WHITE PAPER

Fast assessment of adsorbable organically bound halogens (AOX) in waters

Determination of AOCl, AOBr, AOI, and AOF with combustion ion chromatography (CIC) according to DIN 38409-59

Adsorbable organically bound halogens (known as AOX) are the sum of numerous halogenated organic compounds that are adsorbable on activated carbon. Many of these organic halogen compounds and their degradation products pose serious risks to human health and the environment. Therefore, AOX must be monitored to ensure adequate water quality as well as to trace its sources and investigate the efficiency of AOX removal techniques in water treatment processes. Historically, this sum parameter was determined by microcoulometric titration as per DIN EN ISO 9562 or EPA 1650. However, AOX comprises AOCl, AOBr, and AOI, which cannot be determined individually. The new DIN 38409-59 describes a procedure of adsorption and analysis via combustion ion chromatography (CIC) to determine AOCl, AOBr, AOI, the sum parameter CIC-AOX$_{(Cl)}$, as well as AOF – a monitoring parameter for per- and polyfluorinated alkyl substances (PFASs), which are of increasing global concern.

INTRODUCTION

AOX (adsorbable organically bound halogens) is a complex index covering the sum of halogenated organic compounds that are adsorbable on activated carbon. This sum parameter was historically determined via microcoulometric titration [**1,2**]. By definition (based on the technical setup outlined later in this article), AOX covered adsorbable organically bound chlorine, bromine, and iodine, but not fluorine. Many of these AOX compounds and their degradation products pose serious risks to human health and the environment as they possess toxic, mutagenic, and carcinogenic characteristics, are ubiquitous and persistent, and accumulate in the food chain [**1–5**].

– ORIGIN AND SOURCES

The concept of the sum parameter AOX was already introduced back in 1976 as a way to quickly quantify organohalogens in wastewater, drinking water, and natural water sources. Later on it was extended to include some insoluble compounds in sewage sludge and sediments, often referred to as «AOX-S18» (absorbed organically bound halogens) [**1,2**]. The first naturally derived halogenated organic compounds were already isolated in 1896 [**6**], their numbers increasing following the development of appropriate separation and identification techniques [**7**]. To date, more than 5000 naturally derived organohalogens have been discovered – predominantly organochlorine (50.6%) and organobromine (44.8%), with only minor fractions of organoiodine and organofluorine (4.6%) [**1,7**].

Some organohalogens can be produced naturally by living organisms, although the negative effects of these compounds are rather low, while organohalogens produced by industrial and other anthropogenic means impart a much larger adverse impact, as they are widespread and persistent [**7,8**]. The industrial revolution, which began in the second half of the 18th century, increased the extensive production of halogenated organics, such as chlorinated hydrocarbons or other organic compounds including PCBs (polychlorinated biphenyls), DDT (dichlorodiphenyltrichloroethane), and atrazine, which were used for agricultural, industrial, and domestic purposes [**1**].

In the 1980s, public awareness began to rise along with the fear of negative impacts from AOX in the environment. This was mainly due to the discovery of chlorinated organics and dioxins found in pulp and paper mill effluents [**1,9**], but also from witnessing the tragic aftermath of the Seveso disaster [**10**] along with the long-lasting consequences of using «Agent Orange» in the Vietnam war [**11**].

Major anthropogenic sources of AOX include industrial emissions like wastewater effluents from pulp and paper manufacturers, the textile dyeing and finishing industry, and production plants creating synthetic organohalogens and their end-products (e.g., flame retardants or PCBs). Drinking water disinfection processes, wastewater treatment plants, and municipal wastewaters are also significant sources for AOX [**1,12 and references therein**]. Agricultural runoff is a diffuse and difficult to handle AOX source from which a range of herbicides, insecticides, and pesticides are present – many of which have already been banned in different countries (e.g., atrazine [**13**] and DDT [Stockholm Convention, DDT national law of Germany]). In hospital wastewater, it is especially iodinated organics, iodinated X-ray contrast media, pharmaceuticals, and disinfectants such as triclosan [**3**] that contribute to AOX levels [**1,4,14**].

In recent years, more attention has been paid to organofluorine compounds, especially «forever chemicals» such as PFASs (per- and polyfluorinated alkyl substances). This class of over 10,000 chemicals is persistent, accumulating in the environment as well as in our bodies. Many PFASs pose extensive risks to both environmental and human health [**15 and references therein**]. Targeted analysis of specific compounds in this class is time consuming, complex, and expensive, while the determination of the sum parameters adsorbable organically bound fluorine (AOF) for waters and extractable organically bound fluorine (EOF) for solids or biological matrices have been widely adopted in the scientific community to study and monitor organofluorines [**15 and references therein**].

The overall formation and degradation cycle of AOX is complex (**Figure 1**) and shows the widespread distribution and wide-range transport of some organic halogens.

– REGULATIONS

AOX as a sum parameter provides a valuable solution for laboratories to assess, trace, monitor, and control sources and sinks, as well as to explore the efficiency of removal technologies for organohalogens. For example, organohalogens are listed as potential pollutants in the German Water Management Act, which is based on the EU Water Framework Directive

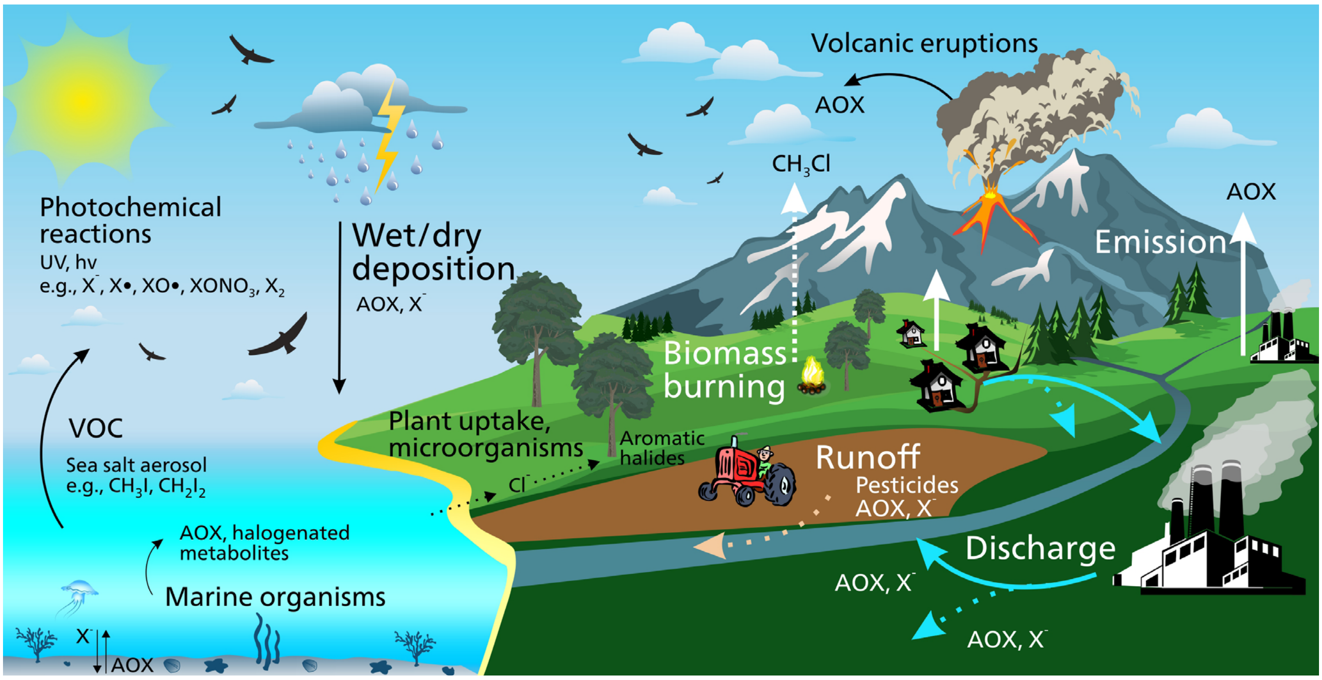

Figure 1. Both natural and anthropogenic sources contribute to AOX presence in the environment. Aside from natural sources including volcanism, microbial activity, and marine turnover (e.g., volatile organic compounds (VOC)), **it is anthropogenic pollution that increases AOX levels most significantly**. Industrial emissions, discharge from industrial and domestic wastewater treatment plants, and runoff from agricultural areas are just some of the ways these myriad compounds find their way into the environment. Figure adapted from Xu et al. [**1**].

(2000/60/EC). AOX is regulated for wastewaters directly in the German Wastewater Ordinance (AbwV) and the German Wastewater Tax Act (AbwAG), and for solid waste in the German Sewage Sludge Ordinance (AbfKlärV). The thresholds for AOX discharge are set to 100 µg/L or 10 kg/year (AbwAG), and the limit for sewage sludge is fixed at 400 mg/kg dry weight (DIN 38414-18, DIN 16166). Wastewater handling requirements vary for certain industries and are regulated with annual load values as well as maximum concentrations within the AbwV.

– PROGRESSION OF ANALYTICAL METHODOLOGY

Until now, all requirements for measuring AOX in waters and wastewaters have been based on the analysis method proposed by Kühn in 1974, involving AOCl, AOBr, and AOI [**16**]. In brief, the organically bound halogens in acidified samples are enriched on activated carbon while inorganic halogens are rinsed away with an acidified nitrate solution, and finally the adsorbed organically bound halogens are determined microcoulometrically subsequent to a mineralization step (combustion) [**17**]. The high affinity of chloride, bromide, and iodide to silver cations introduced during titration with silver nitrate causes the formation of precipitates and allows the total quantification of AOX stated as chloride [**1**]. However, silver-fluoride complexes are highly soluble and were thus not accounted for in any AOX determination according to Kühn [**1,16**], nor the standardized procedure for waters outlined in DIN EN ISO 9562. A similar approach is found in EPA 1650.

A new and promising approach for the determination of organically bound fluorine, chlorine, bromine, and iodine (AOF, AOCl, AOBr, and AOI) was enabled through mineralization of these substances by automatic combustion and subsequent analysis with ion chromatography (CIC). While titration of the mineralized organohalogens (that had been adsorbed on activated carbon) only allows determination of AOX as chloride, ion chromatographic separation after pyrohydrolytic combustion facilitates individual detection of the halogens chloride, bromide, and iodide, as well as fluoride. With this approach, new prospects were made possible and were standardized within the recently published DIN 38409-59. In this norm, individual AOX species can be resolved (e.g., to investigate AOX sources and control specific treatment processes) while the sum parameter AOF can be used for monitoring PFASs [**15**]. By adding up AOCl, AOBr, and AOI, including the respective mass correction, the sum parameter $\text{CIC-AOX}_{(Cl)}$ (expressed as chloride) can also be assessed. Due to the novelty of this approach, $\text{CIC-AOX}_{(Cl)}$ is not yet equivalent to AOX as defined in past regulations.

EXPERIMENTAL

Together with the automatic sample preparation unit (APU sim) from Analytik Jena, Metrohm provides a fully automated analytical solution to fulfill the specifications for CIC-AOX$_{(Cl)}$ from AOCl, AOBr, and AOI, as well as AOF determination as outlined in the new standard DIN 38409-59.

In short, the general working principle begins with preconcentration of adsorbable organically bound halogens on activated carbon, followed by automatic combustion and analysis of the individual halogens by ion chromatography (**Figure 2**). Without any extra effort, the sum parameters of AOX and now AOF can be determined from the measured halogen concentrations. However, the sample preparation for AOX (i.e., AOCl, AOBr, and AOI) and AOF determination (according to DIN 38409-59) differs slightly. An advantage of the new DIN 38409-59 is that it allows the determination of AOF and also the determination of adsorbable organically bound halogens as individual sum parameters. The analytical and instrumental details for these analyses are described in the next section.

– SAMPLE PREPARATION: ADSORPTION OF ORGANOHALOGENS

The overall sample preparation procedure (i.e., preconcentration and adsorption of organohalogens) resembles that of DIN EN ISO 9562, as the adsorption on activated carbon is a key point for both methods.

While it is crucial for AOF determination that the samples have a neutral pH to avoid adsorption of inorganic fluorine to the activated carbon, sample acidification is mandatory for measurement of the other organically bound halogens (similar to the procedure in DIN EN ISO 9562). For AOX (i.e., AOCl, AOBr, and AOI) determination, samples need to be acidified with nitric acid to pH <2 prior to the preconcentration step (**Table 1**). As AOF determination is also within the scope of DIN 38409-59, the preparation for these samples requires neutralization. This is accomplished by addition of sodium nitrate (see related Metrohm Application Notes and DIN 38409-59; **Table 1**).

The adsorption of the organically bound halogens is performed as an automated sample preparation step using the APU sim system from Analytik Jena (**Figure 2**). Although the overall procedure involves several steps, automation and simultaneous handling

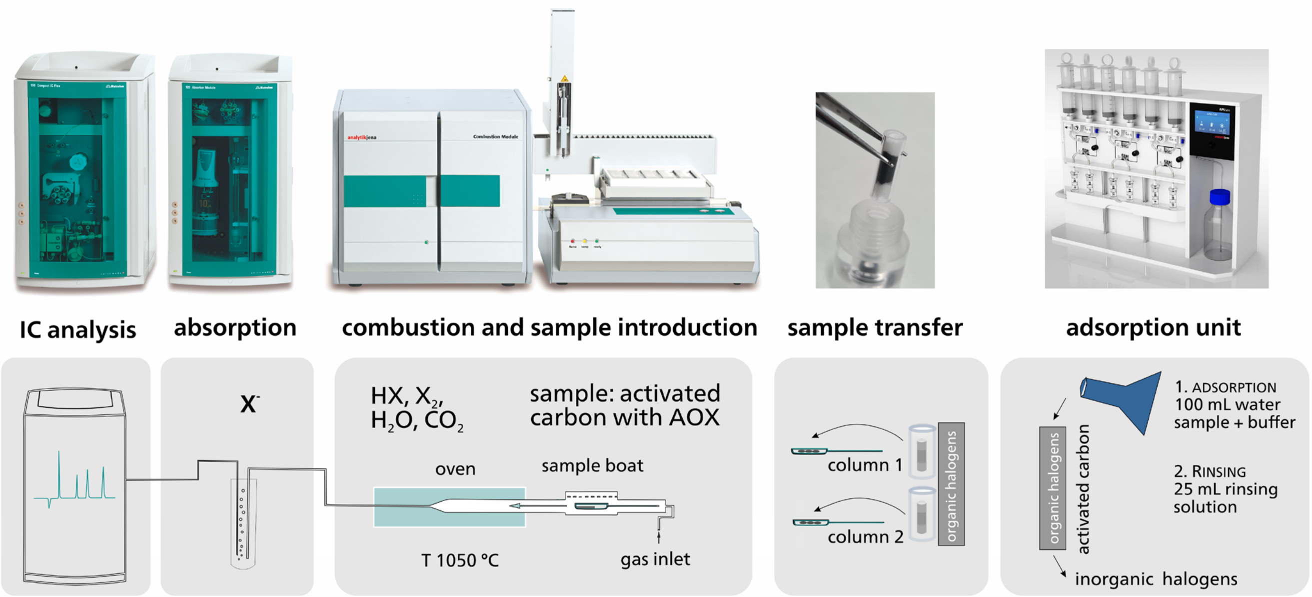

Figure 2. APU sim (Analytik Jena, top right) for automated and standardized adsorption of up to six samples in parallel. Combustion ion chromatograph setup (top left) comprised of an IC (930 Compact IC Flex) and absorber module (920 Absorber Module) with the combustion module (Analytik Jena) consisting of a combustion oven with Auto Boat Drive (ABD) and an autosampler (MMS 5000) (from left to right). After the sorption and preconcentration step, the activated carbon is loaded into combustion boats and automatically analyzed by CIC. Volatilized halogens enter the absorber tube with the argon/oxygen stream as shown in the lower figure. The transfer of samples and standards into the sample loop is performed using the Metrohm intelligent Partial Loop Injection Technique (MiPT), giving full flexibility to reliably inject volumes from 4 to 200 µL. The outcome is a clean IC chromatogram which is used for determining the content of the organohalogens in the original sample.

Table 1. Parameters for AOF and AOX sample preparation.

	AOF	AOCl, AOBr, AOI
pH	Neutralized	Acidified to pH <2 with nitric acid
Buffer	0.5 mL 2 mol/L sodium nitrate	0.5 mL 2 mol/L sodium nitrate, acidified with nitric acid
Sample volume	100 mL	
Rinsing solution	25 mL	
	0.01 mol/L sodium nitrate	0.01 mol/L sodium nitrate, acidified with nitric acid
Absorption columns	Two activated carbon tubes (disposable, from Analytik Jena)	
	402-880.616	402-880.610
Flow rate APU sim	3 mL/min	

of up to six samples makes this a standardized preparation method with excellent repeatability and a high sample throughput of about 60 samples per workday (less than 45 minutes per batch of six samples).

In brief, two columns filled with activated carbon (at least 50 mg for each column) connected in series are flushed with 100 mL sample with a flow rate of 3 mL/min. The organically bound halogens adsorb to the activated carbon (special disposable columns used for AOF and AOX determination, respectively, see **Table 1**), while inorganic halogens are rinsed off with an additional 25 mL of rinsing solution (**Table 1**).

After the automated sample preparation, the complete content of both columns is transferred into two separate ceramic boats (not mandatory, one boat can also be used for combustion) for CIC analysis.

– COMBUSTION IC ANALYSIS

After the activated carbon is transferred to the ceramic sample boats, each boat is individually combusted at a temperature of 1050 °C in the presence of argon and oxygen using the combustion module (**Figure 2**, **Table 2**). The combustion occurs pyrohydrolytically, therefore a water stream is essential as it converts the halogens into their hydrogenous forms. With a Dosino, Metrohm's automated and precise liquid handling tool, ultrapure water is added at a rate of 0.2 mL/min during the combustion step. Automatic sample handling (**Figure 2**) allows users to run up to 35 samples in a row, reducing manual laboratory work to a minimum.

Table 2. Parameters for the combustion process (Combustion Module from Analytik Jena).

Parameter	Setting
Carrier gas, argon (4.8)	100 mL/min
Carrier gas, oxygen (5.0)	300 mL/min
Oven temperature	1050 °C
Post-combustion time	120 s
Water inlet	0.2 mL/min

The organohalogens from the 100 mL sample adsorbed on the activated carbon are volatilized during the combustion step. Then they are transported into the absorber solution (ultrapure water) with an argon/oxygen gas stream and transferred into the liquid phase (**Table 2**).

With the help of Dosinos, the aqueous sample is transferred to the IC for analysis. In addition to the fully automated combustion, absorption, and liquid handling steps, further automation features can improve the efficiency of Metrohm CIC for AOX analysis:

- The 941 Eluent Production Module enables automated eluent production and almost unattended operation of the IC system.

- Application of the Metrohm intelligent Partial Loop Injection Technique (MiPT) allows both automatic calibration and variable-volume sample injections, adjustable depending on the halogen content of the sample.

- The intelligent MagIC Net software allows logical and nested operation in addition to the usual instrument control and data handling features of chromatographic data software programs. By applying high-low calibration, the results are automatically assigned to the appropriate calibration range. Accurate data calculation is guaranteed and extra work for further sample dilutions is rendered unnecessary.

Ion chromatographic separation of the halogens is achieved on a Metrosep A Supp 5 - 250/4.0 column in combination with the A Supp 5 Guard/4.0. AOF (i.e., fluoride) elutes in less than seven minutes [**15**]. For AOX (i.e., chloride, bromide, and iodide), the analyte peaks are entirely resolved in under 25 minutes (**Figure 3**).

Automatic system calibration with MiPT is performed using inorganic anion standards for fluoride, chloride, bromide, and iodide (1 g/L standard solutions, TraceCert® from Sigma-Aldrich, **Table 3**).

To check the overall performance of the AOF and AOX determinations, organic reference standard solutions with varying concentrations (4-fluorobenzoic acid, 4-chlorobenzoic acid, 4-bromobenzoic acid, and 4-iodobenzoic acid, equating to 3–19 µg/L AOF, 20–90 µg/L AOCl, and 2–9 µg/L AOBr and AOI) are applied. To ensure the validity of the overall procedure is checked, these standards are treated in the same way as the samples: preconcentrated on activated carbon using the APU sim and then automatically analyzed with the CIC setup.

In contrast to the analysis of halogens and sulfur in aqueous samples, the procedure for AOX and AOF is slightly more complex. It requires special sample boats and special charcoal (e.g., fluoride-free materials for AOF determination, **Table 3**), to guarantee a low background and to avoid cross-contamination. Nevertheless, blank measurements using preconcentrated ultrapure water are essential for the required blank correction (**Equation 1**) to ensure correct and reliable results for CIC-AOX$_{(Cl)}$ and AOF.

RESULTS

Individual concentrations for AOCl, AOBr, and AOI, as well as AOF for the neutralized samples are calculated according to **Equation 1**. The individual results for both adsorption columns are added together and must be corrected for the column blank values and the respective volumes (**Equation 1**).

Table 3. Parameters for absorption and ion chromatographic detection.

Parameter	Setting
Absorber solution	Ultrapure water
Initial volume of absorber solution	2.0 mL
Dosing rate of absorber solution	0.2 mL/min
Separation column	Metrosep A Supp 5 - 250/4.0 + Metrosep A Supp 5 Guard/4.0
Column temperature	55 °C
Eluent	2.8 mmol/L Na_2CO_3 Automatic production with the 941 Eluent Production Module
Eluent flow rate	0.8 mL/min
Suppression	MSM + MCS + STREAM* Regeneration with 500 mmol/L H_2SO_4
Detection	Suppressed conductivity
Calibration and calibration range	0.01–0.5 mg/L fluoride, chloride, bromide, and iodide Injected via MiPT (4–200 µL)

* **MSM** = Metrohm Suppressor Module, **MCS** = Metrohm CO_2 Suppressor, **STREAM** = Suppressor Treatment of Reused Eluent After Measuring

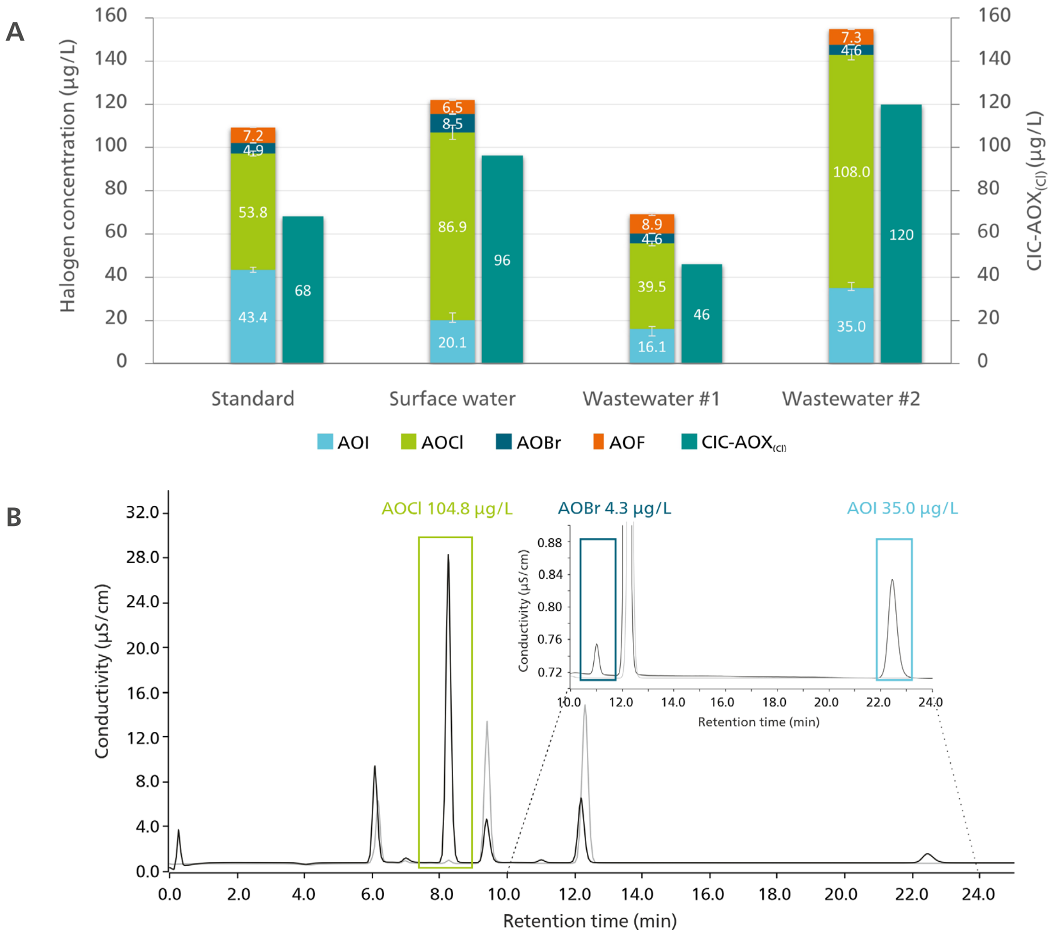

Figure 3. (A) Mass fractions of the individual adsorbable organically bound halogens (AOF, AOCl, AOBr, and AOI) and CIC-AOX$_{(Cl)}$ (the sum concentration of the mass fractions for AOCl, AOBr, and AOI) shown for different water samples and a standard solution. (B) Chromatogram overlay of the blank and wastewater #2 sample for the determination of AOCl, AOBr, and AOI from adsorption step 1 (i.e., adsorption column #1, **Figure 2**). To calculate CIC-AOX$_{(Cl)}$, the blank value is subtracted from the individual fractions according to **Equation 1**. The concentrations of bromine and iodine from the blank determination were found to be 0. No organohalogens were adsorbed at all on column #2 (**Figure 2**), highlighting the efficiency of adsorption column #1 to retain the major CIC-AOX$_{(Cl)}$ fraction.

$$c(X_{ads}) = \left(c(X^-)_{IC} \times \frac{V_{Abs}}{V_{Smpl}}\right) - \left(c(X^-_{BW})_{IC} \times \frac{V_{AbsBW}}{V_{SmplBW}}\right)$$

Equation 1.

$c(X_{ads})$	Mass concentration of individual adsorbable organically bound halogens (with X = Cl, Br, I, and F) in µg/L
$c(X^-)$	Halogen concentration in the sample's absorption solution (with X = Cl, Br, I, and F) in µg/L
V_{Abs}	Final volume of the absorption solution in L
V_{Smpl}	Volume of sample used for adsorption; always 0.1 L
$c(X^-_{BW})$	Halogen concentration in the absorption solution of the blank in µg/L
V_{AbsBW}	Final volume of the absorption solution of the blank in L
V_{SmplBW}	Volume of blank solution used for adsorption; always 0.1 L

$$c(CIC\text{-}AOX_{(Cl)}) = c(AOCl) + c(AOBr) \times 0.4437 + c(AOI) \times 0.2794$$

Equation 2.

$c(CIC\text{-}AOX_{(Cl)})$	Sum concentration of adsorbable organically bound halogens in µg/L as mass concentration based on chloride

Due to the special materials required, the sensitive analysis of the halogens with suppressed conductivity detection, and despite the complex sample preparation procedure, blank values were quite low and only measurable for fluoride and chloride (**Table 4**). The overall detection limits (LODs based on the calculation in DIN 32645) were less than 0.5 µg/L for AOBr, AOI, and AOF, and approximately 1.4 µg/L for AOCl. While LODs for AOBr and AOI were calculated using the calibration curve as no blank value was detected, LODs for AOCl and AOF were based on the blank method (**Table 4**). Ultimately, the described approach fits the scope of DIN 38409-59 (**Table 4**) and is even more sensitive.

During the DIN validation process, a range of water samples was analyzed by different laboratories using a setup similar to the one described earlier in this White Paper. The complete dataset will be published on the Water Chemistry Society website (wasserchemische-gesellschaft.de) once the norm is released, showing the validity and robustness of the overall procedure as a routine method. **Figure 3** shows the results for three selected samples and a check standard.

Table 4. Blank, LOD, and DIN scope for the determination of adsorbable organically bound halogens. LODs were determined following DIN 32645. For AOBr and AOI, LODs were determined using the calibration curve as no blank values were found. For AOF and AOCl, the blank method was applied (DIN 32645).

	Blank value (µg/L)	LOD (DIN 32645) (µg/L)	Scope of DIN application (µg/L)
AOF	1.1	0.38	≥2
AOCl	2.6	1.36	≥10
AOBr	0	0.24	≥1
AOI	0	0.47	≥1

In each case shown in **Table 4**, four replicates were prepared and analyzed. CIC-AOX$_{(Cl)}$ contents (as the mass corrected sum of AOCl, AOBr, and AOI) span a range of 46 to 120 µg/L. The higher values correspond to wastewater #2 and the surface water sample, showing the diversity of pollution sources (**Figure 3A**).

Using IC as an analytical technique, it is now possible to not only determine the sum parameter CIC-AOX$_{(Cl)}$, but also to measure individual fractions contributing to the AOX contents. In all water types, adsorbable organically bound chlorine made up the largest fraction of AOX (**Figure 3**, 85–90%), while the contributions of organoiodine (21–35%) and organobromine (4–10%) were less predominant.

AOF contents ranged from 7–9 µg/L (**Figure 3**). The RSDs for replicate analyses were less than 5%, which proves the excellent method performance (**Figure 3**). With this straightforward, fast, and reliable procedure, monitoring of PFASs (as AOF) is possible as an initial step before more complex analytical techniques (e.g., LC-MS/MS or GC-MS) are used for a more detailed view of individual PFASs.

CONCLUSION

The world of organohalogens is so varied that measuring sum parameters enables more insight into their sources, transport pathways, and also particularly vulnerable regions in a very simple manner. Complex targeted analysis (using e.g., LC-MS/MS or GC-MS) can resolve some individual halogenated organic compounds in a second stage of determination for deeper investigations.

With the new DIN 38409-59, a reliable and fast method is now offered to accurately determine the sum parameter CIC-AOX$_{(Cl)}$, including its individual fractions AOCl, AOI, and AOBr, as well as AOF. Standardization and high throughput of the adsorption procedure with the APU sim unit (six samples in parallel) along with the fully automated CIC analysis (including automatic calibration via MiPT and automatic data calculation) make this an ideal solution for routine laboratory analysis. The additional determination of individual AOX fractions and AOF with high sensitivity extends the application of the standardized procedure to monitor AOF and to investigate the individual sources for AOX without any extra work.

Overall, the entire validated procedure profits from its easy, straightforward, and standardized handling, the precise determination of the analytes, automatic calculation of results, and a low maintenance, single-manufacturer setup.

Automation of the method improves repeatability, accuracy, and reliability of the results. This also saves valuable laboratory time regarding liquid handling, standard and eluent preparation, and allows 24/7 analysis – from which every laboratory, whether research, routine, or governmental lab – can profit.

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