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APPLICATIONS OF ION CHROMATOGRAPHY

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DEVELOPMENT OF A NEW METHOD FOR MONOSACCHARIDE AND SIALIC ACID QUANTIFICATION IN SIALYLATED POLYSACCHARIDES BY HPAEC–PAD

By Francesca Merangolo, Sara Giannini, Massimiliano Gavini, Stefano Ricci, and Cristiana Campa

In the production of a vaccine against Group B streptococcus (GBS), a key quality control step is to measure the concentration of the polysaccharides that create an immunogenic response against three GBS serotypes. This concentration can be measured indirectly by determination of sialic acid on the purified polysaccharides. This study describes the development of a chromatographic method using high-pressure anion-exchange chromatography with pulsed amperometric detection (HPAEC–PAD), which takes advantage of the weakly acidic nature of carbohydrates to give highly selective separations at high pH using a strong anion-exchange stationary phase. This chromatographic method was shown to provide greater accuracy and precision for the investigated samples than the colorimetric assay used previously.

Group B streptococcus (GBS) is a major cause of infections in neonates and important as well as among pregnant women and immunocompromised adults. Protection from GBS infection in neonates is associated with the presence of naturally acquired maternal antibodies to the type-specific capsular polysaccharides (CPS) of these organisms. GBS capsular polysaccharides are important virulence factors and anti-capsular antibodies have been shown to correlate with protection from the disease (1). There are several GBS serotypes that mainly differ in the structure of their CPS. From an epidemiology point of view, serotypes Ia, Ib, II, III and V are the most common.

Novartis Vaccines is developing a GBS vaccine, currently in Phase II, that includes serotypes Ia, Ib, and III. The antigens are glycoconjugate molecules obtained with a conjugation reaction between cross reacting material (CRM_{197}) protein and CPS of the above-cited GBS serotypes. The structures of three GBS serotypes are shown in Figure 1.

Each CPS is obtained in a complex process starting with bacterial fermentation and subsequent steps of purification, which produces at the end a purified high-molecular-weight polysaccharide that undergoes an oxidation reaction with sodium metaperiodate, followed by conjugation to the carrier protein CRM_{197}.

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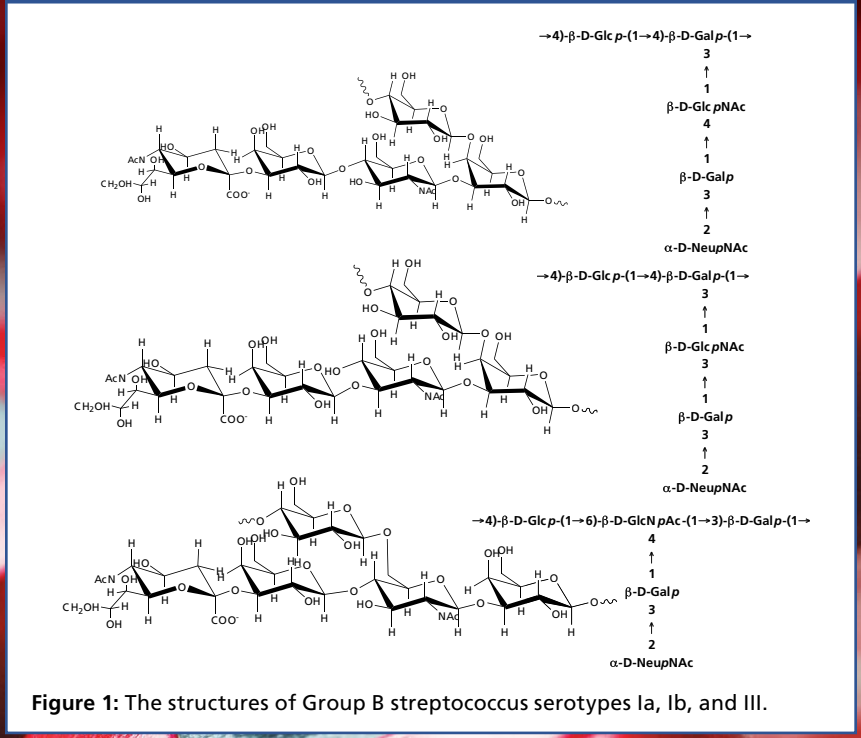

Figure 1: The structures of Group B streptococcus serotypes Ia, Ib, and III.

The process of fermentation and purification that generates the purified CPS includes various steps to eliminate impurities such as proteins and other components.

The relevant steps have to be monitored to check the efficiency of polysaccharide recovery. For this reason an important test applied is the determination of polysaccharide amount, measured as "saccharide titer."

Determination of sialic acid performed on the purified polysaccharide makes it possible to estimate the concentration of sialic acid residues and, by a conversion factor, the concentration of polysaccharide. Therefore, it is important to develop a suitable method for the determination of sialic acid, because this concentration is a critical quality attribute for the polysaccharide purification and conjugation process and ultimately to ensure immunogenic response of the vaccine.

In the concept of Quality by Design, analytical method development is driven by a predefined analytical target profile (ATP). The purpose of the ATP is to ensure that the test methods are selected and developed based on quality attribute requirements. The staff responsible for product and process development generates a list of those requirements for the expected method performance, taking into consideration the method purpose, expected sample and matrix interference, and the testing range for each required application. Additionally, method requirements including method performance indicators (such as accuracy, specificity, precision, range, throughput, and cycle time) should be listed. These target requirements, if prospectively defined, will allow the analytical team to develop the most suitable methods to measure quality attributes. In this context, analytical methods should be challenged and optimized during product and process development to achieve performance as close as possible to the target, exploiting opportunities that new technologies offer over time.

Table I lists the ATP for the saccharide content in GBS polysaccharides. Please note that the ATP is set independently of the available technologies, because it is predefined to continuously challenge the analytical status quo until the desired performance is achieved.

This article shows the evolution of the analytical strategy aimed at the determination of sialic acid resulting from the hydrolysis of Group B streptococcus polysaccharides, passing from a simple colorimetric to a chromatographic method, with the purpose of meeting the optimum requirements of the ATP. In particular, the following methods were explored over time:

- Colorimetric assay (2), which is based on the reaction of sialic acid with resorcinol in the presence of hydrochloric acid and rameic ions, with subsequent formation of a colored compound with a maximum

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Quality Attribute	Selectivity and Specificity	Sensitivity	Range	Accuracy	Precision	Cycle time	Throughput
Saccharide content	Able to selectively detect the desired polysaccharides independently of the matrix and potential known contaminants	**Minimum :** 5.00 µg/mL sialic acid = 16.42 µg/mL saccharide **Optimum:** 1.00 µg/mL sialic acid = 3.28 µg/mL saccharide	**Minimum:** 5-25 µg/mL sialic acid **Optimum:** 1-10 µg/mL sialic acid	**Minimum:** 80–120% **Optimum:** 90–110%	**Minimum:** CV 12% **Optimutm:** CV 8%	**Minimum:** 12 h **Optimum:** 5 h	**Minimum:** 15 samples per session **Optimum:** 30 samples per session

Table I: Analytical target profile that corresponds to the saccharide content in the GBS polysaccharide

absorbance at 564 nm. The absorbance at this wavelength is directly proportional to the amount of the reducing sugar (2).

- High pressure anion exchange chromatography with pulsed amperometric detection (HPAEC–PAD), which takes advantage of the weakly acidic nature of carbohydrates to give highly selective separations at high pH using a strong anion-exchange stationary phase (3). Sialic acid residues are in the lateral chain of the polysaccharide and are quantitatively released by hydrolysis under proper conditions. Then, during the detection step following anion-exchange separation, the sialic acid monomers are electrocatalytically oxidized at the surface of the gold electrode by application of a positive potential. The current generated is proportional to the carbohydrate concentration, and therefore sialic acid can be detected and quantified.

Materials and Methods

Colorimetric Assay

Fermentation process intermediates were ultrafiltered with a Microcon YM-30 Millipore filter with a nominal molecular weight limit of 30 kDa. A calibration curve of 5–25 µg/mL of sialic acid was prepared with commercial N-acetylneuraminic acid. Resorcinol–hydrochloric acid reagent was mixed and both standards and samples were heated at 100 °C for 20 min. After cooling, the absorbance was measured at 564 nm with a UV spectrophotometer.

Chromatographic Assay

All the samples were analyzed with the chromatographic method without pretreatment by ultrafiltration. A standard curve of 1–10 µg/mL of N-acetylneuraminic acid was prepared with commercial N-acetylneuraminic acid. Samples were diluted in water; 1 N hydrochloric acid was added both to samples and standards (to obtain a final concentration of 0.05 N hydrochloric acid) and heated for 1 h at 75 °C. These hydrolysis conditions were optimized to obtain the maximum release of monosaccharide sialic acid from the polysaccharide and the least degradation of the monomeric sialic acid. After cooling for 30 min, 0.75 N sodium hydroxide was added (to obtain a final concentration of 0.06 N sodium hydroxide); samples and standards were filtered through a filter with a 0.45-µm pore size and analyzed. For the analyses, Thermo Scientific Dionex ICS5000 and ICS3000 instruments were used, equipped with an electrochemical detector in the pulsed amperometric mode with a gold working electrode and an Ag/AgCl reference electrode. CarboPac PA1 (carbohydrate) and CarboPac PA20 Fast Sialic Acid (sialic acid) columns, both from Thermo Fisher Scientific, were used in the study.

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Analysis using the carbohydrate column: Eluents C, D, and B were 100 mM sodium hydroxide, 100 mM sodium hydroxide–100 mM sodium acetate, and 100 mM sodium hydroxide–500 mM sodium nitrate, respectively. The separation conditions were as follows: isocratic at 70:30 C–D for 11 min (equilibration and analyte elution), gradient from 10:90 B–C to 40:60 B–C in 5 min (strong wash), and isocratic at 70:30 C–D for 10 min (equilibration). The run time was 30 min for each run. A 20-µL volume of sample was injected for both standards and samples. To detect sialic acid, a triple-potential waveform was applied using the following settings: E_1 = 0.05 V, t_1 = 400 ms; E_2 = 0.75 V, t_2 = 200 ms; E_3 = –0.15 V, t_3 = 400 ms. Integration occurs from 200 to 400 ms during E_1 application. A nondisposable Au electrode and Ag-AgCl reference electrode were used. A CarboPac PA1 guard column was used in addition to the analytical column.

Analysis using the sialic acid column: Eluents A and B were 100 mM sodium hydroxide and 100 mM sodium hydroxide– 300 mM sodium acetate, respectively. The separation conditions were as follows: isocratic at 10% B–A for 1 min (equilibration), gradient from 10% B–A to 100% B in 1 min (analyte elution), 100% B for 0.5 min (strong wash) and 10% B for 1.5 min (equilibration), for a total run time of 4 min. With this method, 5-µL volumes of sample and standards were injected. In the process using the carbohydrate column, the triple carbohydrate waveform was used for the detection, as described in the previous section, with a nondisposable Au electrode and Ag-AgCl reference electrode. No guard column was necessary for this method.

Results

Colorimetric Assay

A colorimetric assay was the first method applied for the saccharide quantification of GBS polysaccharide samples. Simplicity and time of execution (about 2 h) are the advantages of this kind of analysis, but for samples with complex matrices the assay time increases from 2 h to about 5 h (because of the need for sample ultrafiltration, as described above in the "Materials and Methods" section). During a study of validation parameters, the assay showed good performance on samples without complex matrices in terms of repeatability, linearity, and accuracy. Samples that needed the ultrafiltration step showed higher performance variability in terms of accuracy and precision: It was noted that the loss of material during the ultrafiltration step could be different between lots and also between analyses performed at different times. Finally an issue of the colorimetric method is represented by the sensitivity: Below 5 µg/mL the absorbance measurement lacks precision and the linearity of the standard curve decreased in the lower range. The main parameters investigated are reported in Table II.

Sample	Parameters						
	Selectivity and Specificity	Sensitivity	Range	Accuracy	Precision	Cycle time	Throughput
Samples with no complex matrices (no ultrafiltration needed)	Not possible to separate interfering contaminants	5 µg/mL	5–25 µg/mL	95–100%	2–5%	2 h	20
Samples with complex matrices (ultrafiltration needed)	Not possible to separate interfering contaminants	5 µg/mL	2–25 µg/mL	82–88%	5–15%	5 h	20

Table II: Analysis of performance parameters of the colorimetric method for samples with and without ultrafiltration

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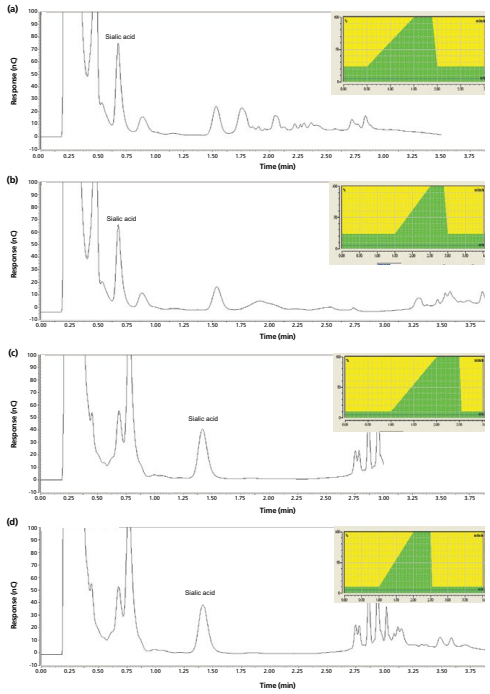

Figure 2: Comparison of different chromatographic conditions for the determination of sialic acid. Yellow and green colors in the inset plots correspond to eluents 100 mM sodium hydroxide and 100 mM sodium hydroxide–300 mM sodium acetate, respectively. Note that the chromatograms in (a) and (c) were obtained with 3-min runs, and the chromatograms in (b) and (d) were obtained with 4-min runs.

HPAEC–PAD Method

The HPAEC–PAD quantification method provides better sensitivity than the colorimetric assay, with good method performance down to 1 µg/mL of sialic acid. Moreover, with this approach including an optimized chromatography step, it is possible to separate the analyte peak (sialic acid) from other sugars with a polysaccharide structure (galactose, glucose and N-acetylglucosammine) and from the matrix contaminants, providing high selectivity without the need for ultrafiltration.

The chromatography step using with the carbohydrate column presents the disadvantage of the relative length of the analysis (30 min), which corresponds to typical run times of chromatographic methods for IC standard analytical columns.

To overcome the weakness of the cycle time described for the carbohydrate column, the sialic acid column was explored. This column has been developed specifically for sialic acid applications, and it allows quantitative analysis of this monosaccharide with high throughput (4 min for each chromatographic run compared to 30 min for the carbohydrate column). The use of the sialic acid column significantly decreases the time of analysis, shortening chromatographic runs almost 10-fold.

Initially the chromatographic conditions for the sialic acid column were optimized with the aim of eluting the analyte (sialic acid) separate from the other sugars and matrix components. The investigated conditions are reported in Figure 2, which also shows the respective chromatograms.

The conditions used in Figure 2d allow the analyte to be eluted with good resolution and well separated from the matrix; moreover the extension of the reconditioning step after regeneration makes the baseline stable. The same conditions resulted in suitable separations for a purified sample and for samples with complex matrixes.

Several injection volumes were tested, and a 5-µL sample volume provided the best performance in terms of peak resolution, repeatability, and linearity with the commercial sialic acid. This reduced injection volume — 5 µL of sample compared to the 20 µL required when using the carbohydrate column — is an advantage when working with samples that are limited in availability. The smaller injection volumes also increase the working electrode lifetime. In addition, the halved flow rate and the shortened chromatographic runs that are possible when using the sialic acid column reduce eluent consumption, with a considerable cost saving. We estimate that the needed eluent volume for each analysis is reduced from about 500 mL for the carbohydrate column to about 30 mL for the sialic acid

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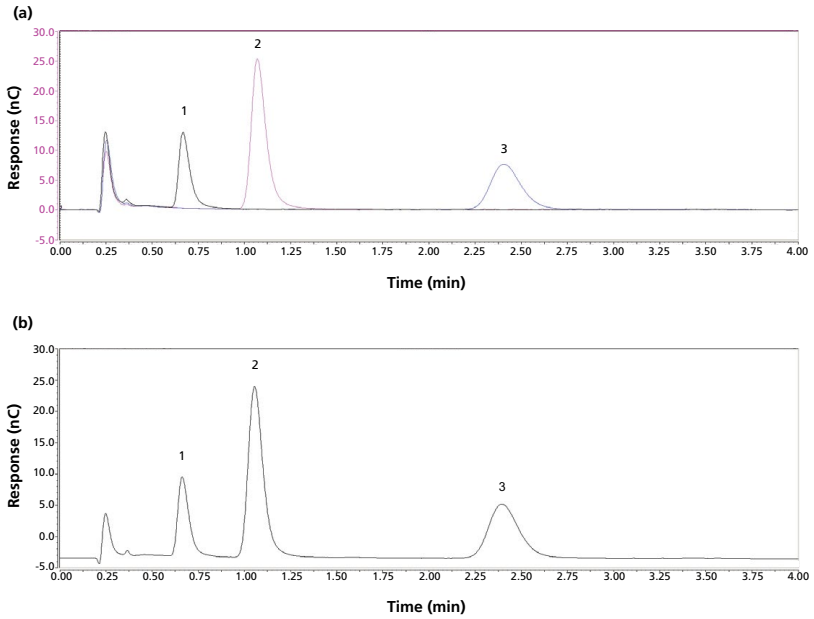

Figure 3: Chromatograms obtained using the sialic acid column showing (a) an overlay of sialic acid, KDO, and glucuronic acid solutions and (b) a mix of the same analytes. Mobile phase: 100 mM sodium hydroxide–75 mM sodium acetate for 4 min (isocratic). Peaks: 1 = sialic acid, 2 = KDO, 3 = glucuronic acid.

column. Finally, because no precolumn is used with the sialic acid column and the flow is relatively low, back pressure is reduced with a consequent stress reduction for the chromatographic pump.

After method selection, method validation was performed, ensuring compliance with predefined ATP requirements.

Finally, some operational aspects aimed at failure monitoring are being explored. A system suitability test is being developed to check the performance of both instrument and column before execution of the analysis. The work is still ongoing (because the sialic acid column has been on the market for just a few months and there are no available references for system suitability), but we describe our initial results below.

At first the same solution typically used for the carbohydrate column — a mix of galactose, fructose, and sucrose— was tested, but none of the screened chromatographic conditions could provide well resolved and separated peaks. Such results are expected because the sialic acid column is not tailored for baseline separation of a mixture of neutral sugars. As a consequence, a mixture of acidic sugars was explored (a mixture of sialic acid, KDO (3-deoxy-D-manno-oct-2-ulosonic acid), and glucuronic acid). Different chromatographic conditions were tested. The most efficient setup was achieved using isocratic elution with 100 mM sodium hydroxide–75 mM sodium acetate for 4 min (Figure 3).

This mixture has been injected during each validation analytical session and is also analyzed any time an analysis is performed. Data are being collected to verify the precision of elution times (both intra- and intersession), to define an acceptance criterion to be included in the standard procedure, based on a robust evaluation of different instruments, column lots, and ages (up to several months).

Conclusions

The HPAEC–PAD methods provide increased performance with respect to the colorimetric assay in terms of accuracy and precision, for the investigated samples. Additionally, the HPAEC–PAD methods provide greater selectivity and specificity, which are dependent on the chromatography and detection conditions. Moreover, the linearity range shifts from 5–25 µg/mL for the colorimetric assay to 1–10 µg/mL for the chromatographic methods. After testing different sample volumes for both the colorimetric and chromatographic methods, it was determined that 5 µg is the lowest quantity that can be analyzed with the colorimetric assay, whereas for HPAEC–PAD the sensitivity increases to 20 ng of sialic acid for the carbohydrate column (with injection of 20 µL) and 5 ng for the sialic acid column (where the injection volume is 5 µL).

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Parameter	Colorimetric Sialic Acid Method		HPAEC–PAD with a Carbohydrate Column		HPAEC–PAD with a Sialic Acid Column	
	Obtained Value	ATP Requirement	Obtained Value	ATP Requirement	Obtained Value	ATP Requirement
Selectivity and Specificity	Analyte not separated from other components; ultrafiltration needed for complex matrix phases	Met upon sample ultrafiltration	Analyte peak separated from the other matrix components	Fully met, no ultrafiltration	Analyte peak separated from the other matrix components	Fully met, no ultrafiltration
First calibration curve point (amount based on the analyzed volumes)	5 µg/mL (5 µg)	Minimum requirement met	1 µg/mL (20 ng)	Optimum requirement met	1 µg/mL (5 ng)	Optimum requirement met
Range	5–25 µg/mL	Minimum requirement met	1–10 µg/mL	Optimum requirement met	1–10 µg/mL	Optimum requirement met
Accuracy	82–100% (prevalidation data)	Minimum requirement satisfied	97–108% (prevalidation data)	Optimum requirement met	95–99% (validation data)	Optimum requirement met
Precision	2–10% Possible variability between different lots and analyses performed over a long time (prevalidation data)	Minimum requirement met	CV 3–10% (prevalidation data)	Minimum requirement met	CV 3% (validation data)	Optimum requirement met
Cycle Time	2 h (no ultrafiltration) 5 h (ultrafiltration required)	Optimum requirement met	10–12 h	Minimum requirement met	4–5 h	Optimum requirement met
Throughput	Up to 20	Optimum requirement met	Up to 15	Optimum requirement met	Up to 30	Optimum requirement met
Sample volume required for the analysis	1 mL	—	20 µL	—	5 µL	—

Table III: Comparison between performance parameters of the colorimetric sialic acid assay and the chromatographic method using carbohydrate and sialic acid columns

In addition, using the column developed specifically for sialic acid analysis ensures significantly high throughput, with a very short time needed for equilibration and wash steps, and with an overall chromatographic run duration of 4 min.

Table III shows a comparison of the three different methods applied for the quantification of saccharide in GBS polysaccharide samples. Based on the results reported above, HPAEC–PAD using a sialic acid column was considered the most reliable approach for the quantitation of saccharide in GBS polysaccharides type Ia, Ib and III, and is fully compliant with ATP requirements. The method was also successfully validated, as discussed above.

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Derivatization-Free Carbohydrate Analysis

Separate and Quantify Carbohydrates

- HPAE – an IC technique known as high-performance anion-exchange which gives highly selective separations of carbohydrates
- PAD – pulsed amperometric detection permits direct quantification of non-derivatized carbohydrates in the pmol to fmol range

Superior Separations with HPAE

HPAE chromatography can be used to separate analytes that can be ionized under high pH conditions. Carbohydrates typically have pKas in the range of 12–13. Once the pH rises above the pKa of the analyte, it becomes ionized in solution by using hydroxide-based eluents. With the development of highly cross-linked, ethylvinyl benzene-divinyl benzene pellicular resins that have broad pH stability (0 to 14), it is feasible to perform separations at high pH conditions. The columns' nonporous resins have small anion-exchange microbeads carrying the anion-exchange functional groups which are permanently attached electrostatically to a larger cation-exchange resin particle. The nonporous nature of the resin minimizes band-broadening and imparts highly effective separations of a wide variety of carbohydrates.

Sensitive, Selective PAD Detection

With PAD it is possible to detect underivatized analytes, with the potential variations known as a waveform. The variations result in oxidizing and reducing conditions on the electrode surface, which in turn causes the oxidation of analytes bound to the working electrode surface. Pulsed amperometry detects only compounds that contain functional groups which become oxidized at the detection voltage employed. This detection is sensitive and highly selective for electroactive species, since many potentially interfering species cannot be oxidized or reduced, and are not detected. Also, neutral or cationic sample components in the matrix elute in, or close to the void volume of the column. As a result, the carbohydrate components of interest are not impacted even if the neutral or cationic sample components are oxidized.

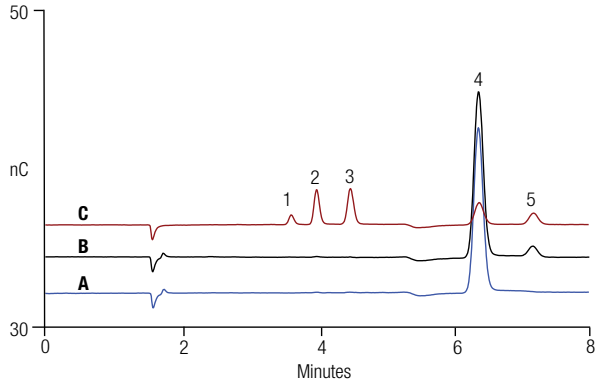
Innovative Solutions

HPAE-PAD can be performed on the Thermo Scientific™ Dionex™ ICS-5000⁺ HPIC™ system with standard bore (4 mm i.d.) microbore (2 mm i.d.) or capillary (0.4 mm i.d.) column formats. HPAE-PAD can also be performed in a capillary scale on the Thermo Scientific Dionex ICS-4000 HPIC system. Additionally, the Thermo Scientific™ Dionex™ CarboPac™ column family offers a selection of columns, each for a different class of compounds. Combined with PAD, these columns support reliable techniques to provide high-resolution separations of glycoprotein oligosaccharides, sialic acids, and complex carbohydrates.

Column:	Dionex CarboPac SA10-4µm and guard columns, 4 mm
Eluent Source:	Dionex EGC 500 KOH cartridge
Eluent:	4 mM KOH
Flow Rate:	1.45 mL/min
Inj. Volume:	10 µL
Column Temp.:	35 °C
Detection:	PAD, Au on PTFE disposable, Four-Potential Carbohydrate waveform
Gasket:	0.002" thick PTFE
Ref. Electrode:	pH-Ag/AgCl
Sample Prep.:	Carrez digestion, centrifuge, filter, Dionex OnGuard IIA cartridge

Sample: A: 100-fold diluted raw, unpasteurized milk
B: Sample A + 0.5 mg/L lactulose
C: 0.5 mg/L carbohydrate standard

Peaks:	A	B	
1. Sucrose	—	—	mg/L
2. Galactose	—	—	
3. Glucose	—	—	
4. Lactose	3.75	3.77	
5. Lactulose	—	0.48	



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APPLICATION OF CAPILLARY ION CHROMATOGRAPHY TO

ENVIRONMENTAL SAMPLES

By Caterina Giuriati and Maria Cristina Cristofori

Capillary ion chromatography is a new format for ion chromatography (IC). The benefits of the capillary scale are low solvent usage and waste generation coupled with improved mass sensitivity with conductivity detection. In this article, we explore the use of capillary IC for analyzing samples of environmental origin with varying matrix concentrations, such as ultrapure water, drinking water (surface and ground water), wastewater and emissions from power plants and industrial factories. The analysis methods were in compliance with EPA and official Italian methods. Also, quantification methods followed UNI EN ISO 17025 quality assurance rules. We also explored the use of 4-µm particle diameter packed ion-exchange columns that showed rapid separations and improved throughput. Additionally, we also demonstrate the utility of the capillary IC method for determining total chloride after combustion using an oxygen bomb setup. Overall, this article demonstrates the utility of the capillary IC technique for analyzing samples of environmental origin.

The capillary format of operation, combined with a scaled-down, capillary-compatible version of the ion chromatograph, is one of the most recent innovations in ion chromatography. Minimizing the dead volume of many of the components of the ion chromatograph became a paramount need to support operation at the capillary flow rate of 0.01 mL/min.

One of the key values of the capillary scale of operation that was attractive to us was the ability to leave the system on all the time, which is possible with the capillary format because of its low eluent consumption and consequently low waste production of about 5 L per year. Continuous operation of the system ensures that the system is available for on-demand analysis. Unlike other formats of operation, there is no need for conditioning or reconditioning the system before analysis. Another area of interest was to explore the use of 4-µm particle diameter (d_p) packed columns for faster analysis. We evaluated the capillary approach with a variety of column chemistries and samples.

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Instrumentation and Analytical Set-up

Chromatographic analysis was performed using an ICS 5000+ system (Dionex) with the following configuration: dual pump module hybrid (DP), eluent generator (EG), detector chromatography module (DC), and IC Cube, which integrates all the capillary consumables used in the capillary system. The system was configured as a dual-channel ion chromatograph supporting conventional standard bore scale and the capillary scale of operation.

Several column chemistries were evaluated, as indicated in the discussion and in the figures.

Our preliminary evaluation focused on characterizing the baseline noise, background, and drift of the capillary system. The typical baseline noise obtained with this method was at the <0.2 nS/cm level, which was well below our expectations. Our analytical system background noise when operated at 1 mL/min flow rate was typically at the <1 nS/cm level. The typical background of <1 µS/cm for the capillary system was comparable to the background obtained with the analytical system. The background drift was 0.6 nS/cm for the capillary system and was also slightly lower than the typical background drift of <1 nS/cm for the standard-bore analytical system. The lower noise resulted in improved detection limits of the capillary system given that for a proportional amount of sample injected into the capillary system the signal remained the same, but the signal-to-noise ratio (S/N) improvement was fivefold as a result of the lower noise of the capillary system relative to the analytical system. The system was also tested for linearity and a correlation coefficient between 99.9 and 99.99% was obtained using a standard comprising a standard test mixture of seven anions. This linearity was comparable to the performance of the standard-bore analytical system except that the capillary system could be calibrated with a standard at a lower dilution level thus expanding the dynamic range of operation. After this preliminary evaluation of the key parameters real-world samples were evaluated in the capillary scale of operation.

APPLICATION OF CAPILLARY ION CHROMATOGRAPHY TO ENVIRONMENTAL SAMPLES

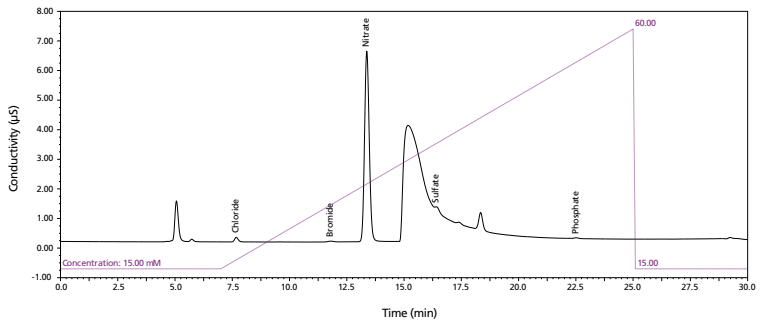

Figure 1: Chromatogram of a stationary emission sample (that is, from power plants and industrial factories) showing a large carbonate peak interfering with sulfate quantitation. Column: 250 mm X 2 mm Dionex IonPac AS19 with a 50 mm X 2 mm AG19 guard column; eluent: potassium hydroxide; gradient: 15 mM (0–7 min), 15–60 mM (7–25 min); flow rate: 0.25 mL/min; suppressor: 2-mm Dionex ASRS 300; current: 11 mA; sample loop volume: 100 µL; temperature: 30 °C.

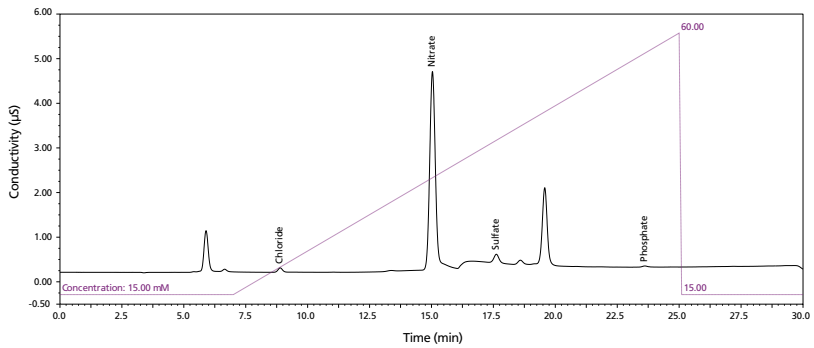

Figure 2: Chromatogram of a stationary emission sample using a carbonate removal device. Column: 250 mm X 0.4 mm Dionex IonPac AS19 with a 50 mm X 0.4 mm AG19 guard column; eluent: potassium hydroxide; gradient: 15 mM (0–7 min), 15–60 mM (7–25 min); flow rate: 0.01 mL/min; suppressor: Dionex ACES 300; current: 5 mA; sample loop volume: 0.4 µL (internal loop); temperature: 30 °C.

Application to Real-World Samples and Matrices

Determination of HCl, HF, NO_x and SO_x in emissions from power plants and industrial factories (1), as prescribed by Italian environmental law, is a major focus of our laboratory. Italian regulations of stationary emissions (2) prescribe the use of a strong base solution for the sampling step. The presence of the strong base can pose challenges during analysis. For example, dissolved carbon dioxide in the emissions is directly converted to carbonate anion in the base and this can interfere with the analysis. We evaluated various IC columns and devices to minimize sample manipulation while achieving the best accuracy in such a challenging matrix. One example of this effort is shown in Figure 1, where we show the analysis of a stationary sample by a direct injection method and the interference caused by the presence of the carbonate peak. The integration of the sulfate peak in a sloping baseline became challenging. A carbonate removal device was used to remove the carbonate peak, which is present as carbonic acid. In Figure 2 we show the analysis of a similar sample using the same column chemistry but under capillary conditions. In this case we show the results obtained using a capillary carbonate removal device, and the sulfate peak can be easily quantified by this approach.

APPLICATION OF CAPILLARY ION CHROMATOGRAPHY TO

ENVIRONMENTAL SAMPLES

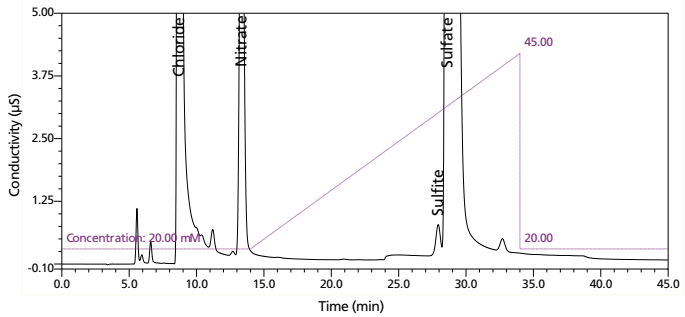

Figure 3: Sulfite determination in a wastewater sample. Column: 250 mm X 0.4 mm Dionex IonPac AS25 with a 50 mm X 0.4 mm AG25 guard column; eluent: potassium hydroxide; gradient: 20 mM (0–14 min), 20–45 mM (14–34 min), 45–20 mM (34–40 min); flow rate: 0.01 mL/min, suppressor: Dionex ACES 300; current: 10 mA; sample loop volume: 0.4 µL (internal loop); temperature: 30 °C.

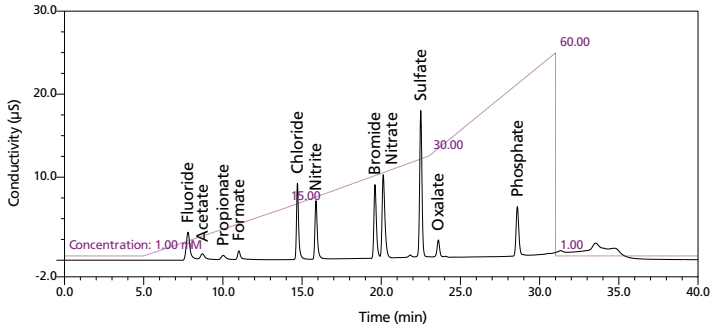

Figure 4: Analysis of a standard mixture comprising organic and inorganic anions. Column: 250 mm X 0.4 mm Dionex IonPac AS1-HC with a 50 mm X 0.4 mm AG11-HC guard column; eluent: potassium hydroxide; gradient: 1 mM (0–5 min), 1–15 mM (5–14 min), 15–30 mM (14–23 min), 30–60 mM (23–31 min), 60–1 mM (31–40 min); flow rate: 0.01 mL/min; suppressor: Dionex ACES 300; current: 20 mA; sample loop volume: 0.4 µL (internal loop); temperature: 30 °C.

Another challenging application is the determination of sulfite in wastewater samples. The key challenge here is the need to quantitate low levels of sulfite in the presence of high levels of sulfate ion. We used a capillary column that was designed for sulfur speciation applications. In Figure 3 we show the analysis of 0.1 ppm sulfite in the presence of 100 ppm of sulfate using a capillary format of operation. It was possible to obtain good quantitation of low levels of sulfite in this wastewater matrix.

We also investigated the use of columns packed with 4-µm particles. Given the smaller particle size, we anticipated improved efficiency and resolution using this column. We investigated the use of this capillary column to pursue analysis of a standard comprising inorganic anions and organic acids. Excellent chromatographic efficiencies and resolution were observed under our gradient conditions, as shown in Figure 4. The same conditions were used to analyze a

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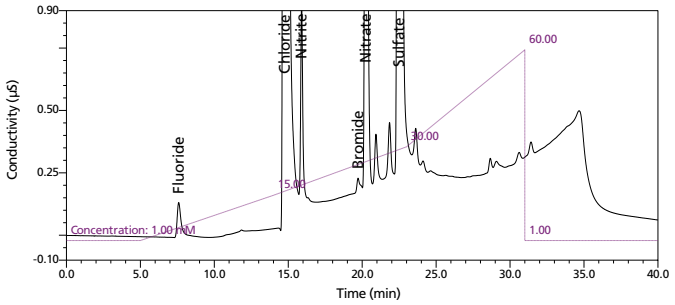

Figure 5: Analysis of a surface water sample. Column: 250 mm X 0.4 mm Dionex IonPac AS11-HC with a 50 mm X 0.4 mm AG11-HC guard column; eluent: potassium hydroxide; gradient: 1 mM (0–5 min), 1–15 mM (5–14 min), 15–30 mM (14–23 min), 30–60 mM (23–31 min), 60–1 mM (31–40 min); flow rate: 0.01 mL/min; suppressor Dionex ACES 300; current: 20 mA; sample loop volume: 0.4 µL (internal loop); temperature: 30 °C.

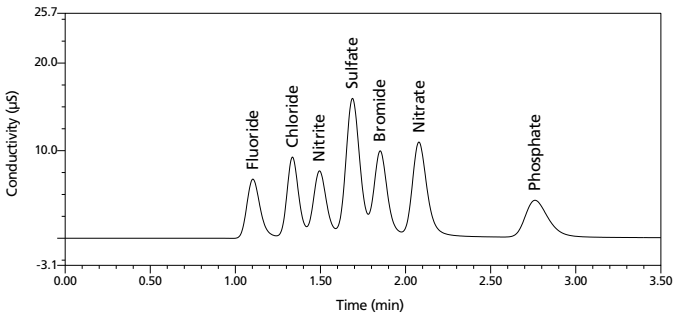

Figure 6: Ultrafast analysis of a seven-anion standard sample. Column: 150 mm X 0.4 mm Dionex Ion Pac AS18-4µm with a 35 mm X 0.4 mm AG18-4µm guard column; eluent: 33 mM potassium hydroxide (isocratic); flow rate: 30µL/min; suppressor: Dionex ACES 300; current: 15 mA; sample loop volume: 0.4 µL (internal loop); temperature: 30 °C.

surface water sample, as shown in Figure 5. Note that low levels of bromide ion were easily analyzed using this approach. These columns were also evaluated for achieving fast analysis. Figure 6 shows separation of a standard mixture of seven anions using a capillary column operated at a flow rate of 30 µL/min. All seven anions were resolved in <3 min using this method.

The same method was also used to analyze a polymer sample after combustion with a Mahler bomb (3). The key objective of this work was to determine the chlorine content of the polymer, and this proved feasible using this capillary

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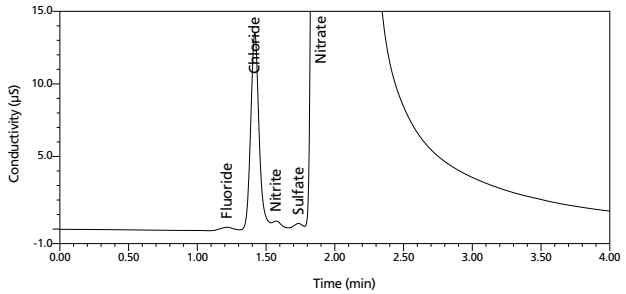

Figure 7: Analysis of a polymer sample after combustion. Analysis conditions are similar to those used in Figure 6.

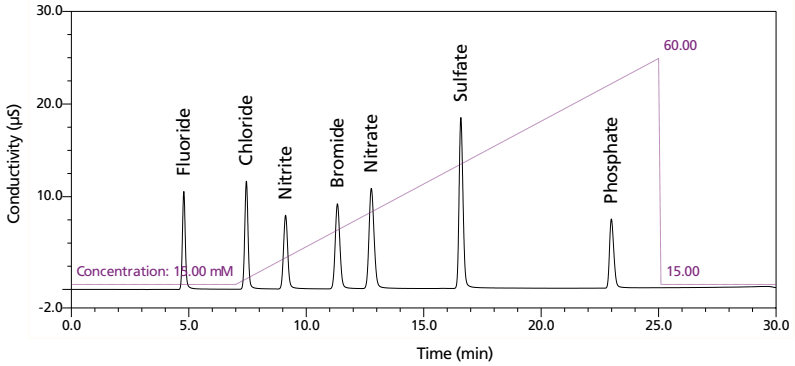

Figure 8: Analysis of a seven-anion standard sample. Column: 250 mm X 0.4 mm Dionex IonPac AS19-4µm with a 50 mm X 0.4 mm AG19-4µm guard column; eluent: potassium hydroxide; gradient: 15 mM (0–7 min), 15–60 mM (7–25 min), 60–15 mM (25–30 min); flow rate: 0.01 mL/min; suppressor: Dionex ACES 300; current: 11mA; sample loop volume: 0.4 µL (internal loop); temperature: 30 °C.

column. We were also able to obtain good resolution of inorganic anions such as nitrite (due to incomplete oxidation) and sulfate, as shown in Figure 7.

We also evaluated an anion-exchange column packed with 4-µm particles that showed improved efficiencies over a comparable column sharing the same chemistry packed with 7.5-µm particles. Under isocratic conditions, chloride peak efficiency was 15,585 plates (calculated following the *European Pharmacopeia [EP]* method), compared to 9835 plates (EP) for the standard column, as shown in Figure 8. Applications run on the columns packed with 7.5-µm d_p columns were transferred directly to the 4-µm version of the anion-exchange column.

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Analyte	Obtained Value (mg/L)	Expected Value (mg/L)	Number of Laboratories	Median (mg/L)	Z-Score
Chloride	5.93	2–40	18	5.95	-0.10
Nitrate	13.4	1–20	20	13.4	0.00
Nitrite	0.37	0.5–10	17	0.37	0.00
Fluoride	1.54	2–40	17	1.5	0.5
Sulfate	1	2–40	14	0.9	0.2

Table I: Results from proficieny testing of a groundwater sample

Analyte	Obtained Value (mg/L)	Expected Value (mg/L)	Number of Laboratories	Median	Z-Score
Chloride	409	100-1000	35	413	-0.10

Table II: Results from proficiency testing of a wastewater sample

Compliance with Regulatory Standards and Accreditation Requirements

In our evaluation we observed that our approach met the performance requirements of the US EPA Methods 300.0 (4) and 300.1 (5).

ACCREDIA is the Italian national accreditation body appointed by the Italian state for laboratory accreditation in compliance with the international standards of the series ISO 17000 (17025) and the harmonized series of EN 45000 European norms. Our laboratory is an accredited laboratory. In all the analyses where capillary ion chromatography was used, we evaluated our Quality Assurance Project Plan to ensure it was complaint with UNI EN ISO 17025 (6) criteria using proficiency tests, reference materials, and control charts (7,8).

An example of this testing is shown in Table I, which presents the results from the proficiency testing of a ground water sample. The observed values for the analysis for all the peaks of interest showed good agreement with the median values obtained from a number of laboratories. The Z score, which is measure of deviation of the results from the assigned value, provides feedback on the quality of the results. The values obtained showed excellent performance of the capillary method for this analysis. Table II shows the results from proficiency testing of a wastewater sample for chloride. Again, we obtained excellent performance, as demonstrated by the small deviation of the obtained value from the median and the Z score.

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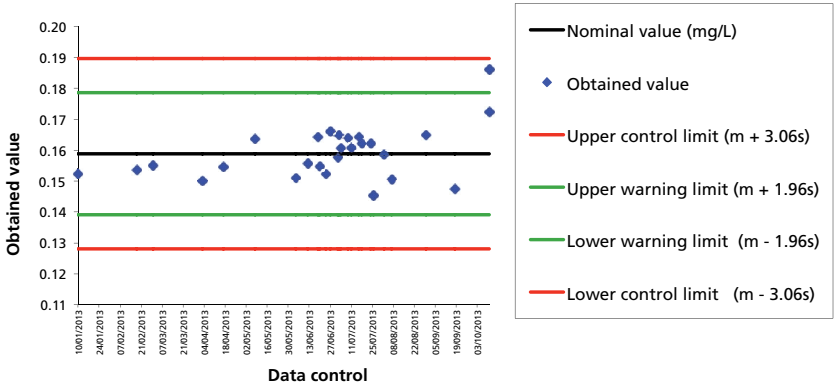

Figure 9: Control chart showing the observed value for fluoride in a surface water sample tracked over a period of several months.

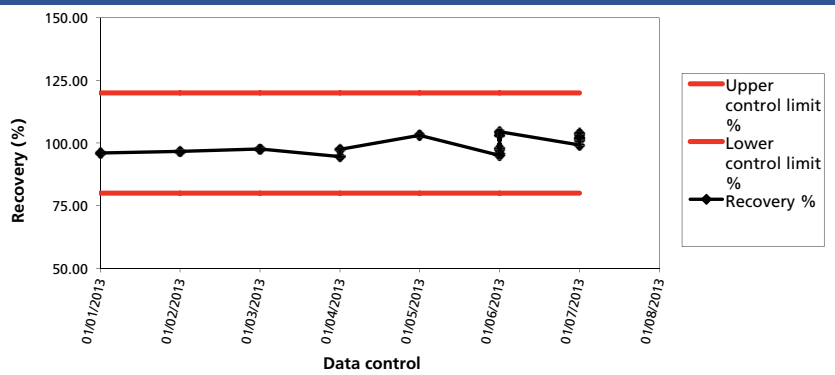

Figure 10: Control chart showing fluoride recovery from a surface water sample tracked over a period of several months.

To evaluate method robustness and accuracy, a control chart was used. Figure 9 shows the obtained values for fluoride using a certified reference material (Environment Canada Mississippi-03) within the upper and lower control values. Clearly the values obtained were within ±3σ criteria, indicating that the method was robust and provided consistent analysis. The analyte recovery for fluoride was also tracked, as shown in Figure 10. The values met the EPA 300.1 method requirements of ±20%. Overall, these results indicate that the capillary ion chromatography approach met all the quality requirements in our laboratory and provided excellent performance.

Conclusions

The utility of capillary ion chromatography for analyzing a variety of samples of environmental origin has been demonstrated here. The performance of the approach was either comparable to or in some cases better than that of classical standard bore or microbore ion chromatography methods in terms of sensitivity and reliability. We also demonstrated the utility of columns packed with 4-µm particles for fast analysis without compromising the separation and the quantitation.

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Capillary Ion Chromatography—Bringing a new level of speed and resolution

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Improve Sensitivity and Selectivity

The combination of a first-dimension standard bore column (i.d. 4 mm) with a second-dimension capillary column (i.d. 0.4 mm) permits lower detection limits in the low ng/L range. Combining columns with disparate stationary phase properties is especially important for high-ionic-strength samples. This precludes the need for equilibration and dramatically reduces waiting time, including sample preparation.

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The Thermo Scientific™ Dionex™ ICS-5000⁺ and ICS-4000 HPIC™ Systems are the world's first capillary IC systems on the market. The Dionex ICS-5000+ operates at standard bore, microbore and capillary flow rates at pressures up to 5000 psi enabling it to also perform high-resolution and fast IC analysis with the latest 4 µm particle-size columns. The Dionex ICS-4000 capillary HPIC system is solely dedicated to capillary IC and provides all the benefits of high-pressure capabilities for high chromatographic resolution. The Dionex ICS-5000⁺ and ICS-4000 systems are designed to take advantage of small particle IC columns for faster and improved separations.

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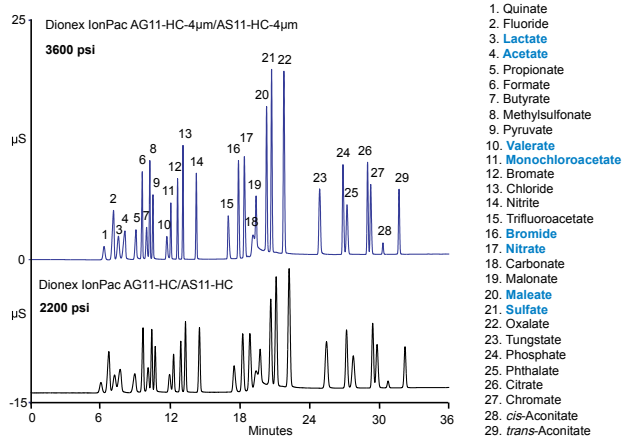

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EXPANDING THE APPLICATIONS OF ION CHROMATOGRAPHY

Ion chromatography is a mature technique for many applications, such as the analysis of drinking water. Some researchers, however, believe it can do more. Pavel Nesterenko of the Australian Centre for Research on Separation Science (ACROSS) at the University of Tasmania, in Australia, has been on the forefront of this effort. He recently talked to *LCGC* about this work.

You have done a lot of work with ion chromatography (IC), and in particular applying IC methods to novel areas. Are there common threads among the applications you have worked on, in terms of why you chose them or why IC is a suitable method for them? If so, what are those common threads?

Nesterenko: Incidentally, this month we are celebrating the 40th anniversary of the establishment of ion chromatography — the first paper by Small, Stevens, and Bauman, entitled "Novel Ion-Exchange Chromatographic Method Using Conductivity Detection," was submitted for publication in *Analytical Chemistry* on December 5, 1974 (1). Today we can say that IC is a mature analytical technique, and the classic types of IC applications, such as determination of inorganic anions in drinking water, have become standard analytical protocols in EPA and ASTM methods.

One clear trend in the development of new applications for IC can be described as a move to the area of biochemical and pharmaceutical analysis, with an enormous diversity of new analytes. The second trend is related to the determination of more common inorganic ions, but in very complex samples such as brines, seawater, organic solvents, and nonaqueous battery electrolytes. In the latter case we have to use either sophisticated sample pretreatment techniques, or new advanced IC technologies and nonstandard approaches. The latter include approaches such as chelation IC, capillary IC, two-dimensional chromatography, hyphenation with more selective detection systems, and use of postcolumn reactions. Obviously, there are no serious alternatives to IC for the separation of inorganic ions, especially anions and different ionic species, except for capillary zone electrophoresis (CZE). However, CZE is usually efficient only for direct (without sample pretreatment) analysis of relatively simple samples.

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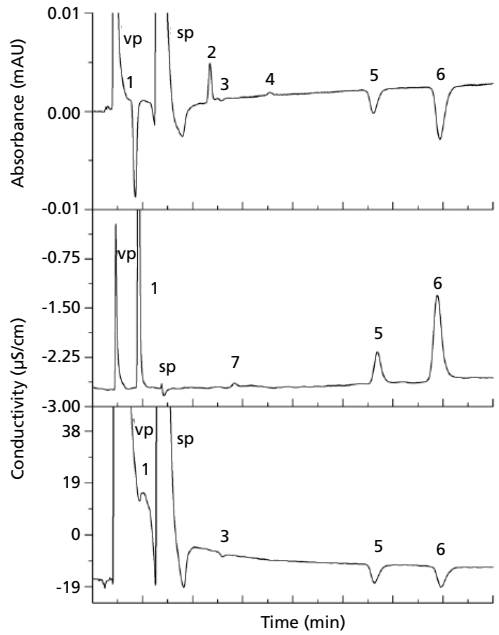

Figure 1: Comparison of the three detection methods: (a) UV detection at 215 nm, (b) suppressed conductivity, and (c) non-suppressed conductivity. Column: IonPac AS14A; flow rate: 1 mL/min. Sample: (a) 10 µL KPF6 (10 mmol/L); (b) and (c) 50 µL KPF6 (10 mmol/L). Peaks: 1 = F–; 2), 3 = difluorophosphate; 4, 7 + unidentified; 5 = monohydrogenphosphate; 6 = monofluorophosphate. Adapted with permission from reference 4.

Have the challenges you faced in developing the methods for these various applications been similar?

Nesterenko: The answer is more "no" than "yes." The challenge of analytical method development depends on sample properties including sample volume, the concentration of target analytes, and the complexity of the matrix. Obviously, the use of capillary IC is more suitable if you have a limited volume of extremely precious sample such as 1.5-million-year-old Antarctic ice collected at the depth of 2–3 km. Columns with selective functional groups should be used in chelation IC for the determination of traces of alkaline earth metal in 4 M sodium chloride brines (2). The simultaneous determination of anions and cations can be accomplished with zwitterionic ion exchangers, anion- and cation-exchange columns connected in series, or mixed-bed columns. More often, we encounter analytical tasks where the goal can be achieved only with a mixed-mode retention mechanism, so the hydrophobicity of ion-exchangers plays a more important role in the analysis of hydrophobic organic ions. An example of such a task is the separation of pharmaceutical ingredients and byproducts (3). Frequently, it is impossible to use suppressors in combination with conductivity detectors, so direct conductivity or other detection options must be considered. For example, for analysis of hexafluorophosphate salts, photometric detection at 215 nm provided more information on products of hydrolysis than suppressed and nonsupressed conductivity (Figure 1). At the same time, decomposition of hexafluorophosphate-containing ionic liquids is a serious problem for the development of energy storage devices, so all possible impurities and decomposition products must be detected at low concentrations. This is just a short list of recent analytical tasks that have required nonstandard applications of IC methods.

In a recent study, you developed an IC method for determining fluoride ions in urinary stones. What advantages does this method present over alternative approaches?

Nesterenko: The possible effect of high intake of fluoride from treated drinking water or of fluorinated toothpaste ingestion on the formation of kidney stones is under intensive dispute in society and in social networks. The formation of kidney stones is a very complex process requiring consideration of multiple factors influencing their growth. The main limitation of existing techniques for kidney stone analysis is an individual determination of stone-forming anions like oxalate, phosphate, urate, as well as fluoride. Fluoride is usually determined in stones using UV–vis spectrophotometry, potentiometric titration, or ion-selective electrodes. The obvious advantage of the IC method is that it makes it possible to do simultaneous determination of several anions from a small volume of the sample, so quantitative analysis of very small stones can be performed, thus

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helping clinicians to choose the right treatment strategy to prevent formation of renal stones.

You have also developed a chelation IC method for the direct determination of transition metals — such as Mn(II), Cd(II), and Zn(II) — in shellfish, to study the bioaccumulation of metal contaminants. Why is IC a better approach to this problem than atomic spectroscopy methods like inductively coupled plasma atomic emission spectrometry (ICP-AES) or ICP-mass spectrometry (ICP-MS)? How did the results obtained with the chelation IC method compare with those obtained using ICP-MS?

Nesterenko: No doubt, ICP-AES and ICP-MS are very powerful analytical tools for the determination of metals in shellfish at very low concentration levels. They exhibit limits of detection (LODs) that are 100–1000 times lower than those of chelation IC with photometric detection. However, the high cost of intense argon plasma for ICP ionization during measurement is still a limiting factor for many researchers monitoring the environment. Also, ICP-AES and ICP-MS instruments are not portable and cannot be used on board a ship or in field expeditions. Finally, the concentration of metals detected in mussel tissue digest in the above-mentioned work (5) was quite high (mussels were collected in a contaminated area next to a zinc smelter plant) and the determined concentrations of Cd(II), Mn(II), and Zn(II) varied around 5, 30, and 400 ppm, respectively. Clearly, there was no need to use powerful spectroscopic methods in this case and we simply tried to avoid "firing a canon" (ICP-MS) to "kill a sparrow" (a sample with high metals content). It should be noted that the results obtained with chelation IC matched the data of ICP-MS analysis of the same samples very well. So, the simplicity, compactness, and cost-effectiveness are the key advantages of chelation IC for this application.

What were the biggest challenges in developing an IC method for determining metals in shellfish? How did you overcome those challenges?

Nesterenko: The samples of digested shellfish have elevated salt concentrations or ionic strength, so the determination of trace metals is similar to the analysis of seawater or brines. Traditional IC columns cannot be used for this task because of a lack of separation selectivity based on electrostatic interactions, high sensitivity to the salt matrix, and subsequent overloading of chromatographic columns. Here, chelation IC has a strong advantage because the retention of transition metal ions is proportional to the stability of the complexes formed between the metals and the functional groups immobilized at the surface of the stationary phase. Because stability constants of complexes are independent of ionic strength, the retention of transition metals is insensitive to the high concentration of salt in the eluent, which, in turn, allows direct injection of brines, seawater, and shellfish samples into chromatographic column.

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You have used ion-exchange chromatography with pulsed amperometric detection (IEC-PAD) to study sugars in dissolved organic matter, to investigate how microbes contribute to the composition of that organic matter. What in particular can IEC-PAD methods contribute to this area of study?

Nesterenko: Dissolved organic matter (DOM) of marine or freshwater origin is the largest carbon reservoir with the mass of 750 gigatons, containing, probably, hundreds of thousands of various organic substances from major classes of compounds, such as amino acids, peptides, proteins, lipids, sterols, DNA, RNA, materials derived from linear terpenoids carboxylic-rich alicyclic molecules, sugars, and amino sugars. Fractionation, characterization of fractions and, ideally, isolation and identification of new substances from DOM is a real challenge for separation scientists. At the Australian Centre for Research on Separation Science (ACROSS), in collaboration with the Central Science Laboratory of the University of Tasmania, we have a strong team of experts working on a Discovery Project funded by the Australian Research Council. To get maximum information on DOM fractions or on separate chemical classes within DOM, we need to explore the possibilities of diverse separation techniques including normal-phase and reversed-phase modes of high-performance countercurrent chromatography, size-exclusion chromatography (SEC), reversed-phase HPLC, hydrophilic interaction liquid chromatography (HILIC), gas chromatography (GC), IC, argentation chromatography, and 2D combinations of methods with orthogonal selectivity. Of course, to maximize the amount of collected information, each separation system is equipped with various detectors such as photometric, refractive index, evaporative light scattering, or mass spectrometry detectors. IEC-PAD is just one of many approaches, and this was the first time IEC-PAD was applied to the analysis of DOM (6).

What were you able to achieve with your method?

Nesterenko: The anion-exchange column used in this work (CarboPac PA1, Thermo Scientific) is specially designed and calibrated for the separation of monosaccharides, disaccharides, and some oligosaccharides using potassium hydroxide as the eluent. Under these conditions, carbohydrates can be detected at low concentration levels by PAD. As expected, the presence of some carbohydrates was recorded for samples collected at 10-m and 60-m depths in the Irish Sea, while the sample collected at the top of the water column contained higher concentrations of carbohydrates. More interestingly, we obtained three characteristic chromatographic peaks for the freshwater, coastal, and offshore seawater samples. This confirms the hypothesis that some DOM has a microbial origin.

What were the challenges in developing that method?

Nesterenko: It is always a challenge to analyze a very complex mixture of

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unknown compounds and inject it onto a chromatographic column. The exact composition of DOM, as well as adsorption or retention properties for most of the substances contained in the DOM, is unknown, so there is a high probability that some of the components of the injected DOM, such as carboxylic-rich alicyclic molecules, can irreversibly modify the surface of the stationary phase, particularly for an ion-exchange column. Luckily, the chromatographic column used in these experiments is still in a good operation condition after an intensive wash.

Another application you took on was the determination of iron species using chelation IC. In what context is the determination of Fe(II) and Fe(III) species important?

Nesterenko: The chemical properties of these two redox forms of iron are very different, but in the nature both forms plays an essential role as micronutrients.

Why is this type of analysis so difficult?

Nesterenko: Formally, there is no problem to separate Fe(II) and Fe(III) in IC using differences in charged species. However, it is extremely difficult to keep the original concentration ratio for these two labile redox forms unchanged during separation, because the forms are very sensitive to the various factors, such as the material of the chromatographic column, possible carryover effects, the composition of the eluent, and the air — which can oxidate Fe(II). Also, as soon as you try to keep one oxidation form of iron intact, the other form may be influenced; as a result, separation conditions must be carefully tuned.

How did you overcome the challenges of this analysis?

Nesterenko: The stabilization of redox forms of iron can be achieved by the addition of proper complexing reagents, usually dipicolinic acid, to the eluent. We proposed the use of chelidamic acid for this purpose and got superior stabilization of redox forms during separation.

You applied the chelation IC technique to the direct analysis of iron species in fuel ethanol. What results did you achieve?

Nesterenko: In general, the advantage of chelation IC over traditional IC for the determination of iron and other metals in organic solvents is the insensitivity of chelation or coordination interactions to the polarity of the solvent as compared to simple ion-exchange or electrostatic interactions. This means that in chelation IC we can inject fuel ethanol directly onto the chromatographic column. In this work (7) we found only traces of copper in the provided bioethanol samples, but potentially we can determine both redox forms of iron.

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You have also developed methods for the determination of strontium in seawater. First, why do we need to study strontium in seawater?

Nesterenko: There is a growing interest to the determination of elements having radiotoxic isotopes such as ^{90}Sr or ^{137}Cs and ^{134}Cs in seawater. This is related to the aftermath of the Fukushima Daiichi nuclear accident on March 11, 2011, which is the largest nuclear disaster since Chernobyl disaster in 1986. As you may recall, an earthquake followed by a tsunami caused damage to a nuclear station, resulting in a massive uncontrolled release of radioactive water to the ocean. Radioactive isotopes slowly spread through the ocean, and their presence was recorded for the first time in water off the shore of Northern California in November 2014. The detected concentrations are extremely low (about 1000 times lower than the drinking water limit set by the U.S. EPA) but it is still very important to keep monitoring the relevant elements in the environment. By the way, according to the data announced by the Tokyo Electric Power Company (TEPCO) at a press conference in August 2014, approximately 5 billion Bq of ^{90}Sr has flowed into Pacific on a daily basis during this year.

Another strong demand for the determination of strontium in seawater and in seawater microorganisms is connected with growing emissions of carbon dioxide and global warming. The consequences of these emissions include ocean acidification, which affects coral skeleton growth and calcification of seawater microorganisms. To understand these processes, the accurate determination of concentration changes of calcium and strontium in seawater is required. Of course, everyone is now concerned about the future of the Great Barrier Reef in Australia and of beautiful coral reefs in the Caribbean.

Last, but not least, it is extremely difficult to determine strontium in seawater (8). Due to serious atomic and molecular isobaric interference from argon used as plasma gas and calcium present at elevated concentrations in seawater samples, the application of ICP-MS to the determination of strontium is not suitable. Actually, the official protocol "ASTM D3352-08a standard test method for determination of strontium ion in brackish water, seawater, and brines" recommends the application of atomic absorption spectroscopy (AAS) following sample pretreatment. This method covers the concentration range of 5–2100 mg/L strontium. So, the limit of quantitation (LOQ) in this method is just below the 8.1-mg/L typical concentration of this metal ion in seawater.

In this work, how did you handle the challenge of measuring metal ions in such a high salt matrix?

Nesterenko: It was the determination of strontium in seawater that clearly demonstrated in full the advantages of high-performance chelation ion

EXPANDING THE APPLICATIONS OF ION CHROMATOGRAPHY

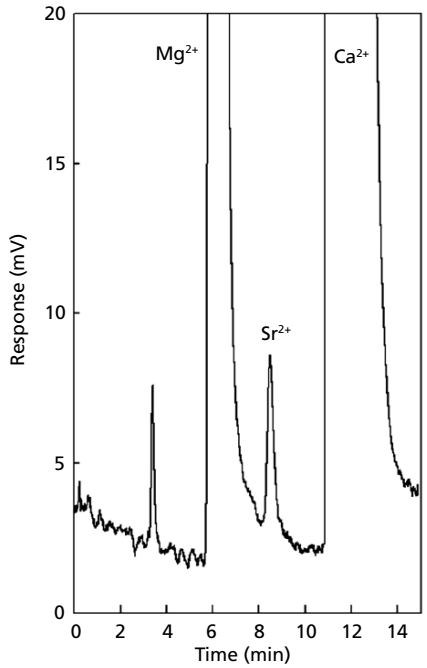

Figure 2: Chromatogram of a seawater sample (salinity standard IAPSO, batch P149). Column: two 100 mm X 4.6 mm silica monolithic columns with HEIDA functional groups are connected in series; eluent: 2 mmol/L glycolic acid, 0.25 mol/L sodium chloride, pH 5.11; injection volume: 4 µL; flow rate: 1 mL/min; detection: photometric at 570 nm after postcolumn reaction with o-cresolphtalein complexone. Adapted with permission from reference 8.

chromatography. As mentioned above, chelation as a retention mechanism in chelation IC is insensitive to the presence of sodium and potassium salts in samples, so we used direct (no sample dilution!) injections of 4 µL of filtered seawater onto a monolithic silica column with covalently bound functional groups of N-(2-hydroxyethyl)-iminodiacetic acid (HEIDA). As shown in Figure 2, the chromatographic peak of strontium is nicely separated from the peaks of magnesium and calcium, which are present at much higher concentrations (the ratio of Mg^{2+}/Sr^{2+} is ~140 and Ca^{2+}/Sr^{2+} is ~ 65). Importantly, the LOD and LOQ of the developed method compare favorably to those of a standard official AAS protocol. With some modifications this method was applied for monitoring Mg^{2+}/Ca^{2+} ratio in seawater (9) and for the determination of organically bound and inorganic magnesium in calcareous skeletons of marine planktonic organisms (10).

What can be done with IC methods for this work that cannot be achieved with other approaches, such as ICP-MS?

Nesterenko: Good question. Technically, ICP-MS has limited possibilities for analysis of brines, seawater, and some organic solvents. Isobaric interference is another limitation for the determination of some elements by ICP-MS, as discussed above for strontium. Definitely, there are limited possibilities to carry out speciation analysis without separation of species. Not to mention the impossibility of using ICP-MS with very small samples where target analytes are present in low concentrations. However, ICP-MS and ICP-AES are powerful detection techniques and in combination with IC they can provide unique information about the chemical composition of the samples.

What do you plan to do next in your work in IC?

Nesterenko: My research interests have always been connected with the development of new stationary phases and selective adsorbents for various IC applications. Recently, we reported on a preliminary investigation on the adsorption properties of glyphosate-bonded silica (11), which can be used in chelation IC. New adsorbents, based on a core–shell format of silica, also appear to be an interesting area to explore in terms of novel applications and solutions to existing challenges. For example, fast isocratic separation of 13 rare earth elements was achieved in 12 min using only a 5-cm-long column (Figure 3). One more direction is the development of new generation of ion-exchange materials based on synthetic diamonds. Diamonds have a unique combination of excellent characteristics to be used at high pressures and temperatures and in chemically harsh separation conditions, so I believe that the application of a diamond-based absorbent will dramatically expand the possibilities of various LC techniques, as recently discussed (12).

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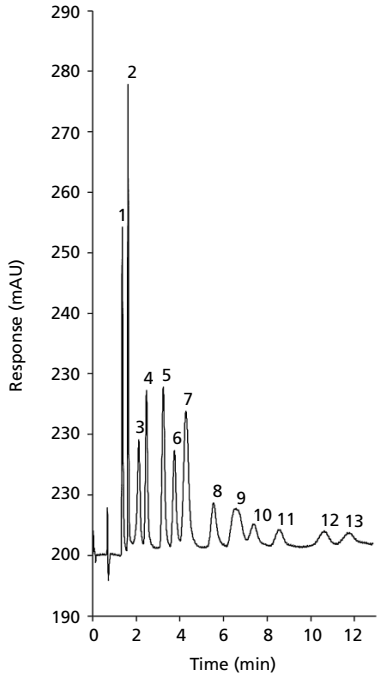

Figure 3: Separation of rare earth elements on a 50 mm X 2.1 mm, 1.7-µm dp HEIDA-silica core–shell column.Eluent: 12 mM HNO3, 0.75 M KNO3; flow rate: 0.9 mL/min; column temperature: 70 °C; injection volume: 10 µL; detection: absorbance at 650 nm after postcolumn reaction with 1.5 × 10–4M arsenazo III. Peaks: 1 = La(III), 2 = Ce(III), 3 = Pr(III), 4 = Nd(III), 5 = Y(III), 6 = Sm(III), 7 = Gd(III)/Eu(III), 8 = Tb(III), 9 = Dy(III)/Ho(III), 10 = Er(III), 11 = Tm(III), 12 = Yb(III), 13 = Lu(III). Adapted with permission from reference 13.

Regarding future applications of IC, there is a very strong demand among marine scientists for the determination of various ions at trace levels in seawater with a particular interest in speciation. The perfect definition for this target can be found in the textbook *Principles and Applications of Aquatic Chemistry* by Morel and Hering (13). They wrote, "The elucidation of the chemical speciation of trace elements in natural waters is probably the greatest remaining challenge to analytical chemists; the objective is to demonstrate and quantify the existence of fractions of chemical constituents as picomolar concentrations of perhaps ephemeral species." Clearly, the majority of these species are charged, so IC must be considered as the first option for their determination. This objective was formulated more than twenty years ago, but it remains very difficult analytical task even for comprehensive IC.

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