Investigation on the extent of organic contamination in high purity water using UHPLC equipped with ultrasensitive diode array detector and LC-MS

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Introduction

- Advances and developments in HPLC systems have resulted to unprecedented levels of sensitivity.
- Such advances and developments imposed more stringent requirements in reagent and solvent purity.
- High purity water plays a critical role in reversed phase HPLC separations.
- 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" or "mystery" peaks.
- In this study, reversed phase HPLC with an ultrasensitive diode array detector and mass spectrometry were used to
  - Investigate the quality of water from a water purification system after the pre-treatment step and the after the polishing step
  - Investigate and compare the quality of fresh ultrapure water and LC-MS grade bottled water

Experimental

HPLC experiments were carried out using an Agilent 1290 Infinity LC system.

Instrument	Agilent 1290 Infinity LC system
Column	ZORBAX, SB C18, 50 x 2.1, 1.8µm
Enrichment	Flow: 1 mL/min, Solvent A Time: 60 minutes
Gradient elution	Solvent A: test Water Solvent B: Acetonitrile 0 min - 5% B, 5 min - 95% B
DAD	210/4 nm, data rate 80 Hz, 10 mm DAD standard max-light cartridge
Flow	1 mL/min
Injection volume	0 µL

Direct infusion experiments were carried out using an Agilent accurate-mass quadrupole time-of-flight mass spectrometer

Instrument	Agilent 6530 Accurate-Mass Q-TOF featuring Agilent Jet Stream Thermal Focusing technology
Polarity	Positive, 100 – 1000 m/z,
Solvent	Water
Flow	200 µL/min

Acetontirile and bottled water were LC-MS grade. Fresh ultrapure water was obtained from a Milli-Q® Integral system (Figure 2A).

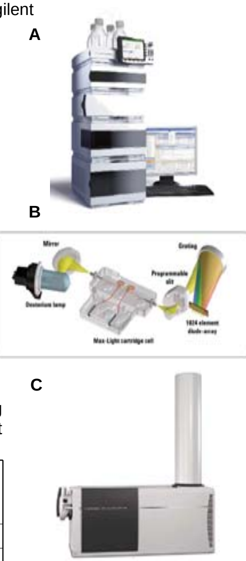

Figure 1.
(A)Agilent 1290 Infinity LC;
(B) Max-light cartridge;
(C) Agilent 6530 Q-TOF

Production of ultrapure water for HPLC and LC-MS

High purity water suitable for HPLC and LC-MS should have very low organic contaminants, 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 2B.

A

B

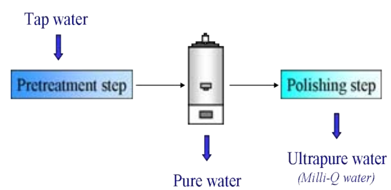

Figure 2. (A) Milli-Q Integral; (B) Schematic of a water purification system

The ***pre-treatment 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 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 purifier, is also bacteria-free and free of particulates larger than the pore size.

Results

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

Pure water, which is obtained after tap water has gone through reverse osmosis and electrodeionization, is not clean enough for UHPLC use, as can be seen in Figure 3A. 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), as Figure 3B shows. Direct infusion experiments also reveal that pure water has significantly more ionizable organic contaminants compared to ultrapure water (Figure 4).

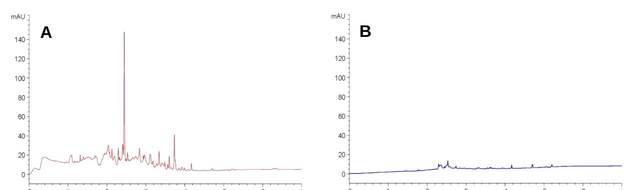

Figure 3 : Chromatograms (210 nm) of (A) pure water (purified from tap water), and (B) ultrapure water that have been enriched for one hour.

Results *(con't)*

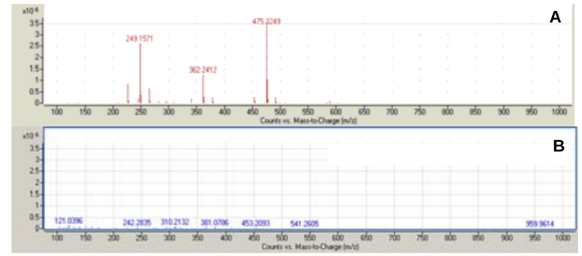

Figure 4: Mass spectra from the direct infusion of (A) pure water (purified from tap water), and (B) ultrapure water

Fresh ultrapure water is an excellent and reliable source of water for UHPLC and LC-MS mobile phase preparation. The other common is bottled water. LC-MS grade water is of higher purity than HPLC-grade. Comparison between fresh ultrapure water and LC-MS grade bottled water by directly infusion show more intense peaks in the LC-MS grade water (Figure 5).

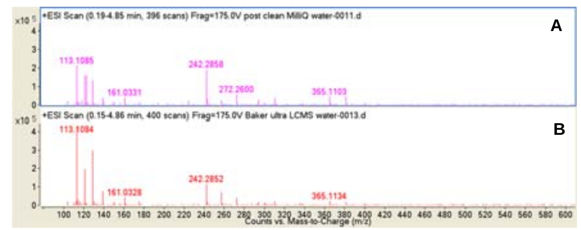

Figure 5 : Mass spectra from the direct infusion of (A) ultrapure water, and (B) LC-MS grade bottled water

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 water on demand, with the added benefits of being able to check the quality of the water being delivered via online resistivity and TOC monitors.

Conclusions

- Analyses of pure water using an HPLC equipped with ultrasensitive diode array detector and by mass spectrometry show that after pre-treatment of tap water by reverse osmosis and electrodeionization, the purified water still contains significant amount of organics.
- Similar analyses of ultrapure water show that the polishing step is extremely effective at reducing organic contaminants, making fresh ultrapure water suitable to use for LC-MS experiments.
- Comparison of the mass spectra indicate that fresh ultrapure water is comparable in quality to LC-MS grade bottled water.