



WHITE PAPER

Measuring organic acids and inorganic anions with ion chromatography mass spectrometry

Two robust and sensitive analytical techniques – ion chromatography (IC) and mass spectrometry (MS) – can be combined, forming a more powerful technique: IC-MS. Superior to other analytical techniques, this multi-parameter method determines various organic acids and inorganic anions in one run.

This White Paper focuses on selected IC-MS applications for the straightforward identification and quantification of organic acids and inorganic anions in different matrices. Some applications discussed include measuring trace levels of anions in solvents, ionic impurities (e.g., adipate, bistriflimide, tetrafluoroborate, trifluoromethanesulfonate, or hexafluorophosphate) in organic matrices, low concentrations of short-chain aliphatic organic acids

(e.g., shikimic acid) next to high amounts of oxalic or citric acid in beverages, oxyhalides in drinking water, and determination of low amounts of glycerate and glycolate in urine without common disturbances of inorganic anions like chloride which are commonly found in such a matrix.

High sensitivity and selectivity of MS combined with the excellent separation capability of IC shows the superiority of IC-MS over UV/VIS or conductivity, since analytes can be resolved and detected at low concentration levels even in complex matrices and/or the presence of interfering ions.

INTRODUCTION

The combination of ion chromatography and mass spectrometry (IC-MS) results in a robust, sensitive, and selective technique for the determination of ionic compounds like organic acids, inorganic anions, or oxyhalides [1].

Ion chromatography separates different ions—even different ionic forms of the same element (speciation)—and it can reduce interferences from the sample matrix. Classical conductivity detection can be complemented with mass-selective detection to guarantee analyte identity and to achieve even lower detection limits. Hyphenated IC-MS provides accurate and sensitive analysis even at a lower chromatographic resolution when components are quantified by their specific m/z signal.

Suitable sample preparation removes interfering substances prior to injection on the IC and improves analyte detection limits. Full automation of this crucial step reduces manual labor and guarantees reproducible and reliable results. These benefits are achieved with Metrohm Inline Sample Preparation (MISP), such as Inline Ultrafiltration or Inline Dialysis. MISP allows the analysis of water samples as well as chemicals and organic solvents from e.g., semiconductor industries, liquid products from the food and beverage sector, or clinical samples like urine in a straightforward and simple way.

Native IC drivers allow users to control and operate the IC equipment with the same software as the MS (including autosamplers and Dosinos). Metrohm offers drivers for Waters™ Empower™ and Agilent OpenLab CDS (Metrohm IC Driver for Empower, Metrohm IC Driver for OpenLab CDS).

This White Paper highlights several example applications showing the analysis of organic acids and inorganic anions in different matrices using IC-MS. The following topics will be covered:

- impurity analysis in high quality chemicals
 - weak organic acids in NMP, methanol, ethanol, 2-propanol, and hydrogen peroxide
 - organic acids and inorganic anions in organic matrices
- quality control of beverages
 - organic acids in soft drinks
 - oxyhalide impurities in bottled water
- organic acids in urine

IMPURITY ANALYSIS IN HIGH QUALITY CHEMICALS

In production processes such as manufacturing semi-conductors, solutions and equipment must be free of any impurities for the best results. Process monitoring and tight quality control are mandatory for all chemicals involved. Among other parameters, the concentration of inorganic anions must stay below certain limits [2–3].

Traces of organic acids are considered impurities and can interfere in various industrial processes such as manufacturing microchips. The ultrapure water used for these processes needs to fulfill the highest purity standards. Also, organic solvents as well as other chemicals must contain no or only minimal amounts of organic acids for optimal production throughput [4]. IC-MS is a suitable analytical technique to check high quality chemicals for traces of organic acids and inorganic anions during incoming inspection and during process control. Thanks to automated Metrohm Inline Matrix Elimination, ions in many different matrices can be determined with the same analysis method.

– WEAK ORGANIC ACIDS IN NMP, METHANOL, ETHANOL, 2-PROPANOL, AND HYDROGEN PEROXIDE

Trace levels of anions and weak organic acids were analyzed by IC-MS in 1-methyl-2-pyrrolidinone (NMP), methanol, ethanol, 2-propanol, and hydrogen peroxide (H_2O_2). Automatic preconcentration and matrix elimination (MISP) of the samples allowed robust and reproducible quantification of all components without any matrix interferences. The Metrosep A Supp 7 - 250/4.0 column was used for IC separation. Standard anions and five organic acids were separated within 30 minutes with a binary high-pressure gradient. After sequential suppressed conductivity detection (Figure 1), an MS with electrospray ionization (ESI) was connected as a second independent detector to gain more information from the samples (Figure 2). The conductivity signal is beneficial for monitoring the major ionic compounds. The MS signal (single ion recording) is suitable for reliable identification of the analytes and their quantification in the $\mu\text{g/L}$ range.

An example of the analysis of ethanol is shown in Figure 2. The components acetate, propionate, oxalate, sulfate, nitrate, and chloride were detected with concentrations in the range of 1–50 $\mu\text{g/L}$.

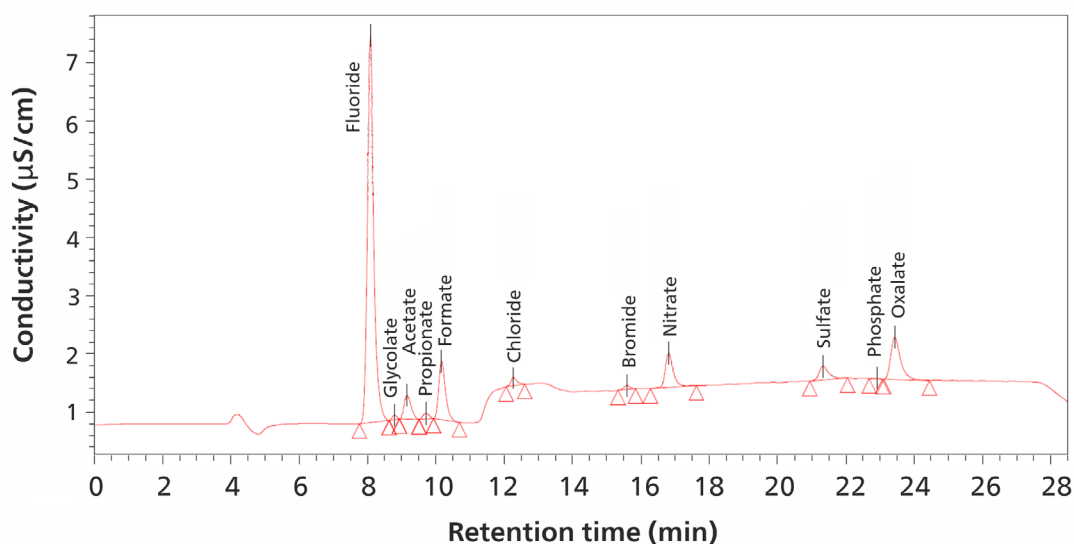


Figure 1. Conductivity signal of fluoride (400 µg/L), glycolate (40 µg/L), acetate (160 µg/L), propionate (80 µg/L), formate (160 µg/L), chloride (10 µg/L), bromide (20 µg/L), nitrate (10 µg/L), sulfate (40 µg/L), phosphate (400 µg/L), and oxalate (160 µg/L) in 250 µL of ultrapure water. Separation was performed on a Metrosep A Supp 7 - 250/4.0 column (eluent: sodium carbonate/acetonitrile gradient, flow rate 0.8 mL/min, column temperature 45 °C). The software used was Waters Empower CDS.

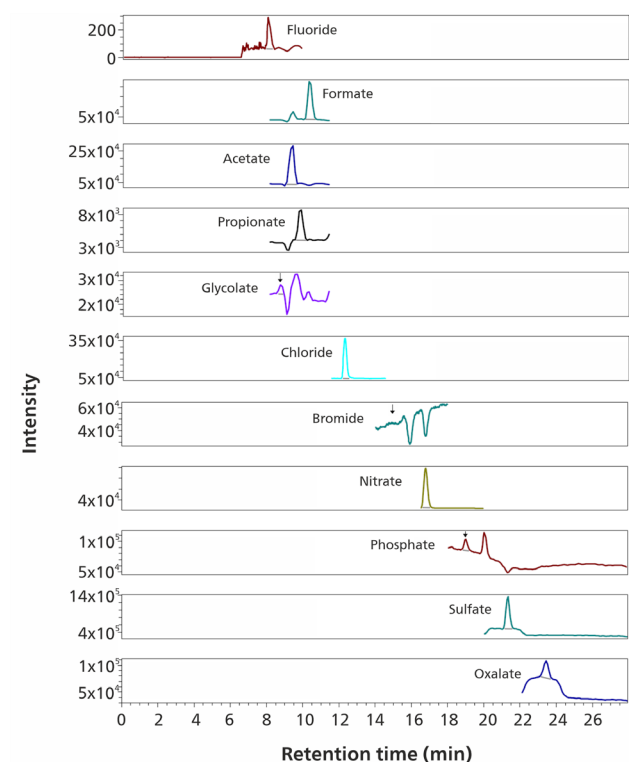


Figure 2. Analysis of 1 mL ethanol for fluoride (m/z 39), glycolate (m/z 75), acetate (m/z 59), propionate (m/z 73), formate (m/z 45), chloride (m/z 35), bromide (m/z 79), nitrate (m/z 62), phosphate (m/z 79), sulfate (m/z 97), and oxalate (m/z 89) (selected ion recordings). Separation was performed on a Metrosep A Supp 7 - 250/4.0 column (eluent: sodium carbonate/acetonitrile, high-pressure gradient, flow rate 0.8 mL/min, column temperature 45 °C). The software used was Waters Empower CDS.

Concentrations of fluoride, glycolate, bromide, and phosphate were below the calibrated range (<4 µg/L, <0.4 µg/L, <0.2 µg/L, and <4.4 µg/L, respectively). The formate concentration was determined to be above 160 µg/L.

Traces of some impurities were also detected in samples of NMP, methanol, 2-propanol, ethanol, and hydrogen peroxide (30%), as shown in **Figure 3**. Automated matrix elimination allows the same method to be used for various organic solvents and even for reactive solutions like hydrogen peroxide.

– ORGANIC ACIDS AND INORGANIC ANIONS IN ORGANIC MATRICES

To reduce matrix interferences and increase the lifetime of analytical columns, organic matrices require sample preparation prior to their analysis.

In most of these cases, MISP offers a way to automate sample preparation. Dosinos perform all liquid handling steps since they are the ideal tools for precise aspiration and dosing, allowing users to perform other important laboratory tasks. In the following application example, an appropriate volume of a proprietary sample was injected onto a preconcentration column (PCC), then the sample matrix was removed by Inline Matrix Elimination (MiPCT-ME).

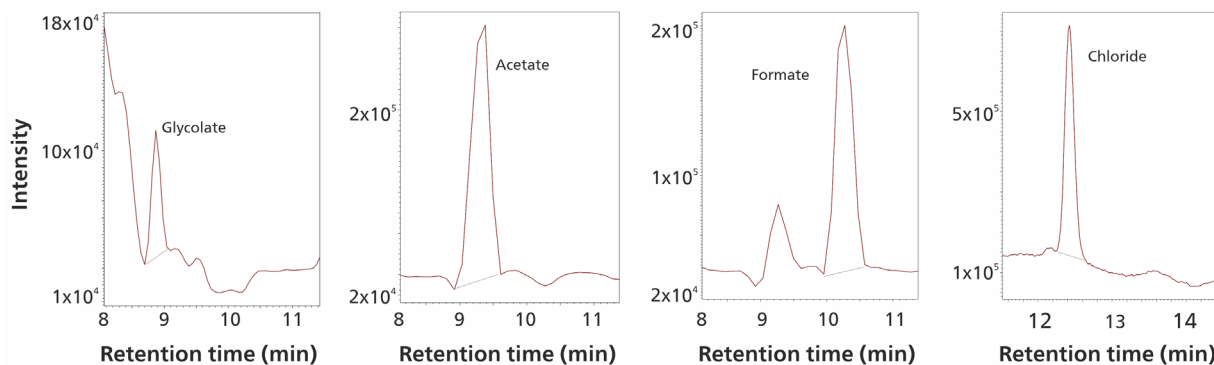


Figure 3. From left to right: determination of glycolate (<0.4 $\mu\text{g/L}$) in 1-methyl-2-pyrrolidinone (NMP), acetate (>166 $\mu\text{g/L}$) in ethanol, formate (<1.8 $\mu\text{g/L}$) in 2-propanol, and chloride (>10 $\mu\text{g/L}$) in hydrogen peroxide (30%). The sample volume was 1 mL. Selected ion recording (SIR) traces were recorded with Waters Empower CDS.

Inorganic anions (fluoride, chloride, bromide, nitrate, nitrite, phosphate, sulfate, triflate (trifluoromethanesulfonate), tetrafluoroborate, hexafluorophosphate, and bistriflimide) as well as anions of organic acids (glycolate, acetate, formate, propionate, adipate, succinate, and sebacate) were separated on a Metrosep A Supp 7 - 250/4.0 column using a high-pressure binary gradient. Parallel detection of suppressed conductivity and selected m/z signals offer the maximum certainty about the analysis results (**Table 1**). Agilent OpenLab CDS software was used to operate both the Metrohm instrument and the single quadrupole mass spectrometer (Agilent InfinityLab LC/MSD with API-Electrospray source). In a feasibility study, it was shown that these organic acids can be detected at trace levels of 5 $\mu\text{g/L}$. Adipate is shown as an example in **Figure 4**.

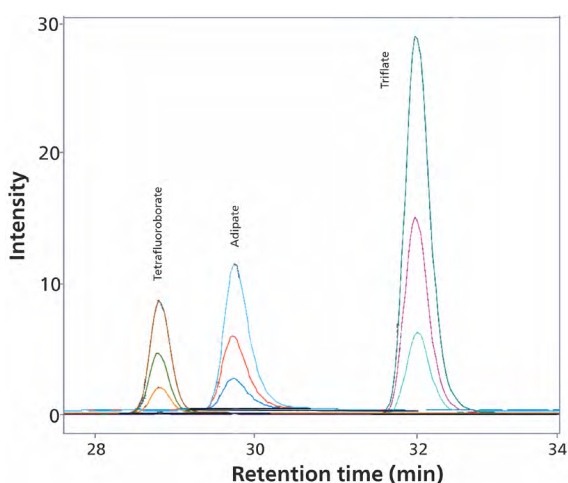


Figure 4. Selected ion monitoring of adipate eluting between tetrafluoroborate and trifluoromethanesulfonate (triflate) at three concentration levels (10 $\mu\text{g/L}$, 25 $\mu\text{g/L}$, and 100 $\mu\text{g/L}$), separated on a Metrosep A Supp 7 - 250/4.0 column (eluent: sodium carbonate/acetonitrile gradient, flow rate 0.8 mL/min, column temperature 55 $^{\circ}\text{C}$, sample volume 200 μL). The software used was Agilent OpenLab CDS.

Table 1. Seven organic acids and eleven inorganic anions, each with m/z signal and retention time as determined on a Metrosep A Supp 7 - 250/4.0 column (eluent: sodium carbonate/acetonitrile gradient, flow rate 0.8 mL/min, column temperature 55 $^{\circ}\text{C}$).

Analyte	m/z	Retention time [min]
Fluoride	19	14.1
Glycolate	75	15.6
Acetate	59	16.5
Propionate	73	17.9
Formate	45	18.1
Chloride	35	20.9
Nitrite	46	22.1
Bromide	79	23.7
Nitrate	62	24.6
Phosphate	79	28.0
Tetrafluoroborate	87	28.7
Sulfate	97	28.8
Succinate	117	29.4
Adipate	145	29.8
Triflate	149	31.9
Sebacate	201	33.6
Hexafluorophosphate	145	47.4
Bistriflimide	280	67.2

In total, 18 components were analyzed in a 200 μL sample of proprietary nature, and their limits of quantification were found to be in the range of 5 $\mu\text{g/L}$. Three different concentrations of hexafluorophosphate (**Figure 5**) and bistriflimide (**Figure 6**) are shown to illustrate the low levels able to be measured with the previously described IC-MS setup.

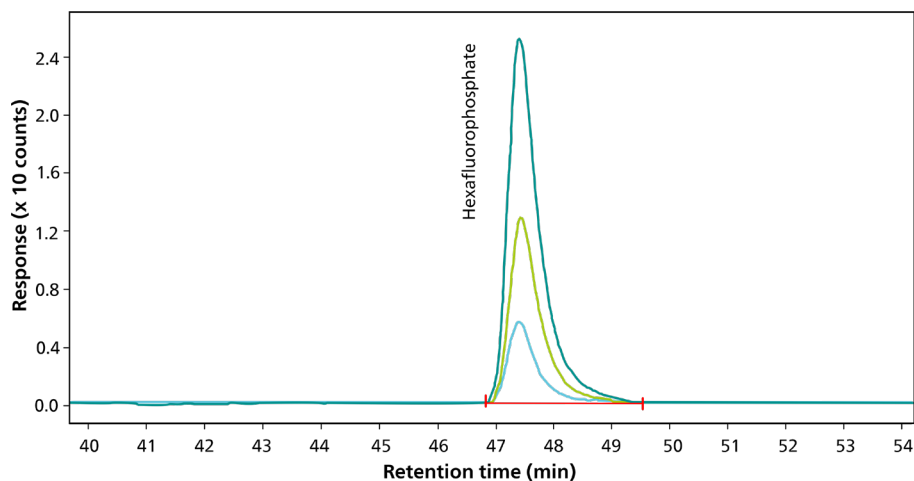


Figure 5. Hexafluorophosphate (m/z 145) measured at concentrations of 10 $\mu\text{g/L}$, 25 $\mu\text{g/L}$, and 50 $\mu\text{g/L}$ with electrospray selected ion monitoring (ES-SIM), separated on a Metrosep A Supp 7 - 250/4.0 column (eluent: sodium carbonate/acetonitrile gradient, flow rate 0.8 mL/min, column temperature 55 $^{\circ}\text{C}$, sample volume 200 μL). The software used was Agilent OpenLab CDS.

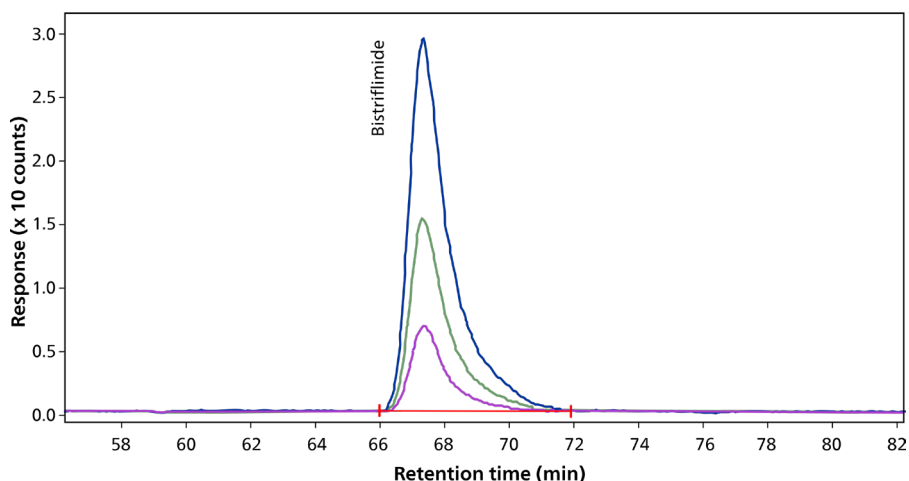


Figure 6. Bistriflimide (bis(trifluoromethane)-sulfonimide, m/z 280) measured at concentrations of 10 $\mu\text{g/L}$, 25 $\mu\text{g/L}$, and 50 $\mu\text{g/L}$, separated on a Metrosep A Supp 7 - 250/4.0 column and detected with ion monitoring in negative mode (eluent: sodium carbonate/acetonitrile gradient, flow rate 0.8 mL/min, column temperature 55 $^{\circ}\text{C}$, sample volume 200 μL). The software used was Agilent OpenLab CDS.

Table 2 shows that concentrations of chloride, bromide, nitrite, nitrate, phosphate, and sulfate between 1–50 $\mu\text{g/L}$ were detected with the applied setup. Fluoride was quantified down to 250 $\mu\text{g/L}$. With a mass-to-charge ratio (m/z) of 19, it is a rather small molecule, which explains its lower signal intensity compared to the other ions listed in **Table 2**.

Table 2. Mass signals (SIM in negative mode as m/z) and detection limits of seven inorganic anions. Limits of detection were estimated by signal-to-noise evaluation. Deprotonated phosphate and sulfate have the same mass-to-charge ratio; however, they are well separated by ion chromatography.

Analyte	m/z	Limits of detection	
		Concentration ($\mu\text{g/L}$) with 20 μL injection volume	Amount (ng)
Fluoride	19	250	5
Chloride	35	10	0.2
Nitrite	46	50	1
Bromide	79	5	0.1
Nitrate	62	1	0.02
Phosphate	97	10	0.2
Sulfate	97	25	0.5

– ORGANIC ACIDS IN SOFT DRINKS

Analysis of organic acids in soft drinks is of interest as they influence flavor and mouthfeel [5–7]. Citric, malic, and tartaric acids are frequently used as acidifying agents in foods, while acetic acid has been shown to have antimicrobial effects [8]. Lactic acid is used in food processing as an antimicrobial agent, for curing and pickling, to adjust the pH, or as a flavoring agent [9–11]. For organic acids in general, there are no limits with respect to an acceptable daily intake according to the FAO (Food and Agriculture Organization of the United Nations) [12]. However, the organic acid profile in foods and beverages can be used as an indicator to track adulteration. IC-MS is an appropriate analytical method to quantify organic acids such as citric, shikimic, malic, quinic, tartaric, and fumaric acids in food matrices [12].

A fast and robust IC-MS method was developed for the quantification of the aforementioned organic acids as well as a suite of inorganic anions based on seven soft drinks (Table 3). Within a short run time (32 minutes), this method precisely detects co-eluting organic acids with MS (Figure 7). Due to its high capacity and symmetric peaks, the Metrosep A Supp 19 is the ideal separation column for samples with complex matrices such as those found in the food and beverage sector.

Automatic calibration was done using MiPT (Metrohm intelligent Partial-Loop Injection Technique) and quadratic fits for MS. Most calibrations covered a concentration range of 4–200 µg/L. Analytes with a lower sensitivity (fluoride, shikimate, formate, propionate, nitrite, and fumarate) were quantified down to 10 or 20 µg/L. Correlation coefficients (R^2) for the calibration were found to be acceptable (0.997–0.999). Chloride, nitrate, sulfate, and citrate were detected in all soft drinks analyzed in this application example. Nitrite, bromide, glutarate, and adipate were not detected in any of the samples.

This easy-to-use IC-MS method for the determination of organic acids in apple spritzer, flavored mineral water, soda, and other soft drinks demonstrated adequate selectivity and sensitivity regarding the detection of co-eluting compounds.

Table 3. Mass traces (m/z) and retention times for 17 organic acids and seven inorganic anions analyzed in one run with IC-MS.

Analyte	m/z	Retention time [min]
Quinate	191	5.1
Fluoride	39	5.2
Shikimate	173	5.5
Glycolate	75	5.7
Lactate	89	5.7
Acetate	59	6.1
Propionate	73	6.3
Formate	45	6.5
Pyruvate	87	6.7
Chloride	35	8.0
Nitrite	46	9.8
Bromide	79	11.0
Nitrate	62	12.4
Phosphate	79	15.3
Sulfate	97	16.7
Malate	133	17.2
Malonate	103	17.4
Glutarate	131	17.8
Tartrate	149	17.9
Succinate	117	18.2
Adipate	145	18.1
Oxalate	89	18.9
Fumarate	115	22.2
Citrate	191	27.8

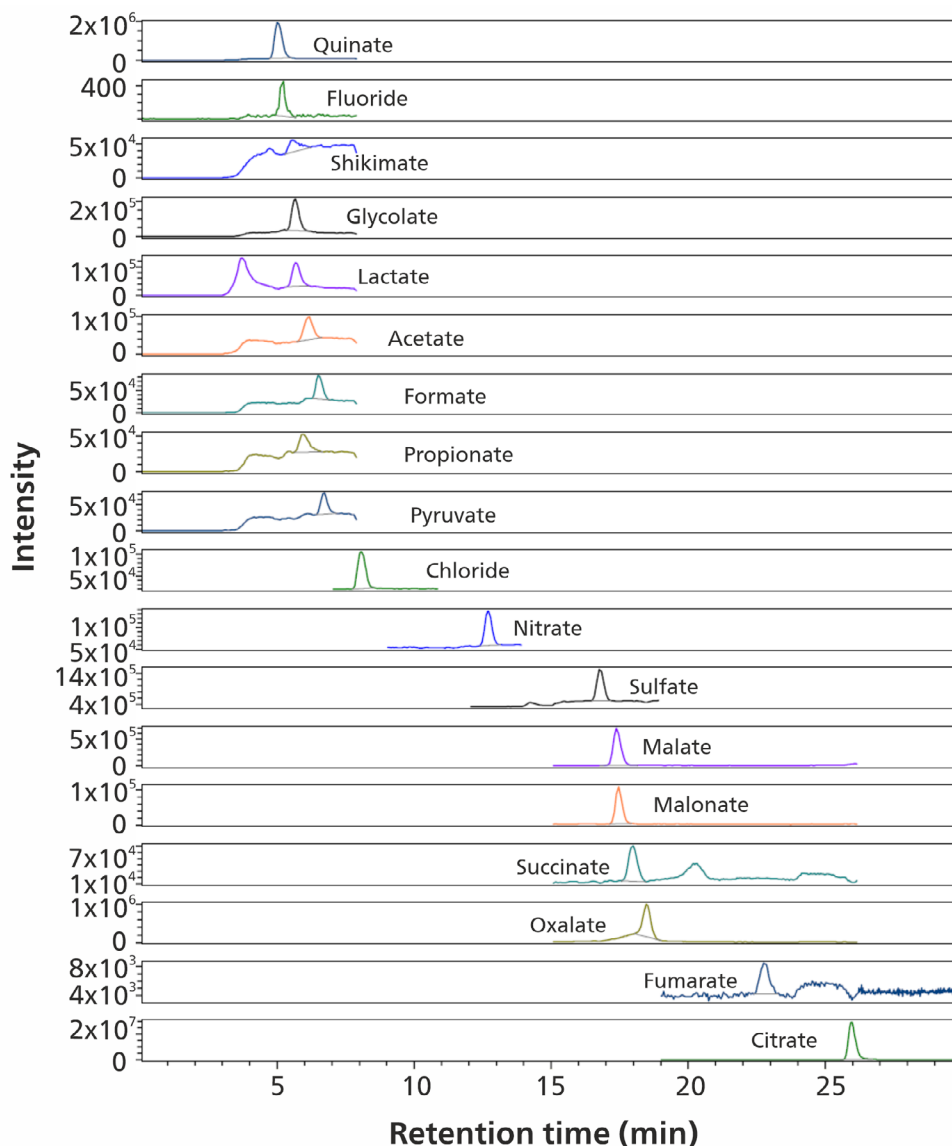


Figure 7. Analysis of iced tea (diluted 20-fold) containing quinate (3.98 mg/L), fluoride (0.35 mg/L), shikimate (0.18 mg/L), glycolate (1.11 mg/L), lactate (0.29 mg/L), acetate (0.52 mg/L), formate (0.69 mg/L), propionate (0.96 mg/L), pyruvate (0.22 mg/L), nitrate (0.12 mg/L), sulfate (3.43 mg/L), malate (1.34 mg/L), malonate (0.18 mg/L), succinate (0.05 mg/L), and fumarate (1.13 mg/L). Nitrite, bromide, phosphate, glutarate, tartrate, and adipate were below the calibrated range. Chloride, oxalate, and citrate were above the calibrated range. Separation was performed on a Metrosep A Supp 19 - 150/4.0 column (eluent A: 8 mmol/L sodium carbonate, 0.25 mmol/L sodium hydrogen carbonate, and 10% (v/v) methanol; eluent B: 80 mmol/L sodium carbonate, 2.5 mmol/L sodium hydrogen carbonate, and 10% (v/v) methanol; flow rate 0.75 mL/min, column temperature 60 °C). The sample volume was 10 μ L. The software used was Waters Empower CDS.



– OXYHALIDE IMPURITIES IN BOTTLED WATER

To provide clean drinking water, suppliers disinfect their water with chlorine, chlorine dioxide, chloramine, and ozone [13]. These disinfectants along with some natural components already present in the water can form toxic disinfection byproducts (DBPs). Ozonation, for example, can transform naturally occurring bromide into bromate, which is a potential carcinogen [14–16]. Chlorite and chlorate can be generated by chloramine treatment and also pose health risks [17]. According to the US Environmental Protection Agency (US EPA), limits in drinking water are set at 10 µg/L for bromate and 1 mg/L for chlorite [18]. Only 3 µg/L of bromate is allowed to be present in bottled natural mineral and spring waters disinfected by ozonation [19]. Chlorate in drinking water is currently not regulated in the United States but is limited to 0.7 mg/L by the World Health Organization (WHO).

Ion chromatography with conductivity detection is the method of choice to monitor oxyhalides in water treatment processes [20]. Thanks to sufficient peak resolution with high-capacity anion exchange columns, analytes can be separated efficiently from matrix components and from each other. However, conductivity detection is limited when it comes to resolving and sensitively detecting low levels (µg/L) of oxyhalides next to high concentrations (mg/L) of inorganic anions. Mass spectrometry as an additional (secondary) detection technique makes the analysis more powerful. With additional MS detection, the sensitivity for oxyhalides is increased, and peak identification is easier and more accurate with the selective mass signals. Even peaks with poor resolution can be readily quantified with their mass signal (when they have different m/z values). Chromatogram times can be shortened to the point where a minimum resolution exists between the peaks, increasing the sample throughput while offering the same or even better quantification quality using IC-MS.

For drinking water quality control, it is essential to quantify the previously mentioned harmful oxyanions in low quantities next to the higher concentrations of other common naturally present anions. Separation on a microbore 2 mm Metrosep A Supp 7 column allows the fast determination (12 minutes) of the oxyhalides bromate, chlorite, and chlorate at ng/L levels in addition to the common inorganic anions fluoride, chloride, bromide, nitrate, phosphate, and sulfate in tap water (Figure 8). The low flow rate required for the 2 mm IC column enables a direct connection of the conductivity detector to the MS

without any flow splitter. As adequate sensitivity using IC-MS is reached for the susceptible oxyanions, there is no need for post-column addition of a make-up solution. Thus, the setup remains easy and robust. Internal standards like ^{18}O -labeled bromate can be used to reach even higher precision in matrix-loaded samples.

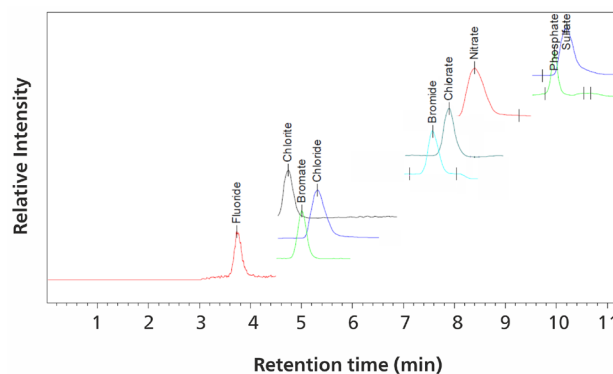


Figure 8. The overlay shows m/z traces of 5 µg/L chlorite, 5 µg/L chlorate, 5 µg/L bromate, 100 µg/L bromide, 100 µg/L fluoride, 100 µg/L phosphate, 5 mg/L chloride, 5 mg/L nitrate, and 5 mg/L sulfate in ultrapure water, separated on a Metrosep A Supp 7 - 150/2.0 column (eluent: sodium carbonate/acetonitrile gradient, flow rate 0.25 mL/min, column temperature 45 °C). The sample volume was 40 µL. The software used was Waters Empower CDS.

An additional benefit of this method, especially for routine labs, comes from using a low volume sample pick-up technique (Metrohm intelligent Pick-up Technique, MiPuT). Only the required amount of sample is precisely transferred and injected into the IC. The volume can be chosen individually for each sample. Furthermore, automatic calibration is performed from one stock standard by using different injection volumes. Therefore, not only low sample volumes can be handled and quantified within a short run time, but also the time usually needed for standard preparation is minimized, making this method even more efficient and attractive for routine high-throughput analysis.

Oxyhalides were calibrated in the range of 15 pg up to 0.5 ng (absolute amounts) by injecting different volumes from one standard solution using MiPuT (Figure 9). Limits of quantification were in the ng/L range (e.g., 25 ng/L bromate with 100 µL sample volume; 2.5 pg bromate, Figure 10). Repeatability and spike studies (Table 4, Figure 11) showed the method performance for analysis of water samples.

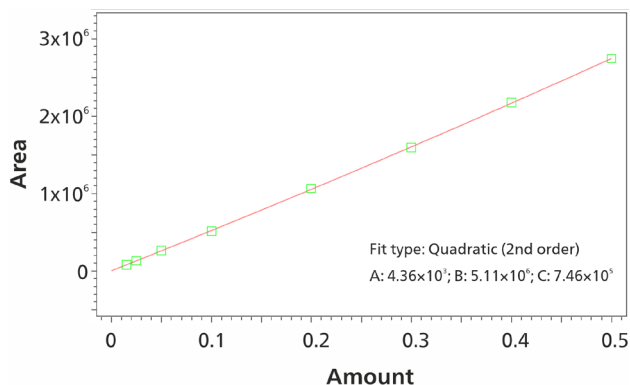


Figure 9. Linear calibration for 15 pg to 0.5 ng bromate with MiPuT by injecting 3–100 μ L from one standard solution (5 μ g/L bromate). The software used was Waters Empower CDS.

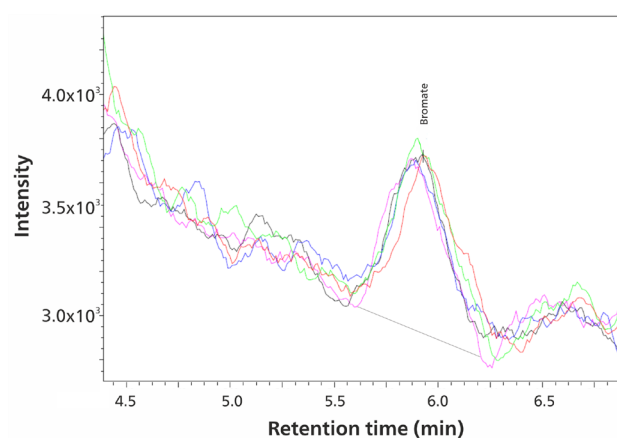


Figure 10. Detection of 25 ng/L bromate with 100 μ L sample volume (2.5 pg bromate). Overlay shows the selected ion recording of five replicate analyses. The software used was Waters Empower CDS.

Table 4. Repeatability study as performed with a 50 μ L check standard composed of 0.5 μ g/L chlorite, 0.5 μ g/L chlorate, 0.5 μ g/L bromate, 10 μ g/L bromide, and 10 μ g/L phosphate.

Analyte	Number of injections	Area RSD [%]	Height RSD [%]
Chlorite	12	25	8
Chlorate	12	8	5
Bromate	12	2	3
Bromide	6	6	5
Phosphate	12	5	3

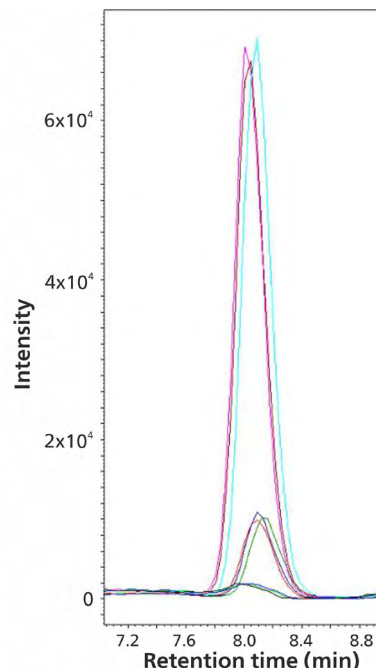


Figure 11. Tap water containing approximately 10 mg/L chloride and nitrate and approximately 5 mg/L sulfate spiked with chlorate (spiking level 1: 1 μ g/L, and level 2: 5 μ g/L). The software used was Waters Empower CDS.

In addition to bromate, chlorite, and chlorate, perchlorate detection is also crucial for quality control of drinking water, as described in EPA method 332.0 [21]. Perchlorate is a contaminant that interferes with the uptake of iodine into the thyroid and is linked to brain damage in newborns and infants. For more details, refer to IC-MS [22] and IC-MS/MS [23] application notes from Agilent.

Combining low level analysis of iodine species with oxyhalides and inorganic anions demonstrates the power of IC-MS once more. Iodide is an essential micronutrient and used in the synthesis of thyroid hormones. Iodide is oxidized to iodate in oxidative drinking water treatment, which avoids the formation of potential toxic disinfection byproducts [24]. The Metrosep A Supp 7 column makes it possible to separate iodide and iodate in the same run next to oxyhalides and inorganic anions. Automatic sample preparation (e.g., [Inline Ultrafiltration](#)) reduces the manual workload and allows robust analysis of surface and drinking water samples, even when they contain particles. Quantification was accomplished down to 0.25 μ g/L for bromate and perchlorate, down to 0.5 μ g/L for bromide, iodate, and iodide (Figure 12), and down to 5 μ g/L for chlorite and chlorate.

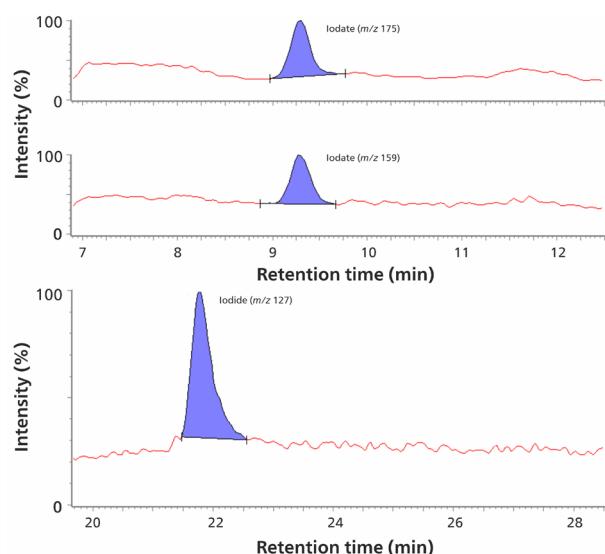


Figure 12. Determination of iodate (0.5 µg/L) as *m/z* 175 and *m/z* 159 (upper panel), and iodide (0.5 µg/L) as *m/z* 127 (lower panel). The analytes were separated on a Metrosep A Supp 7 - 250/4.0 column (eluent: 1.2 mmol/L sodium carbonate, 40% (v/v) acetonitrile, flow rate 0.8 mL/min, column temperature 45 °C). The sample volume was 100 µL. The software used was MagIC Net and Waters MassLynx™.

It is not only water quality laboratories, but also forensic investigations that require sensitive analysis of oxyhalides and inorganic anions. More details can be found in Metrohm Application Note [AN-M-002](#) [25].

MEDICAL APPLICATIONS: ORGANIC ACIDS IN URINE

Determination of the organic acid levels in body fluids is important to help diagnose several diseases. In hospitals, they are regularly measured in blood plasma or urine. For example, oxalate in urine may indicate hyperoxaluria [26]. Glyceric acid is measured in pediatric patients to screen for inborn errors of amino acid metabolism. This rare disorder of D-glyceric aciduria is caused by mutations in the gene encoding the enzyme D-glycerate kinase. The resulting D-glycerate kinase deficiency affects several metabolic pathways, which leads to neurological symptoms and excretion of D-glyceric acid [27].

In the following application example, glycerate, glycolate, oxalate, and citrate were determined in urine (**Figure 13**). Due to the high-resolution power of the Metrosep A Supp 19 column, interferences from chloride or other matrix components are reduced to a minimum. Inorganic anions like chloride and sulfate can also be quantified in the same run. Isocratic elution with a single high-pressure pump guarantees a simple setup, robust method performance, and easy handling. Post-column addition of other chemicals was unnecessary, further simplifying the setup.

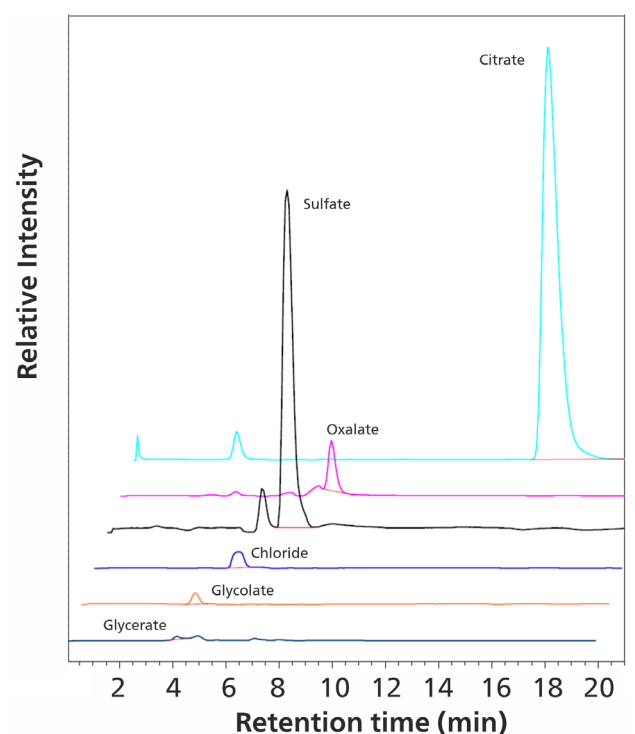


Figure 13. Mass signals of a urine sample (diluted 10-fold) containing 180 ng glycolate (1.8 mg/L; 2 nmol), 860 ng oxalate (8.6 mg/L; 10 nmol), and 28.7 µg citrate (287 mg/L; 150 nmol). Glycerate was not detected. Separation was performed on a Metrosep A Supp 19 - 150/4.0 column (eluent: 30 mmol/L sodium carbonate, 1 mmol/L sodium hydrogen carbonate, and 10% (v/v) methanol; flow rate 0.75 mL/min, column temperature 60 °C). The sample volume was 100 µL. The software used was Waters Empower CDS.

The samples were sterile-filtered, diluted 10-fold with boric acid (2% v/v), and injected with MiPT (Metrohm intelligent Partial-Loop Injection Technique). After separation on the Metrosep A Supp 19 column, the analytes were detected with conductivity and MS within 23 minutes (**Figure 13**). Organic acids were well-separated from inorganic anions with high concentrations (e.g., chloride) which enabled precise quantification (**Figure 14**).

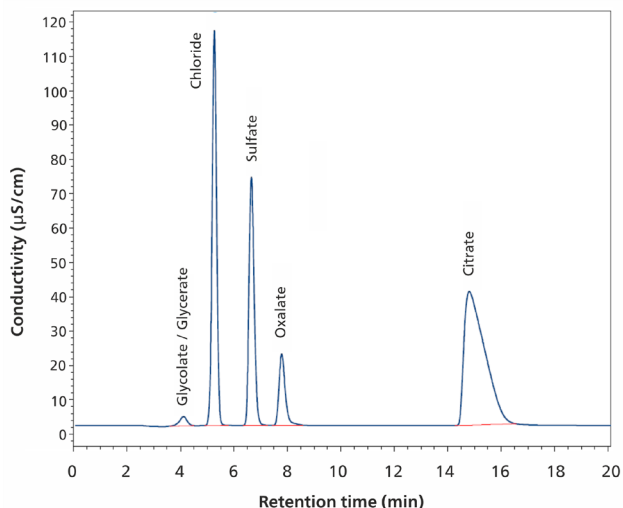


Figure 14. Conductivity signal of a standard containing 400 ng glycerate (2 mg/L; 3.8 nmol), 200 ng glycolate (1 mg/L; 2.7 nmol), 1 µg oxalate (5 mg/L; 11.2 nmol), 20 µg citrate (100 mg/L; 104.7 nmol), 2 µg chloride (10 mg/L; 57.1 nmol), and 2 µg sulfate (10 mg/L; 20.6 nmol). Separation was performed on a Metrosep A Supp 19 - 150/4.0 column (eluent: 30 mmol/L sodium carbonate, 1 mmol/L sodium hydrogen carbonate, and 10% (v/v) methanol; flow rate 0.75 mL/min, column temperature 60 °C). The sample volume was 100 µL. The software used was Waters Empower CDS.

Calibration was in the mg/L range (2–100 mg/L glycerate, 1–50 mg/L glycolate, 5–250 mg/L oxalate, and 100–5000 mg/L citrate) and correlation coefficients (R^2) were >0.999 for glycerate and glycolate, and >0.98 for oxalate and citrate (MS signal, quadratic fit through zero). The anions chloride and sulfate were calibrated by using their conductivity signals as they exhibited a more linear correlation than with the MS signal. In the range of 10–500 mg/L the correlation coefficients (R^2) were >0.9999 for both chloride and sulfate (conductivity, linear fit).

CONCLUSION

Liquid chromatography with mass spectrometry (LC-MS) is a well-established analytical technique with a broad application field. IC-MS is the method of choice when it comes to ionic analytes and/or matrices with high salt contents. IC instrumentation from Metrohm excels with:

- its robust suppression technique
- an inert metal-free flow path
- automated inline sample preparation techniques (MISP)
- a wide variety of reliable ion-exchange columns with great separation power

IC-MS combines and enhances the benefits from the individual techniques of ion chromatography and mass spectrometry [28–30]. Multiple anions including organic acids (e.g., acetate or formate) as well as small inorganic anions (e.g., chloride and bromate) are sensitively and selectively determined in one run.

MISP additionally improves the method efficiency by automating the sample preparation and making even complex matrices suitable for the analysis with sensitive high-end detectors. Not only does MISP reduce human error and manual labor, it also helps save time and money—especially in high-throughput laboratories.

References

- [1] *Application of IC-MS and IC-ICP-MS in Environmental Research*; Michalski, R., Ed.; John Wiley & Sons, Inc: Hoboken, NJ, USA, 2016. DOI:10.1002/9781119085362
- [2] Vanatta, L. E. Ion-Chromatographic Determination of Seven Common Anions in Electronic-Grade, Water-Miscible Solvents. *Journal of Chromatography A* **2008**, *1213* (1), 70–76. DOI:10.1016/j.chroma.2008.09.047
- [3] Figi, R.; Schreiner, C.; Bleiner, D. Systematic Investigations of Plastic Vials Concerning Their Suitability for Ultratrace Anion Analysis in High-Purity Industrial Applications. *Microchim Acta* **2005**, *150* (3), 199–209. DOI:10.1007/s00604-005-0355-2
- [4] Vanatta, L. E. Determination of Low-ppt Levels of Acetate, Propionate, and Formate in Semiconductor-Grade Deionized Water via Ion Chromatography. *Journal of Chromatographic Science* **2010**, *48* (7), 533–536. DOI:10.1093/chromsci/48.7.533
- [5] Quitmann, H.; Fan, R.; Czermak, P. Acidic Organic Compounds in Beverage, Food, and Feed Production. In *Biotechnology of Food and Feed Additives*; Zorn, H., Czermak, P., Eds.; Advances in Biochemical Engineering/Biotechnology; Springer: Berlin, Heidelberg, 2014; pp 91–141. DOI:10.1007/10_2013_262
- [6] Tung, C. M.; Pettigrew, J. *Critical Review of Acidifiers*; Pork Checkoff; NPB #05-169; University of Illinois, 2006; pp 1–48.
- [7] Theron, M. M.; Lues, J. F. R. *Organic Acids and Food Preservation*, 1st ed.; CRC Press: Boca Raton, 2010. DOI:10.1201/9781420078435
- [8] Dauthy, M. E. *Fruit and Vegetable Processing - Chapter 5 General Procedures for Fruit and Vegetable Preservation*; FAO agricultural services bulletin; Food and Agriculture Organization of the United Nations: Rome, 1995.
- [9] EFSA Panel on Biological Hazards (BIOHAZ); EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). Scientific Opinion on the Evaluation of the Safety and Efficacy of Lactic Acid for the Removal of Microbial Surface Contamination of Beef Carcasses, Cuts and Trimmings. *EFSA Journal* **2011**, *9* (7), 2317. DOI:0.2903/j.efsa.2011.2317
- [10] Graefe, G.; Holten, C. H. Lactic Acid, Properties and Chemistry of Lactic Acid and Derivatives. *Starch* **1973**, *25* (1), 34–34. DOI:10.1002/star.19730250108
- [11] 21 CFR 184.1061 -- Lactic acid. <https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-184/subpart-B/section-184.1061> (accessed 2022-08-11).
- [12] Food and Agriculture Organization of the United Nations (FAO). General Standard for Fruit Juices and Nectars (CODEX STAN 247-2005).
- [13] U.S. Environmental Protection Agency, Office of Water. Drinking Water Treatment, 1999.
- [14] *Disinfection By-Products in Drinking Water: Detection and Treatment*, 1st ed.; Prasad, M. N. V., Ed.; Emerging contaminants and micropollutants in the environment; Butterworth-Heinemann, an imprint of Elsevier: Kidlington, Oxford Cambridge, MA, 2020.
- [15] *Bromate in Drinking-Water: Background Document for Development of WHO Guidelines for Drinking-Water Quality*; WHO/SDE/WSH/05.08/78; World Health Organization (WHO): Geneva, 2005; pp 1–24.
- [16] *Disinfectants and Disinfectant By-Products*; Environmental Health Criteria 216; World Health Organization (WHO): Geneva, 2000; p 529.
- [17] *Chlorite and Chlorate in Drinking-Water: Background Document for Development of WHO Guidelines for Drinking-Water Quality*; WHO/SDE/WSH/05.08/86; World Health Organization (WHO): Geneva, 2005; pp 1–31.
- [18] U.S. Environmental Protection Agency (US EPA). *National Primary Drinking Water Regulations*. <https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations> (accessed 2022-09-19).
- [19] European Commission. *Drinking water legislation - Environment - Council Directive 98/83/EC on the quality of water intended for human consumption*. https://ec.europa.eu/environment/water/water-drink/legislation_en.html (accessed 2022-09-19).

- [20] Traces of Bromide and Bromate in Drinking Water by IC-MS, Determination of the Method Detection Limit (MDL). *Metrohm AG, AN-M-004* **2006**.
- [21] U.S. Environmental Protection Agency (US EPA). Method 332.0: Determination of Perchlorate in Drinking Water by Ion Chromatography with Suppressed Conductivity and Electrospray Ionization Mass Spectrometry.
- [22] Mathew, J.; Gandhi, J.; Hedrick, J. The Analysis of Perchlorate by Ion Chromatography/Mass Spectrometry. *Agilent Technologies, Inc., 5989-0816EN* **2004**.
- [23] Mathew, J.; Gandhi, J.; Mohsin, S.; et al. Quantitative Analysis of Perchlorate by Ion Chromatography MS/MS. *Agilent Technologies, Inc., 5989-7907EN* **2008**.
- [24] Allard, S.; Nottle, C. E. Ozonation of Iodide-Containing Waters: Selective Oxidation of Iodide to Iodate with Simultaneous Minimization of Bromate and I-THMs. *Water Res* **2013**, 47 (6), 1953–1960. **DOI:10.1016/j.watres.2012.12.002**
- [25] Chlorite, Chlorate, and Perchlorate in Explosion Residue Using IC/MS Coupling. *Metrohm AG, AN-M-002* **2005**.
- [26] Blau, N. Oxalate, Glycolate, Glycerate, Sulfate, and Citrate. In *Laboratory Guide to the Methods in Biochemical Genetics*; Blau, N., Duran, M., Gibson, K. M., Eds.; Springer: Berlin, Heidelberg, 2008; pp 233–243. **DOI:10.1007/978-3-540-76698-8_13**
- [27] Sass, J. O.; Fischer, K.; Wang, R.; et al. D-glyceric aciduria is caused by genetic deficiency of D-glycerate kinase (GLYCKT). *Hum Mutat* **2010**, 31 (12), 1280–1285. **DOI:10.1002/humu.21375**
- [28] Ruth, K. An Introduction to Ion Chromatography Mass Spectrometry (IC-MS). *Metrohm AG, WP-066EN* **2021**.
- [29] Ruth, K. Measuring Inorganic Cations and Amines with Ion Chromatography Mass Spectrometry (IC-MS). *Metrohm AG, WP-082EN* **2022**.
- [30] Ruth, K. Simple Determination of Haloacetic Acids (HAAs) in Potable Water with Ion Chromatography Hyphenated to Mass Spectrometry. *Metrohm AG, WP-075EN* **2021**.

Further related Metrohm literature

Sample Preparation Techniques for IC. **8.108.5070**

Coupling of ion chromatography and plasma mass spectrometry. **WP-008**

A strong combination – Coupling of Metrohm ion chromatography and mass spectrometry **8.000.5250**

Metrohm meets Empower 3 – Ion chromatography with the Chromatography Data Software (CDS) from Waters **8.102.5004**

IC Driver for Agilent OpenLab CDS – A perfect combination: Metrohm Ion Chromatography and OpenLab CDS Software **8.102.5007**

Metrohm Application Finder: Selection of IC-MS Applications

Contact

Dr. Katinka Ruth

Metrohm International Headquarters; Herisau, Switzerland

info@metrohm.com