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Analysis of hydrogen cyanide in Platform 1 aerosol

PRODUCT TESTING LABORATORY AND GOVERNANCE

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1. ANALYSIS OF HYDROGEN CYANIDE IN PLATFORM 1 AEROSOL

1.1. Abstract

The aerosol samples are generated with a linear smoking machine and collected in two impingers filled with 1 M NaOH solution. Cyanide is then derivatized by reaction with 2,3-naphthalene-dicarboxaldehyde (NDA) and taurine.

The extracts are analyzed by High Performance Liquid Chromatography (HPLC) using tandem mass spectrometry detection with an electrospray (HESI) interface in negative ionization mode. The HPLC system is equipped with a column Macherey-Nagel, EC Nucleodur C-18 Gravity, 75 x 4.6 mm, 3 µm.

Results are expressed as µg/item for P1.

1.2. Applicability

The method described is used for the determination of hydrogen cyanide in platform 1 (P1) aerosols under ISO and Health Canada (HC) conditions, as well as under alternative smoking regimens.

1.3. Reagents

- Potassium cyanide (KCN)
- Isotopic potassium cyanide ($K^{13}C^{15}N$, 99% ^{13}C, 98% ^{15}N)
- 2,3'-naphthalenedicarboxaldehyde (NDA), 98%
- Taurine
- Boric acid
- Acetonitrile, LC-MS purity grade
- Sodium hydroxide
- Formic acid, LC-MS purity grade
- Ultrapure water
- Argon

1.4. Aerosol generation

P1 items are conditioned in opened pack for at least 48 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 under ISO and HC smoking regimens and collected in two impingers filled each with 20 mL of a 1 M NaOH solution and 60 g of Ø 4 mm glass marbles. The collection conditions for the different smoking regimes are summarized in Table **1**.

At the end of the smoking step, impingers are removed from the smoking machine and their contents are mixed in a Filtrona tube and sent to the analytical laboratory for derivatization and analysis.

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

1.5. Sample preparation

5 mL of the aerosol extracts are filtrated with a 0.45 µm filter using a disposable syringe.

The derivatization is performed directly in a clear vial, by mixing 1000 µL of derivatization solution, 20 µL of internal standard (ISTD) stock solution and 20 µL of the filtrated aerosol extract. At least 30 minutes are then allowed to the reaction to take place.

Table 2: Sample derivatization and measurement solution preparation

Sample type	Derivatization solution (µL)	Aerosol extract (µL)	ISTD stock solution (µL)	Derivatization time (min)
P1 samples and P1 blanks	1000	20	20	30

1.6. Derivatization solution preparation

The derivatization solution is prepared by dilution of NDA and taurine stock solutions in a borate buffer solution.

1.7. ISTD solution preparation

An ISTD stock solution is prepared by dissolution of isotopically labelled potassium cyanide ($K^{13}C^{15}N$) in a 1 M NaOH solution.

The final ISTD solution is prepared by dilution of the ISTD stock solution with a 1 M NaOH solution.

1.8. Calibration solutions preparation

Seven standard (STD) solutions are prepared by dilution of a concentrated KCN stock solution with a 1 M NaOH solution.

The range of concentrations covers the range relevant for analysis and is provided in Table 3.

Table 3: HCN calibration standards typical concentrations

Compound	STD1 (µg/mL)	STD2 (µg/mL)	STD3 (µg/mL)	STD4 (µg/mL)	STD5 (µg/mL)	STD6 (µg/mL)	STD7 (µg/mL)
HCN	0.118	0.308	0.520	0.710	4.258	7.806	11.355

The standard level 4 is also used as quality check

Standard measurement solutions are prepared following the same procedure described for samples measurement solutions and summarized in Table **2**.

1.9. Instrumental Conditions

The samples are analyzed by liquid chromatography using tandem mass spectrometry detection (LC-MS/MS) following tables below:

Table 4: Chromatographic Conditions for Determination of hydrogen cyanide

Column	Macherey-Nagel, EC Nucleodur C-18 Gravity, 75 x 4.6 mm, 3 µm
Guard-column	Macherey-Nagel, EC 4/3 Nucleodur C-18 Gravity, 3 µm
Mobile Phase	60% (1% Formic Acid in water) / 40% ACN (% v/v)
Flow rate	0.8 mL/min
Flow mode	Isocratic
Column oven temperature	35°C
Injection volume	10 µL
Loop volume	10 µL
Syringe speed	8 µL/s
Wash and flush solvent	Acetonitrile / water 70/30 % v/v
Run time	7 min
Sample tray temperature	5 °C

Table 5 Mass spectrometer settings

Parameter	Description
Ionization source	HESI
Ionization polarity	Negative
Scan type	SRM
Acquisition time	7 min
Capillary temperature	380 °C

1.10. Testing procedure

The following typical analytical sequence is used for the determination of hydrogen cyanide:

- 3 conditioning injections (sample matrix)
- Calibration curve (STD 1 to 7)
- Smoked blanks
- Samples
- After every 5 samples, inject a quality check (STD level 4)
- Wash injection (acetonitrile)

1.11. Verification of results

1.11.1. Calibration curve

A calibration curve is used to quantify the unknown samples using the response ratio of analyte to the internal standard. The peak area ratio is applied to generate the curve. The linearregression is calculated automatically by the software LC Quan software. Specific information about regressions are provided in Table 6:

Table 6: Parameters used for calibration curves

Compound	Internal standard	Regression type	Weighting factor
HCN	$K^{13}C^{15}N$	Quadratic	1/x

1.11.2. Quality Check

The validity of the calibration is continuously verified during the batch analysis by analysing the calibration control standard injections. Each control standard must be within ±10% of its theoretical value.

1.12. Example Chromatograms

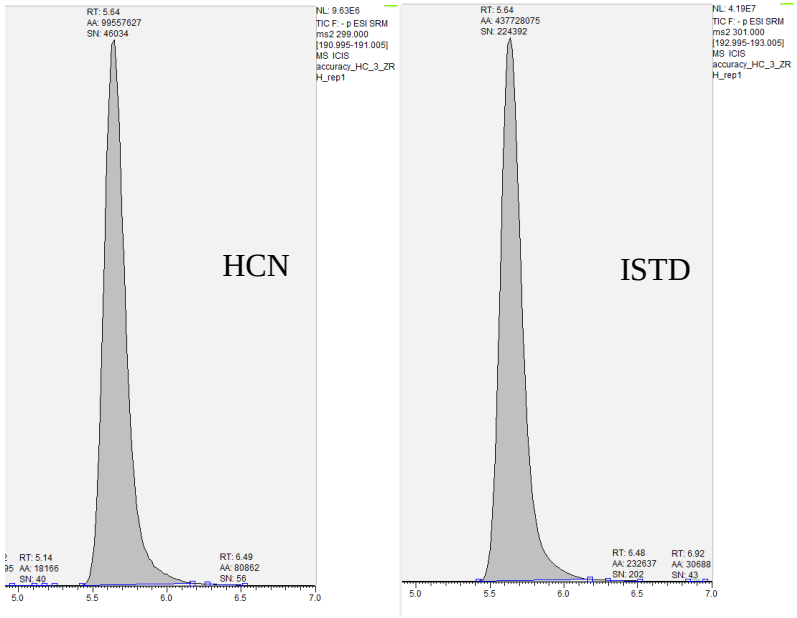

Figure 1: Example of Chromatogram for P1 aerosol extract samples

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

The lower limit of quantification (LLOQ) are determined using the accuracy profiles. Indeed, the intersect point of the beta-expectation tolerance intervals with the acceptance intervals correspond to the smallest quantity measurable with the desired accuracy.

The limit of detection (LOD) is calculated from the LLOQ value.

$$LOD = LLOQ / 3.3$$

Detailed results are provided in Table 7.

Table 7 LOD, LLOQ and ULOQ for P1 samples

	P1, HC smoking regimen		
Compound	**LOD [µg/item]**	**LLOQ [µg/item]**	**STD1 [µg/item]**
Hydrogen cyanide	0.062	0.205	0.944

1.14. 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 smoking days using different standard solutions preparation.

IP and r values for P1 are depicted in Table 8.

Table 8: Repeatability r and Intermediate precision IP for P1 samples (HC Regimen)

	P1, HC regimen		
Compound	**Mean conc [µg/item]**	**r [µg/item]**	**IP [µg/item]**
Hydrogen cyanide	4.00	0.89	3.38

1.15. 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