Ultrapure Water for Nano-LC^n/MS^n Analyses

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Introduction

Analysis of biological samples by MS is challenging due to the limited amount of sample available for analysis, the very low concentration of analyte, and the potential for interference from sample matrix. The advent of nano-LC^n/MS^n offers a solution to these limitations. The nL/min flow rate creates much smaller droplets that are more readily desolvated and result in higher MS sensitivity. In addition, lower detection limits are achieved, less sample is required, and there can be an increased tolerance to chemical interferences compared to conventional LC flow rates.[1-4] Interfacing nano-LC to MS utilizes emitters/sprayers that have tips with inner diameters of ~1-30 µm. This extremely small spraying orifice is susceptible to clogging.[1] It is thus important that only the highest purity solvents are used. In this work, ultrapure water is used as the aqueous solvent in the nano-LC^n/MS^n analysis of neuropeptides, using several combinations of nanobore columns and emitters. By exploring representative results from column and emitter combinations, this presentation gives a proof of concept that ultrapure water from a highly efficient water purification system is suitable for nano-LC^n/MS^n work, and is free of contaminants that could potentially clog nanobore columns and emitters.

Results and Discussion

Plugging of nanobore columns and/or emitters translates to wasted time and resources for researchers. To avoid this, one has to choose a robust emitter and use the highest purity solvents and buffers. In this work, several nanobore columns and emitters were tested by using them continuously for 18 hours (> 100 inj.) at a flow rate of 0.90 µL/min. The results shown were obtained from a typical nanobore column-emitter configuration (Waters Atlantis® dC18 (75 µm x 100 mm, 3 µm) AND New Objective PicoTip SilicaTip (360 µm OD, 10 µm tip ID). All nanobore columns and emitters tested did not indicate plugging, as illustrated by mass chromatograms (Figure 1) and calibrations plots (Figure 2) that remained unchanged over the 18-hr test. The variety of configurations tested were able to deliver precise quantitative results (RSD's 5 %) for batches of hundreds of test solutions. Visual inspections of the emitters showed no signs of plugging (Figure 3).

1. No changes observed in mass chromatograms of neuropeptide biomarkers

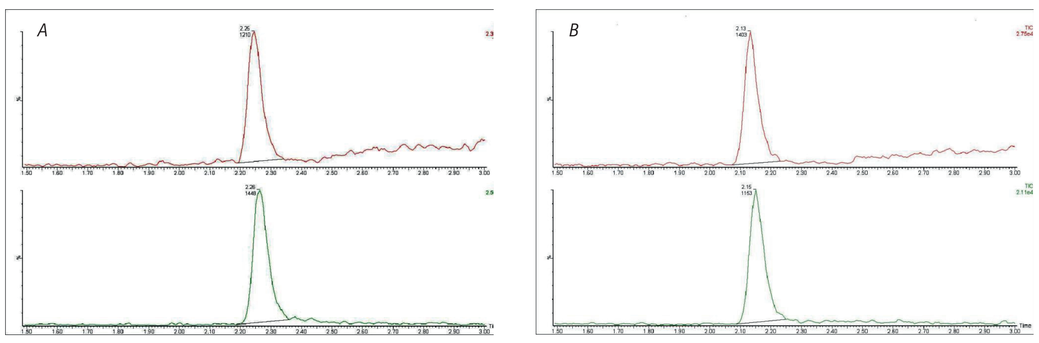

Figure 1. Mass chromatograms of LE (A) and ME (B) at 60 pg/mL concentrations. Red trace corresponds to injection #5, green trace is injection #111. There are no significant changes in peak area, t_R, and peak shape that indicates signal deterioration which could be caused by clogging of the nanobore column and/or emitter. The same observation is true for ANG (chromatogram not shown).

2. No significant differences in calibration plots

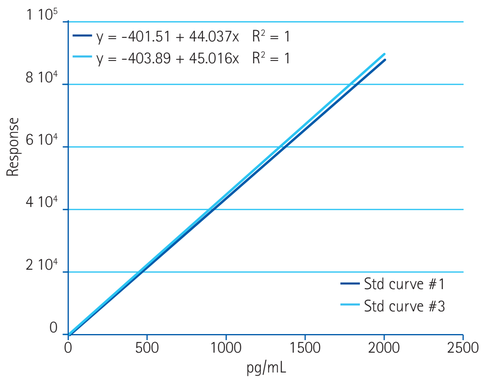

Figure 2. Standard calibration curves for LE. Standard curve #3 was obtained about 100 runs after standard curve #1 was obtained. The calibration curves remain unchanged with similar LLQs of 10 pg/mL for LE and ME and 150 pg/mL for ANG. Similar curves were obtained for ME and ANG.

3. No clogging of emitters observed

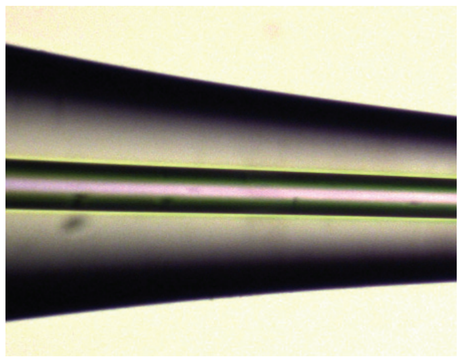

Figure 3. Photomicrograph of a used 0.10 µm tip ID emitter. No apparent clogging can be seen. Similarly, other used emitters did not exhibit clogging. The mobile phase was free of contaminants that would have clogged the emitters.

In another set of experiments (data not shown), a single Monospray emitter from GL sciences was used over a month long period in which various nanobore columns from different vendors were placed in line with the emitter. Towards the end of the month, the initial column used in the experiment was placed back in line to confirm the integrity of the emitter. The peak shapes and retention times were similar to the ones obtained at the beginning of the month-long experiment. The flow rate used in this work (1 µL/min) pushed the limits of the columns and emitters. Even at such high flow rates (for nano-scale work), the columns and emitters tested did not show signs of deterioration due to plugging when using water from a EMD Millipore water purification setup.

References

1. Lee, S.S.H., et. al. *Rapid Commun. Mass Spectrom.* 2005, 19, 2671-2680.
2. Koerner, T., et. al. *Anal. Chem.* 2004, 76, 6456-6460.
3. Corkery, L., Pang, H. J. *Am. Soc. Mass Spectrom.* 2005, 16, 363-369.
4. Kelly, R.T., et. al. *Anal. Chem.* 2006, 78, 7796-7801.

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Results and Discussion (Con't)

4. A highly efficient combination of technologies to produce ultrapure water

The high purity water used in this work was produced via a combination of purification technologies (Figure 4):

- Reverse osmosis (RO): Removes > 95 % of ions, organics, particulates, and bacteria.
- Electrodeionization (EDI): Removes remaining ions using ion exchange resins that are continuously regenerated by an electric current.
- High grade ion exchange resins and activated carbon: Further reduce the concentration of ions and organics.
- Dual wavelength UV lamp: Oxidizes organic contaminants, reducing the levels to 5 ppb.
- 0.22 µm final filter

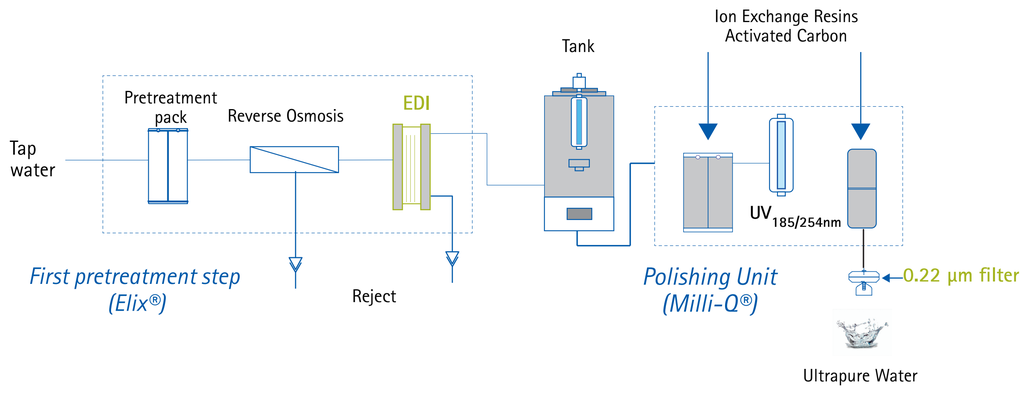

Figure 4. Water purification technology that produces ultrapure water suitable for nano-LC/MS.

Experimental

Instrumentation:

Waters Acquity UPLC (0.90 µL/min achieved by splitting the flow). Waters Quattro Premiere XE equipped with a nanospray source (SIR or MRM, ESI$^+$).

Analytical column and emitter combinations tested:

- Waters Atlantis® dC18 (75 µm x 100 mm, 3 µm) AND New Objective PicoTip SilicaTip (360 µm OD, 10 µm tip ID)
- Waters Atlantis® dC18 (75 µm x 100 mm, 3 µm) AND GL Sciences Monospray (360 µm OD, 10 µm tip ID)
- Waters Symmetry (75 µm x 100 mm, 3 µm) AND Water emitter (no taper tip, 90 µm OD, 20 µm tip ID)
- New Objective Picofrit packed with Inertsil C18 (75 µm x 50 mm, 3 µm)
- GL Sciences Monospray C18 (100 µm x 50 mm, monolith packing)

Mobile phase:

A : Ultrapure water containing 0.2 % Acetic Acid (Ultratrace, BDH) and 1 % Acetonitrile (UV grade, B&J),
B : 100 % Acetonitrile (UV grade, B&J)

Gradient table:

Time	% A
0.00	90.0
0.50	90.0
2.50	50.0
2.60	50.0
3.00	10.0

Time	% A
3.01	00.0
3.50	00.0
3.51	90.0
5.00	90.0

Neuropeptide biomarker standards:

Angiotensin (ANG), Leu-Enkephalin (LE), Met-Enkephalin (ME). Injection volume was 1 µL.

Injection sequence for results shown:

- Standard calibration curve 1
- 40 repeated injections of a standard solution
- Standard calibration curve 2
- 40 repeated injections of a standard solution
- Standard calibration curve 3

Optical microscopy:

Olympus BH-2, carried out by Mr. David Bell (Bioprocess Division, EMD Millipore Corp, Bedford, MA)

Conclusion

- Photomicrographs of used emitters did not reveal any clogging and reproducible data was obtained over numerous analyses involving the use of nanospray emitters and ultrapure water.
- These results demonstrated that ultrapure water from an efficient water purification system is suitable for nano-LC^n/MS^n work. It is free of contaminants that could cause plugging of the emitters and/or nanobore columns.
- Care must be taken not to introduce impurities from other sources and to this end high purity buffers and organic solvents similarly must be utilized to prevent clogging of the emitters and/or nanobore columns.

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany