

Determination of Organic Acids in Beer Samples Using a High-Pressure Ion Chromatography System

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Key Words

Dionex IonPac AS11-HC-4 μ m, Dionex ICS-5000+, HPIC, Beer, Fermentation, Organic Acids

Goal

Demonstrate high resolution chromatographic separations in beer samples using a 4 μ m resin anion-exchange separation column on a high-pressure modular ion chromatography (IC) system.

Introduction

Beer production has been of interest since the beginnings of civilization with brewing processes advancing along with society.¹ Beer is a complex sample matrix that contains numerous components including proteins, carbon dioxide, carbohydrates, inorganic anions and cations, aldehydes, organic acids, and ethanol. These can be passively introduced from the minerals in the water, extracted from the brewing ingredients, generated in the fermentation process, or added to achieve a desired characteristic flavor.² The brewmaster will often select specific grains, hops, and yeast to formulate a particular beer. Mashing is the first stage of brewing beer followed by soaking barley grains in warm water, resulting in the hydrolysis of the grain starches into glucose, maltose, and other oligo- and polysaccharides. Sweet wort is the solution that is separated from the mash and cooked with hops producing a hopped wort solution. The liquid is then separated from the wort, cooled, and fermented with yeast to generate alcohol. At the end of fermentation, the beer may be treated again with hops or minerals to achieve a desired appearance or taste, then cooled and bottled. Although beer samples are a challenging matrix, the analysis of fermentation broths and products of fermentation broths were demonstrated in several publications from 1995 to 2009.²⁻⁵ Since then, there have been numerous advancements in ion chromatography, including several generations of column chemistry, instrumentation, and Reagent-Free™ (RFIC™) eluent generation and eluent generation consumables. More recently, column chemistries with 4 μ m particles and high-pressure ion chromatography systems have been introduced to advance the separation technology even further.



Organic acids are end products of yeast fermentation critical to the flavor of beer, but are also products of bacterial fermentation that introduce a sour flavor, either purposely or unintentionally due to spoilage. The 4 μ m resin particle Thermo Scientific™ Dionex™ IonPac™ AS11-HC-4 μ m anion-exchange column is a high resolution, high-capacity column optimized for organic acids in complex matrices, ideal for analysis of beer samples. At standard flow rates, this 4 μ m resin particle column operates above 3000 psi, which necessitates the use of a high-pressure-capable system such as the Thermo Scientific™ Dionex™ ICS-5000+ HPIC™ system.

This Technical Note presents the advantages of the 4 μ m particle-size Dionex IonPac AS11-HC-4 μ m column combined with the Dionex ICS-5000+ HPIC system for optimal separation of organic acids and inorganic anions in beer samples using electrolytically generated hydroxide eluent with and without an organic modifier.

Equipment

Dionex ICS-5000+ Reagent-Free HPIC system

- Dionex ICS-5000+ SP/DP Pump module
- Dionex ICS-5000+ EG Eluent Generator module with high-pressure degasser module
- Dionex ICS-5000+ DC Detector/Chromatography module
- Thermo Scientific Dionex AS-AP Autosampler
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS), version 6.8 or 7.1
- For external water suppressor mode when using methanol in the eluent:
 - Thermo Scientific Dionex AXP Auxiliary Pump with two extra 4 L bottles or
 - Thermo Scientific Dionex SRS Pressurized Bottle Installation Kit and an extra 4 L pressurized bottle

The consumables are listed in Table 1.

Table 1. Consumables list.

Product Name	Type	Dionex Part Number
Dionex EGC 500 KOH cartridge	Anion Eluent Generator cartridge*	075778
Dionex CR-ATC 500 column	Anion electrolytic trap column*	075550
Dionex HP Degas Module	Degas module*	075522
Dionex HP Fittings (blue)	Bolts*/Ferrules*	074449/ 074373
Applications using External Water Mode		
Dionex AXP pump With two 4 L bottles (order two)	Preferred configuration	063973 039164
Dionex SRS Pressurized Bottle Installation Kit	Alternative configuration. Includes: One 4 L bottle SRS Installation kit 25 psi regulator	038018 039164 039055 038201
4 L water bottle	An extra bottle for external water mode	039164
Applications using 4 mm i.d. Columns		
Dionex IonPac AG11-HC-4µm, 4 × 50 mm column	Anion guard column	078034
Dionex IonPac AS11-HC-4µm, 4 × 250 mm column	Anion separation column	082313
Dionex AERS 500 suppressor	Anion suppressor for 4 mm i.d. columns	082540
Applications using 2 mm i.d. Columns		
Dionex IonPac AG11-HC-4µm, 2 × 50 mm column	2 mm Anion guard column	078036
Dionex IonPac AS11-HC-4µm, 2 × 250 mm column	2 mm i.d. Anion separation column	078035
Dionex AERS 500 suppressor	Anion suppressor for 2 mm i.d. columns	082541

* High-pressure device

Reagents and Standards

- 18 MΩ-cm resistivity degassed deionized water
- Methanol, ACS Grade (Fisher Scientific)
- ACS Grade reagents (Fisher Scientific)

Samples

- U.S. Domestic beer samples

Conditions

Column:	Dionex IonPac AS11-HC-4µm and guard columns
Eluent Source:	Thermo Scientific Dionex EGC 500 KOH cartridge
Eluent:	Potassium hydroxide
Gradient:	See Figures
Inj. Volume:	See Figures
Flow Rate:	See Figures
Inj. Volume:	See Figures
Temperature:	30 °C
Detection:	Suppressed conductivity, Thermo Scientific™ Dionex™ AutoSuppression™ Device, Thermo Scientific™ Dionex™ AERS™ 500 Anion Electrolytically Regenerated Suppressor, recycle mode or external water mode (3–5× eluent flow)
Background Conductance:	< 1.0 µS-cm ⁻¹
Peak-to-Peak Noise:	< 3 nS
System Backpressure:	< 4500 psi

Standard and Sample Preparation

Deionized water at 18 MΩ-cm resistivity or better was used in eluent and standard preparation and as the diluent for diluting samples. Individual stock standard solutions of 1000 mg/L were prepared gravimetrically from the reagents and deionized water. A mixed standard solution was prepared by diluting the individual stock standard solutions into a 100 mL volumetric flask with deionized water. Calibration standards were prepared similarly by diluting the stock standards in deionized water.

The methanol organic modifier was degassed by vacuum filtration with applied ultrasonic agitation. Sonicate the sample for 20–30 minutes and if the carbonate peak is relatively large and is interfering with other analytes, the sample can be sonicated for a longer period of time. Degassed methanol was added to a second 2 L eluent bottle on Channel B under inert atmosphere and was introduced at the proportioning valve.

The beer samples were degassed by vacuum filtration with applied ultrasonic energy, diluted with 18 MΩ-cm resistivity deionized water, and filtered with a syringe filter (0.20 µm) before injection. Dilution factors are stated in Figures 6 and 7.

Tip: To achieve the best chromatography with high-pressure IC, it is important to use high pressure connectors and ferrules (see Table 1) for all connections prior to the suppressor. The high-pressure Dionex ICS-5000+ IC system is designed to operate from 2000 to 5000 psi when using RFIC eluent generation. The installation instructions are the same when running eluent with or without methanol in most cases. Only the differences are highlighted here for clarification.

Configuring the System for Eluent Generation Without Methanol

To plumb the consumables and modules of the Dionex ICS-5000+ HPIC system in recycle mode, follow the flow diagram in Figure 1. Install and hydrate the Dionex EGC 500 KOH cartridge, Thermo Scientific Dionex CR-ATC 500 Continuously Regenerating Anion Trap Column, and Dionex AERS 500 suppressor according to the product manual instructions.⁶⁻⁸ Install and flush the columns 30 min with eluent directed to waste according to the instructions in the column manual.⁹ Complete the installation according to flow diagram. In this example, the suppressor is configured in electrolytic AutoSuppression recycle mode. Use high-pressure connectors and ferrules (blue, P/N listed in Table 1) for all of the fittings from the pump to prior to the suppressor. Standard pressure fittings can be used for the suppressor and detector connections. Detailed instructions are described in Thermo Scientific Technical Note (TN) 129: Configuring High-Pressure IC Systems for Analytical Flow Rates, the product manuals, and the instrument installation and operator's manuals.¹⁰⁻¹²

Configuring the System for Eluent Generation With Methanol

The system, columns, and suppressor (eluent side) are compatible with methanol, however the suppressor should be run using the regenerant flow stream in external water mode to prevent contamination from methanol hydrolysis and potentially damage to the suppressor. To plumb the consumables and modules of the Dionex ICS-5000+ HPIC system in external water mode, follow the flow diagram in Figure 2. Degassed methanol is introduced through Channel B in the SP/DP gradient pump proportioning valve.

Install and hydrate the Dionex EGC 500 KOH cartridge, Dionex CR-ATC 500 column, Dionex AERS 500 suppressor according to the product manual instructions.⁶⁻⁸ Configure the external water mode using the instructions in the Thermo Scientific Dionex ERS 500 Suppressor manual.⁸ Install and flush the columns 30 min with eluent according to the instructions in the column manual.⁹ Complete the installation according to the flow diagram. Use high pressure connectors and ferrules (blue) for all of the fittings from the pump to prior the suppressor. Standard pressure fittings can be used for the suppressor and detector connections. Detailed instructions are described in TN 129, the product manuals, and the instrument installation and operator's manuals.¹⁰⁻¹²

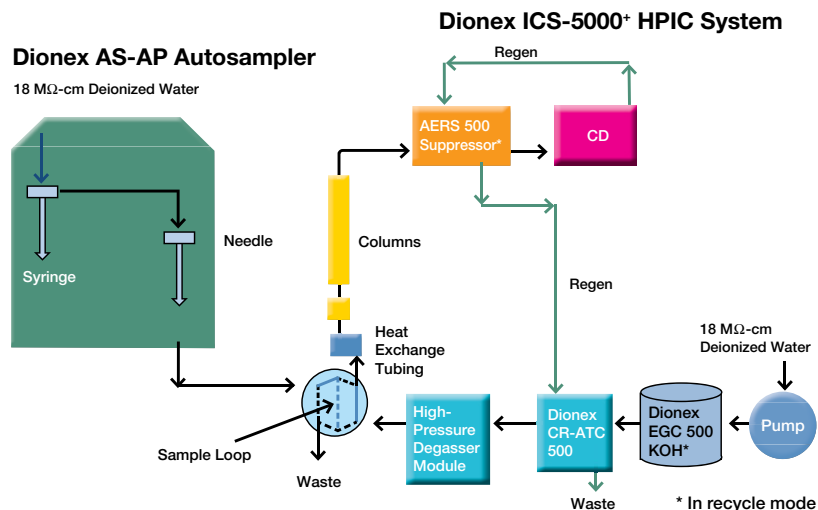


Figure 1. Flow diagram for the Dionex ICS-5000+ HPIC system using recycle mode.

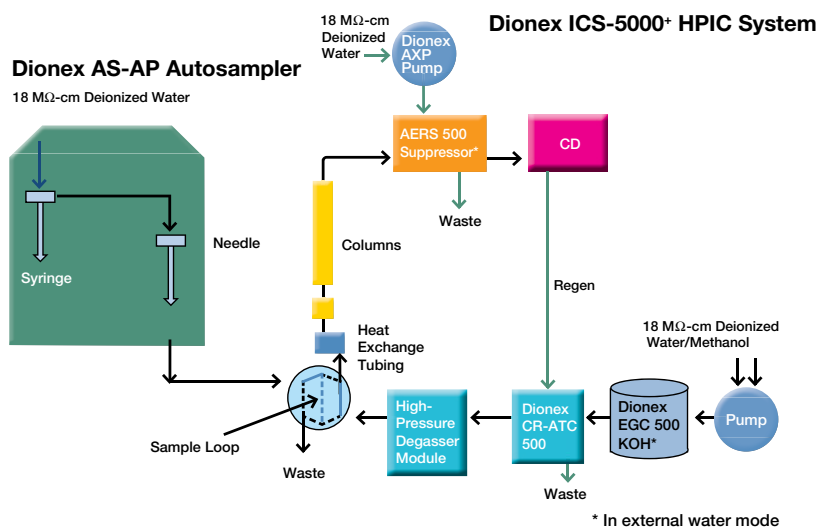


Figure 2. Flow diagram for the Dionex ICS-5000+ HPIC system using external water mode.

Results and Discussion

The Dionex IonPac AS11-HC column is a high-capacity column optimized for organic acid separations. The new 4 μm particle format (the Dionex IonPac AS11-HC-4 μm) has very efficient separation of analytes as compared to the previous 9 μm particle version, resulting in more accurate quantifications and therefore more reliable results.

Figure 3 shows the gradient separation of 40 inorganic and organic acid anions using electrolytically generated potassium hydroxide from 1 to 55 mM KOH on a 2 $\times$ 250 mm column. All the selected analyte peaks are baseline or nearly baseline resolved and elute within 45 min at 0.38 mL/min. This new platform of columns packed with 4 μm resin particles has higher system backpressures when run at standard flow rates (~3800 psi in this example), necessitating use of the Dionex ICS-5000+ HPLC system.

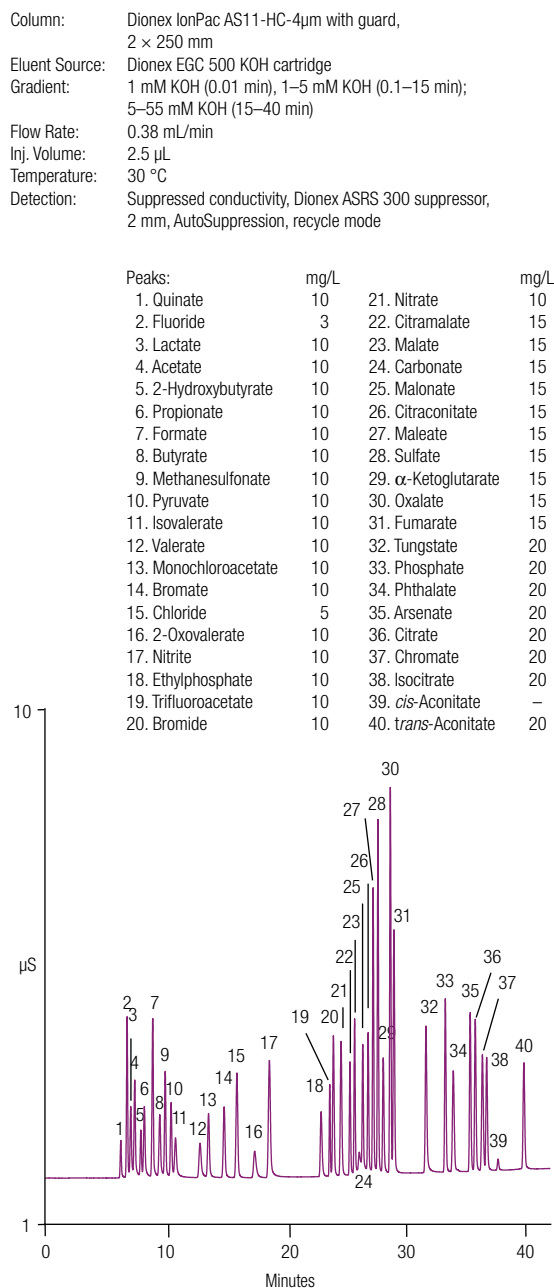


Figure 3. Separation of 40 organic and inorganic anions on a 2 mm i.d. column set.

In Figure 4, the chromatograms of 29 analytes are directly compared on the 9 μm particle (Chromatogram B) and 4 μm particle (Chromatogram A) versions of the Dionex IonPac AS11-HC 4 mm i.d. column. In these examples, the analytes in a 10 μL injection were separated using electrolytically generated hydroxide eluent from 1 to 60 mM KOH at 1.5 mL/min within 35 min. As expected, the results show significantly higher resolutions using the 4 μm particle size column, exhibiting higher signal-to-noise ratios than on the 9 μm particle column.

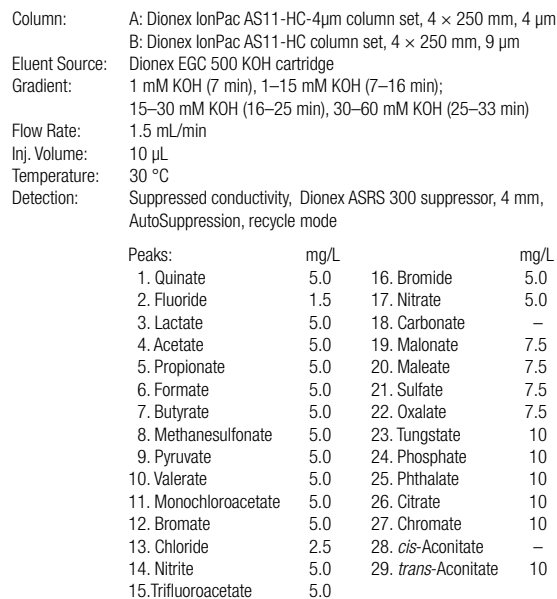


Figure 4. Comparison of 4 μm (A) and 9 μm (B) Dionex IonPac 4 mm i.d. columns.

Table 2. Improved resolutions using 4 μm particle size columns (Figure 4).

Resolution (R_s (EPI))	Lactate (3)-Acetate (4)	Formate (6)-Butyrate (7)	Valerate (10)-Monochloroacetate (11)	Methanesulfonate-Pyruvate (9)	Phosphate (24)-Phthalate (25)
4 μm column	1.90	2.31	1.62	2.00	2.39
9 μm column	1.17	1.46	1.12	1.32	1.42

* Figure 4 data

Table 2 summarizes the resolutions of critical peak pairs on both columns. In general, there was a 40 to 50% improvement in resolution of critical peak pairs on the new 4 μm column. This column improved the resolution for lactate/acetate, formate/butyrate, valerate/monochloroacetate, methanesulfonate/pyruvate, and phosphate/phthalate.

Changing the Selectivity with an Organic Modifier

To improve the resolution of critical peak pairs, methanol was added as an organic modifier. Introducing the organic modifier into the eluent changes the hydrophobicity of the column, thereby changing the selectivity, changing the elution order and resolution of some of the critical peak pairs. These changes in selectivity are demonstrated in Figure 5, where methanol is used as the organic modifier. In these chromatograms, 38 analytes were separated using electrolytically generated potassium hydroxide eluent from 1 to 60 mM KOH at 1 mL/min on a 4 mm Dionex IonPac AS11-HC-4 μm column. To generate chromatogram A, degassed methanol was introduced into the gradient at the proportioning valve. Additionally, the suppressor is configured in external water mode. As expected for conductivity detection, the chromatograms show that the peak responses were lower with the less polar eluent that contains methanol, than with a fully aqueous eluent.

Incorporating methanol into the eluent changed the column selectivity, resulting in: changes in elution order, resolving previously co-eluting peaks or in some cases, losing baseline resolution of previously resolved peaks (Table 3). For example, succinate (Peak 22) and malate (Peak 23) are fully resolved only when using methanol, whereas phosphate (Peak 32) and phthalate (Peak 33) peaks switched elution order and had lower resolution when using methanol in the eluent. Thus, selective critical peak pair resolution can be targeted with the use of organic modifiers.

Column: Dionex IonPac AS11-HC-4 μm and guard column, 4 $\times$ 250 mm
 Eluent Source: A: Channel A: Water, Channel B: Methanol
 A, B: Dionex EGC 500 KOH cartridge
 Gradient A: 1 mM KOH, 10% methanol (10.7 min),
 1–15 mM KOH, 10–20% methanol (10.7–24 min)
 15–30 mM KOH, 20% methanol (24–37.3 min)
 30–60 mM KOH, 20–10% methanol (37.3–50.6 min)
 Gradient B: Same as Gradient A without methanol
 Flow Rate: 1.0 mL/min
 Inj. Volume: 10 μL
 Temperature: 30 $^{\circ}\text{C}$
 Detection: Suppressed conductivity, Dionex ASRS 300 suppressor, 4 mm, AutoSuppression, external water mode, 10 mL/min

Peaks:	mg/L		mg/L
1. Quinate	10	20. Nitrate	10
2. Fluoride	3	21. Glutarate	10
3. Lactate	10	22. Succinate	15
4. Acetate	10	23. Malate	15
5. Glycolate	10	24. Carbonate	15
6. Propionate	10	25. Malonate	15
7. Formate	10	26. Tartrate	15
8. Butyrate	10	27. Maleate	15
9. Methanesulfonate	10	28. Sulfate	15
10. Pyruvate	10	29. Oxalate	15
11. Valerate	10	30. Fumarate	15
12. Galacturonate	10	31. Tungstate	20
13. Monochloroacetate	10	32. Phosphate	20
14. Bromate	10	33. Phthalate	20
15. Chloride	5	34. Citrate	20
16. Nitrite	10	35. Chromate	20
17. Trifluoroacetate	10	36. Isocitrate	20
18. Sorbate	10	37. <i>cis</i> -Aconitate	—
19. Bromide	10	38. <i>trans</i> -Aconitate	20

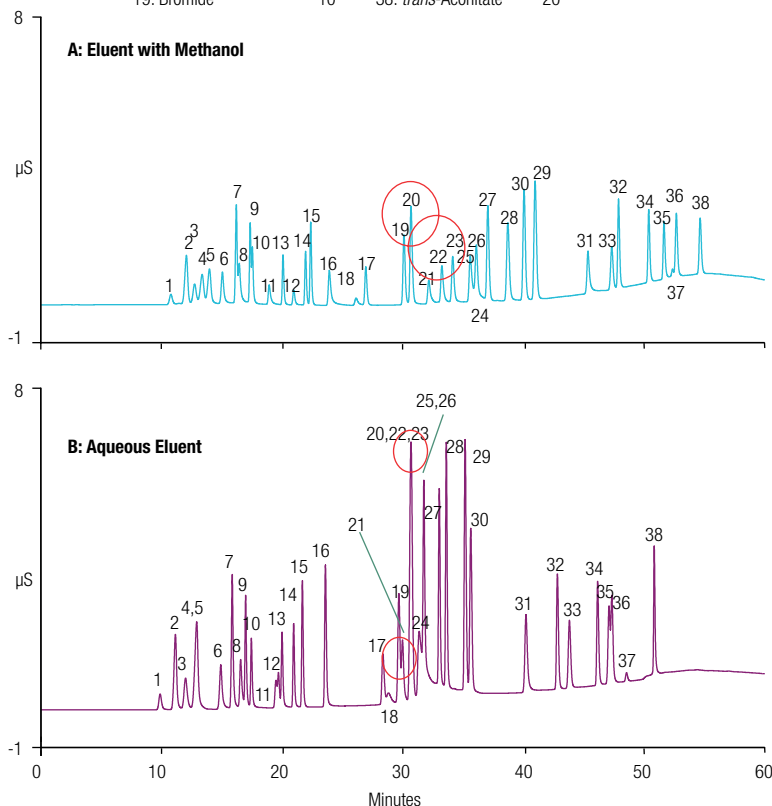


Figure 5. Using an organic modifier to improve selectivity and resolution.

Table 3. Changes in selectivity using methanol as an organic modifier.

Improved Resolution*	Decreased Resolution*	Change in Elution Order*
Acetate (4), glycolate (5)		
	Formate (7), butyrate (8)	
	Methanesulfonate (9), pyruvate (10)	
Valerate (11), galacturonate (12), monochloroacetate (13)		Galacturonate (12), monochloroacetate (13)
Trifluoroacetate (17), sorbate (18)		Trifluoroacetate (17), sorbate (18)
Bromide (19), glutarate (21)		
Glutarate (21), succinate (22), malate (23)		
	Carbonate (24), malonate (25)	
Malonate (25), tartrate (26)		
Oxalate (29), fumarate (30)		Oxalate (29), fumarate (30)
	Phosphate (32), phthalate (33)	Phosphate (32), phthalate (33)
Chromate (35), isocitrate (36)		
	isocitrate (36), <i>cis</i> -iconitate (37)	isocitrate (36), <i>cis</i> -iconitate (37)

* Peak numbers in parenthesis

Beer Analysis

In Figure 6, three U.S. domestic lager beers were analyzed on a 4 mm column using a gradient potassium hydroxide separation at 1.5 mL/min with a system backpressure of ~4000 psi. Although the samples are the same type of beer, there are subtle differences. Sample A has the highest concentrations of chloride, phosphate, and citrate of the three samples, where as Sample C has the highest concentration of pyruvate and sulfate. The analysis results suggests that Sample A may have higher hops flavoring because phosphate and citrate are often used to increase buffering capacity needed to produce higher hops beers.^{1,2} Succinate, malate (Peak 7), and tartrate (not shown), which are peaks of interest in beer analysis, are not resolved with this gradient.

Column: Dionex IonPac AS11-HC-4 μ m with guard, 4 $\times$ 250 mm
 Eluent Source: Dionex EGC 500 KOH cartridge
 Gradient: 1 mM KOH (-5–8 min), 1–15 mM KOH (8–18 min), 15–30 mM KOH (18–28 min), 30–60 mM KOH (28–38 min), 60 mM KOH (38–45 min)
 Flow Rate: 1.5 mL/min
 Inj. Volume: 10 μ L
 Temperature: 30 $^{\circ}$ C
 Detection: Suppressed conductivity, Dionex ASRS 300 suppressor, 4 mm, AutoSuppression, recycle mode
 Beer Samples: A: Lager, B: Lager 2, C: Light Lager
 Sample Prep: Degas, 5-fold dilution

Peaks:
 1. Quinate
 2. Fluoride
 3. Lactate
 4. Acetate
 5. Pyruvate
 6. Chloride
 7. Succinate + Malate
 8. Carbonate
 9. Sulfate
 10. Oxalate
 11. Fumarate
 12. Phosphate
 13. Citrate
 14. Isocitrate
 15. *cis*-Aconitate
 16. *trans*-Aconitate

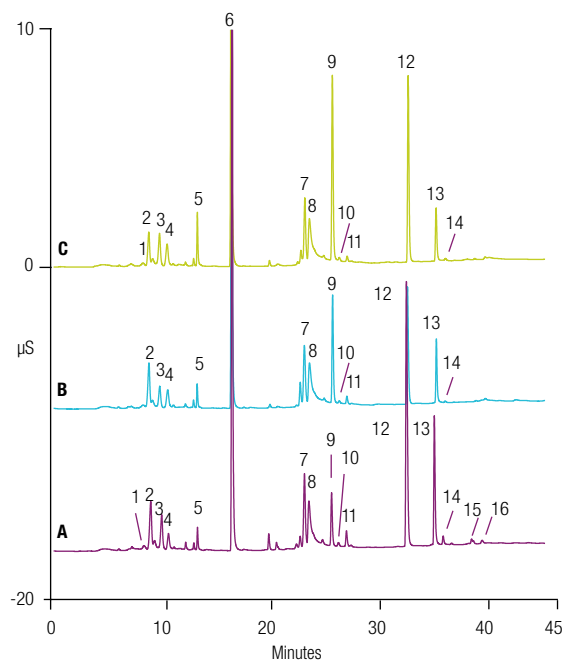


Figure 6. Analysis of beer samples on a 4 mm i.d. column.

Figure 7 demonstrates the improved separations of succinate, malate, and tartrate in Sample 2 lager beer (Chromatograms A and B) using a methanol-KOH gradient. The analytes in both the standard (Chromatogram C) and the beer samples were separated using gradient conditions from 1 to 60 mM KOH and from 2 to 10% methanol on a 2 mm column at 0.38 mL/min. At these conditions, the system backpressure is ~4000 psi. Butyrate is an important analyte because it is associated with bacterial fermentation and is therefore an indicator of spoilage. Butyrate (Peak 7) was only found in the standard and the beer spiked with butyrate.

Column: Dionex IonPac AS11-HC-4 μ m set, 2 $\times$ 250 mm
 Eluent Source: Channel A: Water
 Channel B: Methanol
 Dionex EGC 500 KOH cartridge
 Gradient: 1 mM KOH, 2% methanol (8 min),
 2–10% methanol (8–8.1 min)
 1–15 mM KOH, 10% methanol (8–18 min),
 15–30 mM KOH, 10% methanol (18–28 min),
 30–60 mM KOH, 10% methanol (28–38 min)
 Flow Rate: 0.38 mL/min
 Inj. Volume: 2.5 μ L
 Temperature: 30 $^{\circ}$ C
 Detection: Suppressed conductivity, Dionex ASRS 300 suppressor, 4 mm,
 AutoSuppression, recycle mode
 Sample Prep.: 5-fold dilution
 Samples: A: Lager beer 2; Sample, B: Sample A plus 10 ppm butyrate;
 C: Standard

Peaks (Standard):	mg/L		mg/L
1. Quinate	5	12. Succinate	10
2. Fluoride	3	13. Malate	10
3. Lactate	5	14. Tartrate	10
4. Acetate	5	15. Sulfate	10
5. Propionate	5	16. Fumarate	10
6. Formate	5	17. Oxalate	10
7. Butyrate	5	18. Phosphate	15
8. Pyruvate	10	19. Citrate	15
9. Chloride	5	20. Isocitrate	15
10. Bromide	5	21. <i>cis</i> -Aconitate	—
11. Nitrate	5	22. <i>trans</i> -Aconitate	15

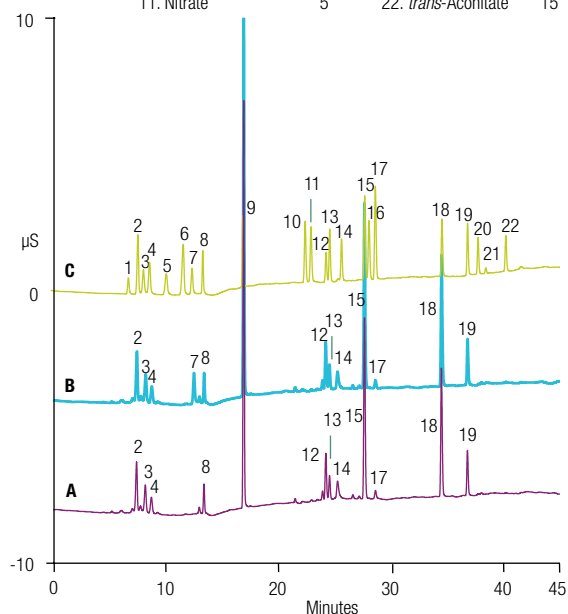


Figure 7. Beer analysis using an organic modifier to change column selectivity.

Conclusion

- The 4 μ m particle size Dionex IonPac AS11-HC-4 μ m column is the ideal column to separate a wide range of organic acids and inorganic anions present in complex sample matrices such as brewed beer.
- This column has similar performance on all formats, 4 mm, 2 mm, and 0.4 mm (capillary) which was demonstrated here on the 2 mm and 4 mm i.d. columns. The capillary format (not discussed here) of the Dionex IonPac AS11-HC-4 μ m can be run on the high pressure capillary systems, the Dionex ICS-5000+ HPIC modular system and the Thermo Scientific Dionex ICS-4000 IC dedicated capillary system.
- The smaller resin particles in this column generate system backpressures > 3000 psi at standard flow rates necessitating the use of the Dionex ICS-5000+ HPIC analytical system.
- This combination of 4 μ m particle size high-capacity column and HPIC system provides a robust platform to operate smaller-particle-sized columns under elevated backpressures, yielding highly efficient separations with improved resolution of critical peak pairs.
- Here 14 or more analyte peaks in lager-style domestic beer samples were separated using hydroxide gradients and hydroxide-methanol gradients to separate succinate, malate, butyrate, and tartrate.

For more information on beverage analysis using 2 mm and 4 mm i.d. columns, refer to Thermo Scientific Application Notes AN 143, AN 182, and AN 273. For beverage analysis on capillary columns, review Application Briefs AB 117, AB 127, and AB 135, and Technical Notes TN 118 and TN 119.^{14–20}

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