Identification and Quantification of 22 Common Anions in Pharmaceuticals in a Single Run Using HPIC with Suppressed Conductivity and Charge Detection

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Overview

- An anion analysis method was developed to identify and quantify multiple counter and impurity ions
- The method described uses a capillary HPIC system with suppressed conductivity (CD) and charge (QD) detectors and a Thermo Scientific™ Dionex™ IonPac™ AS11HC-4µm capillary column. The method separates 22 common pharmaceutical anions in a single run.
- The counter ion profile of an allergy drug was analyzed using the method. Chloride was quantified with high sensitivity (LOQ = 0.004 mg/L) and a large linear range (0.1 to 500 mg/L).

Introduction

Ion analysis is important for the pharmaceutical industry because many active pharmaceutical ingredients (APIs) exist in their salt form. Pharmaceutical products are strictly regulated by the United States Food and Drug Administration (U.S. FDA) and other regulatory agencies, and must be tested for composition to verify their identity, strength, quality and purity.[1] The importance of ion analysis for the pharmaceutical industry has increased in recent years, not only because ion analysis is required for the final products in their salt form, but also ion analysis is becoming more critical for early stage drug development. Identification and quantification of ions is used in material quality control and counter ion selection during early stage drug development. The monitoring of material quality has become increasingly critical due to increased outsourcing of API synthesis. The APIs received from vendors may be contaminated with different counter ions during their synthesis, and therefore, have different physicochemical (solubility, crystallinity, hygroscopicity) and pharmacokinetic properties; Additionally, selecting a proper counter ion to improve API solubility and stability has become a increasing critical step in formulation development due to a trend that new drug-like compounds are less and less aqueous soluble.

Conventional HPLC is not suitable for these ion analysis, because they are typically small ions that lack a chromophore and are not retained by HPLC column. Ion chromatography (IC) with suppressed conductivity detection (CD) is an established and sensitive method used for ion determination.[2,3] IC has been used previously in U.S. Pharmacopeia (USP) methods for single ion analysis of final pharmaceutical products. However ion analysis method is still needed to provide both counter and impurity ion profiles for APIs and formulations, as demanded by recent pharmaceutical developments.

In this application, 26 anions, which cover the majority counter and impurity ions in pharmaceutical samples, were selected for study with the objective to identify and quantify the analytes in a single run. The counteranions were separated on a high-capacity and high efficiency, 4 µm particle ion-exchange columns, facilitated on a Reagent-Free™ IC (RFIC™) high-pressure™ capable capillary IC system (HPIC™). The counteranions were detected by suppressed conductivity detection (CD) and the Thermo Scientific Dionex Charge Detector (QD). The resulting method is powerful and easy-to-use tool applicable for the pharmaceutical industry.

Experimental

Standards and Sample Preparation

Stock standards: Twenty-six individual stock standards (~1000 mg/L) were prepared by dissolving reagents for: chloride, gluconate, acetate, glycolate, formate, pyruvate, glucuronate, nitrate, bromide, glutarate, succinate, malate, tartrate, malonate, benzoate, maleate, sulfate, fumarate, phosphate, citrate, tosylate, benzenesulfonate, lactate, fluoride, nitrite, and trifluoroacetate in 18 MΩ-cm resistivity deionized water.
Mixed standard: Prepare the mixed standard from the 1000 mg/L anion stock standards and deionized water.
Chloride calibration standards: Dilute the 1000 mg/L chloride stock solution appropriately with deionized water to make nine chloride calibration standards ranging from 0.1 to 500 mg/L.

Samples: An over-the-counter (OTC) allergy drug with API as a chloride salt was purchased from a local pharmacy. Samples were prepared by dissolving one tablet in 1000 mL of deionized water and then filtering with a 0.20 µm IC syringe filter prior to injection to remove insoluble particles. Some filtered samples were diluted with deionized water.

Ion Chromatography Conditions

Instrument:	Thermo Scientific Dionex ICS-5000$^+$ HPIC capillary system
Column:	Dionex IonPac AS11-HC-4µm, 0.4 × 250 mm
Eluent Source:	Thermo Scientific Dionex EGC-KOH capillary cartridge
Gradient:	1.5–2 mM KOH (0–5 min), 2–8 mM (5–8 min), 8–16 mM (8–26 min), 16–70 mM (26–32 min), 70 mM (32–38 min).
Flow Rate:	0.0150 mL/min
Inj. Volume:	0.40 µL
Column Temp.:	30 °C
IC Cube Temp.:	15 °C
Detection:	CD: Suppressed conductivity detection, AutoSuppression™ Thermo Scientific™ Dionex™ ACES™ 300 Anion Electrolytic Suppressor, recycle mode
	QD: Charge detection, 6V

Data Analysis

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software.

Figure 1 shows the schematic of the flow diagram of the HPIC system used for this application. It is a high-pressure capillary IC system (HPIC) with CD / QD detectors. The capillary IC components are configured in close proximity in Thermo Scientific™ Dionex™ IC Cube™ module (Figure 2) to minimize the flow path.

FIGURE 1. Schematic of capillary HPIC system with dual detectors.

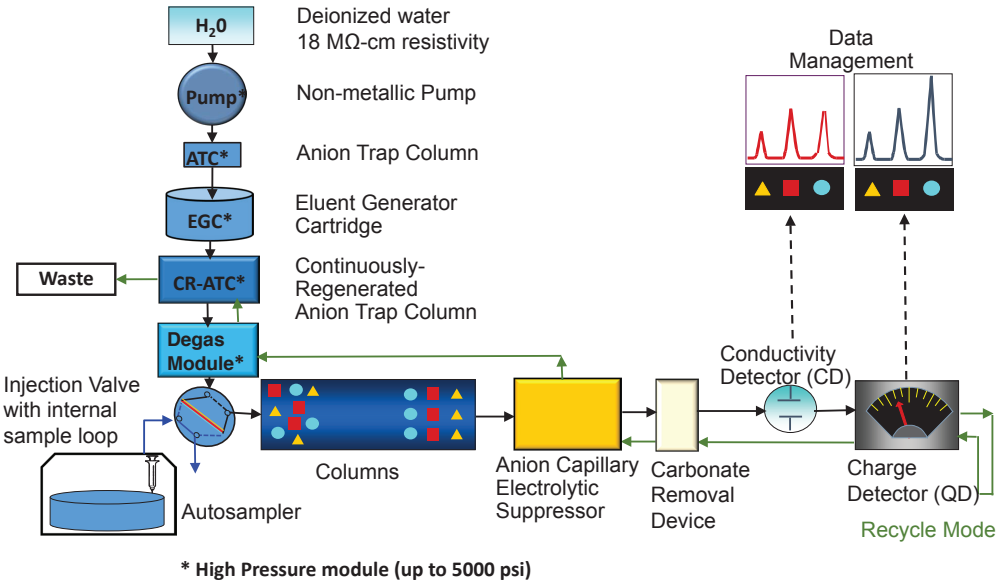
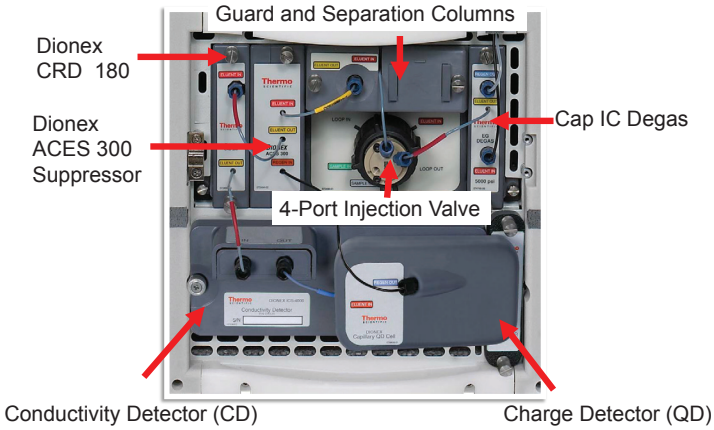


FIGURE 2. Thermo Scientific Dionex IC Cube module.

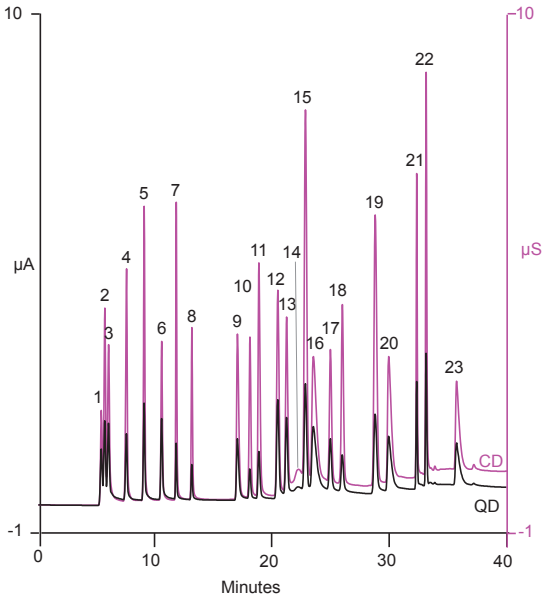


Results and Discussion

Twenty-six of most commonly used counter ions and commonly seen impurity ions in pharmaceutical samples-were included in this study. The Dionex IonPac AS11-HC-4µm capillary column was selected for its ability to separate various organic and inorganic acids in a wide range of products including-fruit juices and wine.[4] The 4µm resin particle technology has further improved the resolution and increased the column efficiency. As can be seen in Figure 3, 22 of 26 organic and inorganic anions were separated in a single run in less than 40 minutes. Four anions coelute with the other ions of interest, and therefore removed from the mixed standard.

The QD detector is an excellent orthogonal complement for the CD detector. Because each ion had a characteristic retention time, CD response, and QD response (Figure 3), all ions were identified and quantified with confidence using the two detectors.

FIGURE 3. Chromatogram of an anion standard mix.



Peaks	Ret. T (Min)	CD (µS-Min)	QD (µA-Min)	Conc. (mg/L)
1.Gluconate	5.45	0.324	0.210	7.5
2. Lactate	5.75	0.667	0.324	6.8
3. Acetate	6.08	0.657	0.345	9.6
4. Formate	7.62	0.792	0.238	8.5
5. Pyruvate	9.12	0.823	0.327	9.1
6. Galacturonate	10.60	0.487	0.298	11.1
7. Chloride	11.86	0.670	0.134	2.1
8. Nitrite	13.20	0.458	0.096	2.1
9. TFA	17.12	0.774	0.313	8.5
10. Bromide	18.19	0.599	0.110	4.2
11. Nitrate	19.97	0.975	0.199	4.8
12. Glutarate	20.50	1.003	0.481	8.6
13. Succinate	21.25	0.779	0.346	8.5
14. Carbonate	-	-	-	
15. Tartrate	22.86	1.979	0.565	10.3
16. Benzoate	23.64	1.203	0.612	18.6
17. Maleate	24.99	0.631	0.249	6.6
18. Sulfate	26.01	0.829	0.191	3.4
19. Fumarate	28.82	1.587	0.489	7.5
20. Benzenesulfonate	30.05	1.040	0.469	17.7
21. Phosphate	32.29	0.660	0.289	7.8
22. Citrate	33.09	0.999	0.358	11.3
23. Tosylate	35.80	0.700	0.355	13.3

Coelution: Gluconate/Fluoride, Acetate/Glycolate, Succinate/Malate, and Tartrate/Malonate

Chloride is the most prominent counter ion used in pharmaceuticals. The application example reported here uses the method to analyze an allergy drug in which both APIs are chloride salts.

Figure 4-1 shows the chromatograms of a water blank, an allergy drug tablet dissolved in 1000 mL water, and its 5-fold dilution. The results confirm that the drug has a clean counter ion profile with mainly chloride and trace amount of acetate, nitrite, nitrate and sulfate.

Figure 4-2 compares the CD and QD chromatograms of the undiluted sample. An additional unknown anion (peak 3) detected by QD illustrates the advantages of having two detectors that are based on different technologies. For some analytes, CD can be used to detect and quantify them, but with QD they are opaque or minimally detected and vice versa.

FIGURE 4 Anions in an allergy drug tablet.

Figure 4-1 Chromatograms using suppressed conductivity (CD) detection.

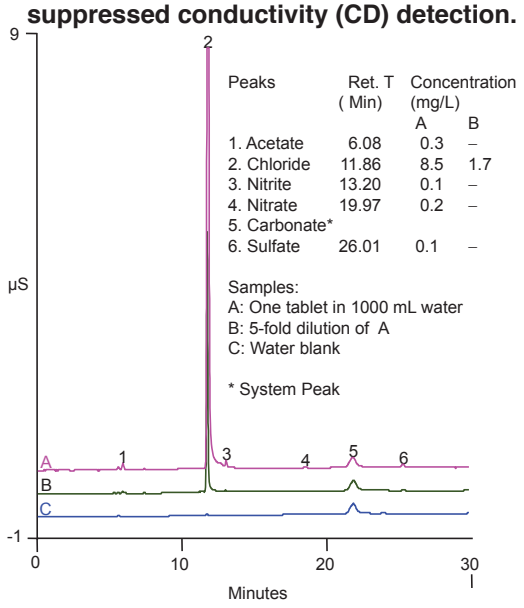
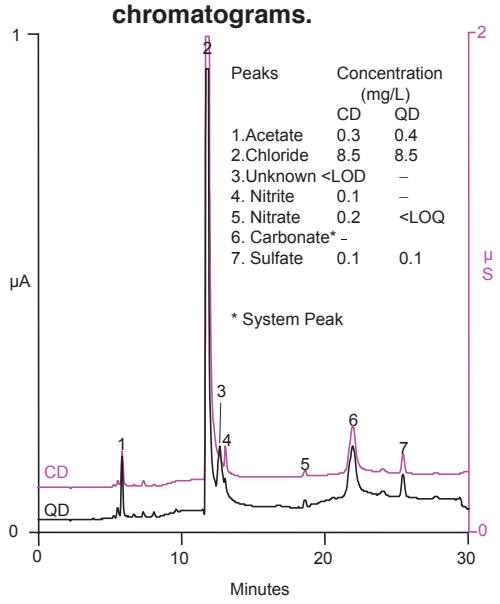


Figure 4-2 Comparison of CD and QD chromatograms.



To quantify chloride, nine chloride standards, from 0.1 to 500 mg/L, were used for the calibration. Figure 5 shows the CD calibration curve of chloride with a good linearity, r^2 = 0.9999. IC with suppressed conductivity detection (CD) is very sensitive with LOQ = 0.004 mg/L.

FIGURE 5 Calibration curve of chloride from 0.1 to 500 mg/L.

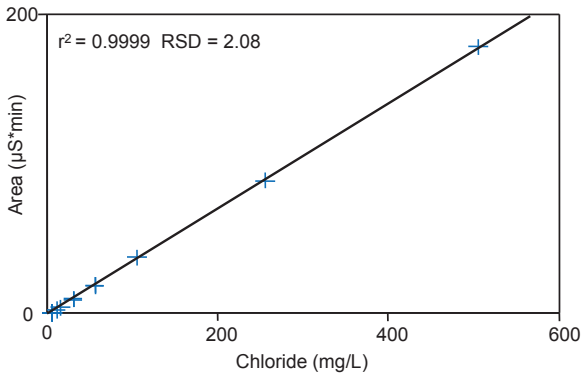


Table 1 summarizes the results from the analysis five tablets of an allergy drug. The chloride results using CD (8.32 mg/tablet) agree with the drug label. The concentrations from QD were higher (8.36 mg/tablet), possibly attributable to the interference of unknown peak 3.

TABLE 1. Chloride in allergy drug tablets.

Tablet No.	Weight (g/tablet)	Chloride (mg/tablet)		
		CD	QD	Label
1	0.710	8.50	8.47	
2	0.726	8.45	8.29	
3	0.692	7.93	8.02	
4	0.710	8.30	8.42	
5	0.730	8.39	8.61	
Average	**0.714**	8.32	8.36	**8.32**
RSD	**2.14**	**2.76**	**2.66**	

Conclusion

- A versatile and easy to use anion analysis method was developed for the pharmaceutical industry which is suitable for the study of both counter and impurity ion profiles for APIs and formulations.
- Using a capillary HPIC system with CD and QD detectors, and a Dionex IonPac AS11HC-4µm capillary column:
  - Twenty-two common pharmaceutical anions were separated in a single run.
  - Multiple counter ions in drug products were easily identified and quantified with confidence, which facilitated the profiling of this allergy drug.
  - For chloride, this method had a low limit of quantification (0.004 mg/L) and a large linear range (0.1 to 500 mg/L).

References

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