Analysis of ammonia in Platform 1 Aerosol

PRODUCT TESTING LABORATORY AND GOVERNANCE

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1. ANALYSIS OF AMMONIA IN PLATFORM 1 AEROSOL

1.1. Abstract

The aerosols are generated on a linear smoking machine and collected using a mixed trapping approach; Cambridge filter pad followed by two micro impingers. The content of the CFP is then combined and exctracted with the content of the impingers as explained in paragraph 1.2.

The extracts are analyzed by Ion Chromatography (IC) using a Dionex column Ion Pac CS16, 3x250mm and a cation conductivity detector.

Results are expressed as µg/item for P1.

The method has been developed from the corresponding CORESTA method for the analysis of ammonia in conventional cigarettes aerosol.

1.1. Applicability

The method described is used to determine ammonia in mainstream aerosol from Platform 1 (P1) under Health Canada (HC) and International Organization for Standardization (ISO) smoking conditions, as well as under alternative smoking regimens.

1.1. Reagents

- Sulfuric acid
- Eluent: Methanesulfonic acid (MSA)
- Ammonium Standard Solution for IC about 1000 µg/mL, certified
- Fresh deionized water

1.2. Aerosol generation

P1 items are conditioned in climatized chamber for at least 48 hours at target conditions of 22 ± 1°C and relative humidity of 60 ± 3% before used for aerosol generation.

Cambridge filters are conditioned for at least 12 hours at target conditions of 22 ± 1°C and relative humidity of 60 ± 3% before used for aerosol generation.

The aerosol samples are generated on a linear smoking machine (Borgwaldt KC or Cerulean) under ISO and HC smoking regimens and collected using a mixed trapping approach, combining a Ø 44 mm Cambridge filter pad and two micro impingers filled with 10 mL of a 0.1N H_2SO_4 solution. The collection conditions for the different smoking regimes are summarized in Table **1**.

At the end of the smoking process, the content of the two impingers and the glass fiber Cambridge filter pad are transferred into a 50mL PP tube. Then, each impinger is rinsed with 10 mL of deionized water, which are transferred in the same 50mL PP tube containing the trapping solution.

Four replicates for each sample are generated. Two blanks are smoked each smoking day to ensure that no contamination is carried out. The first blank is smoked before the first aerosol collection, while the second one is smoked at the end of the smoking day.

Table 1: Aerosol Collection Condition

Regimen	Accumulation number	Puff number	Regimen Condition [puff volume/Puff duration/Puff Interval] [ml/s/s]
ISO	5	6	35/2/60
HC	5	12	55/2/30

1.3. Samples preparation

The 50 mL PP tubes, containing the glass fiber Cambridge filter pad and a combined impinger and rinsing solution, are shaked for about 30±5 minutes on a shaker device; afterwards, approximately 5 mL of the aerosol extracts are filtered through a 0.45 µm PVDF filter with an attached disposable syringe into 15 mL PP tubes and into autosampler vials.

If the expected concentration is out of the calibration range depicted in Table 2, aerosol extracts can be diluted with a 0.05N H_2SO_4 solution. In this case, the same dilution is applied to the corresponding blanks.

1.4. Calibration solutions preparation

1.4.1. Primary Ammonium Stock Solution

The primary ammonium stock solution (20 µg/mL) is prepared by dilution of the certified ammonium stock solution (1000 mg/L) with a 0.05N H_2SO_4 solution.

1.4.2. Calibration Solutions

Seven standard (STD) solutions are prepared by dilution of the primary ammonium stock solution. The range of concentrations covers the range relevant for analysis and is provided in Table 2.

Table 2: Ammonium working standatd ranges (µg/mL)

Level	Theoretical NH_4^+ conc. (µg/mL)	Theoretical NH_3 conc. (µg/mL)
1	0.20	0.189
2	0.55	0.519
3	1.00	0.944
4	1.75	1.653
5	2.50	2.361
6	3.25	3.069
7	4.00	3.778

The standard level 4 is also used as quality check.

1.5. Chromatographic Conditions

The samples are analyzed by Ion Chromatography (IC) following tables below:

Table 3: Chromatographic Conditions for Determination of ammonia

Column	CS16, 3.0 x 250 mm, column Thermo
Pre-column	CG16, 3.0 x 50 mm, pre-column Thermo
Column temperature	40°C
Column compartment temperature	20°C
Auto-sampler tray temperature	10°C
Detector Cell temperature	35 °C
Injection volume	25 µL
Suppressor current	55 mA
Injection mode	PushFull
Flow	0.36 mL/min
Injection speed	fast

Table 4: Gradient conditions

Time (min)	MSA Concentration (mM)
0.0	32
10.0	32
10.2	52
13.8	52
14.0	32
22	32
22	Stop run
22	Post run

1.6. Testing procedure

The following typical analytical sequence, is used for the determination of ammonia:

- 4 conditioning injections (e.g. 3R4F matrix, STD level 1)
- Suitability test (one injection of STD level 1)
- Blank standard (0.05N H_2SO_4 solution)
- Calibration curve (STD 1 to 7)
- Extraction solution (0.1N H_2SO_4 solution used for aerosol generation, diluted with deionized water by the Aerosol Collection Laboratory (ACL)
- 2 smoked blanks
- After every 4 samples, inject a quality check (STD level 4)
- Always end an analytical sequence with a quality check, a wash injection (0.05N H_2SO_4 solution) and a "standby" injection

1.7. Verification of results

1.7.1. Calibration curve verification

A calibration curve is used to quantify the unknown samples using the peak area of ammonium. The peak area is applied to generate the curve. The quadratic regression with 1/x weighting factor is calculated automatically by the software.

The validity of the calibration is continuously verified during the batch analysis by ensuring that the injected quality check (STD level 4) is within ±15% of its theoretical value.

1.7.2. System suitability test

A suitability test is performed after the system conditioning. The acceptance criteria are summarized in Table 5.

Table 5: Acceptance criteria for analysis of ammonia

	Acceptance limits
Peak width at 50%	$0.126 \text{ min} \leq x \leq 0.374 \ min$
Minimum peak area	$\geq 0.116 \ \mu S/min$

1.8. Example Chromatograms

An example chromatogram of ammonia in P1 samples is provided in Figure 1.
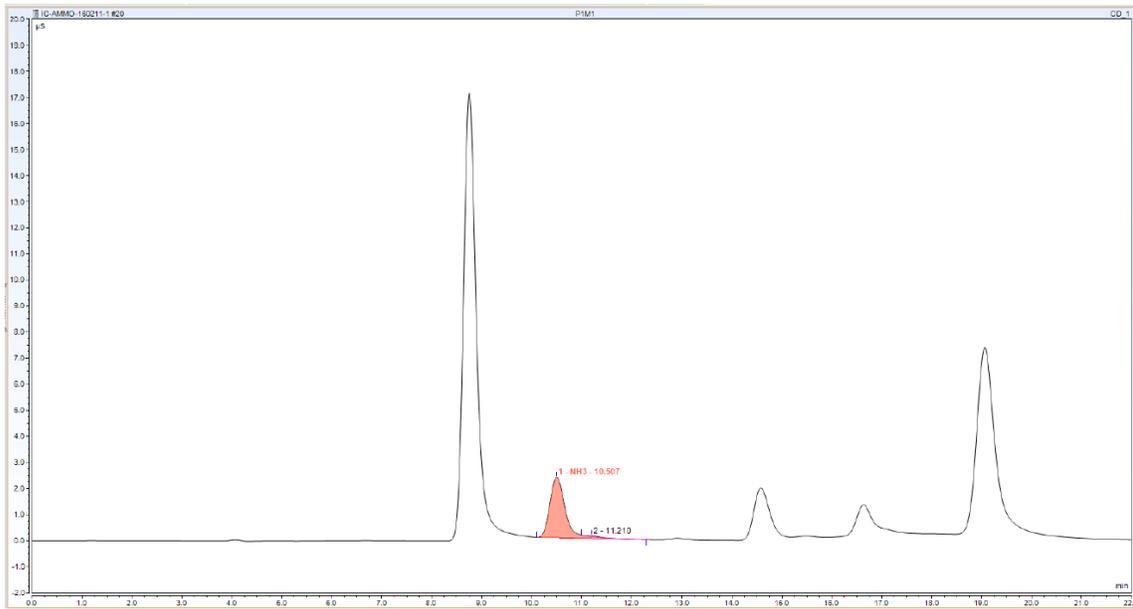
Figure 1: Example Chromatogram of ammonia in P1 aerosol exctract samples

1.9. Limit of Detection (LOD) / Lower Limit of Quantitation (LLOQ)

The LOD and LLOQ were both calculated in terms of the standard deviation of the lowest calibration level (5 injections by using different calibration standard preparations analyzed on 5 different days).

$$LOD = 3 \times SD_{level1}$$

$$LLOQ = 10 \times SD_{level1}$$

Level 1 is the lowest calibration level.

Detailed results are provided in Table 6.

Table 6: Limits of Detection and Quantitation (HC and ISO regimens)

Matrix	HC			ISO		
	LOD	LLOQ	ULOQ	LOD	LLOQ	ULOQ
	(µg/item)	(µg/item)	(µg/item)	(µg/item)	(µg/item)	(µg/item)
P1	0.0103	0.0345	30.2	0.0103	0.0345	30.2

ULOQ = upper limit of quantitation (highest calibration level)

1.10. Repeatability limit (r) and Intermediate precision limit (IP)

$$r = 2 \cdot \sqrt{2} \cdot s_r$$

$$IP = 2 \cdot \sqrt{2} \cdot s_{IP}$$

s_r is the standard deviation of repeatability.

s_{IP} is the standard deviation of intermedidate precision.

Repeatability limit and intermediate precision limit are determined during four different days using different smoking machines for the aerosol generation and standard solutions preparation. Different operators are involved in both aerosol generation and analysis.

Table 7: Repeatability r and Intermediate precision IP (HC and ISO regimens)

Compound	HC			ISO		
	r	IP	Mean	r	IP	Mean
	(µg/item)	(µg/item)	(µg/item)	(µg/item)	(µg/item)	(µg/item)
P1	0.966	1.60	13.4	1.14	1.24	2.36

1.11. NORMATIVE REFERENCES

- ISO 3308:2000 – Routine analytical cigarette smoking machine – definitions and standard conditions
- ISO 3402:1999 – Tobacco and tobacco products – atmospheres for conditioning and testing