The Importance of Water Quality in HPLC and LC-MS Analyses

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Introduction

Optimized HPLC analyses require the use of high purity solvents and reagents. While chromatographers take great care in the selection of salts and organic solvents used in mobile phase preparation, they often take water quality for granted. The presence of trace organics in the water used in the eluent may lead to poor long-term results. Over time, column efficiency may deteriorate, leading to a decrease in resolution and peak tailing. The presence of trace organics in the water could significantly affect the results of LC-MS analyses. This is especially true if the organic contaminants are easily ionizable and have masses close to the analytes of interest.

Aim of the study

The purpose of this study was to investigate the influence of water quality on HPLC and LC- MS analyses. Commercially-available HPLC-grade bottled water was compared to water purified by a water-purification system delivering freshly-produced high purity water on- demand. A mixture of 7 drugs was separated using a gradient of acetonitrile and either bottled water or freshly purified water. Separate yet identical columns were used for the two different mobile phases. The analysis was performed repeatedly (1 310 times), and chromatograms were compared.

Experimental Methods

Drug mixture

The following chromatographic drug standards were purchased from Alltech as 1 mg/mL solutions in methanol:

- Acetaminophen (1)
- Acetazolamide (2)
- Phenobarbital (3)
- Carbamazepine (4)
- Phenytoin (5)
- Secobarbital (6)
- Nabumetone (7)

A mixture containing 0.200 µg/mL of each drug was prepared.

Mobile phase

Water and acetonitrile were used as mobile phases. There were two sources of water: HPLC-grade water (Fisher) and ultrapure water from a Milli-Q® Advantage A10 (Merck Millipore) with a Millipak®-20 Express 0.22 µm (Merck Millipore) as a final filter. Ultrapure water was delivered fresh from the Milli-Q® Advantage A10 system prior to use. Acetonitrile was HPLC-grade (Fisher). Mobile phases were sonicated prior to use.

Instrumentation

The HPLC system was from Waters: 510 HPLC pumps, 717 plus autosampler, and a 996 Photodiode Array Detector set at 190 – 400 nm. Columns were SymmteryShield RP18 from Waters, 3.5 µm, 4.6 x 150 mm.

Procedure

The drug mixture was filtered through 0.45 µm 13 mm Millex®-LCR (Merck Millipore) filter units prior to 25 µL injections. The following gradient profile (water: acetonitrile) was used to separate the components of the mixture:

- 80 % to 30 % water for 15 min
- 30 % to 80 % water for 1 min
- hold at 80 % water for 4 min

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Results and Discussion

- The mixture was injected into each of the column 1 310 successive times. The HPLC separation of the 7 drugs was satisfactory for the length of the experiment, regardless of the type of water used in the experiments (Figure 1)

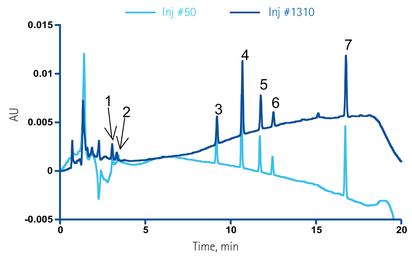


Figure 1. Chromatogram of acetaminophen (1), acetazolamide (2), phenobarbital (3), carbamazepine (4), phenytoin (5), secobarbital (6), nabumetone (7) at 214 nm with HPLC-grade bottled water and acetonitrile as mobile phase

- When HPLC-grade bottled water was used to prepare the eluent, the baseline drifted in the positive direction as the number of analyses performed increased (Figure 2A). The baseline moved minimally when ultrapure water was used (Figure 2B).

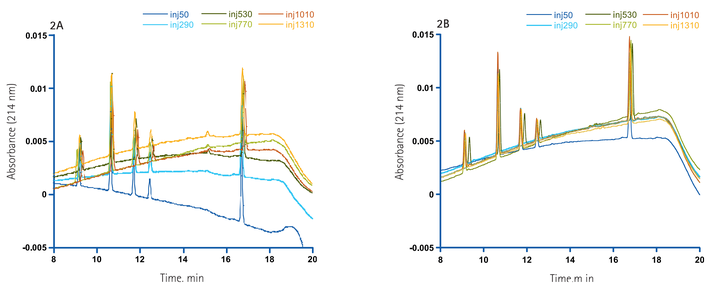


Figure 2. Chromatograms at 214 nm using (A) HPLC-grade bottled water, (B) ultrapure water with TOC < 5 ppb in the mobile phase.

- A drift in baseline was also observed at 254 nm when HPLC-grade water was used in the eluent (Figure 3A and B).
- With time, a ghost peak appeared around 5 min when HPLC-grade bottled water was used (Figure 3A). No ghost peak appeared when ultrapure water was used (Figure 3B).

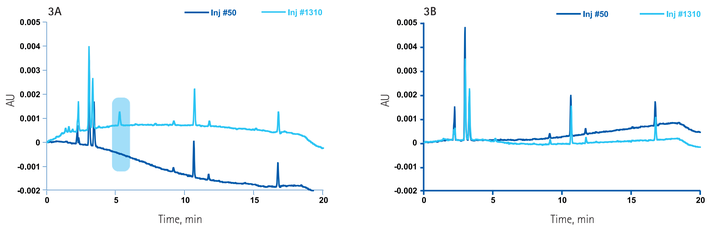
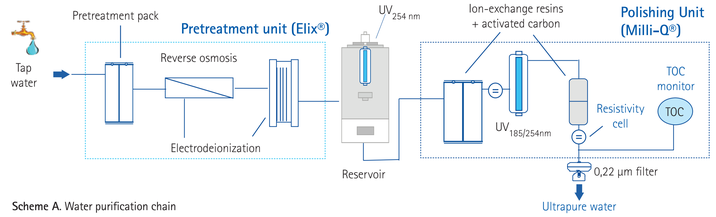


Figure 3. Chromatograms at 254 nm with (A) HPLC-grade water, (B) ultrapure water with TOC < 5 ppb as mobile phase. Note the appearance of a ghost peak in chromatogram A.

- The ghost peak may be due to the organic contaminants concentrating at the head of the column, then being released. The drift in baseline may also be due to organic impurities present in the eluent prepared with the bottled water.
- Using freshly delivered ultrapure water with low TOC levels provides an advantage over HPLC-grade water by providing a stable baseline free of ghost peaks, even after 1 310 injections.

Water purification

A combination of purification technologies was used to produce ultrapure water from tap water on demand:



Scheme A. Water purification chain

The ultrapure water used for this study was free of ions (the resistivity was 18.2 MΩ.cm at 25°C), and contained less than 5 ppb of total organic carbon (TOC).

UV Photo-oxidation

- HPLC protocols usually recommend using HPLC-grade bottled water for the preparation of mobile phases. This type of water has low UV absorbance and is free of particulates. However, there are seldom specifications as to the level of organic impurities (TOC level). Such impurities may prove detrimental in HPLC analyses, especially in gradient runs. They may concentrate at the head of the column, and eventually elute as ghost peaks. This could pose difficulty or ambiguity in the interpretation of the chromatogram. Additionally, organic impurities may result in elevated baselines, and affect assay sensitivity.

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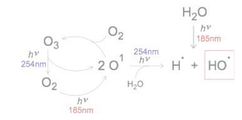
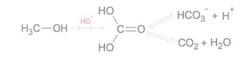


Scheme B. Generation of hydroxyl radical



Scheme C. Oxidation of methanol

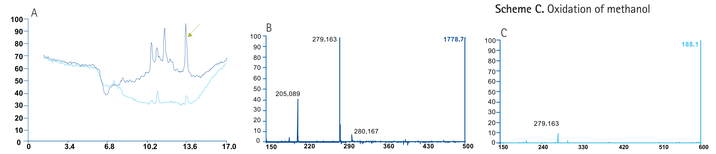


Figure 4. (A) Mass chromatogram of ultrapure water with and without UV photo-oxidation. (B) Mass spectrum of the peak at 13.1 min for water without UV photo-oxidation (TOC = 12 ppb). (C) Mass spectrum of the peak at 13.1 min for water treated with UV photo-oxidation (TOC = 4 ppb).

Conclusion

Using freshly produced ultrapure water with low TOC (< 5 ppb) to prepare mobile phases helps to reach and maintain good chromatographic performance. Unlike bottled HPLC-grade water, the use of such high quality water minimizes baseline drifts and limits the appearance of ghost peaks in HPLC. This is important for the unambiguous identification of peaks and for the quantitation of the analytes. Using an efficient pre-treatment system, followed by a polishing step that involves UV photo-oxidation provides excellent removal of organic impurities from water.