be classified as an anion. As a contaminant, silica is a special case. It is weakly charged and exists in different forms. Monomeric silica comprises a single silica atom, dimeric silica is made up of two atoms, and polymeric silica is composed of many silica atoms, as well as silica sheets and silica crystal forms. Silica therefore encompasses a multitude of contaminants with common behaviors but also small differences. These differences make silica difficult to remove from water.

Removing ionic contaminants from laboratory water avoids positive bias, interferences, and variations in the ionic strength of solutions during analysis. Ionic contaminants can also affect chromatographic results. In high-performance liquid chromatography (HPLC) and ion chromatography (IC), ionic contaminants can result in additional peaks, increased background levels that can affect sensitivity, and artificially increased values.

Ionic contamination can also be an issue in HPLC analyses involving mass spectrometry detectors, especially when using electrospray positive (ESI +) mode. Instead of providing the conventional [M+H]+ response, sodium and potassium ion contamination can result in [M+Na]+ peaks (called sodiated peaks) and [M+K]+ peaks. These unintended peaks produce incorrect mass assignments. Achieving multiple peaks for a single molecule makes data analysis more complicated and difficult.

Dissolved organic substances, which contain carbon atoms, are another source of water contamination. Organic substances can have a natural origin. For example, when leaves fall on the ground in autumn, rain water starts dissolving them. In time, components of the leaves and wood are released and dissolve in the rain water. The long cellulose chains and smaller molecules such as lignin monomers, gallic acid, and catechin (a component of the tannins in the wood) vary widely in nature and in molecular weight.

Natural water may also be contaminated by dissolved organic substances such as the following generated by human activities:

- Solvents called volatile organic compounds (VOC), such as perchlorethene, used in the dry cleaning of clothing
- Molecules such as benzene found in gasoline
- Pesticides such as herbicides,

fungicides, and insecticides used by the farming industry and in gardening
- Polyaromatic hydrocarbons (PAHs), which are molecules released, for example, from coal tar used as a pavement sealcoat
- Polychlorobiphenyls (PCBs), a persistent industrial contaminant whose use has been forbidden since 1976
- Plasticizer substances added to polymers to make them softer, for example, bisphenol-A (BPA), widely used with polyvinyl chloride (PVC), a polymer used to make most of the plastic pipes used to carry tap or deionized water

As with ionic contaminants, organic contaminants must be removed in order to avoid positive bias and interferences during organics analyses. Organic contaminants can also affect chromatography by coating stationary phases. This results in slower mass transfer (shifting retention times), reduced resolution, baseline issues, and shorter column lifetimes.

Particulates and colloids contaminants can be divided into two classes: hard particulates (such as sand and clay) and soft particulates (such as vegetal debris). Hard and soft particulates can offer protection against ultraviolet rays and chemical agents, while acting as a surface for bacteria to adhere to. Particulates and colloids are typically between 0.1 and 100 µm. Hard and soft particulates can slowly dissolve in natural water, generating additional inorganic and organic contaminants.

Colloids (such as colloidal silica) are stable suspensions of organic and inorganic particulates (0.1 to 0.001 µm in size). The particulates in colloids usually have the same electric charge, and the repulsion prevents the liquid from settling (unless a coagulating agent of the opposite charge is added). Hard particulates can damage or impair the operation of laboratory instruments such as pumps and injectors, and soft particulates can plug chromatography columns and frits. Particulates can also act as a shelter for microorganisms and may operate as a catalyst to accelerate certain reactions.

Microorganisms such as bacteria and viruses are also common natural water contaminants. Bacteria vary in size and shape. Escherischia coli, a microorganism commonly found in the human intestine are 2 µm, while Pseudomonas diminuta, is the smallest bacteria. When grown in a very poor culture medium with stirring, Pseudomonas diminuta grows as a sphere 0.27 µm in diameter. Because it is the smallest bacterium known, this microorganism is typically used to challenge membrane filters for bacteriological retention.

Bacteria can be a nuisance even when dead because they release byproducts that can cause problems with scientific experiments. Gram negative bacteria can release pyrogens from their walls, and other bacteria can release nucleases

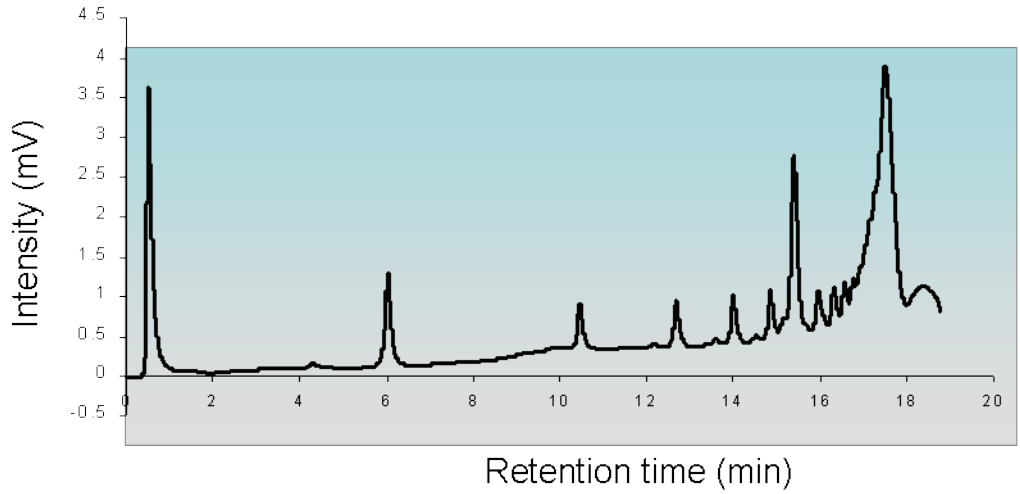

Figure 1: DNA Separation Column: Injection 1

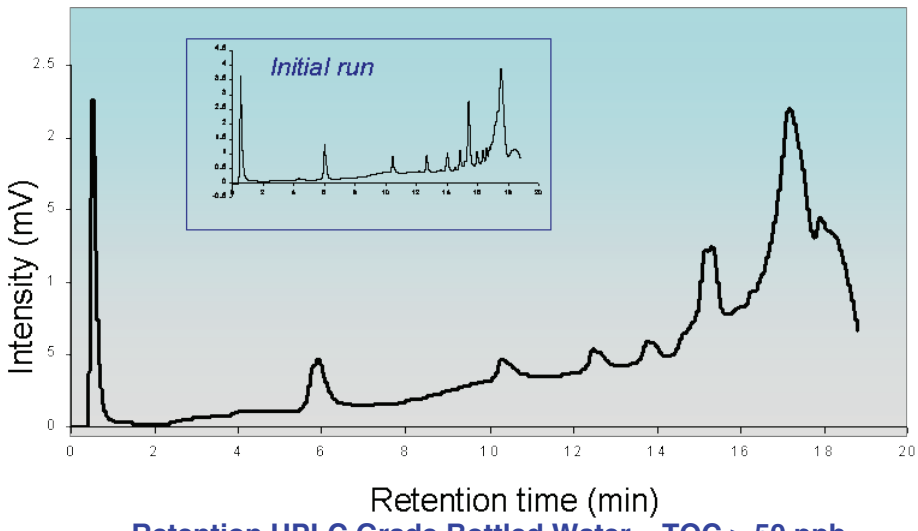

Figure 2: DNA Separation Column:– Injection 1950

such as RNases and DNases. Bacteria and byproducts need to be removed from laboratory water sources because they are sources of ions and organic contaminants that can alter biological experiments such as cell cultures. Bacteria byproducts such as pyrogens and nucleases can affect cell operation and

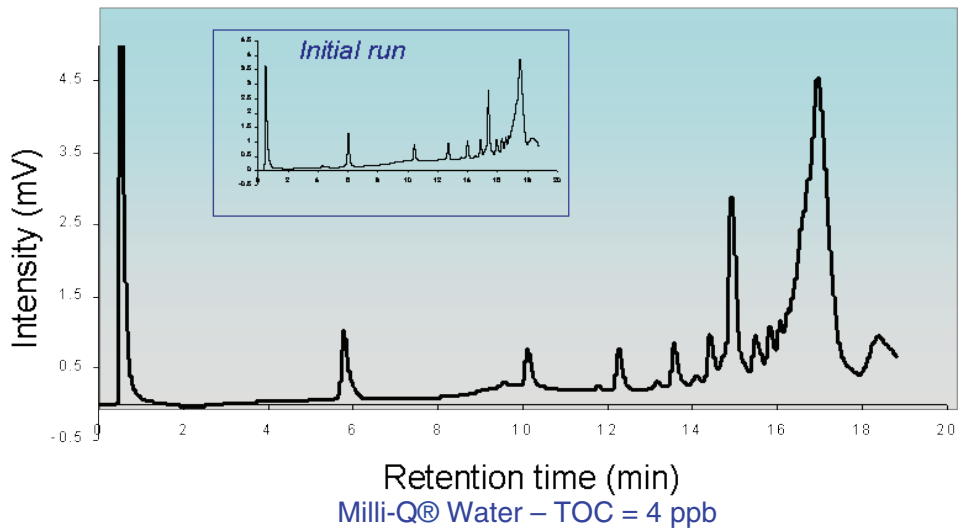

Figure 3: DNA Separation Column:– Injection 6400

molecular biology mechanisms. Bacteria also effect chromatography in much the same way as organic contaminants.

Dissolved gases are also natural water contaminants. The primary gases dissolved in water are typically the main constituents of the air that is in equilibrium with the water source: oxygen, nitrogen, and carbon dioxide (which is in equilibrium with carbonic acid). The solubility of these gases is a function of temperature. Another common dissolved gas contaminant is radon, a water-soluble gas produced by the decay of radium isotopes. It is naturally found in ground water and results from granite formations, phosphate deposits, and uranium deposits. In addition to being a contaminant, radon may also cause human health problems, including cancer.

Other gases can dissolve in rain water and contribute to its acidification, including sulfide dioxide (SO2, from combustion plants, industrial processes, transportation, and natural origins), nitrates (originating primarily from road transportation and combustion plants), and ammonia (NH3, typically originating from agriculture activities). Removing dissolved gases from water may be required for certain experiments to avoid the formation of bubbles. Bubble formation can affect the measure of optical density or result in outgassing, which can cause pump and detector problems in chromatography.

Water Monitoring Techniques

Conductivity is the most accepted means of determining the purity of the water. By definition, conductivity is a material's

DNA Sep Column	Water Source	TOC (ppb)	Number of Injections
1	Bottle Brand B	777	408
2	Bottle Brand E	100	555
3	Bottle Brand C	87	2103
4	Bottle Brand A	32	1235
5	Bottle Brand D	16	2167
6	Milli-Q® Gradient	4	6394
7	Milli-Q® Gradient	4	10685

Bottle = HPLC Grade Bottled Water – Various Brands

Figure 3: DNA Separation Column:– Injection 6400

ability to conduct electricity. However, since Type 1 (ultrapure) water itself does not conduct electricity, conductivity, is by proxy a measurement of all of the ions that remain after the purification process. As the conductivity value becomes very small and approaches the requirements of Type 2 (approaching pure) water, the purified water is said to be characterized by resistivity (the inverse of conductivity). Resistivity is a technique that attempts to assert the absence of ions in Type 1 (ultrapure) water. Resistivity detects ions present in water with a high degree of sensitivity: A concentration as low as 1 ppb (1ug/L) of a salt causescause a significant drop in the value of resistivity. Assuming that water fully and equally dissociates intoto H+ and OH– in equilibrium (resulting in a pH of 7), the theoretical conductivity of a solution of H+ and OH– is 0.055 uS/cm at 25°C, or 18.2 MOhm x cm at 25°C. For this reason, Type 1 (ultrapure) is often referred to as 18.2 MOhm x cm water.

The conductivity of a material is directly related to the temperature; conductivity increases—and resistivity decreases—as temperature rises. The formula used to calculate conductivity is directly related to the temperature of the material so a normalized or compensated value is often reported in order to have a standard point of reference. Typically this temperature is 25°C. Therefore, while 18.2 MOhm x cm water is the accepted value for Type 1 (ultrapure) water, it is only valid if it is measured at or compensated to 25°C. Since many laboratory experiments can be affected by ions, it is important to measure resistivity to assess the presence of ions.

Because there can be multiple sources of organic contamination and because it can be difficult to completely removeremove trace organics due to theirthe complex nature, it is important to ensure that the concentration of organics in water remains low and constant over time. This can only be achieved if a trusted measure of total organic carbon (TOC) is regularly performed on the water prior to use. **Figure 1** through **Figure 3** demonstrate the effects TOC can have on a chromatographic separation. Using HPLC-grade bottled water with a TOC greater than 50 ppb, resolution can be seen to degrade significantly (**Figure 1** and **Figure 2**) over the course of approximately 2000 injections. However using Milli-Q® water, which has a TOC of 4 ppb, the separation remains virtually

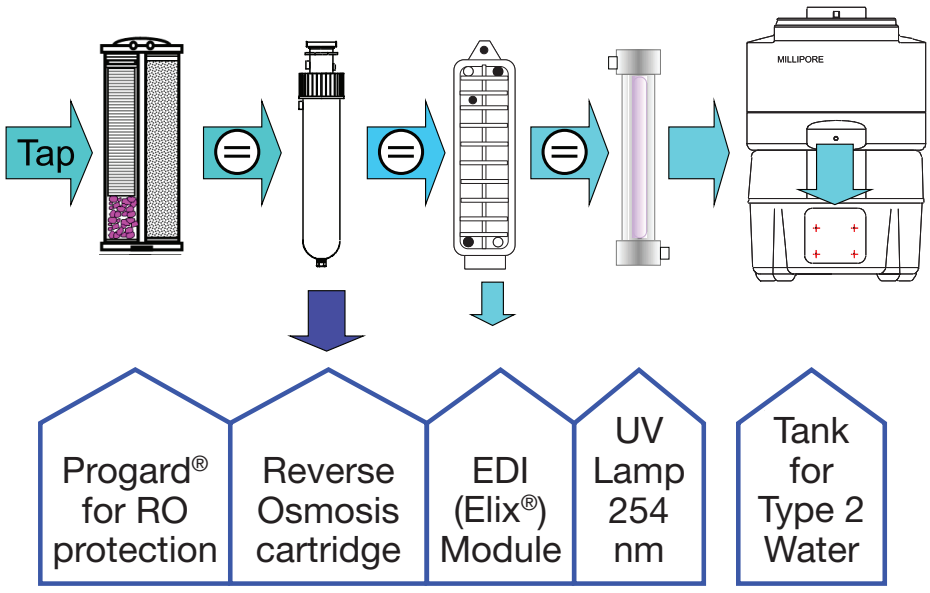

Figure 4: Water Purification Techniques: Pure Water

unchanged after more than 6000 injections. Table 1, which compares the effects of TOC levels on column lifetime for bottled water and Milli-Q® water, further illustrates this point.

Water Purification Techniques

The production of Type 2 (pure) water from tap water is achieved using a series of purification technologies, as illustrated in **Figure 4**. Tap water entering a water purification system such as the Milli-Q® Integral system first passes through a Progard pretreatment cartridge, whose goal is to protect the reverse osmosis cartridge. The pretreatment cartridge contains activated carbon to remove chlorine, polyphosphate to prevent calcium carbonate precipitation, and a filter to retain particulates.

The conductivity of the treated water is then measured before it enters the reverse osmosis cartridge. The reverse osmosis cartridge removes more than 97% of the ions in tap water, as well as 99% of bacteria, particulates, and organics larger than 200 Dalton. A scanning electron microscope picture of a reverse osmosis membrane cross-section is shown in **Figure 5**. The membrane is separated into two layers: a one-micron-thick active layer that removes contaminants on top, and a thicker (100 µm) porous support underneath that provides mechanical support to the thinner active layer. The reverse osmosis membrane is able to reject contaminants using two combined mechanisms related

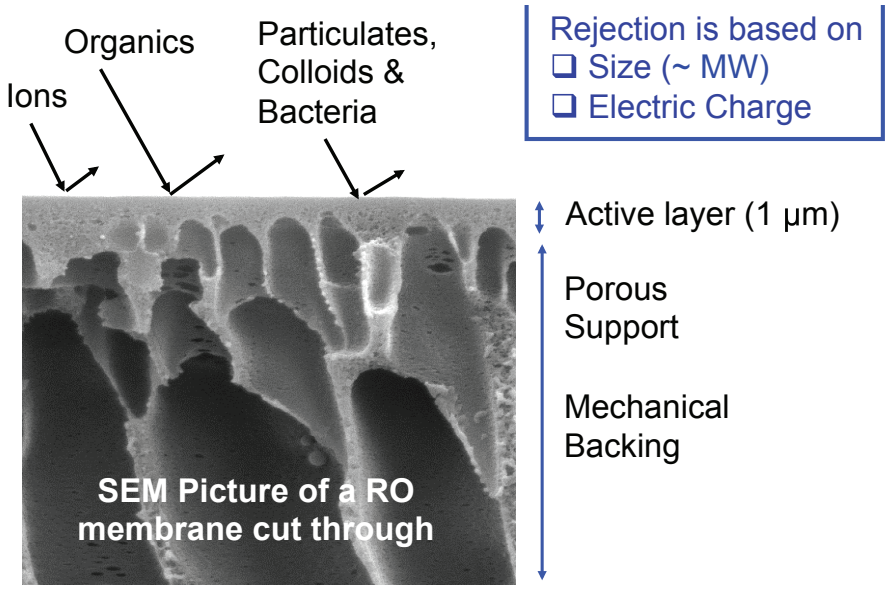

Figure 5: RO Membrane: Structure & Properties

to the size and electric charge of the contaminants. The reverse osmosis membrane therefore effectively rejects ions, organics (even if they are not electrically charged), particulates, colloids and bacteria.

The rejection performance of the reverse osmosis membranes depends on the nature of their material; most are made of cellulose acetate or polyamide. Rejection performance also depends on the manufacturer's process and quality. For instance, in the RiOs™ 5 water purification system, the reverse osmosis cartridge is fed by a flow rate of 47 L/hour to produce 5 L/hour permeate water. Consequently, the cartridge rejects 42 L/hour. However, of the 42 L/hour rejected by the cartridge, the system sends only 15 L/hour to the drain and recycles 27 L/hour upstream of the reverse osmosis cartridge. As a result, the system only needs an additional 20 L/hour of tap water to provide the 47 L/hour required to feed the reverse osmosis cartridge.

At the outlet of the reverse osmosis cartridge, a conductivity meter measures the quality of the water produced and the reverse osmosis cartridge rejection efficiency.

The permeate water then enters the Elix® module, which removes most of the remaining ions in order to produce Type 2 (pure) water with a resistivity greater than 5 ^{MOhm}/cm, and a TOC greater than 30 ppb. A resistivity meter at the outlet of the EDI module measures the quality of the water

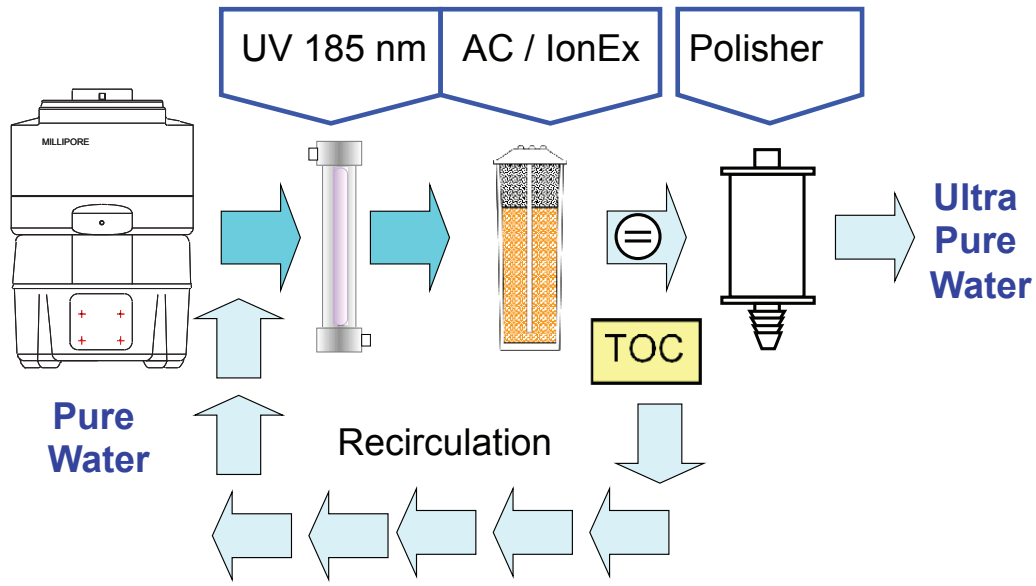

Figure 6: Purification Techniques to Ultrapure Water

produced by the Elix®. Elix®'s capabilities are based on electrodeionization technology developed and patented by EMD Millipore that uses permanent regeneration by a weak electric current. Elix® modules can last for several years and the water does not need to be softened upstream. The resulting Type 2 (pure) water is stored in a reservoir and can be either used directly or further processed to produce Type 1 (ultrapure) water.

The production of Type 1 (ultrapure) water from Type 2 (pure) water is achieved using the system outlined in **Figure 6**. The Type 2 (pure) water first flows through a 185 nm UV lamp module to oxidize organics into electrically charged organic ions and to destroy bacteria.

The water then moves through a Quantum™ cartridge, which contains activated carbon and an ion-exchange resin to remove the remaining traces of inorganic and organic ions to produce Type 1 (ultrapure) water. The synthetic activated carbon, produced from the pyrolysis of styrene beads, has a small (<150 Å) pore size, has a surface area of 1100m2/g, and is relatively homogeneous. Consequently, adsorption kinetics are rapid for low-molecular-weight organics, and very few mineral ions are released. Activated carbon cartridges are used in these polishing cartridges mainly to remove trace organics. Small organic molecules diffuse inside the pores and link to binding sites by weak van der Waals forces. The quality of the Type 1

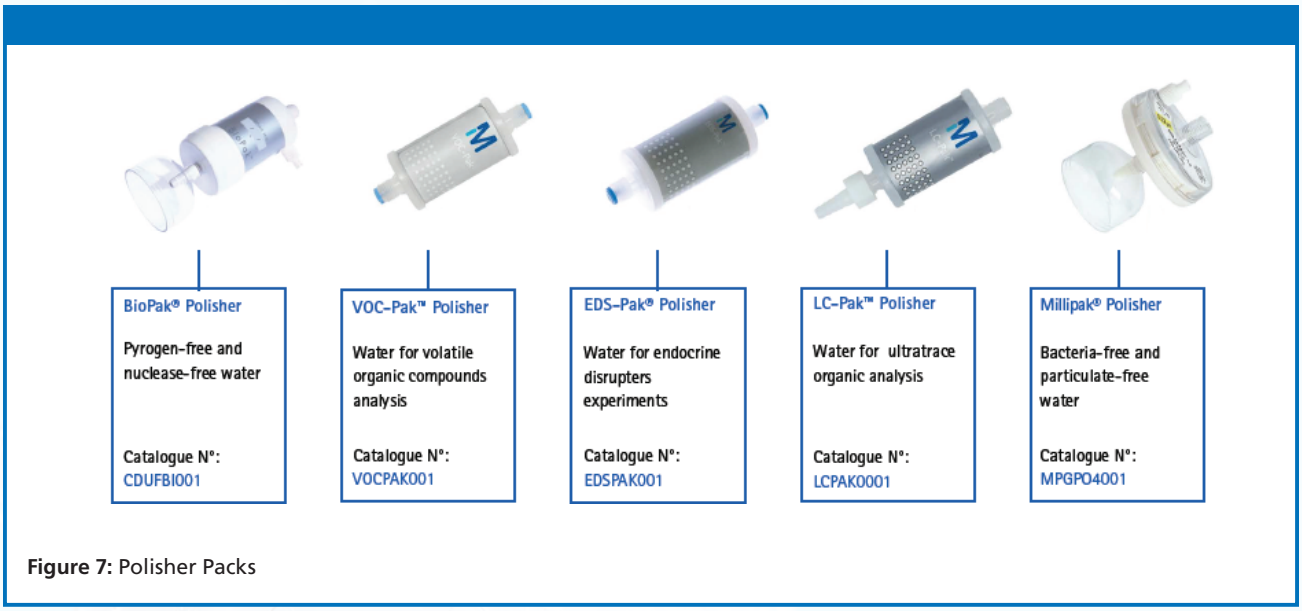

Figure 7: Polisher Packs

(ultrapure) water produced is monitored by an inline resistivity meter and an online TOC monitor. Typically, the sum of all ions is lower than 1 ppb and the sum of all organics is lower than 5 ppb.

The produced Type 1 (ultrapure) water can then be dispensed though a Q-POD® with a POD-Pak polisher (**Figure 7** at the outlet in order to further adjust the water quality to meet the user's needs. The POD-Pak polisher can be adapted as needed for each application.

Selecting, Operating, and Maintaining a Water System

Several parameters are important to consider when choosing a laboratory water system. Quantity is a practical consideration, but perhaps more important is the quality of the water needed—Type 2 (pure) water or Type 1 (ultrapure) water. If Type 1 (ultrapure) water is required, the type, sensitivity, and calibration of the resistivity and TOC monitors are key to ensuring water quality. Daily usage should also be considered, as well as ergonomy, bench space, volumetric dispensing, and flow rate (so that the water level can be accurately adjusted). A laboratory water system should also have intuitive interface.

Quality assurance (ISO9001 certification and cGMP compliance) is also important. Certificates for calibration, conformity, and consumables quality must be available, and established norms of ASTM (resistivity) and USP (resistivity and TOC suitability tests) must be followed. CE certification, cUL certification, and a consumables validation guide should also be included.

Qualification, installation, calibration, maintenance, repair, and technical services should be provided. Operating costs over the system's lifespan—not only

the original capital expenditure—should be considered. Finally, impact on the environment should be considered. During routine operation, storage of Type 1 (ultrapure) water should be avoided, as it can be quickly and easily contaminated. For best results, avoid storing water in carboys. Produce water on demand to prevent container extractables and bacteria buildup. Unnecessary connections—such as tubing at the outlet after the consumable filter—should be avoided. Manufacturer-recommended maintenance schedules should be followed for consumable replacements to reproducibly obtain high water quality. Since purified water is the most common reagent found in most laboratory facilities, a properly configured and maintained laboratory water system is critical to the success of almost every laboratory experiment. The appropriate grade of water is essential for everything from washing glassware to buffer preparation and cell culture analyses to complex analytical techniques such as HPLC or LC-MS. Appropriate purified water ensures the success of projects and maintain productivity.

In 1890, Merck KGaA, Darmstadt, Germany established its U.S. presence in New York City. Today, the company employs approximately 5,000 people in the United States across three businesses—Healthcare, Performance Materials and Life Science—that together are asking very big questions and tackling some of the world's most pressing scientific challenges. As Merck KGaA, Darmstadt, Germany's Life Science business, EMD Millipore is proud to be part this legacy of discovery and innovation. With the company's other businesses in the United States, we're celebrating our collective 125-year U.S. history and the boundless curiosity behind our past—and future—success. We invite you to join us in this celebration and learn how you can be part of our journey of discovery. Because Smarter, Together describes how we collaborate not only internally, but also with our customers, vendors and partners across the global life science industry.

Jean-Francois Pilette
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