

# Tapping technology to advance your ion chromatography methods

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## Goal

Highlight the enhancement of ionic determinations that have resulted from innovations in ion chromatography (IC) technology.

## Introduction

Over the last 45 years, IC system and column technology innovations have improved many IC applications for greater ease of execution, accuracy, consistency, and sensitivity. What follows are some of the analyte determinations that have benefited from these innovations.

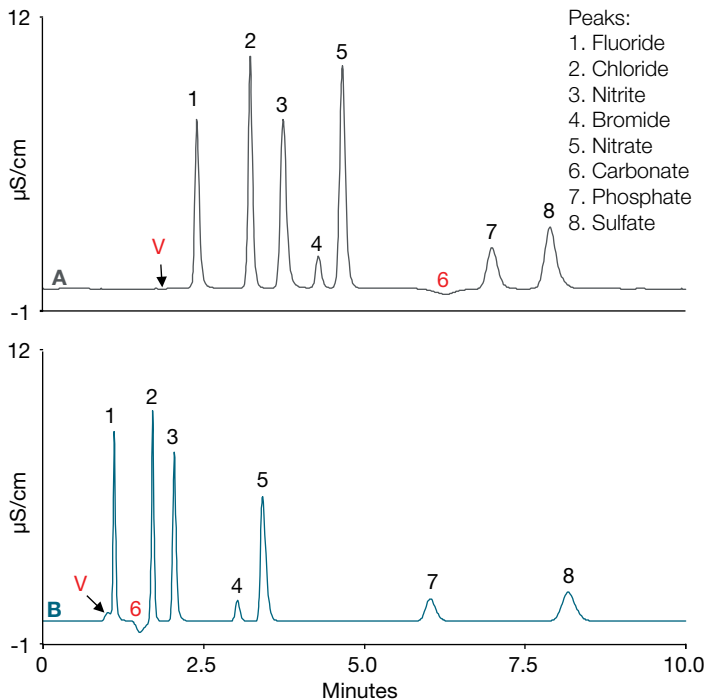
## Inorganic anions

The most widely used IC methods are U.S. Environmental Protection Agency (EPA) Methods 300.0 and 300.1 Part A, which were developed to determine the concentration of inorganic anions in drinking water that are hazardous to human health when present beyond established limits. These methods are required for compliance monitoring as part of the Clean Water and Safe Drinking Water Acts.<sup>1</sup>



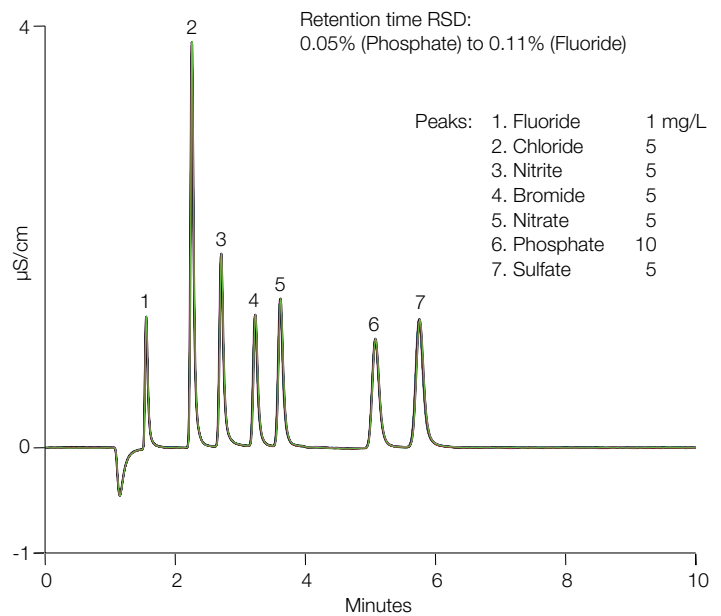
The column specified initially in U.S. EPA Method 300.0 Part A was the Thermo Scientific™ Dionex™ IonPac™ AS4 column and later the Thermo Scientific™ Dionex™ IonPac™ AS4A-SC column. These columns resolve the seven standard inorganic anions in under 8 minutes, applying isocratic conditions with a carbonate/bicarbonate buffer at a 2 mL/min flow rate. A recently published method achieves comparable results using a 1 mL/min flow rate.<sup>2</sup> This 50% reduction in eluent usage was accomplished using the Thermo Scientific™ Dionex™ IonPac AS29-Fast-4 $\mu$ m anion-exchange column. The Dionex IonPac AS4A-SC column has a particle size of 13  $\mu$ m, while the Dionex IonPac AS29-Fast-4 $\mu$ m column is based on 4  $\mu$ m particles. The benefit of the lower particle-size is a 6-fold increased chromatographic efficiency.

Consequently, a shorter column (150 mm instead of 250 mm) can be used to achieve equivalent separation (Figure 1). At the same time, the column capacity increases due to the new hyper-branched polymer used (126  $\mu\text{eq}$  vs 20  $\mu\text{eq}/4$  mm columns). The amount of sample and matrices that can be loaded also increases. The separation of fluoride from the water-dip is improved, and carbonate now elutes between nitrate and phosphate.



**Figure 1. Comparison of separation of common anions with the Dionex IonPac AS29-Fast-4 $\mu\text{m}$  (A) and Dionex IonPac AS4A-SC columns (B). V indicates the void volume.**

Today, instrument configurations are available that take advantage of Reagent-Free IC (RFIC), where only deionized water is needed to prepare eluents *in situ* and to automatically regenerate suppressors. Automated eluent generation and the continuous regeneration of the suppressors are based on inline electrolytic processes. RFIC systems eliminate the variability of manually prepared eluents and regenerants, delivering high purity eluent of precisely controlled concentration (Figure 2).



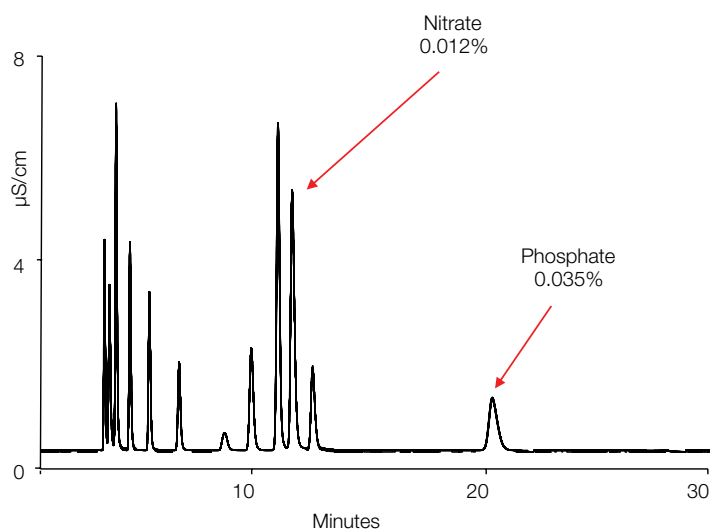
**Figure 2. Overlay of 100 consecutive runs showing the reproducibility of an RFIC system.** Separation of seven common anions on a 4 mm Thermo Scientific™ Dionex™ IonPac™ AS22-Fast-4  $\mu\text{m}$  column using a Thermo Scientific™ Dionex™ EGC 500  $\text{K}_2\text{CO}_3$  cartridge and a Thermo Scientific™ Dionex™ EPM 500 device.

The use of an electrolytically regenerated suppressor provides an additional advantage, avoiding the preparation of regenerant. In its most user-friendly configuration, the neutralized column effluent is used as feed solution for the suppressor's electrolytic regeneration (recycle mode). No additional pumps are needed, thus simplifying operation. At the same time, the initial investment in the instrument is lower.

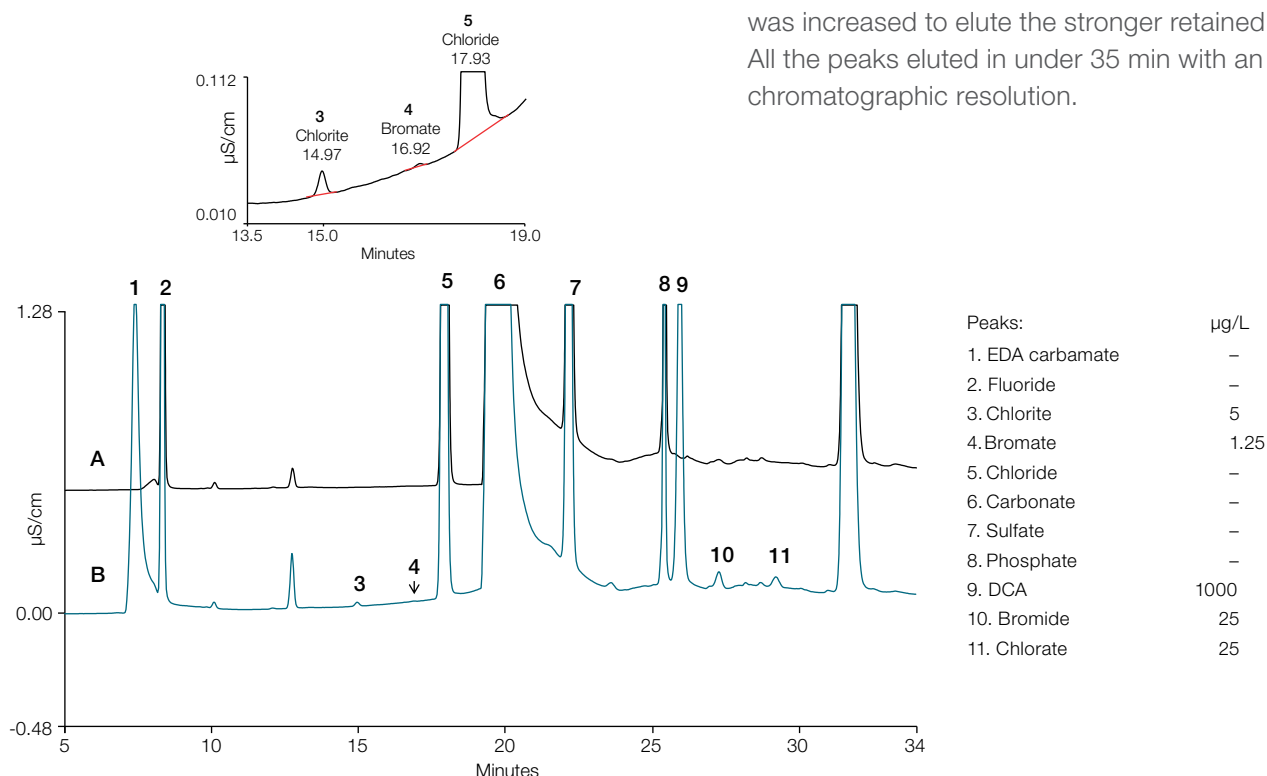
In 2002 the Statistics and Analytical Support Branch (SASB) and the Office of Ground Water and Drinking Water's Technical Support Center (OGWDW/TSC) determined that the use of hydroxide eluents falls within the method flexibility allowed in U.S. EPA Methods 300.0 and 300.1.<sup>3</sup> They concluded that the improved method performance obtained by using hydroxide eluents is acceptable for compliance monitoring under the Clean Water Act and Safe Drinking Water Act.

However, hydroxide eluents' manual preparation requires training and a rather costly supply of helium for proper degassing of the water and blanketing the final eluent. These steps are needed to prevent the uptake of carbon dioxide from the surrounding atmosphere. The changing ionic composition of the eluent would affect the reproducibility and accuracy of the analytical approach. The solution to this is to use a RFIC system with the electrolytic generation of hydroxide eluents and

the continuous regeneration of the suppressors (Figure 3). With this, hydroxide-eluents became more prevalent. They offer improved peak capacity through gradient elution, increased sensitivity through reduced background conductivity and lower noise, and the use of columns with new chromatographic selectivities. No external degassing with helium is needed, and the use of hydroxide eluents and gradients became as simple as “Just add Water”.



**Figure 3. Reproducibility of a KOH RFIC anion system.** Overlay of 20 consecutive runs of inorganic anion standards with the Thermo Scientific™ Dionex™ IonPac™ AS18 column showing the retention time Relative Standard Deviation (RSD) for nitrate and phosphate.



**Figure 4. Drinking water fortified with DBPs, bromide, DCA, and EDA carbamate; A) Unfortified sample, and B) Fortified sample.**<sup>4</sup>

## Oxyhalides

To protect drinking water from potentially dangerous microorganisms, it is typically treated with chemical disinfectants such as chlorine, chlorine dioxide, chloramine, and ozone. These chemicals can react with naturally occurring organic matter and inorganic material in source waters to produce disinfection byproducts (DBPs) potentially harmful to consumers. DBPs include inorganic oxyhalides such as chlorite, chlorate, and bromate. Trace determinations of these oxyhalides could be particularly challenging if ethylenediamine (EDA) were added to samples and standards to preserve chlorite and bromate integrity.<sup>1</sup> The formation of EDA carbamate products from reaction with CO<sub>2</sub> introduces an artifact that is not easily resolved from fluoride using the recommended, high-capacity, hydroxide-selective, anion exchange columns, the Thermo Scientific™ Dionex™ IonPac™ AS27, and the Thermo Scientific™ Dionex™ IonPac™ AS19 columns. This limited resolution has recently been addressed using the high-capacity, hydroxide-selective Thermo Scientific™ Dionex™ IonPac™ AS30 column. It was specifically developed to resolve the EDA carbamate artifact and fluoride, carbonate and sulfate, and dichloroacetate (DCA, a surrogate anion) from potentially interfering matrix anions (Figure 4).

Initially, an electrolytically generated KOH gradient, starting with low ionic strength, was applied to resolve the early eluting peaks. Following that, the KOH concentration was increased to elute the stronger retained analytes. All the peaks eluted in under 35 min with an optimized chromatographic resolution.

## Haloacetic acids

Haloacetic acids (HAAs) are DBPs that can develop during the drinking water purification process. They are a human health concern that has prompted the U.S. EPA to set limits and create U.S. EPA Method 557. This method describes the direct determination of nine HAAs along with bromate and dalapon in drinking water samples using IC hyphenated to mass spectrometry (MS).<sup>5</sup> Hyphenating IC with MS is possible due to the desalting provided by a continuously regenerated membrane-based suppressor before the MS. The high pH eluent (KOH) is converted to water, allowing the direct combination with MS. The run time of the original method using the Thermo Scientific™ Dionex™ IonPac™ AS24 column was approximately one hour. The use of the newly developed Thermo Scientific™ Dionex™ IonPac™ AS31 column lowers the run time while maintaining the separation of the 9 HAAs, bromate, and dalapon. The cycle time is decreased by 39% (Figure 5), reducing costs through lower reagent consumption and improved sample throughput.

In addition to the improved chromatographic selectivity, the column capacity facilitates a large loop injection to optimize sensitivity. The separation of the HAAs from the main anions (chloride, nitrate, sulfate, and carbonate) is best at 15 °C. It is the temperature recommended to stabilize thermolabile HAAs to obtain accurate results.<sup>4</sup>

## Organic acids

Anion exchange chromatography with suppressed conductivity detection provides selective and sensitive determination of short-chain organic acids. The analysis of more challenging, complex sample matrices benefits from the complementary use of an MS detector. The additional level of selectivity and sensitivity provided enhances analyte identification confidence and provides lower detection limits. A recently developed method describes the use of the Thermo Scientific™ Dionex™ IonPac AS11-HC-4µm column in combination with the Thermo Scientific™ ISQ™ EC single quadrupole MS to determine aliphatic and unsaturated organic acids in pharmaceutical solutions.<sup>7</sup> When IC is paired with MS detection after suppressed

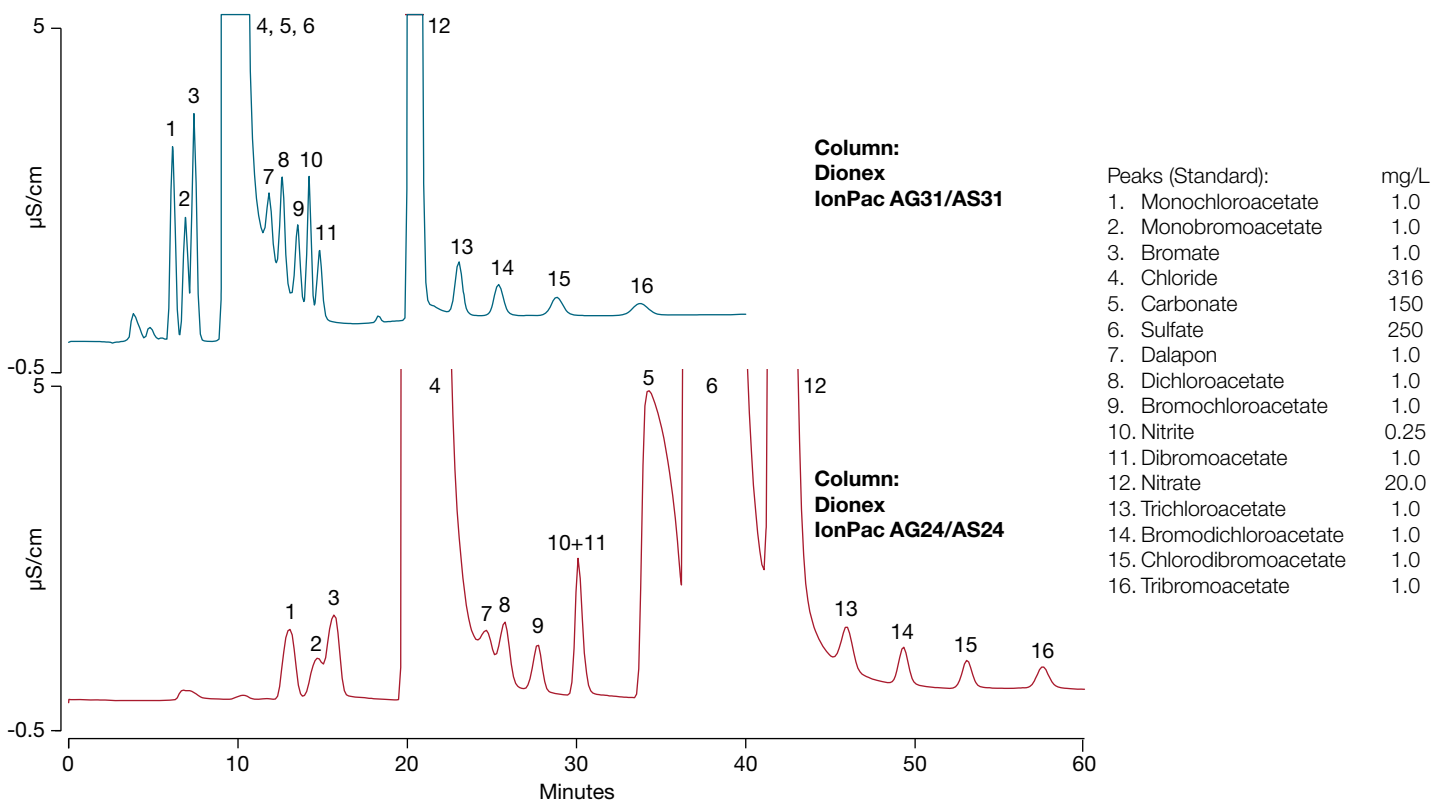
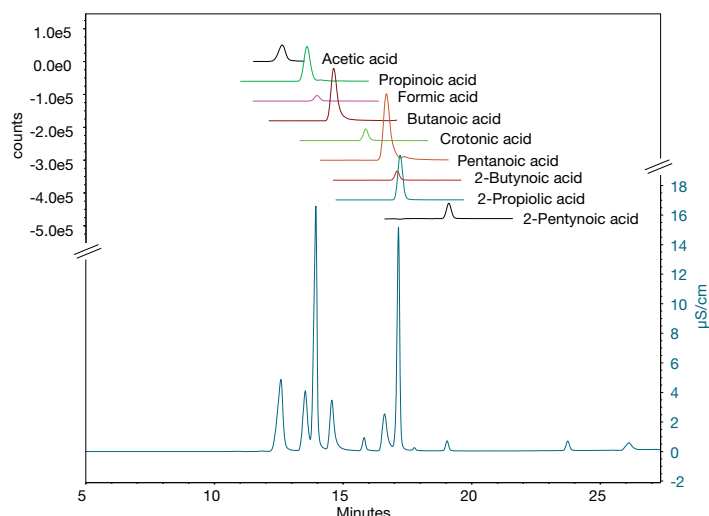


Figure 5. Separation of haloacetic acids, dalapon, and bromate in U.S. EPA Method 557 Laboratory Synthetic Sample Matrix (LSSM) on the Dionex IonPac AS31 and Dionex IonPac AS24 columns with conductivity detection.<sup>6</sup>

conductivity detection, the analytes' baseline resolution in the conductivity channel is not always required for an accurate analyte quantification (Figure 6). Highly efficient, high capacity columns enhance the matrix tolerance needed to determine low concentrations of organic acids in complex samples. The availability of integrated electronic consumables monitoring with the Thermo Scientific™ Dionex™ Integrion™ and ICS-6000 IC systems automates the performance documentation further streamlining the workflow by eliminating another manual process.



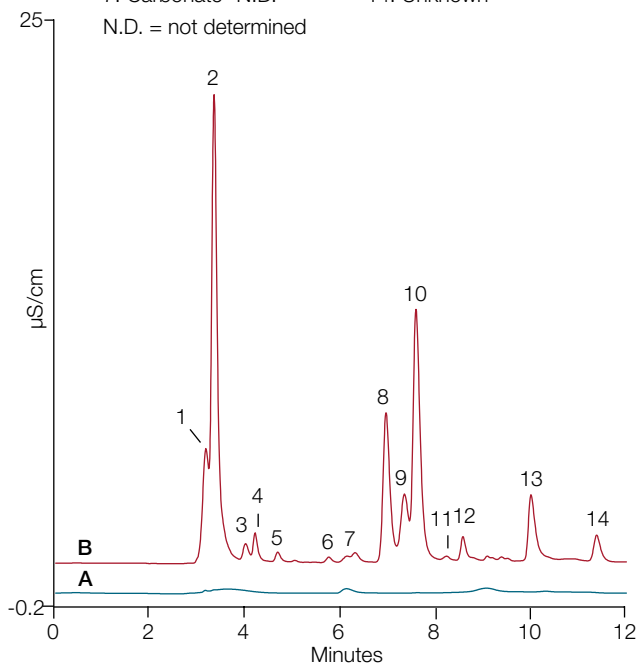
**Figure 6. Conductivity (dark blue) and selected ion monitoring (SIM) chromatograms of all analytes (500 µg/L).** Note that the signals are offset.

### Trace anions

A critical task of industries that process high purity water or other high purity solutions is the trace monitoring of potentially damaging (e.g., corrosive) trace contaminants. One example is the determination of trace anions in caustic solutions. Their analysis would typically require high dilution to prevent column overload, thereby limiting trace analysis. Such matrices can easily be analyzed by automatically neutralizing the sample using electrolytic suppressors during the sample loading step.<sup>8</sup> The setup significantly reduces the run time to 12 min using the Thermo Scientific™ Dionex™ IonPac AS18-4µm column (Figure 7). If needed, improved separation of fluoride from acetate and formate is achieved using the high capacity Thermo Scientific™ Dionex™ IonPac AS28-4µm column at the expense of slightly increased run time.

Peaks:	A	B	
1. Acetate	N.D.	8. Bromide	564 µg/L
2. Formate	N.D.	9. Unknown	-
3. Chloride	23.1 µg/L	10. Sulfate	515
4. Unknown	-	11. Nitrate	N.D.
5. Unknown	-	12. Unknown	-
6. Unknown	-	13. Phosphate	354
7. Carbonate	N.D.	14. Unknown	-

N.D. = not determined



**Figure 7. A) Deionized water, and B) Trace anions in 0.1 M tetrabutylammonium hydroxide (TBAOH) ACS grade solution.**

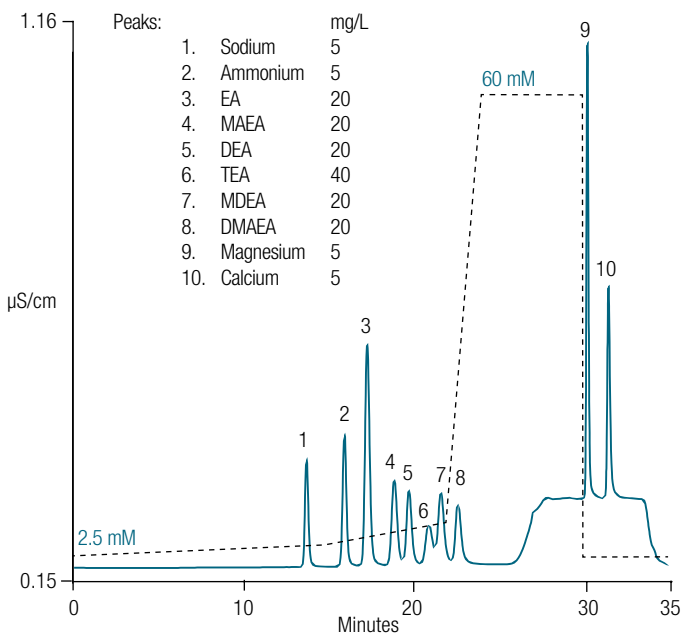
The use of columns with an inner column diameter of 2 mm instead of 4 mm columns resulted in a quadrupled mass-sensitivity. At the same time, the eluent consumption was reduced by 75%. (Table 1).

**Table 1. Comparison of parameters for IC column formats.**

	Analytical	Microbore
Column i.d. (mm)	4	2
Flow rate (mL/min)	1	0.25
Injection volume (µL)	10	2.5
Eluent consumption/waste generated (L/month)	43.2	10.8
Best suited for	<ul style="list-style-type: none"> <li>Established procedures with specific requirements that cannot be readily changed</li> </ul>	<ul style="list-style-type: none"> <li>Standard applications</li> <li>Reduced eluent consumption and waste generation</li> <li>Low-level determinations</li> </ul>

## Neutralizing amines

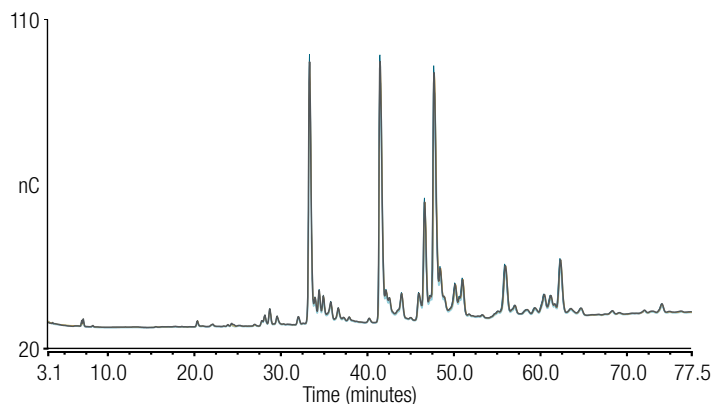
In the processing of oil and gas, amines play a critical role as the primary active ingredient in scrubbing solutions that remove CO<sub>2</sub> and H<sub>2</sub>S impurities that can lead to corrosion and environmental damage. Treatment typically uses percent concentrations of alkanolamines to neutralize the “sour” gas impurities. When the neutralizing capacity is deemed inefficient, the amine solutions are regenerated and stripped of elemental sulfur. Dissolved salts (heat-stable amine salts) remain, building up over time, resulting in higher maintenance costs and increased corrosion. Determinations of the amine concentrations and the heat-stable amine salts are challenging in the concentrated alkanolamine solutions. The challenge of variability in the composition of the neutralizing amine, degradation products, and salt is addressed by the newly developed Thermo Scientific™ Dionex™ IonPac CS20 column. This cation exchange column has three types of cation-exchange functional groups: carboxylates, phosphonates, and sulfonates. Injecting a sample volume of 0.4 μL, inorganic cations, ammonium, and alkanolamines in the concentration range from a few μg/L to 1250 mg/L can be determined in the diluted neutralizing amine solution.<sup>9</sup> The electrolytically generated methanesulfonic acid gradient reduces the run time, maximizes the separation, and the low noise baseline, a result of suppressed conductivity detection, improves the trace determination (Figure 8).



**Figure 8. Separation of a cation and alkanolamine standard; methanesulfonic acid eluent concentration used is indicated by the dashed line.**

## Complex carbohydrates

Glycoprotein characterization and glycosylation profiling are essential tasks in the development and production of biopharmaceutical proteins. A commonly used technique for obtaining this information is high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD). One of the challenges encountered using this technique is reproducible eluent preparation. As noted previously, the development of RFIC has markedly increased consistency and reduced the labor required. Glycoprotein characterization presents a unique challenge for RFIC in that the eluent cannot be produced from a single chemical solution. The solution to this is a Thermo Scientific™ Dionex™ ICS-6000 system equipped with two eluent generation cartridges (Dual EGC mode). The two eluent generator cartridges (EGCs) are configured in series to electrolytically generate eluent, consisting of potassium hydroxide/methanesulfonic acid (KOH/MSA). Using the Dual EGC mode combined with a 1-mm Thermo Scientific™ Dionex™ CarboPac PA200 column, reproducible, high-resolution gradient separations of complex carbohydrates can be obtained (Figure 9).



**Figure 9. Overlaid chromatograms of five consecutive injections of N-linked oligosaccharides released from human α1-acid glycoprotein (AGP).<sup>10</sup>**

**Table 2. Benefits from IC innovations.**

Description	Feature	Impact	Labor/cost savings	Higher throughput	Ease-of-use	Method flexibility
Column	↓ Particle size (4 μm)	↑ column efficiency → ↑ resolution, ↓ run time		✓		✓
	↑ Particle porosity	↑ capacity → ↑ sample load	✓			
	Surface functionalization	Δ selectivity			✓	✓
	↓ Diameter	↓ Eluent consumption, waste	✓			✓
Eluent	RFIC system	↑ consistency	✓		✓	✓
	Gradients	Peak sharpening, ↓ run time		✓		✓
	Hydroxide	Minimal background conductivity → ↑ sensitivity				✓
Suppressor	Electrolytic regeneration	No regeneration solution preparation needed	✓		✓	
Consumable monitoring	Automated performance tracking	↓ manual logging of consumables usage and data	✓		✓	
MS detection	Mass selectivity	Specific analyte detection in complex matrices	✓		✓	✓
Inline sample preparation	Automation	No manual sample treatment needed	✓	✓	✓	

↑ = increased, ↓ = decreased, → = enables, Δ = changed

## Conclusion

As illustrated by the examples above, practically any IC application can be simplified, improved, and optimized for its ease-of-use. Costs are reduced, and the methods become more flexible through the numerous innovations in IC instrumentation, column and consumable chemistry, and automation (Table 2).

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