Application Note 1073

Determination of Ammonia in Sodium Bicarbonate

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Suppressed Conductivity, High-Capacity Cation-Exchange Column, Dionex IonPac CS16 Column, Hemodialysis Solution, Ion Chromatography

Introduction

Sodium bicarbonate, or sodium hydrogen carbonate, is used in a wide variety of pharmaceutical and food products. One of the main pharmaceutical uses of sodium bicarbonate is for hemodialysis. For hemodialysis-grade sodium bicarbonate, the quality and purity requirements are stringent. Ammonia is one of the impurities that is assayed in sodium bicarbonate because the presence of elevated levels of ammonia in the blood stream can have adverse effects.

In the current United States Pharmacopeia-National Formulary (USP-NF) sodium bicarbonate monograph, ammonia is measured by a colorimetric assay. This assay is based on the reaction of ammonia with phenol under alkaline conditions (pH 11–12) in the presence of alkaline hypochlorite (bleach) and sodium nitroferricyanide (a strong oxidizing agent) that results in the formation of a blue/green-colored complex (indophenol blue).[1] The acceptance criteria is "no blue color develops" in 1 h upon addition of the sodium bicarbonate test solution to phenol, sodium nitroferricyanide, sodium citrate, and sodium hypochlorite. This correlates to a 0.002% limit for ammonia in sodium bicarbonate.[2] However, this assay is subjective, requires caution in handling and storing the strong oxidizing agent, and requires that waste not be exposed to acidic conditions that can liberate poisonous cyanide gas.

This study describes an ion chromatography (IC)-based method for the determination of ammonia (as ammonium) in sodium bicarbonate. The method performance characteristics—linearity, precision, accuracy, ruggedness, and the limits of detection (LODs) and quantitation (LOQs)—were measured according to the requirements described in the USP General Chapter <1225> guidelines. The USP General Chapter <1225>, Validation of Compendial Methods, describes the performance characteristics that must be considered to validate

a method submitted to the USP.[3] This informational chapter harmonizes, to the extent possible, with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology that address analytical procedures included as part of registration applications submitted within the European Commission, Japan, and the USA.[4]

Goal

To develop a sensitive and robust IC-based assay for ammonia in sodium bicarbonate; this assay must meet the detection limit criteria of 0.002% for ammonia in sodium bicarbonate.[1,2]

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Equipment and Software

- Thermo Scientific™ Dionex™ ICS-5000+ HPIC™ IC system, capable of supporting high-pressure IC, including:
  - SP Single Pump or DP Dual Pump
  - EG Eluent Generator
  - DC Detector/Chromatography Compartment with CD Conductivity Detector
- Thermo Scientific Dionex AS-AP Autosampler
- Thermo Scientific Dionex EGC III Methanesulfonic Acid (MSA) Eluent Generator Cartridge (P/N 074535) with a Thermo Scientific Dionex CR-CTC II Continuously Regenerated Cation Trap Column (P/N 066262) with 25 µL injection loop
- Thermo Scientific™ Dionex™ CSRS™ 300 Cation Self-Regenerating 2 mm Suppressor (P/N 064557) or Dionex SC-CSRS 300 2 mm Salt-Converter Cation Self-Regenerating Suppressor (P/N 067529)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software

Reagents and Standards

- Deionized (DI) water, 18 M -cm resistance or better
- Ammonia Standard, 1000 mg/mL in water (Fisher Scientific P/N US-ICC-101)

Conditions

Columns:	Thermo Scientific™ Dionex™ IonPac™ CG16 Guard, 3 × 50 mm (P/N 079931) Dionex IonPac CS16 Analytical, 3 × 250 mm (P/N 059596)
Eluent:	7 mM MSA for 26 min, 70 mM MSA from 26.1 to 34 min, re-equilibrate to 7 mM from 34.1 to 40 min
Flow Rate:	0.43 mL/min
Inj. Volume:	25 µL
Column Temp:	40 °C
Detector Temp:	30 °C
Detection:	Suppressed conductivity, Dionex CSRS 300 Cation Self-Regenerating Suppressor (P/N 064557), autosuppression recycle mode, current setting 36 mA
Back Pressure:	2600 psi
Background Conductance:	<0.250 µS
Noise:	~0.1–0.2 nS

Sample Preparation

Ammonia Standards

Prepare all standards using DI water with at least 18 M -cm resistivity. Prepare standards gravimetrically by making appropriate dilutions of a 1000 mg/L ammonia standard with DI water. Store standard solutions at 4 °C when not in use.

Sodium Bicarbonate

In the current USP monograph for sodium bicarbonate, the ammonia assay test solution is a 25 mg/mL sodium bicarbonate solution in water.[1] For the IC assay described here, further dilute the 25 mg/mL sodium bicarbonate solution (prepared as per the current USP monograph) an additional 25-fold in DI water to obtain a 1.0 mg/mL test solution.

General Design of the Robustness Study

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small variations in the procedural parameters. To study the robustness, determine the response of a defined standard solution (1.0 mg/mL sodium bicarbonate solution spiked with 0.02 mg/L ammonia) under these typical variations of analytical conditions:

- Mobile phase concentration (±2 mM MSA)
- Flow rate (±10%)
- Column temperature (±2 °C)
- Column (column sets from two different production batches)

Results

Separation and Determination of Ammonia

To determine ammonia in sodium bicarbonate, a high-capacity carboxylate-based cation-exchange column was used—the Dionex IonPac CS16 column, which is specially designed for samples with disparate concentration ratios of adjacent eluting cations (such as sodium and ammonia). The 25 mg/mL sodium bicarbonate solution (prepared as per the current USP monograph) was further diluted 25-fold in DI water to obtain a 1.0 mg/mL test solution that was used for assay validation. Not diluting the sample would overload the Dionex IonPac CS16 column, despite its high capacity.

Figure 1 shows the separation of ammonia in a 1.0 mg/mL sodium bicarbonate solution spiked with 0.02 mg/L ammonia. The acceptance criteria for ammonia in the current European Pharmacopeia monograph is 0.002%,[2] which the authors expect correlates to the color-determination test in the USP monograph. This separation is challenging due to the 50,000:1 concentration ratio of sodium to ammonia. A low concentration (7 mM) of MSA is used to achieve maximum resolution of ammonia relative to sodium. Ammonia elutes at 24.5 min with a peak resolution of 5.5 (relative to sodium) and a peak asymmetry of 1.2. A higher concentration of MSA (70 mM) is then used to elute more strongly bound cations before re-equilibrating to 7 mM. This method uses a column temperature of 40 °C because the selectivity of the column for maximizing peak efficiencies is optimized at that temperature.

Accuracy

Method accuracy was verified by determining recoveries of ammonia in spiked samples over three consecutive days. The USP General Chapter <1225> guidelines recommend that accuracy be determined using a minimum of nine measurements (i.e., three concentration levels and three replicates of each concentration).[3] A 1 mg/mL sodium bicarbonate solution was spiked with 0.02, 0.1, and 0.5 mg/L ammonia. The average recovery for ammonia ranged from 77 to 85% (Table 1).

Precision

The precision of an analytical procedure is typically expressed as the RSD of a series of measurements. It is determined by assaying a sufficient number of aliquots of a sample that have undergone the complete analytical procedure from sample preparation to final test. The 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). The retention time RSDs were <0.06 and the peak area RSDs ranged from 0.8 to 2.4 (Table 2). If using manually prepared mobile phases, the precisions—especially retention time precision—likely will not be as low as when using electrolytically generated eluent.

Column:	Dionex IonPac CG 16 Guard (3 × 50 mm) Dionex IonPac CS16 Analytical (3 × 250 mm)
Eluent:	7 mM MSA 26 min, 70 mM from 26.1 to 34 min, re-equilibrate to 7 mM for 6 min
Flow Rate:	0.43 mL/min
Inj. Volume:	25 µL
Temp:	40 °C
Detection:	Suppressed conductivity, Dionex CSRS 300 Cation Self-Regenerating Suppressor, recycle mode
Samples:	A. Sodium bicarbonate (1.0 mg/mL) spiked with 0.02 mg/L ammonium B. Sodium bicarbonate (1.0 mg/mL) not spiked C. DI water
Peaks:	1. Sodium 2. Ammonium

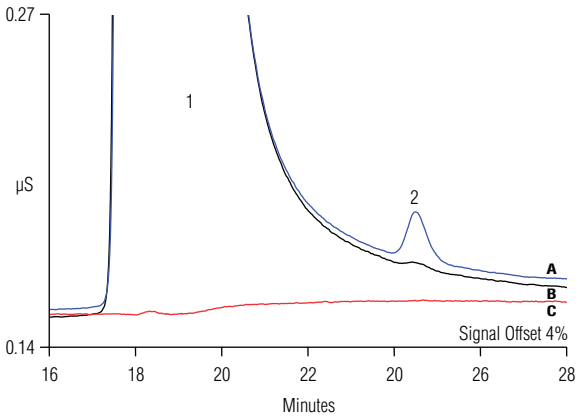

Figure 1. Determination of ammonia in sodium bicarbonate.

Table 1. Recovery for ammonia in sodium bicarbonate.

Spike Level (mg/L)	Recovery (%)
0.02	85
0.1	77
0.5	84

Table 2. Precisions for ammonia in 1 mg/mL sodium bicarbonate.

Ammonia Concn (mg/L)	Retention Time (min)	Retention Time RSD	Peak Area (µS*min)	Peak Area RSD
0.02	24.470	0.06	0.0139	2.36
0.1	24.471	0.04	0.0500	1.10
0.5	24.413	0.02	0.2561	0.81

Detection Limit

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 1:10. Currently, the USP defines S/N as 2H/h, where H is the peak height from the middle of the noise band to the top of the peak. The value of h is the "difference between the largest and smallest noise values observed equal to at least five times the width at the half height of the peak and, if possible, situated equally around the peak of interest" or the "range of the noise in a chromatogram obtained after injection or application of a blank, observed over a distance equal to at least five times the width at half height of the peak in the chromatogram obtained with the prescribed reference solution, and if possible, situated equally around the place where this peak is found."[5]

According to the proposed recommendation outlined in Hinshaw and Dolan's article, S/N should be measured as S/Np-p, with S defined as measurement of peak height from the middle of a noise band to the highest point of the peak (this is synonymous with H), and Np-p defined as peak-to-peak baseline noise, noise measured over ≥five times peak width measured at half of the peak height.[6] Using this proposed S/N measurement criteria, the LOD and LOQ for ammonia in sodium bicarbonate were 0.001 and 0.003 mg/L, respectively.

Linearity

To establish linearity of an impurity in a drug substance or a finished product, the ICH recommends a minimum of five concentrations ranging from 50 to 120% of the acceptance criteria.

Weak bases like ammonia 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 0.9998 using a quadratic curve-fitting function for ammonia in the range of 0.02–2 mg/L. If a linear calibration is desired, use the Dionex SC-CSRS 300 2 mm Salt-Converter Cation Self-Regenerating Suppressor or narrow the concentration range.[7,8]

Robustness

The robustness data using this IC method for sodium bicarbonate is summarized in Table 3. Under the different conditions evaluated, peak asymmetry for ammonia ranged from 1.2 to 1.3 and resolution of ammonia (relative to sodium) ranged from 5.17 to 5.69. The data demonstrates that this is a robust assay for determining ammonia, in spite of the high sodium-to-ammonium ratio.

Table 3. Robustness for ammonia in sodium bicarbonate.

Conditions	Sodium	Ammonium			
	Retention Time (min)	Retention Time (min)	Relative Retention Time (relative to sodium)	Resolution (relative to sodium)	Asymmetry
Flow Rate 0.43 mL/min, 40 °C, 7 mM MSA	18.85	24.46	1.4	5.50	1.22
Flow Rate 0.38 mL/min, 40 °C, 7 mM MSA	21.25	27.54	1.0	5.51	1.27
Flow Rate 0.48 mL/min, 40 °C, 7 mM MSA	16.88	21.83	1.3	5.24	1.31
Flow Rate 0.43 mL/min, 38 °C, 7 mM MSA	19.04	24.83	1.3	5.69	1.18
Flow Rate 0.43 mL/min, 42 °C, 7 mM MSA	18.68	24.11	1.3	5.37	1.26
Flow Rate 0.43 mL/min, 40 °C, 5 mM MSA	25.19	32.88	1.3	5.55	1.25
Flow Rate 0.43 mL/min, 40 °C, 9 mM MSA	15.28	19.64	1.3	5.17	1.31

Conclusion

For sodium bicarbonate, the cation at high concentration is sodium, which elutes close to ammonia and therefore makes its determination challenging. Nevertheless, the high-capacity cation-exchange Dionex IonPac CS16 column—specially designed for samples with disparate analyte concentrations—has demonstrated its ability to quantify low amounts of ammonia in the presence of a high concentration of sodium. This IC assay is capable of determining ammonia at its limit (in the current monograph) in sodium bicarbonate. In addition, automation features such as the electrolytically generated mobile phase and self-regenerating suppressor contribute to the robustness and sensitivity of this method. Only water is used to generate the mobile phase and no hazardous chemicals are used in this IC assay for ammonia in sodium bicarbonate. The precisions (retention time RSD <0.1 and peak area RSD <2), accuracy (average recovery 88–117%), LOD, and robustness of the assay meet the analytical performance characteristics recommended by USP General Chapter <1225>.

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