Efficient Separations Obtained by Using Smaller Particle Size Analytical Columns with a High-Pressure Ion Chromatography System

Barbara Shao, Frank Hoefler, Maria Rey, and Linda Lopez,
Thermo Fisher Scientific, Sunnyvale, CA, USA

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Overview

Purpose: This poster demonstrates smaller-particle-size (4 µm) analytical columns combined with high-pressure ion chromatography to achieve efficient separations, fast analyses, and high throughput.

Methods: Inorganic anions in fruit juice, wine, or environmental and mineral water samples are separated using Thermo Scientific Dionex IonPac AS11-HC-4µm, AS18-4µm, or CS19-4µm anion-exchange smaller particle size resins in prototype standard and microbore column formats, combined with a modified Thermo Scientific Dionex ICS-5000 HPIC system, with prototype high-pressure pumps, eluent generator cartridges, and degas modules, allowing operation in all column formats at 5000 psi system backpressures. Current eluent suppressors are compatible with high-pressure systems, and are operated using AutoSuppression™, either in recycle mode or external water mode, with detection by suppressed conductivity. The advantages of better separation of ions in complex samples and faster analysis are presented.

Results: Inorganic anions in municipal and mineral water samples are separated within 4 min with good baseline resolution using a prototype 4 µm particle size Dionex IonPac™ AS18-4µm microbore (2 mm i.d.) format analytical column; multiple inorganic anions and organic acids are resolved in single runs in fruit juice and white wine samples, using a prototype standard (4 mm i.d.) format Dionex IonPac AS11-HC-4µm column. Several of the separated components showed enhanced baseline resolution using the 4 µm particle-size columns relative to their 9 µm counterparts with the same stationary phase. Inorganic cations are separated within 8 min with high peak efficiency and high resolution using a prototype Dionex IonPac CS19-4µm 4 mm i.d. column.

Introduction

Typical ion chromatography systems and analytical columns in standard 4 mm and microbore 2 mm formats operate below a maximum pressure of 3000 psi using well-established Reagent-Free Ion Chromatography (RFIC) system techniques. A smaller-particle-size analytical column, however, requires high-pressure system capability. The Dionex ICS-5000 Capillary HPIC™ system can operate continuously at up to 5000 psi using eluent generation. The modified system used here extends the high system backpressure capabilities to the entire flow rate range of the Dionex ICS-5000 system.

Running any columns at increased flow rates may cause a decrease in peak efficiencies. However, the superior chromatographic fidelity achieved using the new 4 µm columns, including the Dionex IonPac AS11-HC-4µm, AS18-4µm, and IonPac CS19-4µm, minimizes such losses, to provide better separation of ions in complex matrices and can provide faster analysis times and higher throughput. Higher peak efficiencies can be achieved, even when running at faster flow rates, providing shorter analysis times and increased productivity. These columns are currently available in 0.4 mm i.d. formats, but 2 and 4 mm i.d. columns are in development, and prototypes are demonstrated here.

Methods

Sample Preparation
The samples were diluted before analysis without other pretreatment

Ion Chromatography

Equipment and Data Analysis
Dionex ICS-5000 system, modified for high-pressure operation
Dionex IonPac AS11-HC-4µm, prototype 4 × 250 mm analytical column
Dionex IonPac AS18-4µm, prototype 2 × 150 mm analytical column
Dionex IonPac CS19-4µm, prototype 4 × 250 mm analytical column
Thermo Scientific Dionex Chromeleon Chromatography Data System software

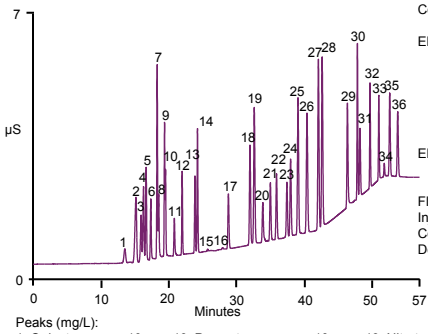
Conditions
The conditions are described in the figures.

Results

Prototype 4 mm i.d. Dionex IonPac AS11-HC-4µm Analytical Column

Small-particle-size columns such as the Dionex IonPac AS11-HC-4µm can improve baseline resolution and separation of organic acids and inorganic anions (see Figures 1 and 2 and Table 1); the high capacity and high efficiency of this analytical column permit separation of a large number of organic acids and inorganic anions in complex sample matrices such as wine or fruit juice samples (see Figures 3 and 4). This ion-exchange column uses smaller resin particles for more efficient separations resulting in more accurate peak integration and more reliable results; therefore, a large number of inorganic anions and organic acids in fruit juice and white wine samples can be resolved in a single run using a hydroxide gradient.

FIGURE 1. Determination of organic acids and inorganic anions in standard mixture using a prototype Dionex IonPac AS11-HC-4µm, 4 × 250 mm column.

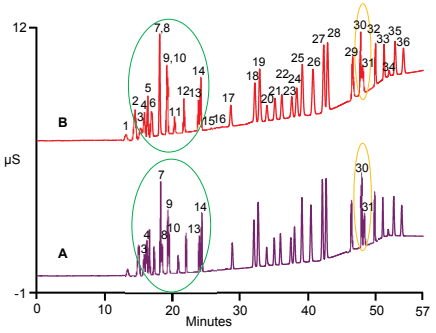


Column:	Dionex IonPac AS11-HC-4µm, 4 × 250 mm prototype
Eluent:	1 mM KOH with 10% methanol for 10.7 min, 1–15 mM KOH with 10% to 20% methanol in 13.3 min, 15 to 20 mM KOH with 20% methanol in 9.7 min, 30 to 60 mM KOH with 20% to 10% methanol in 10.0 min
Eluent Source:	Prototype high-pressure Thermo Scientific Dionex EGC KOH
Flow Rate:	1.0 mL/min
Inj. Volume:	10 µL
Column Temp.:	30 °C
Detection:	Suppressed conductivity, Thermo Scientific Dionex ASRS 300, 4 mm, AutoSupression, external water mode

Peaks (mg/L):

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

FIGURE 2. The Dionex IonPac AS11-HC smaller-particle-size (4 µm) analytical column provides improved resolution over the AS11-HC bigger-particle-size (9 µm) analytical column, including improved separation for monovalent carboxylic acids, such as lactate and acetate, and better baseline resolution for bromate/chloride, formate/butyrate, methylsulfonate/pyruvate, and phosphate/phthalate (see Table 1).

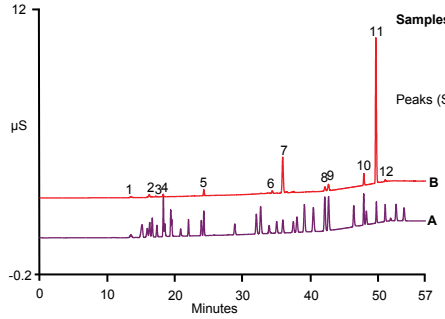


Column A:	Dionex IonPac AS11-HC-4µm, 4 × 250 mm prototype
Column B:	Dionex IonPac AS11-HC, 9 µm, 4 × 250 mm
Eluent Source:	Prototype high-pressure Dionex EGC KOH
Eluent:	1 mM KOH with 10% methanol for 10.7 min, 1–15 mM KOH with 10% to 20% methanol in 13.3 min, 15 to 20 mM KOH with 20% methanol in 9.7 min, 30 to 60 mM KOH with 20% to 10% methanol in 10.0 min
Flow Rate:	1.0 mL/min
Inj. Volume:	10 µL
Column Temp.:	30 °C
Detection:	Suppressed conductivity, Dionex ASRS™ 300, 4 mm, AutoSupression, external water mode
Peaks:	See Figure 1

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

	Lactate (P3)	Bromate (P7)	Formate (P9)	Methyluslfonate (P13)	Phosphate (P30)
Resolution, 4 µm column	1.25	1.10	0.99	1.68	1.49
Resolution, 9 µm column	1.16	None	None	1.26	1.08

FIGURE 3. Determination of organic acids and inorganic anions in orange juice by using a prototype Dionex IonPac AS11-HC-4µm column with high-pressure ion chromatography (IC conditions refer to Figure 1).

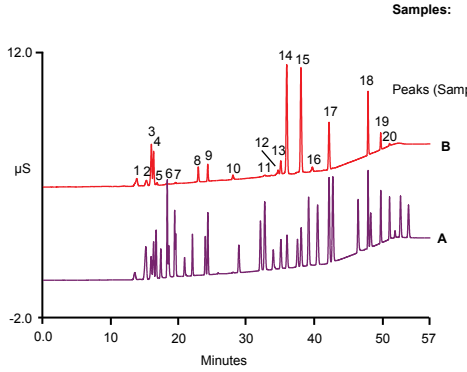


Samples:
A: Standard mix (peak names refer to Figure 1)
B: Orange juice (1:40 dilution with DI water)

Peaks (Sample B):

1. Quinate	3.4 mg/L
2. Acetate	2.5
3. Unknown	--
4. Formate	1.3
5. Chloride	1.3
6. Succinate	0.5
7. Malate	42.7
8. Sulfate	1.8
9. Oxalate	3.0
10. Phosphate	3.0
11. Citrate	165.5
12. Chromate	2.1

FIGURE 4. Determination of organic acids and inorganic anions in white wine using a Dionex IonPac AS11-HC-4µm, 4 × 250 mm prototype anion-exchange column (for IC conditions refer to Figure 1).



Samples: **A: Standard mix (peak names refer to Figure 1)**
B: White wine (1:40 dilution with DI water)

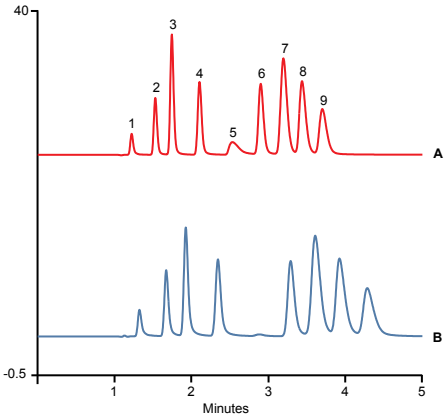
Peaks (Sample B):		mg/L
1.	Quinate	0.1
2.	Fluoride	2.1
3.	Lactate	21.3
4.	Acetate	7.2
5.	Glycolate	0.5
6.	Formate	1.1
7.	Pyruvate	0.8
8.	Unknown	--
9.	Chloride	1.6
10.	Sorbate	11.3
11.	Nitrate	0.8
12.	Unknown	--
13.	Succinate	5.9
14.	Malate	51.4
15.	Tartrate	43.5
16.	Unknown	--
17.	Sulfate	9.3
18.	Phosphate	19.2
19.	Citrate	6.2
20.	Chromate	0.9

Prototype 2 mm i.d. Dionex IonPac AS18-4µm Analytical 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 waters using a manually prepared carbonate-based eluent and suppressed conductivity detection. Later, the development of the Dionex IonPac AS18 hydroxide-selective columns permitted use of RFIC system methods in accordance with U.S. EPA Method 300.0 (A). Reagent-Free IC systems can electrolytically generate eluent inline by just adding water, which improves selectivity and reproducibility, and eliminates errors and labor to prepare hydroxide eluent manually.

Now Reagent-Free IC methods can be employed on the Dionex IonPac AS18 chemistry using 4 µm particle-sized columns for maximum performance. Inorganic anions that commonly exist in environmental water or drinking water samples can be analyzed using a Dionex IonPac AS18-4µm analytical column with an HPIC system using well-established RFIC system techniques. The modified high-pressure system combined with prototype smaller-particle-size columns permit fast run times without compromising resolution also with 2 and 4 mm columns (Figure 5).

FIGURE 5. Without sacrificing the baseline resolution and column efficiency, a shortened run time was obtained by using a prototype smaller-particle-size column (4 µm) compared to a larger-particle-size (7.5 µm) column for the separation of nine anions.

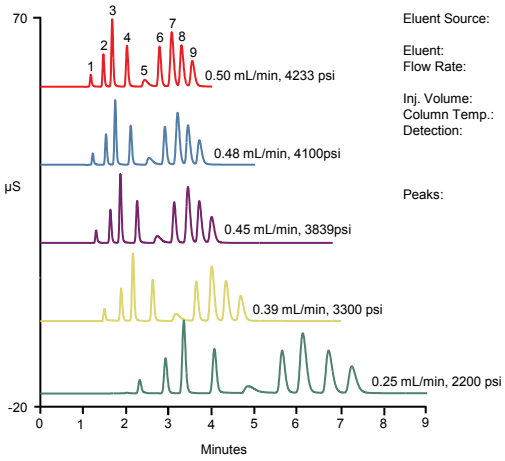


Column:	A) Dionex IonPac AS18-4µm 2 × 150 mm prototype B) Dionex IonPac AS18-Fast, 7.5 µm, 2 × 150 mm
Eluent Source:	A) Prototype high-pressure Dionex EGC KOH B) Dionex EGC III KOH
Eluent:	23 mM KOH
Flow Rate:	0.45 mL/min
Inj. Volume:	5 µL
Column Temp.:	30 °C
Detection:	Suppressed conductivity, Dionex ASRS 300, 2 mm, 24 mA

Chromatograms:
A: Under 3839 psi, 4 µm particle-size column was operated on a modified Dionex ICS-5000 system
B: Under 2200 psi, 7.5 µm particle-size column was operated on a Dionex ICS-5000 system

Peaks:		
1.	Fluoride	0.5 mg/L
2.	Chlorite	5.0
3.	Chloride	3.0
4.	Nitrite	5.0
5.	Carbonate	--
6.	Bromide	10.0
7.	Sulfate	10.0
8.	Nitrate	10.0
9.	Chlorate	10.0

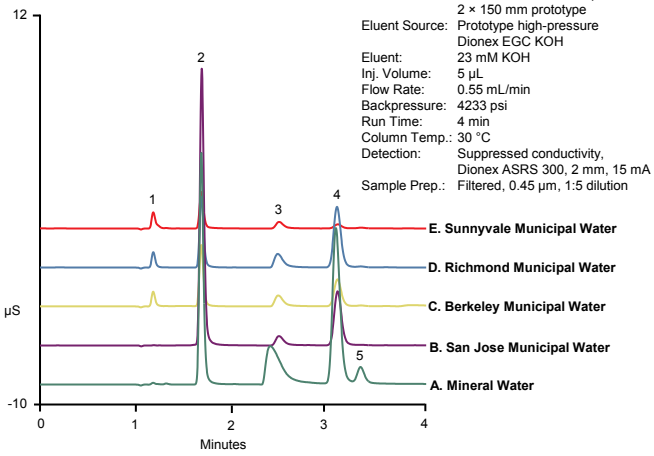
FIGURE 6. Determination of nine inorganic anions using a prototype Dionex IonPac AS18-4µm, 2 × 150 mm anion-exchange column. At a high flow rate of 0.50 mL/min, a high backpressure of 4233 psi resulted with a run time of only 4 min with good baseline resolution.



Column:	Dionex IonPac AS18-4µm, 2 × 150 mm prototype
Eluent Source:	Prototype high-pressure Dionex EGC KOH
Eluent:	23 mM Potassium hydroxide
Flow Rate:	0.25, 0.35, 0.39, 0.45, and 0.50 mL/min
Inj. Volume:	5 µL
Column Temp.:	30 °C
Detection:	Suppressed conductivity, Dionex ASRS 300, 2 mm, recycle mode, 15 mA

Peaks:	
1. Fluoride	0.5 mg/L
2. Chlorite	5.0
3. Chloride	3.0
4. Nitrite	5.0
5. Carbonate	20.0
6. Bromide	10.0
7. Sulfate	10.0
8. Nitrate	10.0
9. Chlorate	10.0

FIGURE 7. Determination of inorganic anions in water samples using a prototype Dionex IonPac AS18-4µm 2 × 150 mm column. Five anions in municipal or mineral water samples were eluted within 4 min on the 4 µm particle size column, emphasizing the fast sample runs possible with this column.



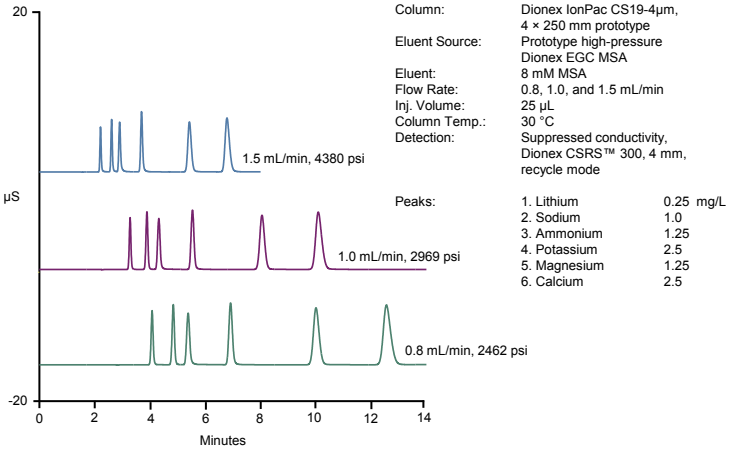
Column:	Dionex IonPac AS18-4µm, 2 × 150 mm prototype
Eluent Source:	Prototype high-pressure Dionex EGC KOH
Eluent:	23 mM KOH
Inj. Volume:	5 µL
Flow Rate:	0.55 mL/min
Backpressure:	4233 psi
Run Time:	4 min
Column Temp.:	30 °C
Detection:	Suppressed conductivity, Dionex ASRS 300, 2 mm, 15 mA
Sample Prep.:	Filtered, 0.45 µm, 1:5 dilution

Peaks:

	A	B	C	D	E	
1. Fluoride	- -	- -	0.40	0.40	0.50	mg/L
2. Chloride	7.47	36.25	1.94	2.27	1.19	
3. Carbonate	116.03	60.10	19.30	25.35	8.84	
4. Sulfate	20.72	28.33	3.46	7.94	0.50	
5. Nitrate	2.48	- -	- -	- -	- -	

Smaller Particle Size Analytical Column for Cation Analysis

FIGURE 8. This application demonstrates resolution of the six cations within 7 min using a prototype Dionex IonPac CS19-4µm 4 × 250 mm 4 µm particle-size analytical column at high flow rates. Fast sample runs with high peak efficiency and resolution are possible with the 4 µm particle size column; additionally, it demonstrates the requirement of high pressure (4380 psi) capability of HPIC at a flow rate of 1.5 mL/min.



Conclusion

The smaller resin particles produce a backpressure over 3000 psi at increased flow rates with the Dionex IonPac AS18-4µm analytical column prototype, and at standard flow rates with the Dionex IonPac AS11-HC-4µm and CS19-4µm analytical column prototypes. This requires a new, high-pressure analytical system. The modified Dionex ICS-5000 system provides the platform to operate these smaller-particle-size columns under high back pressure, yielding highly efficient separations for high resolution or fast analyses and higher throughput.

▪The Dionex IonPac AS11-HC-4µm analytical column provides improved separation for monovalent carboxylic acids and improved resolution between formate and butyrate, methylsulfonate and pyruvate, bromate and chloride, and phosphate and phthalate, compared to the standard 9 µm particle-size column.

▪The Dionex IonPac AS18-4µm analytical column provides efficient anion separations at higher flow rates thereby increasing throughput for environmental and mineral water samples.

▪Fast sample runs with high peak efficiency and high resolution of cations and amines are possible with the Dionex IonPac CS19-4µm column.

References

For more information regarding Thermo Scientific Dionex IonPac AS18, IonPac AS11-HC and IonPac CS19 analytical columns, refer to follow Thermo Fisher Scientific application notes and website.

1.Thermo Fisher Scientific. *Application Note 154, Determination of Inorganic Anions in Environmental Water Using a Hydroxide-Selective Column.*

2.Thermo Fisher Scientific. *Application Note 143, Determination of Organic Acids in Fruit Juices.*

3.http://www.dionex.com/en-us/products/columns/ic-rfic/cation-packed/ionpac-cs19/lp-110108.html

Acknowledgements

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