2016

A rapid and reliable technique for N-nitrosodimethylamine analysis in reclaimed water by HPLC-photochemical reaction-chemiluminescence

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Publication Details

Fujioka, T., Takeuchi, H., Tanaka, H., Nghiem, L. D., Ishida, K. P. & Kodamatani, H. (2016). A rapid and reliable technique for N-nitrosodimethylamine analysis in reclaimed water by HPLC-photochemical reaction-chemiluminescence. Chemosphere, 161 104-111.

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Keywords
nitrosodimethylamine, analysis, reclaimed, water, rapid, hplc, reliable, photochemical, reaction, chemiluminescence, technique, n

Disciplines
Engineering | Science and Technology Studies

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Revised manuscript submitted to

Chemosphere

June 2016

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Abstract

A fast and reliable analytical technique was evaluated and validated for determination of *N*-nitrosodimethylamine (NDMA) formation and rejection by reverse osmosis (RO) membranes in potable water reuse applications. The analytical instrument used in this study is high-performance liquid chromatography (HPLC), photochemical reaction (PR) and chemiluminescence (CL) – namely HPLC-PR-CL. Results reported here show that HPLC-PR-CL can be used to measure NDMA with a similar level of accuracy compared to conventional and more time-consuming techniques using gas chromatography and tandem mass spectrometry detection in combination with solid phase extraction. Among key residual chemicals (i.e. monochloramine, hydrogen peroxide and hypochlorite) in reclaimed wastewater, hypochlorite was the only constituent that interfered with the determination of NDMA by HPLC-PR-CL. However, hypochlorite interference was eliminated by adding ascorbic acid as a reducing agent. Direct injection of ultrafiltration (UF)-treated wastewater samples into HPLC-PR-CL also resulted in an underestimation of the NDMA concentration possibly due to interference by organic substances in the UF-treated wastewater. Accurate determination of NDMA concentrations in UF-treated wastewater was achieved by reducing the sample injection volume from 200 to 20 µL, though this increased the method detection limit from 0.2 to 2 ng/L. In contrast, no interference was observed with RO permeate. These results suggest that RO membranes could remove part of substances that interfere with the NDMA analysis by HPLC-PR-CL. In addition, RO treatment experiments demonstrated that HPLC-PR-CL was capable of evaluating near real-time variation in NDMA rejection by RO.

Keywords: chemiluminescence; *N*-nitrosodimethylamine (NDMA); *N*-nitrosamines; potable water reuse; real-time analysis; reverse osmosis.

1. Introduction

Potable water reuse has become increasingly important in countries and regions where fresh water sources are limited due to prolonged drought and rapid urbanisation (Shannon et al., 2008; Burgess et al., 2015; Lafforgue and Lenouvel, 2015). Potable water reuse refers to the use of reclaimed wastewater as a source of drinking water. A major challenge to implementing potable water reuse is the ubiquitous occurrence of trace organic chemicals (TrOCs) in reclaimed water that could pose a potential threat to public health (Lampard et al., 2010; Debroux et al., 2012; Linge et al., 2012; Scott et al., 2014). These TrOCs include pharmaceuticals, pesticides, endocrine disrupting compounds, and disinfection by-products (Luo et al., 2014).

In many potable water reuse schemes, reverse osmosis (RO) is used specifically for the removal of salts and TrOCs (Drewes and Khan, 2011). Nevertheless, some small and neutral TrOCs can permeate through RO membranes. A notable example is *N*-nitrosodimethlyamine (NDMA, $C_2H_6N_2O$) – which is a probable human carcinogen (USEPA, 1993). NDMA has a small molecular size (molecular weight of 74 g/mol) and is uncharged in aqueous solution (Fujioka et al., 2012). Detection of NDMA in RO permeate at concentrations higher than the California regulatory notification level (NL) of 10 ng/L (CDPH, 2015) has been frequently reported in the literature (Plumlee et al., 2008; Poussade et al., 2009; Farré et al., 2011; Fujioka et al., 2013b). Thus, an ultraviolet (UV) photolytic process or UV-advanced oxidation process (Stefan and Bolton, 2002; Sharpless and Linden, 2003; Lee et al., 2005) are routinely used to further reduce NDMA concentration to below the regulated value for potable reuse (Plumlee et al., 2008; Poussade et al., 2009). Given the need for NDMA monitoring for water quality compliance and process optimisation, there have been many

efforts to develop fast, reliable, and cost effective analytical techniques for determining the NDMA concentration in reclaimed water.

The most common analytical technique used for the determination of NDMA concentrations in aqueous samples is gas chromatography and tandem mass spectrometry detection (GC-MS/MS) preceded by solid phase extraction (SPE) for sample concentration (Munch and Bassett, 2004). A combination of SPE followed by high-performance liquid chromatography (HPLC) separation and tandem mass spectrometry (MS/MS) detection can also be used for NDMA analysis (Plumlee et al., 2008). Both SPE-GC-MS/MS and SPE-HPLC-MS/MS allow for the determination of NDMA in water at part-per-trillion (ng/L) levels but require a large volume (e.g. 200–1000 mL) to make very concentrated extracts (e.g. >1,000 fold) through SPE (McDonald et al., 2012). Moreover, the addition of isotope-labelled NDMA into each sample as a surrogate is necessary to compensate for losses of NDMA that occur during sample preparation (i.e. SPE and evaporation). As a result, NDMA analysis by either SPE-GC-MS/MS or SPE-HPLC-MS/MS is labour intensive, expensive and can take several hours. To overcome issues associated with sample preparation, Kodamatani et al. (2009) has developed a photochemical reaction (PR) - chemiluminescence (CL) method to determine NDMA concentration in drinking water. This innovative method involves direct injection of a small volume of aqueous sample (200 μL) followed by HPLC separation and PR-CL quantification. Briefly, in this HPLC-PR-CL method, the sample first undergoes chromatographic separation, followed by the photolysis of NDMA to form peroxynitrite and then quantification by chemiluminescence.

The reclaimed water matrix is more complex than that of drinking water. Wastewater-derived organic compounds may persist in the reclaimed water, depending on the level of treatment. Additionally, for disinfection and oxidation purposes, chemicals such as chloramine, hydrogen peroxide and hypochlorite are often added to reclaimed water and may remain in

the RO feed, RO permeate, and the final product water at concentrations in the range of several mg/L. These chemicals can potentially interfere with the photolytic process, hindering NDMA analysis by HPLC-PR-CL. Thus, evaluating and eliminating these potential interferences is essential to successful application of the innovative HPLC-PR-CL analytical method for NDMA monitoring of reclaimed water.

The benefits of adapting the fast and simple HPLC-PR-CL method for NDMA analysis in potable water reuse applications are significant in long-term plant operation. For example, it could be used to identify the cause(s) of variation in NDMA rejection by RO that occur during long-term system operation (Bellona et al., 2008; Fujioka et al., 2013b). Unlike the conventional SPE-GC-MS/MS technique, HPLC-PR-CL does not require a complex and time consuming sample preparation step. Thus, real-time analysis for process monitoring and optimisation at full-scale level is potentially possible, and increasingly sought after as the industry moves towards direct potable reuse. In addition, such a small sample volume requirement in HPLC-PR-CL (i.e. 200 µL) will allow for detailed investigations of the fate of NDMA including NDMA formation and removal during water reclamation at laboratory scale. In fact, the limitation of providing a large number of samples for SPE-GC-MS/MS analysis significantly limited the number of sampling occasions in a previous laboratory-scale fouling study (Fujioka et al., 2013a).

This study aimed to establish an HPLC-PR-CL analytical method that is fast and reliable for NDMA analysis during potable water reuse. The interference of common oxidants including monochloramine, hydrogen peroxide and hypochlorite was systematically evaluated. Countermeasures to eliminate the interference from these chemicals and organic substances in reclaimed water were developed. Through NDMA formation studies, NDMA concentrations determined using HPLC-PR-CL were validated against values obtained from

the conventional SPE-GC-MS/MS technique. Investigations of changes in NDMA rejection during RO fouling events were also performed.

2. Materials and methods

2.1. Chemicals

Analytical-grade NDMA solution with concentration of 100 mg/L was purchased from Ultra Scientific (Kingstown, RI, USA) and used as the standard for the HPLC-PR-CL method. An NDMA stock solution was prepared at 1 mg/L in pure methanol. Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) from Wako Pure Chemical Industries (Tokyo, Japan) was used for HPLC-PR-CL. A luminol stock solution was prepared at 20 mM in a 0.5 M carbonate buffer. Hydrogen peroxide, sodium hypochlorite (to represent hypochlorite), ammonium chloride and sodium hydroxide were of analytical grade (Wako Pure Chemical Industries, Tokyo, Japan). These four chemicals were used to evaluate their influence on HPLC-PR-CL. Ascorbic acid and sodium thiosulfate (Wako Pure Chemical Industries, Tokyo, Japan) were used to quench chloramine in treated wastewater. Treated wastewater was collected from the permeate stream of a pilot-scale UF system housed at a municipal wastewater treatment plant (WWTP) in Japan, where the secondary effluent is fed to the UF system. The wastewater treatment consisted of screen, sedimentation and bioreactor processes. The pilot-scale UF system was equipped with one HFU-2020 membrane module (Toray Industries, Inc., Tokyo, Japan) with a nominal pore size of 0.01 µm.

2.2. RO treatment system

Low pressure RO membranes – namely ESPA2 and ESPAB – were supplied by Nitto/Hydranautics (Osaka, Japan). These are thin-film composite RO membranes with an active skin polyamide layer on top of a microporous polysulfone supporting layer, which was

further supported by a polyester backing layer. The ESPA2 membrane is commonly used in water recycling applications (Fujioka et al., 2012), while the EPSAB has been used in the second stage of seawater desalination applications for boron removal (Tu et al., 2010).

The laboratory-scale RO treatment system used in this investigation was comprised of a reservoir, a feed pump (KP-12, FLOM, Tokyo, Japan) and stainless steel membrane cell (Iwai Pharma Tech, Tokyo, Japan) (**Supplementary Material, Figure S1**). The stainless steel membrane cell can hold a circular flat sheet membrane coupon with effective surface area of 36.3 mm^2. The feed solution temperature was controlled in the feed reservoir via a stainless steel heat exchanging coil connected to a temperature control unit (NCB-500, Tokyo Rikakikai, Tokyo, Japan).

2.3. Analytical techniques

2.3.1. HPLC-PR-CL

The HPLC-PR-CL technique that was first reported by Kodamatani et al. (2009) for determining N-nitrosamine concentrations in drinking water was further developed and adapted for this study. In this method, peroxynitrite ($ONOO^-$) forms through photochemical reaction of NDMA with UV irradiation. $ONOO^-$ then reacts with luminol and induces strong chemiluminescence. Each sample in this method underwent three steps: (a) the separation of NDMA with an octadecylsilyl (ODS) column as part of HPLC, (b) photolysis of NDMA with UV light irradiation to form peroxynitrite, and (c) chemiluminescence detection (**Figure 1**). The system was comprised of a DGU-$20A_3$ degasser (Shimadzu), a SIL-20AC autosampler, a CTO-20AC column oven (40 °C), a coupled Capcell Pak C_{18} MGII column (5 µm, 4.6 mm i.d., 250 mm + 100 mm length, Shiseido, Tokyo, Japan), a CL-2027 chemiluminescence detector (JASCO, Tokyo, Japan), a Chromato-PRO data processor (Runtime Instruments, Kanagawa, Japan), and a homemade photochemical reactor consisting of a low-pressure

mercury lamp (15 W, CL-15, National, Tokyo, Japan). Eluent solution comprised of 10 mM phosphate buffer (pH 7) with 1% methanol was fed to the instrument at 1.5 mL/min flow rate (**Figure 1**). A specific sample volume (200 µL) was injected into the system unless otherwise stated. Thereafter, a pH conditioning eluent containing 40 mM Na_3PO_4 was introduced at 0.5 mL/min. Following the photochemical reactor, a 0.05 mM luminol solution in 0.5 M carbonate buffer (pH 10) was added at 0.5 mL/min.

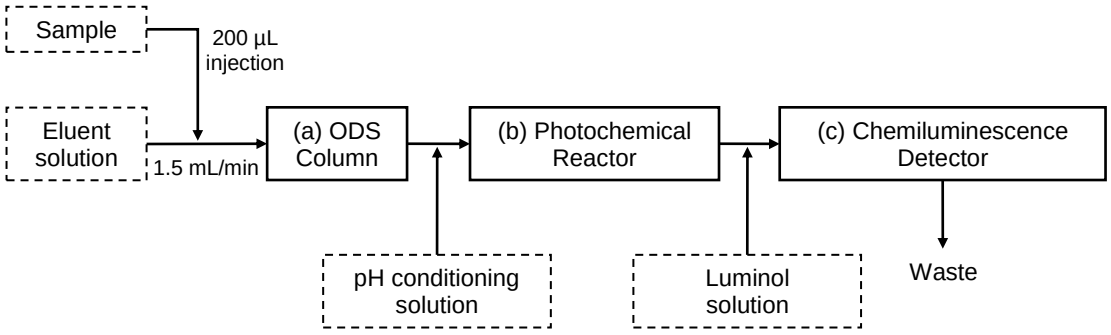

Figure 1: Schematic diagram of the automated HPLC-PR-CL system.

A chromatogram of NDMA-containing samples using HPLC-PR-CL is presented in **Figure 2**. For the first 2.5 min prior to the sample reaching the detector, chemiluminescence occurs as a result of reaction between luminol and active oxygen species. These active oxygen species are assumed to be generated from eluent components (e.g. methanol, water, and dissolved oxygen gas) (Kodamatani et al., 2009). The chemiluminescence continues to occur even after the chemiluminescence of the injected sample takes effect. Thus, the peak levels observed at 0–2.5 min can be considered as the baseline of the HPLC-PR-CL method. NDMA concentrations were determined based on the peak height at 8.1 min. The method detection limit (MDL) was calculated using the U.S. Environmental Protection Agency Method Detection Limit procedure found in Title 40 Code of Federal Regulations Part 136 (40CFR 136, Appendix B, revision 1.11).

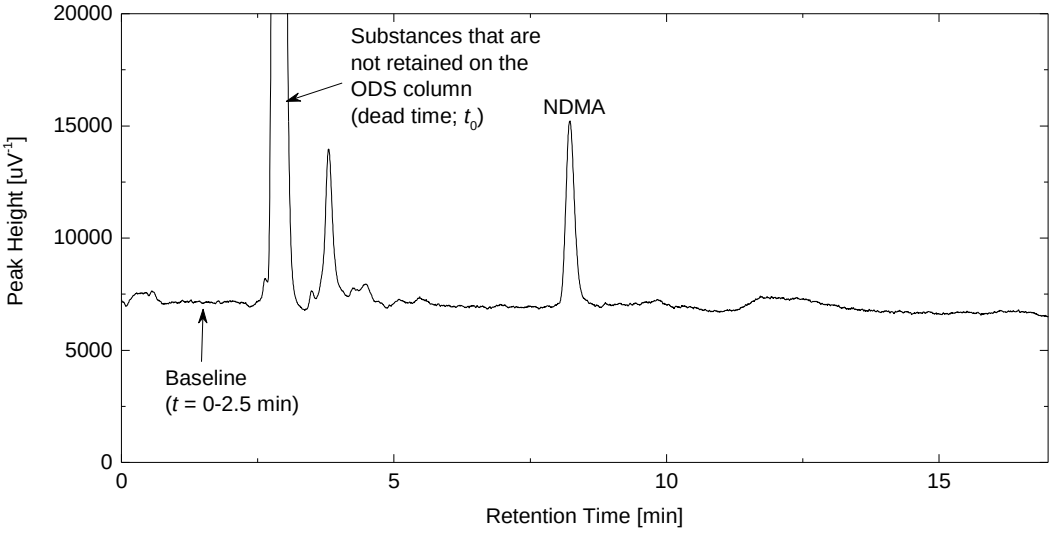

Figure 2: Representative HPLC-PR-CL chromatogram of a pure water sample containing NDMA (10 ng/L, injection volume 200 μL).

2.3.2. SPE-GCMS/MS

NDMA concentrations were also determined using SPE-GC-MS/MS. Full details of the SPE-GC-MS/MS method used in this study were previously reported (Yoon et al., 2013); thus, a brief description of this method has been provided below and in **Supplementary Material, Figure S2**. An analytical-grade NDMA (EPA8270, Supelco, Bellefonte, PA, USA) solution (2,000 μg/mL) was prepared in methanol. Deuterated NDMA (*N*-nitrosodimethylamine-d_6) was obtained from CDN Isotopes (Pointe-Claire, PQ, Canada) and deuterated toluene (toluene-d_8) was purchased from Supelco (Bellefonte, PA, USA) for SPE-GC-MS/MS. Individual stock solutions of the standards were prepared at 1 mg/L in dichloromethane (Wako Pure Chemical Industries, Tokyo, Japan) and stored at -20 °C. A surrogate stock solution containing 1 mg/L of NDMA-d_6 was prepared in methanol (Wako Pure Chemical Industries, Tokyo, Japan) and stored at -20 °C in the dark.

Prior to SPE, the surrogate stock solution was added to each sample (200 mL) to make up NDMA-d_6 concentration of 100 ng/L. The addition of the isotope accounts for analytical variability that occurs during sample preparation and extraction, allowing for accurate quantification of NDMA concentrations. Each sample was cleaned using a Sep-Pak NH2 cartridge (Waters, MA, USA) followed by SPE with a Sep-Pak AC-2 cartridge (Waters, MA, USA) at 10 mL/min flow rate for NDMA extraction. NDMA trapped in each cartridge was eluted with 2 mL of pure dichloromethane and the eluate was concentrated prior to instrumental analysis. To correct for fluctuations in the GC-MS/MS apparatus, toluene-d_8 was added as an internal standard just before injection of the samples into the system. NDMA concentrations were quantified using a Varian 450 gas chromatograph coupled with a Varian 300 triple quadrupole mass spectrometer (Varian, Tokyo, Japan). Only samples with NDMA-d_6 recovery of 70–120% were considered valid, and these NDMA concentrations were averaged for each specific condition.

2.4. Experimental protocol

2.4.1. Effects of chemicals on HPLC-PR-CL analysis

Solutions containing monochloramine, hydrogen peroxide, and hypochlorite were prepared at a concentration of 0.1–10 mM. Monochloramine solution was prepared by dosing 0.12 M sodium hypochlorite into 0.1 M NH_4Cl (pH 8), and the concentration was adjusted to 0.1 mM (as equivalent chlorine). Hypochlorite solution was prepared at 1 mM (as equivalent chlorine). In each monochloramine, hydrogen peroxide, and hypochlorite solution, NDMA stock solution was dosed to achieve a concentration of 10 ng/L. Each chemical solution was then analysed by HPLC-PR-CL.

2.4.2. NDMA formation

NDMA formation tests using the UF-treated wastewater were performed with chloramination times up to 6 h. The experimental procedures fundamentally follow previous studies focusing on NDMA formation potential (Mitch and Sedlak, 2002; Mitch et al., 2003; Yoon et al., 2011). The UF-treated wastewater was pre-conditioned with 10 mM phosphate buffer and the pH was adjusted to 6.9. Chloramination was then performed in a 1.0 L amber glass bottle by adding 100 mL of the 20 mM chloramine (as equivalent chlorine) stock solution into 900 mL of the conditioned UF-treated wastewater for an initial concentration of 2 mM monochloramine (as equivalent chlorine). Each sample was kept in the dark at 25 °C with vigorous shaking using a mechanical shaker (Shaker NR-80, TAITEC, Saitama, Japan) for specified chloramination durations (i.e. 1, 2, 3, and 6 h). The residual chloramine concentration was measured using Hypochlorite Test Kits (HACH, CO, USA) at the end of the NDMA formation test. Sodium thiosulfate solution (up to 4 mM) was added to the reaction bottle to quench residual chloramine.

2.4.3. RO treatment

Prior to each experiment the RO membrane was conditioned with Milli-Q water at 1,500 kPa until the permeate flux stabilised, and then replaced with UF-treated wastewater spiked with 80–90 ng/L NDMA. The RO treatment system was operated at constant flux at 60 L/m^{2}h. Although full-scale water recycling plants typically adapt permeate flux of about 20 L/m^{2}h (Fujioka et al., 2012), 60 L/m^{2}h was selected here to accelerate membrane fouling for subsequent NDMA rejection examination. Throughout the operation of the RO system changes in transmembrane pressure (TMP) were monitored and recorded as the membrane fouled. Feed temperature was maintained at 20 °C. Sampling of the RO feed and permeate

was conducted periodically throughout the experiment. The RO treatment experiments were terminated after 8 h.

3. Results and discussion

3.1. Effects of chemicals on HPLC-PR-CL analysis

In the presence of 1 mM NaOCl, a strong peak appeared at a retention time (*rt*) of 8.3 min which was very close to the peak of NDMA (*rt* = 8.1 min), making the determination of NDMA concentration impossible (**Figure 3**). Based on the early retention time, the unidentified constituent/substance is assumed to be more hydrophilic than NDMA. Hypochlorite is an oxidant that reacts with luminol and could interfere with chemiluminesence-based HPLC-PR-CL analysis. Identification of the source of the strong peak at 8.3 min was beyond the scope of this study but will be a subject of future studies.

To eliminate the influence of hypochlorite, a reducing agent – ascorbic acid – was added to each sample to quench hypochlorite prior to the analysis (**Figure 3**). This resulted in a single peak of NDMA allowing for quantification. It is noted that the addition of ascorbic acid does not influence the chromatogram of NDMA (Supplementary Material Figure S3) and a negative peak at 2.5–4.0 min in **Figure 3** was due to the presence of ascorbic acid, as demonstrated in **Supplementary Material Figure S4**. The results reported here indicate that the quenching step (e.g. addition of ascorbic acid) eliminates the impact of hypochlorite and enables NDMA to be measured at the ng/L level in hypochlorite-containing samples. It should be noted that quenching residual chlorine is typically performed for NDMA analysis as a standard protocol (Plumlee et al., 2008; Farré et al., 2011).

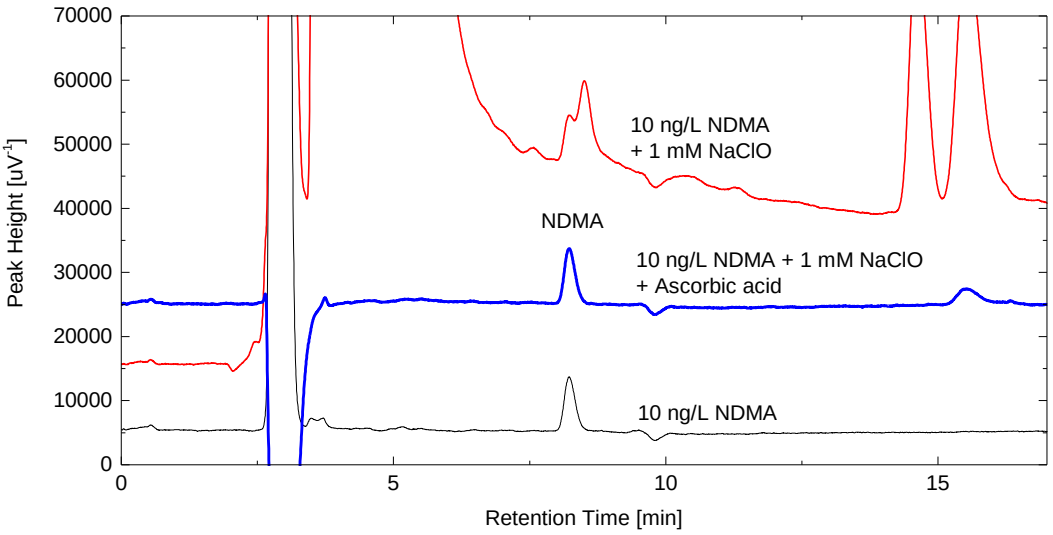

Figure 3: Effect of hypochlorite on the determination of NDMA concentration in a pure water by HPLC-PR-CL (NDMA concentration = 10 ng/L, Sodium hypochlorite concentration = 1 mM (as equivalent chlorine)). Ascorbic acid was dosed at 10 mM prior to the analysis.

Unlike hypochlorite, the presence of monochloramine and hydrogen peroxide in water samples did not have an impact on the determination of NDMA concentrations. These results are summarised in **Supplementary Material Figures S5**. Water samples with 0.1 mM monochloramine did not interfere with NDMA peak (*rt* = 8.1 min). Similarly, samples containing 1 mM hydrogen peroxide did not interfere with NDMA peak, allowing for the determination of NDMA at 10 ng/L. These results indicate that HPLC-PR-CL does not require any pretreatment for the analysis of water samples containing monochloramine and hydrogen peroxide up to 0.1 mM and 1 mM, respectively.

3.2. Analysis in treated wastewater

3.2.1. HPLC-PR-CL with UF-treated wastewater

NDMA analysis using HPLC-PR-CL was performed on the UF-treated wastewater to evaluate the influence of organic substances from treated wastewater. NDMA was spiked into

the UF-treated wastewater at a concentration of 50 ng/L, and the influence of substances in the UF-treated wastewater was evaluated through a recovery of the spiked NDMA dose. As a result, the peak height of NDMA at 8.1 min was 84% recovery relative to the pure water matrix when the standard sample volume (i.e. 200 µL) of the UF-treated wastewater was injected into the HPLC-PR-CL system **(Figure 4a)**. Investigations into the cause of the suppressed peak revealed that when the injected sample reached the detector ($rt \geq 2.5$ min), the chemiluminescence baseline intensity dropped below that of the initial baseline intensity (rt = 0–2.5 min). The reduction in the peak height after 3 min as compared to the initial baseline could be due to the substances in the UF-treated wastewater. These substances may block sample exposure to UV or supress the chemiluminescence by consuming active oxygen species. Identification of the substances was beyond the scope of this study.

To reduce the interference of the overall chemiluminescence peak height and minimise the drop in the baseline after 3 min relative to the initial baseline intensity (rt = 0–2.5 min), the sample injection volume was reduced from 200 to 20 µL. As predicted, the chemiluminescence intensity around the NDMA peak (rt = 8.1 min) was near the same level as the initial baseline (rt = 0–2.5 min) **(Figure 4b)**. Accordingly, the recovery of NDMA improved from 84% (injection volume = 200 µL) to 94% (injection volume = 20 µL), indicating almost no interference from substances in the UF-treated wastewater with the reduced injection volume. The countermeasure was also successfully validated at lower NDMA concentrations (10 and 20 ng/L) **(Supplementary Material Figures S6 and S7)**. However, the reduction in sample injection volume increased the MDL of NDMA from 0.2 ng/L to 2 ng/L. Thus, the strategy of reducing injection volume is only valid for the determination of samples with an NDMA concentration >2.0 ng/L.

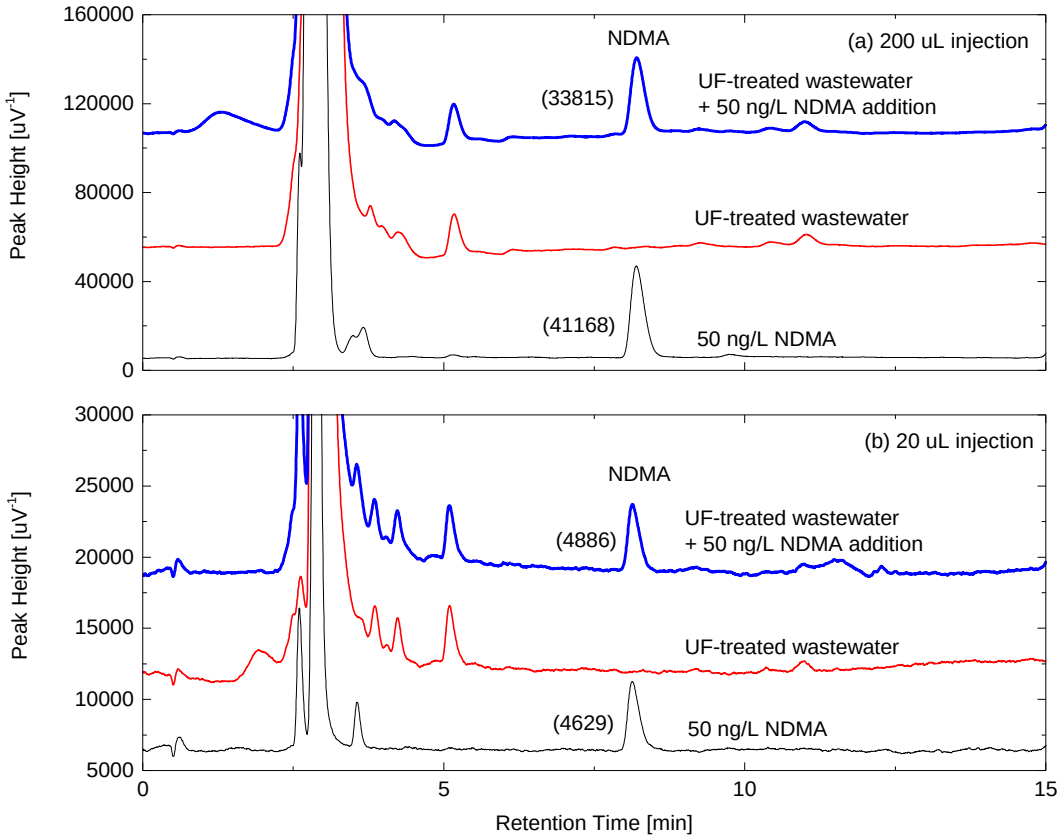

Figure 4: Analysis of NDMA concentration (50 ng/L) in the UF-treated wastewater using the HPLC-PR-CL analysis with sample injection volume of (a) 200 µL and (b) 20 µL. The values in brackets indicate the NDMA peak height.

3.2.2. HPLC-PR-CL with RO permeate

A similar evaluation was also performed using RO permeate from wastewater treated with an ESPA2 membrane. A drop of the baseline after the sample entered the chemiluminescence component (*rt* = >3.0 min) was not observed with the standard injection volume (i.e. 200 µL) **(Figure 5)**, and a very high recovery of NDMA (97%) was achieved. These results indicate that RO membranes are capable of removing the substances that caused a reduction in the chemiluminescence baseline for UF permeate. Thus for RO permeate samples, the standard analytical procedure is likely adequate to accurately determine NDMA concentrations.

In typical water reclamation system for potable water reuse, hydrogen peroxide is added to RO permeate as part of UV-based advanced oxidation process. The final product water may also be chlorinated before distribution to the end-users. Thus, the interference from these oxidants in RO permeate was also evaluated here. The effect of hydrogen peroxide in RO permeate was negligible regardless the addition of this and other reducing agents **(Supplementary Material Figures S8)**. When hypochlorite is quenched with reducing agents, no interference was observed **(Supplementary Material Figures S9)**. It is noted that the effect of monochloramine in RO permeate was not evaluated here, since NDMA formation through chloramination in actual wastewater including RO permeate does not allow for accurate investigations due to formed NDMA during chloramination. Alternatively, in the following section chloraminated water samples were quenched and the effect of reducing agent addition on HPLC-PR-CL was evaluated by comparing results using SPE-GC-MS/MS which is not affected by the presence of reducing agents.

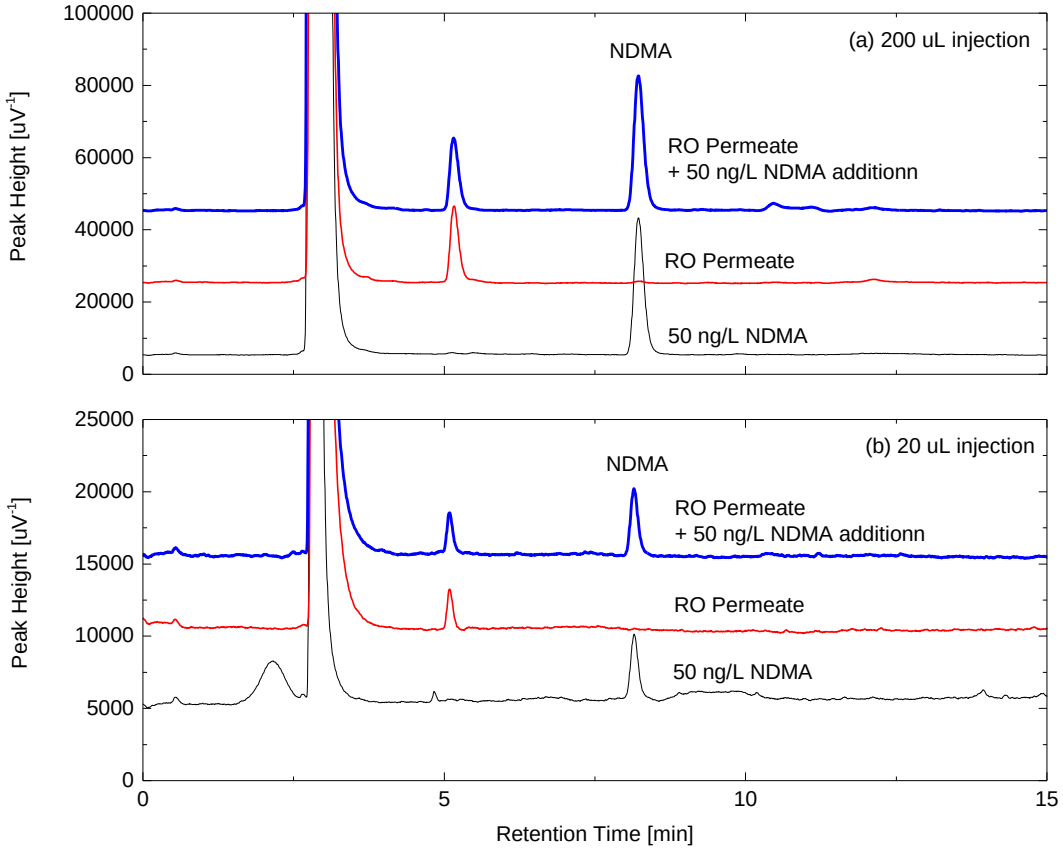

Figure 5: Analysis of NDMA concentration (50 ng/L) in the RO permeate using the HPLC-PR-CL analysis with sample injection volume of (a) 200 µL and (b) 20 µL.

3.2.3. Evaluation of NDMA formation using HPLC-PR-CL and SPE-GC-MS/MS

NDMA formation tests were performed through the chloramination of the UF-treated wastewater for specific reaction periods (0–6 h) at 25 °C, and NDMA concentrations of these samples were determined using both HPLC-PR-CL and SPE-GC-MS/MS. The reaction periods of up to 6 h were determined based on preliminary experiments which showed only a slight increase in NDMA concentration when the chloramination time increased further. The standard deviation (S.D.) for SPE-GC-MS/MS was calculated based on validation samples that have surrogate recovery of 70–120%. Although monochloramine in these samples were quenched by the addition of sodium thiosulfate shortly after the sampling, the solutions

quenched by sodium thiosulfate did not interfere with the NDMA peak by HPLC-PR-CL as demonstrated in **Supplementary Material Figure S5a**.

Overall, the analytical results using HPLC-PR-CL were consistent with those using SPE-GC-MS/MS **(Table 1)**. No NDMA was detected above the detection limit in the original UF-treated water samples. NDMA was identified by both analytical methods at a chloramination period of 1 h. As chloramination progressed further, the NDMA concentrations gradually increased, reaching over 100 ng/L after 6 h of contact time. The determination of NDMA concentrations by HPLC-PR-CL appeared to be more precise and stable than the SPE-GC-MS/MS measurements. In fact, standard deviations of three samples by the HPLC-PR-CL were significantly smaller than those by SPE-GC-MS/MS **(Table 1)**. This is not surprising given the variability in recovery typical for SPE. The MDL of the HPLC-PR-CL method in UF-treated wastewater was 2 ng/L, which was lower than that of the SPE-GC-MS/MS method (2.5 ng/L) **(Supplementary Material Table S10)**. Considering the 10 ng/L NL of NDMA, the fast HPLC-PR-CL method provides advantages for NDMA analysis as an alternative to SPE-GC-MS/MS.

Table 1: NDMA concentrations during chloramination determined by HPLC-PR-CL and SPE-GC-MS/MS.

Chloramination	HPLC-PR-CL [ng/L]		SPE-GC-MS/MS [ng/L]	
period [h]	Mean	S.D.	Mean	S.D.
0	N.D.	N.A.	N.D.	N.A.
1	11.9	± 0.3 (n = 3)	11.1	± 2.8 (n = 4)
2	53.8	± 0.7 (n = 3)	55.9	± 17.0 (n = 4)
3	77.9	± 1.0 (n = 3)	66.1	N.A. (n = 1)
6	107.3	± 0.6 (n = 3)	102.7	± 8.7 (n = 3)

S.D. Standard deviation.

N.D. Non-detect. Feed concentration was below method detection limits.

N.A. Not available.

3.3. Impact of fouling on NDMA rejection

Changes in NDMA rejection were tracked during RO treatment by performing frequent samplings and analysis by HPLC-PR-CL. NDMA concentrations in the permeate gradually decreased as RO treatment with ESPA2 and ESPAB membranes operated on UF-treated wastewater progressed **(Figure 6)**. These results indicate that fouling allowed less NDMA to permeate through these RO membranes, resulting in an increase in NDMA rejection **(Figure 6)**. For instance, during the 8 h treatment period, NDMA rejection by the ESPA2 membrane gradually increased from 40% to 49%, while TMP increased from 1.45 to 1.76 MPa. Similarly, an increase in NDMA rejection by the ESPAB membrane from 68% to 76% was observed with a TMP increase from 1.74 to 1.97 MPa. The increasing trend in NDMA rejection in response to membrane fouling was consistent with a previous study (Fujioka et al., 2013a) where the formation of a cake-like fouling layer on the membrane surface was suggested to hinder diffusion of NDMA into membrane. The results reported here demonstrated that HPLC-PR-CL can provide an accurate profile of NDMA concentrations at ng/L levels and NDMA rejection during RO fouling events.

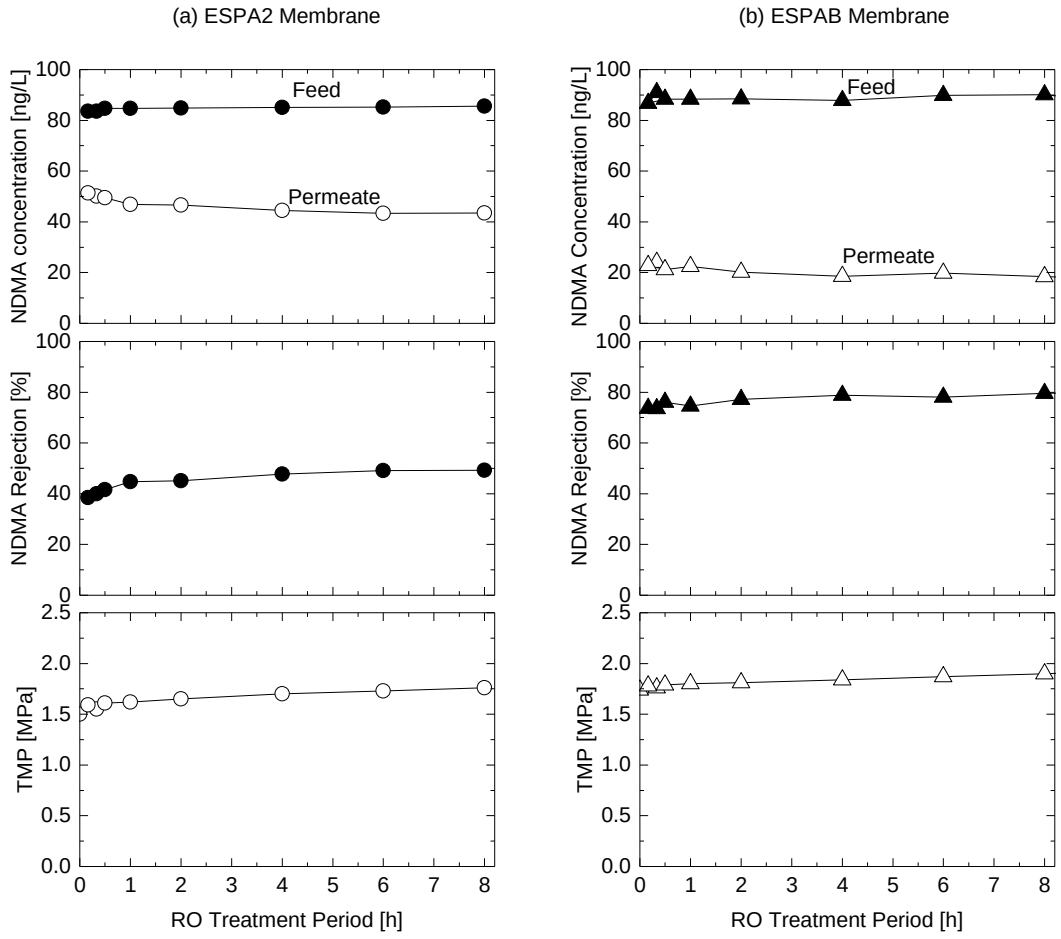

Figure 6: NDMA concentrations in the feed and permeate, NDMA rejection and TMP during RO treatment of UF-treated wastewater with (a) ESPA2 and (b) ESPAB membranes (Permeate flux = 60 L/m^{2}h, Feed solution temperature = 20 °C).

4. Conclusions

A fast and reliable HPLC-PR-CL method for the determination of NDMA concentrations in potable water reuse was evaluated and validated using studies of NDMA formation and rejection by RO membranes. No interference of monochloramine (up to 0.1 mM) and hydrogen peroxide (up to 1 mM) on the NDMA analysis was observed. The interference of hypochlorite-containing water samples was eliminated by quenching the hypochlorite with reducing agents such as ascorbic acid and thiosulfate. The interference of substances in the UF-treated wastewater on the NDMA analysis was countered by reducing the sample

injection volume from 200 to 20 µL, though this deteriorated the NDMA detection limit from 0.2 to 2 ng/L. Comparison of analytical results with SPE-GC-MS/MS revealed that HPLC-PR-CL can be used as an alternative technique. In addition, the results suggest that HPLC-PR-CL can be used in laboratory studies of NDMA due to the convenience of the small sample volume requirement and rapid analysis time. To improve the method detection limit of NDMA in treated wastewater by HPLC-PR-CL, further investigation into the source of the inhibitors and their elimination are needed.

5. Acknowledgements

The authors gratefully acknowledge staff from Orange County Water District (CA, USA) for their advice and valuable information regarding full-scale water reclamation plants.

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A rapid and reliable technique for *N*-nitrosodimethylamine analysis in reclaimed water by HPLC-photochemical reaction-chemiluminescence

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SUPPLEMENTARY MATERIAL

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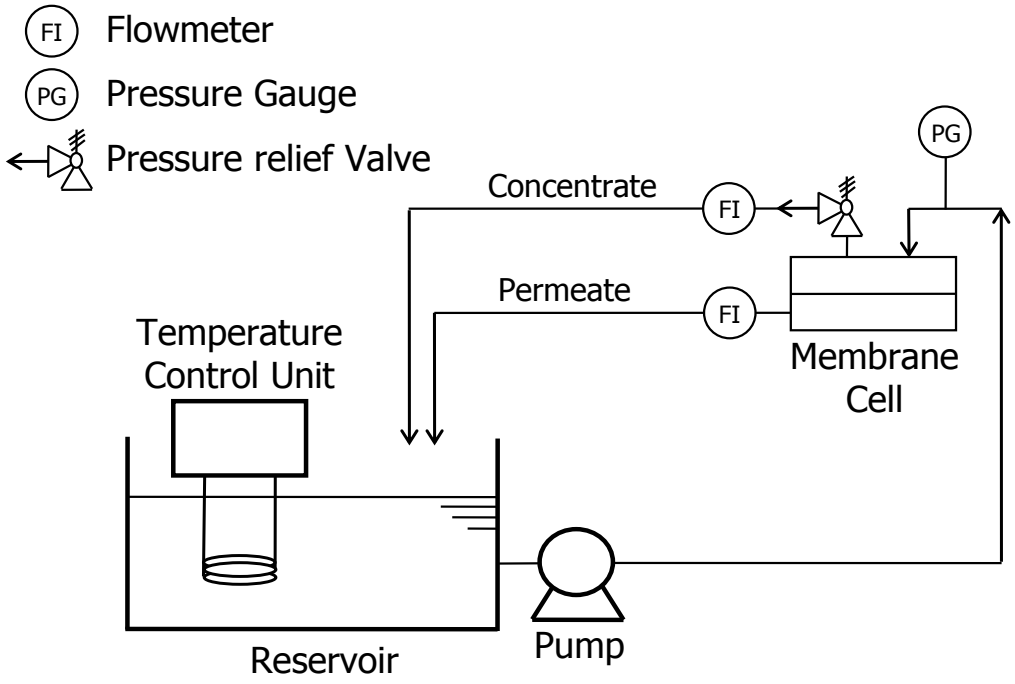
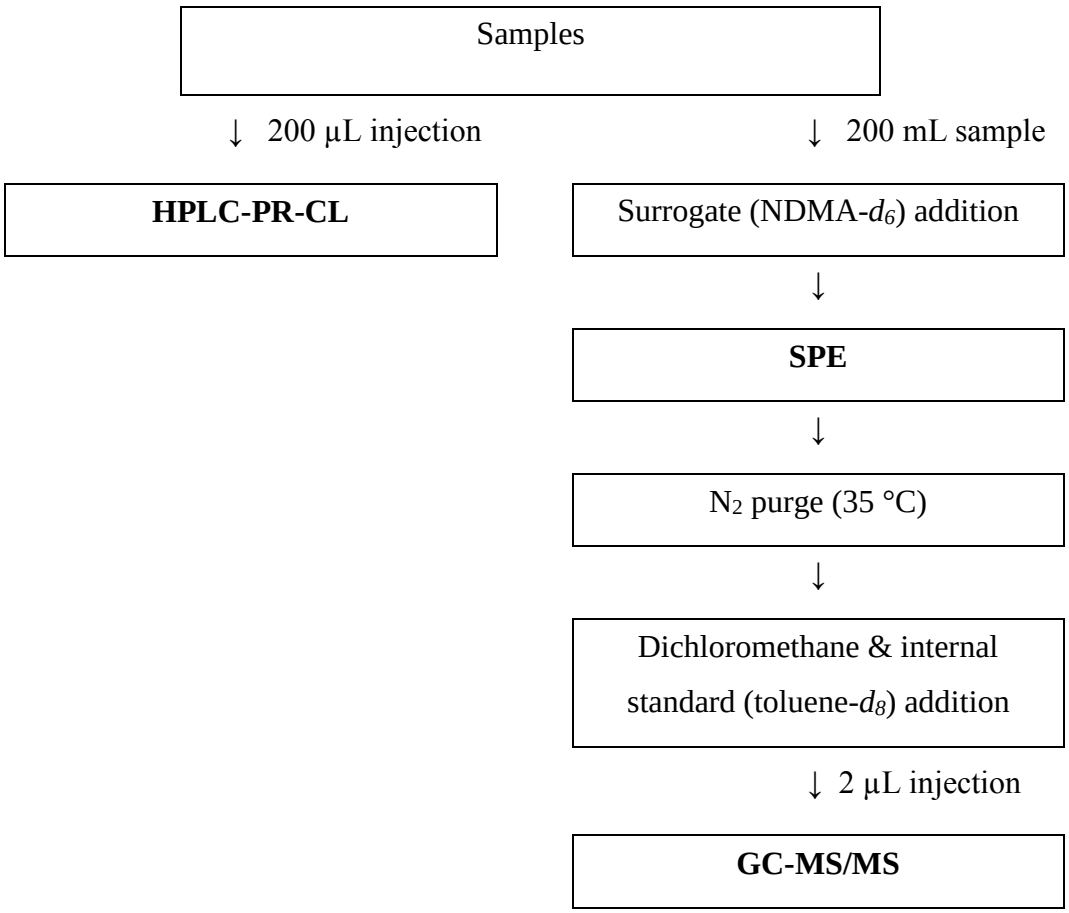


Figure S1: Schematic diagram of the cross-flow RO filtration system.

6



7 **Figure S2:** Flowchart of NDMA analytical techniques: (a) HPLC-PR-CL and (b) SPE-GC-
8 MS/MS.

9

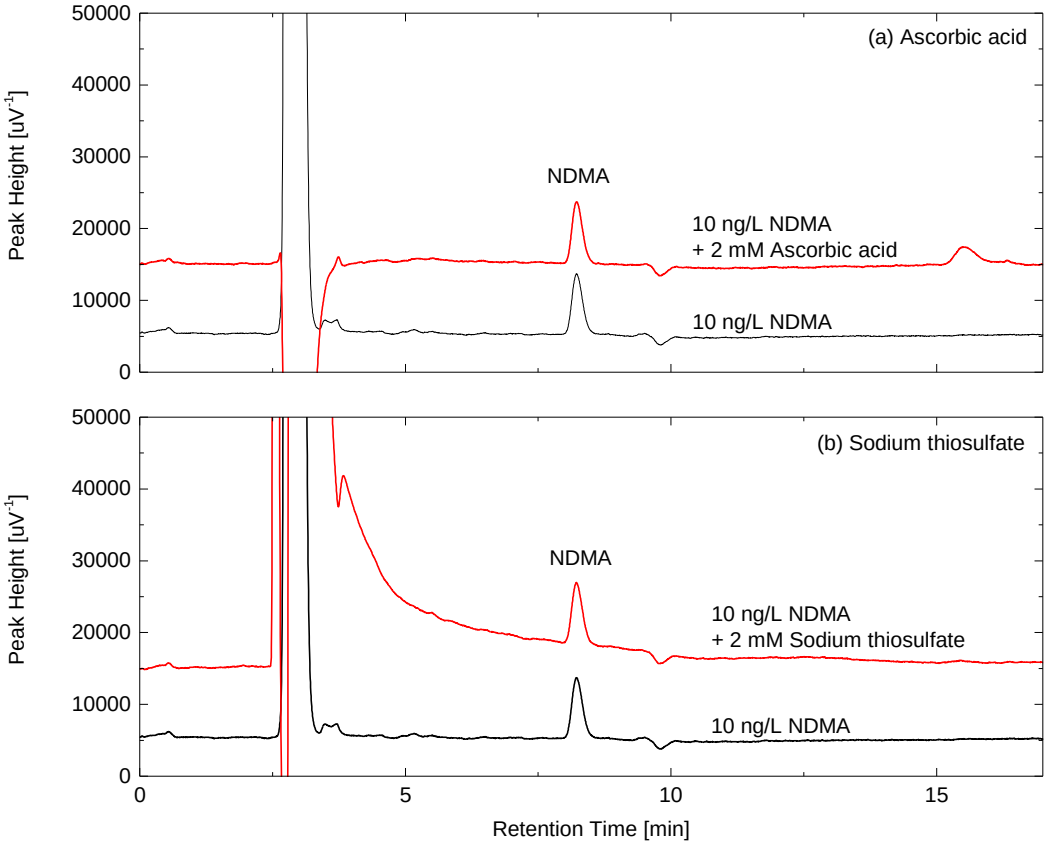
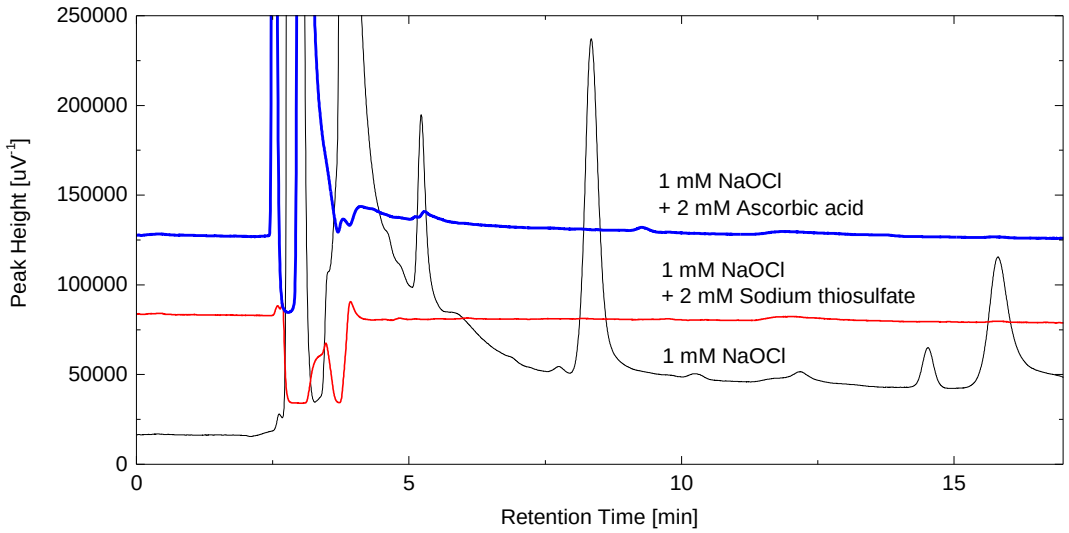


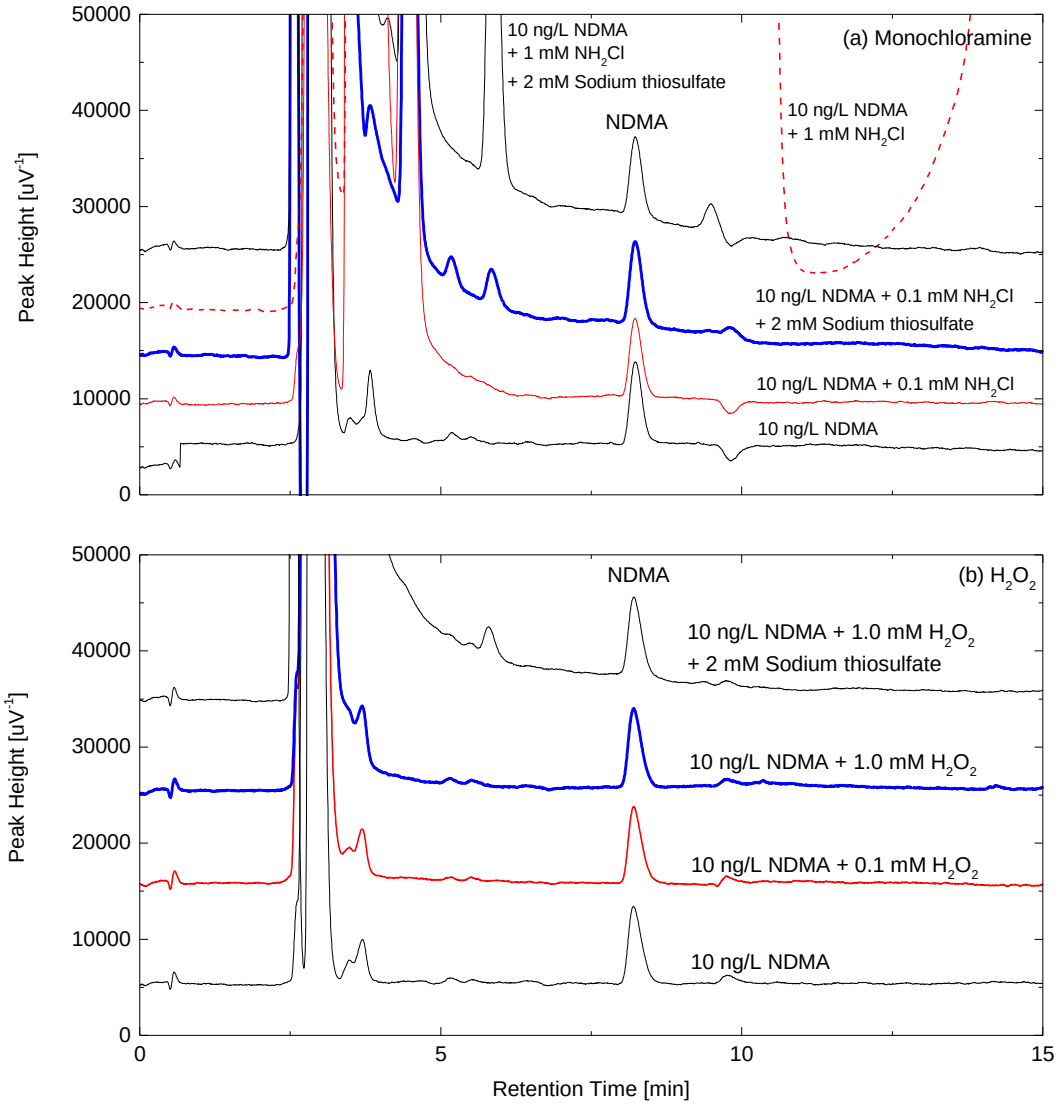
Figure S3: Chromatogram of NDMA with (a) ascorbic acid and (b) sodium thiosulfate using HPLC-PR-CL.



14

15 **Figure S4:** Chromatogram of 1 mM (as equivalent chlorine) sodium hypochlorite solution
16 with reducing agents (2 mM ascorbic acid and 2 mM sodium thiosulfate) using HPLC-PR-CL.
17 No NDMA was added in the solutions.

18



19

20 **Figure S5:** Effect of (a) monochloramine (0.1 mM as equivalent chlorine) and (b) hydrogen
21 peroxide (0.1 and 1 mM) in pure water matrix on the determination of NDMA concentration
22 using HPLC-PR-CL (NDMA = 10 ng/L).

23

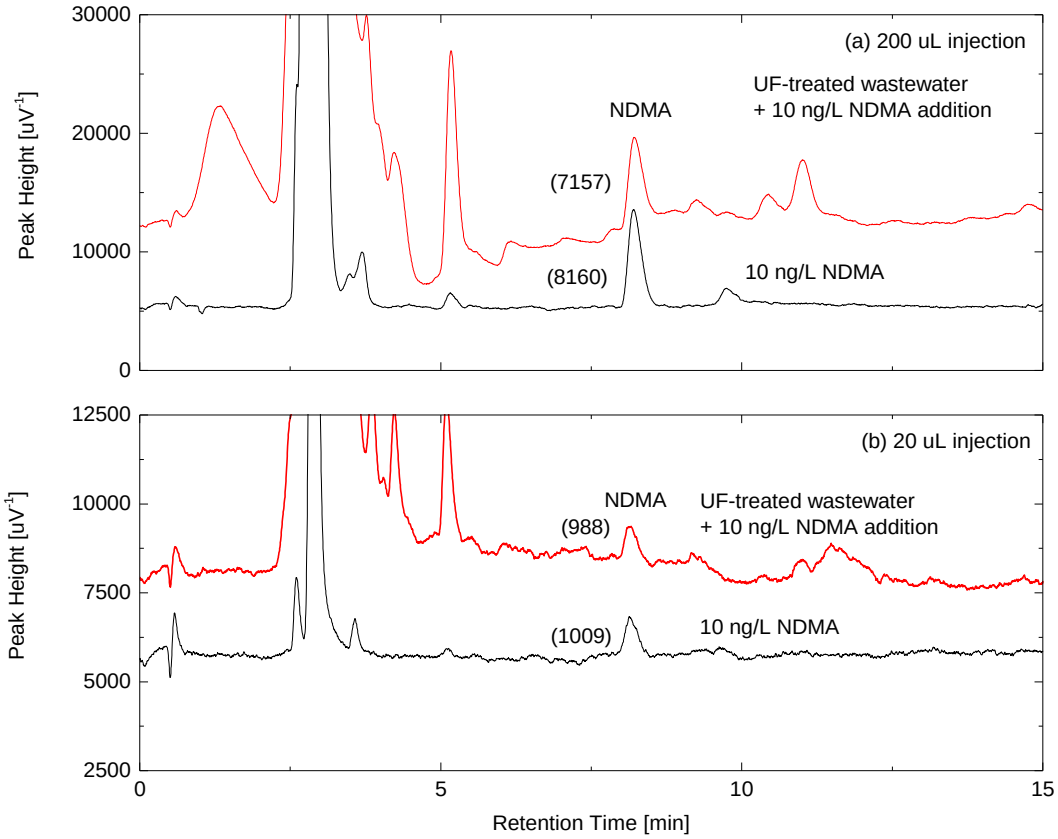


Figure S6: Analysis of NDMA concentration (10 ng/L) in UF-treated wastewater using the HPLC-PR-CL analysis with sample injection volume of (a) 200 µL and (b) 20 µL. The values in brackets indicate the NDMA peak height. The recovery of NDMA at 200 µL injection volume was 81%, while that at 20 µL injection volume was 98%.

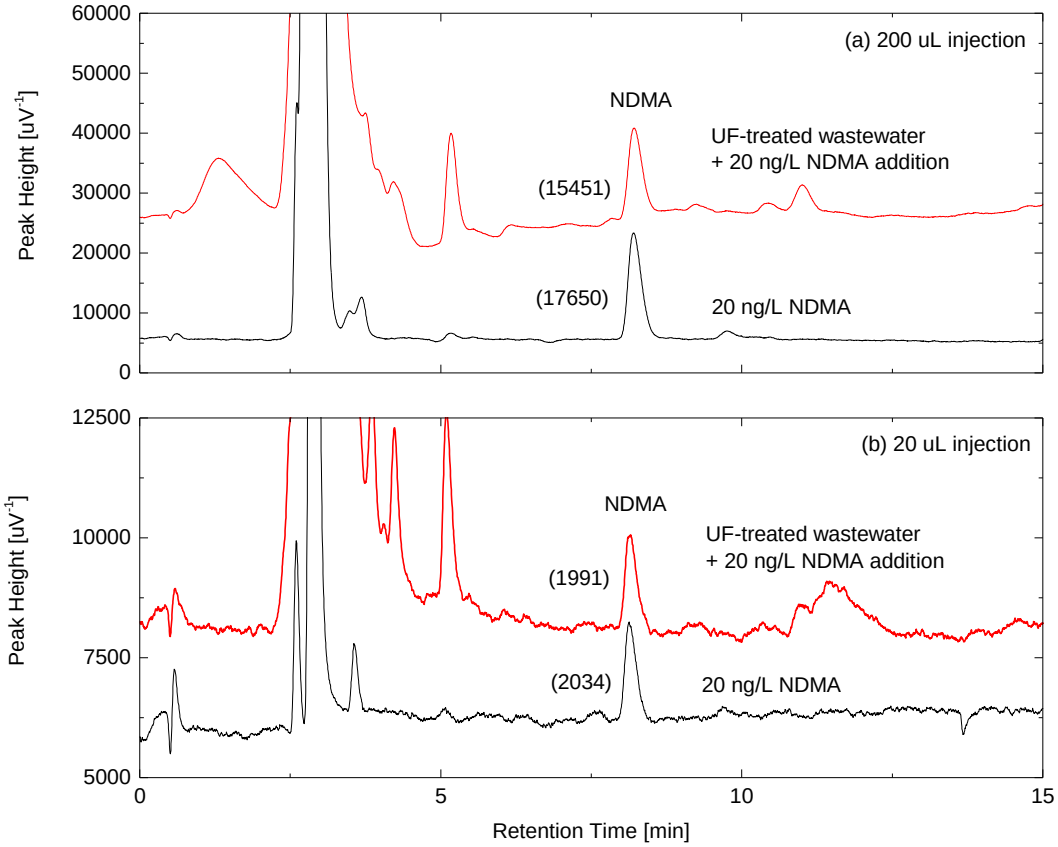


Figure S7: Analysis of NDMA concentration (20 ng/L) in UF-treated wastewater using the HPLC-PR-CL analysis with sample injection volume of (a) 200 µL and (b) 20 µL. The values in brackets indicate the NDMA peak height. The recovery of NDMA at 200 µL injection volume was 87%, while that at 20 µL injection volume was 100%.

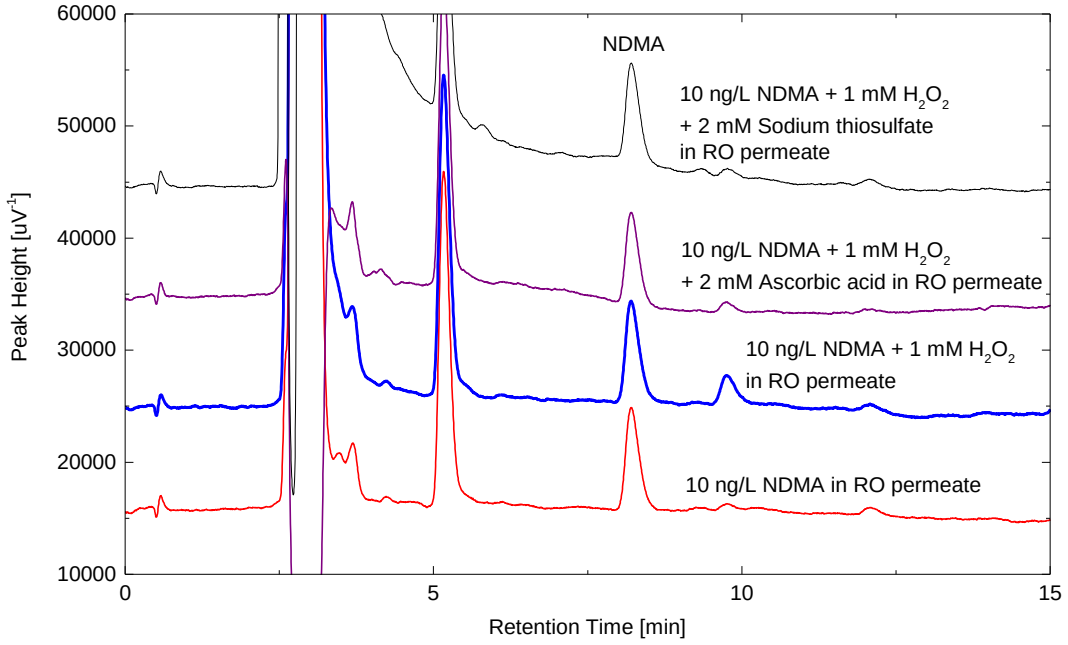


Figure S8: Effect of hydrogen peroxide on the determination of NDMA concentration in RO permeate by HPLC-PR-CL (NDMA concentration = 10 ng/L, hydrogen peroxide concentration = 1 mM, Sodium thiosulfate = 2 mM, Ascorbic acid concentration = 2 mM).

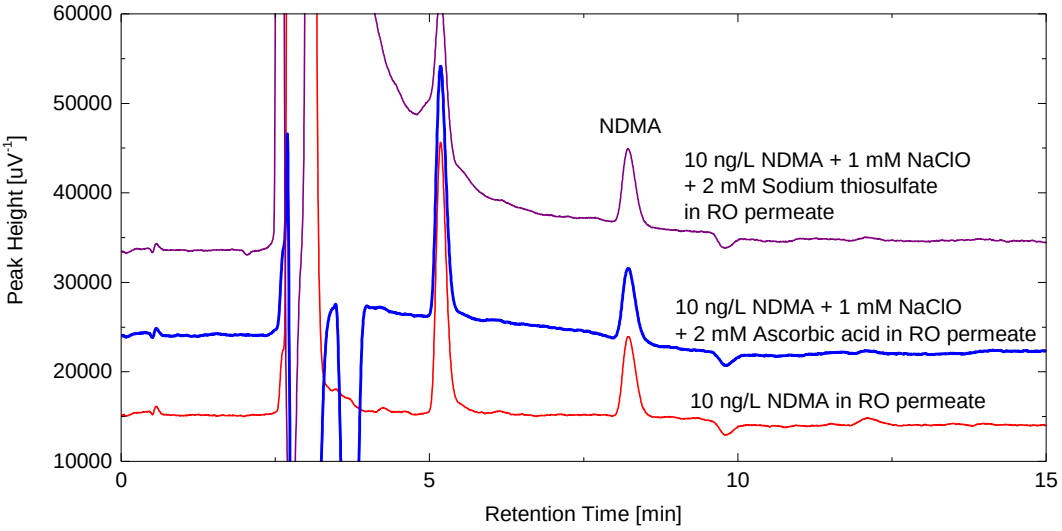


Figure S9: Effect of sodium hypochlorite on the determination of NDMA concentration in RO permeate by HPLC-PR-CL (NDMA concentration = 10 ng/L, Sodium hypochlorite concentration = 1 mM (as equivalent chlorine), Sodium thiosulfate = 2 mM, Ascorbic acid concentration = 2 mM).

Table S10: Method quality parameters of HPLC-PR-CL and SPE-GC-MS/MS in UF-treated wastewater.

Method	Calibration Range [ng/L]	R^2	Method Detection Limit [ng/L]	Instrument Detection Limit [pg] (on Column)
HPLC-PR-CL	10 – 1000	0.998	2.0	0.04
SPE-GC-MS/MS	10 – 250	0.994	2.5	3.8