Analysis of nitrobenzene in Platform 1 Aerosol

PRODUCT TESTING LABORATORY AND GOVERNANCE

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1.1. Abstract

The aerosols are generated on a linear smoking machine and collected using Cambridge filter pad (CFP) followed by a micro impinger filled with 10 mL of cyclohexane with internal standard. The content of the CFP is then combined and exctracted with the content of the impinger and delivered to the analytical laboratory. Solid phase extraction is performed to clean-up the samples before analysis.

The extracts are then analyzed by Gas Chromatography with Mass Spectrometry detection (GC-MS) using a column DB-17 MS 30 m x 0.25 mm ID x 0.15 µm film thickness.

Results are expressed as ng/item for P1.

1.1. Applicability

The method described is used to determine nitrobenzene in aerosol from Platform 1 (P1) under Health Canada (HC) and International Organization for Standardization (ISO) smoking conditions.

1.1. Reagents

- Nitrobenzene
- Nitrobenzene-^{13}C6
- Cyclohexane
- Ethyl Acetate

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 under ISO or HC smoking regimens and collected using a Cambridge filter pad and a micro impinger filled with 10 mL of extraction solution (cyclohexane with internal standard). The collection conditions for the different smoking regimes are summarized in Table **1**.

At the end of the smoking process, the content of the glass fiber Cambridge filter pad is extracted with the extraction solution of the impinger into a Filtrona tube. The sample is then sent to the analytical lab for 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

1.3. Samples preparation

1.3.1. Solid Phase Extraction (SPE) elution pattern

Samples need to be cleaned-up by Solid Phase Extraction (SPE) before to be quantified. In order to collect only the fraction containing the compound of interest, the SPE elution pattern has to be evaluated. Therefore, the test described hereunder is performed per SPE cartridge batch and for mentholated and non-mentholated product.

First of all, the sample extract is concentrated to around 0.3 mL by a nitrogen flush using a turbovap with a 50°C water bath. In the mean time, a SPE cartridge is conditioned with 5 mL of ethyl acetate and 5 mL of cyclohexane. After that, the concentrated extract is loaded on the cartridge and 12 portions of 1 mL are sequentially eluted with cyclohexane. Each portion is then concentrated to arounf 0.30 mL with the turbovap and transferred into a glass vial for GC-MS analysis. Nitrobenzene areas obtained by GC-MS analysis are plotted as a function of the portion number in order to identify the fraction of the SPE elution containing the compound of interest.

1.3.2. Sample clean-up by SPE

The SPE is performed for all the sample extracts, following the procedure described in paragraph 1.3.1: extracts concentration with turbovap, SPE cartridge conditioning with ethyl acetate and cyclohexane, collection of the elution fraction containing the compound of interest followed by evaporation of the eluted fraction using a turbovap and transfer of the concentrated fraction into a glass vial for GC-MS analysis.

1.4. Extraction solution

The extraction solution consists in a cyclohexane solution containing nitrobenzene-$^{13}C6$ as internal standard.

1.5. Calibration solutions preparation

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

Table 2: Nitrobenzene calibration standards typical concentrations

	Nitrobenzene conc.
Level	(ng/mL)
1	0.10
2	0.20
3	0.50
4	1.0
5	2.0
6	3.0
7	4.0

The standard level 5 is also used as quality check.

1.6. Instrumental Conditions

The samples are analyzed by Gas Chromatography (GC) with mass spectrometry detection (MS) following tables below:

Table 3: Instrumental Conditions for Determination of nitrobenzene

Column	DB-17 MS 30 m x 0.25 mm ID x 0.15 µm film thickness
Oven temperature	50°C, 2 min 20°C/min→130°C, 0 min 10°C/min→150°C, 0 min 30°C/min→210°C, 5 min
Total program time	15 min
Injection	3 µL, pulsed splitless, 180 KPa, 0.7 min
Injectior temperature	180 °C
Tansfer line temperature	250 °C
Ion source temperature	180 °C
Quadrupole temperature	150 °C
Gas	Helium
CI gas	1% NH_3 in N_2

1.7. Testing procedure

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

- Conditioning injection (e.g. sample)
- Calibration curve (STD 1 to 7)
- Extraction solution (cyclohexane with internal standard)
- 2 smoked blanks
- After every 5 samples, inject a quality check (STD level 5)
- Always end an analytical sequence with a quality check

1.8. Verification of results

1.8.1. Calibration curve verification

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 linear regression is calculated automatically by the software Mass Hunter. Specific information about regressions are provided in Table 4:

Table 4: Parameters used for calibration curve

Compound	Internal standard	Regression type	Weighting factor
nitrobenzene	nitrobenzene-^{13}C6	linear	$1/x^2$

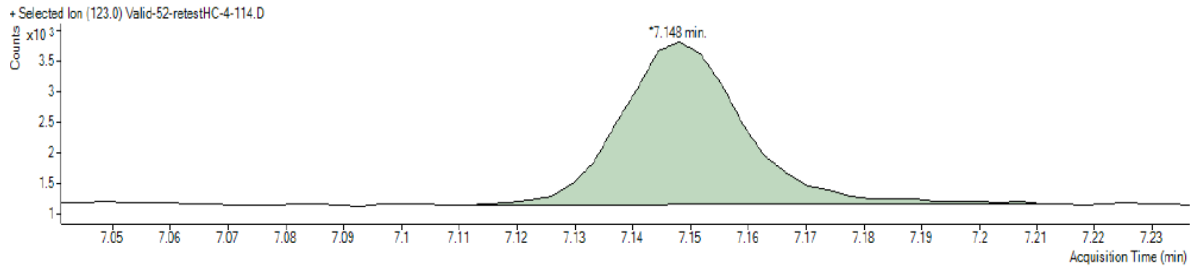
1.8.2. Quality check

The validity of the calibration is continuously verified during the batch analysis by ensuring that the injected quality check (STD level 5) is within ±15% of its theoretical value.

1.9. Example Chromatograms

An example chromatogram of nitrobenzene in P1 samples is provided in Figure 1.

Nitrobenzene:



Nitrobenzene-^{13}C6:

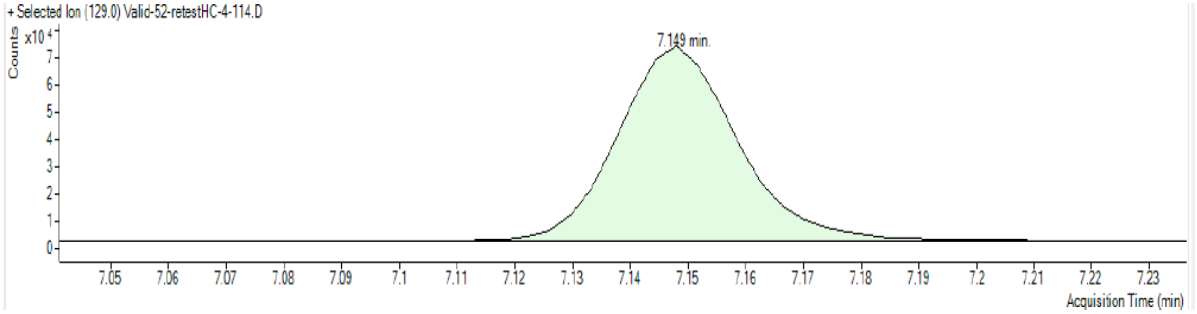


Figure 1: Example Chromatogram of nitrobenzene in P1 aerosol exctract samples

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

The LOD and LLOQ were both calculated in terms of the standard deviation of five different measurements of the lowest calibration standard.

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

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

Level 1 is the lowest calibration level.

Detailed results are provided in Table 5.

Table 5: Limits of Detection and Quantitation (HC smoking regimen)

Compound	P1, HC smoking regimen		
	LOD	LLOQ	STD 1
	(ng/item)	(ng/item)	(ng/item)
Nitrobenzene	0.0016	0.0051	0.020

1.11. 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 standard solutions preparation. Different operators are involved in the analysis.

Table 6: Repeatability r and Intermediate precision IP (HC regimen)

Compound	P1, HC smoking regimen		
	r	IP	Mean
	(ng/item)	(ng/item)	(ng/item)
Nitrobenzene	0.0127	0.0255	0.0903

1.12. 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