Application Note 1047

Simultaneous Determination of Tartaric Acid and Tolterodine in Tolterodine Tartrate

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Key Words
Acclaim Trinity P1 Column, Pharmaceutical, API, Counterion, HPLC, Charged Aerosol Detection

Goal
To design a simultaneous determination of tartaric acid and tolterodine in a tolterodine tartrate drug product

Introduction
Tolterodine tartrate is a medication that is used to treat urinary urgency, thereby reducing the frequency of passing urine and urinary incontinence. Tolterodine, a quaternary ammonium compound, is the active pharmaceutical ingredient (API) and tartrate is the counterion in tolterodine tartrate. It is important for pharmaceutical companies to ensure that a drug product contains the appropriate amount of its API. This is usually validated with a high-performance liquid chromatography (HPLC)-based assay because many APIs are paired with a counterion to form the drug substance. With knowledge of the ratio of API to counterion, the API amount in both a drug substance and a drug product can be checked by assaying the counterion.

Counterions are typically assayed by ion chromatography (IC). Previously, Thermo Scientific™ Application Note 1002 demonstrated that use of either a hydroxide eluent with a Thermo Scientific™ Dionex™ IonPac™ AS20 column or a carbonate/bicarbonate eluent with a Dionex IonPac AS22 column would enable tartrate to be successfully assayed in a tolterodine tartrate drug product.

This study reports an HPLC method in which the API and counterion of a drug product are determined in a single injection. Tolterodine and tartrate from a tolterodine tartrate capsule are separated on a Thermo Scientific™ Acclaim™ Trinity™ P1 column. This trimode column allows separation in the reversed-phase, weak anion-exchange, or strong cation-exchange mode, or any combination of the three modes. The unique construction of the Acclaim Trinity P1 column provides spatial separation of these three modes, allowing each to be independently controlled. After separation, tolterodine and tartrate are detected by a Thermo Scientific™ Dionex™ Corona™ *ultra*™ Charged Aerosol Detector. This detector detects semivolatile and nonvolatile analytes regardless of whether they contain a chromophore, thus making it ideal for determination of counterions, many of which lack a chromophore.

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Equipment

- Thermo Scientific™ Dionex™ UltiMate™ 3000 LC system, including:
  - SRD-3600 Integrated Solvent and Degasser Rack
  - DGP-3600RS Dual Ternary Rapid Separation Pump System
  - WPS-3000RS Rapid Separation Wellplate Sampler
  - TCC-3000RS Rapid Separation Thermostatted Column Compartment
- Corona *ultra* Charged Aerosol Detector
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software version 6.80, SR9 or higher

Reagents and Standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistivity or better
- Ammonium Acetate (CH_3COONH_4, Fisher Scientific P/N A637-500)
- Acetic Acid, Glacial, Certified ACS (CH_3COOH, Fisher Scientific)
- Acetonitrile, HPLC Grade (CH_3CN, Fisher Scientific)
- Tolterodine L-tartrate, ≥98% ($C_{22}H_{31}NO{\cdot}C_4H_6O_6$, Sigma-Aldrich®)

Chromatographic Conditions

Columns:	Acclaim Trinity P1, 3 µm Guard, 3.0 × 10 mm (P/N 071390) Acclaim Trinity P1, 3 µm Analytical, 3.0 × 100 mm (P/N 071387)		
Mobile Phase:	A: 5% 0.2 M NH_4OAc, pH 4/52% Water/ 43% CH_3CN B: 80% 0.2 M NH_4OAc, pH 4/20% CH_3CN		
	Time (min)	A (%)	B (%)
	-5.0	100	0
	0.0	100	0
	1.5	100	0
	2.0	0	100
	8.0	0	100
Flow Rate:	0.8 mL/min		
Inj. Volume:	50 µL		
Column Temp:	35 °C		
Detection:	Corona *ultra* Charged Aerosol Detector		
Nebulizer Temp:	30 °C		
Data Collection Rate:	5 Hz		
Filter Constant:	Medium		

Preparation of Solutions and Reagents

0.2 M Ammonium Acetate (NH_4OAc), pH 4

Place 15.9 g ammonium acetate in a 1000 mL beaker, dissolve with 900 mL DI water, and adjust to pH 4 with acetic acid. Transfer the solution into a 1000 mL volumetric flask and bring to volume with DI water.

Mobile Phase A, 5% 0.2 M NH_4OAc, pH 4/ 52% Water/43% CH_3CN

Mix 50 mL 0.2 M NH_4OAc, 520 mL DI water, and 430 mL CH_3CN in a 1000 mL bottle. Filter the solution with a 0.2 µM filter.

Mobile Phase B, 80% 0.2 M NH_4OAc, pH 4/ 20% CH^3CN

Mix 800 mL 0.2 M NH_4OAc with 200 mL CH_3CN in a 1000 mL bottle. Filter the solution with a 0.2 µM filter.

Tolterodine Tartrate Stock Standard Solution, 1000 mg/L

Place 0.01 g of tolterodine tartrate in a 10 mL volumetric flask, dissolve in DI water, and bring to volume with DI water.

Working Standard Solutions

Add the appropriate volumes of 1000 mg/L tolterodine tartrate stock standard solution into separate 10 mL volumetric flasks and bring to volume with DI water. The volumes of tolterodine tartrate stock standard solution used for the preparation of working standard solutions are shown in Table 1.

Sample Preparation

The drug sample was purchased from a Bangkok, Thailand pharmacy. Five capsules containing tolderdine tartrate were each opened and weighed to find the average weight of the contents of a capsule. This average weight was used for sample preparation. The data are shown in Table 2.

Table 2. Weight of the contents of a tolterodine tartrate capsule.

Capsule No.	Weight of Capsule Content (g)
1	0.191
2	0.194
3	0.191
4	0.195
5	0.184
Average	**0.191**

Table 1. Preparation of working standard solutions.

Concentration Level	Volume of 1000 mg/L Tolterodine Tartrate Stock Standard Solution for 10 mL Preparation (mL)	Tolterodine Tartrate Concentration (mg/L)	Tolterodine Concentration (mg/L)	Tartrate Concentration (mg/L)
1	0.15	15	10.3	4.7
2	0.30	30	20.5	9.5
3	0.45	45	30.8	14.2
4	0.60	60	41.1	18.9

After weighing, grind the contents to a fine powder and place 0.191 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 drug product label indicates that a capsule contains 4 mg of tolterodine tartrate. Based on the label and sample preparation process, the concentration of tolterodine tartrate, tolterodine, and tartrate after sample preparation will be 40.0, 27.4, and 12.6 mg/L, respectively.

Prepare the spiked sample in the same manner and add 1.2 mL of 1000 mg/L tolterodine tartrate stock standard solution to the sample before dissolution. The concentration of added tolterodine tartrate, tolterodine, and tartrate will be 12, 8.2, and 3.8 mg/L, respectively.

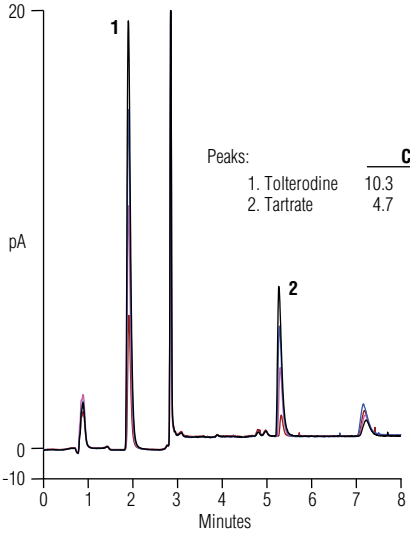
Results and Discussion

Separation and Detection

Tolterodine is quaternary ammonium compound and has tartrate, a carboxylic acid, as its counterion. A conventional C18 reversed-phase (RP) column can be used to separate and determine tolterodine, but it is unlikely to provide adequate retention of tartrate under the necessary conditions due to tartrate's polarity. An anion-exchange column is more suitable for tartrate. Thus, a stationary phase capable of delivering multiple separation modes in a reproducible manner is ideal for determining a hydrophobic analyte and a charged analyte in the same injection. The Acclaim Trinity P1 column has these capabilities. It is a trimode column with RP, strong cation-exchange, and weak anion-exchange (WAX) separation modes. For this application, tolterodine is retained primarily by the RP mode and tartrate primarily by the WAX mode.

Figure 1 shows the separation of tolterodine and tartrate. The method required 13 min per analysis (an additional 5 min for column equilibration) using ammonium acetate and acetonitrile mobile phases. These volatile mobile phase components allow the analytes to be detected with a Corona *ultra* Charged Aerosol Detector, making this method compatible with mass spectrometry detection. Because a Corona *ultra* Charged Aerosol Detector will detect all semivolatile and nonvolatile components, it is important to establish a blank to understand what peaks may arise from the mobile phase and the water used to prepare the sample. Figure 2 shows such a blank, and a comparison with Figure 1 reveals there are no peaks that will interfere with the determinations of tolterodine and tartrate.

Columns:	Acclaim Trinity P1, 3 µm Guard, 3.0 × 10 mm Acclaim Trinity P1, 3 µm Analytical, 3.0 × 100 mm
Eluent :	A: 5% 0.2 M NH_4OAc, pH4/52% Water/43% CH_3CN B: 80% 0.2 M NH_4OAc, pH4/20% CH_3CN
Gradient:	Time (min) -5 0 1.5 2 8 A 100 100 100 0 0 B 0 0 0 100 100
Flow Rate:	0.80 mL/min
Inj. Volume:	50 µL
Column Temp:	35 °C
Tray Temp:	10 °C
Detection:	Corona *ultra* Charged Aerosol Detector
Nebulizer Temp:	30 °C
Data Collection Rate:	5 Hz
Filter Constant:	Medium
Sample:	Calibration Standard



Peaks:	Concentrations				
1. Tolterodine	10.3	20.5	30.8	41.1	mg/L
2. Tartrate	4.7	9.5	14.2	18.9	

Figure 1. Overlay chromatograms of the calibration standards of tolterodine and tartrate.

Columns:	Acclaim Trinity P1, 3 µm Guard, 3.0 × 10 mm Acclaim Trinity P1, 3 µm Analytical, 3.0 × 100 mm
Eluent :	A: 5% 0.2 M NH_4OAc, pH4/52% Water/43% CH_3CN B: 80% 0.2 M NH_4OAc, pH4/20% CH_3CN
Gradient:	Time (min) -5 0 1.5 2 8 A 100 100 100 0 0 B 0 0 0 100 100
Flow Rate:	0.80 mL/min
Inj. Volume:	50 µL
Column Temp:	35 °C
Tray Temp:	10 °C
Detection:	Corona *ultra* Charged Aerosol Detector
Nebulizer Temp:	30 °C
Data Collection Rate:	5 Hz
Filter Constant:	Medium
Sample:	Water

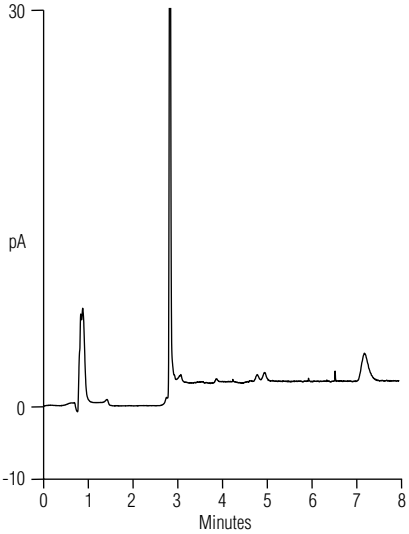


Figure 2. Chromatogram of a DI water blank.

Method Calibration

The method was calibrated before the sample analysis using four concentrations of the standards. The calibration range was configured using the estimated concentration of tolterodine tartrate after sample preparation to keep the sample concentration in the middle of the calibration range. The method showed a linear relationship between analyte concentration and peak area for both tolterodine and tartrate. The coefficients of determination (r^2) of tolterodine and tartrate were 0.9990 and 0.9998, respectively. Figure 1 shows the overlay of chromatograms of the working (calibration) standards. Table 3 shows the concentrations of the working standards and calibration results.

Sample Analysis

The drug sample's label states that each capsule contains 4 mg of tolterodine tartrate. After sample preparation, five sample injections were made to determine the amounts of tolterodine and tartrate in the sample. The measured concentrations were then compared to the calculated concentrations, based on the label and subsequent sample preparation. The results showed that tolterodine and tartrate concentrations were 97.5% and 98.4% of that stated on the label, respectively.

Method accuracy was determined by spiking known amounts of tolterodine and tartrate into the sample before sample preparation. The recovery results of tolterodine and tartrate are 96.3 and 94.7%, respectively. The RSDs of the sample and spiked sample injections were between 0.49 and 1.06%. Sample and recovery results are shown in Table 4, and Figure 3 shows the overlay of chromatograms of the sample and spiked sample.

Columns:	Acclaim Trinity P1, 3 µm Guard, 3.0 × 10 mm Acclaim Trinity P1, 3 µm Analytical, 3.0 × 100 mm
Eluent :	A: 5% 0.2 M NH_4OAc, pH4/52% Water/43% CH_3CN B: 80% 0.2 M NH_4OAc, pH4/20% CH_3CN
Gradient:	Time (min) -5 0 1.5 2 8 A 100 100 100 0 0 B 0 0 0 100 100
Flow Rate:	0.80 mL/min
Inj. Volume:	50 µL
Column Temp:	35 °C
Tray Temp:	10 °C
Detection:	Corona *ultra* Charged Aerosol Detector
Nebulizer Temp:	30 °C
Data Collection Rate:	5 Hz
Filter Constant:	Medium
Sample:	a. Drug sample b. Spiked drug sample

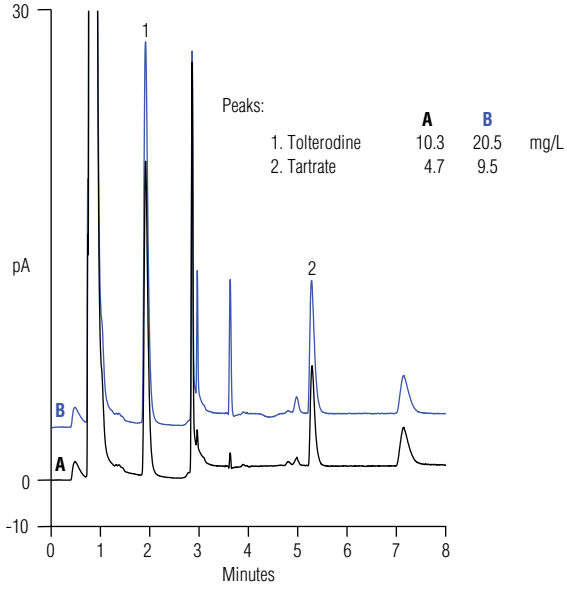


Figure 3. Overlay of chromatograms of the drug sample and the spiked drug sample.

Table 3. Working standard concentrations and calibration results.

Analyte	Concentration (mg/L)				Results			
	Level 1	Level 2	Level 3	Level 4	Points	r^2	Offset	Slope
Tolterodine Tartrate	15	30	45	60	—	—	—	—
Tolterodine	10.3	20.5	30.8	41.1	12	0.9990	0.2179	0.1286
Tartrate	4.7	9.5	14.2	18.9	12	0.9998	-0.4460	0.1429

Table 4. Sample and recovery results.

Injection No.	Found Concentration in Sample (mg/L)		Found Concentration in Spiked Sample (mg/L)	
	Tolterodine (Expected Conc 27.4 mg/L)	Tartrate (Expected Conc 12.6 mg/L)	Tolterodine (Spiked Conc 8.2 mg/L)	Tartrate (Spiked Conc 3.8 mg/L)
1	26.9	12.4	34.5	16.2
2	26.9	12.5	34.3	16.0
3	26.8	12.4	34.7	15.9
4	26.6	12.4	34.5	15.8
5	26.5	12.4	34.7	16.0
Average	26.7	12.4	34.6	16.0
RSD	0.62	0.49	0.52	1.06
Assay (%)	97.5	98.4	—	—
Recovery (%)	—	—	96.3	94.7

Conclusion

This study demonstrates an accurate and reproducible HPLC method for the single-injection determination of tolterodine and tartrate—the API and its counterion—in an encapsulated drug product containing tolterodine tartrate. The method requires only 13 min per analysis using an Acclaim Trinity P1 column and Corona *ultra* Charged Aerosol Detector.

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