2017

Determination of Glycerin and Menthol in Platform 1 aerosol

PRODUCT TESTING LABORATORY AND GOVERNANCE

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1. DETERMINATION OF GLYCERIN AND MENTHOL IN PLATFORM 1 AEROSOL

1.1. Abstract

The aerosol samples are generated by mean of a 20 port linear smoking machine. The mainstream aerosol is collected on a 44mm Cambridge filter pad (CFP).

Glycerin trapped in the CFP is extracted using an in-situ approach and menthol compound using a non in-situ approach. In both the cases, the target compound is extracted with isopropanol containing internal standards (ISTDs), n-heptadecane and ethanol.

The extracts are analyzed by Gas Chromatography (GC) with Flame Ionization Detector (FID). The GC system is equipped with a column RTx-35 30 m x 0.25 mm ID x 1 µm film thickness.

Results are expressed as mg/item for P1.

1.2. Applicability

The method described is used for the determination of glycerin and menthol in mainstream aerosol from Platform 1 (P1) under Health Canada Intense (HCI) and International Irganization for Standardization (ISO) smoking conditions.

1.3. Reagents

- Glycerin
- Menthol
- Extraction solution (Isopropanol with ethanol and n-heptadecane)
- Helium
- Hydrogen

1.4. Aerosol generation

For analysis of glycerin, 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. Mentholated items are conditioned separately in closed packs to avoid menthol losses and cross-contamination.

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

The aerosol samples are generated on a linear smoking machine (LM20X from Borgwaldt KC or equivalent) under ISO or HC smoking regimens and collected on a Cambridge filter pad. Number of accumulations, volume of extraction solution and in-situ or non in-situ extraction depend on the study plan.

Four replicates for each sample are generated. No blanks are required for these two compounds.

For glycerin analysis, aerosol generation is collected on modified metallic (Ø 44 mm) Cambridge filter pad holder which supports in situ extraction methodology needed for the aerosol collection.

For menthol analysis, aerosol generation is collected on plastic filter holder containing the CFP). After the aerosol collection, the CFP is placed in a Filtrona tube to perform the non in-situ extraction (or just the extraction).

The Linear smoking machine should be operated following recommendations from suppliers and/or local procedures. The aerosol collection must start after the end of the pre-heating phase (20 seconds).

The recommended number of P1 per port under the two smoking regimens is detailed in Table **1**.

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
HCI	3	12	55/2/30

1.5. Extraction solution preparation

The extraction solution contains approximately 0.3 mg/mL of n-heptadecane and approximately 2 mg/mL of ethanol in isopropanol. This solution is used for sample extraction and for standard solutions preparation.

1.6. Samples preparation

Directly after the aerosol generation, samples are extracted using non in-situ or in-situ approaches (paragraphs 1.6.1 and 1.6.2, respectively).

After extraction, samples are diluted according to Table 2 before being transferred into GC-vials for analysis.

Table 2: Dilution factors for different types of samples

	Glycerin	Menthol
P1	10x	NA
P1 with menthol	NA	1x

1.6.1. Non in-situ extraction

For menthol analysis, the Cambridge Filter Pad (CFP) is placed in a Filtrona tube directly after aerosol generation and 20mL of extration solution is added. The sealed Filtrona tube is then shaked at 200 rpm for at least 60 minutes.

1.6.2. In-situ extraction

Directly after aerosol generation, the metallic filter holder containing the CFP is placed on an in-situ extractor and an empty syringe is connected on the top of the filter holder. Then, a syringe containing 10 mL of extraction solution is connected to the bottom of the filter holder and the in-situ extractor system is started; the instrument generates a constant back and forth flow cycle of the extraction solution through the metallic filter holder for about 30 minutes. The extraction solution is then collected in a Filtrona tube and sealed.

1.7. Calibration solutions preparation

1.7.1. Glycerin and menthol stock solutions

Glycerin stock solution (2.5 mg/mL) is prepared by dissolution of glycerin in the extraction solution. Similarly, menthol stock solution (4.0 mg/mL) is prepared by dissolution of menthol in the extraction solution.

1.7.2. Calibration standards

Five standard (STD) solutions are prepared in the extraction solution by dilution of the stock solutions. The ranges of concentrations cover the ranges relevant for analysis and are provided in Table 3.

Table 3: Typical standard solution concentrations for glycerin and menthol

Standard level	Glycerin conc. [mg/mL]	Menthol conc. [mg/mL]
1	0.05	0.02
2	0.10	0.08
3	0.25	0.20
4	0.50	0.40
5	1.00	0.80

The standard level 3 is also used as quality check.

1.8. Instrumental Conditions

The samples are analyzed by Gas Chromatography (GC) with Flame Ionization Detector (FID) following tables below:

Table 4: Chromatographic conditions for the determination of glycerin and menthol

Column	Capillary column RTx-35 30 m x 0.25 mm ID x 1 µm film thickness
Run time	12.67 min
Injector	Split/Splitless (mode CAP/SSL)
Injection source	Auto-sampler
Injection volume	1 µL
Injection speed	Fast
Wash solvent	Isopropanol
Carrier Gas	Helium
Carrier Gas flow	1.2 mL/min
Detector	Flame Ionization Detector
Detector temperature	300°C
H_2	40.0 mL/min
Air	350.0 mL/min
Offset	5.0 mV
Oven temperature program	110°C, 1 min
	10°C/min → 150°C, 2 min
	30°C/min → 230°C, 3 min

1.9. Testing procedure

The following typical analytical sequence is used for the determination of glycerin and menthol:

- 3 conditioning injections (STD level 5)
- Suitability test (STD level 3)
- Calibration curve (STD 1 to 5)
- Monitors and samples
- After every 20 samples, inject three quality checks (STD level 3)
- Sequence ends with three quality checks (STD level 3)

1.10. Verification of results

1.10.1. 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 linear regression is calculated automatically by TotalChrom software. Specific information about regressions are provided in Table 5.

Table 5: Parameters used for calibration curves

Compound	Internal standard	Regression type	Weighting factor	R^2
Glycerin	n-heptadecane	linear	1/x	≥ 0.995
Menthol	n-heptadecane	linear	none	≥ 0.999

1.10.2. Quality Check

The validity of the calibration is continuously verified during the batch analysis by analysing the calibration control standard injections. At least two of the three quality checks must be within ±10% of its theoretical value.

1.11. Example Chromatograms

An example chromatogram of glycerin and menthol in standard solution 5 is provided in Figure 1, while an example of chromatograms for a P1 sample with menthol is provided in Figure 2.

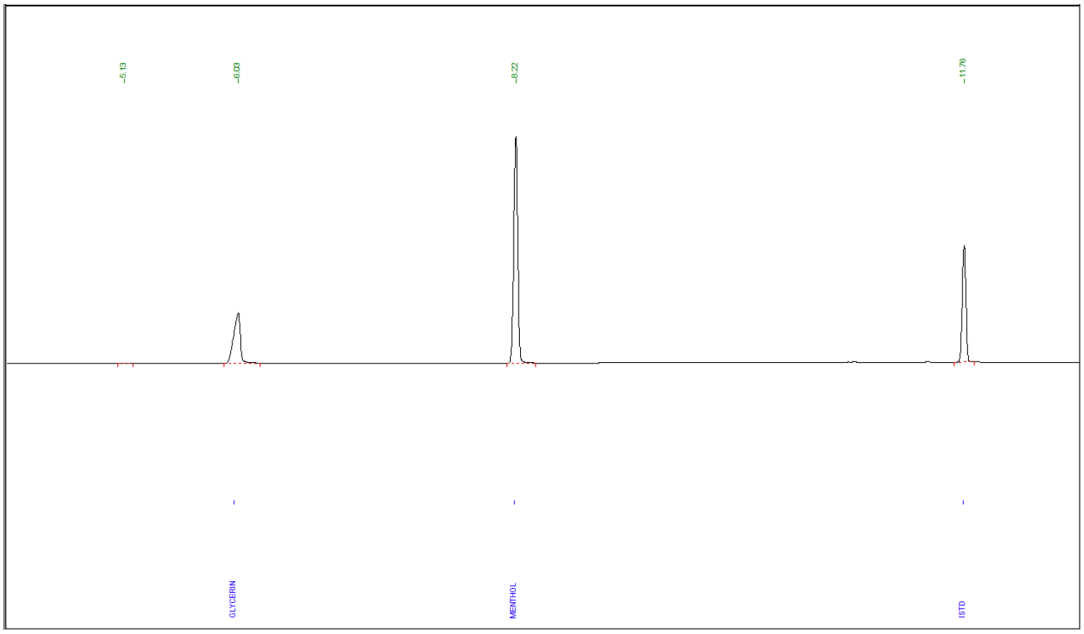

Figure 1: Example chromatogram of standard level 5

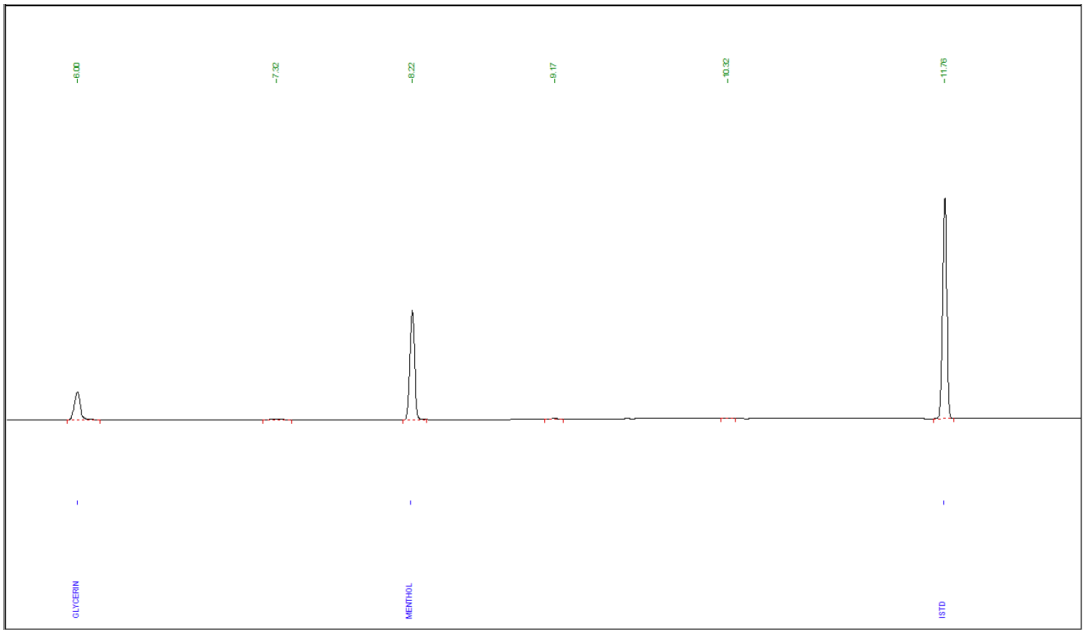

Figure 2: Example chromatogram of P1 samples with menthol

Typical retention times for glycerin, menthol and the ISTDs are provided in Table 6.

Table 6: Typical Retention Times for Target and ISTD Compounds (minutes)

Target		**Internal Standards**	
Glyerin	6.00	*n*-heptadecane	11.80
Menthol	8.20		

1.12. 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 values are provided in Table 7

Table 7: Limits of Detection and Quantitation (HCI and ISO Regimes)

Compound	**P1**					
	HCI			**ISO**		
	LOD	**LLOQ**	**ULOQ**	**LOD**	**LLOQ**	**ULOQ**
	(mg/stick)	(mg/stick)	(mg/stick)	(mg/stick)	(mg/stick)	(mg/stick)
Glycerin	0.073	0.240	0.833	0.017	0.058	0.200
Menthol	0.032	0.105	0.333	0.008	0.025	0.080

1.13. 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 8: Repeatability r and Intermediate precision IP (HCI and ISO Regimes)

Compound	**P1**					
	HCI			**ISO**		
	r	**IP**	**Mean**	**r**	**IP**	**Mean**
	(mg/stick)	(mg/stick)	(mg/stick)	(mg/stick)	(mg/stick)	(mg/stick)
Glycerin	1.076	1.076	4.080	0.287	0.304	1.778
Menthol	0.297	0.297	2.915	0.251	0.347	1.691

1.14. 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
- ISO 8243:2006 – Cigarettes - Sampling