Ultrapure water for biomedical LC analysis

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Introduction

- While detection limits are brought ever lower, results obtained at the trace level using hyphenated techniques rely on quality and purity of the reagents used to prepare mobile phases and buffers. Because of its wide utilization and because of the volumes used in sample preparation and liquid chromatography, water is particularly important and extreme care must be taken with its quality.
- Studies presented here focus on the impact of organic contamination on column lifetime and on trace analysis of peptide analysis.
- Denaturating HPLC performances were compared when various water qualities were used to study single nu-cleotide polymorphism (SNP). Results show a large increase of column lifetime when high purity water freshly produced by a water purification system was used. Results were related to the organic contamination of bottled water.
- Trypsic digests of BSA were analyzed using LC-ESI-MS-MS. High purity water was utilized and results highlight the importance of low organic contamination to reach low levels of detection.

Water purification technologies and monitoring

Technologies

- **Reverse Osmosis**: filtration technique that removes > 95 % of ions, organics, particulates and bacteria.
- **Electrodeionization**: Technique specifically dedicated to ion removal using ion exchange resins that are continuously regenerated by an electric current.
- High grade **Jetpore® ion exchange resins** to remove ions to the trace level
- **UV 254 nm for germicidal effect**
- Dual wavelength UV lamp (185 + 254 nm) for **photo-oxidation process**: addresses issues due to organic contaminants (TOC) by oxidizing organic molecules to CO_2. The hydroxyl radical generated by the UV radiation is the reactive intermediate.

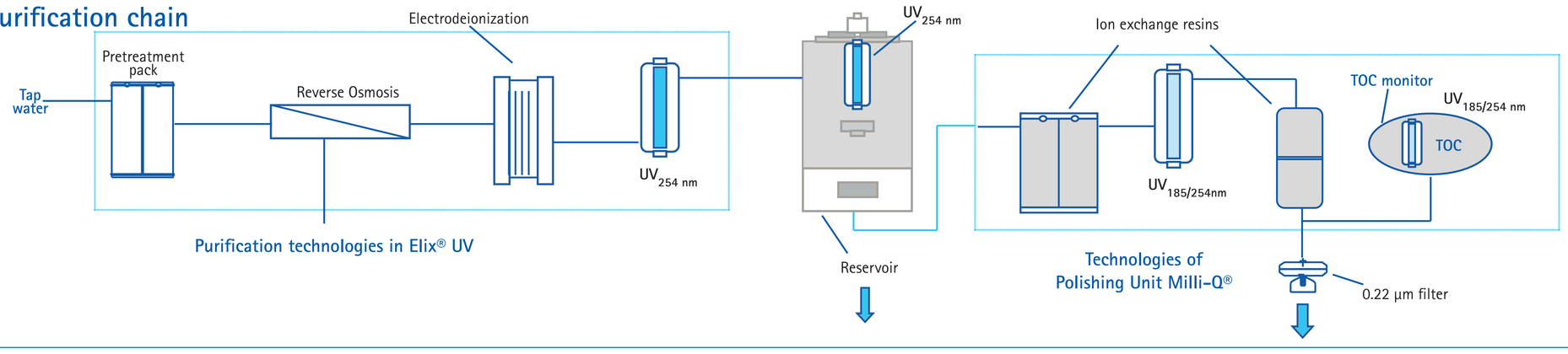
Monitoring

- on-line TOC analyzer. Monitoring is based on UV photo oxidation principle, followed by measurement of the resulting CO_2 formed during the oxidation process (4).
- co-axial resistivity cell designed to measure the overall ionic contamination.

Water quality parameters

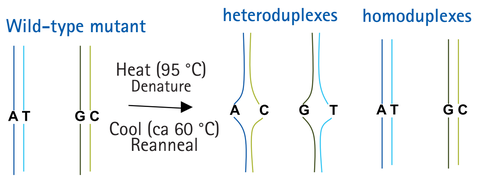
- Resistivity 18.2 MΩ.cm
- TOC < 5 ppb
- Bacteria < 1 cfu/mL

Water purification chain



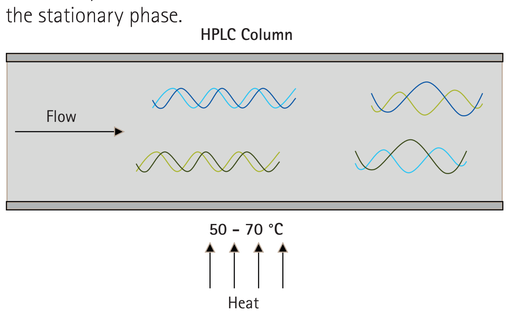
Single nucleotide analysis using denaturating HPLC

Denaturating HPLC is a reverse phase ion pairing HPLC method used to study single polymorphism in DNA molecules. This method resolves hetero duplex and homo duplex of DNA fragments (200 – 1000 base pairs) by differences in size and by differences in helical composition induced by temperature modulated denaturation (4).

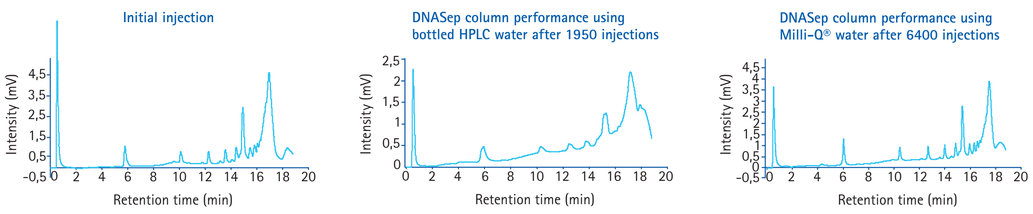


Hybridization of wild-type and mutant DNAs creates mixtures of DNA species corresponding to base pair variations.

A column temperature typically in the range of 50 °C to 70 °C is maintained to induce a partial denaturation. The thermally less stable heteroduplexes denaturate more extensively and, therefore, are retained a shorter time on the stationary phase.



Comparison of bottled HPLC water and Milli-Q® water



Ref: S. Mabic and I. Kano. Impact of purified water quality on molecular biology experiments. Clin. Chem. Lab. Med. 2003, 41 (4).

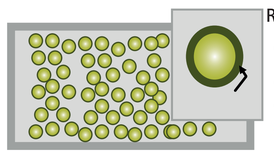
DNASep column	Water Source	Number of injections
1	Bottled HPLC	1235
2	Bottled HPLC	408
3	Bottled HPLC	2103
4	Bottled HPLC	2167
5	Bottled HPLC	555
6	Milli-Q®	6394
7	Milli-Q®	10695

Table 1 : Number of injections with various brands of bottled water and with MilliQ® water

Water Source	Organics as TOC (in ppb)
Brand A	100
Brand B	87
Brand C	777
Brand D	16
Brand E	32
MilliQ® Gradient	4

Table 2 : Organics in various water types

The column lifetime is correlated to organic contamination of the water used to prepare the mobile phase.



Organics foul ion exchange resin by blocking active sites and altering retention.

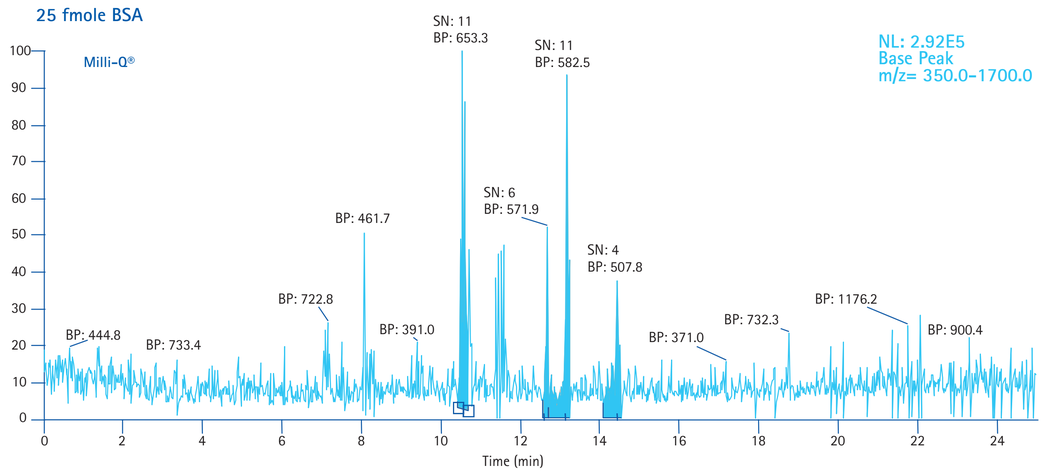
Material and methods

The eluent contains the cationic triethylammonium (TEA) ion (0.1 M) which interacts with the negatively charged phosphate groups on DNA and also with the hydrophobic surface of the particles in the column. The TEA ion can be described as a bridging molecule between DNA and the column. As the mobile phase is made progressively more organic, the DNA fragments are eluted in order of size. It is an "electrophoresis like" separation of DNA fragments.
LC System WAVE®, **Transgenomic, Omaha, NE** Fragment analysis system.
DNASep column
Temperature 50 – 60 °C
Buffer A (0.1 M TEA, Buffer B (0.1 M TEA + 25 % acetonitrile)
Flow 0.9 mL/min
Gradient 40 % to 72 % buffer B over 16 min.
Standard used: DNA standard ladder consisting of fragments varying by 100 bp from 100 bp to 1500 bp.

Analysis of trypsic digestion of BSA using LC-MS/MS

Conditions

Mobile phase A: water, acetonitrile, formic acid (98:2:0.1)
Mobile phase B: water, acetonitrile, formic acid (10:90:0.1)
Flow rate 1 mL/mn
Gradient [A 95%, B 5%] to [A 15%, B 85 %]
Nano LC equipment: Magic 2002 (Michron BioResources, Inc)
Column: Magic C18 (Michron BioResources Inc)
MS equipment: Ion trap MS/MS LCQ (ThermoQuest)
MS data search: Mascot (Matrix Science Ltd)



Amino acid sequence coverage of 25% by database search.

Comparison between bottled HPLC grade water and high purity water (Milli-Q® water) in terms of signal/noise ratio (S/N) of mass peaks (Table 3). The two types of water were used to prepare mobile phases and run the LC. Quantities of BSA analyzed were 25 fmoles in both cases.

m/z	Elution time	S/N Bottled water	S/N High purity water
653.5	10.5 min	8	11
572.0	12.6 min	2	6
582.5	13.3 min	4	11
507.8	14.5 min	2	4

Table 3

Better signal/noise ratio are obtained with high purity water, containing very low levels of organic contamination, TOC (Total Organic Carbon) < 5 ppb. The background contamination is reduced with ultrapure water.

Experimental results provided by Glaxo Smithkline

Conclusion

- It is important to use high purity water with a very low TOC level to prepare samples and run the LC.
- The level of organics is efficiently decreased by UV photo-oxidation, combined with activated carbon and ion exchange resins.
- Additionally, the use of an ultrapure water system with on line TOC (Total Organic Carbon) analysis is the best way to monitor these organic contaminants at the point-of-use.
- Validation and qualification also allow to certify the good quality of ultrapure water used in critical experiments.

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