ELGA VEOLIA

Guaranteeing pure water for your application

Contents

1. Introduction

When it comes to laboratory work, scientists make use of water in a wide range of processes throughout the day. For those working at the limits of analytical detection, pure water is an essential reagent and it becomes increasingly important to use the highest quality available for your application. Maintaining an adequate level of water purity is vital for producing consistent technical performance and reliable results.

With nearly 80 years experience exclusively in water purification systems for laboratories, ELGA have a range of Type I and Type I+ water purification systems specifically designed to provide ultrapure water with extremely low levels of organic and elemental contamination at parts per trillion (ppt) or sub-ppt levels. This level of purity is achieved through innovative system design rather than a reliance on expensive accessories and end-point filters that can have adverse effects on water quality if not used in the right way.

Ultimately the success of your work in the lab is heavily dependent on the quality of your reagents, including pure water, and the reliability of your experiments. This document highlights the contaminants your applications may be affected by, and demonstrates how our systems meet the limits of analytical detection to guarantee your ultrapure water. It will also provide you with the tools needed to make sure that your water supply delivers the performance you need to produce consistent results.

2. Ultrapure water for trace element analysis

Two factors are of particular importance for trace element analysis: protection of the sample from contamination, and the accuracy of the measurement. Elements and compounds that may be present in purified water in the parts per billion (ppb) range or lower, could have detrimental effects particularly if they interact with the samples, active media or system components.

Scientific instrumentation is continuing to become increasingly more sensitive in an attempt to enhance both data accuracy and precision, and as such demand that solutions have virtually zero levels of additional elements or ions. With detection resolutions now down into the parts per trillion (ppt), any impurities can lead to errors in blanks and calibration samples, or artificially high sample concentrations.

Methodology

The ELGA purification systems were operated over a 2 month period and the product water was dispensed to simulate typical usage patterns (60 liters / day). Samples were taken and analyzed for elements by high resolution inductively coupled plasma mass spectrometry (ICP-MS) and for silica by colorimetry.

Results & Analysis

Analysis of the ultrapure water dispensed shown in Table 1, demonstrates that the values all fall below the limits of detection of the sensitive analytical instruments for elemental analysis. Freedom from trace contaminants and ultrapure water with high resistivity (18.2 MΩ.cm) is shown to meet the demands for high sensitivity and accurate results.

Element	Symbol	Isotope	Water from ELGA system (ppt)	Element	Symbol	Isotope	Water from ELGA system (ppt)
Aluminium	Al	27	<1	Molybdenum	Mo	98	<0.5
Antimony	Sb	121	<0.2	Nickel	Ni	60	<2
Arsenic	As	75	<2	Niobium	Nb	93	<1
Barium	Br	138	<0.5	Osmium	Os	190	<2
Beryllium	Be	9	<3	Platinum	Pt	195	<5
Bismuth	Bi	209	<0.2	Potassium	K	39	<5
Boron	B	10	<10	Rhenium	Re	187	<3
Cadmium	Cd	114	<0.5	Rubidium	Rb	85	<1
Calcium	Ca	40	<2	Ruthenium	Ru	102	<2
Cerium	Ce	140	<1	Scandium	Sc	45	<5
Cesium	Cs	133	<1	Selenium	Se	82	<500
Chromium	Cr	52	<1	Silicon	Si	28	<25
Cobalt	Co	59	<0.5	Silver	Ag	109	<0.5
Copper	Cu	63	<1	Sodium	Na	23	<2
Gallium	Ga	71	<0.5	Strontium	Sr	87	<0.2
Germanium	Ge	74	<1	Tantalum	Ta	181	<3
Gold	Au	197	<5	Thallium	Tl	205	<1
Iridium	Ir	193	<2	Thorium	Th	232	<1
Iron	Fe	56	<2	Tin	Sn	120	<0.5
Lead	Pb	208	<0.2	Titanium	Ti	48	<0.5
Lithium	Li	7	<0.2	Tungsten	W	186	<1
Magnesium	Mg	24	<1	Uranium	U	238	<2
Manganese	Mn	54	<0.5	Vanadium	V	51	<0.2
Mercury	Hg	202	<5	Zinc	Zn	66	<2

Table 1: *Levels of elemental impurities detected in water from ELGA Type I+ systems. Analysis of water from these systems clearly demonstrates that results meet the demands of trace element analysis instrumentation.*

3. Purity for advanced chromatography applications

Chromatography is often used in many labs due to its relative simplicity, excellent reliability and broad applicability in a wide range of research areas and industries. As mentioned in the previous section, recent improvements in the sensitivity of advanced analytical techniques, such as liquid chromatography mass spectrometry (LC-MS), ultra and high performance liquid chromatography (UHPLC and HPLC respectively), require water of the highest purity to ensure clean output of data. Good chromatographic performance is highly dependent on the purity of the water used, particularly when you are looking to detect in the parts per billion (ppb) range, but it is often taken for granted.

Organic compounds in water are likely to be the most influential water contaminant as they compete with the analyte in the mobile phase. This reduces the effective levels retained in the column, resulting in a reduction in sensitivity. Dissolved gases, particles, colloids, bacteria and organic compounds can all have detrimental effects on results by either producing higher background values or interfering with the analysis directly.

Commercially available HPLC grade water typically supplied in bottles are often used in the lab, but when used as an eluent can give significantly inferior results. An alternative to bottled water is to use a water purification system which monitors the quality of the water throughout the process and direct to the point of use.

Methodology

In order to verify that ultrapure water produced from an ELGA purification system matched or exceeded the quality of commercially available bottled water, 50 ml water samples were pre-concentrated on a C18 reverse phase chromatography column with a water/acetonitrile gradient of 0-100% at 5% per minute and a flow rate of 2 ml per minute with UV detection at 254 nm. The samples were then eluted and analyzed by LC-MS (PDA, single quadrupole).

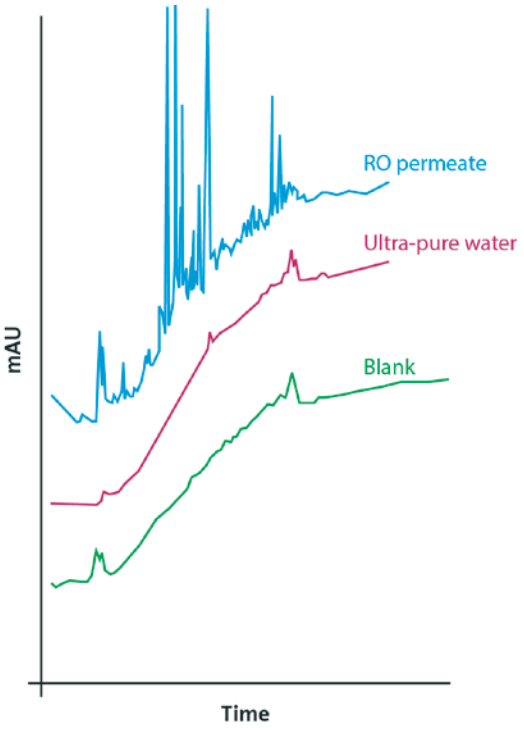


Figure 1. *Trace gradient HPLC of primary grade and ultrapure water.*

Results and analysis

The two diagrams below demonstrate the change in the absorbance of ultrapure water over a period of 60 minutes. Figure 2 compares the absorbance (mAU) of bottled HPLC water with ultrapure water from an ELGA purification system. The poorer performance of the bottled water may be due to contamination of the water through storage introducing leachables or bacterial growth, or through the addition of common solvents such as methanol, acetonitrile or acetic acid.

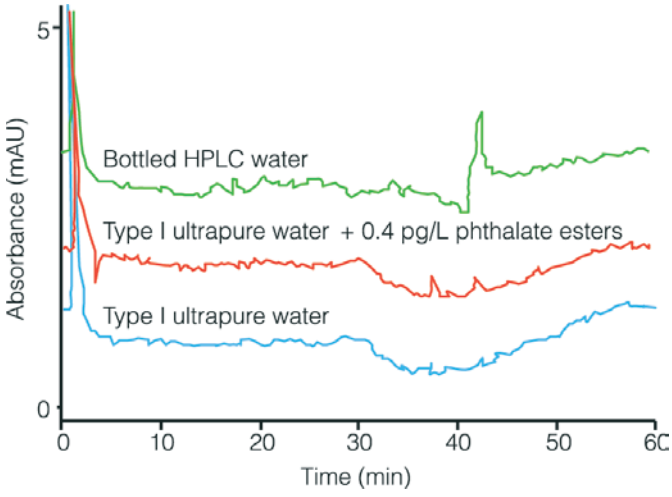


Figure 2. *Comparison of the absorbance (mAU) of bottle HPLC water with ultrapure water.*

4. Effective removal of Volatile Organic Compounds (VOCs)

Volatile Organic Compounds (VOCs) are organic-based molecules that have high vapor pressure at room temperature. This characteristic, which results from a low boiling point, can lead to a large number of molecules evaporating or sublimating from the liquid or solid form of the compounds respectively, to enter the surrounding air. The presence of VOCs in ground water is particularly concerning, due to their association with health problems.

As such, when analyzing aqueous samples for VOCs the quality of the water is critical as it should be free from bacteria and organic compounds. The presence of these in the water can degrade the VOCs and produce organic by-products or interfere with the analyses, resulting in increased background noise and erroneous or enlarged peaks. Typically, analysis of VOCs is conducted by gas chromatography (GC) often coupled with mass spectrometry (GC-MS).

Methodology

The systems were operated over a 2 month period and the product water was dispensed to simulate typical usage patterns (60 liters / day) using a PURELAB® Chorus 1 and PURELAB flex 3. Samples were taken and analyzed by a purge and trap gas chromatography mass spectrometry (GC-MS) according to EPA 524 for VOCs.

Results and Analysis

Table 2 demonstrates the trace levels of volatile and semi-volatile organic impurities in ultrapure water. Typically, levels of impurities all fall below the limits of detection: <0.05µg/l for VOCs. These levels are consistent with a Total Organic Carbon (TOC) value of <1µg/l. Ultrapure water is produced for lab use, suitable for all analytical applications. No VOCs were detected.

Compound	Detection Limit (ppb)	Quantification Limits (ppb)
benzene	0.05	< 0.05
bromobenzene	0.05	< 0.05
bromochloromethane	0.05	< 0.05
bromodichloromethane	0.05	< 0.05
bromoform	0.05	< 0.05
bromomethane	0.05	< 0.05
n-butylbenzene	0.05	< 0.05
sec-butylbenzene	0.05	< 0.05
tert-butylbenzene	0.05	< 0.05
carbon tetrachloride	0.05	< 0.05
chlorobenzene	0.05	< 0.05
chloromethane*	0.05	< 0.05
chloroethane	0.05	< 0.05
chloroform	0.10	< 0.10
2-chlorotoluene	0.05	< 0.05
4-chlorotoluene	0.05	< 0.05
dibromochloromethane	0.05	< 0.05
1,2-dibromo-3- chloropropa-ne (DBCP)	0.05	< 0.05
1,2-dibromoethane	0.05	< 0.05
dibromomethane	0.05	< 0.05
dichlorodifluoromethane*	0.05	< 0.05
1,2-dichlorobenzene	0.05	< 0.05
1,3-dichlorobenzene	0.05	< 0.05
1,4-dichlorobenzene	0.05	< 0.05
1,1-dichloroethane	0.05	< 0.05
1,2-dichloroethane	0.05	< 0.05
1,1-dichloroethene	0.05	< 0.05
cis-1,2-dichloroethene	0.05	< 0.05
trans-1,2-dichloroethene	0.05	< 0.05
1,1-dichloropropane	0.05	< 0.05
1,2-dichloropropane	0.05	< 0.05

Compound	Detection Limit (ppb)	Quantification Limits (ppb)
1,3-dichloropropane	0.05	< 0.05
1, 3, 5-Trichlorobenzene	0.05	< 0.05
2,2-dichloropropane	0.05	< 0.05
1,1-dichloropropene	0.05	< 0.05
cis-1,3-dichloropropene	0.05	< 0.05
trans-1,3-dichloropropene	0.05	< 0.05
ethylbenzene	0.05	< 0.05
hexachlorobutadiene	0.05	< 0.05
isopropylbenzene	0.05	< 0.05
4-isopropyltoluene	0.05	< 0.05
dichloromethane	0.05	< 0.05
naphthalene	0.05	< 0.05
n-propylbenzene	0.05	< 0.05
styrene	0.05	< 0.05
1,1,1,2-tetrachloroethane	0.05	< 0.05
1,1,2,2-tetrachloroethane	0.05	< 0.05
tetrachloroethene	0.05	< 0.05
toluene	0.05	< 0.05
1,2,3-trichlorobenzene	0.05	< 0.05
1,2,4-trichlorobenzene	0.05	< 0.05
1,1,1-trichloroethane	0.10	< 0.10
1,1,2-trichloroethane	0.05	< 0.05
trichloroethene	0.05	< 0.05
trichloromonofluoromethane	0.05	< 0.05
1,2,3-trichloropropane	0.05	< 0.05
1,2,4-trimethylbenzene	0.05	< 0.05
1,3,5-trimethylbenzene	0.05	< 0.05
o-xylene	0.05	< 0.05
m-xylene	0.05	< 0.05
p-xylene	0.05	< 0.05
vinyl chloride	0.05	< 0.05

Table 2. *Comparison of the detection limit for GC-MS (ppb or µg/l) with the quantification limits and levels of VOCs detected in water from ELGA Type I+ systems (ppb or µg/l). All analyses were completed with a PURELAB Chorus 1 unless stated as * PURELAB flex 3. Analysis of water from these systems clearly demonstrates results meeting the demands of GC-MS.*

5. Optimizing water for Life Science applications

For Life Science applications such as histology, immunohistochemistry, PCR, in-vitro fertilization, cell cultures and more, ensuring that the water used is free from biologically-active species is essential. Concerns have recently been raised that purified water being used in labs may contain endotoxins, RNase, DNase or bacteria. Impurities in the water, both of the biological and non-biological varieties, can lead to poor quality data and unreliable conclusions.

Polymerase chain reaction (PCR) is wholly dependent on the action of DNA polymerases that amplify single stranded DNA 'templates' producing vast numbers of additional copies. Bacterial contamination can lead to the introduction of artifacts in mounted samples in histology applications. The successful outcome of these procedures depends on the quality of the water used for sample and solution preparation.

Methodology

The systems were operated over a 3 month period and the product water was dispensed to simulate typical usage patterns (60 liters / day). During this period a product water sample was taken and analyzed as follows:

DNase and RNase

Samples were measured using a cleavable fluorescent-labeled substrate. A modified oligonucleotide was degraded by the nuclease in question and a fluorescent probe released. This fluorescence was detected and compared to a calibration using a spectrofluorometer.

Bacteria

Samples were regularly taken and analyzed for total viable counts by filtration and incubation on Reasoner's 2A agar (R2A) at 27°C for 5 days.

Protease

Protease was measured by incubating the sample for 24 hours with FTC-Casein at 37°C. The reaction is stopped with Trichloroacetic acid (TCA) and precipitated FTC-Casein then removed by centrifuging. An assay buffer was added and the fluorescent intensity measured at 525nm using 490nm excitation. The intensity of the fluorescence is directly proportional to the total protease activity in the sample. The method was calibrated using Trypsin.

Results and Analysis

Table 3 demonstrates the trace levels of bacteria, pyrogens, DNase, RNase and endotoxins in ultrapure water. All levels of impurities fell below the limit of detection, ensuring suitability for analytical, clinical, pharmaceutical and molecular biological applications. Low levels of bacteria are maintained by the use of UV light, filtration and regular sanitization. Ultrafiltration removed endotoxins and RNase.

Product Water Specification	
Pyrogen Level (EU/ml)	< 0.001
RNases (ng/ml)	< 0.003
DNases (ng/µl)	< 0.1
Bacteria (CFU/ml)	< 0.1
Protease (ng/ml)	< 1*

Table 3. *Quantification limit and product water specification of organic material detected from an ELGA Type I+ system. *Chorus 1 Life Science limit for protease is <0.1 ng/ml.*

6. Endocrine Disruptors and Phthalates

Endocrine Disrupting Compounds (EDCs) are chemicals that interfere with the endocrine system, typically by imitating the action of various hormones. The health effects of EDCs are wide ranging and vary from problems in cognitive development and the triggering of cancer, to development complications. Even low levels of EDCs can induce chronic effects, making their toxicological assessment particularly difficult. Due to practical implications, a large percentage of safety testing relies on using doses that are typically lethal.

There are a number of chemicals which are suspected of having endocrine disrupting properties, such as pesticides, pharmaceuticals, compounds used in the manufacturing of plastics and synthetic hormones. Recent legislation in the monitoring of EDCs has created the need for fast, reliable and highly sensitive techniques for detection, including high performance liquid chromatography (HPLC). This technique, which is one of the most commonly used instruments in the lab, makes use of ultrapure water throughout the analytical process. In addition to the standards of water required for trace elemental analysis, the ultrapure water also needs to be free of EDCs to ensure that there is no background noise or false positives.

Methodology

The systems were operated for several months with no particular usage pattern. New consumables were fitted and each system was operated for two weeks dispensing 25 liters of water per day before taking samples for analysis. For Bisphenol A and Nonylphenol, the samples were analyzed by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). For the other analytes, the samples were analyzed using a Dionex HPLC system with Phenomenex 00F-4251-E0 column and PDA detector. Between 10 and 100ml of sample were loaded onto the system before running a gradient method of ultrapure water and acetonitrile (MeCN) (95% to 10% UPW) over 30 minutes. Absorbance at 205nm was measured using the Photodiode Array (PDA).

Results and Analysis

Tables 4 demonstrates the suitability of the water purification systems in providing ultrapure water free from EDCs and phthalates, and are specifically validated for efficient removal of Bisphenol A (BPA), diethyl phthalate and di-n-butyl phthalate. The chromatogram signal in Figure 3 corroborates this data, demonstrating that measured BPA concentrations for the samples were below the detection limit of the analytical technique.

EDC tested	Product water (ppt)
Bisphenol A	< 5
Butyl benzyl phthalate	< 5
Diethylhexyl phthalate	< 130**
Diethyl phthalate	< 25
Dimethyl phthalate	< 35*
Di-n-butyl phthalate	< 25
Nonylphenol	< 25

Table 4. *Measurement of known EDCs with the levels of impurities detected in water from an ELGA Type I+ system. * raised detection limit (only samples of 10ml possible) ** raised detection limit (interference peak over-lapping analyte peak).*

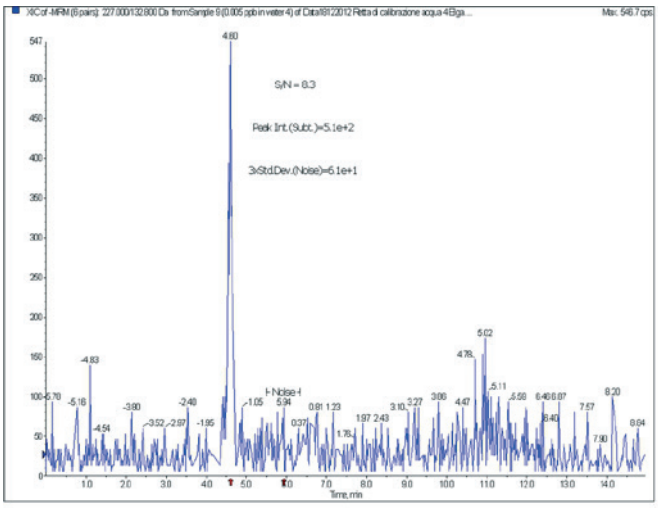


Figure 3. *Bisphenol A ion chromatogram signal for 5ppt standard in ultrapure water.*

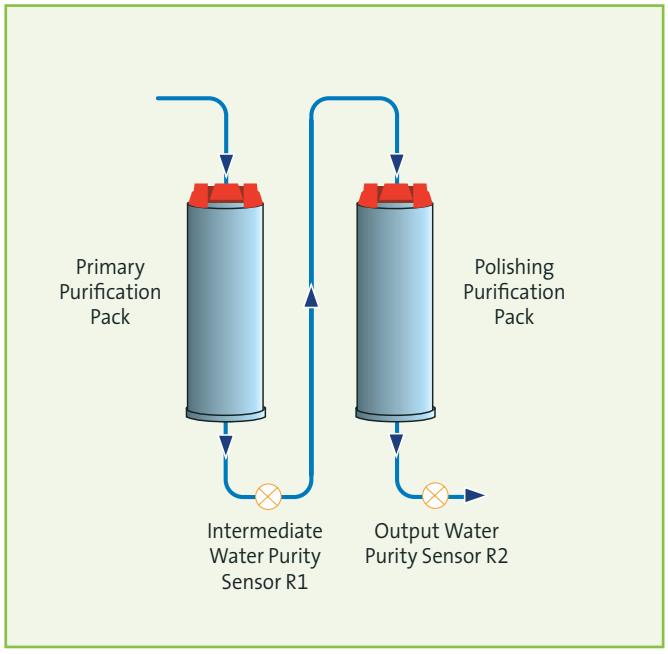
7. Our technologies

All of the applications highlighted demonstrate the requirement for high quality water with low traces of both organic and inorganic species. This level of purity can be achieved through innovative system design rather than a reliance on bottled water, expensive accessories and end point-of-use filters, which could have adverse effects on your water quality if not used in the correct way.

In addition to the technologies within the water purification systems, the method of dispensing can also have impact on your work. Accurate and low volume dispensing means that you can take the exact quantity of ultrapure water that you need at that moment, avoiding the need to fill large containers that will inevitably degrade the purity of the water and affect your results.

PureSure® Technology

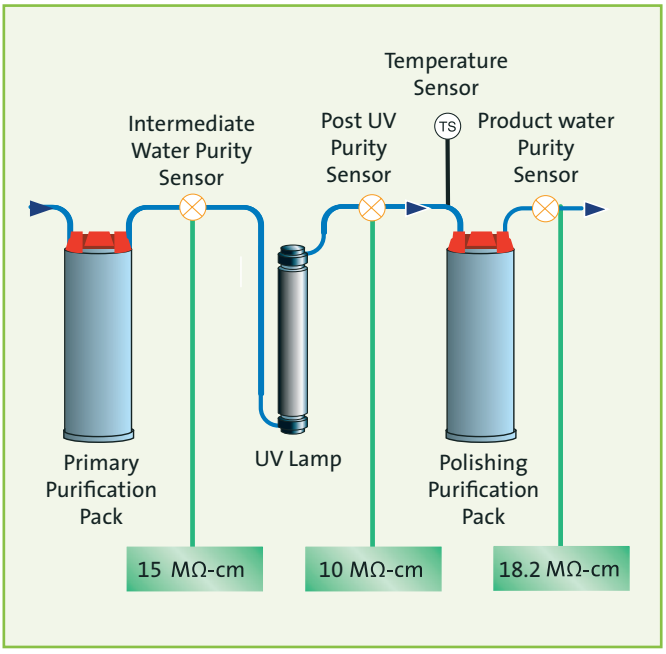
In all deionization processes there is a risk that weakly ionized impurities will elute from the resins and into your application as they approach exhaustion. After a period of use, the ion exchange capacity of a purification pack starts to be exhausted and these compounds are released first, even before the resistivity has fallen below 18.2 MΩ-cm. PureSure technology prevents this.



This double purification pack and monitoring system ensures accurate results with uninterrupted work flow, by retaining any organics and silica that may be released by the first purification pack as it approaches exhaustion, and providing you with the time to change the consumable without impacting on your results. Not only does it help to guarantee your water purity, you have increased security and advanced warning of usable purification pack life.

Real Time TOC Monitoring

Total Organic Carbon (TOC) is a universal indicator of the presence of organic impurities. By constantly monitoring TOC you can be assured of the overall organic purity of the water. Real-time TOC monitoring provides you with a reliable and fast indication of organic purity by identifying heavy organic loading on the system before dispensing the water and potentially affecting your analyses.



Integrated Filters

PURELAB Chorus provides integrated ultrafiltration and microfiltration systems.

This integrated system enables a more effective total system approach, combining technologies such as RO, ion exchange, UV and micro- or ultrafiltration to provide ultrapure water rather than relying on a single point-of-use system.

The major benefits of this over typical POU filters:

- Levels of bacteria or endotoxins can be further filtered or recycled before use, as integrated filters are cleared of impurities by recirculating ultrapure water through them
- Extra security and peace of mind as monitoring of the final water quality is possible

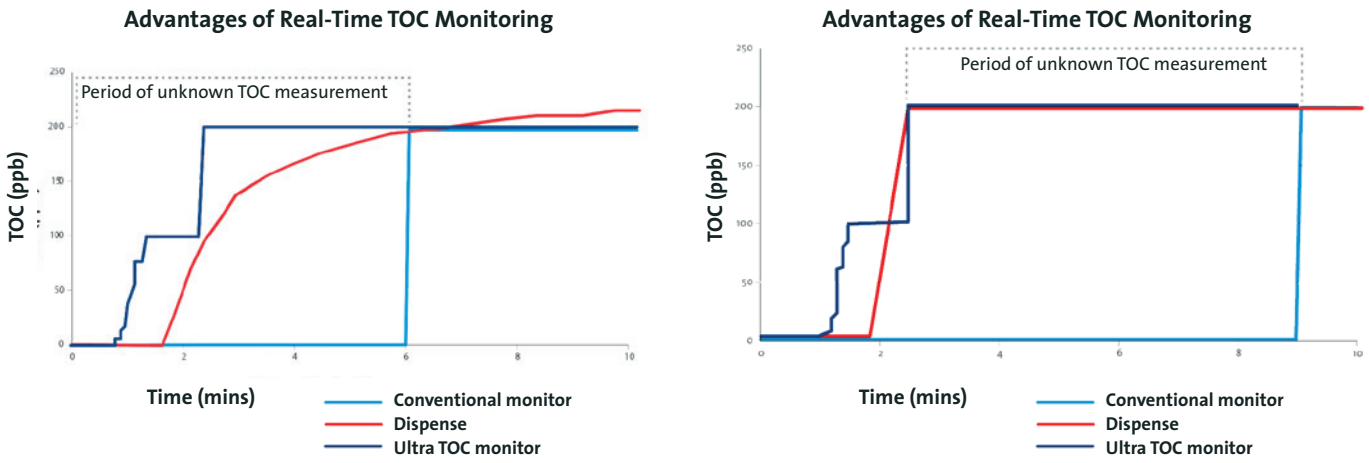


Figure 4. *Detection of a sudden change in TOC level.*

8. Conclusion

Whatever application you are undertaking in your lab, lack of appropriate water purity can have serious implications for your results.

Point-of-use filters are often offered as a 'peace-of-mind' solution to further clean the water at the end of the water treatment process. The activated carbon they contain effectively removes organics. However, the drawback of these filters is that they introduce inorganic impurities back into the product water. Activated carbon by its very nature has a high surface area and even the highest grades of carbon will release trace levels of elemental impurities back into the application. As the filter is used at the end of the water treatment process, there is no deionization process to remove these impurities and no monitor to ensure that you are not dispensing contaminated water.

It is important that you are sure that the water you are using is as pure as you think it is. The best way to ensure you have the correct water purity is through continuous sampling, ideally in real-time. In-line systems built into the purification process are the best option for obtaining the most accurate data. Integrated filters also guarantee a more effective total system approach. As ultrapure water is produced in a multi-stage process, there is often the need for multi-stage monitoring in order to ensure a high degree of consistency with regard to purity. By measuring ions and organics in a truly continuous fashion, you can be certain that your results will not be adversely affected by possible spikes in contaminant levels.

9. Working with ELGA

We hope that the information provided here has given you a valuable insight into how important water purity monitoring to point of use is for your application.

If you would like to know more on this subject, please contact our team of experts. ELGA has been working exclusively in water purification for almost 80 years, making us the world leader in water treatment for the lab.

As an organization we are committed to ensuring that those working in the lab receive the highest quality of professionalism and water possible. Whatever your water needs, get in touch now to see how we can help you make sure all the work you carry out in the laboratory is productive and rewarding.

Tel: +44 (0) 203 567 7300
Fax: +44 (0) 203 567 7205
Email: info@elgalabwater.com **Website:** www.elgalabwater.com

ELGA LabWater
Lane End Industrial Park • High Wycombe • HP14 3BY • United Kingdom
tel: +44 (0) 203 567 7300 • fax: +44 (0) 203 567 7205
info@elgalabwater.com • www.elgalabwater.com