

Fast Separations of Counter Ion in an Allergy Drug Tablet Using High-Pressure Capillary IC

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Key Words

Ion Chromatography, IonSwift MAX 200, HPIC, ICS-5000+, Pharmaceutical, Chloride

Goal

Demonstrating the rapid separation and quantification of a pharmaceutical counter ion.

Introduction

Many drugs are manufactured in a salt form to provide solubility, bioavailability, and stability. Counter ion determinations are important to confirm the correct stoichiometry and formula weight of the drug but also to test for drug impurities. High Pressure Capillary ion chromatography is the latest advancement in ion chromatography instrumentation. Typically with an Reagent-Free™ ion chromatography (RFIC™) system, the system pressure is limited to < 3000 psi because of the limitations of materials in the RFIC accessories. Now with the upgrade to high pressure, the Thermo Scientific™ Dionex™ ICS-5000+ HPIC™ capillary system can operate at system pressures < 5000 psi. This advance in technology allows increased capillary flow rates with all the same advantages as standard pressure capillary IC, resulting in low consumption of water (30 to 40 mL/d of water) and low waste generation. In capillary IC, the system can remain on without a loss in resources, i.e., capillary IC is always on and ready for analysis. These advantages result in:

- Greater ease-of-use
- Longer eluent generator cartridge life
- Lower cost of ownership

Additionally, with the increase in mass sensitivity, comparable results are achieved as with standard bore system using a sample injection of only 0.4 µL. In a previous study published in AB 136, counter ions were determined on a water extract of a pharmaceutical drug used to allergies using standard pressure capillary IC.¹



In this study, inorganic anions from an allergy treatment pharmaceutical tablet dissolved in water are separated by anion-exchange chromatography on a capillary size Thermo Scientific™ Dionex™ IonSwift™ MAX 200 monolith IC column. The Dionex IonSwift MAX 200 column is designed with a monolith backbone and optimized with Thermo Scientific™ Dionex™ IonPac™ AS19 anion-exchange chemistry. This column was selected for these high pressure cap IC experiments because of the high efficiency and high flow rate characteristics. In these experiments, the chloride counteranion was separated at 10, 20, and 25 µL/min flow rates using an electrolytically-generated gradient. At 25 µL/min flow rate conditions, the system backpressure approaches 4500 psi which is well within the new high pressure tolerances while reducing the run time from 20 to 8 min. The analytes are detected by suppressed conductivity detection as they elute from the column, using the Thermo Scientific™ Dionex™ ACES™ Anion Capillary Electrolytic Suppressor, specifically optimized for capillary IC.

Equipment

Dionex High Pressure ICS-5000⁺ HPIC Reagent-Free capillary IC system

- ICS-5000⁺ SP Single Pump or DP Dual Pump module
- ICS-5000⁺ EG Eluent Generator module
- ICS-5000⁺ DC Detector/Chromatography module with Thermo Scientific™ Dionex™ IC Cube™ and high pressure degas cartridge
- Thermo Scientific Dionex AS-AP Autosampler
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data system (CDS)

Reagents and Standards

- 18 M -cm degassed deionized water
- Thermo Scientific Dionex Combined Seven Anion II Standard (Dionex P/N 057590)

Samples

Pharmaceutical tablet prescribed for the treatment of seasonal allergies

Conditions

Columns:	Dionex IonSwift MAX 200, 0.25 × 250 mm
Eluent Source:	Thermo Scientific Dionex EGC KOH capillary cartridge with Thermo Scientific Dionex capillary CR-ATC Continuously Regenerated Anion Trap Column
Gradient:	2 to 50 mM KOH
Flow Rate:	A: 10 µL/min B: 20 min C: 25 µL/min
Dionex IC Cube Temp.: *	30 °C
Compartment Temp.:	15 °C
Inj. Volume:	0.4 µL
Detection:	Suppressed conductivity, Dionex ACES 300, Thermo Scientific Dionex CRD 200 Carbonate Removal Device (Capillary), recycle mode, A: 8 mA; B: 15 mA; C: 18 mA
Background Conductance:	0.5–0.8 µS conductance
Noise:	< 0.3 nS
System Backpressure:	A: 1900 psi; B: 3700 psi, C: 4500 psi

*The Dionex IC Cube heater controls the separation temperature by controlling the column cartridge temperature. The original term of “column temperature” refers to the temperature in the bottom DC compartment which is not used for capillary IC. The part numbers of the consumables for this method are shown in Table 1.

Table 1. Consumables list.

Product Name	Type, Capillary	Dionex Part Number
Dionex EGC-KOH	Eluent Generator cartridge	072076
Dionex CR-ATC	Electrolytic trap column	072078
Dionex IonSwift MAX-200	Separation column	075889
Dionex CRD 200	Carbonate removal device	072054
Dionex ACES	Suppressor	072052
Dionex HP fittings (blue)	Bolts / Ferrules	074449 / 074373
EG Degas HP cartridge	High pressure degas cartridge, up to 5000 psi	074459
Dionex AS-AP autosampler vials	Package of 100, polystyrene vials, caps, blue septa	074228
Thermo Scientific™ Dionex™ OnGuard™ RP II cartridge	Sample preparation, pkg of 48	057084

Standard and Sample Preparation

The Dionex Seven Anion II Standard was diluted appropriately for calibration. The sample was a 60 mg tablet from a prescription allergy pharmaceutical drug, ground to a powder with a mortar and pestle and mixed thoroughly. Approximately 20 mg of the ground tablet was added to 20 mL of deionized water, and heated and stirred for 30 min at 80 °C. The sample mixtures were then cooled to room temperature, filtered with a 0.45 µm, IC syringe filter, Dionex OnGuard RP filter, and diluted 1:10 with deionized water prior to analysis.

Tip: It is important to use 18 M -cm resistivity, deionized water for standards, eluent, and autosampler flush solution. It is recommended to degas the deionized water intended for eluent. (An appropriate degassing method is vacuum filtration.) Using deionized water with resistivity less than 18 M -cm can reduce sensitivity, introduce contamination, and affect calibration, thereby resulting in inaccurate quantification. Results can vary and contamination introduced from samples can affect the chromatography.

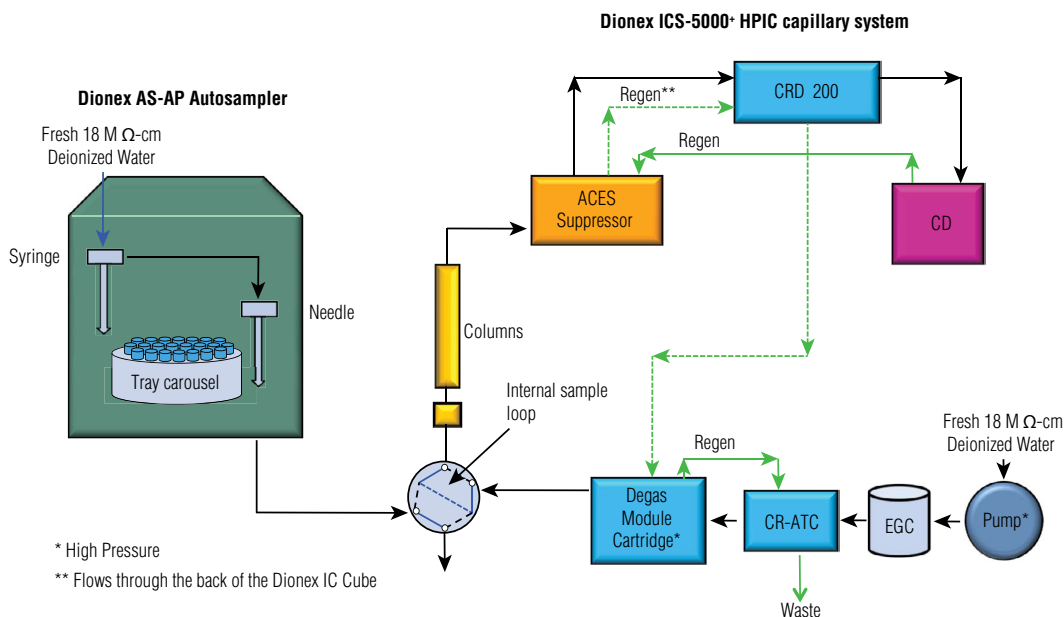
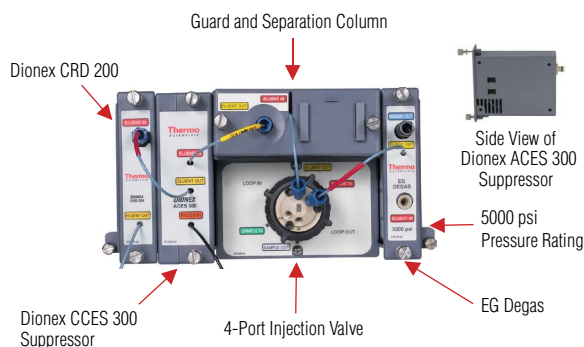


Figure 1. Flow diagram.

Instrument Setup and Installation

Tip: To achieve the best chromatography with capillary IC, it is important to minimize void volumes between connections by using precision cut tubing, high pressure connectors and fittings (colored blue), and by seating the ferrule > 2 mm above the end of the tubing. These tips are thoroughly discussed in “TN 113: Practical Guidance for Capillary IC”.² Extra care should be used to prevent introducing air into any of the consumables or tubing by observing a steady liquid flow before installing the next device in line. The high pressure Dionex ICS-5000⁺ HPIC capillary IC system is designed to operate continuously up to 5000 psi which results in very low noise. To setup this application, plumb the consumables and modules of the Dionex ICS-5000⁺ HPIC system, according to Figure 1.

Install and hydrate the Dionex EGC-KOH capillary cartridge and Dionex CR-ATC trap column. Install the EG Degas cartridge, Dionex CRD 200 cartridge, and the Dionex ACES Anion Capillary Electrolytic Suppressor capillary devices into the Dionex IC Cube (Figure 2). Hydrate the devices according to the product manuals and Section 3.18 of the Dionex ICS-5000 installation manual.¹⁻⁵ Install the columns and complete the configuration according to Figure 1. Detailed instructions are described in TN 131, the product manuals, and the instrument installation and operator’s manuals.¹⁻⁵

Figure 2. Dionex ICS-5000⁺ IC Cube .

Results and Discussion

Counter anions in drug formulations can include inorganic cations, inorganic anions, and organic acids. Therefore, to determine counteranions in this allergy pharmaceutical drug, the Dionex IonSwift MAX 200 was selected for its high resolution anion-exchange chemistry and fast separations of oxyhalides and inorganic anions. Although this sample contains only chloride, this column is suitable for separations of inorganic anions, oxyhalides, and some organic acids.

To determine the analyte concentrations, we determined the peak area response to concentration by calibrating with duplicate injections of the 50-, 100-, and 200-fold diluted Dionex Combined Seven Anion II Standard. A linear regression curve was used for the fluoride, chloride, nitrite, nitrate, bromide, sulfate, and phosphate peaks, resulting in linear coefficients of $r^2 > 0.999$.

Here we demonstrate the effects of flow rate on the 5-fold diluted extract from the allergy tablet (Figure 3). By just increasing the flow rate from 10 to 25 $\mu\text{L}/\text{min}$ using the high pressure Dionex ICS-5000+ HPIC capillary IC capable of operating at 5000 psi, the elution time of single analyte - chloride is reduced from 7 min to 2.5 min.

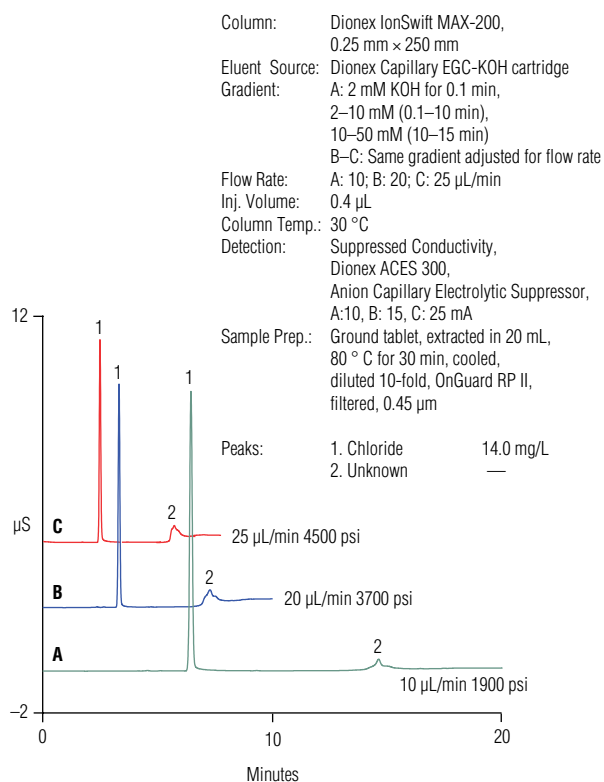


Figure 3. Fast separations of counter anions in an allergy pharmaceutical drug tablet using high pressure capillary IC.

Conclusion

This application demonstrates the advantages of high pressure capillary IC using the high efficiency Dionex IonSwift MAX 200 capillary size anion-exchange monolith column to provide high sample throughput by simply increasing the flow rate on a high pressure capable Dionex ICS-5000+ HPIC capillary IC, resulting in saving time and money.

For additional information on counter ion determinations in pharmaceutical drugs by high pressure and standard pressure capillary IC, please refer to AB 136, Thermo Fisher Scientific poster *High Pressure Capillary Ion Chromatograph for the Fast Separation of Pharmaceutical Relevant Inorganic Anions and Cations*, and Pharmaceutical Capillary IC Applications in Dionex Capillary IC Library website.^{6–8} Counter ion determinations using standard bore and microbore IC are thoroughly discussed in AN 116, AN 164, AN 190, and AN 210 and De Borba et al poster.^{9–13}

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