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Ultrapure water for liquid chromatography–mass spectrometry studies

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Abstract

Improvements in trace enrichment techniques combined with the sensitivity of mass spectrometry offer enhanced opportunities to analyze ever lower concentrations of drugs, metabolites, pesticides or environmental pollutants. To perform HPLC and liquid chromatography–mass spectrometry (LC–MS) analyses under optimum conditions, the water used for mobile phase preparation needs to be highly purified and delivered on demand. Indeed, both UV photodiode array detection and MS detection methods are sensitive to organic contaminants (total organic carbon, TOC), and the water quality has a direct impact on the achievable detection limits. The benefits of UV photooxidation on TOC reduction for LC–MS studies were highlighted using electrospray ionization MS detection by comparing HPLC-grade bottled water, freshly produced $UV_{185/254}$-treated water, and freshly produced non-UV-treated water.

Keyword: Water

1. Introduction

Liquid chromatography (LC) has become routine equipment in a large variety of laboratories, including environmental, pharmaceutical, forensic, clinical and research facilities. Due to the needs of collecting increasing amounts and types of data on samples analyzed and on compounds detected or quantified, mass spectrometry (MS) has been added to photodiode array UV detection (DAD) in many types of applications [1–3]. Because analyses are performed at ever lower concentrations, performances of the LC–MS instruments and columns, as well as purity of the solvents occupy a prominent role in the analytical process. Indeed, to focus on searching for $pg\,l^{-1}$ concentrations of a drug metabolite in urine or on extracting environmental pollutants [4–6] in water, the best operational conditions have to be reached consistently. While attention is naturally drawn to achievable performances and limits of detection when an instrument is acquired or used for critical studies, the purity and the quality of the solvents, including water, are often under-estimated [7]. Water in LC analysis is used throughout the experiment, from eluent or buffer to sample preparation, e.g. involving SPE, and standard dilution, to column rinsing and blanks. It is therefore essential to use high purity grade water.

To produce high purity water, a combination of technologies [8] is utilized to remove major contaminants initially present in tap water. Ionic and organic contaminants are efficiently removed using processes such as reverse osmosis and electrodeionization in combination with high grade ion exchange resins, activated carbon and UV photo-oxidation process [9,10].

Data presented in this study highlight the importance of using freshly produced ultrapure water for HPLC and LC–MS, and show the effect of UV photo-oxidation on water quality. Special attention was brought to the effect of organic contaminants, referred to as total organic carbon (TOC). Three water qualities were analyzed and compared using DAD and MS detection: HPLC-grade glass bottled water (A); freshly produced water by an ultrapure water purification system including a UV photo-oxidation step (B); and freshly produced water with the same purification system with no UV photo-oxidation process (C).

2. Experimental

2.1. Solvents

Bottled HPLC-grade water used was Chromanorm water (Prolabo, VWR, Fontenay-sous-Bois, France), and HPLC-grade acetonitrile (J.T. Baker, Deventer, The Netherlands).

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2.2. Equipment

The LC–MS equipment used throughout the experiment was from Waters, Milford, MA, USA, including an LC Alliance 2695, and a photodiode array Model 2996 detector. Computer for data acquisition and system control was equipped with the Empower software.

The Milli-Q Gradient (Millipore, Billerica, MA, USA) water purification system was equipped with a built-in dual-wavelength (185 nm + 254 nm) low-pressure mercury UV lamp either turned on or off, with a built-in on-line TOC A10 monitor and with Q-Gard1 (Millipore) pretreatment and Quantum EX (Millipore) polisher cartridges. Resistivity of water delivered was 18.2 MΩ cm (measured in-line and compensated to 25 °C). The Milli-Q Gradient was fed by an Elix pretreatment water purification system, combining reverse osmosis and electrodeionization processes.

2.3. Column liquid chromatography

The column selected was an X-Terra MS (C_{18}, 50 mm × 2.1 mm, 2.5 μm). For the elution, a linear gradient of 100% water to 100% acetonitrile was applied in 30 min and the HPLC pump was delivering the gradient eluent at 0.25 ml min^{-1}. The solvents of elution were water freshly produced from a Milli-Q Gradient A10 system with UV on or off, bottled HPLC water, and acetonitrile.

2.4. Mass spectrometry

A single quadrupole Mass Detector ZQ (Waters) with the mass range set at *m*/*z* 150–600, the electrospray ionization (ESI) at 0.5 V and ionization temperature of 125 °C, was used, with a Dynolite-type photomuliplier at 650 V. Source and desolvation gas was nitrogen.

2.5. Sample preparation

Water samples were collected in glass bottles previously cleaned with permanganate at 90 °C and thoroughly washed out with ultrapure water. The waters were analyzed immediately after sampling in order to avoid external contamination. For type C water, 10 l of water were dispensed with UV lamp disconnected before taking the sample in order to regenerate the water in the recirculation loop. Trace-enrichment method (pre-concentration): volumes of 15 ml (Condition 1) or 45 ml (Condition 2) of each water type were concentrated on the top of the column with an equilibration mode at 100% water before starting the gradient.

3. Results

3.1. Photodiode array detection

HPLC with DAD detection of the three types of water was studied first. Chromatograms corresponding to types A (bottled HPLC) and B (UV-treated, freshly produced) waters were compared at 254 nm (Fig. 1). About 13 background peaks were detected for type A water and five peaks for type B water. Additional peaks in type A water were mostly earlier-eluting peaks, between 10 and 15 min, corresponding to more polar contaminants. Peak intensities and baseline absorbance were higher in type A water.

Types B (UV-treated, freshly produced) and C (non-UV-treated, freshly produced) water qualities were also

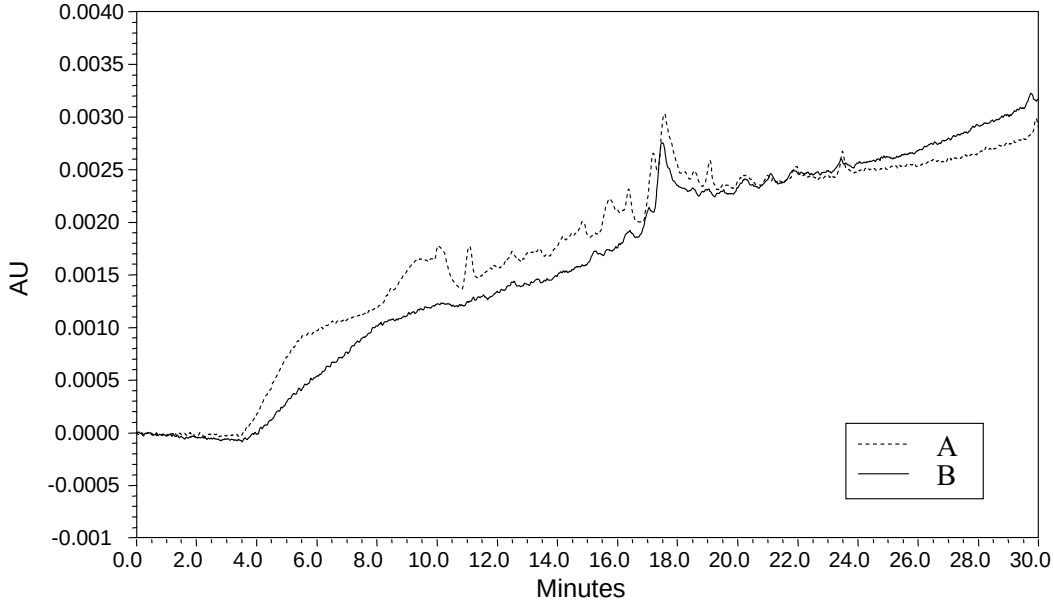

Fig. 1. Comparison of UV spectra at 254 nm of waters A (HPLC-grade bottled) and B (freshly produced UV-treated) (Condition 1).

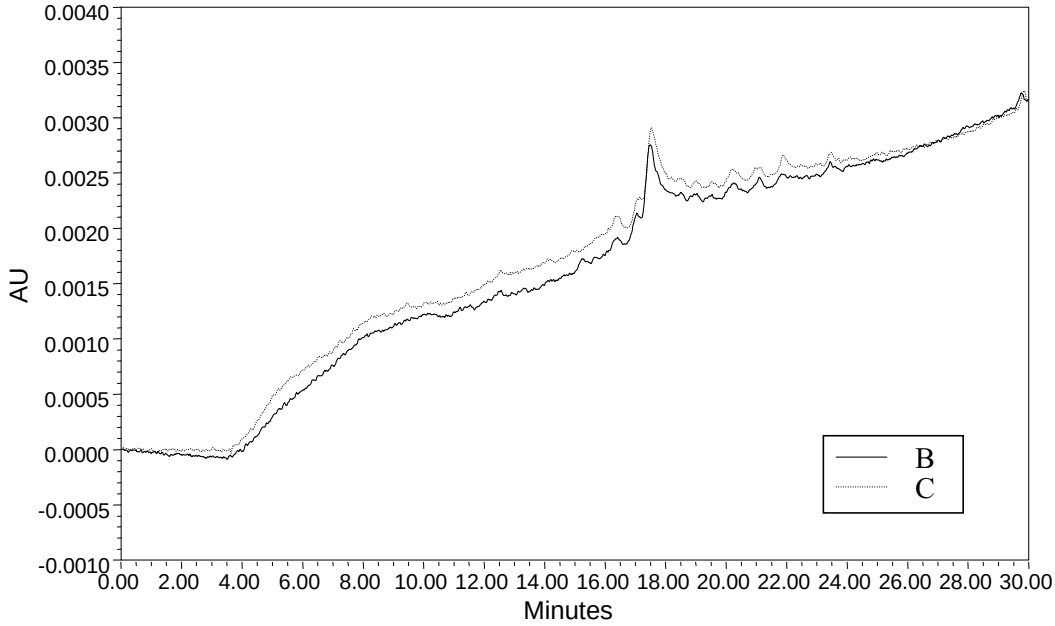

Fig. 2. Comparison of UV spectra at 254 nm of waters B (freshly produced UV-treated) and C (freshly produced non-UV-treated) (Condition 1).

compared at 254 nm (Fig. 2). The differences in the graphs between types B and C waters were not as obvious as with bottled HPLC water (Type A), but the general level of the baseline UV-absorbance was slightly higher for type C water.

To assess whether the peaks seen on chromatograms were coming from the water or from the system itself, samples of waters B and C were pre-concentrated on the column for different periods of time (1–3 h). All the peak heights increased proportionally to the volume loaded on the column, except for the peak eluting at 17.5 min, which intensity remained constant irrespective of the pre-concentration time (data not shown). This suggests that this contaminant was not water-born.

3.2. Mass spectrometry detection

The reconstructed total-ion-current chromatograms (RTICCs) of the three types of water were compared (Fig. 3). Type A water had clearly the highest baseline

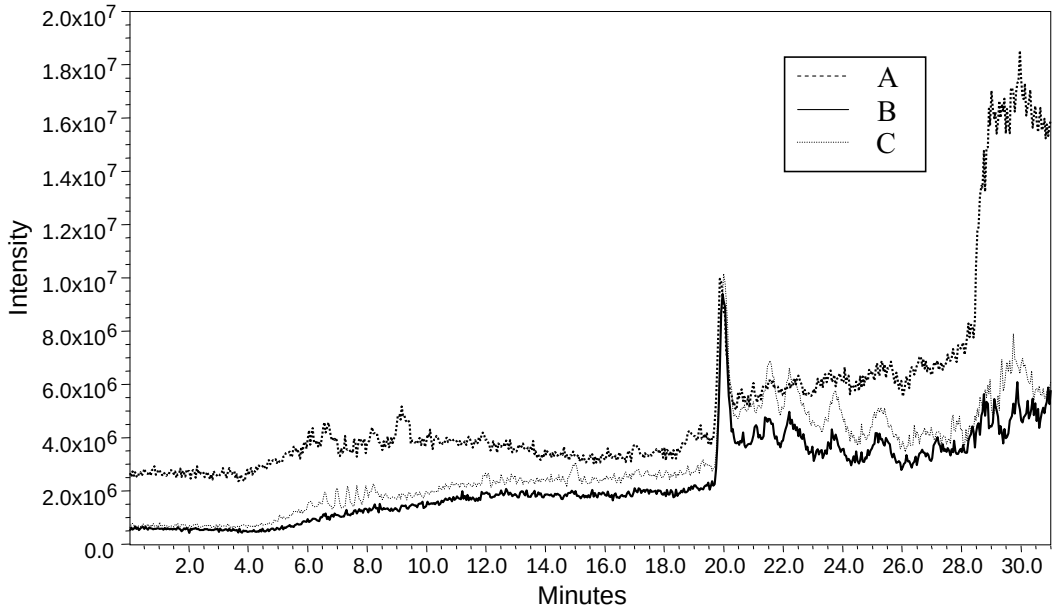

Fig. 3. Comparison of reconstructed total-ion current chromatograms of waters A (HPLC-grade bottled), B (freshly produced UV-treated) and C (freshly produced non-UV-treated) (Condition 1).

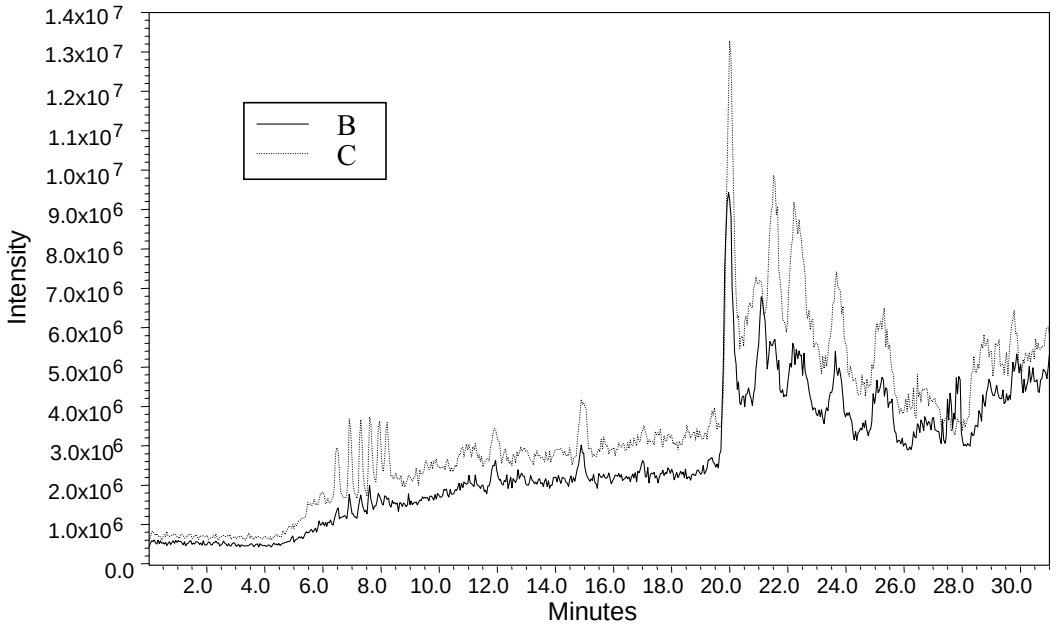

Fig. 4. Comparison of RTICCs of waters B (freshly produced UV-treated) and C (freshly produced non-UV-treated) (Condition 2).

intensity. Types B and C water showed less differences, although type C was above type B level of intensity. The TOC levels of types B and C waters measured on-line with a built-in TOC monitor were, respectively, 3 and 6 $\mu g\,l^{-1}$.

LC–MS analyses were then performed in Condition 2, where water was concentrated three times more on the top of the column (45 ml pre-concentrated volume). The RTICCs of types B and C waters were shown in Fig. 4. The levels of the peaks were much higher in intensity for the water purified without the UV photo-oxidation step (Type C). Mass spectra corresponding to both water types were obtained (Figs. 5 and 6, for types C and B water, respectively) after processing the RTICC at 14.99 min.

When the range of intensities of the mass spectra (Figs. 5 and 6) is considered, it appears that the maximum intensity was divided by a 6.7 ratio from 120 000 for type C water to 18 000 for type B water. This difference in background intensity is related to the deactivation of the UV lamp in the purification system.

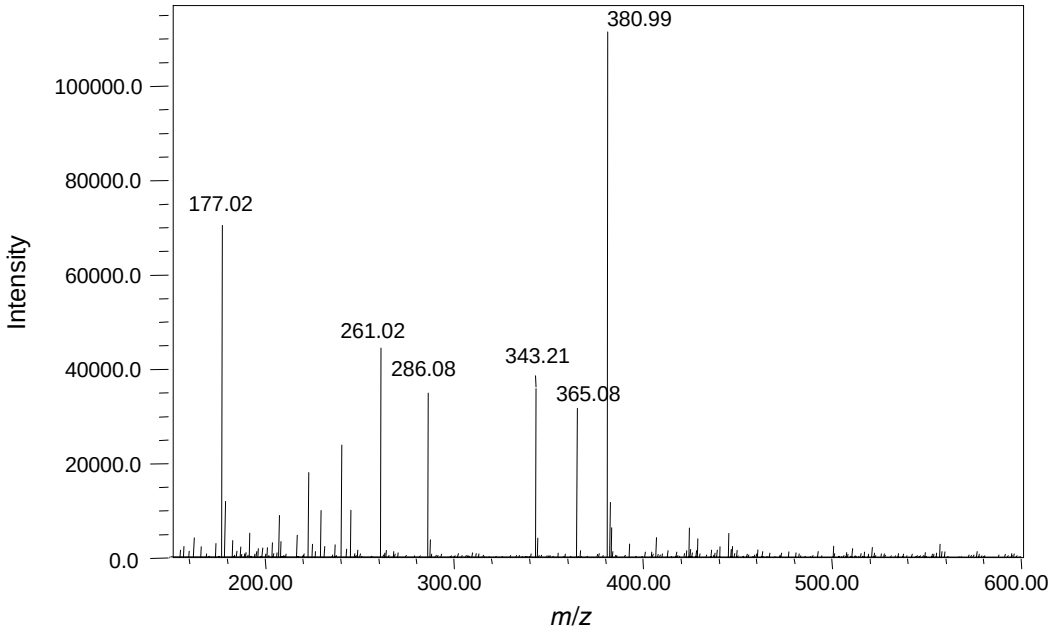

Fig. 5. Mass spectrum of water C (freshly produced non-UV-treated) at 14.99 min (Condition 2).

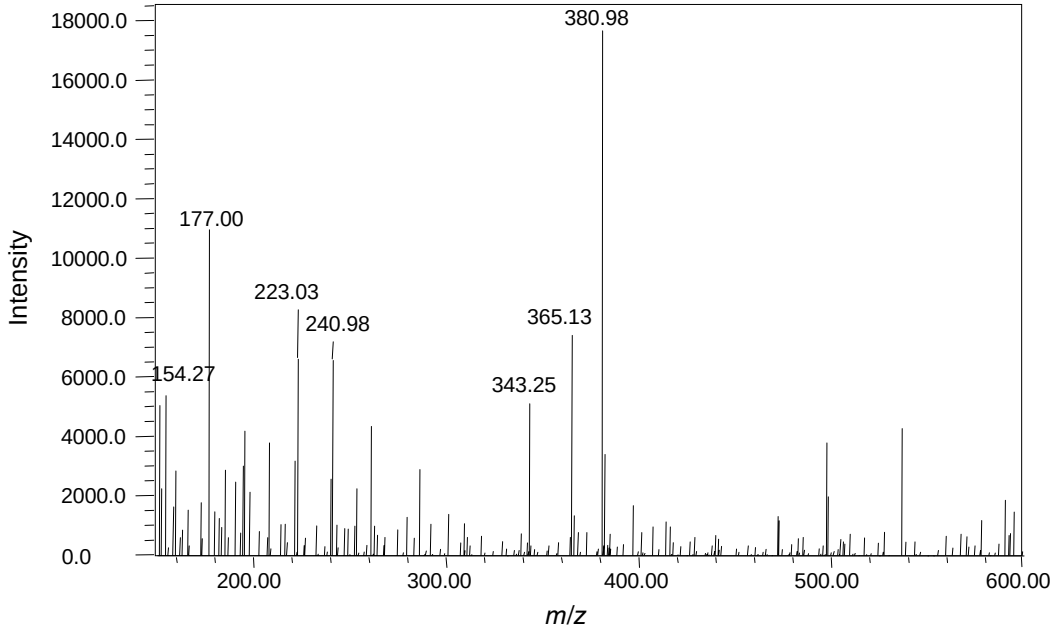

Fig. 6. Mass spectrum of water B (freshly produced UV-treated) at 14.99 min (Condition 2).

4. $UV_{185/254\,nm}$ photooxidation process

The UV photo-oxidation process in water purification systems uses a dual wavelength low pressure mercury UV lamp in quartz sleeves and irradiating at 185 and 254 nm [10]. The combination of both wavelengths induces the generation of hydroxyl radicals ($OH^{\bullet}$) from dissolved oxygen and water, through singlet oxygen and ozone [9]. This hydroxyl radical has a very high oxidation potential (2.80 V), and reacts with organic molecules to induce the breaking of covalent carbon–carbon bonds. Chain radical reactions, as well as the combination of intermediary radi-

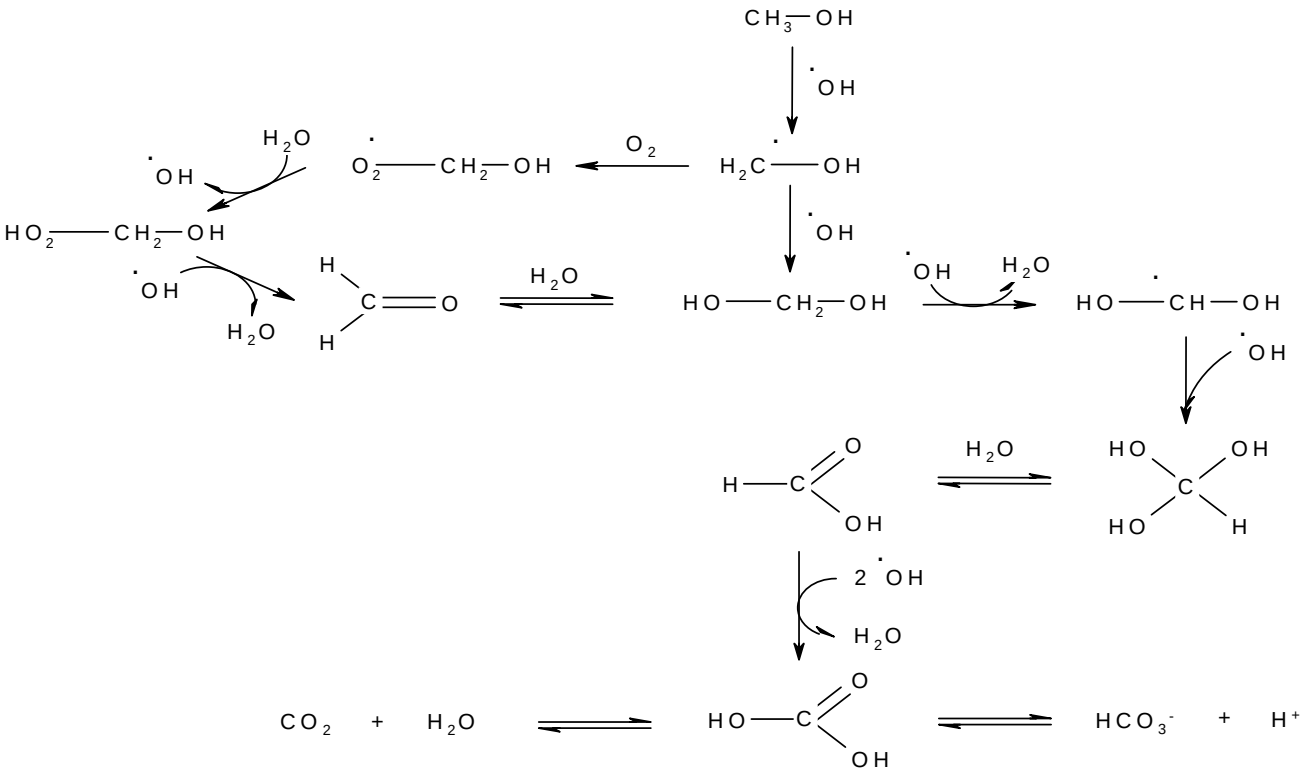

Fig. 7. Possible chemical pathway accounting for oxidation of methanol.

cals with water molecules, result in the generation of CO_2, the end product of organic molecules oxidation. Possible chemical reaction pathways are proposed to account for UV photo-oxidation of methanol (CH_3OH), chosen as an example of organic molecules (Fig. 7). The CO_2 formed reacts with water molecules to generate carbonic acid, which is in equilibrium with bicarbonate and carbonate ions. In high purity water, the pH is close to 7 [11], and the main species present is hydrogencarbonate (HCO_3^-). This ion is efficiently removed by ion exchange resins present in water purification systems. The TOC levels in ultrapure water purified using photo-oxidation technology is typically below $5\,\mu g\,l^{-1}$, when an adapted pretreatment system is installed.

5. Discussion

Purified water is one of the major components of LC eluents, and it can have a major impact on analyses, both because of the volumes used and because remaining contaminants can interact with the detection of analytes present as trace levels. Processes using pre-concentration steps prior to LC–MS analysis [12] are particularly sensitive to traces of organic contaminants originating from the water used to run the LC systems. It is particularly important, therefore, to use high purity grade water for studies involving sensitive assays as well as routine experiments.

In this study, three types of purified water underwent trace enrichment and were separated via LC prior to analysis using two different detection methods: DAD and MS. Different initial conditions of trace enrichment enabled to assess the effect of the UV photo-oxidation step installed in the purification system.

DAD enabled to show differences in the level of purity between all three water types. Bottled water clearly contained more contaminants, resulting in more peaks and higher background level (Fig. 1). These results were consistent with previous experiences and findings regarding water storage. Water quality, indeed, degrades on storage, even using good storage conditions [13]. Contamination is likely to originate from the handling of the bottled water, rather than from the glass itself.

Differences between freshly produced type B (UV-treated) and type C (non-UV-treated) waters were more subtle. Although type C water seemed to be giving a little more background absorbance, the number and the height of peaks remained in the same range.

Results obtained with ESI-MS confirmed that a good quality of water was all the more important when lower detection levels were achieved. Impact of contaminants coming from the mobile phase becomes more prominent, and as could be anticipated, the RTICCs showed more differences between the three water qualities than DAD. Background intensity of type A (bottled HPLC) water chromatogram was about three times higher (Fig. 3) than the backgrounds of freshly produced waters. It becomes harder in such conditions to detect very low concentrated analytes in the baseline and to process the data to find an exploitable mass spectrum. Using purer water in the mobile phase allows to see many more peaks on the baseline, and to reach lower detection limits.

While little differences were observed between types B and C water using DAD, the effect of the presence or absence of UV lamp was more significant with mass spectrometry detection. Additional polar molecules were detected in water purified without UV photo-oxidation (Fig. 4) and the background intensity was always greater. These trace level of organics are likely to originate from the water purification system itself. Example of known molecules leaching from plastics [14] include antioxidant plastic additives in polyethylene tubing such as the 2,6-di-*tert*-butyl-1,4-benzoquinone or dialkyl phthalate esters. A UV photo-oxidation step within an ultrapure water purification system reduces contamination due to organic molecules, which enables obtaining lower baselines and increases the S/N. Quantification becomes more accurate and easier to achieve.

Establishing a correlation between the level of TOC monitored on-line and the effect on LC-ESI-MS is really interesting. Indeed the difference of TOC for the two freshly produced water types (B, $3\,\mu g\,l^{-1}$, and C, $6\,\mu g\,l^{-1}$) was of $3\,\mu g\,l^{-1}$ only. Even such a small difference in TOC can make a big difference when studying a mass spectrum. A ratio of 6.7 was observed when the peak at retention time 14.9 min was analyzed. Hence, UV photo-oxidation process, as well as the monitoring of the TOC, seem all the more important to incorporate in water purification systems dedicated to produce water for HPLC and LC–MS. Monitoring the TOC ensures that there is no deviation in the organic quality of the water and ensures to always work in optimum operational conditions [15,16]. Furthermore, it is adamant that researchers should check their water sources routinely by running full-procedure blanks. The UV photo-oxidation purification step is not only important for mass analysis, it is also interesting to eliminate organic contaminant that pollute the columns by creating a column blocking, which results in high back-pressure and variations in retention times. Using high purity water suitable to LC–MS water increases the lifetime of columns [17].

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