WHO TobLabNet
Official Method
SOP 15

Standard Operating Procedure for Determination of Nicotine, Glycerol and Propylene Glycol Content in the Tobacco of Heated Tobacco Products

World Health Organization

Standard operating procedure for determination of nicotine, glycerol and propylene glycol content in the tobacco of heated tobacco products. WHO TobLabNet Official Method SOP15

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No.: SOP 15
Date: August 2023

Standard operating procedure for determination of nicotine, glycerol and propylene glycol content in the tobacco of heated tobacco products

WHO TobLabNet Official Method SOP15

Method:	Determination of nicotine, glycerol and propylene glycol content in the tobacco of heated tobacco products (HTPs)
Analytes:	Nicotine (3-[(2S)-1-methylpyrrolidin-2-yl]pyridine) (CAS # 54-11-5) Glycerol (propane-1,2,3-triol) (CAS # 56-81-5) Propylene glycol (propane-1,2-diol) (CAS # 57-55-6) Triacetin (glyceryl triacetate) (CAS # 102-76-1)
Matrix:	Tobacco
Last update:	25 August 2023

FOREWORD

This document was prepared by the No Tobacco Unit of the Health Promotion Department of the World Health Organization and members of the WHO Tobacco Laboratory Network (TobLabNet), as an analytical method standard operating procedure (SOP) for measuring nicotine, glycerol and propylene glycol content in the tobacco of heated tobacco products (HTPs). The method is also applicable for the quantification of triacetin upon proper verification in a laboratory, paying particular attention to the recommended quality control criteria.

INTRODUCTION

To establish comparable measurements for testing heated tobacco products (HTPs) globally, consensus methods are required for measuring the nicotine, glycerol and propylene glycol content in the tobacco used in HTPs. WHO TobLabNet reviewed commonly used procedures for the determination of nicotine, glycerol, and propylene glycol in tobacco of heated tobacco products in order to prepare a procedure as a WHO TobLabNet SOP.

The Conference of the Parties (COP) to the WHO Framework Convention on Tobacco Control (WHO FCTC) at its eighth session (Geneva, Switzerland, 1–6 October 2018) requested the Convention Secretariat to invite WHO and WHO Tobacco Laboratory Network to: "(c) to assess whether the available standard operating procedures for contents and emissions are applicable or adaptable to heated tobacco products; (d) to advise, as appropriate, on suitable methods to measure the contents and emissions of these products"; as outlined in paragraph 2 of decision FCTC/COP8(22) on Novel and emerging tobacco products.

This SOP that was prepared to describe the procedure for the determination of nicotine, glycerol and propylene glycol content in the tobacco of HTPs, was adapted based on WHO TobLabNet SOP 11 [**2.1**], WHO TobLabNet SOP 06 [**2.2**] and the publication by Chen et. al [**2.3**].

1. SCOPE

This method is suitable for the quantitative determination of nicotine, glycerol, and propylene glycol content in the tobacco of HTPs by gas chromatography (GC). This method can also be used to quantitatively determine triacetin in the tobacco of HTPs by GC, however triacetin was not validated by a collaborative trial due to the limited number of reported results. The working range of the method for nicotine is up to 50 mg/g, for glycerol, up to 500 mg/g; for propylene glycol, up to 100 mg/g and for triacetin, up to 100 mg/g (optional).

2. REFERENCES

2.1 World Health Organization. 2021. Standard operating procedure for determination of nicotine, glycerol and propylene glycol in e-liquids, Geneva. Tobacco Laboratory Network, WHO TobLabNet SOP 11.

2.2 World Health Organization. 2016. Standard operating procedure for determination of humectants in cigarette tobacco filler, Geneva. Tobacco Laboratory Network, WHO TobLabNet SOP 06.

2.3 Chen A X, Akmam Morsed F and Cheah, N P. 2021. A Simple Method to Simultaneously Determine the Level of Nicotine, Glycerol, Propylene Glycol, and Triacetin in Heated Tobacco Products by Gas Chromatography Flame Ionization Detection. Journal of AOAC INTERNATIONAL.

2.4 ISO 13276: Tobacco and tobacco products — Determination of nicotine purity — Gravimetric method using tungstosilicic acid, 2021.

3 TERMS AND DEFINITIONS

3.1 *Nicotine, glycerol, propylene glycol and triacetin (optional) content:* Individual amounts of nicotine, glycerol, propylene glycol (and triacetin) in HTP tobacco, expressed as mg/g of HTP tobacco.

3.2 *Heated tobacco products (HTPs):* A product containing tobacco or a tobacco substrate that is designed to be heated by a separate source (e.g., electrical, aerosol, carbon, etc.) to produce a nicotine-containing aerosol, which is then inhaled by users.

3.3 *Laboratory sample*: Sample intended for testing in a laboratory, consisting of a single type of product delivered to the laboratory at one time or within a specified period.

3.4 *Test sample*: Sample of HTPs, taken at random from the laboratory sample. The test sample shall be representative of the laboratory sample.

3.5 *Test portion:* Random portion from the test sample to be used for a single determination. (The amount or volume of the test sample taken for analysis, usually of known weight or volume, IUPAC definition).

Note: The number of test portions analyzed per test sample shall be adapted to sample inhomogeneity.

4. METHOD SUMMARY

4.1 Nicotine, glycerol and propylene glycol are extracted from HTP tobacco filler with a diluent consisting of 70% methanol/30% acetonitrile **[7.7]** **[7.8]** and internal standards **[7.9]** **[7.10]**. The extracts are measured by gas chromatography with flame ionization detection (GC-FID).

4.2 The ratios of the peak areas of analytes (nicotine, glycerol and propylene glycol) and corresponding internal standards, derived from the measurement

of standard solutions with known concentrations, are plotted against the analyte concentration. Calibration curves used to determine the analyte content of each test portion are created by linear regression.

5. SAFETY AND ENVIRONMENTAL PRECAUTIONS

5.1 Follow routine safety and environmental precautions, as in any chemical laboratory activity.

5.2 The testing and evaluation of certain products with this test method may require the use of materials or equipment that could be hazardous or harmful to the environment; this document does not purport to address all the safety aspects associated with its use. All persons using this method have the responsibility to consult the appropriate authorities and to establish health and safety practices as well as environmental precautions, in conjunction with any applicable regulatory requirements, prior to its use.

5.3 Special care should be taken to avoid inhalation or dermal exposure to harmful chemicals. Use a chemical fume hood, and wear an appropriate laboratory coat, gloves and safety goggles when preparing or handling undiluted materials, standard solutions, diluent solutions or collected samples.

6. APPARATUS AND EQUIPMENT

Usual laboratory apparatus, in particular:

6.1 Sonicator configured to hold the vessels in position.

6.2 Gas chromatograph equipped with a flame ionization detector (GC-FID).

6.3 Capillary GC column capable of distinct separation of solvent peaks, the peaks for the internal standard, nicotine, and other tobacco components, e.g., Agilent DB-ALC1 (30 m x 0.32 mm, 1.8 µm).

6.4 Calibrated analytical balance with a readability of 0.0001 g.

6.5 Bulb pipette and syringe pipette, e.g., 1.0 ml to 20.0 ml suitable capacity for sample and standard preparation).

6.6 Class A Volumetric flask (suitable capacity for standard preparation).

6.7 Standard food grinder.

7. REAGENTS AND SUPPLIES

All reagents shall be of at least analytical reagent grade unless otherwise noted. When possible, reagents are identified by their Chemical Abstract Service (CAS) registry numbers.

7.1 Carrier gas: Helium [CAS number: 7440-59-7] of high purity (> 99.999%). Hydrogen [CAS number: 1333-74-0] of high purity (> 99.999%) can be used as an alternative carrier gas.

7.2 Auxiliary gases: Air and hydrogen [CAS number: 1333-74-0] of high purity (> 99.999%) for the flame ionization detector.

7.3 Nicotine (3-[(2S)-1-methylpyrrolidin-2-yl]pyridine) [CAS number: 54-11-5] of known purity not less than 98% **[2.4]**. Nicotine salicylate [CAS number: 29790-52-1] of known purity not less than 98% may be used alternatively.

7.4 Glycerol (Propane-1,2,3-triol) [CAS number: 56-81-5] of known purity not less than 98%.

7.5 Propylene glycol (propane-1,2-diol) [CAS number: 57-55-6] of known purity not less than 98%.

7.6 Triacetin (glyceryl triacetate) [CAS number: 102-76-1] of known purity not less than 98% (optional).

7.7 Methanol, chromatographic purity [CAS number: 67-56-1].

7.8 Acetonitrile, chromatographic purity [CAS number: 75-05-8].

7.9 Internal standard for nicotine and triacetin (optional): *n*-heptadecane (purity > 98% of mass fraction) [CAS number: 629-78-7].

7.10 Internal standard for glycerol and propylene glycol: 1,3-butanediol (purity > 99% of mass fraction) [CAS number: 107-88-0].

8. PREPARATION OF GLASSWARE

8.1 Clean and dry glassware in a manner to avoid contamination.

9. PREPARATION OF SOLUTIONS

9.1 Diluent solution

The diluent solution consists of 70% methanol/30% acetonitrile **[7.7]** **[7.8]** (700 mL methanol plus 300 mL acetonitrile) containing appropriate amounts of internal standards:

Pipette 0.50 mL of *n*-heptadecane **[7.9]** plus 2.00 mL 1,3-butanediol **[7.10]** into a 1-litre volumetric flask.

Dilute to volume (1 litre) with 70% methanol/30% acetonitrile **[7.7]** **[7.8]**, mix thoroughly and transfer the solution into a storage container equipped with features to prevent contamination.

Note: The concentration and/or type of internal standard may be adjusted, keeping in mind the possible effect of internal standards on the sensitivity and selectivity, as well as the linear range of the method.

10. PREPARATION OF STANDARDS

Preparation of the standard solutions as described below is for reference purposes. The preparation of the standard solutions can be adjusted, if necessary.

Solvent and solutions stored at low temperatures shall be allowed to equilibrate to (22 ± 5) °C before use.

10.1 Nicotine standard stock solution (5 g/L)

Weigh approximately 500 mg of nicotine **[7.3]** (or 925 mg nicotine salicylate) to the nearest 0.1 mg into a 100 mL volumetric flask and dilute to volume with the diluent solution **[9.1]**.

Mix thoroughly and store between 0 °C and 4 °C protected from light.

10.2 Glycerol standard stock solution (50 g/L)

Weigh approximately 5000 mg of glycerol **[7.4]** to the nearest 0.1 mg into a 100 mL volumetric flask and dilute to volume with the diluent solution **[9.1]**.

Mix thoroughly and store between 0 °C and 4 °C protected from light.

10.3 Propylene glycol standard stock solution (5 g/L)

Weigh approximately 500 mg of propylene glycol **[7.5]** to the nearest 0.1 mg into a 100 mL volumetric flask and dilute to volume with the diluent solution **[9.1]**.

Mix thoroughly and store between 0 °C and 4 °C protected from light.

10.4 Triacetin standard stock solution (20 g/L) (Optional)

Weigh approximately 2000 mg of triacetin **[7.6]** to the nearest 0.1 mg into a 100 mL volumetric flask and dilute to volume with the diluent solution **[9.1]**.

Mix thoroughly and store between 0 °C and 4 °C protected from light.

10.5 Working standard solutions

10.5.1 Pipette the designated amount of nicotine stock standard solution prepared in **10.1** for the specific standard solution into 100 mL volumetric flasks, as described in Table 1.

10.5.2 Pipette the designated amount of glycerol stock standard solution prepared in **10.2** into the same set of 100 mL volumetric flasks **[10.5.1]**, as specified in Table 2.

10.5.3 Pipette the designated amount of propylene glycol stock standard solution prepared in **10.3** into the same set of 100 mL volumetric flasks **[10.5.1]**, as specified in Table 3.

(Optional) Pipette the designated amount of triacetin stock standard solution prepared in 10.4 into the same set of 100 mL volumetric flasks **[10.5.1]**, as specified in Table 4.

10.5.4 Fill the volumetric flasks to the mark (100 mL) with diluent solution **[9.1]**.

10.5.5 Store the standard solutions, protected from light, at 4–8 °C.

10.5.6 Determine the final nicotine, glycerol, propylene glycol and triacetin (optional) concentrations in the standard solutions from the following equation:

$$\text{Final concentration (mg/mL)} = \frac{x*y}{10000}$$

where:

x is the original weight (in mg) of the component as weighed in **10.1**, **10.2**, **10.3** or **10.4**; and

y is the volume of the stock standard solution (in mL) as pipetted in **10.5.1**, **10.5.2**, **10.5.3** and **10.5.4.**

The final concentrations in the nicotine standard solutions are shown in Table 1, the glycerol concentrations are shown in Table 2, the propylene glycol concentrations are shown in Table 3, and the triacetin concentrations are shown in Table 4 (optional).

Table 1. Concentrations of nicotine in standard solutions

Standard	Volume of nicotine stock standard solution (**10.1**) (mL)	Total volume (mL)	Nominal nicotine concentration in final mixed standard solution (mg/mL)
1	2.0	100	0.1
2	4.0	100	0.2
3	8.0	100	0.4
4	16.0	100	0.8
5	20.0	100	1.0

Table 2. Concentrations of glycerol in standard solutions

Standard	Volume of glycerol stock standard solution (**10.2**) (mL)	Total volume (mL)	Nominal glycerol concentration in final mixed standard solution (mg/mL)
1	1.0	100	0.5
2	4.0	100	2.0
3	8.0	100	4.0
4	16.0	100	8.0
5	20.0	100	10.0

Table 3. Concentrations of propylene glycol in standard solutions

Standard	Volume of propylene glycol stock standard solution (**10.3**) (mL)	Total volume (mL)	Nominal propylene glycol concentration in final mixed standard solution (mg/mL)
1	0.6	100	0.0
2	4.0	100	0.2
3	10.0	100	0.5
4	20.0	100	1.0
5	40.0	100	2.0

Table 4. Concentrations of triacetin in standard solutions (optional)

Standard	Volume of triacetin standard solution (**10.4**) (mL)	Total volume (mL)	Nominal triacetin concentration in final mixed standard solution (mg/mL)
1	0.5	100	0.1
2	1.5	100	0.3
3	2.5	100	0.5
4	5.0	100	1.0
5	10.0	100	2.0

The range of the standard solutions may be adjusted, depending on the equipment used and the samples to be tested, keeping in mind the possible effect on the working range of the method.

All solvents and solutions must be adjusted to room temperature (22 ± 5 °C) before use.

11. SAMPLING

11.1 Sample collection

Sample HTPs to obtain a representative sample, as required by applicable regulation or availability of samples.

11.2 Constitution of test sample

Divide the laboratory sample into separate sales units, if applicable.

Take a representative number of HTP sticks from at least $\sqrt{n}$ **[2.5]** of the individual sales units (e.g., packet, container) for the preparation of the test sample.

12. PRODUCT PREPARATION

12.1 Remove the tobacco from at least one sales unit of the HTP sticks from which the test portion and quality control samples (when applicable) will be formed.

12.2 Combine and homogenize the tobacco of the HTP sticks from **12.1** to constitute at least 2 g.

13. PREPARATION OF THE SMOKING MACHINE

Not applicable.

14. SAMPLE GENERATION

Not applicable.

15. SAMPLE PREPARATION

15.1 Mix and grind the tobacco from **12.2** until it is well homogenized.

15.2 Weigh about 0.2—0.3 g of the well-mixed, ground tobacco sample into a glass extraction vessel.

15.3 Add 10 mL of diluent to the sample.

15.4 Sonicate the vessel for 60 minutes.

15.5 Transfer an aliquot of the sample extract into an autosampler vial, preventing solid particles from entering the vial.

15.6 If the sample is to be stored before analysis, keep it protected from light at 4–8 °C.

16. SAMPLE ANALYSIS

GC with flame ionization detection is used to quantify nicotine, glycerol, propylene glycol and triacetin (optional) in HTPs. The analytes are separated from other potential interference on the column used. Individual analyte concentrations are derived by comparison of the peak area ratios of the unknowns with the peak area ratios of the known standard concentrations.

16.1 GC operating conditions. Example:

GC column: Agilent DB-ALC1 (30 m x 0.32 mm, 1.8 µm), or equivalent
GC parameters:

Oven:	140 °C for 5 mins, 140—180 °C at 40 °C/min, 180 °C for 4 mins, 180—230 °C at 5 °C/min
Injection temperature:	225 °C
Detector temperature:	260 °C
Carrier gas:	Helium at a flow rate of 1.5 ml/min
Injection volume:	1.0 µl
Injection mode:	Split

Note: Adjustment of the operating parameters may be required, depending on the instrument and column conditions as well as the resolution of chromatographic peaks.

Under the above conditions, the expected total analysis time will be about 20 minutes. (The analysis time may be extended to optimize separation).

For informative purposes, Appendix 2 provides an example of GC-mass spectrometry (GC-MS) settings to be used if GC-MS is used as an alternative to GC-FID.

16.2 Expected retention times

The elution order and retention times must be verified before test sample analysis is begun.

Note: For the conditions described in **16.1**, the expected sequence of elution will be propylene glycol, 1,3-butanediol, glycerol, nicotine, triacetin and *n*-heptadecane.

Differences in for example temperature, gas flow rate and age of the column may alter retention times.

16.3 Determination of nicotine, glycerol, propylene glycol and triacetin (optional)

The quality assurance policies and procedures of the specific laboratory will determine the practices and sequence of samples analyzed. This section illustrates an example of practices and sequence of samples analyzed for determining nicotine, glycerol, propylene glycol and triacetin (optional) content in the tobacco of HTPs.

Inject aliquots of the standard solutions and sample extracts under identical conditions.

16.3.1 Condition the system just before use by injecting two 1.0-µl aliquots of a sample solution as a primer.

16.3.2 Inject 1.0 µL diluent solution **[9.1]** and a test standard solution under the same conditions as the samples to verify the performance of the GC system and absence of contamination of reagents used.

16.3.3 Inject an aliquot of each of the combined nicotine, glycerol, propylene glycol and triacetin (optional) standard solutions into the GC.

16.3.4 Assess the retention times and responses (area counts) of the analytes in the standard solutions. If the retention times are similar (± 0.2 min) to the retention times in previous injections, and the responses are within 20% of typical responses in previous injections, the system is ready to perform the analysis. If the responses are outside specifications, seek corrective action according to your laboratory policy.

16.3.5 Record the peak areas of nicotine, glycerol, propylene glycol and triacetin (optional) and the internal standard compounds.

16.3.6 Calculate the peak area ratios (RF) of the analyte peaks to the internal standard peak for each of the analytes in each standard solution, including the solvent blanks according to the following equation.

$$RF = A_{analyte} / A_{IS}$$

where:

RF is the peak area ratio;

$A_{analyte}$ is the peak area of the analyte peak; and

A_{IS} is the peak area of the internal standard peak.

16.3.7 Plot the concentrations of the analytes in the standard solutions (X axis) against the peak area ratios (RFs) (Y axis) as calculated in **16.3.6**.

Note: The calibration functions are expected to be linear over the specified concentration ranges.

16.3.8 Calculate the calibration function ($Y = a + bx$) by linear regression from this data and use both the slope (b) and the intercept (a) of the linear regression for calculation of analytical results. If the coefficient of determination (R^2) is less than 0.99, the calibration should be repeated. Check for individual outliers according laboratory procedures.

16.3.9 Inject 1.0 µl of each of the test portion extracts (**15.5**) and if applicable of the quality control samples **[20.3]** and determine the peak areas with the appropriate instrument software.

Note: See Appendix 1 for representative chromatograms

17. DATA ANALYSIS AND CALCULATIONS

17.1 For each test portion, calculate the ratio (Y_t) of the analyte peak areas to the internal standard peak area.

Note: The analyte peak area ratios (Y_t) obtained for all test portions must fall within the working range of the calibration curve; otherwise, standard solutions or test portions concentrations should be adjusted as necessary

17.2 Calculate the component concentration in mg/mL for each test portion according to the following equation, using the coefficients of the calibration curves determined in **16.3.8**.

$$M_t = (Y_t - a) / b$$

where:

M_t is the concentration of the analyte in the test solution in mg/mL;

Y_t is the ratio of the peak area of the analyte to the peak area of the internal standard;

a is the intercept of the calibration curve obtained by linear regression in **16.3.8**; and

b is the slope of the calibration curve obtained by linear regression in **16.3.8**.

17.3 Calculate the analyte content (m_c) in the tobacco test sample expressed in mg/g tobacco using the following equation:

$$m_c = \frac{M_t \times V_e}{m_o}$$

where:

m_c is the content of the analyte in the test sample, in mg/g;

M_t is the concentration of the analyte in the test solution, in mg/mL;

V_e is the volume of the extraction solution used, in mL; and

m_o is the mass of test portion **[12.1]** in g.

18. SPECIAL PRECAUTIONS

18.1 After installing a new column, condition it by injecting a test sample (tobacco sample) solution under the GC conditions described in **16.1**. Injections should be repeated until the peak areas (or heights) of both the component(s) and the internal standard(s) are reproducible. This may require approximately four injections.

18.2 It is recommended to elute high-boiling-point components from the GC column after each sample set (series) by raising the column temperature to the maximum allowed isothermal temperature of the column for 30 minutes.

18.3 When the peak areas (or heights) observed for the internal standard(s) in a test portion are significantly higher than expected, it is recommended to extract an aliquot of the test portion in a solution consisting of 70% methanol/30% acetonitrile **[7.7]** **[7.8]** without internal standard. This makes it possible to determine whether any component co-elutes with the internal standard, which would bias (artificially lower) results of analysis.

19. DATA REPORTING

19.1 Report individual measurements for each sample evaluated.

19.2 Report results as specified in overall project specifications.

19.3 For more information, see World Health Organization. Standard operating procedure for validation of analytical methods of tobacco product contents and emissions. Geneva, Tobacco Laboratory Network, 2017 (WHO TobLabNet SOP 02) **[2.6]**.

20. QUALITY CONTROL

20.1 Control parameters

Note: If the quality control measurement results are outside the tolerance limits of the expected values, appropriate investigation and action must be taken.

Note: Additional laboratory quality assurance procedures should be carried out in compliance with the policies of the individual laboratory.

20.2 Laboratory reagent blank

To detect potential contamination during sample preparation and analysis, include a determination of diluent solution **[9.1]**. The results should be less than the limit of detection of the specific component.

20.3 QUALITY CONTROL SAMPLE

To verify the consistency of the entire analytical process, analyze a reference or quality control HTP tobacco sample in accordance with the practices of the individual laboratory.

21. METHOD PERFORMANCE SPECIFICATIONS

21.1 Note: Laboratory is encouraged to verify the method in accordance with its quality practices. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD is specified as three times the standard deviation of the mean of blank determinations, and the LOQ is specified as 10 times the standard deviation of the mean of blank determinations. LOD and LOQ are shown in Table 5 (data taken from by single laboratory validation).

Table 5. LOD and LOQ of nicotine, glycerol, propylene glycol and triacetin in HTPs.

Component	LOD (mg/g)	LOQ (mg/g)
Nicotine	0.05	0.2
Glycer	1.4	4.7
Propylene glycol	1.0	1.0
Triacetin	0.2	0.8

Note: The LOD and LOQ values listed in Table 5 are outside the working ranges of this method.

21.2 Laboratory-fortified matrix recovery

The recovery of the analyte(s) spiked into the matrix was used as a surrogate measure of accuracy. The recovery of the components was determined by single laboratory validation. Tea leaves were used for the determination of the recovery because no raw tobacco without nicotine, glycerol, propylene glycol and triacetin was available. If raw tobacco was used for the determination of the recovery, the results would have been outside the working range of the method.

The recovery by single laboratory validation was determined by adding different amounts of nicotine, glycerol, propylene glycol and triacetin standard stock solutions into 10 mL volumetric flasks containing 0.2—0.3 g of tea leaves. After homogenizing the flasks for 60 minutes using a sonicator **[6.1]**, the recovery samples were transferred into autosampler vials. For each of the spiked samples, nicotine, glycerol, propylene glycol and triacetin were determined by analyzing the individual vials in one day. This procedure was duplicated on a second day to provide the results below. The native (not spiked) tea leaves were also analyzed. The recovery is calculated using the following equation and is summarized for nicotine in Table 6, glycerol in Table 7, propylene glycol in Table 8, and triacetin in Table 9.

$$R = 100 \times \frac{c_r - c_n}{c}$$

where:

R is the recovery, in %;

c_r is the concentration of the analyte in the recovery sample, in mg/mL;

c_n is the concentration of the analyte in the native sample, in mg/mL; and

c is the nominal analyte concentration in the recovery sample, in mg/mL.

Table 6. Mean and recovery of nicotine by single laboratory validation

Nicotine					
Day 1			Day 2		
Spiked amount (mg/g)	Mean (mg/g)	Recovery (%)	Spiked amount (mg/g)	Mean (mg/g)	Recovery (%)
5.2	5.0	89.8	5.3	5.2	91.6
20.6	20.2	97.0	21.1	20.8	95.9
41.3	40.8	97.4	42.2	41.0	95.0

Table 7. Mean and recovery of glycerol by single laboratory validation

Glycerol					
Day 1			Day 2		
Spiked amount (mg/g)	Mean (mg/g)	Recovery (%)	Spiked amount (mg/g)	Mean (mg/g)	Recovery (%)
25.1	25.0	98.1	25.2	25.4	100.5
251.0	250.7	98.2	251.4	244.1	95.2
401.6	409.2	101.1	402.3	398.2	97.2

Table 8. Mean and recovery of propylene glycol by single laboratory validation

Propylene Glycol					
Day 1			Day 2		
Spiked amount (mg/g)	Mean (mg/g)	Recovery (%)	Spiked amount (mg/g)	Mean (mg/g)	Recovery (%)
2.5	2.5	98.8	2.6	2.6	98.6
25.3	25.1	98.0	25.5	24.8	95.5
75.8	76.1	100.2	76.6	77.4	100.9

Table 9. Mean and recovery of triacetin by single laboratory validation

Triacetin					
Day 1			Day 2		
Spiked amount (mg/g)	Mean (mg/g)	Recovery (%)	Spiked amount (mg/g)	Mean (mg/g)	Recovery (%)
5.0	4.9	90.6	5.0	5.0	98.8
50.3	49.6	93.3	50.2	50.3	98.4
150.9	153.8	99.7	150.7	151.8	100.4

21.3 Analytical selectivity

The retention time of the analyte of interest is used to verify the analytical selectivity. An established range of ratios of the responses of the analytes to those of the internal standard compounds of quality control tobacco is used to verify the selectivity of the gas chromatographic measurements for an unknown sample.

21.4 Linearity

The nicotine calibration curve established is linear over the concentration range of 0.1—1.0 mg/mL. The glycerol calibration curve established is linear over the concentration range of 0.5—10.0 mg/mL. The propylene glycol calibration curve established is linear over the concentration range of 0.03—2.0 mg/mL. The triacetin calibration curve established is linear over the concentration range of 0.1—2.0 mg/mL (optional).

21.5 Possible interference

The presence of flavourings can cause interference, due to a similar retention time to one of the analytes or internal standard compounds.

22. REPEATABILITY AND REPRODUCIBILITY

An international collaborative study conducted from January 2022 to June 2022, involving 12 laboratories and three heated tobacco product samples, performed according to WHO TobLabNet Method Validation Protocol and this SOP, gave the following values for this method.

The test results were analysed statistically in accordance with ISO 5725-1 [**2.7**] and ISO 5725-2 [**2.8**] to give the precision data shown in Tables 10—12.

Table 10. Precision limits for the determination of nicotine content (mg/g) in the tobacco (substrate) of heated tobacco products

Heated tobacco products	n	$\hat{m}$	Repeatability limit	Reproducibility limit (R)
HTP1	10	13	1	5
HTP2	11	12	0	4
HTP3	11	13	1	5

Table 11. Precision limits for the determination of glycerol content (mg/g) in the tobacco (substrate) of heated tobacco products

Heated tobacco products	n	$\hat{m}$	Repeatability limit	Reproducibility limit (R)
HTP1	11	133	16	33
HTP2	10	108	11	27
HTP3	11	187	17	52

Table 12. Precision limits for the determination of propylene glycol content (mg/g) in the tobacco (substrate) of heated tobacco products

Heated tobacco products	n	$\hat{m}$	Repeatability limit	Reproducibility limit (R)
HTP1	12	3	0	2
HTP2	11	4	0	2
HTP3	9	6	0	1

23. BIBLIOGRAPHY

23.1 United Nations Office on Drugs and Crime. 2009. Guidelines on representative drug sampling. Vienna, Laboratory and Scientific Section (http://www.unodc.org/documents/scientific/Drug_Sampling.pdf).

23.2 World Health Organization. 2017. Standard operating procedure for validation of analytical methods of tobacco product contents and emissions. Geneva, Tobacco Laboratory Network (WHO TobLabNet SOP 02).

23.3 ISO5725-1. 2019. Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions.

23.4 ISO 5725-2. 2019. Accuracy (trueness and precision) or measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.

23.5 Report of a collaborative study for the validation of an analytical method for the determination of nicotine, glycerol and propylene glycol content in the tobacco of Heated Tobacco Products (HTPs) (in press).

ANNEX 1

Typical chromatograms obtained in the analysis of HTP for the determination of nicotine, glycerol propylene glycol and triacetin (optional) in the tobacco of HTP

Fig. A1.1. Example of a chromatogram of a standard solution with nicotine concentration of 0.4 mg/mL, glycerol concentration of 4.0 mg/mL, propylene glycol concentration of 0.5 mg/mL and triacetin concentration of 0.5 mg/mL.

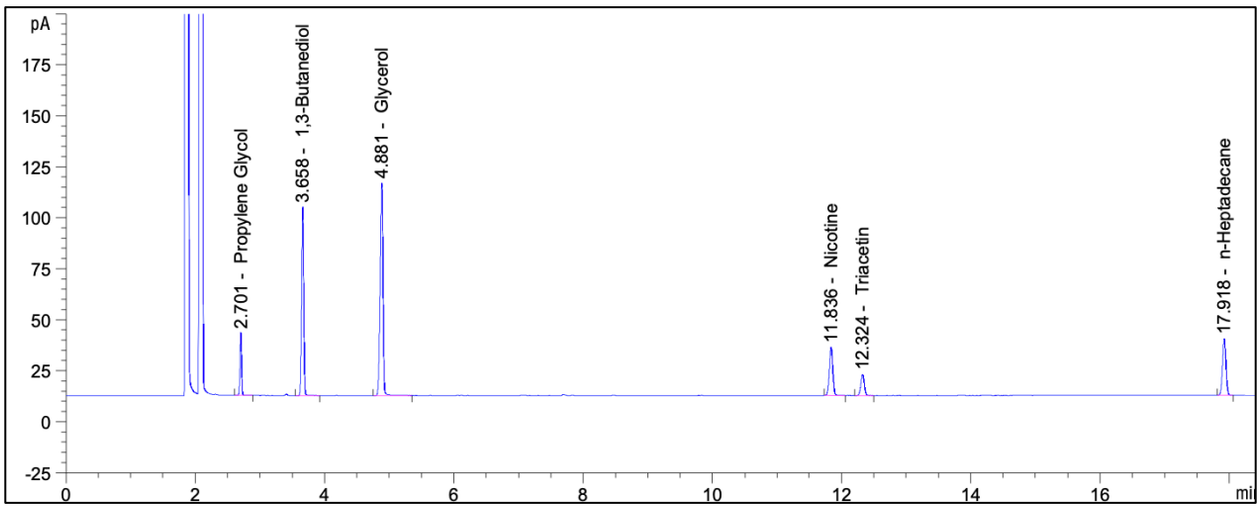
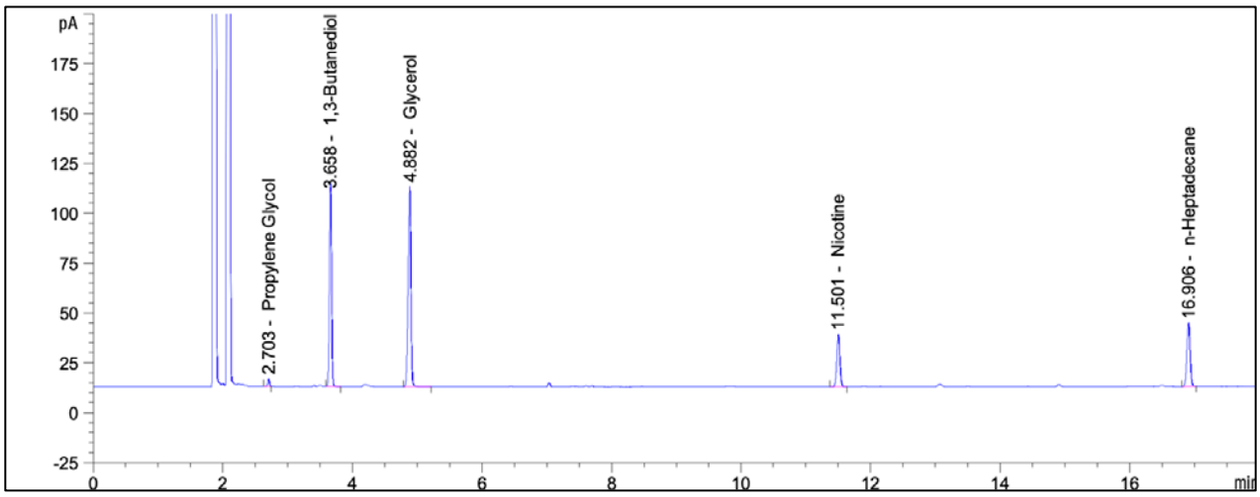


Fig. A1.2. Example of a chromatogram of an extract of HTP tobacco (substrate)



ANNEX 2
GC-MS settings for alternative measurement technique

Specific GC conditions, like carrier gas, flow rate and total run time, can be adapted to specific MS needs.

MS operating conditions:

Transfer line temperature: >= 180°C
Dwell time: 50 msec
Ionization mode: Electron Ionization (electron energy 70 eV)
Detection: propylene glycol: m/z 61 (quantifier ion) 45 (confirmation ion)
glycerol: m/z 61 (quantifier ion) 43 (confirmation ion)
nicotine: m/z 162 (quantifier ion) 133 (confirmation ion)
triacetin: m/z 145 (quantifier ion) 103 (confirmation ion)
quinaldine: m/z 143 (quantifier ion) 128 (confirmation ion)
n-heptadecane: m/z 240 (quantifier ion) 85 (confirmation ion).

Use of this internal standard is not recommended for GC-MS detection due to its low specific mass spectrum.

The recommended internal standard for GC-MS is the deuterated form of the analyte. Alternative internal standards, such as quinaldine or n-heptadecane can be used. The user should verify the performance of the alternative internal standards in own laboratory to ensure the quality control criteria is met.

GC-MS data are not included in the collaborative trial to establish the repeatability and reproducibility data of this method.

This document was prepared by the No Tobacco Unit of the Health Promotion Department of the World Health Organization and members of the WHO Tobacco Laboratory Network (TobLabNet), as an analytical method standard operating procedure (SOP) for measuring nicotine, glycerol and propylene glycol content in the tobacco of heated tobacco products. The method is also applicable for the quantification of triacetin upon proper verification in a laboratory, paying particular attention to the recommended quality control criteria.

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