SafeCREW
ANALYTICAL PROTOCOL #1

ADVANCED ANALYTICAL PROCEDURES FOR THE CHARACTERIZATION OF SULFONATED DISINFECTION BYPRODUCTS IN DRINKING WATER

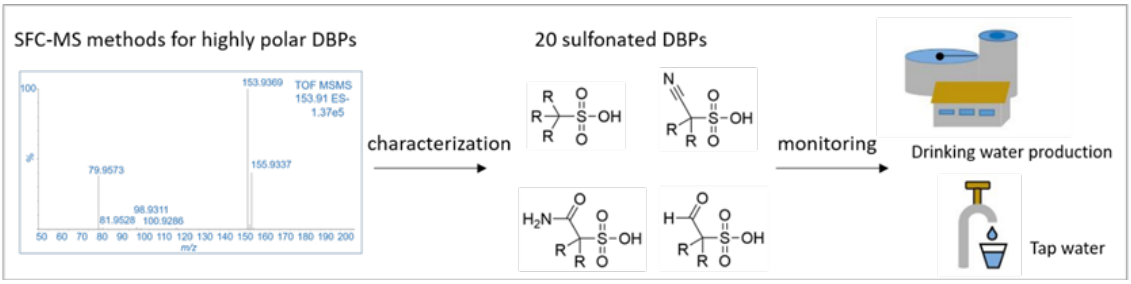

Figure 1 Schematic illustration showing an example mass spectrum and representative structures of sulfonated disinfection byproducts detected in the finished water and tap water

Introduction

Only a small number of disinfection byproducts (DBPs) are regulated so far. However, the majority, including the main toxicity drivers in disinfected water, remain unknown. Traditional methods, such as gas chromatography-mass spectrometry (GC-MS) and reversed-phase liquid chromatography-mass spectrometry (RP-LC-MS), are often ineffective for highly polar contaminants, as these compounds are neither sufficiently volatile for GC analysis nor well retained by reversed-phase columns in RP-LC-MS. To address this limitation, the SafeCREW project has developed a novel analytical approach based on supercritical fluid chromatography-mass spectrometry (SFC-MS) enabling the characterization of previously unknown highly polar sulfonated DBPs in drinking water.

Funded by the European Union

This project has received funding from the European Union´s Horizon Europe research and innovation programme under grant agreement No 101081980. This publication reflects the views only of the author, and the Commission cannot be held responsible for any use which may be made of the information contained therein.

Target Audience

This analytical test protocol targets water utilities and researchers. Utilities can apply it to monitor and manage DBPs in their systems; researchers can use it to design studies on DBP formation mechanism and toxicity.

Scope and Objectives

This guideline covers advanced analytical procedures for sampling, detecting, identifying, and quantifying highly polar sulfonated DBPs. It focuses on complementary chromatographic techniques, high-resolution and targeted mass spectrometry, and sample-enrichment strategies relevant to drinking water.

The objective of this guideline is to provide reliable, sensitive, and practical analytical methods for the characterization of polar DBPs. It aims to support laboratories and researchers in applying state-of-the-art techniques for comprehensive DBP analysis.

Guideline for the Analysis of Sulfonated DBPs in Drinking Water

The chloro- and bromo-analogues of halomethanesulfonic acids, haloacetonitrilesulfonic acids, haloacetamidesulfonic acids, and haloacetaldehydesulfonic acids (20 compounds in total) in drinking water can be reliably characterized using the following procedures.

1) Sampling:

- Use chlorine-demand free amber glass bottles for sample collection.
- Let tap water run for approximately 30 seconds before filling the bottles.
- Do not add quenching reagents (e.g., ascorbic acid), as some sulfonated DBPs degrade in their presence.
- Store the samples at 4 °C and process them immediately after collection to minimize DBP hydrolysis.

Funded by the European Union

This project has received funding from the European Union´s Horizon Europe research and innovation programme under grant agreement No 101081980. This publication reflects the views only of the author, and the Commission cannot be held responsible for any use which may be made of the information contained therein.

2) Sample Enrichment

Freeze-drying is recommended prior to SFC coupled with quadrupole time-of-flight mass spectrometry (QTOF) analysis of sulfonated DBPs to improve signal intensity, as it provides the most consistent recoveries.

Freeze-drying (Preferred Method):

Step 1: Transfer 40 mL sample in a 50 mL centrifuge tube and freeze at –20 °C overnight.

Step 2: Dry in a freeze-dryer at 15 °C and 1.65 mbar until complete.

Step 3: Reconstitute the residue in 400 µL acetonitrile/water (90:10, v/v).

Step 4: Centrifuge at 14000 min^{-1} for 10 min.

Step 5: Transfer the supernatant to LC-MS vials and store at –20 °C for up to 3 months.

Alternative Options:

- Solid phase extraction (SPE) using weak anion exchange (WAX) cartridges: Targeted enrichment of anionic DBPs with cleaner extracts.
- Azeotropic evaporation (water/acetonitrile): Fast but lower enrichment efficiency.

3) SFC-QTOF-Based Characterization of Sulfonated DBPs

Because analytical standards for most sulfonated DBPs are not commercially available, characterization is recommended to be based on signal intensities obtained using SFC-QTOF following freeze-drying enrichment. Semi-quantification can be performed for selected compounds by preparing mixture standards generated through chlorination of cysteine and determining their concentrations using quantitative NMR spectroscopy. This approach enables approximate quantification when needed, and the detailed procedure for this semi-quantification method is published elsewhere (Nihemaiti et al., 2023).

Funded by the European Union

This project has received funding from the European Union´s Horizon Europe research and innovation programme under grant agreement No 101081980. This publication reflects the views only of the author, and the Commission cannot be held responsible for any use which may be made of the information contained therein.

The following bullet points and Table 1 summarize the recommended SFC-QTOF operating parameters.

- Instrument: ACQUITY UPC² system coupled with Synapt GS2 QTOF (negative ESI mode; nitrogen and argon as cone and collision gases, respectively.
- Data Acquisition: Centroid mode, m/z 50–1200, 0.08 s scan time. MSE acquisition to simultaneously collect two data sets: a low-collision-energy scan for parent ions and an elevated-collision-energy scan (15-40 eV) for all fragments.

Table 1: Detailed parameters of SFC-QTOF

Column	BEH column (3.0 mm × 100 mm, 1.7 µm, Waters, Eschborn, Germany)
Column back pressure	2200 psi
Column temperature	55 °C
Mobile phase	(A) CO_2 and (B) methanol/water co-solvent (95:5, by volume) containing 10 mM ammonium formate
Mobile phase gradient	0–0.2 min, 1% B; 8.5 min, 25% B; 12-17 min, 50% B; 17.1-19 min, 1%B
Flow rate	1.3 mL/min
Makeup flow	methanol/water (90:10, by volume) with 0.1% formic acid
Makeup flow rate	0.3 mL/min
Injection volume	5 µL
Capillary voltage	-2 kV
Source temperature	140 °C
Desolvation temperature	550 °C
Cone voltage	20 V
Source offset	40 V
Desolvation gas flow	950 L/h

4) SFC-MS/MS-Based Quantification of Sulfonated DBPs

The analytical standards for dichloromethanesulfonic acid (Cl_2MSA) and dibromomethanesulfonic acid (Br_2MSA) are commercially available; therefore, their quantification can be performed using SFC coupled to triple-quadrupole mass spectrometry by direct injection. As these compounds typically occur in drinking water at low µg/L levels, sample enrichment is not recommended for routine analysis. Quantification can be conducted using an ACQUITY UPC² system coupled with a Xevo TQ-XS (Waters, Germany). The instrumental parameters follow those listed in Table 1, and the corresponding MRM parameters are summarized in Table 2.

Table 2: The MRM parameters applied in SFC-QXS

Compound	Formula	Transition 1 (Tr1)	Transition 2 (Tr2)	Cone Voltage (V)	Collision Energy in (eV)	Ion Mode	Retention Time (min)	Intensity ratio Tr1/Tr2
Cl_2MSA	$CH_2Cl_2SO_3$	162.878 > 79.832	---	6	26	ES–	8.76	1.0
		---	162.878 > 98.765	6	12	ES–	8.76	
Br_2MSA	$CH_2Br_2SO_3$	250.801 > 79.000	---	20	20	ES–	8.98	1.2
		---	252.793 > 81.190	28	18	ES–	8.98	

Funded by the European Union

This project has received funding from the European Union´s Horizon Europe research and innovation programme under grant agreement No 101081980. This publication reflects the views only of the author, and the Commission cannot be held responsible for any use which may be made of the information contained therein.

Conclusion

Procedures outlined in this guideline were tested and evaluated at SafeCREW case study sites in Hamburg, Berlin, Milan and Tarragona. The analytical test protocol proved effective, and detailed results are presented in Deliverable D1.1 Advanced Analytical Procedures for the Characterization of Disinfection Byproducts, where extended methodological descriptions can also be found. Furthermore, these analytical procedures have been successfully applied to investigate the occurrence of sulfonated DBPs in European tap water samples and their formation potentials under different disinfectants, with findings published in peer-reviewed journals (Nihemaiti et al., 2023; Nihemaiti et al., 2026).

References

- Nihemaiti, M., Jou, S., del Val, L., Lange, D. and Ribalta, M., 2024. SafeCREW Deliverable D1.1 Advanced analytical procedures for the characterization of disinfection by-products and methodology for remote sensing of natural organic matter, submitted to EC by 12 July 2024.

- Nihemaiti, M., Icker, M., Seiwert, B. and Reemtsma, T. 2023. Revisiting Disinfection Byproducts with Supercritical Fluid Chromatography-High Resolution-Mass Spectrometry: Identification of Novel Halogenated Sulfonic Acids in Disinfected Drinking Water. Environmental Science & Technology 57(9), 3527-3537. https://doi.org/10.1021/acs.est.2c05536

- Nihemaiti, M., Wullenweber, J., Stefanoni, M., Kahle, L., Lechtenfeld, O.J., Cantoni, B., Antonelli, M., Ernst, M. and Reemtsma, T. 2026. Formation of regulated and novel disinfection by-products during chlorine and chlorine dioxide disinfection of surface water and groundwater. Water Research 290, 124996. https://doi.org/10.1016/j.watres.2025.124996

- European Commission (n.d.). Climate Resilient Management for safe disinfected and non-disinfected water supply systems (SafeCREW, Grant Agreement No. 101081980). CORDIS.DOI 10.3030/101081980

Disclaimer

Funded by the European Union under grant agreement No 101081980. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Coordinated by

DVGW | TUHH Technische Universität Hamburg

Participants

POLITECNICO MILANO 1863

KWB Kompetenzzentrum Wasser Berlin

BDS BioDetection Systems

eurecat

Umwelt Bundesamt

UFZ HELMHOLTZ Zentrum für Umweltforschung

Consorci d'Aigües de Tarragona

TUTECH

MM

National University of Water and Environmental Engineering

Partners

Multisensor

Contact

DVGW

Research Centre TUHH / Institute of Water Resources and Water Supply

c/o Dr. Anissa Grieb

Am Schwarzenberg-Campus 3 (E)

21079 Hamburg

Phone (Office) +49 40 42878-3453

Email anissa.grieb at tuhh.de

Funded by the European Union

This project has received funding from the European Union´s Horizon Europe research and innovation programme under grant agreement No 101081980. This publication reflects the views only of the author, and the Commission cannot be held responsible for any use which may be made of the information contained therein.