Surface water screening for chloroalkane contamination via Bruker *µ*DROP and GC-MS/MS

Environmental protection by rapid target extraction for sensitive and specific determination of chloroalkane pollutants using the EVOQ GC-Triple Quadrupole MS/MS system

Abstract

Modern measures to protect surface waters against known pollutants include strict government regulations and high analytical standards for their sensitive and reliable determination. Sample enrichment methods, often necessary in order to detect pollutants at trace concentration levels, can add significant time and expense to 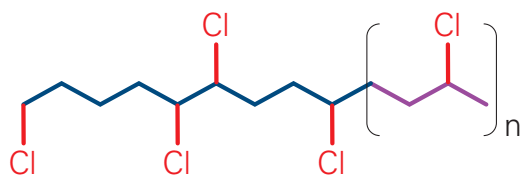required environmental monitoring workflows. The true utility of both preparation and detection technologies may also be challenged by the diversity of targeted compounds, even within the same pollutant class. This note highlights the speed, sensitivity, and specificity of the Bruker *µ*Drop method of sample enrichment in combination with the EVOQ GC-TQ MS/MS system for the detection of short chain chloroalkanes from surface water samples.

Introduction

Chloroalkanes, also known as Polychlorinated n-Alkanes (PCAs) or Chlorinated Paraffins (CPs) have been used as additives to plastics, rubber, and sealants and within specialized coatings for textiles and leathers based on the desirable properties of flame retardancy and improved durable flexibility. These protective features have also been a rationale for their use as a component of

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C10-C13 (short chain - **SCCP**)
C14-C17 (medium chain - **MCCP**)
C>17 (long chain - **LCCP**)

SCCP Isomers														
C/Cl	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**Σ**
10	5	25	60	110	126	110	60	25	5	1	0	0	0	527
11	6	30	85	170	236	236	170	85	30	6	1	0	0	1055
12	6	36	110	255	396	472	396	255	110	36	7	1	0	2080
13	7	42	146	365	651	868	868	651	365	146	42	7	1	4159
											Total number of isomers			**7821**

Figure 1. Structure of chloroalkanes.

lubricants for industrial metal-working applications. However, the environmental and safety risks directly linked to exposure to chloroalkanes have led to their classification as hazardous materials and the implementation of regulatory measures in many parts of the world.

Within the broader chloroalkanes family (Figure 1), Short Chain Chlorinated Paraffins (SCCP) have been identified as a priority hazardous substance. The "persistent, bioaccumulative, and toxic effects" of SCCP exposure have led to strict control on their industrial use within the EU [1], however, decades of past use necessitate caution against unintentional exposure. Passive propagation of SCCPs through the environment may result from the recycling of household and industrial plastics, leaching from soil into groundwater, and domestic wastewater treatment, among other sources.

In recognition of their particular harm to aquatic life, surface waters are protected under Directive 2013/39/EU of the European Commission [2]. In order to meet Environmental Quality Standards (EQS), the annual average of detected SCCPs must be less than 400 ppt (ng/L) (Table 1) and the method LOD should be 30% of lower parametric value, or 120 ppt. As SCCPs

exist as a complex mix of congeners containing 10 to 13 carbons and chlorination rates from 30-70% (w/w), nearly 8000 isomers are possible (Figure 1). Further, any method for reliable detection and quantitation of SCCPs can be challenged by other semi-volatile organic compounds, co-eluting compounds, or other classes of chloroalkanes.

Methods used for the determination of chloroalkanes are traditionally based on GC-ECD (gas chromatography-electron capture detector) or GC-NCI-MS (gas chromatography-negative chemical ionization-mass spectrometry), as they have good sensitivity. However, the response factors of these methods have a strong dependence on the degree of chlorination in the mixtures analyzed. GC-NCI-MS, in particular, does not detect CPs with fewer than five chlorine atoms [3], thus quantitation accuracy for this mixture is unreliable.

A simple, sensitive, and cost-effective approach to quantitative detection of total CPs (the sum of SCCPs and MCCPs) - independent of chlorinated content - can be made using electron ionization (EI)-MS/MS in combination with Bruker µDROP sample preparation. With EI-MS/MS, chloroalkanes signal response is not influenced by carbon chain length, or by the degree of chlorination [4]. Further, the innovative µDROP workflow is well aligned with green analytical chemistry (GAC) principles, extending protective environmental stewardship into the laboratory.

The value of high enrichment (up to 1000 times) and target recovery factors (70-120%) of µDrop workflows have been demonstrated in other aqueous samples and wines [5,6]. The Bruker µDROP method is based on dispersive liquid/liquid microextraction techniques, where three liquid phases are involved: the water sample to analyze (aqueous phase), organic solvents immiscible in

Table 1. Surface water limits for short chain chloroalkanes according to EU Directive 2013/39/EU [2]. AA=annual average; MAC= maximum allowable concentration.

Environmental Quality Standards (EQS) for Priority Substances				
Name of substance	**AA-EQS Inland surface waters (ng/L=ppt)**	**AA-EQS Other surface waters (ng/L=ppt)**	**MAC-EQS Inland surface waters (ng/L=ppt)**	**MAC-EQS Other surface waters (ng/L=ppt)**
C10-C13 chloroalkanes	400	400	1400	1400

the aqueous phase (extractant phase), and organic solvents fully soluble in both the extractants and the aqueous phase (dispersant phase). The dispersant phase bridges the solubility gap between the other two phases and facilitates target extraction and enrichment for downstream analysis.

This work describes the use of the Bruker μDROP method for sample preparation, followed by EVOQ GC-TQ MS/MS for the analysis of a short chain chloroalkane standard in various control and natural (crude) water matrices. Quantitation linearity and accuracy were evaluated, as was method sensitivity, within the framework of current EU detection requirements. The specificity of the method was also tested by the analysis of water samples spiked with both chloroalkanes and a mixture of pesticides. This combination of technologies is uniquely suited to pollutant determination in aqueous samples.

Materials and Methods

Water samples

Four different water matrices were studied: pure (Milli-Q®) water, tap water collected from Móstoles, Madrid (GPS: 40°20′04.3′′ N; 3°52′55.1′′W), water from the Eresma River, Segovia (GPS: 40°95′44.02′′ N; -4°12′11.43′′W), and water collected from the Mediterranean sea at Castelldefels, Barcelona (GPS: 41° 26′32.28′′ N; 1° 98′57.84′′W). The samples were denoted as MQ, TAP, RIVER, and SEA, respectively.

Chloroalkane standards

A short chain chloroparaffin (C10-C13) standard mixture in cyclohexane (51.5% Cl, DRE-A23105100CY-100, Dr. Ehrenstorfer) and an internal standard (PCB 30, 10 μg/mL in cyclohexane, DRE-L20003000CY, Dr. Ehrenstorfer) were purchased from LGC Standards.

Bruker μDROP target extraction and GC-MS/MS analysis

Sample preparation has been previously described [5,6]. Briefly, the SCCP standard mixture and the internal standard were added to 35 mL of water in a 50 mL Bruker centrifuge tube (p/n: 1850435). After mixing (15 seconds), an aliquot of the Bruker μDROP Solvent Extraction Mixture #1 from the (ready-to-use) μDROP kit (p/n: 1845184) was added, and the solution was again mixed (15 seconds). The sample was then centrifuged for 10 minutes at 3000 rpm at room temperature. The μDROP, containing all extracted compounds, was then collected by pipet (Figure 2, inset) and transferred to a GC vial to be placed within the autosampler for automated analysis by the EVOQ GC-TQ system.
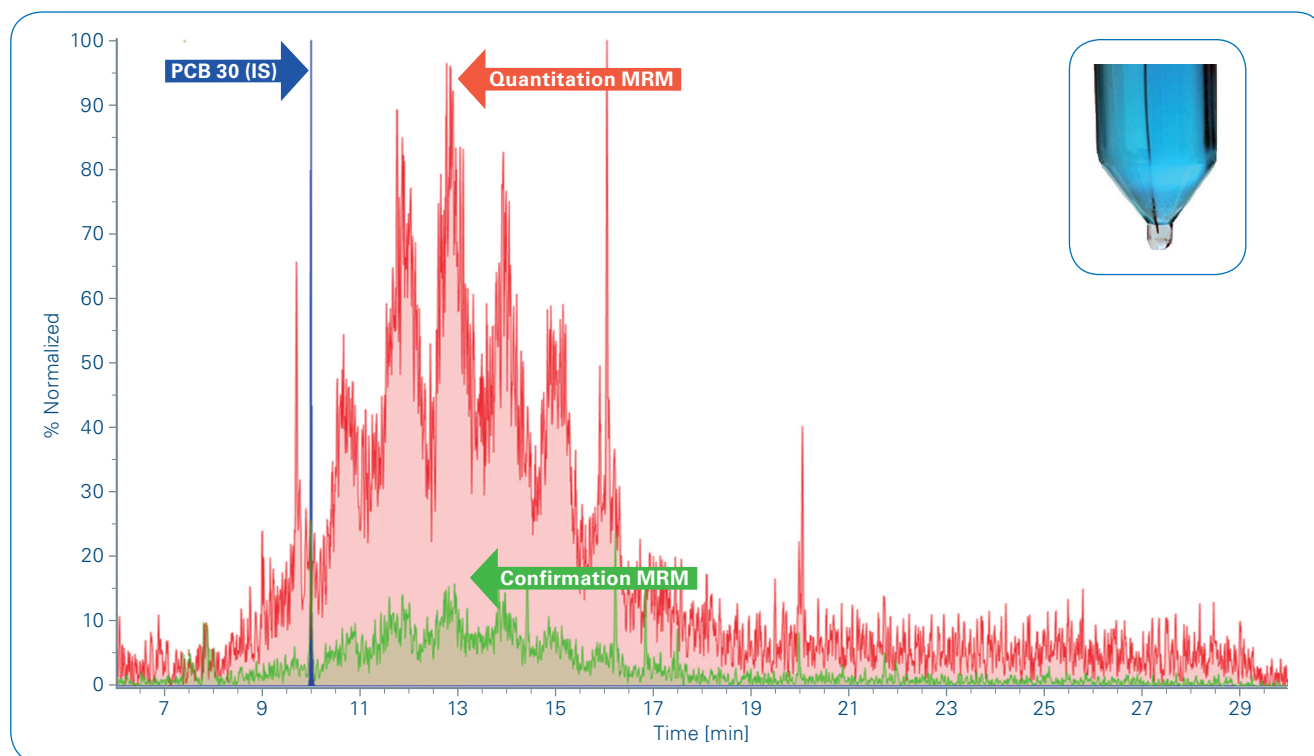

Figure 2. River water sample spiked with the short chain chloroalkanes standard at 25 ppt, extracted using the Bruker μDROP method and analyzed via GC-TQ MS. Inset: Collection of μDrop containing all extracted compounds.

Details of the GC-MS/MS analysis conditions are outlined in Tables 2 and 3. Bruker Compass TQ software (Bruker Daltonics) was used for EVOQ GC-TQ data screening and quantitation. An example MRM chromatogram is shown in Figure 2.

Evaluation of method linearity, sensitivity, and specificity

Water samples were spiked with the chloroalkanes standard mixture at 50, 100, 200, 500, and 1000 ppt prior to extraction using the Bruker µDROP method as described above. An internal standard (PCB 30) was spiked at 500 ppt to all calibration mixtures. Four replicates were prepared in each water matrix.

Method sensitivity was evaluated according to the parameters of the Method Reporting Limit (MRL) and Method Detection Limit (MDL). These values, also referred as LOQ (Limit of Quantitation) and LOD (Limit of Detection), were experimentally determined. For determination of MDLs, water samples spiked with decreasing concentrations of the chloroalkane standard were prepared. A blank control of each water sample, subjected to the same extraction process, was injected following their respective analyses.

To evaluate method specificity, water samples at the lowest calibration level (50 ppt) were spiked with a mixture of more than 100 pesticides (Table 4) at 1000 ppt to evaluate possible interference to the detection and quantitation of SCCPs. The paired water samples were then extracted following the µDROP procedure and analyzed via EVOQ GC-TQ.

Results

Linearity

The EVOQ GC-TQ analysis method following Bruker µDROP extraction had excellent linearity for chloroalkanes determination in all water matrices. The quality criteria established for this method were $R^2 > 0.99$ and RSD < 30%, and both were met for all samples (Figure 3). RSD values were between 9 and 15%, and the method linearity was reliably consistent, independent of the water matrix. The signal response linearity is also well visualized in the overlaid MRM transition panel shown in Figure 4.

Method sensitivity

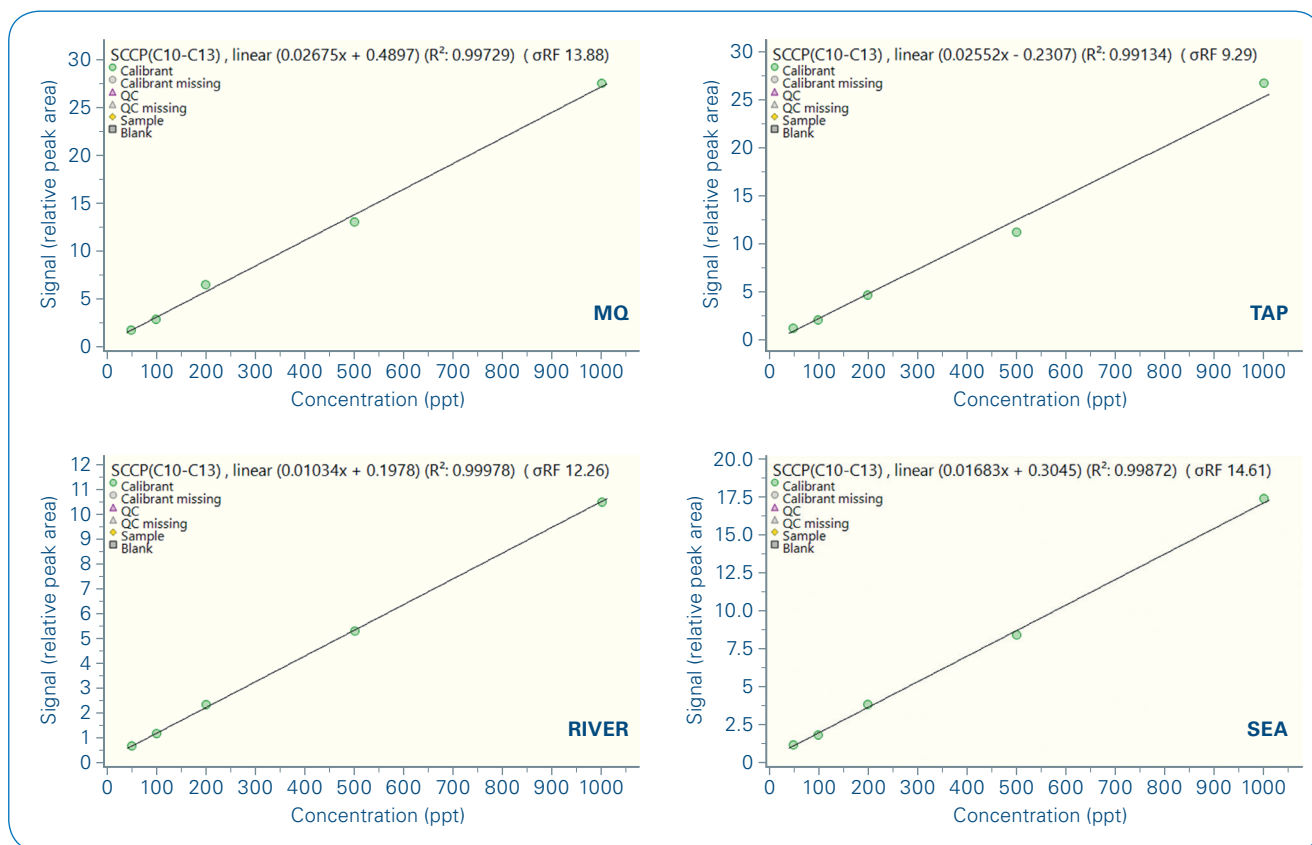Method sensitivity evaluations consider both the sample preparation and

Figure 3. **SCCP calibration linearity** in various water matrices. EU method requirements ($R^2 > 0.99$ and RSD < 30%) were met for all samples.
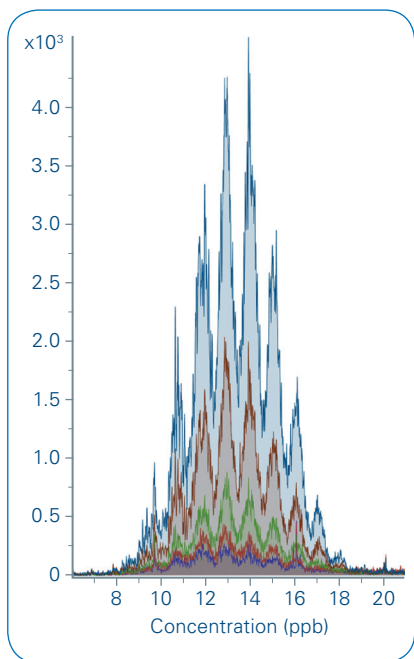
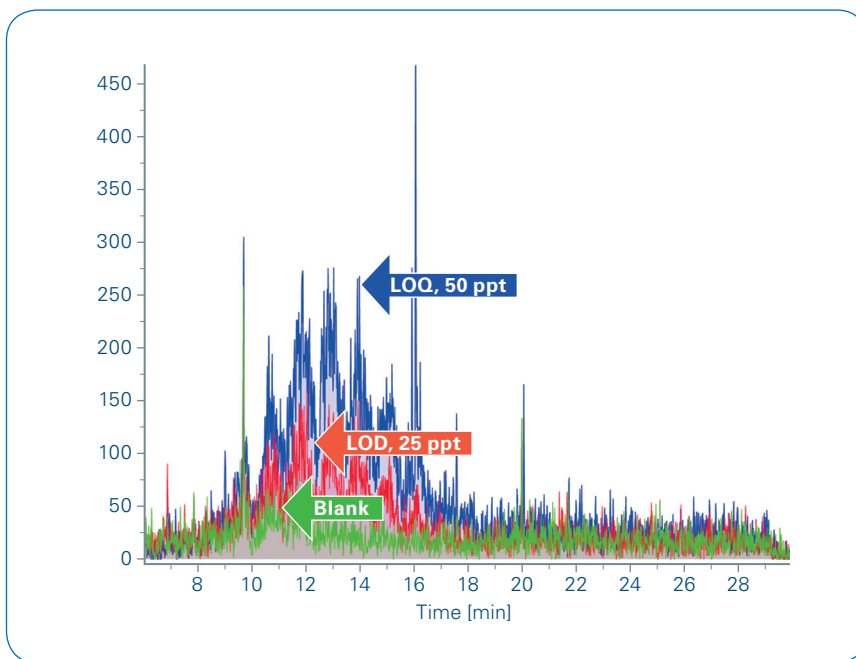*Figure 4. Overlaid MRM calibration chromatogram in a tap water sample.*

*Figure 5. **Confirmation of method sensitivity** for target chloroalkanes. LOQ (MRL) and LOD (MDL) meet current EU regulatory requirements.*

the specific instrument parameters. The Method Reporting Limit (MRL) and Method Detection Limit (MDL) are often used as sensitivity descriptors for modern TQ MS analyses, as noise values near zero hinder the accuracy of statistical calculations solely based on S/N, potentially leading to false positive detections.

In this analysis, the Method Reporting Limit (MRL) was established at 50 ppt, the lowest calibrant level. The lowest concentration at which the chloroalkanes were detectable with S/N values > 10 for both the quantitation and confirmation ions (when compared against the blank) was 25 ppt (Figure 5).

As defined within the current EU regulatory criteria [2], the LOD for chloroalkanes in surface waters is set at 30% of the lower parametric value (400 ppt), or 120 ppt. This detection sensitivity requirement was exceeded by the combined method of Bruker μDrop extraction and EVOQ GC-TQ analysis.

Table 2. Analytical conditions.

Gas Chromatography	
Instrument	**Bruker 436 GC**
Injector	1079 inert, PTV injector
Column	BR-5ms, 30 m x 0.25 mm, 0.25 micron
Total run time	29 min
Column flow	He, 1 mL/min
Autosampler	Bruker 8400
Mass Spectrometer	
Instrument	**Bruker EVOQ Triple Quadrupole**
Ionization mode	EI
Source temperature	280°C
Transfer line temperature	280°C
MS operation mode	MRM
Collision gas	Ar, 2 mTorr
Detector	EDR
Software	Bruker Compass TQ

Table 3. MRM conditions.

N°	Compounds	RT	Quantitation transitions	CE	Confirmation transitions	CE
1	PCB 30 (Internal Standard)	9.97	258 > 186	-25	258 > 151	-50
2	Chloroalkanes	12.86	89 > 63	-15	103 > 67	-10

Table 4. Composition of the pesticide mixture for evaluation of method specificity. Water samples were spiked to a final concentration of 1000 ppt from a stock pesticide mixture in methanol.

N°	Pesticides	N°	Pesticides	N°	Pesticides	N°	Pesticides
1	2-phenylphenol	28	Diazinon	55	Malathion	82	Prothiofos
2	Acetochlor	29	Dichlobenil	56	Mepronil	83	Pyridaben
3	Acrinathrin	30	Dicofol	57	Metalaxyl	84	Pyrifenox
4	Aldrin and Dieldrin	31	Diphenylamine	58	Methacrifos	85	Pyrimethanil
5	Atrazine	32	Difenoconazole	59	Metribuzin	86	Pyriproxyfen
6	Benalaxyl	33	Diflufenican	60	Mirex	87	Quinalphos
7	Benfluralin	34	Diniconazole	61	Molinate	88	Quintozene
8	Bifenazate	35	Disulfoton	62	Myclobutanyl	89	Simazine
9	Biphenyl	36	Disulfoton-sulfoxide	63	Napropamide	90	Tau-Fluvalinate
10	Bifenthrin	37	Endosulfan	64	Nuarimol	91	Tebuconazole
11	Bitertanol	38	Ethion	65	o,p´-DDT	92	Tebufenpyrad
12	Bromophos	39	Ethofumesate	66	Ofurace	93	Tecnazene
13	Bromopropylate	40	Ethoprophos	67	Oxadixyl	94	Tefluthrin
14	Bupirimate	41	Fenpropathrin	68	p,p´-DDE	95	Terbuthylazine
15	Carbophenothion	42	Fenarimol	69	p,p´-DDT	96	Terbutryn
16	Cyfluthrin	43	Fenazaquin	70	Penconazole	97	Tetraconazole
17	Cypermethrin	44	Fenthion	71	Pendimethalin	98	Tetradifon
18	Cyproconazole	45	Fenvalerate	72	Pentachloroanisole	99	Tetramethrin
19	Cyprodinil	46	Flucythrinate	73	Pentachlorobenzene	100	Thiometon
20	Chlordane	47	Fonofos	74	Permethrin	101	Tolclofos-methyl
21	Chlorfenapyr	48	Kresoxim-methyl	75	Phenthoate	102	Transfluthrin
22	Chlorfenvinphos	49	Hexachlorobenzene	76	Piperonyl butoxide	103	Triadimenol
23	Chloropropylate	50	Iprovalicarb	77	Pirimiphos-ethyl	104	Trifluralin
24	Chlorpyrifos	51	Isazofos	78	Pirimiphos-methyl	105	Vinclozolin
25	Chlorpyrifos-methyl	52	Isofenphos	79	Procymidone		
26	Chlorpropham	53	Isofenphos-methyl	80	Profenofos		
27	Chlorthal-dimethyl	54	Lambda-Cyhalothrin	81	Propyzamide		

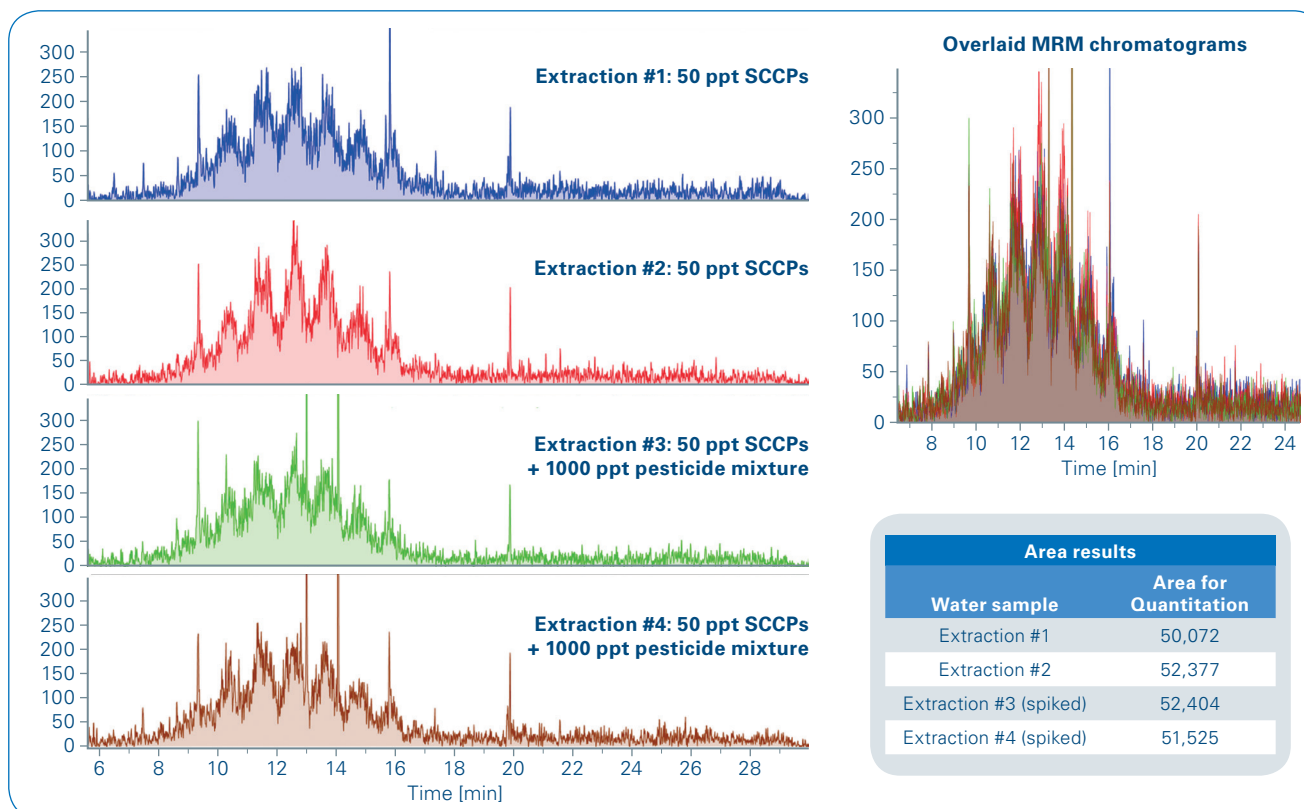| Area results	
Water sample	Area for Quantitation
Extraction #1	50,072
Extraction #2	52,377
Extraction #3 (spiked)	52,404
Extraction #4 (spiked)	51,525

*Figure 6. **Confirmation of method specificity**. Quantitative detection of target SCCPs was unaffected by the presence and simultaneous extraction of other SVOCs pollutants common to surface waters.*

Method specificity

As the Bruker μDrop method extracts all non-polar semi-volatile organic compounds (SVOCs) from water samples in a single droplet, other pollutants may be enriched for analysis as well. No interference in the quantitative detection of chloroalkanes was observed in pesticide-spiked samples using this workflow (Figure 6). Further, alternate GC conditions could be used to (separately) analyze the pesticide mixture from the same collected μDROP (Figure 7).

Discussion

As awareness of the potential environmental damage – both long and short term – from exposure to many chemical classes has increased, analytical tools for confident detection continue to be developed. Methods with high sensitivity and specificity are critical, and methods utilizing green chemistry principles are highly desirable. In addition to procedural speed, simplicity, and scalability, the Bruker μDrop sample enrichment method requires very little energy (with simple pipetting, mixing, and room temperature centrifugation) and generates very little hazardous waste. Coupled with reliable GC-MS analyses on the EVOQ GC-TQ system, chloroalkane pollutants may be easily and reproducibly detected and quantitated in water matrices. Although this study is focused on surface water regulations, this approach is amenable to many aqueous pollutant testing needs, including extractive soil testing and wastewater screening.
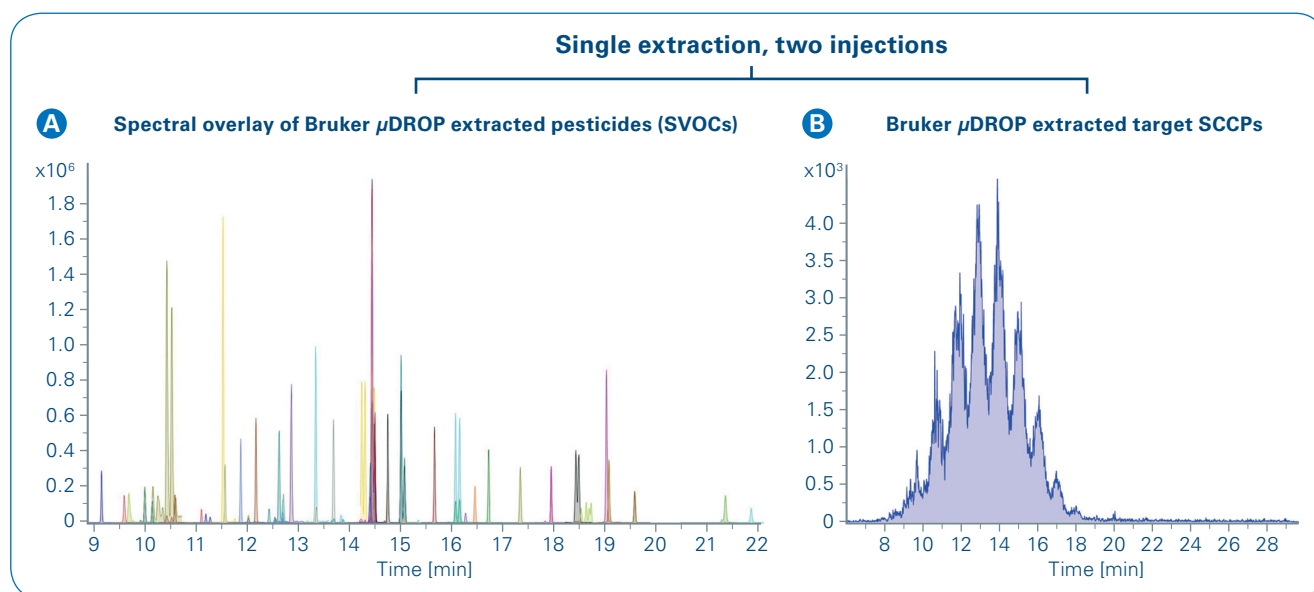

Figure 7. Expanded analytical capabilities for non-polar compounds in aqueous samples. Using Bruker μDROP, both pesticides (A) and short chain chloroalkanes (B) can be examined from the same extracted water sample.

Conclusion

- In this study, Bruker μDrop extraction prior to EVOQ GC-TQ analysis has been shown to be a precise, reliable, and sensitive methodology for the determination of chloroalkane pollutants in various water matrices, meeting current EU criteria for surface water analysis.

- The EI-based GC-TQ workflow permits detection of chloroalkanes regardless of the carbon chain length or degree of chlorination.

- The enrichment power of the Bruker μDrop methodology, together with the ease and speed of sample preparation (approximately 11 minutes), supports sensitive and reproducible detection and quantitation of chloroalkanes at trace concentration levels. Multiple samples may be prepared simultaneously for higher throughput analyses.

- The alignment of the μDROP workflow with modern green analytical chemistry principles offers further advantages to the unique strengths of this combined workflow for pollutant determination in aqueous samples.

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References

[1] Regulation 2019/1021 of the European Parliament and of the Council of 20 June 2019 on persistent organic pollutants. http://data.europa.eu/eli/reg/2019/1021/oj

[2] Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. http://data.europa.eu/eli/dir/2013/39/oj

[3] van Mourik LM, Leonards PEG, Gaus C, de Boer J (2015). *Recent developments in capabilities for analysing chlorinated paraffins in environmental matrices: a review*, Chemosphere, **136**:259-272.

[4] Zencak Z, Reth M, Oehme M (2004). *Determination of total polychlorinated n-alkane concentration in biota by electron ionization–MS/MS.* Anal Chem, **76**:1957–62.

[5] Bruker Application Note LCMS-136: Determination of SVOCs in water samples using the Bruker μDROP method for the EVOQ GC-TQ MS/MS system

[6] Bruker Application Note GCMS-13: Novel multiresidue method for rapid determination of pesticides in wines using GC-MS/MS

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