IC APPLICATION NOTE S-404

Water quality testing with EPA 300.1

Boost lab efficiency with automated eluent generation and single-run analysis of anions and disinfection byproducts

Standard analytical methods like **U.S. EPA Method 300.1 (Parts A and B)**, ISO 10304-1, and ISO 10304-4 ensure compliance when monitoring water contaminants that affect human health. Disinfection byproducts (DBPs) in particular are linked to cancer and reproductive issues [1–5]. EPA 300.1 Part A and ISO 10304-1 outline the analytical requirements for ion chromatographic determination of major inorganic anions. Harmful DBPs (chlorite and chlorate) are included in ISO 10304-4 and EPA 300.1 Part B (plus bromate and dichloroacetic acid / DCAA). Achieving method detection limits (MDLs) for analytes with significant relative concentration differences can be challenging. The **Metrosep A Supp 21** high-capacity anion-exchange column for hydroxide eluents and sequentially suppressed conductivity detection allows for a single-run analysis that meets these needs. The **948 Continuous IC Module, CEP** automates KOH eluent generation. This eliminates manual preparation and ensures stable retention times along with an ultra-low baseline. Along with a **single-standard calibration**, this analytical approach provides a highly efficient, sustainable, reliable solution for water testing labs.

SAMPLE AND SAMPLE PREPARATION

The sample series contained two tap water samples, one artificial tap water sample, and one commercially available mineral water sample. The samples were prepared according to the requirements of U.S. EPA Method 300.1 [1]. As specified, 50 mg/L EDA (ethylenediamine) was added to standards and samples to stabilize chlorite. EDA shifts the sample pH to more basic conditions which guarantees the stability of chlorite.

To assess the recovery, a mixed standard solution (i.e., spike solution, **Table 1**) was added to the water samples. A separate mixed standard (with concentration of anions corresponding to **Table 1**) was fortified with high levels of chloride, nitrate, sulfate, and carbonate (20–500 mg/L each) and early eluting organic acids like glycolate, acetate, and formate (1 mg/L each) to assess matrix compatibility.

To calibrate the ion chromatography (IC) system, a single standard was prepared (**Table 1**) and variable volumes (4–200 µL) were injected using the Metrohm intelligent Partial-Loop Injection Technique (MiPT).

Table 1. Composition of the standard and spike solutions.

Analyte	Standard 1 (µg/L)	Spike solution (µg/L)
Fluoride	100	100
Chlorite	10	5
Bromate	10	5
Chloride	10,000	10,000
Nitrite	20	20
Bromide	20	20
Chlorate	10	5
Nitrate	10,000	10,000
Dichloroacetate	1,000	1,000
Sulfate	10,000	10,000
Phosphate	100	not spiked

EXPERIMENTAL

The samples and standards were directly injected into the IC system equipped with a 948 Continuous IC Module, CEP, and an MiPT setup (**Figure 1**).

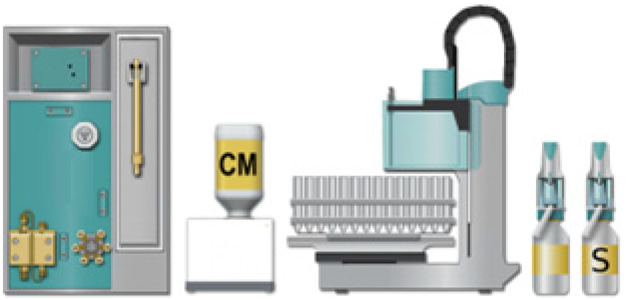

Figure 1. Setup for drinking water testing using a 930 Compact IC Flex with automatic KOH eluent generation (948 Continuous IC Module, CEP), an 858 Professional Sample Processor, and the Metrohm intelligent Partial-Loop Injection Technique (MiPT).

An automated approach (MiPT) is used for the system calibration. By injecting different volumes (4–200 µL) from a single-standard solution (**Table 1**), a highly precise calibration series is created (fluoride, phosphate: 8–400 µg/L; chlorite, bromate, chlorate: 0.8–40 µg/L; chloride, nitrate, sulfate: 0.8-40 mg/L; nitrite, bromide: 1.6–80 µg/L; DCAA: 0.08–4 mg/L).

The samples were injected with a common volume of 50 µL. Each sample was analyzed four times. The results reflect the recovery of the measurements.

Elution of the analytes is done with a hydroxide eluent gradient (18–80 mmol/L KOH, concentrate: 4 mol/L potassium hydroxide solution (Supelco, Merck), **Figure 2**) on the high-capacity Metrosep A Supp 21 column at 45 °C using the 948 Continuous IC Module, CEP.

Continuous hydroxide eluent generation works as follows. On-demand eluent production with the 948 Continuous IC Module, CEP is based on the electrolysis of water. By applying a defined electrical current (the «Faradaic current»), hydroxide (OH^-) ions are formed from the ultrapure water stream at the platinum electrode of the 948 Continuous IC Module's Eluent Production Cartridge (EPC A).

The respective amount of potassium (K^+) ions complements the hydroxide ions. These are delivered from the high-purity potassium hydroxide concentrate (either purchased or self-made at a concentration of 4 mol/L or higher) by passing through the ion-selective membrane. The applied current is constantly monitored to guarantee maximum accuracy, precision, and reproducibility for the defined isocratic or gradient OH$^-$ concentrations. Manual preparation of corrosive and hard-to-handle hydroxide eluents is unnecessary. The issue of carbonate attraction is also minimized, resulting in stable retention times and an ultra-low baseline for determining even trace concentrations of analytes.

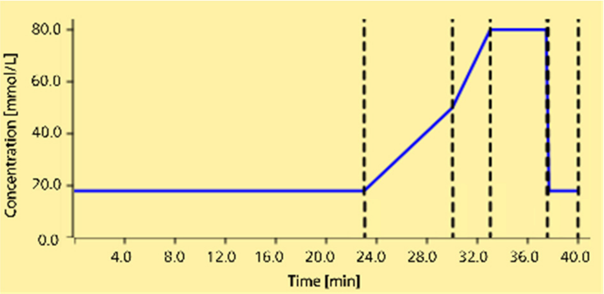

Figure 2. Gradient profile of KOH ensured with the 948 Continuous IC Module, CEP (concentrate: 4 mol/L potassium hydroxide solution, 67109, Supelco, Merck).

Table 2. Chromatographic conditions for the analysis of common anion and DBPs according to U.S. EPA 300.1 A and B.

Gradient (948 Continuous IC Module, CEP)	18–80 mmol/L KOH (Figure 2)
Column/guard column	Metrosep A Supp 21 - 250/4.0 Metrosep A Supp 21 Guard/4.0
Flow rate eluent	0.80 mL/min
Column temperature	45 °C
Recording time	40 min
Injection volume	4–200 µL (MiPT) 50 µL for samples
Suppression	Sequential suppression
MSM	MSM-HC Rotor A, Hydroxide
MSM stepping interval	10 min
Dosino regeneration volume	10 mL (200 mmol/L sulfuric acid)
Detection	Conductivity

The conductivity signal is recorded (IC Conductivity Detector MB) after sequentially suppressed conductivity detection. Therefore, for hydroxide conditions, the designed MSM-HC Rotor A, Hydroxide is used to achieve the best system performance.

RESULTS

Analysis of the target analytes was performed within 40 minutes (**Figure 3**). The analyses for U.S. EPA Method 300.1 Parts A and B are combined in a single IC method and use a common injection volume of 50 µL for the samples.

Although the resolutions between the peaks are not explicitly mentioned within the standard text of U.S. EPA Method 300.1, their values were monitored throughout the complete analysis series. To compare, ISO 10304-1 and ISO 10304-4 require a minimum resolution of 1.3 for proper quantification. Thereby, the resolution between bromide and chlorate, as well as between chlorite and bromate, are the most critical. Throughout a measuring series of 95 samples and standards, including the shown mineral water (**Figure 3**), the resolution between bromide and chlorate was 2.1 on average, and the average resolution between chlorite and bromate, was 1.9—well above the requirements in the ISO standards.

The analyzed water samples contained high concentrations (i.e., mg/L range) of chloride (8.2–10.9 mg/L), sulfate (4.8–13.9 mg/L), and nitrate (3.8–9.6 mg/L). Fluoride (57–72 µg/L), bromide (8–9 µg/L), and chlorate (mineral water, 3 µg/L) were detected in minor concentrations. No chlorite, bromate, phosphate, or DCAA were found in the samples.

The relative standard deviations (RSDs) for repeated water analysis were below 2.5% (with exceptions for chlorite and bromate, <5%), and spiking recoveries of 89–102 % fell within the standard quality criteria, highlighting the repeatability, accuracy, and robustness of the IC method.

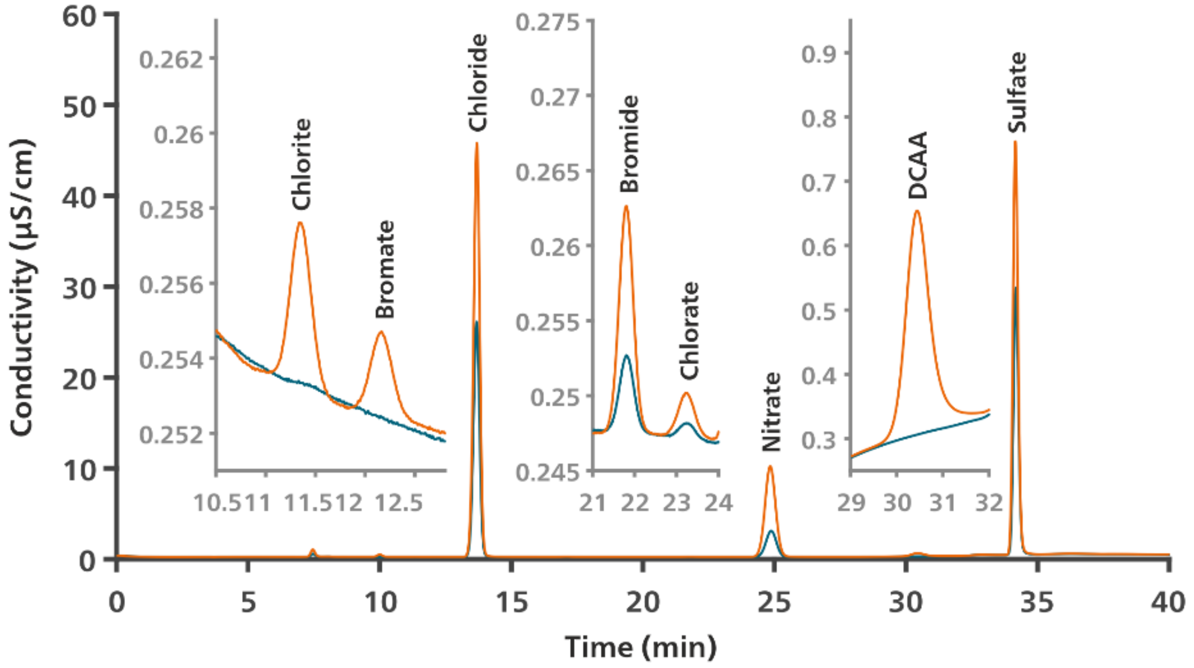

Figure 3. Chromatogram of a mineral water sample analyzed according to U.S. EPA 300.1 Parts A and B [1]. The blue line corresponds to the original water sample (72 µg/L fluoride with a retention time of 7.6 min; 0 µg/L chlorite, bromate, phosphate, and DCAA; 10.9 mg/L chloride; 9 µg/L bromide; 3 µg/L chlorate; 3.8 mg/L nitrate; 13.9 mg/L sulfate). The orange line corresponds to the mineral water sample spiked with a mixed spike solution (Table 1). Gradient elution of the analytes (18–80 mmol/L KOH) was performed on the high-capacity Metrosep A Supp 21 column (injection volume 50 µL) prior to sequentially suppressed conductivity detection.

As matrix compatibility is always an issue for drinking water quality analysis, the effect of several matrix components (e.g., chloride, as shown in Figure 4) was tested to check the separation quality. High chloride concentrations usually affect the peaks of bromate and nitrite and affect proper integration. However, for a concentration of up to 500 mg/L chloride, the bromate and nitrite peaks are well separated from chloride (resolution >3), allowing for accurate quantification.

No disturbances occurred when nitrate (which has an effect on chlorate) was present up to 200 mg/L and sulfate (with an effect on DCAA) was present in concentrations of up to 500 mg/L.

Interferences can also occur from early eluting organic acids (e.g., glycolate, formate, and acetate). Testing showed that even if glycolate – the closest eluting organic acid next to fluoride – was present, no coelution was seen between these two ions and a proper resolution was obtained (2.1).

CONCLUSIONS

This application focuses on separating and measuring high concentrations of inorganic anions, (e.g., chloride, nitrate, and sulfate) along with lower concentrations of disinfection byproducts (DBPs, i.e., bromate, chlorite, and chlorate) and nitrite and bromide in a single analysis run using ion chromatography. Accurate measurement across a wide concentration range requires a detector with high linearity, such as the IC Conductivity Detector MB (linearity range of 0–15,000 µS/cm), a high-capacity column (Metrosep A Supp 21) for appropriate peak separation, and a low baseline with outstanding signal-to-noise ratio to reach the lowest detection limits.

The 948 Continuous IC Module, CEP, in combination with the suppressor designed for hydroxide eluents (MSM-HC Rotor A, Hydroxide), guarantees the best analytical conditions – a high purity eluent, low baseline, and best signal-to-noise ratios. The automatic, almost reagent-free hydroxide eluent production out of a high-purity concentrate (4 mol/L potassium hydroxide solution, Supelco, Merck) and easy, straightforward gradient generation eliminates any manual steps and associated human errors. This provides stable elution conditions, resulting in stable retention times, and is a sustainable, cost-efficient solution for any lab.

The Metrosep A Supp 21 column, designed for hydroxide eluents, enables a high resolution of all analytes required in U.S. EPA Method 300.1 Parts A and B as well as in ISO 10304-1 and ISO 10304-4. Originally, these standards intended for the analysis to be performed with two separate methods to determine the highly concentrated standard anions beside the trace anions accurately. Indeed, many methods lack an appropriate resolution and sufficient sensitivity, and two separate methods are necessary for the analyses, dramatically reducing the sample throughput.

Metrohm offers a very comprehensive way to combine the two parts of EPA 300.1 without any quality losses by using a setup with the Metrosep A Supp 21 - 250/4.0 separation column in combination with automatic high-purity hydroxide eluent generation and conductivity detection after sequential suppression and automatic inline calibration.

With this efficient setup, water laboratories can fulfill regular drinking water standards and increase their overall work efficiency. All-in-all, this is a complete, robust, and efficient solution.

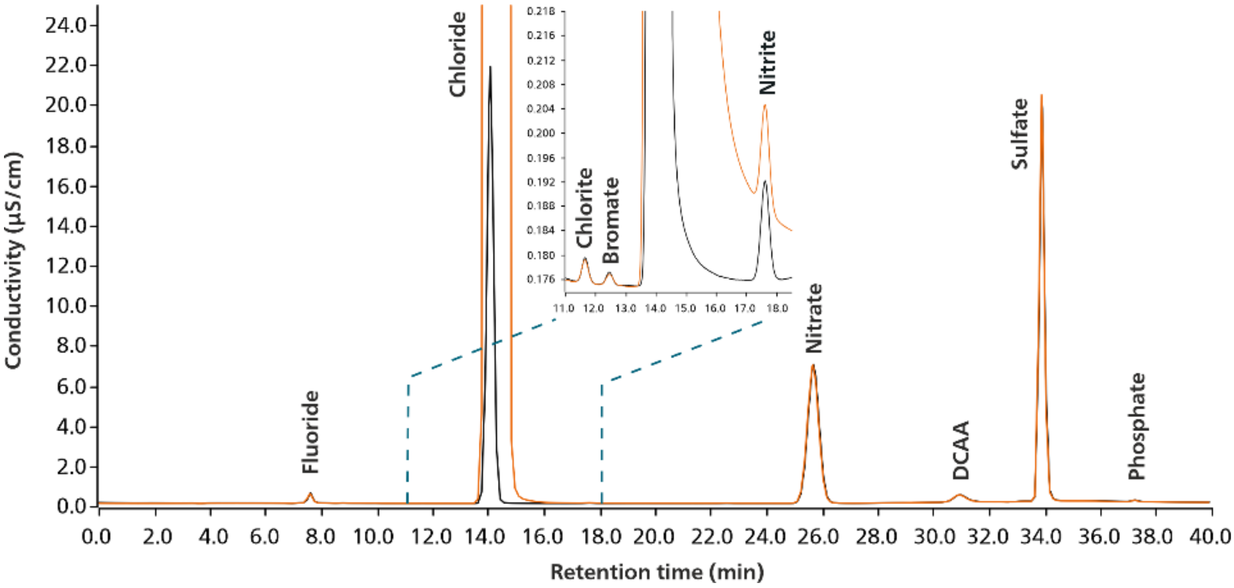

Figure 4. Chromatogram overlay for a mixed standard (**Table 1**, spike solution, black) fortified with 300 mg/L of chloride (orange). Gradient elution of the analytes (18–80 mmol/L KOH), prior to sequentially suppressed conductivity detection, was performed with the high-capacity column Metrosep A Supp 21 (injection volume 50 µL). The recovery of the most influenced ions for high chloride contents yielded 98% for nitrite and 97% for bromate.

REFERENCES

[1] U.S. EPA. U.S. EPA Method 300.1: Determination of Inorganic Anions in Drinking Water by Ion Chromatography, Revision 1.0, 1997.

[2] Boorman, G. A.; Dellarco, V.; Dunnick, J. K.; et al. Drinking Water Disinfection Byproducts: Review and Approach to Toxicity Evaluation. *Environmental Health Perspectives* **1999**, *107*, 207–217. https://doi.org/10.2307/3434484.

[3] Jackson, P. E. Ion Chromatography in Environmental Analysis. In *Encyclopedia of Analytical Chemistry*; Meyers, R. A., Ed.; Wiley, 2000. https://doi.org/10.1002/9780470027318.a0835.

[4] ISO. ISO 10304-4:2022 - Water Quality — Determination of Dissolved Anions by Liquid Chromatography of Ions — Part 4: Determination of Chlorate, Chloride and Chlorite in Water with Low Contamination, 2022.

[5] ISO. ISO 10304-1:2007 - Water Quality — Determination of Dissolved Anions by Liquid Chromatography of Ions — Part 1: Determination of Bromide, Chloride, Fluoride, Nitrate, Nitrite, Phosphate and Sulfate, 2007.

Analytes:	**Halogens – chloride, chlorite, chlorate, bromide, bromate, fluoride; Nitrogen – nitrate, nitrite; Phosphorus – phosphate; Sulfur – sulfate**
Matrix:	**Water – drinking water, tap water, others**
Method:	**Ion Chromatography**
Industry:	**Food & beverage; Environmental**
Standards:	**EPA Method 300.1 Part A; EPA Method 300.1 Part B ISO 10304-1, ISO 10304-4**

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