# Determination of Ammonia in Tobacco Smoke

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## Key Words
Ion Chromatography, Freebasing Nicotine, Dionex IonPac CS19 Column, Cigarette

## Goal
To develop an IC method that can determine ammonia in tobacco smoke and is not subject to interference from small amines

## Introduction
Ammonia (0.01–0.6%) occurs naturally in cured tobacco leaves and is now being used as an additive to enhance flavor and increase the amount of free nicotine in cigarette smoke. This practice is called freebasing of nicotine.[1,2] Typically, the freebased amount equates to 0.2–10 mg ammonia per gram of tobacco or 7–200 µg and 320–450 µg ammonia per gram of smoked tobacco in mainstream and sidestream smoke, respectively (Figure 1).[3]

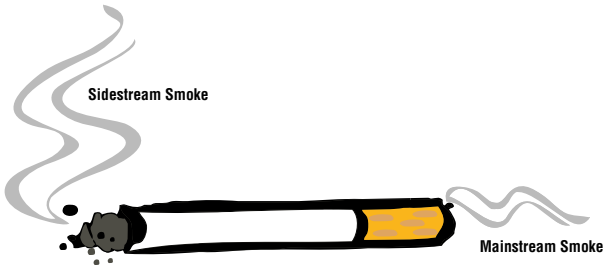


Figure 1. Main- and sidestream smoke from a cigarette.

On June 23, 2009, the U.S. Congress passed the Tobacco Control Act that granted the U.S. Food and Drug Administration (FDA) authority to regulate the manufacture, marketing, and distribution of tobacco products to protect public health. In January 2011, the FDA established a list of harmful and potentially harmful constituents in tobacco products and tobacco smoke.

Ammonia is listed as a respiratory toxicant. This list was published in the Federal Register to announce that beginning June 22, 2012, the Food Drug and Cosmetics Act would require tobacco product manufacturers to submit a list and quantify the constituents (including smoke constituents) identified by the FDA as harmful or potentially harmful to health in each of their tobacco products.[4] Hence, there is a need for a robust and sensitive analytical method for quantifying ammonia in tobacco products and smoke.

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Ion chromatography (IC) has been used for the determination of ammonia (as ammonium) and other cations in tobacco products.[5-9] The CORESTA Analytical group[6] and the Canadian Tobacco Control program[7] have recommended an IC method to determine ammonia in whole tobacco and finished products. The recommended method uses extraction into sulfuric acid solution, followed by separation of ammonium from other cationic species on a Thermo Scientific™ Dionex™ IonPac™ CS12A column, and detection by suppressed conductivity. This column has also been used for the determination of ammonia in mainstream and sidestream smoke.[8,9] However, when using this method for smoke samples, it is not possible to achieve good resolution between ammonium and small amines (e.g., methylamine and ethylamine) that are present in tobacco smoke.

This study describes an IC method that achieves better resolution between ammonium and other amines for the determination of ammonia in tobacco smoke. This improved resolution may be due to the use of the Dionex IonPac CS19 column set. This column was designed to separate common cations, as well as both polar and hydrophobic amines, and has proven to be ideal for measuring ammonia in acid extracts from reference cigarette smoke samples. This method uses a Reagent-Free™ IC (RFIC™) system; therefore, the methanesulfonic acid (MSA) eluent is electrolytically generated, thus eliminating the labor and possible error associated with preparation of eluents. The self-regenerating suppressor also eliminates the labor of preparing a strong base regenerant for the suppressor.

## Equipment

- A Thermo Scientific™ Dionex™ ICS-5000⁺ HPIC™ system, including:
  - SP Single Pump or DP Double Pump
  - EG Eluent Generator
  - DC Detector/Chromatography Compartment, with CD Conductivity Detector
- Thermo Scientific Dionex AS-AP Autosampler
- Thermo Scientific Dionex MSA Eluent Generator Cartridge (EGC III MSA, P/N 074535)
- Thermo Scientific Dionex CR-CTC II Continuously Regenerated Cation Trap Column (P/N 066262)
- Injection Loop, 5 µL
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System CDS software

## Reagents and Standards

- Sulfuric Acid (Fisher Scientific P/N A510-500)
- Thermo Scientific™ Dionex™ Combined Six Cation Standard-I (P/N 040187)
- Ammonium Standard (Fisher Scientific P/N US-ICC-101)
- Deionized (DI) water, 18 MΩ-cm resistance

| Conditions | |
|---|---|
| Columns: | Dionex IonPac CG19 Guard, 2 × 50 mm (P/N 076029)<br>Dionex IonPac CS19 Analytical, 2 × 250 mm (P/N 076028) |
| Eluent: | 5 mM MSA for 7 min, 50 mM MSA from 7.1 to 16 min, 5 mM MSA from 16.1 to 24 min |
| Flow Rate: | 0.25 mL/min |
| Inj. Volume: | 5 µL |
| Column Temp: | 30 °C |
| Cell Temp: | 35 °C |
| Detection: | Suppressed conductivity, Thermo Scientific™ Dionex™ CSRS™ 300 Cation Self-Regenerating Suppressor (P/N 064557), autosuppression recycle mode, power setting 37 mA |
| Backpressure: | 2200 psi |
| Background Conductance: | <0.250 µS |
| Noise: | 1–2 nS |

## Preparation of Solutions and Reagents

### Eluent Solution

Generate high-purity MSA eluent on line by pumping DI water through the Dionex EGC III MSA cartridge. Chromeleon CDS software tracks the amount of MSA used and calculates the remaining cartridge lifetime. If needed, eluents can be manually prepared.

### Sulfuric Acid, 50 mN

Carefully add 2.6 g of concentrated sulfuric acid (96% w/w) to approximately 800 mL of degassed DI water in a 1 L volumetric flask. Dilute to the mark with degassed DI water and mix.

### Ammonium Standards

Gravimetrically prepare standards by making appropriate dilutions of a commercial 1000 mg/L standard with 50 mN sulfuric acid. Store standard solutions at 4 °C when not in use. The standard solutions are stable for approximately 30 days when stored at 4 °C.

### Sample Preparation

Smoke samples were kindly provided by Global Laboratory Services, Inc. of Wilson, NC. Smoke extracts (from reference cigarettes 3R4F) were in 50 mN sulfuric acid. The acid extracts were injected into the IC system without dilution. The samples were kept at 4 °C when not in use.

### Precautions

After installing a new column, flush it with 8 mM MSA for 30 min before connecting to the suppressor.

## Results and Discussion

### Separation

Figure 2 shows the separation of ammonium in typical smoke samples (A and B) and in a 1 mg/L ammonium standard (C) on a Dionex IonPac CG19 (2 × 50 mm) and CS19 (2 × 250 mm) column set with 5 mM MSA. The Dionex IonPac CS19 column is a carboxylate-functionalized cation-exchange column tailored for the separation of common cations and polar amines as well as moderately hydrophobic and polyvalent amines.

Ammonium was well resolved from the low-molecular-weight amines on the Dionex Ion Pac CS19 column (Figure 2), an improvement over the separation of these cations on the Dionex Ion Pac CS12A column (Figure 3). The eluent conditions were optimized for ammonium (Figure 2, peak 2, eluting at ~6 min) to be well resolved from sodium (Figure 2, peak 1) and methylamine (Figure 2, peak 3) with peak resolutions of 1.6 and 1.7, respectively. To elute the other cations, a stronger eluent (50 mM MSA) was used before returning to 5 mM MSA and re-equilibrating the column. Total injection-to-injection time was 24 min. Assignments for the amines and other cations were based on retention times of standards run using the same conditions (data not shown). The amount of ammonium determined in the smoke samples ranged from 1.0 to 2.2 mg/L.

### Precision

Method performance was evaluated with three replicate injections of three ammonium standards ranging from 0.5 to 6 mg/L. Table 1 summarizes the precision data for ammonia in a standard solution and in a typical smoke sample. The calculated retention time (RT) and peak area precisions were ≤0.11% and <1%, respectively, with little difference between the results of the standards and samples.

Table 1. Precision.

| Sample | RSD | |
|---|---|---|
| | RT | Peak Area |
| Ammonium Standard, 0.5 mg/L | 0.09 | 0.76 |
| Ammonium Standard, 1 mg/L | 0.07 | 0.94 |
| Ammonium Standard, 6 mg/L | 0.12 | 0.82 |
| Smoke Samples | 0.11 | 0.28 |

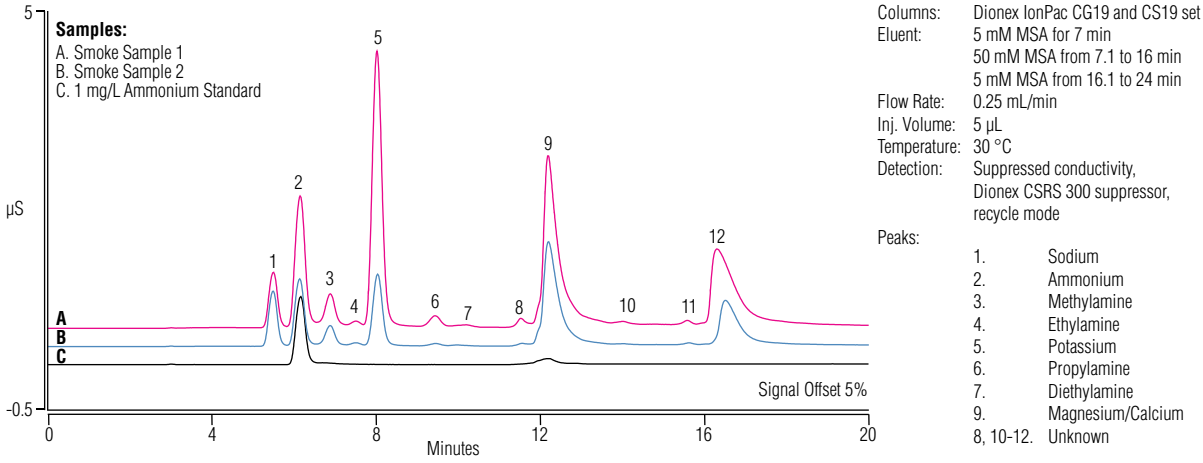


Figure 2. Ammonium in smoke samples (A and B) and in a 1 mg/L standard (C).

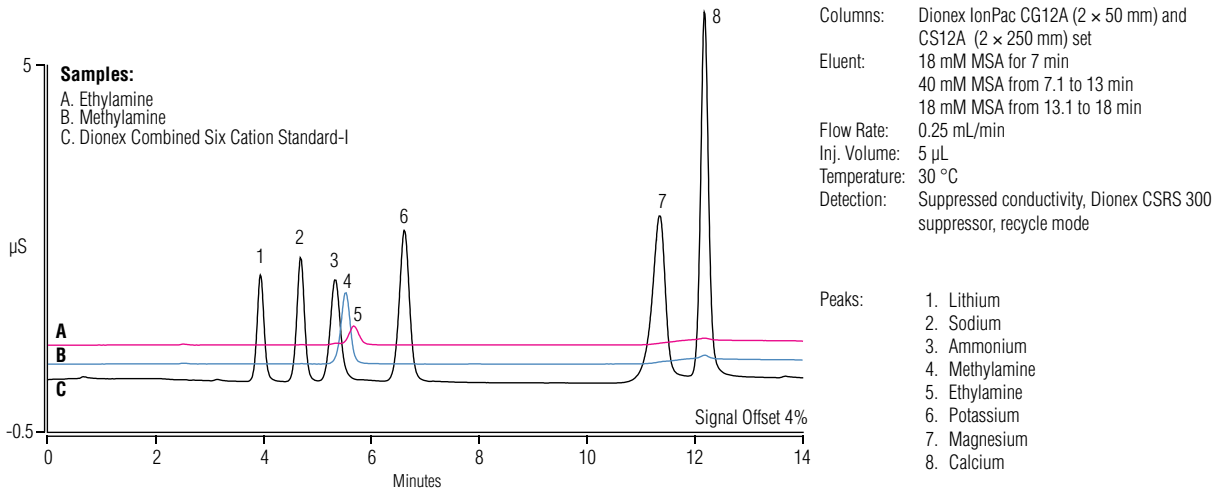


Figure 3. Separation of six cations, methylamine, and ethylamine on the Dionex IonPac CS12A column.

### Calibration Range

The calibration range of ammonium was determined by triplicate injections of the ammonium standard covering a range from 0.01 to 10 mg/L (0.01, 0.025, 0.1, 0.5, 1.0, 2.0, 4.0, 6.0, and 10 mg/L). Weak bases like ammonium are partially dissociated and thus give a nonlinear response as the concentration increases. The coefficient of determination was 0.9993 using a quadratic curve-fitting function for ammonium. For a narrow range (0.01 to 2 mg/L), response can be considered to be linear, and the coefficient of determination for a linear fit in that range was 0.9976.

### Accuracy

The accuracy of the method was evaluated by determining recoveries of ammonium in spiked smoke samples over three consecutive days. The smoke sample was spiked with 1.0 mg/L ammonium. Recoveries were calculated from the difference in response between the spiked and unspiked samples. The average recovery of ammonium ranged from 86 to 115% (Table 2). This demonstrates that this method can be used for accurate determination of ammonium in smoke samples.

### Detection Limit

The detection limit is defined in terms of a signal-to-noise ratio of 3:1 and a limit of quantitation (LOQ) defined by a 10:1 signal-to-noise ratio. Typical baseline noise for this method using the suppressor in the recycle mode is 0.001 µS. The limit of detection and the LOQ for ammonia in tobacco smoke (i.e., concentrations that resulted in peaks that are 3× and 10× that of the noise) are 0.003 and 0.01 mg/L, respectively.

### Robustness

The robustness of the method was tested by evaluating the peak-area and retention-time stabilities of a 1 mg/L ammonium standard injection interspersed with smoke sample injections. Table 3 summarizes the precision and percent change of retention time and peak area for the 1 mg/L standard over 370 injections (including 115 smoke samples and 250 standards on each column). There is a small loss of retention time (1–2%) over the course of the analysis. After 500 or more sample injections, a column cleanup may be required to restore retention time to its starting value.

The variance due to different columns was tested by comparing results from columns from two different lots. Both column sets showed similar retention time for ammonium (6.15 and 6.10 min), and precisions in retention time and peak area.

Table 2. Recovery of samples spiked with 1 mg/L ammonium.

| Day | Ammonium Present (mg/L) | Ammonium Measured (mg/L) | Recovery (%) |
|---|---|---|---|
| 1 | 1.70 | 2.71 | 101 |
| | 1.70 | 2.71 | 101 |
| 2 | 1.64 | 2.59 | 95 |
| | 1.64 | 2.79 | 115 |
| 3 | 1.65 | 2.51 | 86 |
| | 1.64 | 2.58 | 93 |

Table 3. Robustness.*

| Ammonium, 1 mg/L | RT RSD** | Peak Area RSD | Change in RT (min) | % Change in RT |
|---|---|---|---|---|
| Column 1 | 0.6 | 4.5 | -0.06 | -1.0 |
| Column 2 | 0.8 | 3.5 | -0.10 | -1.6 |

* >370 injections (including 115 sample injections) on each column

** The retention time RSD does not truly represent this method because there is a slight negative trend of retention time.

## Conclusion

This study describes an IC-based method for the accurate determination of ammonium in tobacco smoke. The described method achieves better resolution of ammonium from other analytes present in tobacco smoke when compared to other IC methods. The method uses a Dionex IonPac CS19 column set with electrolytically generated MSA gradient. The moderately high capacity of the Dionex IonPac CS19 column allows sample analysis without dilution. The method has high precisions (RSD <0.2% for retention time and <1% for peak area), a low detection limit (0.01 mg/L), and good recoveries (86–115%). Use of the RFIC system allows for continuous operation of the instrument with minimum maintenance. The analyst only needs to add DI water to the RFIC system to analyze samples. Additionally, the 2 mm column format generates less waste and uses less eluent than the typically used 4 mm format.

## Acknowledgement

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