Fast, High-Efficiency Separations on a 4 µm Ion Exchange Phase Using a High-Pressure Ion Chromatography System

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Overview

Purpose: This poster demonstrates that 4 µm columns combined with a high-pressure ion chromatography system achieve efficient separations and fast analyses.

Methods: Inorganic anions in fruit juice, wine, or drinking and waste water samples were separated using a Thermo Scientific™ Dionex™ IonPac™ AS11-HC-4µm column or Dionex IonPac AS18-4µm anion-exchange column in standard and microbore formats. The Dionex IonPac CS19-4µm cation-exchange column was used to separate cation standards. These columns were used with the high-pressure Thermo Scientific™ Dionex™ ICS-5000$^+$ Reagent-Free™ HPIC™ system. Eluent suppressors were operated using the Thermo Scientific™ Dionex™ AutoSuppression™ device, either in recycle mode or external water mode, with suppressed conductivity detection. Elution conditions were as described in the figures.

Results: Multiple inorganic anions and organic acids were separated in single runs of fruit juice and wine samples, using a standard (4 mm i.d.) format Dionex IonPac AS11-HC-4µm column. Several of the separated components showed enhanced resolution of up to 68 % using the 4 µm particle size column relative to the 9 µm counterpart. Inorganic anions in municipal drinking and waste water samples were separated within 5 min with resolution above 1.6 (EP) using a 4 µm particle size Dionex IonPac AS18-4µm microbore (2 mm i.d.) format column. Inorganic cations were eluted in less than 12 minutes by a combination of increased flow rate and an eluent gradient using the Dionex IonPac CS19-4µm, 4 mm i.d. column.

Introduction

Four µm columns separate analytes with greater efficiency, permitting the use of higher flow rates while maintaining resolution and reducing overall run time. Running any column at increased flow rates may cause a decrease in peak resolution. However, the superior chromatographic fidelity achieved using the new 4 µm columns, including the Dionex IonPac AS11-HC-4µm, Dionex IonPac AS18-4µm, and Dionex IonPac CS19-4µm, minimizes such losses, to provide better separation of ions in complex matrices. Faster flow rates reduce run time and increases productivity when multiple samples are being analyzed, but back pressure is also increased, which is typically >3000 psi. To take advantage of the benefits of 4 µm columns, they should be combined with a high-pressure instrument, such as the Dionex ICS-5000$^+$ HPIC system, which can operate continuously at up to 5000 psi using eluent generation.

Methods

Sample Preparation
The samples were diluted with deionized water before analysis without other pretreatment, as indicated in the figures.

Equipment and Data Analysis
Dionex ICS-5000$^+$ system
Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software

Conditions
Columns:
Dionex IonPac AS11-HC-4µm, 4 x 250 mm column
Dionex IonPac AS18-4µm, 2 x 150 mm column
Dionex IonPac CS19-4µm, 4 x 250 mm column
Eluent Source:
Thermo Scientific™ Dionex™ EGC 500 Cartridge for 2 mm and 4 mm columns
Detection: Suppressed conductivity, recycle mode
Thermo Scientific™ Dionex™ SRS™ 300 Self-Regenerating Suppressor

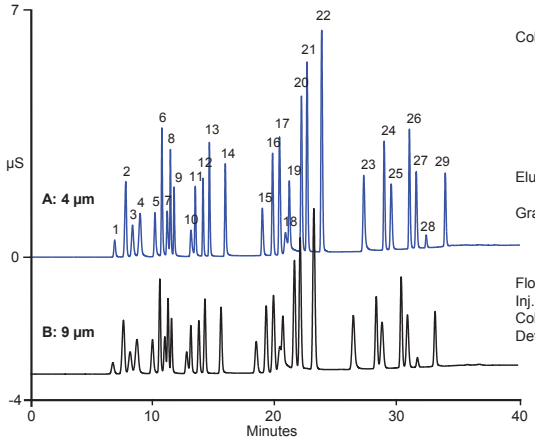
Results

Dionex IonPac AS11-HC-4µm Anion-Exchange Column

Four µm columns such as the Dionex IonPac AS11-HC-4µm column can improve resolution and separation of organic acids and inorganic anions, simplifying peak integration. Figure 1 illustrates improved separation for monovalent carboxylic acids, such as lactate and acetate, and an improvement in resolution for formate, methylsulfonate, valerate, and phosphate of 58, 50, 45, and 68%, respectively (Table 1).

The high efficiency of this column permits separation in a single run of a large number of organic acids and inorganic anions in complex sample matrices such as fruit juice or wine samples (see Figures 2 and 3).

FIGURE 1. The Dionex IonPac AS11-HC-4µm column provides improved resolution over the Dionex IonPac AS11-HC column.



Columns:	A: Dionex IonPac AG11-HC-4µm/ Dionex IonPac AS11-HC-4µm, 4 × 250 mm B: Dionex IonPac AG11-HC, Dionex IonPac AS11-HC, 4 × 250 mm
Eluent Source:	Dionex EGC 500 KOH cartridge
Gradient:	1 mM KOH (0–7 min) 1–15 mM KOH (7–16 min) 15–30 mM KOH (16–25 min) 30–60 mM KOH (25–33 min)
Flow Rate:	1.5 mL/min
Inj. Volume:	10 µL
Column Temp.:	30 ˚C
Detection:	Suppressed conductivity, Dionex ASRS 300, 4 mm, recycle mode

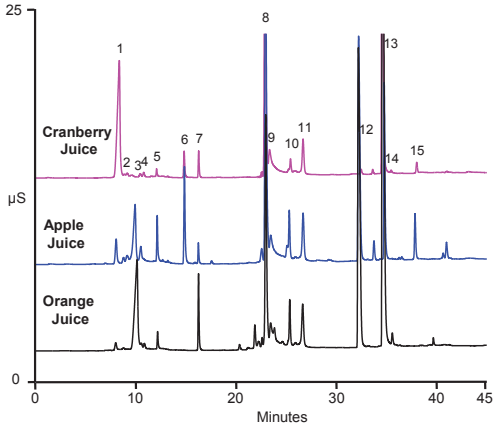
Peaks (mg/L):

1. Quinate	5	9. Pyruvate	5	17. Nitrate	5	25. Phthalate	10
2. Fluoride	1.5	10. Valerate	5	18. Carbonate	–	26. Citrate	10
3. Lactate	5	11. Monochloroacetate	5	19. Malonate	7.5	27. Chromate	10
4. Acetate	5	12. Bromate	5	20. Maleate	7.5	28. *cis*-Aconitate	–
5. Propionate	5	13. Chloride	2.5	21. Sulfate	7.5	29. *trans*-Aconitate	10
6. Formate	5	14. Nitrite	5	22. Oxalate	7.5		
7. Butyrate	5	15. Trifluoroacetate	5	23. Tungstate	10		
8. Methylsulfonate	5	16. Bromide	5	24. Phosphate	10		

TABLE 1. Comparison of resolution obtained on columns with different particle size.

	Lactate (Peak 3)	Formate (Peak 6)	Methylsulfonate (Peak 8)	Valerate (Peak 10)	Phosphate (Peak 24)
Resolution (EP), 9 µm column	1.17	1.46	1.33	1.12	1.42
Resolution (EP), 4 µm column	1.90	2.31	2.00	1.62	2.39
Improvement in resolution (%)	62	58	50	45	68

FIGURE 2. Analysis of organic acids and inorganic anions in cranberry, apple, and orange juice using a Dionex IonPac AS11-HC-4µm column.

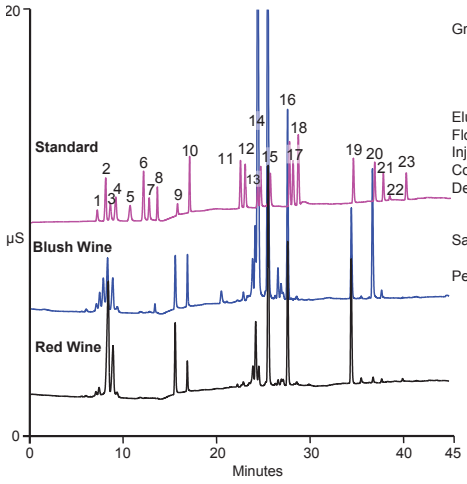


Column:	Dionex IonPac AG11-HC-4µm/ Dionex IonPac AS11-HC-4µm, 4 × 250 mm
Eluent :	1 mM KOH (0-8 min) 1–15 mM KOH (8-18 min) 15–30 mM KOH (18-28 min) 30–60 mM KOH (28-38 min)
Eluent Source:	Dionex EGC 500 KOH cartridge
Flow Rate:	1.5 mL/min
Inj. Volume:	10 µL
Column Temp.:	30 ˚C
Detection:	Suppressed conductivity, Dionex ASRS 300, 4 mm, recycle mode
Sample Prep.:	10-fold dilution

Peaks :

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

FIGURE 3. Analysis of organic acids and inorganic anions in red and blush wines using a Dionex IonPac AS11-HC-4µm column.



Column: Dionex IonPac AG11-HC-4µm/ Dionex IonPac AS11-HC-4µm, 2 × 250 mm
Gradient: 1 mM KOH, 2% MeOH (0–8 min)
1 mM KOH, 10% MeOH (8–10 min)
1–15 mM KOH, 10% MeOH (10–20 min)
15–30 mM KOH, 10% MeOH (20–30 min)
30–60 mM KOH, 10% MeOH (30–40 min)
Eluent Source: Dionex EGC 500 KOH cartridge
Flow Rate: 0.38 mL/min
Inj. Volume: 2.5 µL
Column Temp.: 30 °C
Detection: Suppressed conductivity, Dionex ASRS 300, 2 mm, external water mode
Sample Prep.: 20-fold dilution

Peaks (Standard):

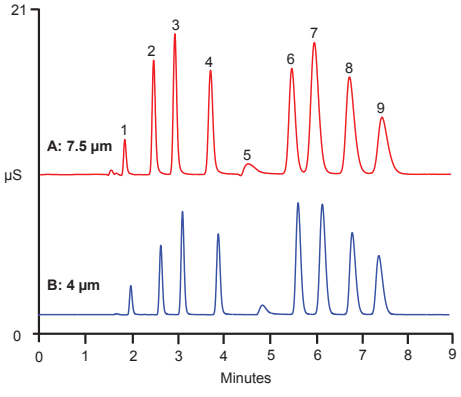
1. Quinate	5 mg/L	14. Succinate	10
2. Fluoride	3	15. Malate	10
3. Lactate	5	16. Tartrate	10
4. Acetate	5	17. Sulfate	10
5. Propionate	5	18. Fumarate	10
6. Formate	5	19. Oxalate	10
7. Butyrate	5	20. Phosphate	15
8. Pyruvate	10	21. Citrate	15
9. Galacturonate	10	22. Isocitrate	15
10. Chloride	5	23. *cis*-Aconitate	--
11. Bromide	5	23. *trans*-Aconitate	15
12. Nitrate	5		

Dionex IonPac AS18-4µm Anion-Exchange Column

In 1993, the U.S. EPA Method 300.0 (A) defined the use of the Dionex IonPac AS4A anion-exchange column to determine inorganic anions in environmental water samples using a manually prepared carbonate-based eluent and suppressed conductivity detection. With the introduction of Reagent-Free Ion Chromatography (RFIC™) systems, hydroxide-based eluents were electrolytically generated inline and used in combination with anion-exchange columns optimized for hydroxide chemistry. RFIC systems deliver reproducible and precise eluent concentrations, eliminating labor, operator-to-operator variability, and potential errors associated with manual eluent preparation.

The Dionex IonPac AS18-4µm column has been developed using Dionex IonPac AS18 column chemistry with 4 µm resin particles, which maintains peak resolution (Figure 4) while permitting faster flow rates for reduced run times (Figure 5). When this column is combined with a reagent-free HPIC system, inorganic anions that commonly exist in waste or drinking water samples are eluted within 5 minutes at a resolution above 1.6 (EP, Figure 6).

FIGURE 4. Peak efficiency and resolution were enhanced by using a 4 µm column to separate nine anions.

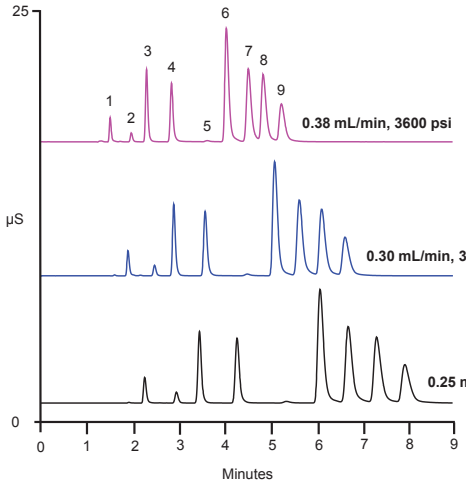


Column: A: Dionex IonPac AS18-Fast, 2 × 150 mm
B: Dionex IonPac AS18-4µm, 2 × 150 mm
Eluent Source: A: Dionex EGC III KOH cartridge
B: Dionex EGC 500 KOH cartridge
Eluent: 23 mM KOH
Flow Rate: 0.25 mL/min
Inj. Volume: A: 5 µL; B: 2.5 µL
Column Temp.: 30 °C
Detection: Suppressed conductivity, Dionex ASRS 300, 2 mm, recycle mode

Peaks:

1. Fluoride	0.5 mg/L
2. Chlorite	5
3. Chloride	3
4. Nitrite	5
5. Carbonate	20
6. Bromide	10
7. Sulfate	10
8. Nitrate	10
9. Chlorate	10

FIGURE 5. Run times were shortened by increasing the flow rate to separate nine inorganic anions using a Dionex IonPac AS18-4µm anion-exchange column.

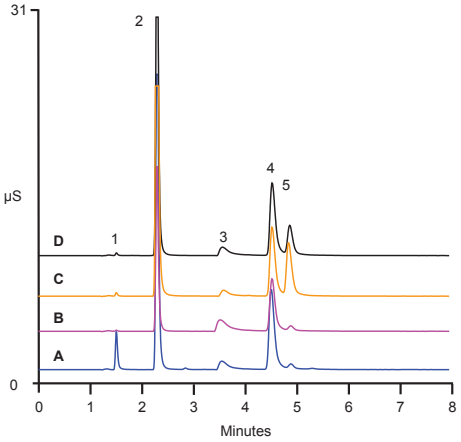


Column:	Dionex IonPac AG18-4µm/ Dionex IonPac AS18-4µm, 2 × 150 mm
Eluent Source:	Dionex EGC 500 KOH cartridge
Eluent:	23 mM KOH
Flow Rate:	0.25, 0.30, 0.38 mL/min
Inj. Volume:	5 µL
Column Temp.:	30 ˚C
Detection:	Suppressed conductivity, Dionex ASRS 300, 2 mm, recycle mode

Peaks:

1. Fluoride	0.5 mg/L
2. Chlorite	1
3. Chloride	3
4. Nitrite	5
5. Carbonate	--
6. Bromide	10
7. Sulfate	10
8. Nitrate	20
9. Chlorate	10

FIGURE 6. Rapid analysis of inorganic anions in municipal drinking and waste water samples using a Dionex IonPac AS18-4µm column.



Column:	Dionex IonPac AG18-4µm/ Dionex IonPac AS18-4µm, 2 × 150 mm
Eluent Source:	Dionex EGC 500 KOH cartridge
Eluent:	23 mM KOH
Flow Rate:	0.38 mL/min
Inj. Volume:	5 µL
Column Temp.:	30 ˚C
Detection:	Suppressed conductivity Dionex ASRS 300, 2 mm, recycle mode
Samples:	A. City 1 Drinking Water B. City 2 Drinking Water C. City 1 Wastewater D. City 2 Wastewater
Dilutions:	A: Undiluted B: 5-fold dilution C, D: 10-fold dilution

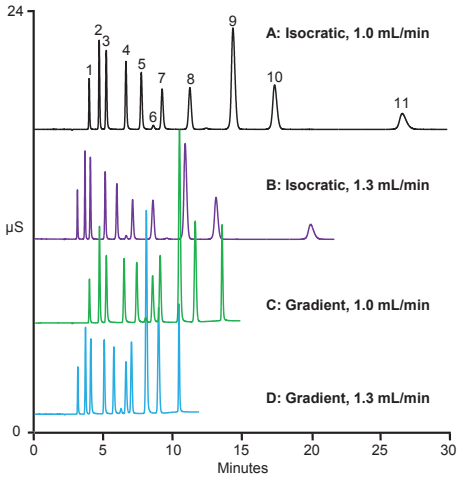
Peaks:

	A	B	C	D	
1. Fluoride	0.86	0.11	0.77	0.56	mg/L
2. Chloride	18.0	36.0	250.0	160.0	
3. Carbonate	- -	- -	- -	- -	
4. Sulfate	12.0	38.0	100.0	110.0	
5. Nitrate	0.57	2.9	84.0	43.0	

Dionex IonPac CS19-4µm Cation-Exchange Column

Four µm resin can also be exploited to resolve cations. Figure 7 illustrates how a Dionex IonPac CS19-4µm column was used to rapidly separate lithium, sodium, ammonium, potassium, rubidium, cesium, magnesium, calcium, strontium, and barium with high resolution. By increasing the flow rate from 1.0 to 1.3 mL/min and using an eluent gradient that ranged from 7 to 20 mM MSA, the run time was reduced from 30 to 12 minutes. The use of a gradient impacted the shape of the barium peak the greatest, decreasing the peak width at 50% by 7-fold compared to isocratic conditions (0.078 vs 0.54 min.).

FIGURE 7. Fast separation of inorganic cations using a Dionex IonPac CS19-4µm column by varying flow rate and elution conditions.



Column:	Dionex IonPac CG19-4µm/ Dionex IonPac CS19-4µm, 4 × 250 mm
Eluent Source:	Dionex EGC 500 MSA cartridge
Eluent:	A: 7 mM MSA (0–30 min) B: 7 mM MSA (0–22 min) C: 7 mM MSA (0–1.5 min) 7–14 mM MSA (1.5–9 min) 14–20 mM MSA (9–10.5 min) 20 mM MSA (10.5–13.5 min) 7 mM MSA (13.5–15 min) D: same as C, adjusted for flow rate
Flow Rate:	A: 1.0; B: 1.3; C: 1.0; D: 1.3 mL/min
Inj. Volume:	25 µL
Column Temp.:	30 ˚C
Detection:	Suppressed conductivity, Dionex CSRS 300, 4 mm, recycle mode

Peaks :

1. Lithium	0.15 mg/L	7. Cesium	1.5
2. Sodium	0.60	8. Magnesium	0.75
3. Ammonium	0.48	9. Calcium	1.5
4. Potassium	1.5	10. Strontium	1.5
5. Rubidium	1.5	11. Barium	1.5
6. Unknown	---		

Conclusions

Four µm resin particles produce highly efficient separations that yield high resolution and fast analyses. Shorter runs times benefit the analysis of large sample numbers, which results in higher throughput. The Dionex ICS-5000$^+$ HPIC system is designed to operate up to 5000 psi using all column formats, making it the ideal companion system for 4 µm particle columns.

- The Dionex IonPac AS11-HC-4µm column provides improved separation for monovalent carboxylic acids and an increase in resolution of formate, methylsulfonate, valerate, and phosphate of up to 68%, compared to the standard 9 µm particle size column.
- The Dionex IonPac AS18-4µm column provides efficient anion separations at higher flow rates thereby decreasing the time required to analyze drinking and waste water samples.
- Increasing flow rate and using an eluent gradient reduced run time from 30 to 12 minutes and decreased the 50% peak widths for some cations by as much as seven-fold with a Dionex IonPac CS19-4µm column.

References

For more information regarding Dionex IonPac AS11-HC and Dionex IonPac AS18 columns, refer to the following Thermo Fisher Scientific application and technical notes:

1. Application Note 143: *Determination of Organic Acids in Fruit Juices,* LPN 1415, Thermo Fisher Scientific, Sunnyvale, CA, 2003.

2. Application Note 154*: Determination of Inorganic Anions in Environmental Water Using a Hydroxide-Selective Column,* LPN 1539, Thermo Fisher Scientific, Sunnyvale, CA, 2003.

3. Fisher, C., Christison, T., Shao, B., Lopez, L. Technical Note 127: *Efficient and Fast Separations of Inorganic Anions in Water Samples Using a 4 µm Particle Size Microbore Column with a High-Pressure Ion Chromatography System.* Available mid-2013.

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