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APPLICATION NOTE 71410

Ion chromatography: A versatile technique for the analysis of beer

Key words
beverage, IonPac AS11, CarboPac PA1, HPAE-PAD, CarboPac PA100, IonPac ICE-AS6, IonPac CS12, maltodextrins

Introduction

Ion chromatography is an efficient technique for the analysis and quantification of ions in solution. Although there are several techniques that have been used for the analysis of beer — including gas chromatography, HPLC, enzyme-based methods, and wet chemical methods — ion chromatography is rapidly becoming the method of choice.

The compounds of interest for the beer industry range — from inorganic ions, organic acids, and hop bittering principles that contribute to the overall taste and bitterness of the beverage — to proteins, carbohydrates, and alcohols that are monitored to determine the extent of fermentation. The finished beer product may be analyzed to determine the concentration of added preservatives and colorants, in addition to ensuring manufacturing authenticity.

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The first step in the beer making process involves soaking barley, and sometimes other grains, in warm water. Enzymes present in the barley break down starch from the grains, producing mostly glucose, maltose, and other oligo- and polysaccharides. This process is called mashing, and the resulting solution is called sweet wort. The sweet wort is then treated with hops, thereby producing hopped wort. Yeast is added and the smaller saccharides are fermented to produce alcohol. Because of the different concentrations, chemical behavior, and molecular mass ranges of the various components in beer, their isolation and determination can be difficult. Ion chromatography, using polymer-based resins, provides a means to monitor many of these important compounds during the brewing process and in the final product.

This application note describes the use of ion-exchange or ion-exclusion chromatography for the determination of five classes of compounds of interest to the brewing industry, including: carbohydrates, alcohols, organic acids, inorganic anions, and inorganic cations. One of two forms of electrochemical detection is used, pulsed amperometry or conductivity detection.

Equipment

A Dionex chromatographic system* consisting of:

- Gradient Pump
- Chromatography Enclosure Electrochemical Detector with pulsed amperometry and conductivity modes
- Eluent Organizer
- PeakNet Chromatography Workstation**

Reagents and standards

- Deionized water, 18.2 MΩ-cm or better

Carbohydrate analysis

- Sodium acetate (P/N 059326)
- Sodium hydroxide solution, 50% (w/w) (Fisher Scientific P/N SS254-500)

Anion analysis

- Sodium hydroxide solution, 50% (w/w)
- Methanol

Alcohol analysis

- Perchloric acid

Cation analysis

- Methanesulfonic acid

Organic acid analysis

- 0.1 M Tetrabutylammonium hydroxide (TBAOH)
- Heptafluorobutyric acid

Preparation of solutions and reagents

1.00 M Sodium acetate

Weigh out 82.0 g of anhydrous sodium acetate and place into a 1 L volumetric flask. Add approximately 600 mL of deionized water (DI) and swirl to dissolve. When the salt has dissolved completely, fill up to the mark with (DI) water. Filter the resulting solution through a 0.2 µm filter.

500 mM Sodium hydroxide

Weigh 974 g (974 mL) of DI water into an eluent reservoir bottle. Degas the water for approximately 10 min. Tare the bottle on the balance and add 40.0 g (26.2 mL) of 50% (w/w) sodium hydroxide directly to the bottle. Quickly transfer the eluent bottle to the instrument and pressurize it with helium or nitrogen.

100 mM Sodium hydroxide

Weigh 992 g (992 mL) of DI water into an eluent reservoir bottle. Degas the water for approximately 10 min. Tare the bottle on the balance and add 8.00 g (5.25 mL) of 50% (w/w) sodium hydroxide directly to the bottle. Quickly transfer the eluent bottle to the instrument and pressurize it with helium or nitrogen.

1.00 mM Sodium hydroxide

Place 990 g (990 mL) of DI water into an eluent reservoir bottle. Degas the water for approximately 10 min. Pipette 10.0 mL of the 100 mM sodium hydroxide solution directly into the bottle. Quickly transfer the eluent bottle to the instrument and pressurize it with helium or nitrogen.

100 mM Methanesulfonic acid

Weigh out 9.61 g of methanesulfonic acid (MSA). Carefully add this amount to a 1 L volumetric flask containing about 500 mL of DI water. Dilute to the mark and mix thoroughly

* Equivalent or improved results can be achieved using the Thermo Scientific™ Dionex™ ICS-6000 system

** Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) Software, version 7.2, can be used

100 mM Perchloric acid
Dilute 8.60 mL of perchloric acid into 992 mL of DI water.

0.8 mM Heptafluorobutyric acid
Heptafluorobutyric acid (perfluorobutyric acid) in 10.0 mL bottles. Dilute the entire contents of one 10.0 mL bottle in 1.00 L to obtain a 77.2 mM stock solution. Dilute 10.4 g of the stock solution in 1.00 L to obtain the 0.800 mM working eluent.

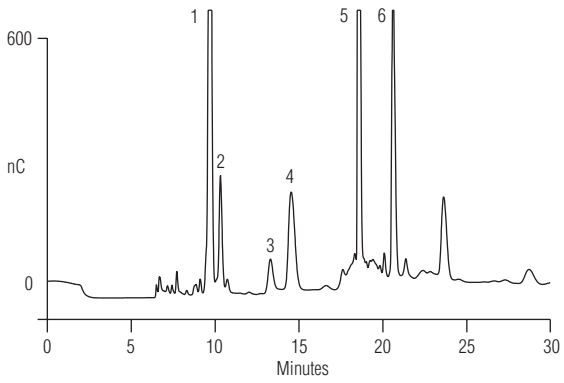
5 mM Tetrabutylammonium hydroxide
Dilute 50 mL of the Dionex 0.1 M TBAOH ion-pairing reagent to 1 L with DI water. Prepare several liters of the regenerant.

Results and discussion
Carbohydrate analysis
Carbohydrates and other species containing hydroxyl functional groups can be detected by measuring the current caused by their oxidation at a gold electrode. A repeating sequence of three applied potentials is used first to oxidize the carbohydrates, and then to clean the electrode of the products of the oxidation reaction by applying a large positive and then a negative potential. This sequence is repeated once every second to eliminate electrode fouling and thereby to ensure a reproducible response. Unless this sequence is performed, the peak heights will steadily decrease as the electrode surface becomes fouled. For the chromatograms shown in Figures 1, 2, and 3, the electrochemical detector was programmed with the waveform shown in Table 1. Integration was performed from 0.2 to 0.4 seconds. Other waveforms that have been developed and tested are described in *Thermo Scientific Technical Note (TN) 21: Optimal Settings for Pulsed Amperometric Detection of Carbohydrates Using the Dionex ED40 Electrochemical Detector* and may provide superior results in some cases.

Table 1. ED40 waveform for the analysis of carbohydrates by ion chromatography using a gold electrode.

Time (s)	Potential (V)
0.00	0.05
0.40	0.05
0.41	0.75
0.60	0.75
0.61	-0.15
1.00	-0.15

Because carbohydrates have pK_as between 12 and 14, they can be separated as anions by ion-exchange chromatography. To drive the oxidation reaction at the working electrode, the eluent must be above pH 12, thus the resins in the Thermo Scientific™ Dionex™ CarboPac™ columns are polymer-based for stability and durability in the pH range 0–14. Using a hydroxide gradient, the sugars are separated on the Dionex CarboPac PA1 column in the following order:



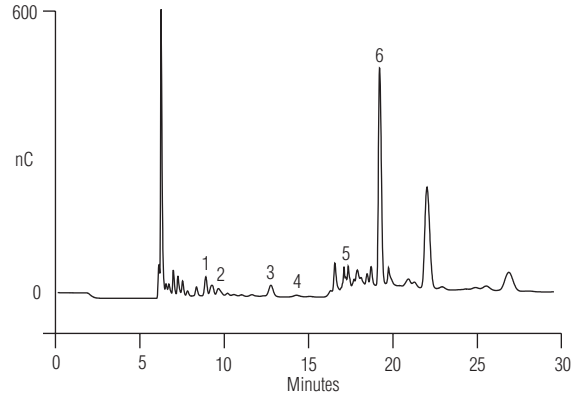
Column: Dionex CarboPac PA1
Eluent 1: Deionized water
Eluent 2: 500 mM Sodium hydroxide
Gradient:

Time	E1	E2	Comments
Initial	99	1	Reequilibrate
5.00	99	1	Inject
6.00	99	1	Back to Load
20.00	91	9	
45.00	0	100	
50.00	0	100	

Flow Rate: 1.0 mL/min
Inj. Volume: 10 µL
Detection: Pulsed amperometry, gold electrode (see Table 1 for waveform)

Peaks:
1. Glucose
2. Fructose
3. Isomaltose
4. Sucrose
5. Maltose
6. Maltotriose

Figure 1. Separation of fermentable sugars in wort by ion-exchange chromatography with pulsed amperometric detection. The sample was diluted 1:10 before injection.



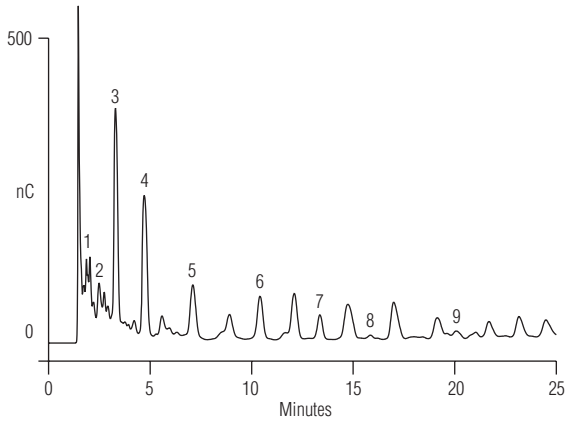
Column: Dionex CarboPac PA1
Eluent 1: Deionized water
Eluent 2: 500 mM Sodium hydroxide
Gradient:

Time	E1	E2	Comments
Initial	99	1	Reequilibrate
5.00	99	1	Inject
6.00	99	1	Back to Load
20.00	91	9	
45.00	0	100	
50.00	0	100	

Flow Rate: 1.0 mL/min
Inj. Volume: 10 µL
Detection: Pulsed amperometry, gold electrode (see Table 1 for waveform)

Peaks:
1. Glucose
2. Fructose
3. Isomaltose
4. Sucrose
5. Maltose
6. Maltotriose

Figure 2. Separation of mono-, di-, and trisaccharides in an American beer by ion-exchange chromatography with pulsed amperometric detection. The sample was diluted 1:10 before injection.



Column: Dionex CarboPac PA-100
Eluent 1: Deionized water
Eluent 2: 500 mM Sodium hydroxide
Eluent 3: 1 M Sodium acetate
Gradient:

Time	E1	E2	E3	Comments
Initial	57	33	10	Reequilibrate
1.50	57	33	10	Inject
6.50	57	33	10	Gradient Start
31.50	42	33	25	Gradient End

Flow Rate: 1.0 mL/min
Inj. Volume: 10 µL
Detection: Pulsed amperometry, gold electrode (see Table 1 for waveform)

Peaks
1. Glucose
2. Maltose
3. Maltotriose
4. Maltotetraose
5. Maltopentaose
6. Maltohexaose
7. Maltoheptaose
8. Maltooctaose
9. Maltodecaose

Figure 3. Separation of malto-oligosaccharides in an American beer by ion-exchange chromatography with pulsed amperometric detection. The sample was diluted 1:10 before injection.

monosaccharides, disaccharides, and trisaccharides. The experimental conditions used for the separations shown in Figures 1 and 2 are presented in each Figure.

The carbohydrates of most importance to the brewing industry are the fermentable sugars. In general, saccharides larger than DP3 are not fermentable; however, they will contribute to the caloric value as well as to the overall flavor of the beer and its ability to form a head. Figure 1 shows the separation of fermentable sugars (≤DP3) in a hopped wort sample. These are the sugars that are converted by yeast to alcohol. If this figure is compared with the separation shown in Figure 2, the difference between the finished beer and a beer during production is evident. As expected, the concentration of fermentable sugars is greater in the wort than in the final beer product.

The complex sugars, starches, and dextrins, which are broken down by enzymes to produce a wort of high fermentability, are built up from glucose sub-units. Maltose is the simplest of the complex sugars and is

formed by two glucose molecules joined together by an a-1,4 linkage. Following convention, chains are named according to the number of glucose units that have been incorporated. Thus, maltotetraose (DP4), for example, is a chain of four glucose units linked together by a-1,4 linkages.

Figure 3 shows the separation of maltose oligosaccharides in beer from DP3 to DP10, using the Dionex CarboPac PA-100 column. Excellent resolution of maltose oligomers up to DP15 is possible, allowing for rapid profiling. As indicated in the experimental conditions listed in Figure 3, the eluent contained sodium acetate in addition to sodium hydroxide. The sodium acetate increases the eluent strength, which reduces the retention time of the oligosaccharides. Separation is possible in the absence of sodium acetate, but the run times are prohibitively long.

Alcohol analysis

Usually, the only two alcohols present in high concentrations in beer are ethanol and glycerol. Glycerol is an important component in beer; it has a considerable effect on flavor and is sweeter than glucose. Ethanol and glycerol can be separated by ion-exclusion chromatography, then detected with pulsed amperometry using the waveform described in Table 2 with integration performed from 0.05 to 0.25 seconds.

Table 2. ED40 waveform for the analysis of alcohols by ion-exclusion chromatography using a platinum electrode.

Time (s)	Potential (V)
0.00	0.30
0.05	0.30
0.25	0.30
0.26	1.25
0.60	1.25
1.61	0.10
1.00	0.10

The Thermo Scientific™ Dionex™ IonPac™ ICE-AS6 Analytical & Guard Column is an ion-exclusion column that separates alcohols. The experimental conditions used for the separation of glycerol and ethanol are represented in Figure 4. Figure 4 shows the separation of ethanol and glycerol in beer by ion-exclusion chromatography using pulsed amperometric detection with a platinum electrode.

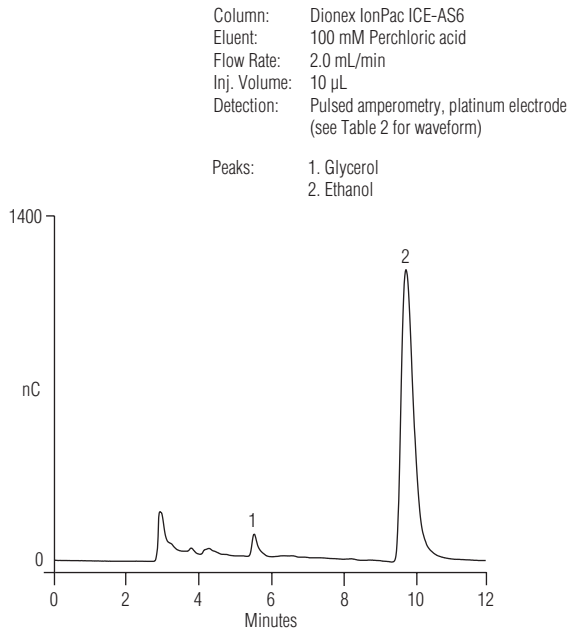


Figure 4. Separation of glycerol and ethanol in an American beer by ion-exclusion chromatography with pulsed amperometric detection. The sample was diluted 1:10 before injection.

Organic acid analysis

The measurement of organic acids, in all phases of beer production, can be used to help track metabolic products of fermentation and to correlate beer flavor trends. One way to separate organic acids is with ion-exclusion chromatography using suppressed conductivity detection. The Dionex IonPac ICE-AS6 column is an ion-exclusion column designed for the efficient separation of low molecular weight aliphatic organic acids including hydroxy-substituted organic acids, in addition to species such as aliphatic alcohols and glycols. Using this separation mechanism, weakly ionized species are separated based on differences in their pK_as. Strong inorganic acid anions are not retained by the stationary phase and elute in the void volume of the column. The standard eluent for use with the Dionex

IonPac ICE-AS6 column is 0.4 mM heptafluorobutyric acid (perfluorobutyric acid). Other monoprotic acids can be used; however, the background conductivity will be higher. The experimental conditions are listed in Figure 5.

Figure 5 shows the separation of a series of organic acids in a stout. The sample was degassed and diluted 1:40 prior to injection. Oxalic and maleic acids are eluted on either side of the 'water dip,' masked by strong acid anions such as fluoride and chloride. Pyruvate, citrate, malate, formate, lactate, acetate, and succinate, however, are all baseline resolved. The presence of acetate may provide evidence of oxidation, while pyruvic acid is present as an intermediate product in the conversion of glucose to alcohol. Lactate is produced by lactic acid bacteria that convert glucose and other sugars to lactic acid, so it is kept to a minimum in most beers. A few unidentified peaks are also resolved.

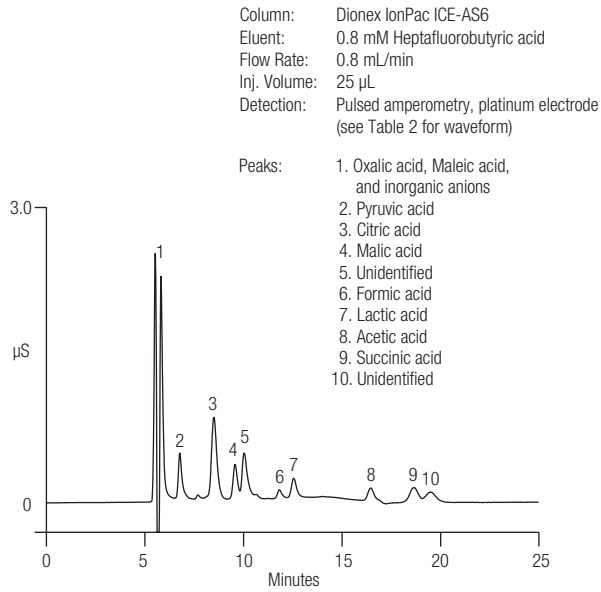


Figure 5. Separation of organic acids in a British stout. The sample was diluted 1:40 before injection.

Inorganic anions

Inorganic anions are introduced into beer from the brewing water and have an important impact on the flavor of beer. Thus, the water can be monitored by ion chromatography to ensure purity and consistency. Despite the deliberate addition of high levels of some anions such as sulfate (Burtonization), excessive amounts of sulfate and chloride, for example, can have a detrimental effect on the flavor of the beer. In addition, high concentrations of other anions such as nitrate (if it is converted to nitrite) can harm the yeast during the fermentation process. Therefore, monitoring the anion profile is an important quality control step in the brewing industry.

Inorganic anions are separated by anion-exchange chromatography and monitored by suppressed conductivity detection; Table 3 lists the experimental conditions. When performing gradient elution on the Dionex IonPac AS11 column, a hydroxide eluent system is used instead of a carbonate eluent because of the low background conductivity of hydroxide. A Thermo Scientific Dionex ATC Anion Trap column should be installed between the gradient pump and the injection valve to minimize baseline shifts resulting from the elution of anionic contaminants in the eluent.

Table 3. Experimental conditions for the separation of inorganic anions in beer using the Dionex IonPac AS11 column.

Column:	**Thermo Scientific™ Dionex™ IonPac™ AS11 Analytical Column, 4 mm Thermo Scientific™ Dionex™ IonPac™ Guard Column, 4 mm Thermo Scientific™ Dionex™ IonPac™ ATC-1 Anion Trap Column**
Eluent 1:	Deionized water
Eluent 2:	1 mM Sodium hydroxide
Eluent 3:	100 mM Sodium hydroxide
Eluent 4:	Methanol
Gradient:	**Time E1 E2 E3 E4** Initial 80 20 — — 3.00 80 20 — — 5.00 66 20 — 14 18.00 42 — 38 20 18.01 80 20 — —
Flow rate:	2 mL/min
Inj. volume:	25 µL
Detection:	Suppressed conductivity, Thermo Scientific™ Dionex™ ASRS™ Anion Self-Regenerating Suppressor, AutoSuppression™ recycle mode

Figure 6 shows the simultaneous separation of a mixture of inorganic and organic anions in an American ale using the Dionex IonPac AS11 column. The sample was degassed and diluted 1:40 prior to injection. The sodium hydroxide concentration in Eluent 2 is weak enough that not only is fluoride eluted after the void, but several weakly retained monovalent organic acids are also resolved. The addition of methanol to the eluent modifies the selectivity of the column for the more hydrophobic anions, thus allowing resolution between succinate and malate and also between tartrate and maleate, which would otherwise coelute. Thus, using the conditions described in Table 3, it is possible to separate not only the strong acid anions, but also a variety of weak organic acids. To obtain a flat baseline for this chromatogram, the baseline subtraction option in the chromatography software was used. Reproducibility for this method is on the order of 0.5% or better for retention times and 2% or better for peak areas with good linearity (r^2 = 0.999) over the range tested (1.5 orders of magnitude).

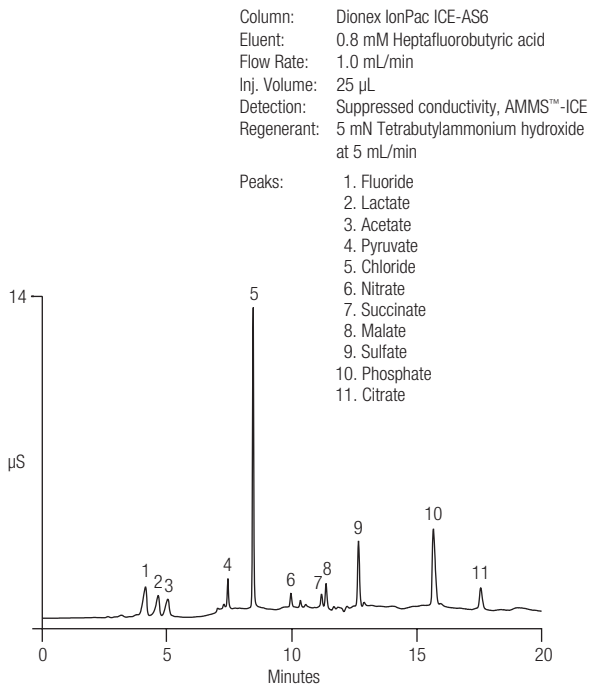


Figure 6. Separation of inorganic anions and organic acids in an American ale by ion-exchange chromatography. The sample was diluted 1:40 before injection.

The first inorganic anion to be eluted is fluoride, which is often added to municipal water supplies to prevent tooth decay and is harmless for brewing purposes. Chloride is eluted next, and at levels above 250 mg/L it has been found to enhance the sweetness of beer. However, it may also hamper yeast flocculation. Nitrate was once thought of as a problem in the brewing process, but it has since been discovered that it is the nitrite produced from nitrate that affects yeast metabolism to cause weak and incomplete fermentation. Sulfate is found naturally in water but imparts a sharp, dry edge to well hopped beers and is therefore kept to a minimum. Finally, phosphate is present in the malt and buffers the mash at a slightly acidic pH.

Inorganic cations

As is the case with the inorganic anions, most of the inorganic cations are introduced into the beer from the water supply. The four most abundant cations in beer are sodium, potassium, calcium, and magnesium. Some of these cations affect the pH of the mash, while others affect the flavor of the beer. Other metals such as lead, copper, and zinc are also monitored to ensure their absence since most of them are poisonous at any significant level. This application note focuses on the alkali and alkaline earth metals.

Inorganic cations are separated by ion-exchange chromatography and monitored by suppressed conductivity detection, as described in Figure 7. The gradient allows for the separation of barium and strontium in addition to the five cations shown in Figure 7. A step change at 5 min from the weak eluent to a stronger eluent allows for the elution of sharp peaks for the divalent cations. The reproducibility of this method is on the order of 0.5% or better for retention times and 2% or better for peak areas. Linearity is good over the range tested (2 orders of magnitude) with a coefficient of determination, r^2 = 0.999 or better, for all analytes except ammonium. If it is not important to monitor for barium or strontium, the conditions can be changed to allow for isocratic elution of the five cations shown in Figure 7 (≤10 min). If isocratic elution is desired, then the concentration eluent should be 20 mM methanesulfonic acid.

Column:	Dionex IonPac CS12 Analytical, 4 mm Dionex IonPac CG12 Guard, 4 mm Dionex IonPac CTC-1 Cation Trap				
Eluent 1:	Deionized water				
Eluent 2:	100 mM Methanesulfonic Acid				
Gradient:	Time	E1	E2	E3	E4
	Initial	84	16	—	—
	5.00	84	16	—	—
	5.01	60	40	—	—
	10.00	60	40	—	—
	10.01	84	16	—	—
Flow Rate:	1.0 mL/min				
Inj. Volume:	25 µL				
Detection:	Suppressed conductivity, Dionex CSRS suppressor, AutoSuppression recycle mode				
Peaks:	1. Sodium 2. Ammonium 3. Potassium 4. Magnesium 5. Calcium				

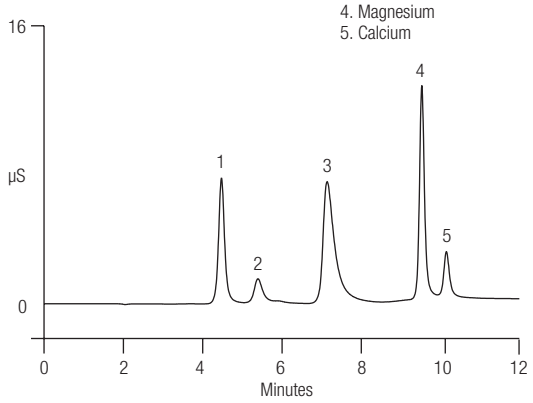


Figure 7. Separation of inorganic cations in a 1:40 dilution of an American lager by ion-exchange chromatography using the Dionex IonPac CS12 column.

Figure 7 shows the separation of the main cations in beer using the Dionex IonPac CS12 column. The sample was degassed and diluted 1:40 prior to injection. Sodium was the first peak to be eluted. At a concentration of 75–150 mg/L, it gives a round smoothness to the beer when combined with chloride. If too much sulfate is present, however, sodium gives an unpleasant harshness to the flavor. Potassium is the next metal to be eluted, and like sodium it can impart a slightly salty flavor to the beer. It also inhibits the action of certain enzymes in mash. Magnesium is an important nutrient for yeast at levels around 10–20 mg/L, but imparts a sharp, bitter-sour flavor at levels much higher than 20 mg/L. Calcium is perhaps the most important metal, since it reacts with phosphate in the malt to lower the pH of the mash and wort. It also assists enzyme action, but has no effect on the flavor of the beer.

Conclusion

Carbohydrates, alcohols, organic acids, and inorganic anions and cations can all be separated on various ion-exchange or ion-exclusion columns and detected by pulsed amperometric or suppressed conductivity detection. Although many of these constituents can be determined individually using unrelated analytical techniques, reduced analysis time and equipment costs can be realized by using one instrument with multi-species capability. Ion chromatography is a versatile technique that meets many of the analytical requirements of the beer making process.

Find out more at **www.thermofisher.com/chromatography**

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