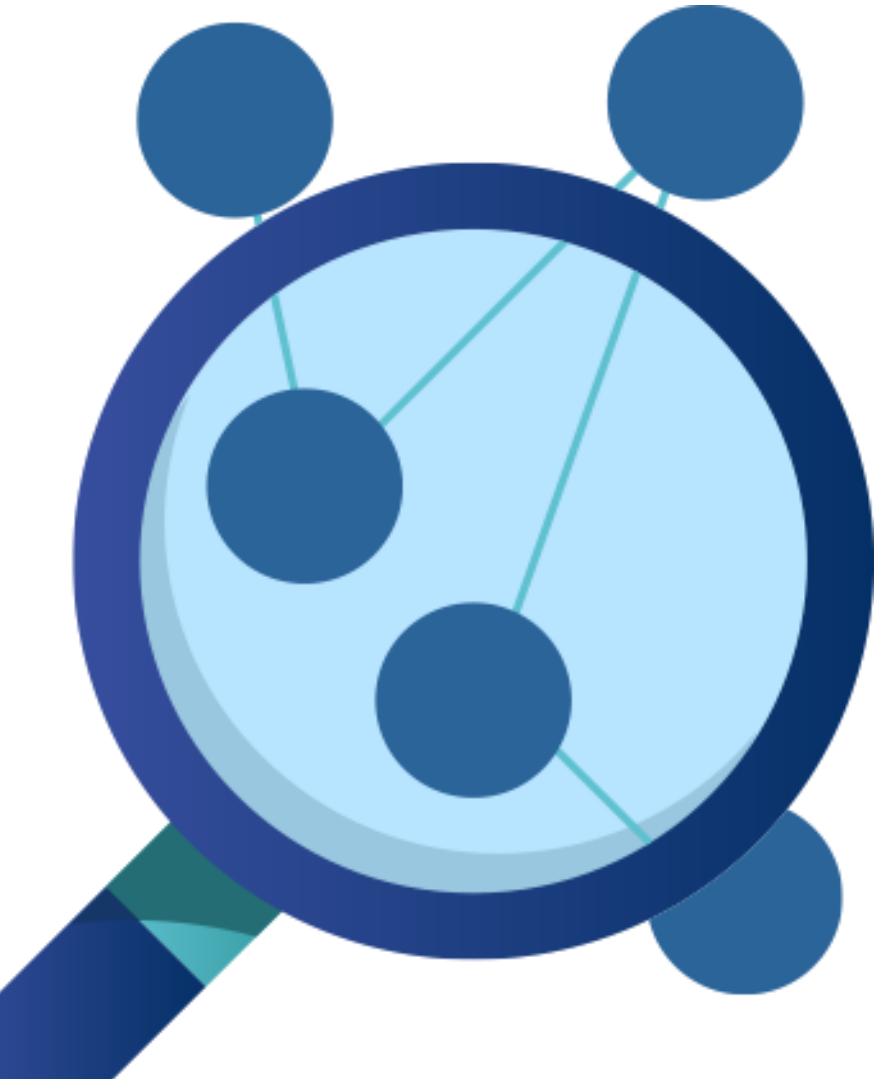
Ref. Ares(2024)887848 - 06/02/2024

Methodology for the sampling and analysis

Deliverable 2.1

WP 2



Author: Maria José Farré

Date: 31.05.2023

Funded by the European Union

GRANT AGREEMENT NUMBER	101081728		
FULL TITLE / ACRONYM	INNOVATIVE TOOLS TO CONTROL ORGANIC MATTER AND DISINFECTION BYPRODUCTS IN DRINKING WATER / intoDBP		
START DATE	01.12.2022	**DURATION**	48 months
END DATE	30.11.2026		
PROJECT URL	intodbp.eu		
DELIVERABLE	**2.1**		
WORK PACKAGE	**2**		
CONTRACTUAL DATE OF DELIVERY	**31.05.2023**		
ACTUAL DATE OF DELIVERY	31.05.2023		
NATURE	**REPORT**	**DISSEMINATION LEVEL**	PUBLIC
LEAD BENEFICIARY	**ICRA**		
RESPONSIBLE AUTHOR	Maria José Farré		
CONTRIBUTIONS FROM	UCY, UNICT		
ABSTRACT	The internal deliverable 2.1 (D2.1) presents the parameters to be measured which include (1) regulated DBPs (THMs, HAAs, chlorite, chlorate and bromate ions); (2) unregulated DBP species such as HANs, HNMs, HAs, KH and NAs, when relevant for the CS; (3) dissolved organic carbon, total organic nitrogen, ammonia, nitrate, bromide, iodide, pH, conductivity, and (for selected samples) adsorbable organic halogen; (4) DBP formation potential under the disinfection conditions relevant for the CS (i.e., chlorine or chloramines, mainly); (5) UV-VIS absorbance spectra, fluorescence and DOM-HRMS fingerprinting.		

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ABBREVIATIONS

Abbreviation	Description
1,1-DCP	1,1-dichloropropanone
1,1,1-TCP	1,1,1-trichloropropanone
1,2DBPd6	1,2-dibromopropane-d6
AC	Activated carbon
AOBr	Adsorbable organic bromine
AOCl	Adsorbable organic chlorine
AOI	Adsorbable organic iodine
AOX	Adsorbable organic halogen
APHA	American Public Health Association
ATL	Ens d'Abastament d'Aigua Ter-Llobregat
BAA	Bromoacetic acid
BCAA	Bromochloroacetic acid
BCAN	Bromochloroacetonitrile
BDCAA	Bromodichloroacetic acid
BDCM	Bromodichloromethane
CANAL	Canal de Isabel II
C-DBP	Carbonaceous DBP
CH	Chloral hydrate
CS	Case study
DBAA	Dibromoacetic acid
DBAN	Dibromoacetonitrile
DBCAA	Dibromochloroacetic acid
DBCM	Dibromochloromethane
DBP	Disinfection byproduct
DBPFP	Disinfection byproduct formation potential
DCAA	Dichloroacetic acid
DCAN	Dichloroacetronitrile
DES	Diethyl sulfate
DIN	German Institute for Standarization
DMP	2,6-dimethylphenol
DMS	Dimethyl sulfate
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DW	Drinking water
DWTP	Drinking water treatment plant
EEM	Excitation emission matrix
EI	Electron ionization
EM	Emission
EX	Excitation
ESI	Electrospray ionisation
FA	Formic acid
GAC	Granulated activated carbon
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
GF/F	Glass fiber/filter
GWS	Group Water Schemes
HA	Haloacetaldehydes

HAA	Haloacetic acid
HAN	Haloacetonitrile
HNM	Halonitromethane
HPLC	High pressure liquid chromatography
HPLC-MS/MS	High pressure liquid chromatography- tandem mass spectrometry
HRMS	High resolution mass spectrometry
ICRA	Fundació Institut Català de Recerca de l'Aigua
IFE	Inner filter effect
IS	Internal standard
ISO	International Organization for Standarization
KH	Haloketone
LC-HRMS	Liquid chromatography HRMS
LC-MS/MS	Liquid chromatography- tandem mass spectrometry
LGW	Laboratory grade water
LDWP	Limassol drinking water plant
LWDN	Limassol water distribution network
MCAA	Chloroacetic acid
MeOH	Methanol
MRM	Multiple reaction monitoring
MS	Matrix spike
MSD	Matrix spike duplicate
MSDS	Material safety data sheet
MtBE	Methyl tert-butyl ether
NA	N-nitrosamine
N-DBP	Nitrogenous DBP
NDBA	N-nitrosodibutylamine
NDMA	N-nitrosodimethylamine
NDMEA	N-nitrosomethylethylamine
NDEA	N-nitrosodiethylamine
NDEAd10	N-nitrosodiethylamine-d10
NDMAd6	N-nitrosodimethylamine-d6
NDPA	N-nitrosodi-n-propylamine
NDPAd14	N-nitrosodipropylamine-d14
NDPhA	N-nitrosodiphenylamine
NIST	National Institute of Standards and Technology
NMOR	N-nitrosomorpholine
NPIP	N-nitrosopiperidine
NPYR	N-nitrosopyrrolidine
PaCl	Polyaluminium Chloride
PDMS	Polydimethylsiloxane
PPE	Personal protective equipment
PPL	Priority pollutant
PTFE	Polytetrafluoroethylene
QC	Quality control
RPD	Relative percent difference
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SRM	Selected reaction monitoring
SUVA	Specific ultraviolet absorbance
TBA-HSO_4	Tetrabutylammonium hydrogensulfate

TBAA	Tribromoacetic acid
TBM	Bromoform (tribromomethane)
TBNM	Tribromonitromethane (bromopicrin)
TCAA	Trichloroacetic acid
TCAN	Trichloroacetonitrile
TCM	Chloroform (trichloromethane)
TCMd	Chloroform-d
TCNM	Trichloronitromethane (chloropicrin)
TD	Thermal desorption
TD-GC-MS	Thermal desorption-gas chromatography-mass spectrometer
TDWTP	Ter drinking water treatment plant
THM	Trihalomethane
TIC	Total ion chromatogram
TOC	Total organic carbon
TON	Total organic nitrogen
UCY	University of Cyprus
UV	Ultraviolet
UV-VIS	Ultraviolet-visible
VDWTP	Valmayor drinking water treatment plant
VOC	Volatile organic compound
WBL	Water Board of Limassol
WDD	Water Development Department

SUMMARY

This deliverable summarizes the sampling strategy and the experimental protocols to carry out the measurements and experiments requested in the intoDBP project.

In particular, the deliverable presents the parameters to be measured which will include (1) regulated disinfection byproducts (DBPs) (trihalomethanes, THMs), haloacetic acids (HAAs), chlorite, chlorate and bromate ions); (2) unregulated DBP species such as haloacetonitriles (HANs), halonitromethanes (HNMs), haloacetaldehydes (HAs), haloketones (KHs) and nitrosamines (NAs), when relevant for the case study; (3) dissolved organic carbon (DOC), total organic nitrogen (TON), ammonia, nitrate, bromide, iodide, pH, conductivity, and (for selected samples) adsorbable organic halogen (AOX); (4) DBP formation potential under the disinfection conditions relevant for the case study (i.e., chlorine or chloramines, mainly) and (5) UV-VIS absorbance spectra, fluorescence and dissolved organic matter high resolution mass spectrometry (DOM-HRMS) fingerprinting.

1. TASK DESCRIPTION

This deliverable is the result of task 2.1 from work package 2. The objective is to design a sampling and analysis methodology, tailored to each case study site, integrating specific requirements and actors involved (municipalities, water authorities, industry, and researchers) to be able to characterize the case study sites.

2. CASE STUDY SITES

The four case study sites of intoDBP are the following:

2.1. CS1-LIMASSOL (CYPRUS)

The Limassol Drinking Water Plant (LDWP) is managed by the Water Development Department (WDD). The plant has been operating since 1994 and its capacity is 80,000 m^3/day and is the water supply for the city of Limassol, villages west of Limassol and the British Base of Akrotiri. The plant receives water from Kouris reservoir. The treatment is pre-chlorination (chlorine)→ Polyaluminium Chloride (PaCl) and polyelectrolyte added in rapid mixer→ flocculation→ clarification (lamella plate)→ sand filters→ treated water reservoir. The Limassol Water Distribution Network (LWDN) is managed by the Water Board of Limassol (WBL). The system serves 110,000 consumers, and the annual average water demand is estimated to be 17 million m^3. The network includes 31 water tanks, 37 pumping stations and approximately 1,150 km of pipes with 100 km of transport pipes and 1,050 km of distribution pipes. Figure 1 shows the diagram of the LDWP and Figure 2 the respective treatment train diagram.

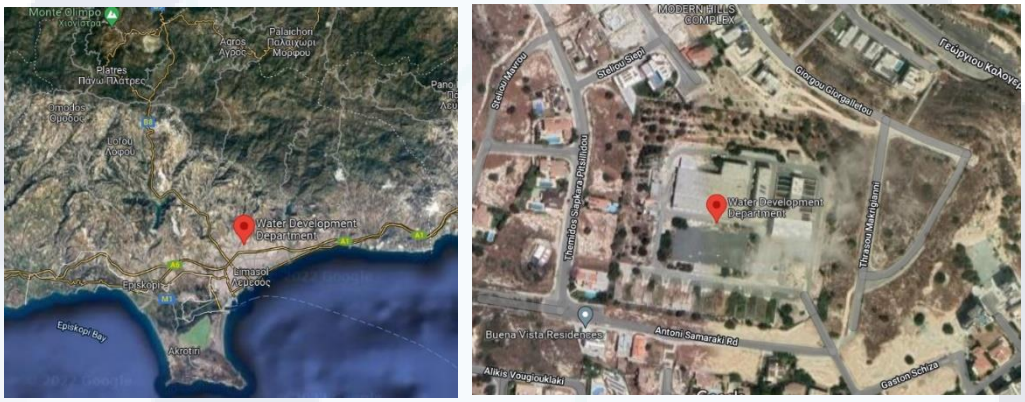


Figure 1: The Limassol Drinking Water Plant (LDWP) location in Cyprus (CS1, 34°42'52.3"N 33°01'58.1"E)

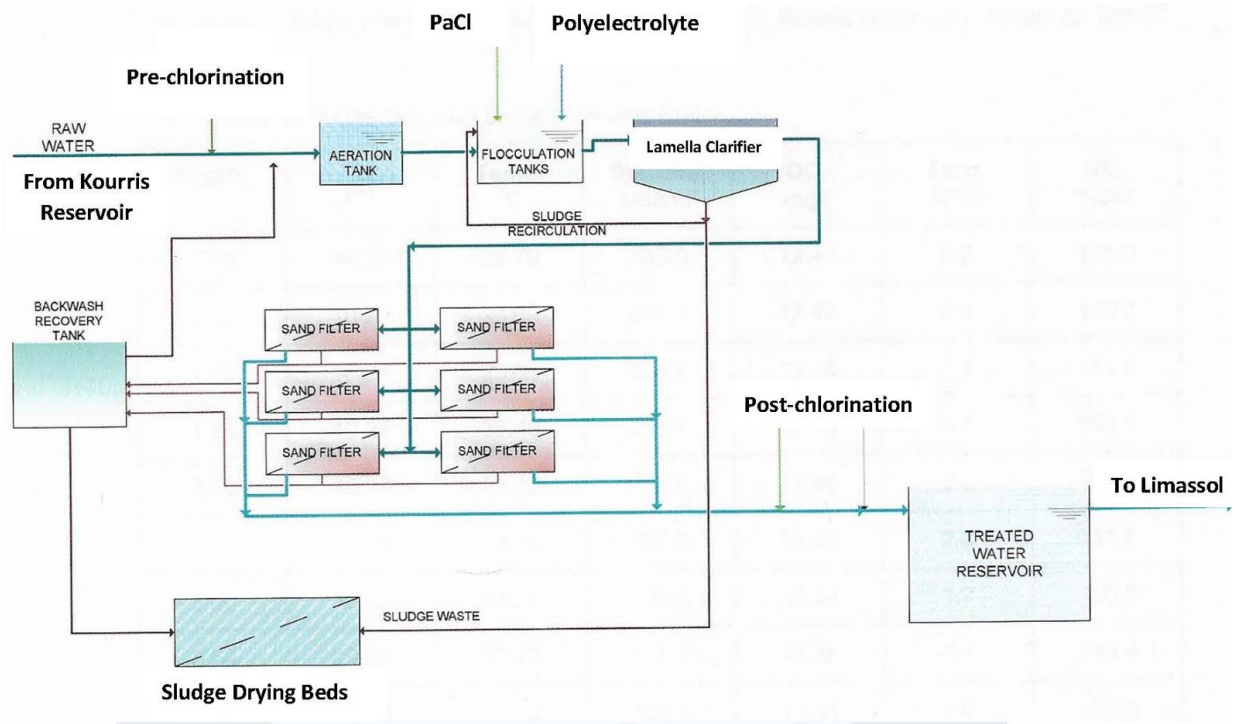


Figure 2: Diagram of the treatment train at the Limassol Drinking Water Plant location in Cyprus (LDWP, CS1)

2.2. CS2- BARCELONA (SPAIN)

The Ter Drinking Water Treatment Plant (TDWTP) is managed by Ens d'Abastament d'Aigua Ter-Llobregat (ATL) and provides drinking water (DW) for the city of Barcelona and metropolitan area. The plant has been operating since 1966 and its capacity is 691,200 m^3/day. The plant receives water from the Ter River catchment after being dammed by a series of 3 dams. The following procedure is performed: pre-oxidation with chlorine dioxide and sodium hypochlorite → coagulation+flocculation → clarification → activated carbon filtration → final disinfection (sodium hypochlorite). The plant can store 557,664 m^3. Figure 3 shows the diagram of the plant, whereas Figure 4 the diagram of the treatment train.

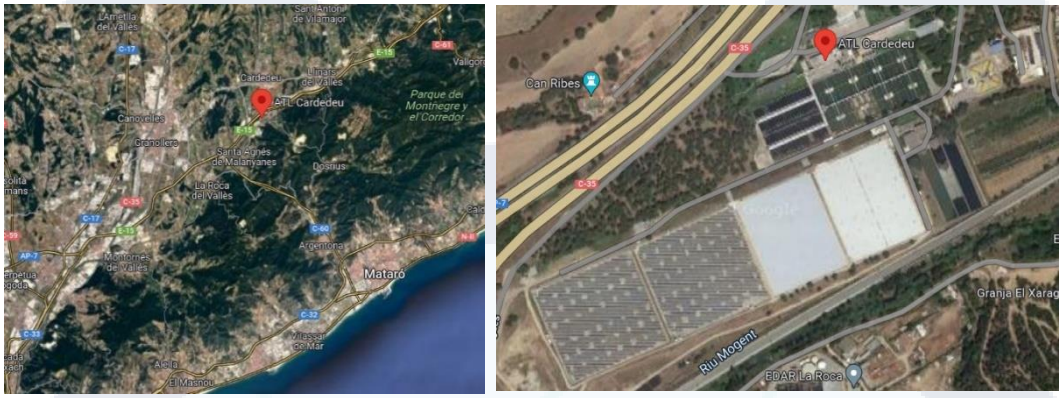


Figure 3: The Ter Drinking Water Treatment Plant in Spain (CS2, 41.6196° N, 2.3599°)

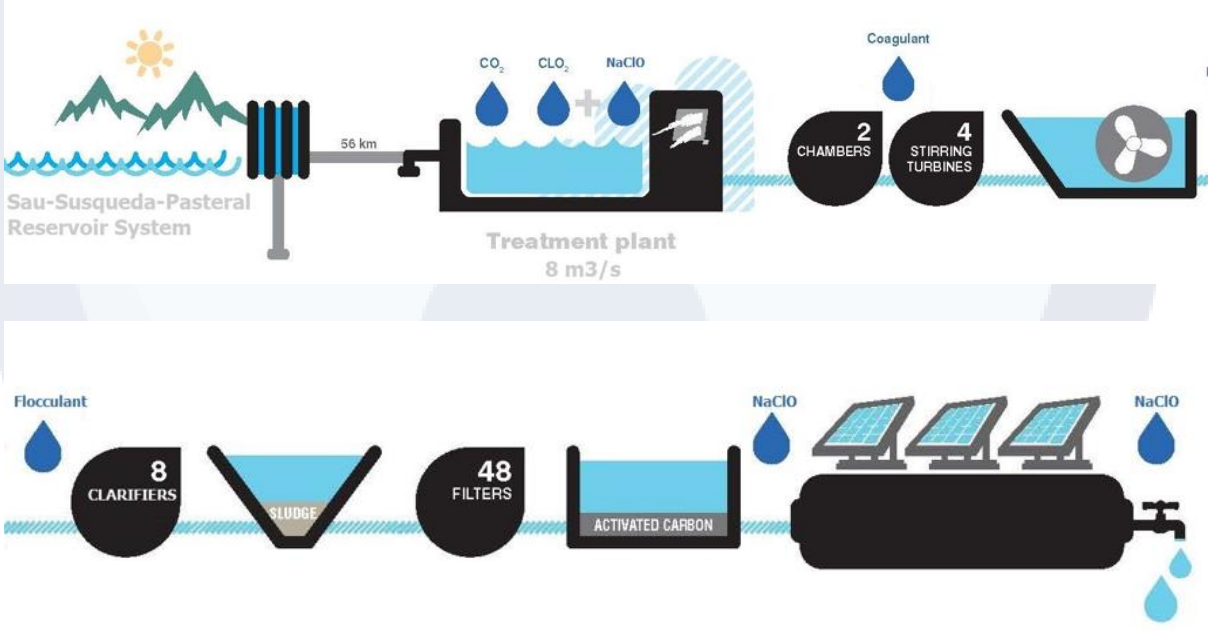


Figure 4: Diagram of the treatment train at the Ter Drinking Water Treatment Plant in Spain (TDWTP, CS2).

2.3. CS3- MADRID (SPAIN)

The Valmayor Drinking Water Treatment Plant (VDWTP) is managed by Canal de Isabel II (CANAL). The plant has been operating since 1976 and its capacity is 1,036,800 m^3/day (second biggest DWTP in Spain). The plant receives water from the Valmayor Reservoir. The treatment consists of pre-oxidation with ozone, chlorine, chlorine dioxide, and permanganate→ coagulation→ flocculation→ micro-sand addition (ballasted settling)→ sand filtration→ ozone/hydrogen peroxide (advanced oxidation process)→ filtration on granular activated carbon disinfection (chloramine). Figure 5 shows the VDWTP diagram and Figure 6 the diagram of the plant.



Figure 5: The Valmayor Drinking Water Treatment Plant in Spain (CS3, 40°31'55.6"N 4°01'52.3"W).

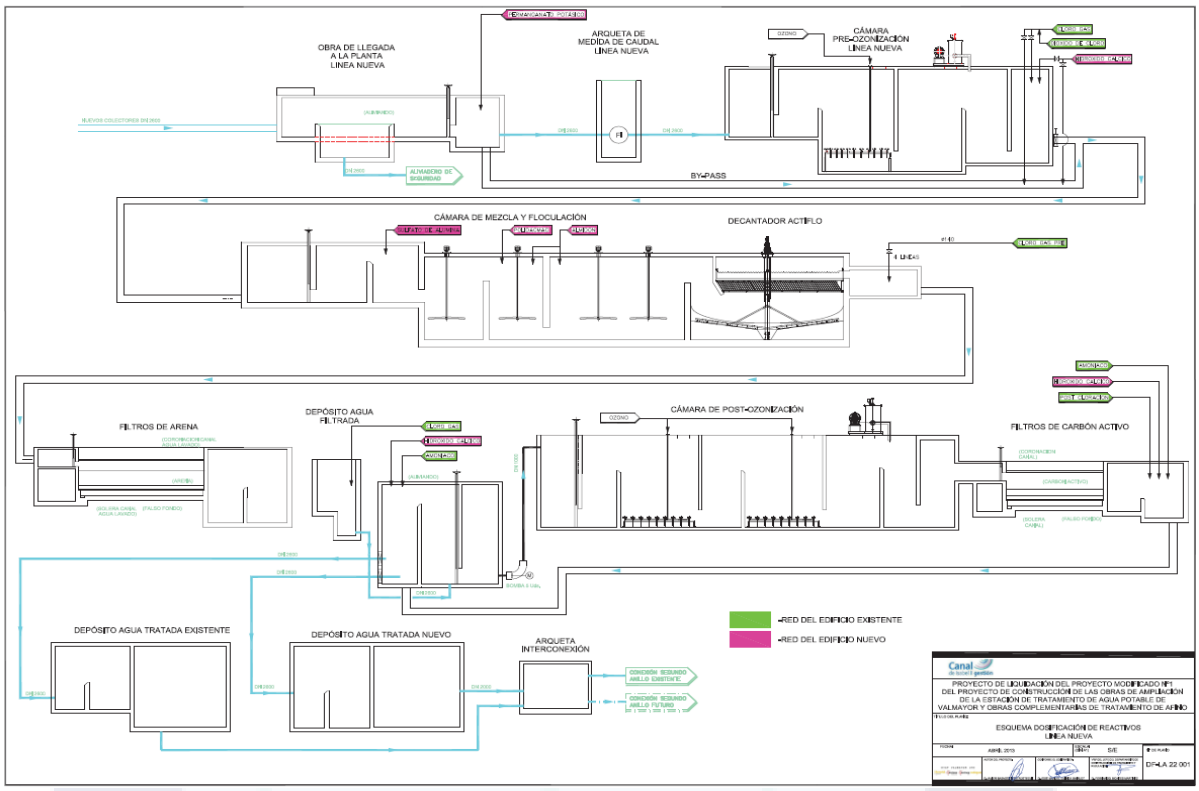


Figure 6: Diagram of the treatment train at Valmayor DWTP (CS3).

2.4. CS4- Co. MAYO (IRELAND)

The CS in Ireland includes a cluster of three small Group Water Schemes (GWS) located in Co. Mayo, supplying rural communities. The utilities are co-operative schemes which are privately managed and are represented by the National Federation of GWS.

The selected GWS are located near to each other in catchments with a high prevalence of peatland. (a) CS4.1: extracts its water from a 63 ha lake serving approximately 850 households with treatment involving clarification in combination with UV and chlorination (b) CS4.2: extracts its water from two interconnected lakes and serves approximately 1900 homes and commercial premises. Treatment involves screening, sand filtration in combination with UV and chlorination (c) CS4.3: abstracts water from a mountain stream which supplies approximately 630 households and uses a treatment combination of media filtration, ozonation and chlorination. Figure 7 shows the Irish CSs. Exact location cannot be shown due to confidentiality agreements.



Figure 7: Area showing the location of Irish CS I in Co. Mayo, Ireland

3. SAMPLING STRATEGY

Our goal with the sampling is to gain a deeper understanding of the current levels of DBP and its precursors at the case study sites, as well as to measure the effectiveness of the intoDBP project in reducing DBP formation. Additionally, we will collect samples for use in other project tasks, including tasks 2.2 and 2.5, from various work packages. In total, we will conduct four sampling campaigns at each case study site, as outlined in Table 1.

Table 1: Sampling periods for the selected CS sites.

Site	Sampling 1 (spring/summer, year 1)	Sampling 2 (autumn/winter, year 1)	Sampling 3 (spring, year 4)	Sampling 4 (autumn, year 4)
CS1 (Limassol)	June 2023	November 2023	June 2026	November 2026
CS2 (Barcelona)	April 2023	September 2023	April 2026	September 2026
CS3 (Madrid)	May 2023	October 2023	May 2026	October 2026
CS4 (Ireland)	June 2023	December 2023		

During each sampling campaign, we will collect samples from several key points, including (1) the source water, (2) treated water prior to pre-oxidation and/or disinfection, (3) treated water, and (4) representative points throughout the network distribution system. We have provided a detailed explanation of the sampling protocols and analytical methodologies for various parameters in the following sections. Please refer to Table 2 for the locations of the samples.

Table 2: Sample location per CS site.

CS 1 (Limassol)	CS 2 (Barcelona)	CS 3 (Madrid)	CS 4 (Ireland)
Raw water	Raw water	Raw water	Raw water 1
After aeration tank	Clarified Post ClO_2	Pre-ozone	Raw water 2
After sand filters	Post AC	Filtered water	Raw water 3
After treated water reservoir (outlet DWTP, sensor 1)	Outlet of the tanks	GAC filtered water	
Outlet of re-chlorination tank (sensor 2)	Distribution 1 (15-25h)	Outlet of the plant	
Inlet of DMA (sensor 3)	Distribution 2 (30-70h)	Distribution 1	
Distribution 1 (sensor 4)	Distribution 3 (70-110h)	Distribution 2	
Distribution 2		Distribution 3	
Distribution 3			

4. MONITORED PARAMETERS

We will measure the following parameters in all samples collected during the campaign. The aim is to obtain a comprehensive characterization of the sampling sites and to quantify any improvements achieved during the lifetime of the project. In addition, we will align specific project tasks with the samplings to optimize our resources.

Dissolved Organic Matter characterization

- Excitation Emission Matrix (EEM) to characterize water throughout the plant and investigate the relation of loss of fluorescence intensity in the different regions after disinfection and formation of DBPs (ICRA).
- Specific Ultraviolet Absorbance (SUVA) to complement the information from EEM as SUVA is currently the best approach to predict C-DBP formation (but it may not be as useful for N-DBP prediction) (ICRA/UCY).
- Fingerprinting with high resolution mass spectrometry (LC-HRMS) to evaluate the regions of the Van Krevelen diagram that change with disinfection and compare to EEM and UV-VIS data (ICRA).
- UV spectra obtained with the on-line s::can sensors. To be supplied by SCAN to develop algorithms for DBP formation potential.

General water quality characteristics, inorganic ions and inorganic DBPs

- Total organic carbon, total organic nitrogen (ICRA/UCY)
- Bromide, iodide, ammonia, nitrate, nitrite (UCY/ICRA)
- pH, conductivity (UCY/ICRA)
- Chlorite (ICRA/UCY), chlorate (ICRA/UCY), bromate (UCY)
- Total hardness as $CaCO_3$ (UCY/ICRA)

DBP analysis – volatile DBPs (regulated and unregulated)

- Trihalomethanes (THMs = trichloromethane, bromodichloromethane, dibromochloromethane, tribromomethane) (ICRA/UCY)
- Haloacetonitriles (HANs = dichloroacetonitrile, trichloroacetonitrile, bromochloroacetonitrile, dibromoacetonitrile) (ICRA/UCY)
- Halonitromethanes (HNMs = tribromonitromethane, trichloronitromethane) (ICRA/UCY)
- Haloketones (HKs = 1,1-dichloropropanone, 1,1,1-trichloropropanone) (ICRA/UCY)
- Haloacetaldehydes (HAs = chloral hydrate) (ICRA/UCY)

DBP analysis – Haloacetic acids

- Regulated haloacetic acids (HAA = bromoacetic acid, chloroacetic acid, dibromoacetic acid, dichloroacetic acid, trichloroacetic acid) (ICRA/UCY)
- Additional HHAs (dibromochloroacetic acid, bromochloroacetic acid, bromodichloroacetic acid, tribromoacetic acid) (ICRA/UCY)

DBP analysis – *N*-nitrosamines

- Nitrosamines (N-nitrosodimethylamine, N-nitrosodibutylamine, N-nitrosodi-n-propylamine, N-nitrosomethylethylamine, N-nitrosodimethylamine, N-nitrosopyrrolidine, N-nitrosopiperidine, N-nitrosodiphenylamine, N-nitrosomorpholine) (ICRA)

Adsorbable organic halogen

- AOX (AOCl, AOBr and AOI) (ICRA)

DBP formation potential tests

- DBP formation potential test will be performed at both ICRA and UCY following the conditions that are relevant for the water utility in terms of type and concentration of disinfectant and hydraulic retention time.

5. EXPERIMENTAL PROTOCOLS

5.1. WASHING OF GLASSWARE FOR DBPs

The following procedure is used for glassware that has come into contact with real water (not Milli-Q or standards) or anything that can cause the formation of DBPs and lead to false positives in analysis.

GLASS

1. Rinse glassware with deionised water
2. Soak glassware overnight in soap (Pyroneg powder – 4 caps into 4 L deionised water)
3. Rinse glassware with deionised water and then methanol
4. Soak in nitric acid (<10%) overnight
5. Nitric acid is recycled
6. Solvent wash with Milli-Q water followed by methanol
7. Dry in oven overnight at 100ºC (optional and not applicable for volumetric material)

NOTE: Care must be taken when using acid. Face shield, butyl II gloves and personal protective equipment (PPE) must be worn. It should be done in fume hood and only when there is someone else in the laboratory.

PLASTICS

1. Rinse plastic with Milli-Q water
2. Soak glassware overnight in soap (Pyroneg powder – 1 cap into 1 L Milli-Q water)
3. Rinse plastic with Milli-Q water and then methanol
4. Allow to dry on paper towels, covered and overnight

5.2. SAMPLING PROTOCOL

Minimum number of samples for case study site characterization are described in Table 2. Additional samples can be taken if considered relevant and agreed among partners.

Glassware and quenching agents to prepare prior to sampling:

- **2.5L** for formation potential tests. NOT QUENCHED but shipped immediately. Used to measure pH and conductivity.
- **2.5L** for UV-VIS measurement, EEM, DOC, TON, NH_4^+, NO_3^-, Br^-, I^-, oxoanions and HRMS. QUENCHED: Add 7 mg/L of sodium sulfite per mg/L of chlorine - if quenched with a prepared dissolution, concentrated solution of sodium sulfite must be prepared fresh the day of sampling. Filter through 0.7 μm glass fibre filter.
- **250 mL** for volatile DBPs and HAAs analysis. QUENCHED: Add 5 mg/L ascorbic acid for each 1mg/L of chlorine – if quenched with a prepared dissolution, concentrated solution of ascorbic acid must be prepared fresh the day of sampling.
- **1x1L** for NDMA measurement (if considered a risk). QUENCHED. Add 2.5 g/L solid sodium sulfite in solid.
- **1x250 mL plastic** for AOX analysis (can be frozen). QUENCHED: Add 7 mg/L of sodium sulfite per mg/L of chlorine - if quenched with a prepared dissolution, concentrated solution of sodium sulfite must be prepared fresh the day of sampling.

Collecting the samples

Before collecting samples, the sample tap should be opened and allowed to run to waste for 2-3 minutes. The flow should then be reduced, the bottle placed at an angle and the water allowed to run down the side. DO NOT FILL AND DISCARD BECAUSE BOTTLES CONTAIN QUENCHING AGENTS. When the bottle is almost full, cap the bottle with the Teflon side of the liner facing inwards. Invert the bottle to mix and then open the cap and completely fill so that no air bubbles remain. Invert to confirm absence of air. Samples should be labelled using pencil.

Sample storage

1. Store samples in the dark at 4-6 ºC until pre-processing starts.
2. Samples cannot be frozen except samples to measure bromate and AOX.
3. Processing should begin within the following timeframes. These timeframes apply to the measurements done after finishing formation potential test too:

    - Formation potential test should start within one week after sampling.
    - UV, EEM and general parameters, samples should be measured as soon as possible to avoid degradation of the samples (ideally within 2-3 days, but if shipment is necessary this time may be delayed. Filtration of sample prior to shipment will improve the stability of the sample).
    - Once filtered (ideally on the day of sampling) DOM should be extracted within one week. Extracts in methanol must be analysed within 28 days after extraction.
    - For volatile DBPs analysis, quenched samples must be extracted within 5 days (ideally 3 days to avoid hydrolysis of HANs). Extracts must be stored at -10 °C or less and protected from light in glass vials with PTFE-lined caps. MTBE extracts must be analysed within 21 days of extraction.

- For haloacetic acids analysis, quenched samples must be extracted within 14 days. Extracts must be stored at -10 °C or less and protected from light in glass vials with PTFE-lined caps. MTBE extracts must be analysed within 21 days of extraction.
- For nitrosamines analysis, quenched samples must be extracted within 14 days of collection. Sample extracts may be stored for up to 28 days after sample extraction, when stored in amber vials at -15 °C or less and protected from light.
- For chlorite and chlorate analysis, quenched samples must be injected within 14 days of collection.

In the following Tables (Tables 3-7), the measured parameters and the sampling preparation steps per CS are presented in detail.

Table 3: Sampling points of each CS.

	2.5L not quenched	2.5L w. sodium sulfite	250 ml w. ascorbic acid	1L w. sodium sulfite	250 ml w. sodium sulfite
CS1 - Limassol					
Raw water	x	x	x		x
After aeration tank	x	x	x		x
After sand filters	x	x	x		x
After treated water reservoir=outlet of DWP (sensor 1)	x	x	x		x
Outlet of rechlorination tank (sensor 2)	x	x	x		x
Inlet of DMA (sensor 3)	x	x	x		x
Distribution 1 (sensor 4)		x	x		x
Distribution 2		x	x		x
Distribution 3		x	x		x
CS2 - Barcelona					
Raw water	x	x	x		x
Clarified post ClO_2	x	x	x		x
Post AC	x	x	x		x
Outlet of the tanks	x	x	x		x
Distribution 1		x	x		x
Distribution 2		x	x		x
Distribution 3		x	x		x
CS3 - Madrid					
Raw water	x	x	x	x	x
Pre-ozone	x	x	x	x	x
Filtered water	x	x	x	x	x
GAC filtered water	x	x	x	x	x
Outlet of the plant	x	x	x	x	x
Distribution 1		x	x	x	x
Distribution 2		x	x	x	x
Distribution 3		x	x	x	x
CS4 - Ireland					
Raw water 1	x				
Raw water 2	x				
Raw water 3	x				

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Table 4: Parameters to be analysed in CS 1.

CS1- Limassol	Actual sample taken from the plant												Formation potential test										
	EEM (s.s.)	UV (s.s.)	DOM (s.s.)	TOC/TN (s.s.)	Inorganic ions (s.s.)	pH, conductivity, hardness	Inorganic DBPs (s.s.)	HAAs (a.a.)	Volatile DBPs (a.a.)	NAs (s.s.)	AOX (s.s.)	FP tests	EEM (s.s.)	UV (s.s.)	DOM (s.s.)	TOC/TN (s.s.)	Inorganic ions (s.s.)	NAs (s.s.)	pH, conductivity, hardness	Inorganic DBPs (a.a.)	HAAs (a.a.)	Volatile DBPs (a.a.)	AOX (s.s.)
Raw water	x	x	x	x	x	x	x	x	x		x	x	x	x	x					x	x	x	x
After aeration tank	x	x	x	x	x	x	x	x	x		x	x	x	x	x					x	x	x	x
After sand filters	x	x	x	x	x	x	x	x	x		x	x	x	x	x					x	x	x	x
After treated water reservoir=outlet of DWTP=sensor 1	x	x	x	x	x	x	x	x	x		x	x	x	x	x					x	x	x	x
Outlet of re-chlorination tank=sensor 2	x	x	x	x	x	x	x	x	x		x	x	x	x	x					x	x	x	x
Inlet of DMA=sensor 3	x	x	x	x	x	x	x	x	x		x	x	x	x	x					x	x	x	x
Distribution 1=sensor 4	x	x	x	x	x	x	x	x	x		x												
Distribution 2	x	x	x	x	x	x	x	x	x		x												
Distribution 3	x	x	x	x	x	x	x	x	x		x												

s.s.: sodium sulfite, a.a.: ascorbic acid

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Table 5: Parameters to be analysed in CS 2.

CS2- Barcelona	Actual sample taken from the plant												Formation potential test										
	EEM (s.s.)	UV (s.s.)	DOM (s.s.)	TOC/TN (s.s.)	Inorganic ions (s.s.)	pH, conductivity, hardness	Inorganic DBPs (s.s.)	HAAs (a.a.)	Volatile DBPs (a.a.)	NAs (s.s.)	AOX (s.s.)	FP tests	EEM (s.s.)	UV (s.s.)	DOM (s.s.)	TOC/TN (s.s.)	Inorganic ions (s.s.)	NA (s.s)	pH, conductivity, hardness	Inorganic DBPs (a.a.)	HAAs (a.a.)	Volatile DBPs (a.a.)	AOX (s.s.)
Raw water	x	x	x	x	x	x	x	x	x		x	x	x	x	x					x	x	x	x
Clarified Post ClO_2	x	x	x	x	x	x	x	x	x		x	x	x	x	x					x	x	x	x
Post AC	x	x	x	x	x	x	x	x	x		x	x	x	x	x					x	x	x	x
Outlet of the tanks	x	x	x	x	x	x	x	x	x		x	x	x	x	x					x	x	x	x
Distribution 1	x	x	x	x	x	x	x	x	x		x												
Distribution 2	x	x	x	x	x	x	x	x	x		x												
Distribution 3	x	x	x	x	x	x	x	x	x		x												

s.s.: sodium sulfite, a.a.: ascorbic acid

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Table 6: Parameters to be analysed in CS 3.

CS3-Madrid	Actual sample taken from the plant												Formation potential test										
	EEM (s.s.)	UV (s.s.)	DOM (s.s.)	TOC/TN (s.s.)	Inorganic ions (s.s.)	pH, conductivity, hardness	Inorganic DBPs (s.s.)	HAAs (a.a.)	Volatile DBPs (a.a.)	NAs (s.s.)	AOX (s.s.)	FP tests	EEM (s.s.)	UV (s.s.)	DOM (s.s.)	TOC/TN (s.s.)	Inorganic ions (s.s.)	NAs (s.s.)	pH, conductivity, hardness	Inorganic DBPs (a.a.)	HAAs (a.a.)	Volatile DBPs (a.a.)	AOX (s.s.)
Raw water	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			x		x	x	x	x
Pre-ozone	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			x		x	x	x	x
Filtered water	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			x		x	x	x	x
GAC filtered water	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			x		x	x	x	x
Outlet of the plant	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			x		x	x	x	x
Distribution 1	x	x	x	x	x	x	x	x	x	x	x												
Distribution 2	x	x	x	x	x	x	x	x	x	x	x												
Distribution 3	x	x	x	x	x	x	x	x	x	x	x												

s.s.: sodium sulfite, a.a.: ascorbic acid

Table 7: Parameters to be analysed in CS 4.

CS4-Ireland	Actual sample taken from the plant												Formation potential test										
	EEM (s.s.)	UV (s.s.)	DOM (s.s.)	TOC/TN (s.s.)	Inorganic ions (s.s.)	pH, conductivity, hardness	Inorganic DBPs (s.s.)	HAAs (a.a.)	Volatile DBPs (a.a.)	NAs (s.s.)	AOX (s.s.)	FP tests	EEM (s.s.)	UV (s.s.)	DOM (s.s.)	TOC/TN (s.s.)	Inorganic ions (s.s.)	NAs (s.s.)	pH, conductivity, hardness	Inorganic DBPs (a.a.)	HAAs (a.a.)	Volatile DBPs (a.a.)	AOX (s.s)
Raw water 1	x	x	x	x	x	x						x	x	x	x					x	x	x	x
Raw water 2	x	x	x	x	x	x						x	x	x	x					x	x	x	x
Raw water 3	x	x	x	x	x	x						x	x	x	x					x	x	x	x

s.s.: sodium sulfite, a.a.: ascorbic acid

5.3. QUALITY CONTROL

Precision is measured as the average and relative percent difference (RPD) of the duplicate analyses of each sample. RPD should be less than 10% otherwise sample has to be flagged as suspect. The coefficient of variation of all the internal standard responses for the complete set of samples must be less than 15%. Individual samples responsible for elevating this value above the threshold should be flagged and considered suspect.

For all analysis a calibration check standard is prepared in the mid-range of the standard calibration curve and is injected every 10 samples. If the detector response for this sample varies more than 10% from the previous injection, all samples analysed between the two injections are flagged for investigation. Each sample bottle set is accompanied by replicate field and travel blanks.

In cases with unknown or mislabelled samples, we will first attempt to determine what sample identification is, based on received samples and shipping list information. If a reasonable idea of the sample is determined, it will be analysed, and those data will be qualified in reports and future data analyses.

5.4. SAFETY

The methods presented here involve the use of chemicals that are known or suspected carcinogens.
Therefore, wear Personal Protective Equipment (PPE) to reduce risk to self (lab coat, gloves, safety glasses, enclosed footwear).
Read material safety data sheet (MSDS) and familiarize oneself with the hazards associated with the chemicals used in this procedure.
Clean work surfaces regularly but immediately after spillage.
Report any incidences or near accidents.
It is advisable that each laboratory prepares their specific risk assessment documents.

5.5. GENERAL WATER QUALITY PARAMETERS AND INORGANIC DBPs

General parameters to characterize water samples include the concentration of bromide, iodide, ammonia, nitrate, dissolved organic carbon (DOC), total organic nitrogen (TON), nitrite, pH, conductivity.

(a) At ICRA the different parameters will be measured using the following equipment:
pH with a pH meter GLP21+ (Crison, Düsseldorf, Germany) and conductivity with a conductivity meter GLP31+ (Crison, Düsseldorf, Germany). Bromide, nitrate, nitrite, ammonium, and other ions such as inorganic DBPs chlorite and chlorate will be analysed by ionic chromatography, on a Dionex ICS-5000 ion chromatography system (Dionex, Sunny Vale, U.S). DOC will be analysed following catalytic oxidation, on a TOC-V CSH (230V) analyser (Shimadzu, Tokyo, Japan), which includes the TNM-1 modules for the total nitrogen determinations. If necessary, bromate will be analysed by commercial laboratory SAILab with an HPLC Agilent 1200 RR and ABSCIEX 3200 QTrap ESI- mass spectrometer.

(b) At UCY the different general parameters will be analysed as follows:
pH will be measured with a pH meter (Model: PL-600) (LABEC, Marrickville, Sydney) and conductivity with a conductivity meter (Model: CD-4318SD) (Lutron Electronics, Coopersburg, PA USA). Total DOC will be measured by an OI Analytical Aurora TOC analyser (Model: 1030) coupled with OI Analytical Auto Sampler (Model: 1088) (Colorado, USA).
Nitrate, nitrite, ammonium, and total hardness as $CaCO_3$ will be measured photometrically using Spectroquant® test kits and an Spectroquant® VIS spectrophotometer (Model: Prove 100). More specifically, the catalogue number of the Spectroquant® test kits and a brief description of the method is provided below:

(a) Nitrate (Catalogue number: 1.09713.0001) / Method: In sulfuric and phosphoric solution nitrate ions react with 2,6-dimethylphenol (DMP) to form 4-nitro-2,6-dimethylphenol that is determined photometrically. The method is analogous to DIN 38405-9.
(b) Nitrite (Catalogue number: 1.14547.0001) / Method: In acidic solution nitrite ions react with sulfanilic acid to form a diazonium salt, which in turn reacts with N-(1-naphthyl)ethylenediamine dihydrochloride to form a red-violet azo dye. This dye is determined photometrically. The method is analogous to EPA 354.1, APHA 4500-NO2- B, DIN EN 26777, and ISO 6777.
(c) Ammonium (Catalogue number: 1.14739.0001) / Method: Ammonium nitrogen (NH_4-N) occurs partly in the form of ammonium ions and partly as ammonia. A pH-dependent equilibrium exists between the two forms. In strongly alkaline solution ammonium nitrogen is present almost entirely as ammonia, which reacts with hypochlorite ions to form monochloramine. This in turn reacts with a substituted phenol to form a blue indophenol derivative that is determined photometrically. Due to the intrinsic yellow coloration of the reagent blank, the measurement solution is yellow-green to green in colour. The method is analogous to EPA 350.1, APHA 4500-NH3 F, ISO 7150-1, and DIN 38406-5.
(d) Total hardness as $CaCO_3$ (Catalogue number: 1.00961.0001) / Method: In neutral solution calcium and magnesium ions react with phthalein purple to form a violet dye that is determined photometrically. The use of a selective masking agent permits a differentiation between calcium and magnesium.

The measuring range of the Spectroquant® tests kits was selected based on the previous measurements of raw water and treated water, which were provided by WDD. It is important to note that Spectroquant® Prove 100 VIS spectrophotometer is preprogrammed for all Spectroquant® test kits and methods. In case the measured ions fall out of the measuring range of the previous test kits, new test kits with the appropriate measuring range will be used.

Bromide, iodide, chlorite, and TON of the UCY samples will be measured at ICRA and the water samples will be shipped with icepacks to ICRA the day following the sampling.

5.6. VOLATILE DBPs with offline liquid-liquid microextraction followed by GC-MS analysis

1. METHOD DESCRIPTION

This method is compiled for the determination of different volatile DBPs in water and is based on an internal method learned in Prof. Howard Weinberg laboratory (University of North Carolina).

Table 8: list of volatiles DBPs included in the method.

Trihalomethanes			Commercial standard
TCM	chloroform	67-66-3	4M8140-U Supelco EPA 501/601 Trihalomethanes Calibration Mix
BDCM	bromodichloromethane	75-27-4	
DBCM	dibromochloromethane	124-48-1	
TBM	bromoform	75-25-2	
Haloacetonitriles			
DCAN	dichloroacetonitrile	3018-12-0	M551B 1ml CLUZEAU Disinfectant byproducts 8 compounds at 5.0 mg/mL in acetone
TCAN	trichloroacetonitrile	545-06-2	
BCAN	bromochloroacetonitrile	83463-62-1	
DBAN	dibromoacetonitrile	3252-43-5	
Haloketones			
1,1-DCP	1,1-dichloropropanone	513-88-2	
1,1,1-TCP	1,1,1-trichloropropanone	918-00-3	
Haloacetaldehydes			
CH	chloral hydrate	302-17-0	
Halonitromethanes			
TCNM	trichloronitromethane (chloropicrin)	76-06-2	
TBNM	tribromonitromethane (bromopicrin)	464-10-8	Toronto Research Chemicals T772600
Internal standard			
1,2DBPd6	1,2-dibromopropane-d6	51209-47-3	3U-D1712 CLUZEAU
TCMd	chloroform-d (optional)	865-49-6	151823 SIGMA-ALDRICH

2. METHOD SUMMARY

30 mL of sample is liquid-liquid extracted with MtBE at pH 3.5. 1 mL sample with internal standard is injected into the GC-MS for analysis.

3. EQUIPMENT

Liquid-liquid extraction

- Clear 60 mL, clean, prewashed glass screw cap sample vials with polytetrafluoroethylene (PTFE)-lined silicone septa. Clean vials by washing with powder detergent solution, rinsing with tap water, and soaking in a 10% ACS-grade HNO_3 bath overnight. The vials should then be rinsed at least three times with tap

water and then rinsed three times with laboratory grade water (LGW) and dried in a 180°C oven for at least 24 hours (optional).
- 50-200 µL Socorex micropipette fitted with clean glass capillary tips
- 100 mL glass volumetric flasks with glass stoppers
- 100 mL amber bottle mounted with 10 mL pump pipetting dispenser containing PFTE transfer line
- 23 cm disposable glass Pasteur pipettes
- Rubber Pasteur pipette bulb
- pH indicator strips pH 0-6
- HPLC amber vials
- HPLC caps
- Vial inserts
- Vortexer

Gas Chromatograph
- Gas chromatograph (GCTrace 1300, Thermo Fisher Scientific) coupled to a mass spectrometer (GC–MS/MS, Thermo Fisher Scientific).

4. REAGENTS

- HPLC water
- Extraction solvent: methyl-tert-butyl ether (Sigma Aldrich, 443808)
- Sodium sulfate (Na_2SO_4)
- Methanol (for rinsing glassware) HPLC grade
- L-ascorbic acid (for quenching residual Cl_2) (Aldrich, 255564)
- Sulfuric acid (for pH adjustment) certified ACS Plus

5. PROCEDURE

BATCH PREPARATION

Internal Standard
- Prepare a primary dilution at 100 µg/mL, depending on the initial concentration, using a micropipette and a volumetric flask containing MtBE, fill to line with MtBE.
- Extracting solution at 50 µg/L or 100 µg/L (depending on what expected concentration of analytes in samples).
- Calculate how much extracting solvent will be needed for all your samples and calibrations (3 mL for each sample and calibration).
- Make from primary dilution and prepare more than needed because there may be bubbles in the dispenser that you need to clear and will need to pump a few times to start out.

Calibration curve
- Prepare a laboratory reagent blank and the laboratory fortified blank at the beginning of each day and analyse on the GC before extracting samples. If QC criteria fail, troubleshoot, and correct the problem, re-analysing these check standards before proceeding to the next step.
- Prepare calibration standards in 100 mL HPLC water according to the range of concentrations expected in the samples.

NOTE: Label all the vials with pencil to avoid chloroforms interferences.

Sample extraction

- Prepare matrix spike (MS) and matrix spike duplicate (MSD) in 25 mL samples. Should be ~2-3 times halogenated volatile levels in samples.
- Measure 30 mL from all calibration standards and samples using a 50 mL measuring cylinder starting from lowest to highest concentration and then follow with the samples all in duplicate and transfer into 60 mL vials. Rinse cylinder 3 times with Milli-Q water and once with sample to be measured next between each. Pour at an angle so halogenated volatiles are not lost through volatilization.
- Adjust all samples and calibrations to approximately pH 3.5 with 0.2 N H_2SO_4 (Amount required for pH adjustment will likely be different for calibrations compared to samples).
- Use remaining 30 mL aliquot from 60 mL vial to determine how much H_2SO_4 will be needed.
- Add 3 mL extracting solvent (containing internal standard) from a solvent dispenser bottle to each 30 mL aliquot. Make sure there are no bubbles in the dispenser addition line.
- Add ~10 g pre-baked sodium sulfate (or high-quality sodium sulfate) to each 30 mL sample/calibration standard (10 g can be measured out in pre-measured marked 10 mL glass beaker). Vortex samples for 1 minute immediately after adding sodium sulfate to avoid clumping. Let samples settle for 5 minutes.
- Using a disposable 23 cm glass Pasteur, transfer ~1.5 mL from the middle of the MtBE layer (top layer) to a vial. Do not transfer any sodium sulfate crystals as they will clog the GC. Cap the vial. Fill three GC vials for each sample (two for analysis, and one backup).
- Store in the laboratory freezer at -15 ºC in a tray covered in aluminium foil if not analysed immediately. Also fill two vials with MtBE and 2 vials of extracting solvent containing MtBE + IS. Analyse within 4 weeks.
- Analyse according to the specified GC method.

Pure standard

- Prepare a non-extracted standard, with all the measured DBPs, in MtBE + IS at 100 ug/ to evaluate the percentage of extraction. An acceptable value is between 70-130.

6. ANALYSIS

Analysis of the extracts is performed with a gas chromatograph (GC Trace 1300, Thermo Fisher Scientific) coupled to a mass spectrometer (GC–MS/MS, Thermo Fisher Scientific). Chromatographic separation was performed using a TG1701MS de Thermo Scientific Column Dimensions: 30 x 0.25 x 0.5 µm. The oven temperature program was as follows: 35 ºC for 5 min, ramp to 100 ºC at 10 ºC /min and then ramp to 200 ºC at 20 ºC /min and held for 1 min. The retention times and the monitored transitions are shown in Table 9.

Table 9: retention times of different DBPs.

Compound	RT (min)	SIM 1 *(m/z)*	SIM2 *(m/z)*
IS-D	4.09	84	86
TCM	4.09	83	85
TCAN	4.90	108	110
BDCM	7.00	83	85
1,1-DCP	8.01	43	83
TCNM	8.08	117	119
DCAN	9.23	74	82
DBCM	9.27	129	127
1,1,1-TCP	10.11	43	125
IS-1,2-DCP-D	10.11	127	129
TBM	11.39	173	171
BCAN	11.53	74	155
DBAN	13.24	118	120

5.7. VOLATILE DBPs with Thermal Desorption- Gas Chromatography MS

1. METHOD DESCRIPTION

A green (no use of solvents) headspace TD-GC-MS method for the detection of all DBPs (THMs, HANs, HNMs, HK, and HA) in a single run is presented.

2. METHOD SUMMARY

Place 10 mL of drinking water samples in a 20 mL headspace glass vial (Agilent). Add 2 g NaCl and 0,5 µL of the 200 ppm solution of internal standard, close with HiSorb septa and allow to reach equilibrium at 25 °C for 24 h. Use polydimethylsiloxane (PDMS) HiSorb probes (Markes International Ltd, UK) that have been pre-conditioned at 260 °C for 25 min under helium flow rate of 75 $mLmin^{-1}$ prior to use. Add a magnetic stirrer to the vial and insert the HiSorb probes through septum. Allow analyte adsorption by continuous magnetic stirring at 1000 rpm for 60 min.

3. EQUIPMENT

- 20 mL headspace vials
- Caps with HiSorb septum
- 1-10 µL Socorex micropipette
- 50 mL glass volumetric cylinder
- 10 mL glass volumetric flask
- Vortex mixer
- Microsyringe 1 mL
- THERMAL DESORPTION - GAS CHROMATOGRAPHY – MASS SPECTROMETRY (TD-GC-MS)
  UNITY-xr thermal desorption (TD) (Markes International Ltd., UK), connected to an Agilent GC-7890B gas chromatograph equipped with a MSD-5977B mass spectrometer (TD-GC-MS)

4. REAGENTS

- Sodium chloride (NaCl)

5. PROCEDURE

Internal Standard

- Prepare a primary solution of 200 µg/mL (200 ppm) depending on the initial concentration using a micropipette and a volumetric flask containing MeOH, fill to line with MeOH.

Table 10: list of trihalomethanes included in the method.

Trihalomethanes			Commercial standard
TCM	chloroform	67-66-3	4M8140-U Supelco EPA 501/601 Trihalomethanes Calibration Mix
BDCM	bromodichloromethane	75-27-4	
DBCM	dibromochloromethane	124-48-1	
TBM	bromoform	75-25-2	
Internal Standard (IS)			
	chlorobenzene-d5	3114-55-4	48086 Sigma-Aldrich

Table 11: retention time of different trihalomethanes and the internal standard.

Compound	EI Ions (*m/z*)
TCM	83, 85, 47, 48, 87, 35, 49
BDCM	83,85, 47, 48, 129, 87
DBCM	129, 127, 131, 48, 47, 91
TBM	173, 171, 175, 91, 93, 252
IS	117, 82, 52, 54, 119, 40

Table 12: different DBPs unregulated DBP species (HANs, KH, HAs, HNMs) and their EI masses.

Haloacetonitriles			Ions
DCAN	dichloroacetonitrile	3018-12-0	74, 82, 76, 84, 47, 35
TCAN	trichloroacetonitrile	545-06-2	108, 110, 73, 47, 82, 112
BCAN	bromochloroacetonitrile	83463-62-1	74, 76, 155, 153, 81, 79
DBAN	dibromoacetonitrile	3252-43-5	120, 118, 199, 79, 81, 201
Haloketones			
1,1-DCP	1,1-dichloropropanone	513-88-2	43, 83, 63, 85, 44, 42
1,1,1-TCP	1,1,1-trichloropropanone	918-00-3	43, 125, 83, 97, 127, 85
Haloacetaldehydes			
CH	chloral hydrate	302-17-0	82, 84, 47, 83, 111, 113
Halonitromethanes			
TCNM	trichloronitromethane (chloropicrin)	76-06-2	117, 119, 82, 121, 47, 84
Internal Standard			
	Chlorobenzene-d5	3114-55-4	117, 82, 52, 54, 119, 40

6. ANALYSIS

The VOCs desorption was performed at 250 °C for 10 min. The desorbed analytes were focused on a graphitized carbon cold trap (Markes International Ltd., UK) at a temperature of -10 °C, under helium carrier gas flow of 40 mL min^{-1}. Then, the cold trap was rapidly heated from -10 °C to 320 °C for 3 min with a split flow of 1:10. A SPB™-624 (60 m x 250 µm x 1.4 µm, Supelco) capillary column was used as the stationary phase; helium (99.999%, Linde) was used as the carrier gas at a flow rate of 1.7 mL min^{-1}. The initial

oven temperature of 45 °C was held for 1 min, ramped to 100 °C at 10 °C min^{-1}, followed by a ramp to 140 °C at 20 °C min^{-1}, held for 1 min, ramped to 180 °C at 3 °C min^{-1} , held for 3 min and then ramped to 220 °C at 20 °C min^{-1}, and held for 1 min. The total analysis time was 28.83 min. The analysis was performed in splitless mode. The temperatures of quadrupole interface, and ion source were set at 150 °C, 250 °C, and 230 °C, respectively. 70 eV EI spectra were recorded in full scan mode between *m/z* 35 to *m/z* 350. The integration of the total ion chromatogram (TIC) peaks was performed using the ChemStation software (Agilent, USA). Finally, NIST 20 library (National Institute of Standards and Technology) was used to identify the eluted compounds.

7. METHOD SUMMARY

The VOCs desorption was performed at 250 °C for 10 min. The desorbed analytes were focused on a graphitized carbon cold trap (Markes International Ltd., UK) at a temperature of -10 °C, under Helium carrier gas flow of 40 mL min^{-1}. Then, the cold trap was rapidly heated from -10 °C to 320 °C for 3 min with a split flow of 1:10. A SPB™-624 (60 m x 250 µm x 1.4 µm, Supelco) capillary column was used as the stationary phase; helium (99.999%, Linde) was used as the carrier gas at a flow rate of 1.7 mL min^{-1}. The initial oven temperature of 50 °C was held for 4 min, ramped to 65 °C at 5 °C min^{-1}, held for 1 min, followed by a ramp to 120 °C at 5 °C min^{-1} , held for 1 min, ramped to 180 °C at 10 °C min^{-1} , held for 2 min and then ramped to 200 °C at 5 °C min^{-1}, and held for 2 min. The total analysis time was 28.83 min. The analysis was performed in splitless mode. The temperatures of quadrupole, interface, and ion source were set at 150 °C, 250 °C, and 230 °C, respectively. EI (70 eV) spectra were recorded in full scan mode from 35 to 350 *m/z*. The integration of the total ion chromatogram (TIC) peaks was performed using the ChemStation software (Agilent, USA). Finally, NIST 20 library (National Institute of Standards and Technology) was used to identify the eluted compounds. An alternative chromatographic column for this analysis would be DB-5MS 30 m × 0.25 mm × 0.25 µm.

5.8. HALOACETIC ACIDS

5.8.1. HALOACETIC ACIDS (UCY)

Concentrations of DBPs in tap water are to be determined using an in-house developed and validated LC-MS/MS method. The method could be modified to reflect the composition of water samples obtained from the water treatment and distribution sites. An alternative column suitable for the anion analysis would be the Waters Anion Polar Pesticide DEA.

1. METHOD DESCRIPTION

Concentrations of disinfection by-products in tap water are to be determined using an in-house developed and validated LC-MS/MS method.

Table 13: list of haloacetic acids and ions included in the method.

Analytes			Commercial standard
ClO_2^-	chlorite ion	7758-19-2	Sigma-Aldrich 71388
ClO_3^-	chlorate ion	16887-00-6	LGC VHG-ICLO3-100
BrO_3^-	bromate ion	15541-45-4	LGC VHG-IBRO3-100
CAA	chloroacetic acid	79-11-8	LGC DRE-A50000518MB
DBAA	dibromoacetic acid	631-64-1	
BAA	bromoacetic acid	79-08-3	
DCAA	dichloroacetic acid	79-43-6	
TCAA	trichloroacetic acid	76-03-9	
DBCAA	dibromochloroacetic acid	5278-95-5	
BCAA	bromochloroacetic acid	5589-96-8	
BDCAA	bromodichloroacetic acid	71133-14-7	
TBAA	tribromoacetic acid	75-96-7	

2. METHOD SUMMARY

An in-house developed single direct injection HPLC-MS/MS method was followed for the determination of chlorite, chlorate, and bromate anions, in addition to the nine (9) haloacetic acids (HAAs) in tap water samples.

3. EQUIPMENT

- Waters Alliance 2695 hyphenated to Waters Quattro Premier XE mass spectrometer fitted with ESI source operated in negative ESI mode
- Waters Symmetry C18, 2.1 x 150 mm, 3.5 um

4. REAGENTS

- Water (LC-MS grade)
- Methanol (LC-MS grade)

- Formic acid (LC-MS grade)

5. PROCEDURE

5.1 HPLC-MS/MS CONDITIONS

Analysis was performed using an Alliance 2696 HPLC module hyphenated to a Quattro Premier XE triple quadrupole mass spectrometer using the following conditions:

Chromatographic Conditions

Column: Symmetry C18 150 x 2.1 mm, 3.5 µm
Flow rate: 0,4 mL/min
Column temperature: 40 °C
Injection volume: 100 µL
Run time: 12,25 min
Autosampler temperature: 10 °C
Mobile phase A: 0,05 % formic acid in water
Mobile phase B: 0,3 % formic acid in 80% methanol

Table 14 : HPLC-MS/MS gradient profile.

Time (min)	% Mobile Phase A (v/v)	% Mobile Phase B (v/v)	Curve
0.00	99	1	1
1.23	99	1	1
3.68	60	40	1
4.29	40	60	1
7.35	0	100	1
9.80	0	100	1
9.86	99	1	1
12.25	99	1	1

Source: ESI-
Source temperature: 120 °C
Capillary voltage: 2.5 kV
Desolvation temperature: 400 °C
Desolvation flow: 500 L/hr
Cone gas flow: 50 L/hr

Table 15: HPLC-MS/MS Multiple Reaction Monitoring (MRM).

Analyte	Precursor (*m/z*)	Products (*m/z*)	Cone (V)	Collision (V)
Chlorite	83.2	67.2 51.2	30 30	15 15
Chlorate	67.1	67.1	30	2
Bromate	126.9	110.9 95.0	30 30	35 35
CAA	93.0 94.8	35.0 37.2	5 5	10 8
DCAA	126.9 128.9	83.1 85.1	30 30	12 12
TCAA	116.8 118.9	35.1 35.1	30 30	10 8
BAA	137.0 138.9	79.0 81.0	30 30	6 5
DBAA	214.9 218.9	170.8 174.8	30 30	12 12
BCAA	171.0 173.1	127.0 129.0	30 30	12 12
BDCAA	160.8 162.9	116.9 118.9	30 30	8 8
TBAA	250.9 252.9	79.2 81.1	20 20	15 12
CDBAA	206.8 208.9	163.0 81.0	30 30	13 13

5.8.2. HALOACETIC ACIDS (ICRA)

1. METHOD DESCRIPTION

This method is compiled for the determination of different HAAs in drinking water.

Table 16: list of haloacetic acids included in the method.

Haloacetic acids			Commercial standard
BAA	bromoacetic acid	79-08-3	49107-U Supelco EPA 552.2 Haloacetic Acids Mix
CAA	chloroacetic acid	79-11-8	
DBAA	dibromoacetic acid	631-64-1	
DCAA	dichloroacetic acid	79-43-6	
TCAA	trichloroacetic acid	76-03-9	
DBCAA	dibromochloroacetic acid	5278-95-5	
BCAA	bromochloroacetic acid	5589-96-8	
BDCAA	bromodichloroacetic acid	71133-14-7	
TBAA	tribromoacetic acid	75-96-7	

2. METHOD SUMMARY

Derivatization method based on EPA (2003) is used for the determination of HAAs in drinking water.

3. EQUIPMENT

- 60-mL clear glass vials with PTFE-lined screw caps
- Autosampler vials
- Graduated conical centrifuge tubes PTFE-lined screw caps
- Pasteur pipettes
- Adjustable volume dispensers
- Pipettes
- Volumetric flasks
- Micro syringes
- Thermostated water bath
- pH paper
- Balance
- Vortexer
- GC Trace 1300, Thermo Fisher Scientific with SPME module coupled to a mass spectrometer (GC–MS/MS, Thermo Fisher Scientific).

4. REAGENTS

- Dimethyl sulfate (DMS)
- Diethyl sulfate (DES)
- Tetrabutylammonium hydrogen sulfate (TBA-HSO_4)
- 2,3-dibromopropionic acid (98%) and 1,2-dibromopropane (97%) used as surrogates

- Methanol
- Sulfuric acid
- Methyl tertbutyl ether
- Anhydrous sodium sulfate
- Copper (II) sulfate pentahydrate

5. PROCEDURE

SAMPLE EXTRACTION
Place into a precleaned 60-mL glass vial:

- 20 mL of the water sample using a 10 mL pipette.
- 50 µL of 400 ppb surrogate to the aqueous sample.
- 1 mL of concentrated H_2SO_4 to adjust the pH to less than or equal to 0.5 using an automatic pipette with filtered tip.
- 9 g of muffled or high quality Na_2SO_4 (to increase the ionic strength due to the precipitation remained at the bottom of the vial). To be added as the final substance.
- Shake the 60 mL glass vial until it dissolves.
- Insert with automated dispenser exactly 2 mL MtBE which contains the Internal Standard at a concentration of 10 ppb.
- Agitate the vial vigorously for three minutes by hand.
- Allow the phases to separate for 5 minutes.

SAMPLE METHYLATION WITH ACIDIC METHANOL

- Transfer 1,5 mL (upper MtBE layer) with a Pasteur pipette to a 15 mL graduated conical centrifuge tube.
- Add 1,5 mL of 10% sulphuric acid in MeOH and cap the 15 mL centrifuge tube. To prepare 100 mL of 10% H_2SO_4 in acid solution: working under fume hood is necessary. In a washbasin, place accumulators, water and a 100 mL flask half-full of approximately 50 mL of MeOH. Add drop by drop 10 mL of concentrated sulfuric acid measured in a graduated cylinder. Finally, fill to line with MeOH.
- Place the centrifuge tubes in a rack and heat them up to 50 ºC by means of a water bath. To ensure that the temperature is maintained, insert a thermometer and 1,5 mL of water in a centrifuge tube. To avoid heat loss, cover lightly the water bath.
- Heat the centrifuge tubes for 2 hours.
- Allow the centrifuge tubes to cool down before removing their caps by placing the vials under tap water.
- Add 3,5 mL of 150 g/L Na_2SO_4 solution with a micropipette to each centrifuge tube. To prepare 100 mL of 150 g/L Na_2SO_4: First 15 g of Na_2SO_4 are weighed using weighing boats. Insert a magnetic stir bar and some millilitres of Fisher water in a beaker and place it on the stirrer. Add and stir slowly the amount of Na_2SO_4 checking that it dissolves completely. Once all the Na_2SO_4 has been incorporated, the solute is transferred to a 100 mL flask. Finally fill to line with Fisher water.
- Vortex each centrifuge tube to ensure that the two phases are evenly mixed and have achieved complete equilibrium.
- Wait for the two phases to settle completely in the 100 mL flask but avoid leaving them for an extended period as this may result in the breaking of chemical bonds.
- Extract the upper phase and discard the lower layer with a long Pasteur pipette, ensuring that no more than 0.3 mL of the aqueous phase is left behind.

- Add 0,5 mL of saturated $NaHCO_3$ solution to each centrifuge tube. To prepare 100 mL of saturated $NaHCO_3$ solution: a transparent vessel containing water and a magnetic stir bar is placed on a magnetic stirrer. $NaHCO_3$ is added incrementally until a precipitate is observed.
- Vortex each centrifuge tube to ensure that the two phases are evenly mixed and have achieved complete equilibrium.
- Transfer 0,5 mL (upper layer) to an auto-sampler vial with a Pasteur pipette. Use the remaining extract to fill another vial to create a duplicate vial.
- Store the extracts in the freezer.

INSTRUMENTAL METHOD

- Operation conditions (retention times, compound separation, and resolution) are set and appropriate relative retention time window for each target and surrogate are established to identify them in the samples.
- Analysis of the extracts is performed with gas chromatography (GC Trace 1300, Thermo Fisher Scientific) coupled to a mass spectrometer (GC–MS/MS, Thermo Fisher Scientific). Chromatographic separation was performed using a TG1701MS de Thermo Scientific Column Dimensions: 30 x 0.25 x 0.5 µm. The oven temperature program was as follows: 35 ºC for 4 min, ramp to 115 ºC at 20 ºC /min hold 1 minute and then ramp to 245 ºC at 40 ºC /min and held for 3 min.

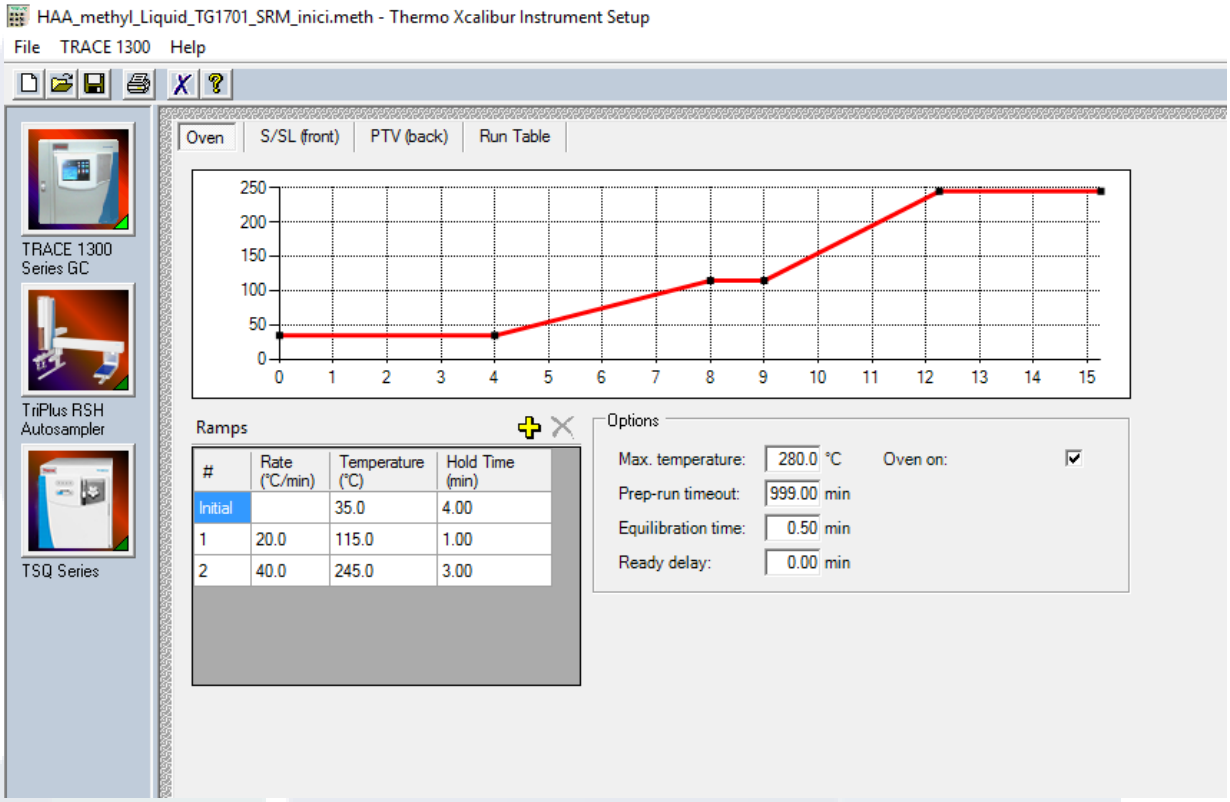
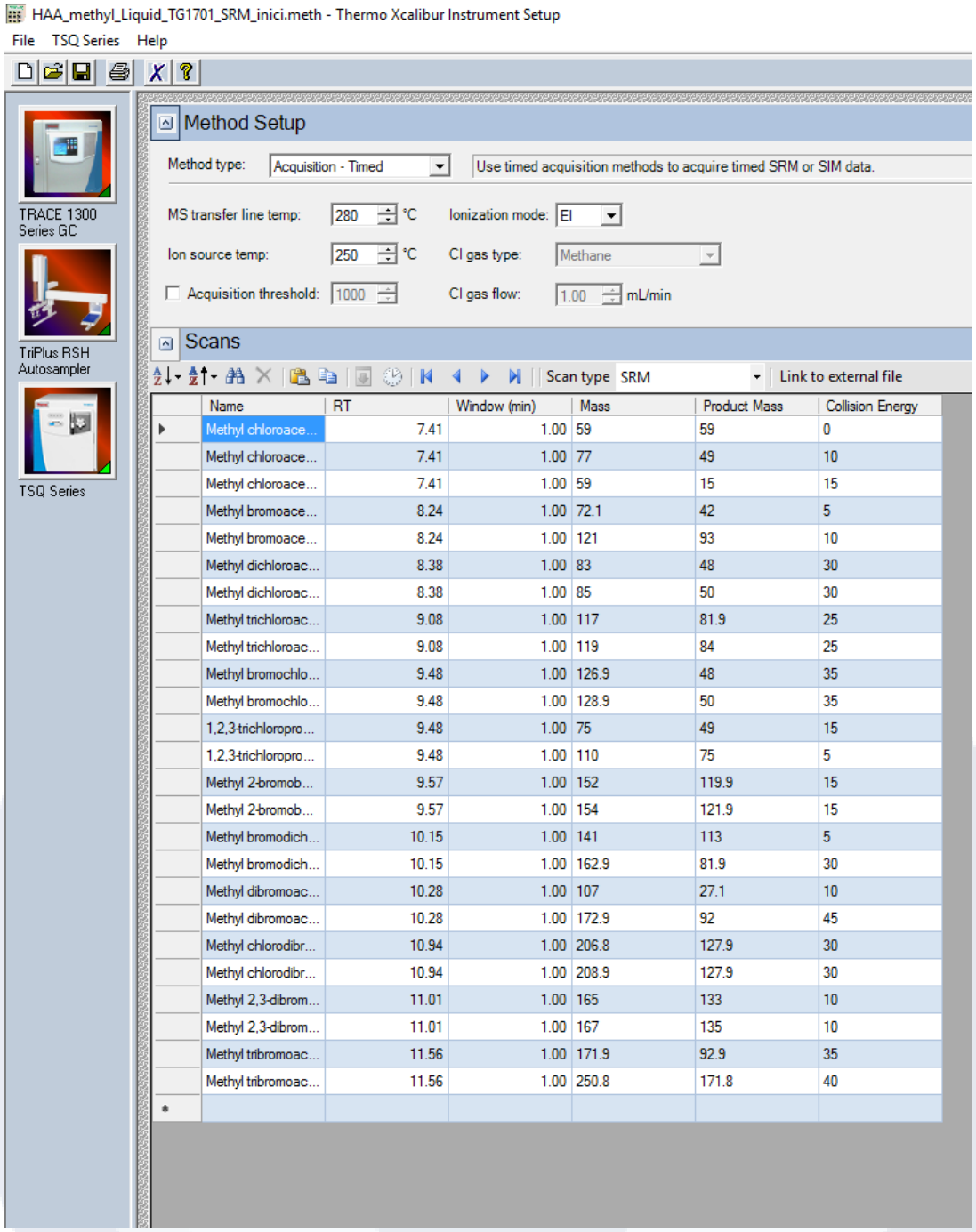


Figure 8. Thermo Xcalibur Software Chormatographic run.



Name	RT	Window (min)	Mass	Product Mass	Collision Energy
Methyl chloroace...	7.41	1.00	59	59	0
Methyl chloroace...	7.41	1.00	77	49	10
Methyl chloroace...	7.41	1.00	59	15	15
Methyl bromoace...	8.24	1.00	72.1	42	5
Methyl bromoace...	8.24	1.00	121	93	10
Methyl dichloroac...	8.38	1.00	83	48	30
Methyl dichloroac...	8.38	1.00	85	50	30
Methyl trichloroac...	9.08	1.00	117	81.9	25
Methyl trichloroac...	9.08	1.00	119	84	25
Methyl bromochlo...	9.48	1.00	126.9	48	35
Methyl bromochlo...	9.48	1.00	128.9	50	35
1,2,3-trichloropro...	9.48	1.00	75	49	15
1,2,3-trichloropro...	9.48	1.00	110	75	5
Methyl 2-bromob...	9.57	1.00	152	119.9	15
Methyl 2-bromob...	9.57	1.00	154	121.9	15
Methyl bromodich...	10.15	1.00	141	113	5
Methyl bromodich...	10.15	1.00	162.9	81.9	30
Methyl dibromoac...	10.28	1.00	107	27.1	10
Methyl dibromoac...	10.28	1.00	172.9	92	45
Methyl chlorodibr...	10.94	1.00	206.8	127.9	30
Methyl chlorodibr...	10.94	1.00	208.9	127.9	30
Methyl 2,3-dibrom...	11.01	1.00	165	133	10
Methyl 2,3-dibrom...	11.01	1.00	167	135	10
Methyl tribromoac...	11.56	1.00	171.9	92.9	35
Methyl tribromoac...	11.56	1.00	250.8	171.8	40

Figure 9. Thermo Xcalibur Software and aspects of GC-MS/MS method set-up.

5.9. N-NITROSAMINES

1. METHOD DESCRIPTION

This method is compiled for the determination of different N-nitrosamines in water and is an adaptation of US EPA method 521 (2005).

Table 17: list of nitrosamines included in the method.

N-nitrosamines			Commercial Standard
NDMA	N-nitrosodimethylamine	62-75-9	502138 Supelco EPA 8270/Appendix IX Nitrosamines Mix certified reference material, 2000 µg/mL each component in dichloromethane
NDBA	N-nitrosodibutylamine	924-16-3	
NDPA	N-nitrosodi-n-propylamine	621-64-7	
NDMEA	N-nitrosomethylethylamine	10595-95-6	
NDEA	N-nitrosodiethylamine	55-18-5	
NPYR	N-nitrosopyrrolidine	930-55-2	
NPIP	N-nitrosopiperidine	100-75-4	
NDPhA	N-nitrosodiphenylamine	86-30-6	
NMOR	N-nitrosomorpholine	59-89-2	
NDPAd14	N-nitrosodipropylamine-d14	621-64-7	Accustandard (M-521-1S)
NDMAd6	N-nitrosodimethylamine-d6	17829-05-9	Accustandard (M-521-SS)
NDEAd10	N-nitrosodiethylamine-d10 (optional)	1219794-54-3	

2. METHOD SUMMARY

1 L of sample is passed through coconut carbon solid-phase extraction (SPE) cartridges. Nitrosamines are eluted with 30 mL dichloromethane and reduced in volume to 1 mL using a nitrogen blow down apparatus. 1 mL sample is transferred to vials and analysed by GC-MS.

3. EQUIPMENT

Solid-Phase Extraction (SPE)

- Top pan balance capable of accurately weighing to 1 g
- Microsyringe 50 µL
- Solid-phase extraction (SPE) columns
- Restek pre-packed coconut carbon cartridges (cat # 26032) SPE for US EPA method 521
- Empty SPE tubes to use as reservoirs, Phenomenex strata (Part # AH0.7002)
- 15 mL tapered graduated glass tubes
- Filter paper
- 3 mm diameter transfer Teflon tubing with seals
- Vacuum manifold
- Vacuum reservoir
- Glass beakers

- Pasteur pipettes
- Vials

Gas Chromatograph -MS/MS

- Gas chromatograph (GC Trace 1300, Thermo Fisher Scientific) coupled to a mass spectrometer (GC–MS/MS, Thermo Fisher Scientific).

4. REAGENTS

- All reagents must be HPLC grade or higher unless otherwise specified
- Methanol
- Dichloromethane
- HPLC Water
- Sodium sulfate (dried at 400ºC overnight)
- Nitric acid for acid bath

5. PROCEDURE

BATCH PREPARATION

Standard Preparation

- Prepare a solution of d6-NDMA and d10-NDEA at 0.5 mg/L in MeOH depending on the initial concentration.
- Prepare a solution of nitrosamine mix at 0.5 mg/L in MeOH depending on the initial concentration.
- Preparation of nitrosamine spike sample: add 100 µL of 0.5 mg/L nitrosamine standard mix to 1 L of HPLC grade water giving a final concentration of 50 ng/L.
- Blank water sample: 1 L of HPLC water.

SPE COCONUT CHARCOAL SPE

- Record the combined mass of sample and sample bottle prior to the start of the extraction on the batch sheet (without cap).
- Spike each sample and blank with 50 µL of surrogate (d6-NDMA and d10-NDEA 50 µL of 0.5 mg/L into 1L sample gives a surrogate standard concentration of 25 ng/L each).
- Set up a vacuum manifold. Line up coconut carbon SPE labelled corresponding to sample number.
- Condition SPE (apply vacuum, approximately 10 mL/min) with:
    - 1 reservoir dichloromethane, carbon must go to dryness
    - 2 reservoirs methanol, carbon must go to dryness
    - 3 reservoirs water (HPLC grade), DO NOT allow carbon to go to dryness. Turn tap off as soon as the water enters the SPE cartridge tube.
- Fill the reservoir with HPLC grade water.
- Apply sample to carbon column (vacuum is required). To do this transfer tubes are placed into the sample bottle and the tubes adaptors placed on top of the corresponding reservoirs. Sample loading rate should be approximately 10 mL/min. Check samples frequently to ensure loading rate is optimum.

NOTE: Ensure that the transfer tubes are clean. Pass through methanol and HPLC grade water before and after use.

- As each individual sample finishes passing through the SPE, close the valve. Once all the samples are through open all valves and dry the SPE, using vacuum, for 1 hour.

ELUTION OF NDMA AND REMOVAL OF WATER FROM THE SAMPLE
Label graduated 2 x 15 mL glass tubes for each sample (1, 1b / 2, 2b / ...).

- To a clean and dry empty 3 mL reservoir insert a filter paper into the bottom and fill with sodium sulfate (dried at 400°C overnight).
- Remove a carbon cartridge from the manifold and attach it, using an adaptor, to the top of the sodium sulfate reservoir. Add an empty reservoir to the top of the carbon cartridge using an adaptor and fill with dichloromethane.
- Place a 15 mL graduated glass tube underneath the cartridge and force the dichloromethane through the carbon cartridge and then the sodium sulfate using a syringe. A total elution volume of 30 mL is required (2 x 15 mL).

NOTE: Elution must be done in a fume hood. Dichloromethane is a highly volatile solvent.

- Record the weight of the sample bottle (without caps) after completion of SPE.

REDUCTION OF VOLUME

- Using a nitrogen blow down, reduce the sample volume to 1 mL. When the tubes have reduced to approximately half combine tube 1 with 1b and continue evaporation to 1 mL.
- Transfer the sample, using a Pasteur pipette into 2 mL amber glass vials and add 25 µg/L of IS d14-NDPAscrew cap ready for analysis by GC-MS.

ANALYSIS
Analysis of the extracts is performed with a gas chromatograph (GC Trace 1300, Thermo Fisher Scientific) coupled to a mass spectrometer (GC–MS/MS, Thermo Fisher Scientific). Chromatographic separation was performed using a TG1701MS de Thermo Scientific Column Dimensions: 30 x 0.25 x 0.5 µm. The oven temperature program was as follows: 35 ºC for 1 min, ramp to 130 ºC at 25 ºC min^{-1} and then ramp to 250 ºC at 20 ºC min^{-1} and held for 2 min. The retention times and the monitored transitions are shown in Table 18. Detection is made by SRM mode. The first transition is used for quantification and the second one for confirmation. Data is processed with TraceFinder software.

Table 18: retention times and monitored transitions.

Compound	R_T (min)	Mass	Transition 1 *(m/z)*	Transition 2 *(m/z)*	Energy 1 (eV)	Energy 2 (eV)
NDMA	4.65	74.1	42	44.1	15	5
NDMA d6	4.67	80.1	46.1	50.1	15	5
NMEA	5.31	88.1	42	71	15	5
NDEA	5.80	102.1	44.1	85.1	10	5
NDEA d10	5.80	112	62.1	94.2	15	5
NDPA	7.00	130.2	43.1	113.2	10	5
NDPA d14	7.00	144	50.1	126.2	10	5
NMOR	7.30	116.1	56	86.1	10	5
NPYR	7.45	100.1	55	70.1	5	5
NPIP	7.60	114.1	84.1	97.1	5	5
NDBA	8.37	116.2	74	99.1	10	5

QUANTIFICATION
The quantification, recovery of surrogates will be considered and corrected, as well as the exact volume extracted.

5.10. ADSORBABLE ORGANIC HALOGENS

1. METHOD DESCRIPTION

This method is compiled for the determination of adsorbable organically bound chlorine, bromine, and iodine in water.

2. METHOD SUMMARY

This method determines adsorbable organically bound chlorine, bromine and iodine by means of combustion and subsequent ion chromatography measurement.
If only dissolved analyte components are to be analysed or undissolved sample components lead to clogging of the adsorption column, the sample shall be filtered through a filter with a pore size of 0,45 µm.

3. EQUIPMENT

- Amber glass bottles
- Volumetric flasks
- Balance
- Pipette
- TXA-04 module
- Channel tubes
- Plastic bottles
- Activated carbon cartridges
- Pre-baked ceramic boats

Combustion Ion Chromatograph

- Mitsubishi AQF-2100H combustion unit
- Mitsubishi GA-210 gas absorption unit
- Ion Chromatograph

4. REAGENTS

- Sodium nitrate
- Nitric acid
- Methanol
- Milli-Q water
- Hydrogen peroxide
- Calibration standards

5. PROCEDURE

BATCH PREPARATION

- Prepare the needed solutions to process the sample:
    - Nitric nitrate stock solution, c (HNO_3) = 0,2 mol for the AOCl, AOBr, and AOI determination.

Dissolve 17 g of sodium nitrate in water in a 1000 mL volumetric flask, add 25 mL of nitric acid and dilute to volume with water. If stored in an amber glass bottle, the solution is stable for six months.

- Nitric nitrate wash solution, c ($NaNO_3$) = 0,001 mol, pH ≈ 1.7 for the AOCl, AOBr and AOI determination

Pipette 5 mL of nitric nitrate stock solution into a 1000 mL volumetric flask and dilute to volume with nitric acid dilution water. Acidified dilution water is made with 100 mL water and acidified with 0,2 mL nitric acid. The solution is stable for 4 weeks if stored in an amber glass bottle.

AOCl, AOBr, AND AOI BLANK DETERMINATION

Take 100 mL of acidified dilution water, add 5 mL nitric nitrate **stock** solution, and analyse in the same way as the sample.

AOCl, AOBr, AND AOI EXTRACTION AND ANALYSIS

- Sample extraction is done with the TXA-04 module.



Figure 10: The *TXA-04 extraction unit.*

- Before starting wash channels 1 and 2 with methanol and Milli-Q water. For that connect the channel tubes to the methanol bottle and press “Wash”. Then connect the channel tubes to the Milli-Q water and press “Wash”.
- Subsequently each channel is washed with 10 mL of the sample that will be passed through.
- Then, load the carbon cartridges to the holder (2 for each holder) and place it in the instrument (TXA-04) and connect the tubes to the carbon cartridges.
- Take 100 mL of the homogenized sample (do not shake it vigorously), add 5 mL nitric nitrate **stock** solution and pass it through the carbon cartridges. In case of dilution, dilute the sample portion up to 100 mL total volume with acidified solution water, add 5 mL of nitric nitrate **stock** solution and process. If dilution is necessary and in case of inhomogeneous samples, do not apply less than 5 mL of the original sample. If the dilution factor exceeds 10, dilute in at least two steps.
- Afterwards, 10 mL of the nitric nitrate wash solution is passed through the cartridges.
- The activated carbon columns (containing the adsorbed organic halogens) are transferred to prebaked ceramic boats, using the appropriate tool, to be combusted using Mitsubishi AQF-2100H Combustion Unit.

- In the gas absorption unit, Mitsubishi GA-210, takes place the absorption of the organic halogens from combustion.
- Then the instrument will automatically inject the sample to the ionic chromatograph.

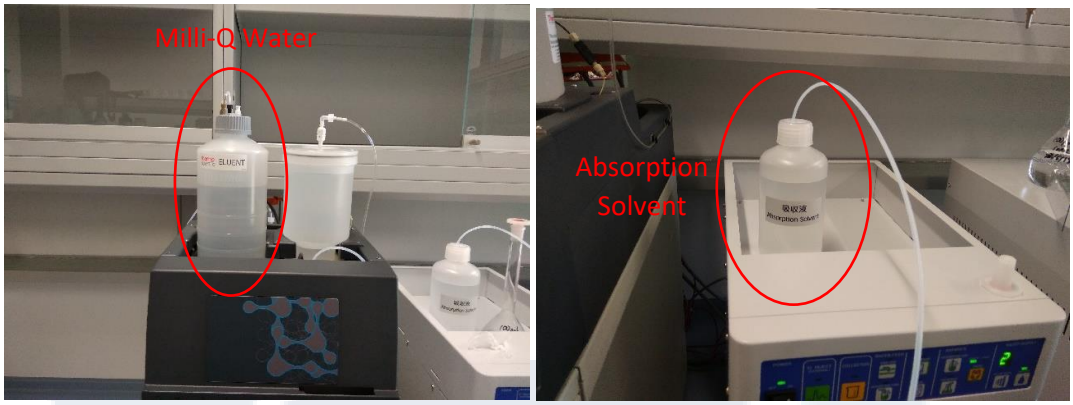


Figure 11: Photos of the tanks to replace Milli-Q water.

- Use of the furnace, and the absorption and chromatography units:
- Before starting using the instrument, replace the Milli-Q water (Eluent), the washing water containers (rinse plastic bottles with Milli-Q water before filling them again with fresh Milli-Q water) and the hydrogen peroxide (Absorption Solvent) at 30 ppm. Also check the waste containers and deposit them properly if they are full.
- Open each gas bottle (O_2 and Ar).
- First switch on the instrument and then the software (Chromeleon Console). Once the software opens, wait 80s and press connect (wait until the green light turns on).
- Then it is needed to prime the system to avoid air going into the column. For that open the valve 90 degrees and press prime and wait 5-10 minutes.



Figure 12: Details of the valve location

- After this time switch off the prime button and close the valve. Then switch on the Compartment_TC, Column_TC and Pump_ECD and wait for the pressure to rise above 1000 psi. After, turn on the electrochemical part above (Suppressor, CR_TC and Eluent Generator Cartridge.
- Wait (around 15 min) until the conductivity (CD and CD Total) is stabilized on the third decimal. Its value should be around 0.6µS.
- At this point, check also that the Cell Heater on the CDet tab is on and at 35 ºC and the Suppressor Current on the Electrolytics tab is set at 186mA.
- Summary of conditions:
    - Flowrate = 1ml min^{-1}
    - Eluent concentration = 30mM
    - Column_TC = 30 ºC
    - Compartment_TC = 15ºC
    - Supressor current = 186 mA
    - Cell Heater Temperature 35 ºC

- In the sequence created there will be the calibration standards followed by three blank determinations (Milli-Q water) and then the samples. Save the sequence.
- Go to the queue tab on the instruments section. Click on “Add”, look for your sequence and open it.
- Do not add a tick mark on the small square to the left of your sequence (startup column). Click on “Ready check”.
- Go back to the “Data” section and press the “Start” button on the upper left side of the screen. The instrument is now ready to inject. You should see the row in the injection list green and the status “running”.
- The injection of the first three Milli-Q water samples (blanks) is done through the GA-210. There is an injection line on the left side of the equipment with the label “STD”. Immerge the injection line in the Milli-Q water and press the “IC inject (external)” button on the GA-210 Gas Analyzer Unit. You will hear the equipment purging the line with the sample and injecting the sample. You must do this manual process for each Milli-Q sample.

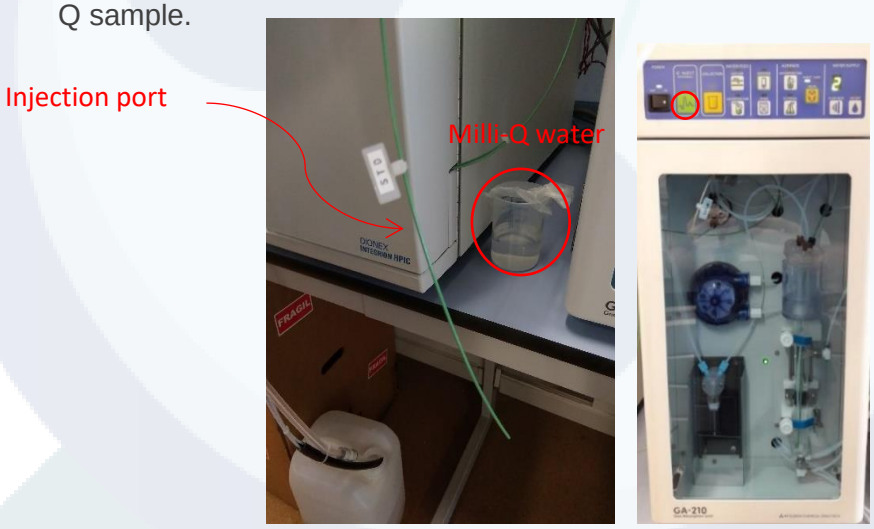


Figure 13: The GA-210 Unit.

COMBUSTION SOFTWARE (NSX-2100)

- Open the two gas bottles before switching on the gas and heater, respectively the red valve and the yellow flame.
- Once the system is heating, make your sequence. In the left bar of the program go to “Open Schedule”. Then press “Open”. The GREEN frame should open with a sequence.

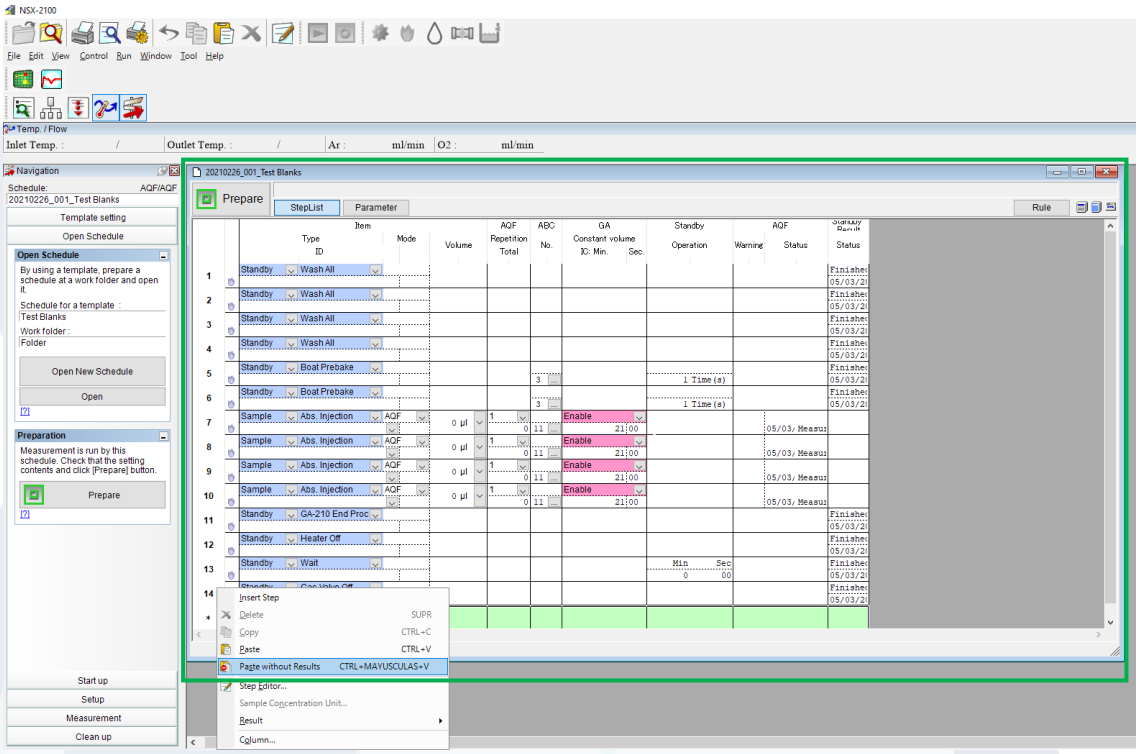


Figure 14: Details of NSX-2100 software.

- Before every measurement do 4 “wash all” and 2 “boat prebakes” (ABC program: 3), afterwards add the samples. End with the “END PROTOCOL”, then “Heater off”, “Wait” and finally “Gas valve off”. Make sure these 4 steps end your sequence.
- Once your sequence is ready press “Prepare” in the upper left corner.
- First load your sample, then to start the sequence, press the blue blinking play button on the left from the red “Heater valve¨.

5.11. DBP FORMATION POTENTIALS

1. METHOD DESCRIPTION

This method is compiled to determine the potential of potable source waters that form DBPs in influence of direct chlorination.

2. METHOD SUMMARY

The required sample volume is chlorinated at standard conditions (pH ≈ 7.0) with an excess of free chlorine and incubated from 1 to 7 days in the dark at 21-25 ºC. At the end of the incubation period, a free chlorine residual of at least 1mg/L is required. DBPs are liquid-liquid extracted and analysed by the GC-MS method. This has been adapted from Standard Methods 5710 B (2018).

3. EQUIPMENT

- Acid washed glassware material (100 mL glass volumetric flasks with glass stoppers, 100-mL beaker)
- Pipette
- Pipette tips
- Incubator (Temperature 21-25 ºC)
- Spectrophotometer HACH
- Free and total Test Kit HACH (LCK cuvette test system)
- 0.7µm glass filter membrane (GF/F)
- Vacuum filtration system

4. REAGENTS

- Phosphate buffer: Dissolve 68.1 g of potassium dihydrogen phosphate (anhydrous) (KH_2PO_4) and 11.7 g of sodium hydroxide (NaOH) in 100 mL of organic-free water (HPLC grade is recommended). For 2500 mL of sample, 5 mL of phosphate buffer is required. Calculate the required buffer solution volume if the volume is different.
- Hypochlorite solution: Calculate the volume of hypochlorite solution required. Prepare a concentrated aqueous sodium hypochlorite (NaOCl) solution using organic-free water. The solution concentration will depend on the quality of the water sample and the desired free chlorine residual after incubation. Verify the solution concentration by analysing free chlorine value using a LCK cuvette test. In case measured value doesn't match the calculated one, correct and use the real value for further calculations.
- Quenchers. Depending on the analysis to be carried out afterwards different quenchers have to be used. See section 5.2.

5. PROCEDURE

CHLORINE DEMAND

- To better estimate the chlorine demand of your sample, the following parameters must be defined: incubation period, free chlorine residual value, Total Organic Carbon

(TOC) and ammonia concentration of the samples. Take into consideration possible interferences such as, organic DBP-precursor material present in reagents and glassware, water exposure to free chlorine before sample collection, among others.

- From previous experiences, for samples with TOC concentrations ≈3 mg/L, the chlorine solution has been dosed in a range of 2-6 ppm for an incubation period of 48h and aiming to obtain a residual chlorine of 0.5-1 ppm.

SAMPLES CHLORINATION

- To increase the likelihood of achieving the desired free chlorine residual, dose at least 3 samples portions providing a range of chlorine concentrations with each chlorine dose differing in increments of 2 ppm. Each sample must have a duplicate in order to have enough volume for the verification of residual chlorine at the end of the incubation period. The doses calculations are based on the real hypochlorite solution concentration.
- For sample chlorination, first, add the buffer solution followed by the sample (3/4 of the volume), the chlorine dose and finish by completely filling the container. Close the container avoiding any headspace or air bubble.
- Add 1 blank (organic-free water) to go through the whole process for quality control purposes.

FORMATION POTENTIAL TEST

- Place the samples inside the incubator for the desired incubation period in the dark at a temperature between 21-25 ºC.

SAMPLE ANALYSIS

- After the reaction period, use the duplicates to measure the free and total chlorine concentration of all the samples. Select the dose which provides the desired free chlorine concentration and separate this batch of samples for reaction quenching.

QUENCHING

- Add the required quenching solution according to the free chlorine concentration value of each sample. Seal again the vials with the screw caps verifying absence of air inside the sample.
- If DBPs are not extracted immediately, store the samples at 4ºC until extraction day (preferably no longer than 3 days). Before beginning extraction process, samples must reach room temperature.

5.12. UV-VIS SPECTRA

1. METHOD DESCRIPTION

This method is compiled for the determination of UV/VIS spectra in drinking water.

2. METHOD SUMMARY

The method to determine UV/VIS spectra in samples of drinking water involves the use of a spectrophotometer to measure the absorbance of UV/VIS light by the water sample. After the sample collection, samples must be filtered with GF/F 0.7 µm.

3. EQUIPMENT

- Glass fiber membrane filters 0.7 µm
- Filter paper
- Optical paper
- UV-Visible ChemStation Software
- UV/VIS Spectrophotometer

4. REAGENTS

- Milli-Q water
- Methanol or ethanol

5. PROCEDURE

BATCH PREPARATION

- Filter the samples with GF/F 0.7 µm as soon as possible after collecting the sample.

Before starting

- Put the samples at room temperature the night before.
- Renew the Milli-Q water every day.
- The cuvette will be manipulated by touching the edges of it or the top, never its sides. This way we will avoid dirtying and scratching it.
- Use the same cuvette for all MilliQ and samples measures. That is why it is important to clean it well. At the beginning of the day, you will pass through distilled water under pressure, then ethanol or methanol, and then Milli-Q. Remove excess water without rubbing the cuvette, just by contact with filter paper. Once dry, clean it with optical paper.
- Turn on the spectrophotometer (Agilent Cary 4000 UV) and the computer.
- Wait 30 minutes for the lamp to warm up.
- Open the spectrophotometer software (*Scan*).
- Check that the parameters are correct:
    - *Setup/ Cary option/ X Mode*
        - Mode: Nanometres
        - Start: 800.00nm

        - Stop: 200,00nm
    - *Baseline:* Baseline Correction
    - *Report:* ASCII (csv)
- Fill cuvette with Milli-Q water and place in the spectrophotometer.
- Click *Baseline*.
- Fill cuvette with sample and place on spectrophotometer.
- When the traffic light is green, click *Start*.
- A window will open in which it will ask you for the name of the Excel file that will contain the results.
- Prior to each measurement, it will ask for the name of each sample. By default, Sample 1, Sample 2 and so on appears, but you can give it whatever name you want.
- Check the absorbance to avoid the Inner Filter Effect (IFE). If the absorbance at 254nm is greater than 0.3 cm-1, may give IFE and the sample may need to be diluted.
- Once the analysis of all samples is done, turn on the equipment, the software and the computer.

5.13. FLUORESCENCE SPECTRA

1. METHOD DESCRIPTION

This method is compiled for the determination of fluorescence spectra in drinking water.

2. METHOD SUMMARY

The method to determine fluorescence spectra in samples of drinking water involves the use of a fluorescence spectrophotometer to measure the fluorescence light emitted by the water sample. After the sample collection, samples must be filtered with GF/F 0.7 µm.

3. EQUIPMENT

- Glass fiber membrane filters 0.7 µm
- Filter paper
- Optical paper
- FL Solutions Software
- Hitachi Fluorescence spectrophotometer

4. REAGENTS

- Milli-Q water
- Methanol or ethanol

5. PROCEDURE

SAMPLE PREPARATION

- Filter the samples with GF/F 0.7 µm as soon as possible after collecting the sample.

Before starting

- Put the samples at room temperature the night before.
- Renew the Milli-Q water every day.
- The cuvette will be manipulated by touching the edges of it, never its sides. This way we will avoid dirtying and scratching it.
- Use the same cuvette for all Milli-Q and samples measures. That is why it is important to clean it well. At the beginning of the day, you will pass through distilled water under pressure, then ethanol or methanol, and then Milli-Q. Remove excess water without rubbing the cuvette, just by contact with filter paper. Once dry, clean it with optical paper.
- First switch on the equipment (Hitachi) and then turn on the computer (software).
- Open the *FL Solutions* software.
- Turn on the fluorimeter.
- Wait 45 minutes for the device to warm up.
- Load the method:
- *Method/General/Load/*Corresponding method.*flm*
- Verify that the method is correct in the *Instrument* tab:

Data Mode: Fluorescence	
EX Start WL: 200	EX Slit: 5
EX End WL: 450	EM Slit: 5
EX Sampling Interval: 3	PMT Voltage: 700
EM Start WL: 250	PMT Voltage 0-100: No
EM End WL: 600	Response: Auto
EM Sampling Interval: 3	Corrected Spectra: Yes
Speed: 12000	Shutter Control: Yes

* Depending on the samples, the excitation (EX) and emission (EM) ranges could be modified. EX (250-450) and EM (250-550).

- Select the location of the results. Click on *Sample*:
  *Sample/Select/Desktop/*Corresponding Folder

- Create a folder with the date YYYYMMDD (e.g., 20230221). Also indicate the name of the file and the name of the sample, which will be the same.
- Once this is done, make a Milli-Q measurement, which will be repeated every 10 samples. The Milli-Q file name will be YYYYMMDD_MQX. Indicate in the name of each sample which Milli-Q measure corresponds to: MQ1, MQ2, etc.
- Fill the cuvette with Milli-Q and put it into the spectrophotometer. Close the lid.
- Click on *Measure.*
- Between sample and sample the cuvette will be cleaned 5 times: the first 4 times with a little Milli-Q water, removing the remains of the previous samples, and the last one with the sample to be processed. Drying will be as indicated at the beginning of this protocol.
- Fill the clean cuvette with the sample and put it into the spectrophotometer, renaming the file again, and then clicking *Measure*.
- Once finished, close the program. It will ask if you also want to turn off the lamp, click accept. For the lamp to cool with the help of the fan, wait about 15-20 min. before turning off the equipment.

5.14. HRMS FINGERPRINTING

1. METHOD DESCRIPTION

This method summarizes the sample extraction protocol for extracting dissolved organic matter for characterization with high resolution mass spectrometry (DOM-HRMS). It has been adapted from Dittmar (2008).

2. METHOD SUMMARY

Dissolved organic matter is extracted with PPL prepacked cartridges and eluted with methanol for preparation to injection in the Orbitrap MS/MS.

3. EQUIPMENT

- 500mg 3 mL PPL column (Agilent Technologies 12105006)
- SPE column rack
- Supply of N_2
- Pre-weighed 2 mL HPLC vials
- Pipette
- Elution tube
- 50 mL volumetric flask
- Acidified MQ/HPLC water

4. REAGENTS

- Methanol
- Formic acid 0.1%

5. PROCEDURE

BATCH PREPARATION

- Filter samples with GF/F 0.7 um. Measure each corresponding volume of sample according to each DOC. Acidify sample with FA to pH=2.
- Weight 2 mL vials (with cap) and label them.
- Clean PPL column with 3 mL methanol.
- Soak column 3 mL methanol and capping both ends. Wait at least 2 hours, ideally overnight.
- Drain and add 3 mL 0.1% formic acid.
- Pass filtered, acidified samples at <3ml/min (ideally 0.5 ml/min i.e. 2/3s per drop). At the end, it is possible to push the last bit of sample through with some air in a syringe.
- Rinse away salts with 3+3 ml 0.1% formic acid.
- Dry the column with N_2 (optimal) 5-10 minutes (you see the colour of the column changing back to sand colour).
- Elute sample with 2 mL methanol into the pre-weighed vial.
- Re-weigh the vial to see the actual sample volume (density of methanol = 0.792 g/mL).
- Store at -20°C ready for mass spectrometry or other analysis.

DETERMINATION OF [SPE-DOC]

- Take 150 uL of your methanol extract (1,5mL of samples 7 and 8) to and 8 mL tube.
- Dry the methanol with N_2. (One morning).
- Re-dilute in 8 mL of HPLC water.
- Sonicate for 15min.
- Measure DOC. Typical efficiency on PPL is 60%.

You should calculate your expected DOC before deciding on how much sample to use and how much water to re-dissolve in – make sure it is in the range of your standards!
[SPE-DOC] is calculated and reported in terms of the original sample concentration (not the concentration in the extract, which is highly concentrated), and extraction efficiency can be reported as 100*[SPE-DOC]/[DOC].

- Write in vials the reported DOC concentration.

ANALYSIS
Samples are injected into the Orbitrap™ (Thermo Scientific) in ICRA´s laboratories. For that a concentration of 500-1000 ppm as carbon is injected. The employed column is a C18 column (50 mm × 2.1 mm Hypersil GOLDTM, pore size 1.9 µm, Thermo Scientific, Lithuania). Gradient elution of water with 0.1% formic acid (A phase) and acetonitrile with 0.1 % formic acid (B phase) is used at a flow rate of 0.300 mL/min, under the following gradient program: 0–3 min 1% B, 3–12 min 99 % B, then maintained 99% B for 2 min. Full scan was made at a 120.000 resolution in the range 200-1000 m/z. RF lens 70%. Acquisition in negative and in profile mode in H-ESI. Internal calibration was used. Sheath gas 10, ion transfer tube 325ºC and vaporizer temperature 350ºC.

6. REFERENCES

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