Determination of Tartaric Acid in Tolterodine Tartrate Drug Products by IC with Suppressed Conductivity Detection

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Application Note 1002

Key Words
Dionex IonPac AS20 Column, Dionex IonPac AS22 Column, Counterion, Drug Product, RFIC, Pharmaceutical

Introduction
Tolterodine is a quaternary ammonium compound used to treat urinary incontinence caused by abnormal bladder contraction. Quaternary ammonium compounds used as drug substances are typically prepared as salts. Pharmaceutical companies assay the counterion in the drug substance to ensure it is present at the appropriate levels. The counterion for tolterodine is tartrate. The counterion assay can also serve as a check of the amount of active pharmaceutical ingredient in the sample. Ion chromatography (IC) is often used for counterion assays.[1]

Although an IC assay for tartrate in tolterodine tartrate drug substance that uses carbonate/bicarbonate eluent has been previously reported,[2] that method requires a minimum of 15 min per analysis (20 min analyses are shown) and the analyst must manually prepare dilute sulfuric acid solution for the suppressor. A faster analysis that requires no preparation of eluents or regenerant should be possible. Shown here is a comparison of the separation and detection of tartaric acid using carbonate/bicarbonate versus hydroxide eluent.

Goal
To develop a better IC method to determine tartrate in tolterodine drug products

Equipment
- Thermo Scientific Dionex ICS-3000 system* including:
  - DP Dual Gradient Pump
  - EG Eluent Generator Module
  - DC Detector/Chromatography Module
  - CD Conductivity Detector
  - AS Autosampler
- Thermo Scientific Dionex Chromeleon Chromatography Data System (CDS) software version 6.80, SR9 or above

*A Thermo Scientific Dionex ICS-5000 or any Reagent-Free™ IC (RFIC™) system may also be used. If using a traditional IC system, eluents must be prepared manually.

Reagents and Standards
- Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistivity or better
- Tartaric acid ($[CHOHCOOH]_2$, 99.5% AR grade, Ajax Finechem)
- Sodium carbonate (Na_2CO_3), 0.5 M, P/N 037162
- Sodium bicarbonate ($NaHCO_3$), 0.5 M, P/N 037163
- Thermo Scientific Dionex 7-Anion Standard, 50 mL (P/N 056933)

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Preparation of Solutions and Reagents

Na_2CO_3, 6 mM/$NaHCO_3$, 1.4 mM Eluent

Weigh 1970.4 g DI water in a 2 L eluent bottle. Add 24 mL of 0.5 M sodium carbonate and 5.6 mL of 0.5 M sodium bicarbonate to the eluent bottle and mix.

KOH, 20 mM Eluent

The EG module produces the eluent using the Thermo Scientific Dionex EluGen EGC II KOH cartridge and DI water supplied by the pump. The eluent concentration is controlled by Chromeleon™ CDS software. The EG degasser requires 14 MPa (2000 psi) system backpressure, which ensures optimal removal of electrolytic gas produced by the EG cartridge. For more information about adding system backpressure, refer to the Dionex ICS-3000 system operator's manual (Document No. 065031) or any other RFIC system operator's manual.

Standard Solutions

Tartaric Acid Stock Standard Solution, 1000 mg/L

Place 0.1 g tartaric acid in a 100 mL volumetric flask, dissolve with DI water, and bring to volume with DI water.

Working Standard Solutions

Add the appropriate volumes of 1000 mg/L tartaric acid stock standard solution into separate 100 mL volumetric flasks and bring to volume with DI water. The volumes of the tartaric acid stock standard solution used for preparation of the working standard solutions are shown in Table 1.

Table 1. The preparation of working standard solutions

Level	Concentration (mg/L)	Volume of 1000 mg/L Tartaric Acid Stock Standard Solution for 100 mL Preparation (mL)
1	5	0.50
2	10	1.00
3	15	1.50
4	20	2.00

Sample Preparation

The drug sample was purchased from a local pharmacy. Five capsules containing tolterodine tartrate were each opened and weighed to determine the average weight of a single capsule. This weight was used to describe the sample preparation below. The average weight and the weight of each sample are shown in Table 2.

After weighing, grind contents to a fine powder. Place 0.184 g of the fine sample powder in a 100 mL volumetric flask. Add 50 mL of DI water and place the volumetric flask in an ultrasonic bath for 5 min. After sonication, bring to volume with DI water. Mix and filter with a 0.45 µm syringe filter before injecting the sample. The label of the drug product shows a nominal value of 4 mg of tolterodine tartrate per capsule. Based on the label and sample preparation process, the concentration of tartrate after sample preparation will be 12.6 mg/L. The calculated concentration of tartrate is used to determine the amount of tolterodine tartrate in the capsule because the molar ratio of tolterodine to tartrate is 1:1.

Prepare the spiked sample in the same manner, with 0.4 mL of 1000 mg/L tartaric acid added to the sample before dissolution.

Table 2. Weight of drug sample in each capsule

Capsule No.	(g/Capsule)
1	0.178
2	0.182
3	0.189
4	0.186
5	0.184
Average	0.184

Chromatographic Conditions

Condition 1	
Column:	Thermo Scientific Dionex IonPac AS20 Analytical, 4 × 250 mm (P/N 063148)
Guard Column:	Dionex IonPac™ AG20 Guard, 4 × 50 mm (P/N 063154)
Eluent Source:	Dionex EluGen EGC II KOH (P/N 058900) with Thermo Scientific Dionex CR-ATC Continuously Regenerated Anion Trap Column (P/N 060477)
Eluent:	20 mM KOH
Flow Rate:	1 mL/min
Pressure:	~2100 psi
Inj. Volume:	10 µL
Column Temp.:	30 °C
Detection:	Suppressed conductivity
Suppressor:	Thermo Scientific Dionex ASRS 300 Anion Self-Regenerating Suppressor, 4 mm (P/N 064554), recycle mode, current 50 mA

Condition 2	
Column:	Dionex IonPac AS22 Analytical, 4 × 250 mm (P/N 064141)
Guard Column:	Dionex IonPac AG22 Guard, 4 × 50 mm (P/N 064139)
Eluent:	6 mM Na_2CO_3/1.4 mM $NaHCO_3$
Flow Rate:	1.2 mL/min
Pressure:	~1850 psi
Inj. Volume:	10 µL
Column Temp.:	30 °C
Detection:	Suppressed conductivity
Suppressor:	Dionex ASRS™ 300, 4 mm (P/N 064554), recycle mode, current 40 mA

Results and Discussion

Separation

Separation and drug product analyses are presented using both hydroxide and carbonate/bicarbonate eluents. An EG module was used for separation with the hydroxide eluent, and manual eluent preparation was used for separation with the carbonate/bicarbonate eluent. Using the EG module or manual eluent preparation are options for both methods; however, EG-based methods will typically provide superior reproducibility.

The Dionex IonPac AS20 and AS22 columns were used with hydroxide and carbonate/bicarbonate eluents, respectively, for the determination of tartaric acid in tolterodine tartrate. Figure 1 shows that tartrate (tartaric acid) is easily resolved from seven common anions using a hydroxide gradient with the Dionex IonPac AS20 column. The method first elutes six of the seven anions along with tartrate, after which the eluent strength is increased to elute the trivalent phosphate. Application of this method to the drug sample (Figure 1, trace B) showed no significant amount of phosphate in the sample; therefore, the method was simplified to an isocratic method using 20 mM KOH (Figure 2). These chromatographic conditions are referred to as Condition 1.

Figure 3 shows separation of tartrate and seven common anions using isocratic elution with a carbonate/bicarbonate eluent on the Dionex IonPac AS22 column. Phosphate is divalent at this pH (8 to 9) and therefore elutes before tartrate. These chromatographic conditions are referred to as Condition 2. This method requires 12 min per analysis and can be accelerated using the Dionex IonPac AS22-Fast column, which is designed for fast, relatively simple separations.

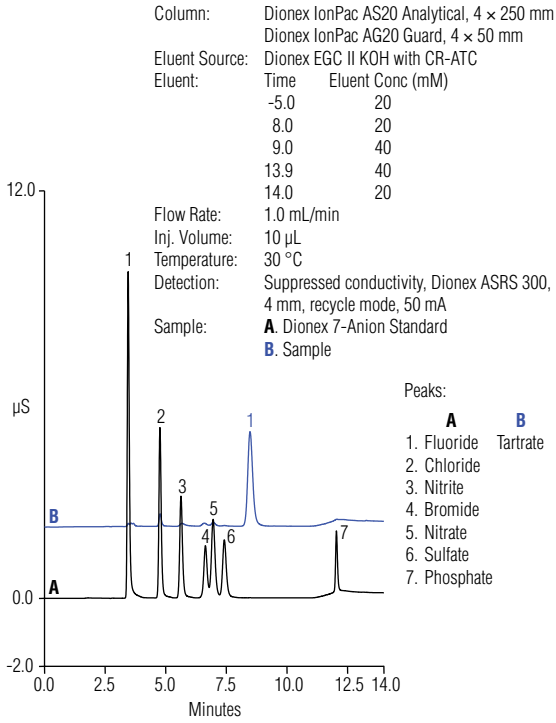

Figure 1. Overlay of chromatograms of gradient elution of the Dionex 7-Anion Standard and sample using the Dionex IonPac AS20 column and hydroxide eluent

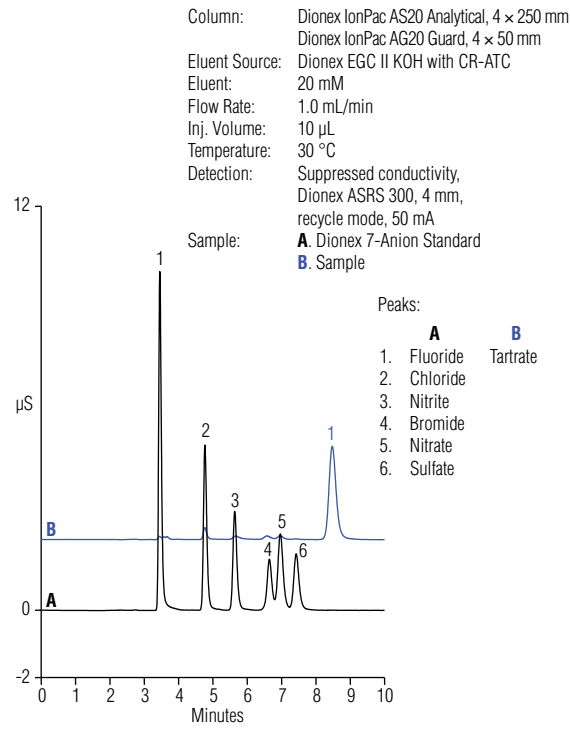

Figure 2. Overlay of chromatograms of the Dionex 7-Anion Standard and sample using Condition 1

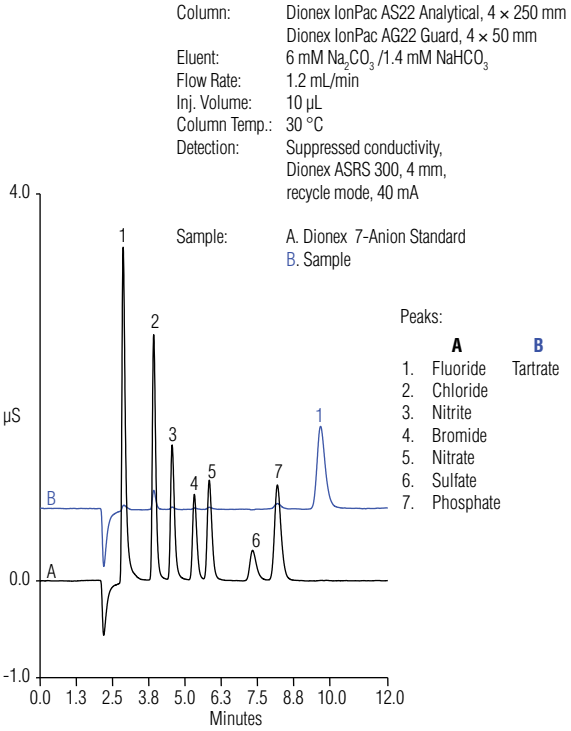

Figure 3. Overlay of chromatograms of the Dionex 7-Anion Standard and sample using Condition 2

Method Calibration

The methods were calibrated before sample analysis using the same working standards. Both methods demonstrated a linear relationship between peak area and concentration. The coefficients of determination (r^2) obtained from Conditions 1 and 2 were 0.9996 and 0.9990, respectively. Method calibration results and working standard concentrations are shown in Table 3.

Sample Analysis

The sample was a drug product (in capsule form) containing tolterodine tartrate. Each capsule was labeled as containing 4 mg of tolterodine tartrate. After sample preparation, five injections of each sample were made for each of the two chromatographic conditions to determine the amount of tartrate in each sample.

Table 4 shows the results of these analyses and Figures 4 and 5 show representative sample chromatograms. Table 4 shows good accuracy and reproducibility for both methods, with tartrate at 98.4% and 96.0% of the label value, respectively. Method accuracy was also determined by spiking a known amount of tartaric acid into the sample before sample preparation to yield an additional 4 mg/L after sample preparation. The recovery results of 100% and 103% for Conditions 1 and 2, respectively, confirm accuracy for both methods. These results are also shown in Table 4. Interestingly, neither the U.S. nor the European Pharmacopeias contains a monograph to instruct on the assay of tolterodine tartrate.

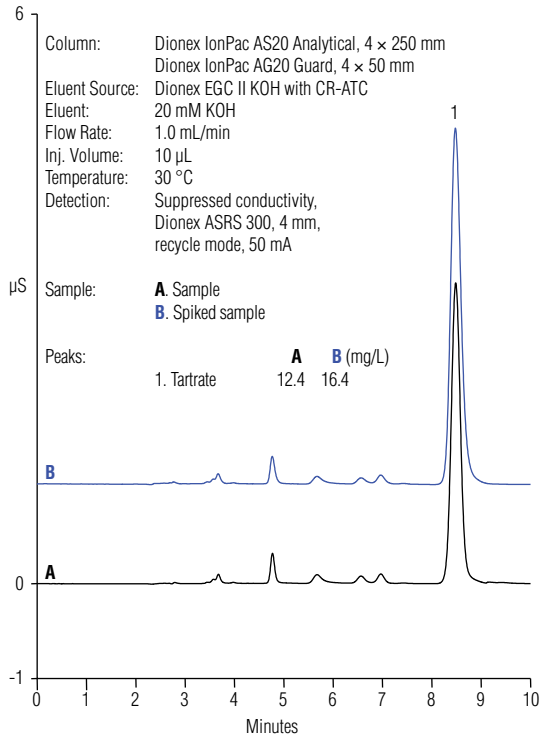

Figure 4. Overlay of chromatograms of a sample and spiked sample obtained using Condition 1

Table 3. Working standard concentrations and calibration results for Conditions 1 and 2

Condition 1								
Analyte	Concentration (mg/L)				Results			
	Level 1	Level 2	Level 3	Level 4	Points	r^2	Offset	Slope
Tartaric acid	5	10	15	20	12	0.9996	0.0061	0.0588
Condition 2								
Analyte	Concentration (mg/L)				Results			
	Level 1	Level 2	Level 3	Level 4	Points	r^2	Offset	Slope
Tartaric acid	5	10	15	20	12	0.9990	-0.0010	0.0232

Table 4. Determination of tartrate in encapsulated drug samples, along with spiked sample recovery results

Injection No.	Condition 1		Condition 2	
	Sample (mg/L)	Spiked Sample (mg/L)	Sample (mg/L)	Spiked Sample (mg/L)
1	12.4	16.5	12.1	16.3
2	12.3	16.4	12.1	16.3
3	12.3	16.3	12.0	16.1
4	12.4	16.4	12.2	16.2
5	12.4	16.5	12.0	16.3
Average	12.4	16.4	12.1	16.2
RSD	0.29	0.56	0.74	0.49
Assay (%)	98.4%	—	96.0%	—

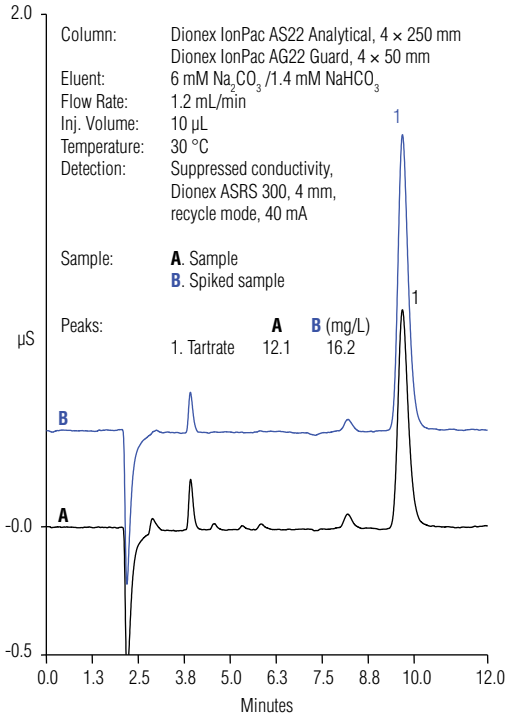

Figure 5. Overlay of chromatograms of a sample and spiked sample obtained using Condition 2

Conclusion

The work shown here demonstrates two IC methods that are accurate and reproducible for determination of tartrate in an encapsulated drug product containing the drug substance tolterodine tartrate. One method uses eluent generation and the other uses manual eluent preparation; however, both methods can be executed using either method of eluent preparation. Both methods use an electrolytic suppressor, so the time and labor associated with preparing dilute sulfuric acid regenerant are eliminated. The RFIC method demonstrates improved accuracy and productivity because both eluent and regenerant are prepared automatically, eliminating the time and effort required to manually prepare these reagents while also reducing the possibility of errors and contamination. The RFIC system method requires only 10 min per analysis. The second method uses a carbonate/bicarbonate eluent and has an analysis time of 12 min.

References

1. Dionex (now part of Thermo Scientific) Application Note 190: Determination of Sulfate Counter Ion and Anionic Impurities in Aminoglycoside Drug Substances by Ion Chromatography with Suppressed Conductivity Detection. LPN1946, Sunnyvale, CA, 2007. [Online] www.dionex.com/en-us/webdocs/61810-AN190_IC_counter_ions_aminoglycosides_28Aug2007_LPN1946.pdf (accessed Feb. 28, 2012).
2. Deshpande, G.R.; Rao, B.M.; Someswararao, N. Quantitative Determination of Tartaric Acid in Tolterodine Tartrate by Ion Chromatography Using Conductivity Detection. *RASAYAN J. Chem.* **2009**, *2*, 101–107.

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