

# Investigating organic contamination in high purity water using UHPLC with ultrasensitive diode array detection and LC/MS

## Application Note

Pharmaceutical and Chemical

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### Abstract

UHPLC systems, such as the Agilent 1290 Infinity LC system, and MS instruments are gaining increased sensitivity. To achieve optimum performance, it is therefore important to run these instruments with ultrapure solvents. This Application Note demonstrates that a carefully planned combination of purification technologies will produce ultrapure water that is suitable for UHPLC and LC/MS analyses from tap water. Guidelines to avoid contamination of ultrapure water are also given.

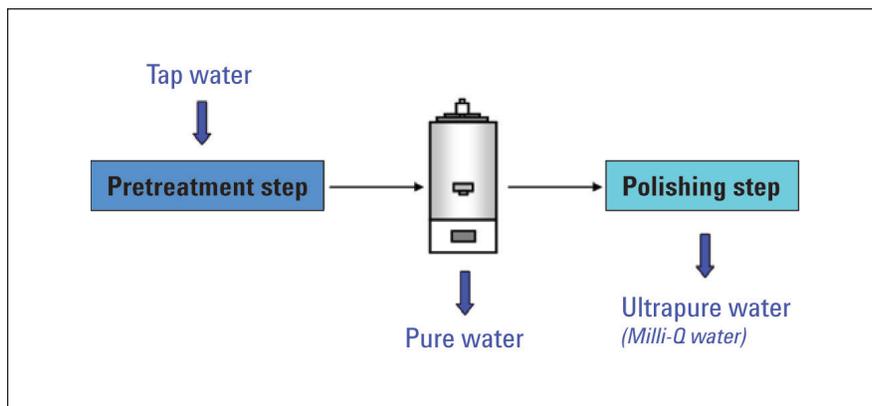


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## Introduction

The recent advances and developments in HPLC and UHPLC systems, like the Agilent 1290 Infinity LC system, have resulted in unprecedented levels of sensitivity. Such advances and developments require that the reagents and solvents used have to be of highest purity. Water plays a critical role in reversed phase HPLC and UHPLC separations and analyses; therefore contamination has to be at the lowest possible level<sup>1</sup>. Organic contaminants in the water used to prepare the aqueous mobile phase accumulate in the column, and could cause problems such as high background noise, drifting baselines, and the appearance of ghost peaks. The water needs to be of high ionic purity to avoid formation of metal adducts. Particles and bacteria should be avoided because they plug column frits. Also, bacteria introduce new contaminants due to their by-products.

In this Application Note, different sources of high purity water for HPLC, UHPLC and LC/MS were investigated using reversed phase UHPLC with an ultrasensitive diode array detector and a QTOF mass spectrometer. High purity water suitable for HPLC, UHPLC and LC/MS should have very low organic contaminants, and should be ion-free, particulate-free, and bacteria-free. The right water purification system should be equipped with purification technologies that are purposely combined to efficiently remove these contaminants, as illustrated in Figure 1. The pretreatment step consists of reverse osmosis and electrodeionization that removes bulk of the contaminants in tap water to produce pure water. The polishing step consists of ion-exchange resins, activated carbon and UV photo-oxidation, to produce ultrapure water that typically is ion-free (resistivity is



**Figure 1**  
Schematic of a water purification system.

18.2 MΩ.cm), has very low organic contaminants (< 5 ppb total oxidizable carbon, TOC), and with a 0.22 μm membrane point-of-use filter, is also bacteria-free and free of particulates larger than the pore size.

## Experimental

### Equipment

UHPLC experiments were carried out using an Agilent 1290 Infinity LC system comprising of a 1290 Infinity Binary Pump, a 1290 Infinity High Performance Autosampler, a 1290 Infinity Thermostatted Column Compartment, and a 1290 Infinity Diode

Array Detector with 10 mm MaxLight standard cell. ChemStation B.04.01 software was used for data acquisition and analysis.

Direct infusion experiments were carried out using an Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight Mass Spectrometer with Agilent Jet Stream Thermal Focusing technology. MassHunter software was used for data acquisition and analysis.

Acetonitrile and bottled water were LC/MS grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 μm membrane point-of-use cartridge (Millipak).

### Method

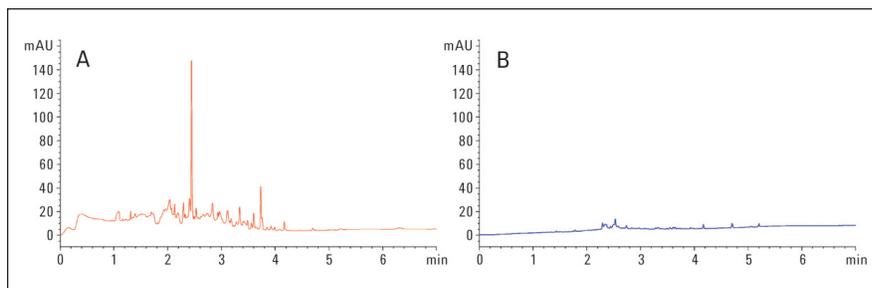
Solvents:	A: Water B: Acetonitrile
Column:	Agilent ZORBAX RRHD, SB C18, 50 × 2.1, 1.8 μm
Enrichment:	Solvent A; 1 mL/min; 60 min
Gradient:	0 min – 5% B, 5 min – 95% B
Flow rate:	1 mL/min
Injection volume	0 μL
UV detection:	210/4 nm, data rate 80 Hz, 10 mm DAD standard max-light cartridge
QTOF:	Positive mode, 100 – 1000 m/z Sheath gas: 350 °C, drying gas: 300 °C, nebulizer: 35 psi V <sub>cap</sub> : 3000V
Infusion flow rate:	200 μL/min

## Results and discussion

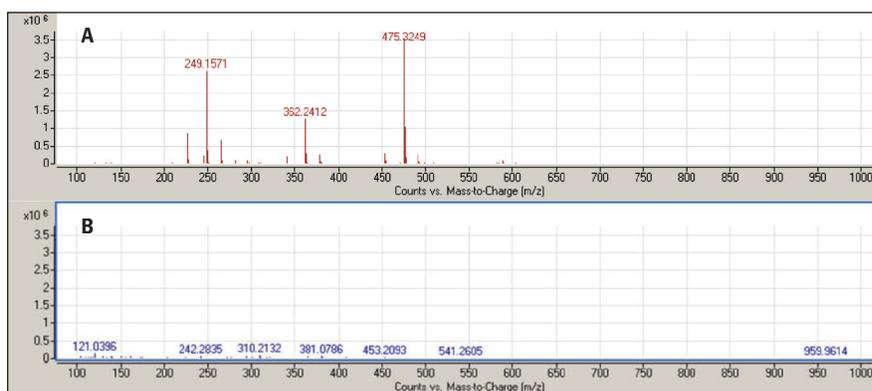
The water samples analyzed by UHPLC were enriched for one hour prior to gradient elution. During this enrichment step, organics in the water accumulate on the column. In the elution step, these trapped organics elute out of the column when the solvent composition is strong enough<sup>2</sup>.

Pure water obtained from tap water has gone through reverse osmosis and electrodeionization has a resistivity that is typically greater than 5 M $\Omega$ .cm at 25 °C and TOC that is less than 30 ppb. As can be seen in Figure 2A, pure water is not clean enough for UHPLC use. The presence of numerous high intensity peaks indicate that this water is significantly contaminated with organics. Further purification by combining ion-exchange resins, synthetic activated carbon, and UV photo-oxidation tremendously improves the quality of the water (ultrapure), with resistivity of 18.2 M $\Omega$ .cm and TOC levels less than 5 ppb. Figure 2B shows the chromatogram of ultrapure water after one hour pre-concentration. It is much cleaner, with very few low intensity peaks. In addition, direct infusion experiments also reveal that pure water has significantly more ionizable organic contaminants compared to ultrapure water (Figure 3). This affirms that the ultrapure water has very low organic contaminants, as the low TOC level (less than 5 ppb) suggests.

Another helpful water quality parameter to consider is resistivity. An 18.2 M $\Omega$ .cm resistivity means that ultrapure water is not a source of metal ions, such as Na<sup>+</sup> or K<sup>+</sup>, that could form adduct peaks. The 0.22  $\mu$ m membrane final filter at the end of the water purification system ensures that the ultrapure water is free of bacteria and particulates. These contaminants are critical in UHPLC and LC/MS.



**Figure 2**  
Chromatograms (210 nm) of (A) pure water (purified from tap water), and (B) ultrapure water that have been enriched for one hour.



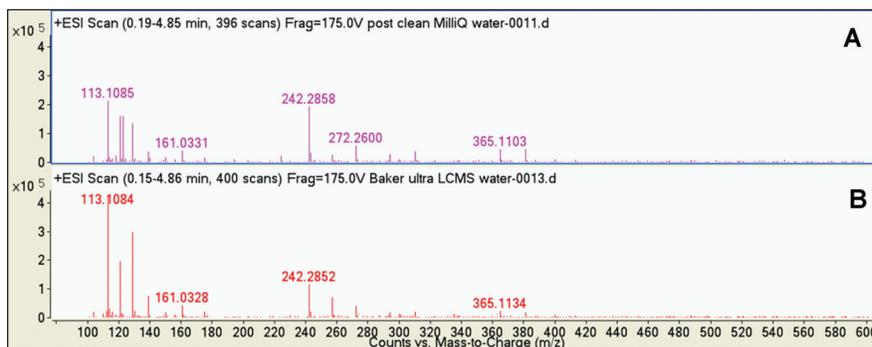
**Figure 3**  
Mass spectra from the direct infusion of (A) pure water (purified from tap water), and (B) ultrapure water.

Fresh ultrapure water is an excellent and reliable source of water for UHPLC and LC/MS mobile phase preparation. The other common source is bottled water. LC/MS grade water is of higher purity than HPLC-grade water. Comparison between fresh ultrapure water and LC/MS grade bottled water by the direct infusion show more intense peaks in the LC/MS grade water (Figure 4). Another disadvantage of bottled water is that once it is opened, contaminants are re-introduced. On the other hand, a water purification system provides fresh ultrapure on demand, with the added benefits of being able to check the quality of the water being delivered by means of online resistivity and TOC monitors.

### Practical guidelines to avoid ultrapure water contamination

Ultrapure water can be easily contaminated, and this can compromise the quality of chromatograms and spectra, and the overall performance of the instrument. To avoid contamination, ensure that:

- The water purification system is properly and regularly maintained.
- Always use fresh ultrapure water to prepare aqueous mobile phases. Discard the first 1 or 2 L before collecting water for UHPLC.
- Use dedicated glassware. Clean the glassware by washing with ultrapure water and ultrapure organic solvent followed by a drying step in a muffle furnace at 400 °C for six hours. Store the cooled bottles capped with aluminum foil until use. Only use a dishwasher in which no detergent is ever used and the final rinse in the dishwasher is DI water.



**Figure 4**  
Mass spectra from the direct infusion of (A) ultrapure water, and (B) LC/MS grade bottled water.

- Do not store ultrapure water. Due to its aggressive nature, ultrapure water will absorb contaminants in the atmosphere and in the container, and it is no longer ultrapure. Once the solvent is exposed to the atmosphere, it becomes contaminated with airborne bacteria, bacterial nutrients, and bacterial by-products.
- Choose the highest grade organic solvent and modifier.
- Do not attach plastic tubing at the end of the system.

The extent of organic contamination can be evaluated by:

- Direct infusion on to a mass spectrometer. Ideally, it should give very low background signals.
- Gradient test: Run a gradient, with an enrichment procedure as described in the Experimental Method part. Less peaks means that the water has less contaminants.

### Conclusion

Solvent purity is crucial in highly sensitive UHPLC and LC/MS measurements. Fresh ultrapure water from an efficient water purification system provides an excellent source of water for mobile phase preparation since it contains very low organic contaminants, which is important for high sensitive UHPLC and LC/MS measurements. It is also free of ions, bacteria, and particles, contaminants that are known to affect the performance of the instruments.

### References

- 1 S. J. Williams, *J Chrom A*, 1052 (2004) 1.
- 2 C. Regnault, I. Kano, D. Darboret, S. Mabic, *J Chrom A*, 1030 (2004) 289.

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