APPLICATION NOTE 73482

Determination of ammonia impurity in potassium bitartrate using ion chromatography

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Goal

To develop a sensitive and robust ion chromatography (IC)-based test for the determination of ammonia impurity in potassium bitartrate to modernize the test in the United States Pharmacopeia's (USP) Potassium Bitartrate monograph

Introduction

Potassium bitartrate is a laxative for medical use and is commonly used with sodium bicarbonate to produce carbon dioxide. The active ingredient is bitartrate. Ammonia is one of the impurities tested in potassium bitartrate. The USP has embarked on a global initiative to modernize many of the existing monographs across all compendia.[1] In the current USP monograph for potassium bitartrate, ammonia is measured by a colorimetric test,[2] which is a non-specific test requiring hazardous reagents. To address the need to update and modernize the ammonia impurity test, we propose a selective and sensitive IC method to replace the colorimetric test for the determination of ammonia impurity in potassium bitartrate. The USP has already replaced a colorimetric test for ammonia in its Sodium Bicarbonate monograph.[3-4]

Our IC method offers a significant improvement to the current impurity test in the USP monograph. The 5 min method uses a Thermo Scientific™ Dionex™ IonPac™ CS16 high capacity column, an electrolytically generated methanesulfonic acid (MSA) eluent, and suppressed conductivity detection on a Reagent-Free™ Ion Chromatography (RFIC™) system. Thermo Scientific™

Chromeleon™ Chromatography Data System (CDS) software allows the analyst to run regulatory compliant analyses in an enterprise environment—from method creation to final reporting. Because the RFIC system requires only deionized (DI) water as the carrier, it significantly simplifies system operation and improves analytical reproducibility. The linearity, precision, accuracy, ruggedness, and the limits of detection (LODs) and quantitation (LOQs) of this 5 min method were validated following the guidelines outlined in USP General Chapter <1225>, Validation of Compendial Methods.[5]

Experimental

Equipment

- Thermo Scientific™ Dionex™ ICS-6000 HPIC system* including:
  - Dionex ICS-6000 DP Pump module
  - Dionex ICS-6000 EG Eluent Generator module with high-pressure degasser module
  - Dionex ICS-6000 DC Detector/Chromatography module
  - CD Conductivity Detector
  - Tablet control
- Thermo Scientific™ Dionex™ AS-AP Autosampler with Sample Syringe, 250 µL (P/N 074306) and Buffer line, 1.2 mL (P/N 074989)

* This method can be run on any Dionex RFIC system, and a SP pump module can be used rather than the DP pump module.

HPIC consumables

- Thermo Scientific™ Dionex™ EGC 500 MSA Eluent Generator Cartridge (P/N 075779)
- Thermo Scientific™ Dionex™ CR-CTC 600 Continuously Regenerated Cation Trap Column (P/N 088663)
- Thermo Scientific™ Dionex™ CDRS 600 Cation Dynamically Regenerated Suppressor (4 mm, P/N 088668)
- Dionex IC PEEK Viper Fitting Kit for Dionex ICS-6000 4 mm systems with CD (Analytical) (P/N 088803)

Software

- Chromeleon Chromatography Data System software version 7.2

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistivity or better
- Thermo Scientific™ Dionex™ Combined Six Cation Standard-II, 50 mL (P/N 046070)
- Ammonia Standard, 1000 mg/L in water (Fisher Scientific P/N US-ICC-101)
- Potassium bitartrate USP reference standard (Sigma-Aldrich P/N 1549840-3G)

Sample

- Potassium L-tartrate monobasic, (puriss., meets analytical specification of Ph.Eur., BP, FCC, 99.5-100.5%), (Sigma-Aldrich P/N 25506-1KG)

IC conditions

Parameter	Value
Columns	Dionex IonPac CG16 Guard, 3 × 50 mm (P/N 079931) Dionex IonPac CS16 Analytical, 3 × 250 mm (P/N 059596)
Eluent source	Dionex EGC 500 MSA Eluent Generator Cartridge with CR-CTC 600 trap column
Eluent	55 mM MSA
Flow rate	0.7 mL/min
Column temperature	40 °C
Detector temperature	20 °C
Injection volume	25 µL (Full Loop)
Detection	Suppressed Conductivity, Dionex CDRS 600 Suppressor (4 mm), recycle mode, Use recommended voltage at constant voltage mode or 113 mA in constant current mode. Note: Do not use a Dionex CDRS 600 Suppressor (2 mm) for this application due to its capacity limit under high flow rate and eluent concentration conditions used here, which are not typical for this suppressor format.
System backpressure	~2600 psi (100 psi = 0.6894 MPa)
Background conductance	~0.3 µS/cm
Run time	5 min

Preparation of solutions and reagents

Note: Do not use glassware to prepare the solutions. Polymeric containers made of high-density polyethylene (HDPE) are recommended.

Ammonia primary dilution solution (ammonia PDS), 1.0 mg/L

Prepare the ammonia PDS by diluting of the 1000 mg/L ammonia standard with DI water. The ammonia PDS is used to prepare calibration standards, the system suitability solution, and the recovery test solutions.

Ammonia calibration standard solution

Calibration standards were prepared by diluting the 1.0 mg/L ammonia PDS to 0.01, 0.02, 0.05, 0.1, 0.12, 0.2, 0.5, and 1 mg/L with DI water. Mix thoroughly and store at 4 °C. These solutions should be prepared on the day of analysis.

USP potassium bitartrate test solution, 1000 mg/L

In the current USP monograph for potassium bitartrate, the ammonia test solution is a 2.5 mg/mL potassium bitartrate solution in water.[2] For the IC impurity test described here, further dilute the 2.5 mg/mL potassium bitartrate solution (prepared as per the current USP monograph) an additional 2.5-fold in DI water to obtain a 1000 mg/L test solution.

System suitability solution, 0.01% ammonia impurity in USP potassium bitartrate

Dilute the USP potassium bitartrate stock solution and ammonia PDS with DI water to make the system suitability solution containing 1 mg/mL of USP potassium bitartrate, and 0.1 mg/L of ammonia. Mix thoroughly and store at 4 °C. This solution should be prepared on the day of analysis.

Recovery test solutions

To make 1000 mg/L USP potassium bitartrate solution spiked with 0.02, 0.1, and 0.5 mg/L of ammonia in triplicate, mix 2.5 mg/mL USP potassium bitartrate, 1.0 mg/L ammonia PDS, and DI water to the corresponding concentrations (Table 1).

Table 1. Preparation of spiked samples for the recovery test (n=3)

Ammonia concentration (mg/L)	0.02	0.1	0.5
2.5 mg/L USP solution (mL)	4	4	4
1 mg/L Ammonia PDS (mL)	0.2	1	5
DI water (g)	5.8	5	1

Sample preparation, 1000 mg/L

Accurately weigh 100.0 mg potassium bitartrate into a 125 mL polypropylene bottle and dissolve in 100 mL (100.00 g) of DI water to make a 1000 mg/L sample test solution.

Robustness study

Following the guidelines in USP General Chapter <1225>, Validation of Compendial Methods,[5] and USP General Chapter <621> Chromatography,[6] the robustness of this method was evaluated by examining the retention time (RT), peak asymmetry, and resolution of the analyte after imposing small variations (±10%) in procedural parameters (e.g., flow rate, eluent concentration, column temperature). The same procedure was applied to two column sets from different lots. The following variations were tested:

- Flow rate at 0.7 mL/min, 0.63 mL/min, and 0.77 mL/min
- Column temperature at 40 ˚C, 36 ˚C, and 44 ˚C
- Eluent concentrations at 55 mM MSA, 49.5 mM MSA, and 60.5 mM MSA

Results and discussion

Separation and detection

The acceptance criterion for ammonia impurity in the current USP monograph for potassium bitartrate is NMT 0.01%. The concentration of potassium bitartrate at 1.0 mg/mL instead of 2.5 mg/mL in the current USP monograph was used for the validation of method performance. Not diluting the sample would overload the Dionex IonPac CS16 column, despite its high capacity.

The separation of ammonium and potassium was achieved within 5 min using a Dionex IonPac CS16 column set with 55 mM MSA at a 0.7 mL/min flow rate. This method uses a column temperature of 40 °C because the selectivity of the column for maximizing peak efficiencies is optimized at that temperature. Figure 1 shows the separation of 0.01% ammonia in a 1.0 mg/mL USP potassium bitartrate solution. Ammonium was well resolved from sodium and potassium present in the USP potassium bitartrate standard and confirmed by the reference standard. The result demonstrates that this method reliably detects 0.01% ammonia in the potassium bitartrate solution.

Column:	Dionex IonPac CG16 Guard, 3 × 50 mm Dionex IonPac CS16 Analytical, 3 × 250 mm
Eluent:	55 mM MSA
Eluent Source:	Dionex EGC 500 MSA cartridge with CR-CTC 600
Temperature:	40 °C
Flow Rate:	0.7 mL/min
Inj. Volume:	25 µL
Detection:	Dionex CDRS 600 suppressor, 4 mm, recycle mode, 113 mA
Samples:	A: 1 mg/mL USP potassium bitartrate spiked with 0.1 mg/L ammonium B: 1 mg/mL USP potassium bitartrate (non-spiked) C: 0.1 mg/L ammonium D: DI water
Peaks:	1. Sodium 2. Ammonium 3. Potassium

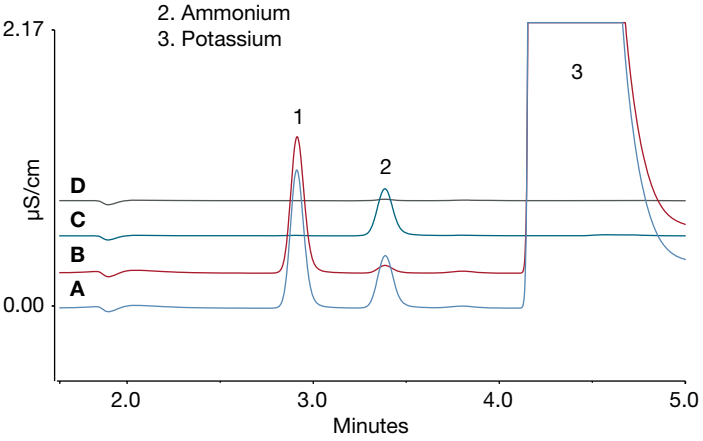

Figure 1. Separation and detection of 0.01% ammonia in potassium bitartrate

Accuracy

Method accuracy was validated by the recovery of spiked ammonia in 1.0 mg/mL USP potassium bitartrate test solution over three concentration levels, with three replicates of each concentration. The 1 mg/mL potassium bitartrate test solution was spiked with 0.02, 0.1, and 0.5 mg/L ammonia, which represented 0.002%, 0.01%, and 0.05%, respectively. The average recovery for ammonia ranged from 96 to 100% (Table 2).

Table 2. Recovery of ammonia in 1 mg/mL potassium bitartrate (n=3)

Spike level (mg/L)	Concentration	Recovery (%)
0.02	0.002%	96
0.1	0.01%	100
0.5	0.05%	97

Precision

The precision of an analytical procedure is typically expressed as the RSD of a series of measurements. It is determined by assessing a sufficient number of aliquots of a sample that have undergone the complete analytical procedure from sample preparation to final test. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines recommend that repeatability be assessed using a minimum of nine determinations covering the specified range for the procedure (i.e., three concentrations and three replicates of each concentration).[7-8] The retention time RSDs were <0.07% and the peak area RSDs ranged from 0.088 to 0.456% (Table 3). If using manually prepared mobile phases, the precisions—especially retention time precision—likely will not be as low as when using electrolytically generated eluent.

Detection limit (LOD) and quantitation limit (LOQ)

The USP/ICH defines LOD in terms of a signal-to-noise ratio (S/N) of 2:1 or 3:1 and LOQ as a ratio of 10:1.[5] Currently, the USP General Chapter <621> Chromatography defines S/N ratio as 2H/h, where H is the height of the peak measured from the peak apex to a baseline extrapolated over a distance ≥5 times the peak width at its half-height; h is the difference between the largest and smallest noise values observed over a distance ≥5 times the width at the half-height of the peak and, if possible, situated equally around the peak of interest.[6] The ICH documents also describe a common approach, which is to compare measured signals from samples with known low concentrations of analyte with those of blank samples.[7-8] The minimum concentration at which the analyte can reliably be detected is established. Using DI water as a blank to measure background noise, the sample signal was determined from the average peak height of seven injections of 0.01 mg/L ammonia standard solution. The LOD and LOQ for ammonia were 0.003 and 0.01 mg/L, respectively.

Linearity

The ICH and the USP General Chapter <1225> guidelines recommend a minimum of five concentrations to establish linearity in an assay.[5] For the determination

Table 3. Retention time and peak area precisions of ammonia in 1 mg/mL potassium bitartrate (n=3)

Spike level (mg/L)	Concentration	Retention time (min)	Retention time RSD	Peak area (µS*min)	Peak area RSD
0.02	0.002%	3.386	0.07	0.0127	0.456
0.1	0.01%	3.388	0.05	0.0422	0.137
0.5	0.05%	3.397	0	0.1738	0.088

of an impurity, the minimum specified range is from 50 to 120% of the acceptance criterion. Weak bases like ammonia, the suppression product of ammonium, are partially dissociated; thus, as their concentration increases, they yield a nonlinear response by suppressed conductivity detection. For this IC method, the coefficient of determination was 1.0 using a quadratic curve-fitting function for ammonia in the range of 0.01–1 mg/L (Figure 2). If a linear calibration is desired, use the Dionex SC-CERS 500 Salt Converter-Cation Electrolytically Regenerated Suppressor or narrow the concentration range.[9]

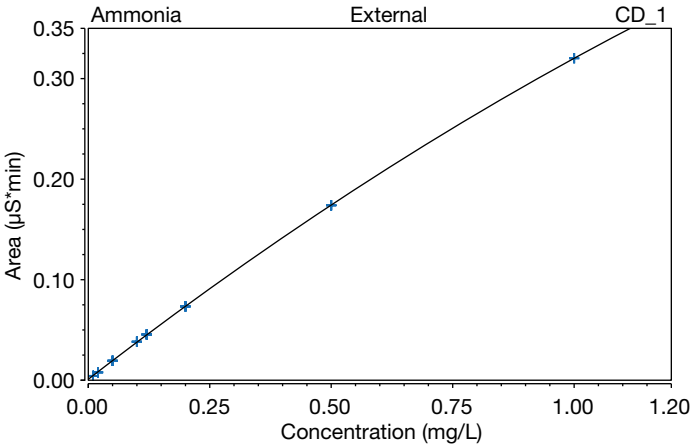

Figure 2. Ammonia response to concentration (0.01, 0.02, 0.05, 0.1, 0.12, 0.2, 0.5, and 1 mg/L)

Sample analysis

The USP monograph requires that potassium bitartrate contains no more than 0.01% ammonia. The USP potassium bitartrate reference standard and Sigma-Aldrich potassium bitartrate were analyzed to determine the ammonia impurity.

Ammonia percentage in potassium bitartrate

Prepare a 1 mg/mL solution of each potassium bitartrate product. Calculate the concentration (mg/L) of ammonia using the calibration curve. Calculate the percentage of ammonia in potassium bitartrate as below:

$$\text{Impurity \%} = \frac{\textit{Calculated concentration of ammonia (mg/L)}}{\textit{1000 (mg/L)}} \times 100$$

Acceptance criteria: no more than 0.01%

As shown in Table 4, both potassium bitartrate solutions had ammonia impurity much lower than the acceptance criterion of NMT 0.01%. Our method reliably quantifies ammonia at the 0.01% level and lower.

Table 4. Ammonia impurity in potassium bitartrate

	USP potassium bitartrate reference standard	Sigma-Aldrich potassium bitartrate
Average	0.001%	0.002%
RSD (n=5)	3.09	0.81

Robustness

The assay robustness was evaluated by measuring the influence of small variations (±10%) in procedural parameters (e.g., flow rate, eluent concentration, column temperature) on the RT, peak asymmetry, and resolution of the analyte on two column sets from two different lots. The peak asymmetry was evaluated using the USP formula. The ammonia resolution was determined relative to the sodium and potassium in a chromatogram using the USP formula.

The system suitability solution was injected three times at each chromatographic condition. The chromatographic resolution between sodium and ammonia was in the range of 3.04–3.42 on Column 1 and 3.04–3.36 on Column 2. The resolution between ammonia and potassium was in the range of 4.03–4.36 on Column 1 and 3.93–4.24 on Column 2. These results indicate the method was robust to both changes in chromatography conditions and column lots (Table 5, next page). The target analyte could be accurately measured under all tested conditions.

Conclusion

The high-capacity Dionex IonPac CS16 column is ideal for impurity testing. This test method is capable of determining ammonia with good accuracy and precision at its limit (in the current USP monograph) in potassium bitartrate. This 5 min method was validated following the guidelines outlined in USP General Chapter <1225>, Validation of Compendial Methods. The method is calibrated in the range 0.01 to 1 mg/L (r^2 = 1.0, quadratic fitting), precise with low retention time and peak area RSDs (<0.07% and 0.088–0.456%, respectively), accurate (96–100% of recovery), and robust for the ammonia test in potassium bitartrate. Compared to the time-consuming colorimetric method in the USP Potassium Bitartrate monograph, this IC-based test executed with an RFIC system offers a simple, accurate, and robust measurement of the analyte without handling hazardous reagents. Therefore, this method is a suitable candidate to replace the existing ammonia colorimetric test in the USP's Potassium Bitartrate monograph.

Table 5. Robustness of the IC-based test for ammonia in potassium bitartrate (n=3)

Parameter		Retention time (min)	Difference (%)	Asymmetry	Difference (%)	Resolution (relative to potassium)	Difference (%)	Resolution (relative to sodium)	Difference (%)
Column 1									
Flow rate	0.70 mL/min	3.39	-	1.15	-	4.20	-	3.21	-
	0.63 mL/min	3.75	10.8	1.14	-0.87	4.26	1.43	3.27	1.87
	0.77 mL/min	3.09	-8.86	1.12	-2.61	4.13	-1.67	3.14	-2.18
Eluent concentration	55.0 mM	3.39	-	1.15	-	4.20	-	3.21	-
	49.5 mM	3.57	5.31	1.13	-1.74	4.36	3.81	3.42	6.54
	60.5 mM	3.24	-4.25	1.15	0.00	4.06	-3.33	3.04	-5.30
Column temperature	40 °C	3.39	-	1.15	-	4.20	-	3.21	-
	36 °C	3.44	1.65	1.10	-4.35	4.36	3.81	3.24	0.93
	44 °C	3.34	-1.48	1.14	-0.87	4.03	-4.05	3.16	-1.56
Column 2									
Flow rate	0.70 mL/min	3.45	-	1.11	-	4.06	-	3.13	-
	0.63 mL/min	3.83	10.83	1.12	0.90	4.14	1.97	3.29	5.11
	0.77 mL/min	3.15	-8.86	1.06	-4.50	4.02	-0.99	3.10	-0.96
Eluent concentration	55.0 mM	3.45	-	1.11	-	4.06	-	3.13	-
	49.5 mM	3.63	5.21	1.10	-0.90	4.24	4.43	3.36	7.35
	60.5 mM	3.31	-4.23	1.05	-5.41	3.95	-2.71	3.04	-2.88
Column temperature	40 °C	3.45	-	1.11	-	4.06	-	3.13	-
	36 °C	3.51	1.65	1.05	-5.41	4.24	4.43	3.21	2.56
	44 °C	3.40	-1.62	1.06	-4.50	3.93	-3.20	3.14	0.32

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