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Comprehensive green analytical methods (TD-GC–MS, HPLC-MS/MS) for monitoring small organic and inorganic disinfection by-products in drinking water

Photini Papaioakeim [a], Efstathios A. Elia [a], Agapios Agapiou [a,b,*]

[a] Department of Chemistry, University of Cyprus, P.O.Box 20537, 1678 Nicosia, Cyprus
[b] Nireas-International Water Research Center, University of Cyprus, P.O. Box 20537, 1678 Nicosia, Cyprus

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ABSTRACT

Disinfection of drinking water is essential for public health protection; however, it can lead to the formation of potentially harmful disinfection by-products (DBPs). This study focuses on the development and optimization of green analytical methodologies for the simultaneous determination of small organic (trihalomethanes (THMs), haloacetic acids (HAAs)) and inorganic (chlorite, chlorate, bromate) DBPs in water. Various sorbent-based microextraction techniques were evaluated, including Thin Film-Solid Phase Microextraction (TF-SPME), Stir Bar Sorptive Extraction (SBSE), and their combined application, in conjunction with thermal desorption-gas chromatography–mass spectrometry (TD-GC–MS) for THMs analysis. Results demonstrated that the combined use of TF-SPME and SBSE offered superior extraction efficiency due to complementary sorption characteristics. Furthermore, high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) methods were developed for the determination of HAAs and inorganic anions without the need for sample pretreatment or derivatization. The optimized methods achieved high sensitivity and linearity, meeting current European regulatory standards (EU 2020/2184), and are suitable for routine monitoring. This work contributes to the advancement of green, efficient, and comprehensive analytical strategies for DBPs surveillance in drinking water.

1. Introduction

Disinfection of drinking water has been a critical public health measure since the late 19th century, significantly reducing waterborne diseases and representing one of the most impactful advancements in modern public health. Commonly used disinfectants include chlorine, ozone, chloramines, and chlorine dioxide, while methods such as UV disinfection and membrane filtration (e.g., desalination) are also gaining wider adoption. Although disinfectants effectively eliminate harmful microorganisms, an unintended side effect is the production of chemical disinfection by-products (DBPs) [1]. These by-products are formed when disinfectants react with naturally occurring organic matter (NOM) or bromide and iodide in water [2,3].

The first identified DBPs in chlorinated drinking water were trihalomethanes (THMs), which include chloroform (trichloromethane, TCM), bromoform (tribromomethane, TBM), bromodichloromethane (BDCM), and dibromochloromethane (DBCM). Discovered in 1974, these compounds were found to be widespread in chlorinated water, with chloroform being linked to cancer in laboratory animals [4,5]. This prompted regulatory actions in the USA and other countries [4]. More recently, epidemiological studies have suggested potential reproductive and developmental risks associated with chemically disinfected water. However, the specific DBPs responsible for these effects remain unidentified [4].

Since 1974, over 500 additional DBPs have been detected in drinking water or through laboratory simulations of disinfectant interactions with NOM. Despite significant research, the complexity of DBPs is not fully understood [6,7]. For instance, more than half of the total organic halide (TOX) content in chlorinated water and a similar proportion of the assimilable organic carbon (AOC) in ozonated water remain uncharacterized. This highlights the ongoing need for research to better understand and mitigate the risks associated with drinking water DBPs [6].

After trihalomethanes, haloacetic acids (HAAs) have been reported as the second most abundant class of DBPs that form during water chlorination [8]. HAAs were first detected in swimming pool water in

* Corresponding author at: Department of Chemistry, University of Cyprus, P.O.Box 20537, 1678 Nicosia, Cyprus.
E-mail address: agapiou.agapios@ucy.ac.cy (A. Agapiou).

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1999 [9]. Replacing non-acidic hydrogens in acetic acid (CH_3COOH) with one or more halogen atoms forms the chemical structure of HAAs. These compounds are formed when water is treated with chlorine, much like THMs. Factors influencing their formation include increased free chlorine concentration, especially >3 mg/L [10], acidic conditions, and hydrophobic NOM, in contrast to hydrophilic NOM [11], which favors the formation of THMs. The hydrophobic fraction of NOM consists of complex high-molecular-weight organic compounds, rich in aromatic content, primarily composed of humic substances. Humic substances are formed from the decomposition of plant material, such as lignin. Lignin, found in plants, is highly resistant to biodegradation but reacts with oxidants like chlorine [12]. On the other hand, hydrophilic NOM consists of acids, proteins, amino acids, and carbohydrates [13–15]. At high temperatures (>23 °C) and elevated pH levels, HAAs may decompose into simpler neutral compounds, such as THMs. The five most extensively studied HAAs are collectively referred to as HAA5 and include the following: monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), monobromoacetic acid (MBAA), and dibromoacetic acid (DBAA) [16]. Additionally, there are four less commonly known HAAs, which are not regulated by current legislation (EU 2020/2184): dibromochloroacetic acid (DBCAA), bromochloroacetic acid (BCAA), bromodichloroacetic acid (BDCAA), and tribromoacetic acid (TBAA). Due to their potential health risks, including carcinogenic and reproductive effects [17,18], regulatory agencies such as the U.S. Environmental Protection Agency (EPA) [19] and the European Union (EU) have set limits on their concentrations in drinking water, with a parametric value of 60 μg/L for HAAs.

In aqueous environments, chlorine dioxide undergoes rapid decomposition, leading to the formation of inorganic DBPs, primarily chlorite (ClO_2^-) and chlorate (ClO_3^-) ions. Among these, chlorite is the most prevalent, as approximately 50–70% of the chlorine dioxide is converted into this by-product. On the other hand, chlorate can also be detected in treated water when sodium hypochlorite is used as a disinfectant, often as a consequence of the degradation of improperly stored hypochlorite solutions [20–22]. Bromide ions, which are occasionally found in certain source waters, can be oxidized to bromate (BrO_3^-) by ozone. As a result, regulations in some countries, require bromate analysis only in cases where ozonation is used as a treatment method. A substantial body of scientific literature has been dedicated to this issue, reinforcing the notion that bromate formation was primarily associated with the ozonation process [23]. However, in 1992, it was reported that bromate ions were also present in sodium hypochlorite solutions, which have long been used as a disinfectant in drinking water treatment. Toxicological studies in animals indicate that chlorite exposure primarily induces oxidative stress, leading to hematological damage such as hemolytic anemia and methemoglobinemia [24]. Additionally, chlorine dioxide and, more significantly, chlorate exposure has been linked to neurobehavioral and neurological impairments, delayed female sexual development, soft tissue abnormalities, and disruptions in thyroid function. As a result, chlorate has recently been identified as one of the most critical unregulated emerging DBPs, warranting further research and regulatory attention [20]. Bromate is classified as possibly carcinogenic to humans [25]. According to the European Union regulations, the maximum allowable concentration for each chlorinated (chlorate and chlorite) inorganic anion is 700 μg/L, whereas for bromate, the limit is set at 10 μg/L [22].

In addition, due to their physicochemical properties, the determination of HAAs in water presents a significant analytical challenge. This class of DBPs has traditionally been measured using analytical approaches based on U.S. EPA methods, which rely on gas chromatography (GC) coupled with electron capture detection (ECD). To enhance the selectivity of these methods, variations incorporating mass spectrometry (MS) detection instead of ECD have also been employed. For example, U. S. EPA Method 551.1 for THMs requires liquid-liquid extraction (LLE) with large volumes of organic solvents [26], while EPA Method 552.2 for HAAs necessitates acidic derivatization followed by GC-ECD analysis [27]. These protocols are not only labor-intensive and time-consuming but also generate significant hazardous waste, contradicting the emerging principles of Green Analytical Chemistry (GAC) [28].

The EU Directive 2020/2184 on drinking water calls for broader identification of small organic and inorganic DBPs. This necessitates the expansion of current analytical methods (such as EPA Method 551.1, LLE for THMs) and the integration of more sustainable complementary techniques for monitoring. Therefore, a method for the detection of THMs in drinking water following the principles of green chemistry, has been previously developed and validated; it was based on a high capacitive sorptive extraction technique (HiSorb) [29]. The novelty of present work was to explore additional sorbent-based microextraction techniques beyond HiSorb, such as Thin Film-Solid Phase Microextraction (TF-SPME) and Stir Bar Sorptive Extraction (SBSE), and to determine whether their combination yields improved results for the extraction of THMs from water [30–32]. Furthermore, two separate methods for the quantitation of HAAs and inorganic anions using HPLC-MS/MS are herein described using a minimal sample volume and without any sample preparation [14].

2. Materials and methods

2.1. Reagents

Methanol (MeOH), acetonitrile (ACN), water, ammonium formate and formic acid (FA) were all of LC-MS grade and purchased from Carlo Erba (Spain) and used for mobile phase and sample preparation. Mixtures of THMs (EPA 501/601, Sigma Aldrich) and HAAs (EPA 552.2, Sigma Aldrich) served as reference standards. Chlorate, bromate and chlorite anion reference solutions were purchased as ion chromatography certified reference material solutions (1 g/L) from CPAChem (Bulgaria). Chlorobenzene-d_5 (2000 μg/mL, Sigma Aldrich) and 1,2-dibromoethane (500 μg/mL, Sigma Aldrich) were used as internal standards (ISs) for THMs analyses.

2.2. Sample preparation

2.2.1. THMs

Aliquots (10 mL) of LC-MS grade water were added to 20 mL screw top headspace (HS) glass vials (Agilent, Santa Clara, California, US) and spiked with the THMs mixture to result in a 50 μg/L concentration of each analyte. Next, 2 g of NaCl (Merck, Darmstadt, Germany), 2 μL of a 100 mg/L chlorobenzene-d_5 (IS_1, 20 μg/L final concentration) and 2 μL of 500 mg/L 1,2-dibromoethane (IS_2, 100 μg/L final concentration) methanol solutions were added, along with a magnetic stirrer. Preconditioned microextraction samplers (250 °C for 45 min, 50 mL/min of nitrogen) were then added to the vial, either TF-SPME (Markes International Ltd., UK) or SBSE (Gerstel Twister®), both using a polydimethylsiloxane (PDMS) sorbent, depending on the extraction approach used. In cases where extraction was performed using a combination of both techniques, both samplers were added simultaneously. The vials were then capped with suitable septum and vial caps (Markes International Ltd., UK) and left for analyte extraction to occur.

2.2.2. HAAs and inorganic anions

Aliquots (10 mL) of LC-MS grade water were transferred to non-sterile conical centrifuge (Falcon) tubes and spiked with the HAAs mixture and each of the inorganic ions of interest at a concentration of 100 μg/L per analyte.

2.3. Analytical methods

2.3.1. Determination of THMs using TD-GC–MS

Optimization of the SBSE and TF-SPME sampling methods involved the investigation of the type of sampling (headspace or direct immersion), and various extraction times (1.5, 2, and 4 h) [29,33]. Triplicate

($n = 3$) analysis of each sample was conducted for each experimental parameter. For the combined techniques, the extraction was performed by exposing the TF-SPME sampler to the sample headspace while immersing the SBSE sampler in the liquid phase.

Briefly, thermal desorption (TD) analysis was performed using a Unity-xr thermal desorber (Markes International Ltd.) connected to an Agilent GC-7890B gas chromatograph and an Agilent MSD-5977B mass spectrometer (TD-GC–MS). The analytical details of the validated TD-GC–MS method have been described in detail elsewhere [29]. A DB5-MS column (30 m × 0.25 mm, 0.25 um, Agilent J&W) was used for all experiments. Three replicates were performed per sample, and the results were expressed as the area ratios of the analyte to IS_2 (1,2-Dibromoethane) area. The evaluation of the experimental parameters was performed in terms of the normalized chromatographic peak area per THM analyte and for total THMs by the normalized sum the total THMs, according to eqs. (1) and (2):

$$\text{THMs Compound} = \frac{A_C}{A_{IS}} \quad (1)$$

$$\text{Total THMs} = \Sigma_{\text{total THMs}} \frac{A_C}{A_{IS}} \quad (2)$$

2.3.2. Determination of HAAs and inorganic anions using HPLC-MS/MS

Two separate chromatographic methods were developed and used for the separation and quantitation of HAAs and inorganic ions, respectively, using a Waters Alliance 2696 Separation module (Waters Corp., UK). For HAAs, separation was achieved using a Waters Symmetry C18 (2.1 × 150 mm, 3.5 um) column held at 45 °C. An injection volume of 100 μL was used, with the samples kept at 10 °C. Mobile phase A was H_2O + 0.05% FA and mobile phase B was 80% MeOH +0.3% FA. A gradient elution was performed with a flow rate of 0.4 mL/min according to the following profile:

Time (min)	% A	% B
0	99	1
1.23	99	1
3.68	60	40
4.29	40	60
7.35	0	100
9.8	0	100
9.86	99	1
12.25	99	1

For inorganic ions, separation was achieved using a Waters Anionic Polar Pesticides (2.1 × 50 mm, 5 um) column held at 50 °C. An injection volume of 100 μL was used with the samples kept at 10 °C. Mobile phase A was H_2O + 50 mM ammonium formate +0.9% FA and mobile phase B was ACN + 0.9% FA. A gradient elution was performed with a flow rate of 0.4 mL/min according to the following profile:

Time (min)	% A	% B
0	10	90
2	40	60
3	40	60
5	10	90

Mass spectrometric analysis was performed using a Waters Quattro Premier XE operated in negative ESI mode with the following parameters: capillary voltage 1 kV, extractor voltage 5 V, desolvation temperature 400 °C, desolvation gas (nitrogen) flow 870 L/h and collision gas (argon) flow 0.4 mL/min. Multiple Reaction Monitoring (MRM) was performed for all analytes with the recorded precursor and product ions as well as associated cone voltages and collision energies shown in Table 1. All HPLC-MS/MS data was acquired and processed using Masslynx 4.1 (Waters Corp., UK).

Table 1
MRM transitions and collision-induced dissociation (CID) fragmentation settings for all analytes investigated using HPLC-MS/MS.

Analyte	Precursor ion (m/z)	Product ion (m/z)	Cone voltage (V)	Collision energy (eV)
BrO_3^-	126.9	95.0	50	20
ClO_2^-	67.1	51.2	20	10
ClO_3^-	83.2	67.2	15	15
MCAA	93.0	35.0	5	10
MBAA	137.0	79.0	5	6
DBAA	214.9	170.8	30	12
DCAA	126.9	83.1	30	12
TCAA	116.8	35.1	30	10
DBCAA	206.8	163.0	5	13
BCAA	171.0	127.0	30	12
BDCAA	160.8	116.9	5	8
TBAA	250.9	79.0	30	15

2.3.3. Blank samples

Procedural blanks consisting of unspiked LC-MS grade water were analyzed with every batch of samples for both analytical techniques. No target analytes were detected in the blanks above the LOD, confirming the absence of carryover or interfering analytes.

2.3.4. Tap water samples

Tap water samples were collected following the protocol described previously [29]. For THMs analysis, 250 mL of tap water was collected in a 250 mL brown bottle; a 10 mL aliquot was then transferred to 20 mL headspace glass vials (Agilent, Santa Clara, California, US). Following the transfer, 2 g of NaCl (Merck, Darmstadt, Germany), 2 μL of a 100 μg/mL of chlorobenzene-d_5 (IS_1) and 2 μL of 500 μg/mL 1,2-dibromoethane (IS_2) methanol solutions were added.

For HAAs and inorganic anions analysis, a 1.5 mL aliquot of the collected tap water sample was transferred to a 2 mL HPLC vial, capped and placed in the autosampler. No further sample preparation steps were used.

3. Results and discussion

3.1. Optimization of TD-GC–MS

The microextraction techniques applied in this study, namely TF-SPME and SBSE, are based on the same fundamental principle as the HiSorb extraction method. This latter technique was employed in our previous study, which served as the methodological foundation for the current work [29]. Nevertheless, the predominantly used PDMS phase exhibits low affinity for compounds with moderate to high polarity (log $K_{ow} < 3$).

The optimization of TF-SPME and SBSE extraction techniques followed a one-variable-at-a-time (OVAT) approach. Toward this, all variables are kept constant except for one, which is varied to study the system's behavior at different levels. Although the OVAT strategy cannot reveal potential interactions between variables and demands numerous experiments, it is straightforward to apply in this type of optimization, given the small number of variables and the low likelihood of significant interactions [29].

3.1.1. Method of extraction

In the optimization of microextraction parameters, comparison includes TF-SPME sampling in the headspace (HS) and direct immersion (DI) sampling. As shown in Fig. 1 (A-B), TF-SPME performed substantially better under HS conditions than DI, particularly for total THMs. This observation can be attributed to the high volatility of THMs, which favors their partitioning into the gas phase. The thin film geometry of the TF-SPME is particularly suited for HS extraction, enabling efficient analyte uptake from the vapor phase with minimal matrix interference [34]. In contrast, SBSE showed negligible differences between HS and DI

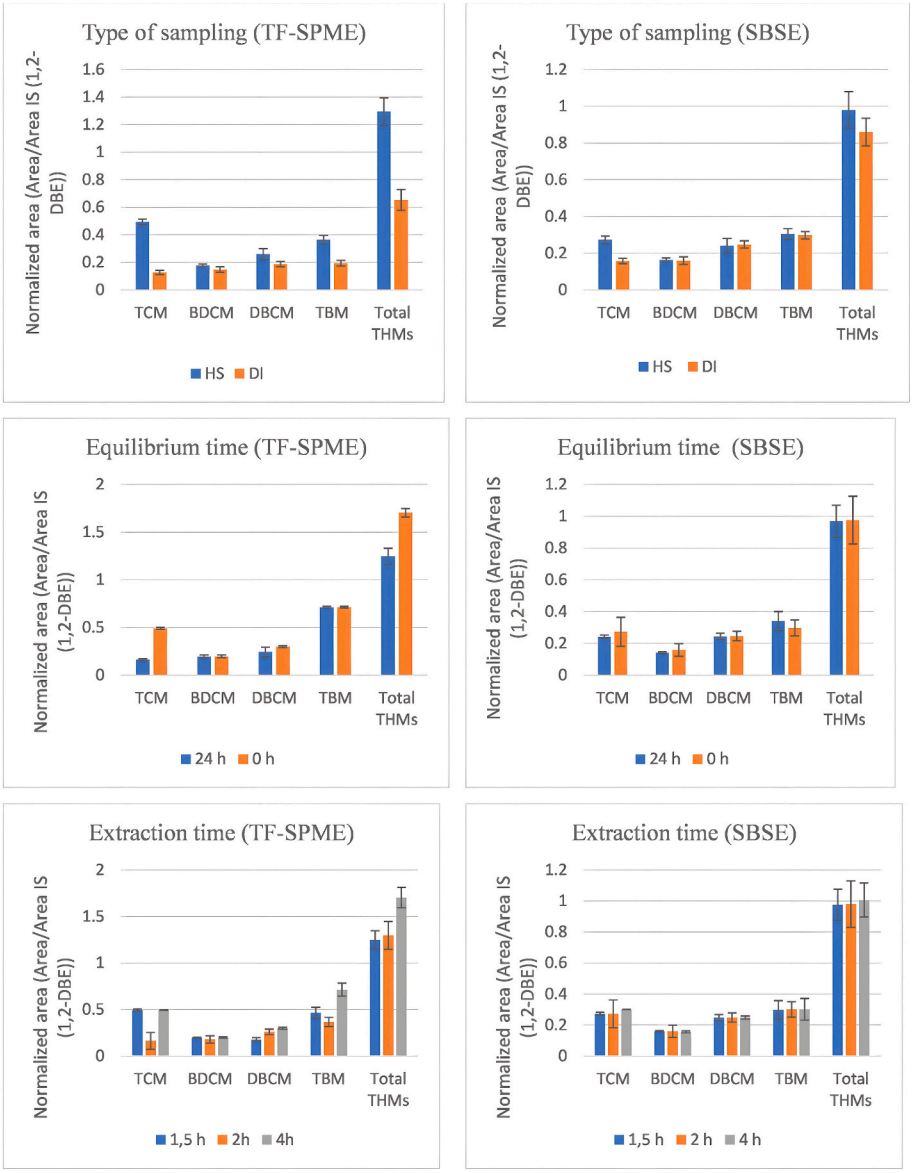

Fig. 1. Optimization parameters for the extraction of THMs using TF-SPME and SBSE: (a, b) Comparison of type of sampling (HS Vs DI), (c, d) Equilibrium time (24 h Vs 0 h), and (e, f) Extraction time (1.5 h, 2 h, 4 h).

sampling, likely due to the higher sorptive capacity and mass of its PDMS coating, which allows efficient extraction directly from the aqueous phase [35].

3.1.2. *Equilibration time*

Subsequently, during the investigation of experimental parameters, the sample equilibration time before heating and stirring was also investigated. In contrast, for HiSorb, the vial is left hermetically sealed at rest to allow equilibrium to occur, after which the rod is placed into the vial [29]. The results indicate that while bromodichloromethane and tribromomethane are unaffected, trichloromethane and dibromochloromethane decrease after a 24-h equilibration (Fig. 1, C—D). A possible explanation for this phenomenon is that these specific THMs were initially absorbed into the sorbent material over the 24-h period, and then when the sample was heated at 60 °C, a portion of them was released back into the sample headspace. This appears to be more pronounced for trichloromethane, which shows the largest difference in results between the two investigated equilibration times. In contrast, SBSE showed negligible differences between HS and DI sampling, likely due to the higher sorptive capacity and mass of its PDMS coating. Based on the observed results, the effect of equilibration time was found to be negligible in both cases. The difference in total THMs recovery between 0 h and 24 h was not substantial enough to justify the extended waiting time. Therefore, a 0 h equilibration time was selected for the subsequent experiments, as it offers comparable analytical performance while significantly reducing total analysis time and improving sample throughput.

3.1.3. *Extraction time*

Finally, the extraction time of TF-SPME and SBSE with simultaneous heating (60 °C) and stirring (1000 rpm) was evaluated (Fig. 1, *E*-F). The effect of extraction time further highlighted the kinetic limitations of TF-SPME, where signal intensity increased progressively with longer extraction durations. This behavior reflects the relatively small volume and surface area of the sorptive phase, requiring extended contact times for sufficient analyte accumulation [36]. SBSE, on the other hand, showed minimal variation in total THMs across the tested extraction times. The large sorptive capacity of the stir bar may allow for rapid equilibrium or even early saturation of the phase, especially for low-molecular-weight and moderately hydrophobic compounds such as

THMs [37]. Overall, the optimal sampling time for TF-SPME appears to be 4 h for all THMs and 1.5 h for SBSE.

3.1.4. Combined use of SBSE and TF-SPME

Finally, the combined extraction technique was tested, where sample agitation was performed using SBSE, while TF-SPME was simultaneously exposed to the headspace. SBSE provides higher volumes of adsorptive material for increased capacity. TF-SPME technique is particularly suitable for on-site sampling due to the structural robustness of the membrane and its ease of transport to remote locations. The geometry of TF-SPME enhances the sampling rate by utilizing a thin extraction phase and a large surface area, resulting in a high surface-to-volume ratio. This feature helps reduce the time required to reach equilibrium while simultaneously increasing the extraction capacity for target molecules [38].

The two extraction tools were subsequently desorbed together in the same tube in the TD. The comparison of the extraction techniques is presented in Fig. 2, where the normalized area (Area/Area$_{IS}$ 1,2-DBE) is shown for the four evaluated approaches. Initially, when comparing the performance of the TF-SPME and SBSE techniques individually, it is evident that TF-SPME exhibits significantly higher extraction efficiency, with a normalized area of approximately 1.7 compared to only ~1.0 for SBSE. This suggests a greater capacity of TF-SPME to extract analytes, possibly due to a larger active surface area or enhanced selectivity of the extraction medium. Interestingly, the combination of the two microextraction techniques (TF-SPME + SBSE) resulted in a further increase in signal, reaching the highest normalized area (~2.0). This indicates a synergistic effect, likely due to the complementary extraction capabilities of the two microextraction tools. While the HiSorb technique previously validated by our group [29] offers superior automation for routine screening, the combined TF-SPME and SBSE approach examined here achieved a normalized extraction efficiency of ~2.0, which is approximately 25% higher than HiSorb (~1.6) and nearly double that of standalone SBSE (~1.0). This demonstrates that for applications requiring maximum sensitivity at trace levels, the synergistic combination of thin-film kinetics and stir-bar capacity provides a distinct advantage. Furthermore, unlike standard EPA Method 551.1 which generates significant solvent waste, this combined microextraction approach remains solvent-free. HiSorb however, offers notable practical advantages in terms of use, potential higher degree of automation and requires less manual intervention compared to the synergistic approach of TF-SPME and SBSE. Therefore, although the highest extraction performance was achieved by the combined use of TF-SPME and SBSE, HiSorb emerges as the most well-rounded technique for routine applications. It provides satisfactory analytical performance while also optimizing time, ease of use, and reproducibility.

The developed combined TF-SPME+SBSE method was also applied to the analysis of tap water samples obtained from the local area. Enhanced recovery of THMs with this combined method, compared to the HiSorb method previously evaluated in spiked water samples, was also observed in real tap water samples. The same samples were analyzed using both the optimized TF-SPME+SBSE method and the HiSorb method for comparison. The chromatographic peaks obtained for the four THMs of interest (TCM, BDCM, DBCM, TBM) using the two developed methods are shown in Fig. 3. For all four THMs, higher relative abundance and larger peak areas were obtained using the combined TF-SPME+SBSE method compared with the HiSorb method, highlighting the advantage of the combined approach.

3.2. Optimization of HPLC-MS/MS

Initially, a single HPLC-MS/MS method for the simultaneous separation and detection of HAAs and inorganic ions was pursued. Trials were performed using a Waters Sunfire C18 (2.1 × 150 mm, 3.5 um) column which has sufficient polar surface activity to interact with both inorganic ions and HAAs, even though these analytes differ in polarity. The Sunfire C18 column, however, failed to retain the most polar analyte which is the chlorite anion, hence attention was shifted in the development and optimization of two separate chromatographic methods; one for HAAs and another for inorganic ions. The initial failure of the C18 column to retain chlorite is attributed to the high polarity and ionic nature of the ClO_2^- anion. C18 stationary phases rely on hydrophobic interactions, which are insufficient for retaining small inorganic anions. The transition to the Anionic Polar Pesticides column, which utilizes anion exchange properties, provided the necessary polar retention and selectivity to separate chlorite anions. There is evidence in the literature that a single chromatographic method can be used for the simultaneous determination of HAAS and inorganic ions [39,40], but the majority of the developed methods use ion chromatography hyphenated to mass spectrometry (IC-MS), or in the few cases of HPLC-MS/MS use, the researchers were not focused on the determination of the chlorite anion [41–43].

Following the change to develop two methods, the HAAs method was based on an Application Note published by Shimadzu Corporation [44], while the inorganic ions method was based on an Application Note published by Waters Corporation [45]. Both were slightly modified to

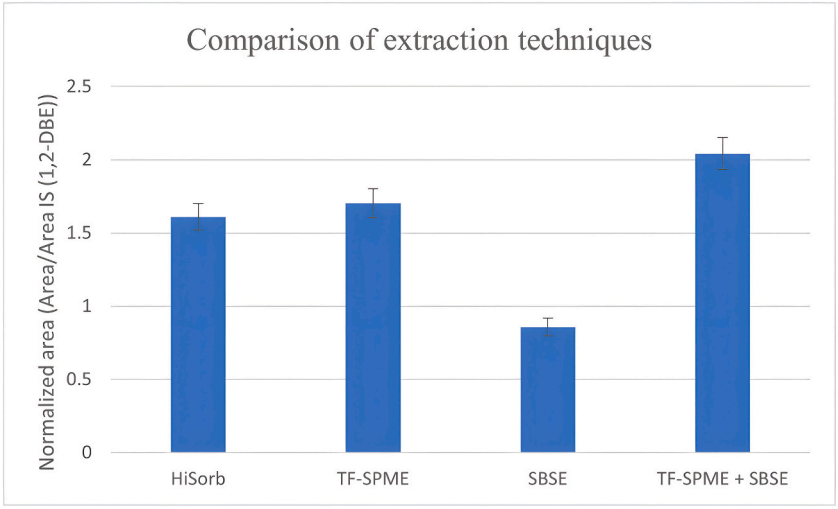

Fig. 2. Comparison of combined SBSE and TF-SPME extraction with each sorptive technique separately for THMs extraction. HiSorb analytical results are shown as well [29].

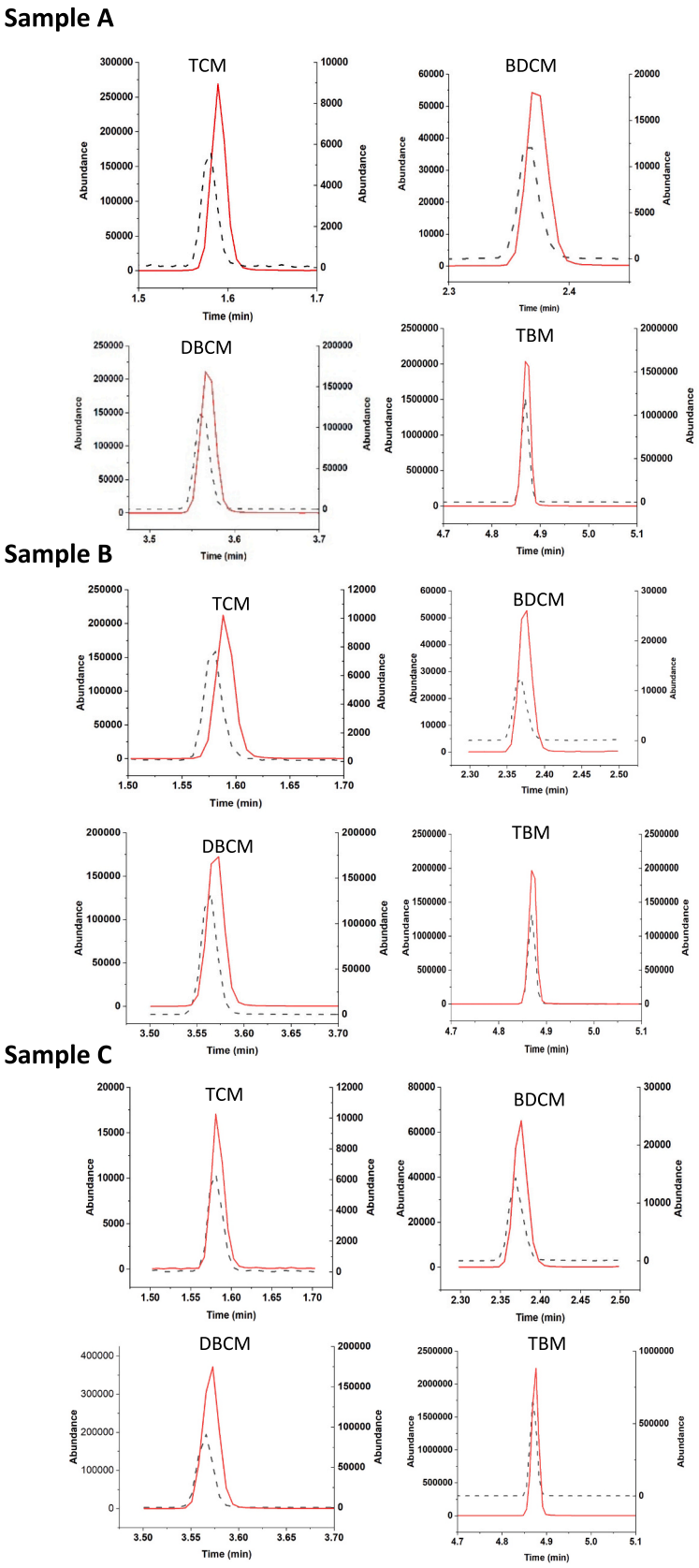

Fig. 3. Chromatographic peaks obtained from the analysis of three local tap water samples using the optimized combined TF-SPME+SBSE and HiSorb methods. An enhancement in relative abundance and peak area for all THMS was observed when using the combined TF-SPME+SBSE (red solid line, left y-axis) method compared to the HiSorb (dashed black line, right y-axis)) method. The total THM concentrations determined using the HiSorb method were: (a) 61.4 μg/mL, (b) 54.2 μg/mL, and (c) 40.1 μg/mL. In the latter samples, the concentrations followed the order TBM > DBCM > BDCM > TCM (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

meet our instrument specifications; typical extracted ion current chromatograms obtained from both methods can be seen in Fig. 4.

Separation for all analytes was achieved using the developed methods without the need for sample preparation steps such as filtration or pre-concentration, even when analytes were spiked in drinking water samples.

3.3. Sensitivity and linearity

Following method development and optimization, the two methods were then tested for sensitivity and linearity for all analytes. The European Union has set a limit of 60 μg/L (summed) for five HAAs (MCAA, DCAA, TCAA, MBAA and DBAA) and a limit of 700 μg/L for each of the chlorinated inorganic ions (chlorate, chlorite), while a limit of 10 μg/L is imposed for bromate. In the case of the HAAs limits, these are only applicable if a disinfection method that can produce these by-products is used. As for the inorganic ions' limits, these are applicable when chlorine dioxide is used as the disinfection product; in all other cases, the limit is reduced to 0.25 mg/L. To allow the use of the developed methods for routine analysis of drinking water samples, the sensitivity of both methods should be such as to meet the new EU Directive's limits (EU 2020/2184). Sensitivity was determined by diluting solutions containing each analyte and recording the obtained chromatographic peak area; the limit of detection (LOD) and limit of quantitation (LOQ) for each analyte can be seen in Table 2. The LOQ was determined experimentally and was the analyte concentration that gave a signal-to-noise ratio of 10; LOD was then calculated as LOQ/3. Following this, an upper quantification limit was set that contains the EU Directive's limit, the linearity of the developed method was tested between the LOQ and this upper quantification limit for each analyte. The obtained calibration curve

Table 2
Results of linearity and sensitivity investigation of inorganic ions and HAAs, including values for LOD, LOQ and R^2 as well as the calibration equation for each analyte.

Analyte	LOD (μg/L)	LOQ (μg/L)	Linear range (μg/L)	Equation	R^2
BrO_3^-	1.7	5	5–500	y = 7.3772× + 109.9	0.99
ClO_2^-	66	200	200–1000	y = 25.075× - 6959.5	0.99
ClO_3^-	1.7	5	5–500	y = 5.3126× + 243.95	0.99
MCAA	7	20	20–1000	y = 82.16× + 342.17	0.99
DCAA	1.7	5	5–1000	y = 152.05× + 177.14	0.99
TCAA	1.7	5	5–1000	y = 189.73× + 26.12	0.99
MBAA	17	50	50–1000	y = 73.89× – 453.63	0.99
DBAA	1.7	5	5–1000	y = 92.593× - 9.7306	0.99
TBAA	1.7	5	5–1000	y = 63.934× – 15.903	0.99
BCAA	3.5	10	10–1000	y = 60.039× - 220.24	0.99
BDCAA	1.7	5	5–1000	y = 652.29× + 4660.7	0.99
CDBAA	1.7	5	5–1000	y = 472.74× + 26.071	0.99

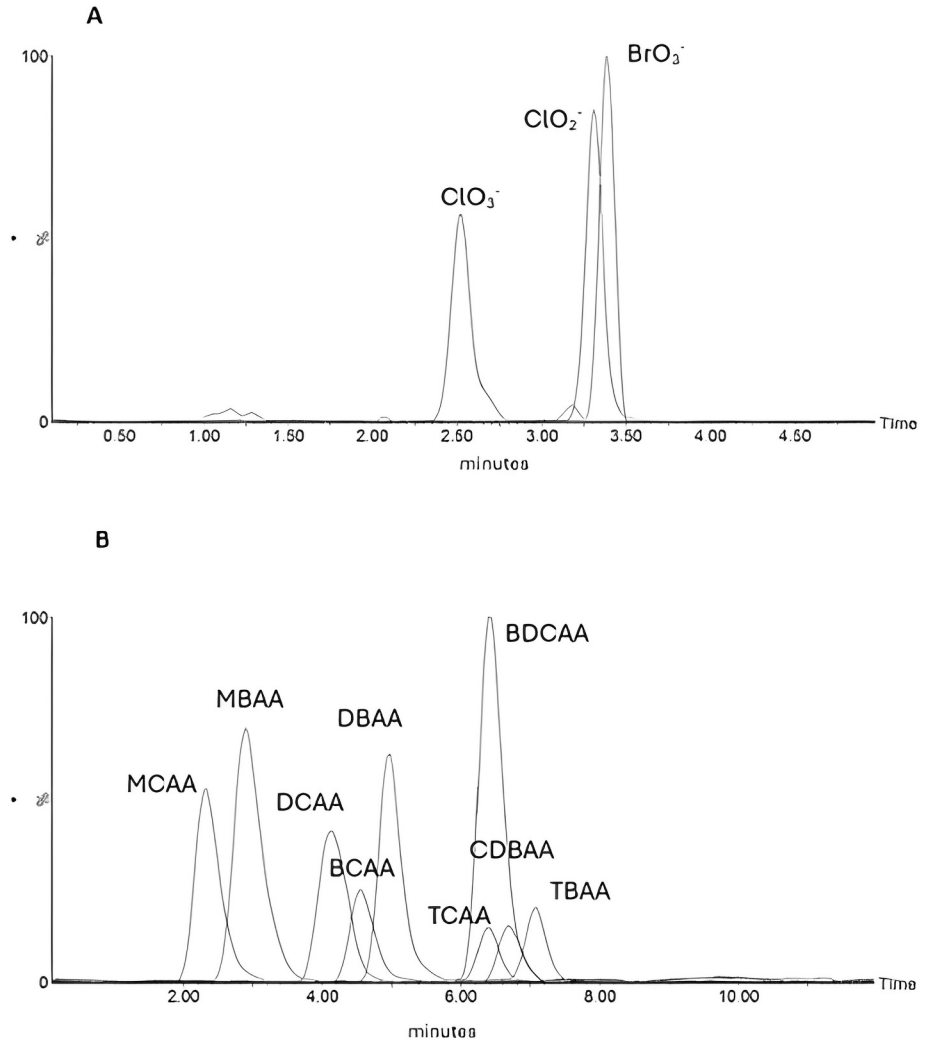

Fig. 4. Combined extracted ion current chromatograms obtained when analyzing spiked water samples with: (a) Inorganic anions, and (b) HAAs at a concentration of 500 μg/L per analyte.

equations and associated linearity ranges can be seen in Table 2. The analytical performance of the developed workflow aligns with the recent paradigm shift toward direct-injection mass spectrometry, offering a balance between sensitivity and operational simplicity [46]. While high-end triple-quadrupole methods, such as the direct-injection LC-MS/MS protocol reported by Planas et al., have achieved sub-μg/L detection limits (LODs 0.01–0.6 μg/L) suitable for trace-level research, they often require high-resolution instrumentation not always available for routine compliance monitoring [47]. Conversely, recent advances in miniaturized portable systems, such as the capillary LC-MS method developed by Mikhail et al., demonstrate the feasibility of green, on-site HAAs analysis but report detection capabilities (e.g., 5.3 μg/L for DCAA) that are comparable to or slightly higher than those presented herein [48]. By achieving LODs in the range of 1.7–17 μg/L using a standard laboratory HPLC-MS/MS setup, the proposed method bridges this gap, providing sufficient sensitivity to meet the EU Directive 2020/2184 limits (60 μg/L), while eliminating the toxic derivatization steps characteristic of legacy GC methods (e.g., EPA 552.2). This confirms that a solvent-free, direct-injection approach can serve as a robust, regulatory-compliant alternative for modern water quality laboratories. The sensitivity achieved for inorganic anions was further contextualized against established regulatory methodologies and recent literature. Traditional ion chromatography methods utilizing conductivity detection (e.g., EPA Method 300.1) typically report detection limits in the range of 10–20 μg/L, which can be insufficient for reliably verifying compliance with strict regulatory standards [49]. While specialized techniques involving post-column reaction (e.g., EPA Method 317.0) offer sub-μg/L sensitivity [49], they necessitate the use of toxic reagents and complex hardware. In contrast, the developed HPLC-MS/MS method achieved an LOD of 1.7 μg/L (LOQ 5 μg/L) for bromate and chlorate. This represents a significant improvement in sensitivity compared to a similar LC-MS/MS workflow, which reported an LOQ of 10 μg/L for these oxyhalides [21]. The current method's performance is also consistent with recent external studies utilizing polar-retention mechanisms [45], confirming that the optimized Anionic Polar Pesticide column provides a robust, high-sensitivity alternative for compliance monitoring.

3.4. Application to tap water samples

Local tap water samples were collected to evaluate the applicability of the developed methods for the routine determination of HAAs and inorganic anions in drinking water. The water samples shown in Fig. 3 were found to contain chlorate anions at concentrations below the legislative limit of 700 μg/mL, with measured levels of 121.9 μg/mL in sample A, 113.6 μg/mL in sample B, and 223.7 μg/mL in sample C. No other HAAs or inorganic anions were detected above the method's LOD.

4. Conclusions

The present study successfully optimized a comprehensive analytical workflow for the determination of regulated and emerging DBPs in drinking water, strictly adhering to the sensitivity requirements of the EU Directive 2020/2184. Regarding volatile THMs, the comparative evaluation of microextraction techniques revealed distinct advantages for different analytical needs. The combined application of TF-SPME and SBSE demonstrated a synergistic effect, yielding the highest extraction efficiency (normalized area ~ 2.0) by coupling the rapid headspace kinetics of the thin-film geometry with the high sorptive capacity of the stir bar. While this combined approach offers maximum sensitivity for trace analysis, the HiSorb technique remains a highly viable alternative for routine high-throughput laboratories due to its superior automation potential.

For the analysis of HAAs and inorganic anions, this work overcomes the limitations of traditional C18 stationary phases, which fail to retain highly polar species like chlorite. By optimizing two separate HPLC-MS/MS methods utilizing an Anionic Polar Pesticide column for inorganic ions, we achieved successful separation and quantification without the need for time-consuming sample pre-treatment or toxic derivatization steps.

The developed methods exhibited excellent linearity ($R^2 > 0.99\%$) and sensitivity, with LODs ranging from 1.7 to 66 μg/L, well below the regulatory parametric values of 60 μg/L for HAAs and 700 μg/L for chlorate. Consequently, this study provides a validated, green analytical toolbox that significantly reduces solvent consumption and analysis time, making it highly suitable for compliance monitoring in modern water quality laboratories.

All developed methods were evaluated for their suitability for the routine determination of THMs, HAAs, and inorganic anion concentrations in local tap water samples. The concentrations determined for each analyte were below the legislative limits for safe drinking water established by the EU and local authorities.

CRediT authorship contribution statement

Photini Papaioakeim: Writing – original draft, Methodology, Investigation. **Efstathios A. Elia:** Writing – review & editing, Validation, Methodology, Conceptualization. **Agapios Agapiou:** Writing – review & editing, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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