Cooperation Centre for Scientific Research Relative to Tobacco

Heated Tobacco Products Sub-Group

CORESTA Recommended Method No. 107

DETERMINATION OF GLYCEROL, PROPYLENE GLYCOL AND NICOTINE IN THE AEROSOL OF HEATED TOBACCO PRODUCTS BY GAS CHROMATOGRAPHIC ANALYSIS

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(March 2024)

0. INTRODUCTION

In 2021, the Heated Tobacco Products (HTP) Task Force (changed to Sub-Group in 2023) carried out a proficiency study to determine and recommend suitable methods for the analysis of glycerol, propylene glycol, nicotine, nitrogen oxide (NO), nitrogen oxides (NOx), carbon monoxide (CO), Aerosol Collected Mass (ACM), and Device Mass Loss (DML) when applicable in HTP products. Seventeen laboratories and four manufacturers participated in the proficiency study. The sub-categories of HTPs include aerosol Heated Tobacco Products (aHTPs), carbon Heated Tobacco Products (cHTPs), and electrically Heated Tobacco Products (eHTPs). After receiving results and running statistics, it was discovered that many of the laboratories were analyzing the samples using similar methodology and yielding comparable results. Most laboratories had developed analytical methods for analytes in HTP aerosol based on the method used for cigarette smoke and/or e-cigarette aerosol testing, consequently, nearly every laboratory ran essentially the same method. After discussion with the participants in the HTP Task Force it was decided that this study could in essence be considered both a proficiency study as well as a collaborative study. As such, the results of this study have been used to create CORESTA Recommended Methods (CRM). Since four laboratories used different analytical methods to assess glycerol, propylene glycol, and nicotine, data from the four laboratories were excluded in the calculation of repeatability and reproducibility values.

1. SCOPE

This method is applicable to analysis of glycerol, propylene glycol, and nicotine in trapped heated tobacco aerosol.

2. NORMATIVE REFERENCES

CORESTA Recommended Method N° 99, 2023, *Definitions and Standard Conditions: Aerosol Generation and Collection for Aerosol Heated Tobacco Products*

CORESTA Recommended Method N° 100, 2023, *Definitions and Standard Conditions: Aerosol Generation and Collection for Carbon Heated Tobacco Products*

CORESTA Recommended Method N° 101, 2023, *Definitions and Standard Conditions: Aerosol Generation and Collection for Electrically Heated Tobacco Products*

3. PRINCIPLE

Aerosol is generated and collected from HTPs in accordance with the conditions indicated in CRM N° 99, 100, or 101, depending on the sub-category of the product. The collected matter is extracted into isopropyl alcohol solution containing internal standard. The glycerol,

propylene glycol (PG), and nicotine content of this solution are determined by Gas Chromatography with Flame Ionization Detection (GC-FID). ACM and DML are determined gravimetrically. Results are expressed as the weight of analyte collected per consumable, or per puff as warranted.

4. APPARATUS

4.1 A routine analytical aerosol generation and capture system (machine) as described in CRM N° 99, 100, or 101 should be used as appropriate.

4.2 Typical laboratory glassware such as volumetric flasks and extraction vessels.

4.3 Mechanical shaker.

4.4 Analytical balance, suitable for measuring to the nearest 0,1 mg.

4.5 A Gas Chromatograph (GC) equipped with a Flame Ionization Detector (FID) and data collection for glycerol, propylene glycol, and nicotine analysis.

4.6 Gas chromatography capillary column, capable of distinct separation of the peaks for the solvent, internal standard, analytes of interest from each other and other components of the sample extract. Typically, a DB-ALC1 (30 m × 0,32 mm × 1,8 µm) may be used. DB-ALC1, Part # 123-9134 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement of this product.

4.7 Gloves (powder-free) and any other personal protective equipment deemed appropriate by the testing laboratory.

5. REAGENTS

5.1 Propan-2-ol (CAS 67-63-0, analytical grade, maximum water content 1,0 mg/mL).

5.2 Internal standard(s): n-heptadecane (CAS 629-78-7), quinaldine (CAS 91-63-4), n-octadecane (CAS 593-45-3), or other appropriate internal standards which have been demonstrated not to co-elute with other peaks and have been found to be consistent for peak area (minimum purity 99 %).

5.3 Gases: hydrogen (CAS 1333-74-0), nitrogen (CAS 7727-37-9), helium (CAS 7440-59-7) and compressed air as necessary for operation of the GC.

5.4 Glycerol (1,2,3-propanetriol, CAS 56-81-5) (minimum purity 99 %) for preparation of standard solutions.

5.5 Propylene glycol (1,2-propanediol, CAS 57-55-6) (minimum purity 99 %) for preparation of standard solutions.

5.6 Nicotine (CAS 54-11-5); (minimum purity 99 %) for preparation of standard solutions. Nicotine salicylate (CAS 29790-52-1; minimum purity 99 %) may also be used.

Protect from moisture, air, and light as indicated by vendor.

5.7 Extraction solvent

Propan-2-ol (5.1) containing an internal standard with a typical concentration of 1 mg/mL.

6. CALIBRATION STANDARDS

Dissolve glycerol, propylene glycol, and nicotine in the solvent (5.7) to produce a series of at least five calibration solutions including blanks, as warranted, whose concentrations cover the range expected to be found in the samples. A typical range of calibration standards for each analyte is listed in Table 1.

Table 1 - Calibration standards

Analyte	Typical range (mg/mL)
Glycerol	0,05 – 2,0
Propylene glycol	0,05 – 2,0
Nicotine	0,05 – 2,0

Note that a blank standard may be warranted for some analytes depending on the anticipated concentration range of the samples.

Store calibration standards at (4 ± 2) °C and protect from light.

7. PROCEDURES

7.1 Gas chromatography

Set up and operate the gas chromatograph and equipment according to the manufacturer's instructions.

Ensure that peaks of interest are well resolved.

Operating conditions, including column type, should be optimized for analyte separation and sensitivity. Typical operating conditions are as shown in Table 2.

Table 2 - Example of operating conditions for gas chromatography

Column	DB-ALC1 (30 m × 0,32 mm × 1,8 µm)[*1]
Carrier Gas	Helium[*2], 3 mL/min
Injection temperature	250 °C
Injection mode	Split, 25:1
Injection volume	1 µL
Oven temperature	90 °C (1 min), 15 °C/min to 120 °C, 40 °C/min to 280 °C (2 min)
Detector	Flame Ionization
Detector temperature	275 °C

[*1] DB-ALC1, Part # 123-9134 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement of this product

[*2] It is also suitable to use hydrogen or nitrogen as a carrier gas.

7.2 Instrument calibration of the gas chromatograph(s)

Inject aliquots of the standard solutions into the gas chromatograph. Record the peak areas or heights of the analytes and the internal standard.

Calculate the ratio of the analyte peak to the internal standard peak from the peak area (or height) data for each of the calibration solutions. Determine the slope (m) and intercept (b) based on linear regression (y = mx +b) for each of the analytes.

The minimum coefficient of determination, R^2 should be 0,995. The signal (peak area or height) obtained for all test portions must fall within the working range of the calibration curve. If test samples are outside of the working calibration range, the range may be extended if the suitability of the calibration is verified and maintained. Alternatively, samples may be diluted with the solvent (5.7) as appropriate.

7.3 Glass fiber filter pad handling

Glass fiber filter pads should be stored in the target atmosphere of the test conditions for a minimum of 24 hours prior to determination of pre-testing weights.

For all operations, the operator shall prevent contamination from the fingers by wearing gloves of a suitable material (4.7). Glass fiber filter pads should be processed immediately. If samples are not analyzed immediately, a glass fiber filter pad holder cap must be installed to prevent water uptake or loss.

7.4 Aerosol collection and sample preparation

Using the relevant CORESTA Recommended Method N° 99, 100, or 101 depending on the sub-category of the samples, condition the samples, set up the machine, and collect the aerosol onto glass fiber filter pads.

Devices should be fully charged and cleaned according to manufacturer's instructions before the analysis.

Typically, the number of eHTPs or cHTPs is at minimum two and at maximum five per replicate for eHTPs and cHTPs. A fresh liquid-containing consumable and an aHTP are used per replicate for aHTPs.

It should be ensured that the capacity of the glass fiber filter pads for aerosol is not exceeded.

Note: It has been reported that the capacity of a 44 mm Cambridge Filter Pad is up to 380 mg of smoke under the Health Canada intense regime for conventional cigarettes (Drake et al, 2012). It has been reported for e-vapour aerosol that the capacity of a 44 mm Cambridge Filter Pad is up to 850 mg (Miller et al, 2016). Based on the results of the proficiency study, the capacity of a 44 mm glass fiber filter pad is up to 280 mg for eHTP aerosol and 360 mg for aHTP aerosol (Ref. HTP-280-CTR).

In addition, after aerosol collection, remove holder assemblies from the collection system and process for determination of ACM (7.5) and DML (7.6) as necessary.

For each glass fiber filter pad, open the holder and remove glass fiber filter pad with forceps. Fold the pad twice with the aerosol side toward the inside of the folds, being careful to handle only the edge of the glass fiber filter pad. Wipe the inside of the filter holder front with two separate quarters of an unused conditioned filter disc and add these to the flask to collect any residual aerosol. Alternatively, wipe the holder with the folded unused side of the glass fiber filter pad. Transfer to a sample vessel for extraction.

Extract the glass fiber filter pads using a fixed volume of the solvent (5.7) of 20 mL for 44 mm pads or 50 mL for 92 mm pads and 30 min of agitation on a mechanical shaker. The volume of solvent may be adjusted to give analyte concentrations in the range of the calibration standards. The extracts must be protected from light.

7.5 Simultaneous determination of ACM

Optionally, ACM may be determined gravimetrically. Glass fiber filter pads in their holder (with or without end caps) and without sample holders are weighed before and after aerosol collection prior to extraction for analyte analysis. Weights should be determined to the nearest 0,1 mg.

Aerosol collected mass is calculated as:

$$m_{ACM} = m_1 - m_0$$

Where:

m = mass

1 = filter pad holder with glass fiber filter pad, after aerosol collection

0 = filter pad holder with glass fiber filter pad, before aerosol collection

Express the test results in mg/consumable, or mg/puff, as indicated by study design.

7.6 Simultaneous determination of heated tobacco product DML (only aHTP)

Optionally, the DML of the aHTP may be determined gravimetrically. The test article should be weighed prior to installation on the collection system. Typically, the sample may be reweighed after a termination of the collection completion (section 7.5 in CRM 99). Mass loss as a change (Δ) is calculated as noted below and reported as a positive value:

$$\Delta m_{htp} = m_b - m_a$$

Where:

m = mass

htp = heated tobacco products, device

a = heated tobacco product and device, after aerosol collection

b = heated tobacco product and device, before aerosol collection

7.7 Determination of analyte content for the samples

Inject one or more aliquots of the sample extracts into the gas chromatograph using the conditions described in section 7.1. Record the peak areas or heights of analytes and the internal standard.

Calculate the mean value of the ratio of the peak area or height of analyte to that of the internal standard for the replicate injections.

The amount of analyte is determined in mg/mL using an internal standard calibration method. Ensure that the values lie within the range of the standards prepared in section 6.

For each analyte, express the test results in mg/consumable, mg/puff, as indicated by study design.

Example calculations are given below:

$$N = \frac{C \times V}{n}$$

Where:

N = Nicotine content (mg/consumable)

C = the concentration obtained from the calibration curve (mg/mL)

V = the volume of extraction solution added to the sample (typically 20 mL)

n = the number of consumables (eHTP, cHTP, or aHTP) per replicate

8. REPEATABILITY AND REPRODUCIBILITY

Results listed in the tables below are based on the study "Proficiency Study for Propylene Glycol, Glycerol, Nicotine, CO, NO, NOx, ACM, and DML in HTP Aerosol" conducted by the Heated Tobacco Products Task Force using two eHTPs, one aHTP, and one cHTP. The statistical analysis was conducted in basic conformance with ISO 5725-2:1994. Since Lab 3, Lab 6, Lab 13 and Lab 18 used different analytical methods to assess propylene glycol, nicotine and glycerol, these four laboratories' data were excluded in the calculation of statistics estimates. All laboratories' data was used for ACM and DML in the calculation of statistics estimates.

Table 3 - Estimates of test sample mean, repeatability and reproducibility for propylene glycol.

Sample	Number of Labs	Mean mg/consumable	r mg/consumable	R mg/consumable
eHTP-1	11	0,45	0,070	0,213
eHTP-2	1	-	-	-
aHTP	6	54,30	12,30	14,03
cHTP	7	0,17	0,034	0,099

Table 4 - Estimates of test sample mean, repeatability and reproducibility for nicotine.

Sample	Number of Labs	Mean mg/consumable	r mg/consumable	R mg/consumable
eHTP-1	12	1,53	0,260	0,538
eHTP-2	9	0,58	0,114	0,275
aHTP	7	1,26	0,338	0,690
cHTP	10	0,78	0,186	0,290

Table 5 - Estimates of test sample mean, repeatability and reproducibility for glycerol.

Sample	Number of Labs	Mean mg/consumable	r mg/consumable	R mg/consumable
eHTP-1	11	4,73	1,229	1,517
eHTP-2	9	4,71	1,208	2,230
aHTP	7	37,96	9,086	25,37
cHTP	9	14,00	2,319	3,545

Table 6 - Estimates of test sample mean, repeatability and reproducibility for ACM.

Sample	Number of Labs	Mean mg/consumable	r mg/consumable	R mg/consumable
eHTP-1	16	45,26	5,148	19,08
eHTP-2	13	27,73	3,449	13,62
aHTP	12	119,82	26,74	63,80
cHTP	13	42,90	8,105	10,80

Table 7 - Estimates of test sample mean, repeatability and reproducibility for DML.

Sample	Number of Labs	Mean mg/consumable	r mg/consumable	R mg/consumable
aHTP	10	127,68	30,26	41,96

9. TEST REPORT

The test report shall give the analyte content of the sample (i.e. per consumable, per puff, etc.). The test report shall also mention all pertinent operating conditions not specified in this document as well as any circumstances that may have affected the result.

10. BIBLIOGRAPHY

[1] CORESTA Recommended Method N° 84 2021, Determination of Glycerin, Propylene Glycol, Water, and Nicotine in the Aerosol of E-Cigarettes by Gas Chromatographic Analysis

[2] CORESTA Heated Tobacco Products Task Force Technical Report, Proficiency Study for Propylene Glycol, Glycerin, Nicotine, CO, NO, NOx, ACM, and DML in HTP Aerosol (September 2022, HTP-280-CTR)

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[4] Miller JH, Wilkinson J, Spicer-Glave S, Flora JW, Gardner WP. E-cigarette aerosol collection: 44 mm Cambridge filter pad capacity. Paper presented at CORESTA Congress, Berlin, 2016, Smoke Science/Product Technology Groups, STPOST 03