WHO TobLabNet
Official Method
SOP 11

Standard Operating Procedure for Determination of Nicotine, Glycerol and Propylene Glycol in E-Liquids

No Tobacco Unit (Tobacco Free Initiative)
Tobacco Laboratory Network (TobLabNet)

World Health Organization

WHO TobLabNet
Official Method

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No.: SOP 11
Date: 31 March 2021

World Health Organization

World Health Organization Tobacco Laboratory Network

Standard operating procedure for method

Determination of nicotine, glycerol and propylene glycol in e-liquids

Method:	Determination of nicotine, glycerol and propylene glycol in e-liquids
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)
Matrix:	e-liquid
Last update:	March 2021

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FOREWORD

This document was prepared by members of the World Health Organization (WHO) Tobacco Laboratory Network (TobLabNet) in cooperation with member laboratories of the European Joint Action on Tobacco Control (JATC) as an analytical method standard operating procedure (SOP) for measuring nicotine, glycerol and propylene glycol in e-liquids.

INTRODUCTION

In order to establish comparable measurements for testing e-liquids globally, consensus methods are required for measuring specific contents of e-liquids. The Conference of the Parties (COP) to the WHO Framework Convention on Tobacco Control (WHO FCTC) at its sixth session (Moscow, Russian Federation, 13–18 October 2014) requested the Convention Secretariat to invite WHO to: (a) prepare an expert report on electronic nicotine delivery systems (ENDS) and electronic non-nicotine delivery systems (ENNDS) for the seventh session of the COP (COP7), with an update on the evidence of the health impacts of ENDS/ENNDS, their potential role in quitting tobacco usage and impact on tobacco control efforts; (b) subsequently assess policy options to achieve the objectives outlined in paragraph 2 of decision FCTC/COP6(9); and (c) consider the methods to measure the contents and emissions of these products.[1]

As nicotine content is limited to a certain concentration in some parts of the world (for example, in the European Union, the maximum nicotine concentration in e-liquids is 20 mg/mL), nicotine is considered a priority component to be measured in e-liquids. Since glycerol and propylene glycol are typically ingredients of e-liquids and can be measured simultaneously with nicotine, these components are included in the SOP.

This SOP was prepared to describe the procedure for the determination of nicotine, glycerol and propylene glycol in e-liquid and based on ISO 20714 [**2.1**].

1 SCOPE

This method is suitable for the quantitative determination of nicotine, glycerol and propylene glycol in e-liquids by gas chromatography (GC). The working range of the method is for 1 to 30 mg/mL nicotine, for 200 mg/mL to 1000 mg/mL propylene glycol and for 200 mg/mL to 1000 mg/mL glycerol.

[1] Decision FCTC/COP6(9).

2 REFERENCES

2.1 *ISO 20714 (en). E-liquid—Determination of nicotine, propylene glycol and glycerol in liquids used in electronic nicotine delivery devices—Gas chromatographic method (ISO 20714:2019, IDT).*

2.2 *ISO 13276: Tobacco and tobacco products—Determination of nicotine purity—Gravimetric method using tungstosilicic acid.*

2.3 *ISO 5725-2: 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.*

2.4 *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)* (https://www.who.int/tobacco/publications/prod_regulation/standard-operation-validation-02/en/, accessed 10 December 2020).

2.5 *United Nations Office on Drugs and Crime. Guidelines on representative drug sampling. Vienna, Laboratory and Scientific Section, 2009* (http://www.unodc.org/documents/scientific/Drug_Sampling.pdf, accessed 10 December 2020).

3 TERMS AND DEFINITIONS

3.1 *Nicotine content*: total amount of nicotine in e-liquid, expressed as mg per millilitre of e-liquid.

3.2 *E-liquid*: liquid or gel which may or may not contain nicotine intended for aerosolization, to be inhaled with an electronic delivery device.

3.3 *Electronic nicotine delivery device system/electronic non-nicotine delivery system*: device used to aerosolize an e-liquid for inhalation.

3.4 *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.

3.5 *Test sample*: product to be tested, taken at random from the laboratory sample. The number of products taken shall be representative of the laboratory sample.

3.6 *Test portion*: random portion from the test sample to be used for a single determination. The number of products taken shall be representative of the test sample.

3.7 *Prefilled e-liquid cartridges*: prefilled e-liquid containers that can be or are connected directly to an electronic nicotine delivery device.

4 METHOD SUMMARY

4.1 After bringing to room temperature, the e-liquid is homogenized.

4.2 The e-liquid is diluted with a diluent consisting of propan-2-ol [**7.6**] and internal standards [**7.7**] [**7.8**].

4.3 The ratios of the peak areas of analytes (nicotine, glycerol, 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 with the appropriate authorities and to establish health and safety practices as well as environmental precautions in conjunction with any existing 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 Positive displacement pipette, applicable for repetitive pipetting 100 µL (e.g. Brand HandyStep Electronic or Eppendorf Repeater stream).

6.2 Positive displacement pipette, applicable for repetitive pipetting 9.9 mL (e.g. Brand HandyStep Electronic or Eppendorf Repeater stream).

6.3 Transparent 20 mL flat bottom sample vials or other suitable flasks.

6.4 3D rotating mixer configured to hold flasks in position (e.g. Stuart Scientific gyro rocker, MiuLab RH-18+ 3D Rotating Mixer).

6.5 Vortex mixer.

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

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

6.8 In case of prefilled e-liquid cartridges: centrifuge tubes, with screw cap, designed for at least 1500 rpm (relative centrifugal force (RCF) ≈ 315) and capable of holding different types of prefilled e-liquid cartridges.

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 Abstracts Service (CAS) registry numbers.

7.1 Carrier gas: Helium [CAS number: 7440-59-7] of high purity (> 99.999%).

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

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 Propan-2-ol [CAS number: 67-63-0], with a maximum water content of 1.0 g/L.

7.7 Internal standard for GC analyses:
Nicotine determination:
n-heptadecane (purity > 98% of mass fraction) [CAS number: 629-78-7].
Quinaldine (purity > 98% of mass fraction) [CAS number: 91-63-4] or other suitable alternatives may be used.

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

8 PREPARATION OF GLASSWARE

Clean and dry glassware in a manner to avoid contamination.

9 PREPARATION OF SOLUTIONS

9.1 Diluent solution

The diluent solution consists of propan-2-ol [**7.6**] containing appropriate amounts of internal standards (0.5 mL/L n-heptadecane / 2 mL/L 1,3-Butanediol).

Pipette 0.50 mL of n-heptadecane [**7.7**] plus 2.0 mL 1,3-Butanediol [**7.8**] into a 1-litre volumetric flask. Dilute to volume (1 litre) with propan-2-ol [**7.6**], 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.

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 and exclude light (maximum storage of four weeks).

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

10.2 Glycerol and propylene glycol standard stock solution (50 g/L)

Weigh approximately 5000 mg of glycerol plus 5000 mg of propylene glycol 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 (maximum storage of four weeks).

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

10.3 Working standard solutions

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

10.3.2 Add the designated amount of the combined glycerol and propylene glycol standard stock solution prepared in **10.2** into the same 100 mL volumetric flasks [**10.3.1**], as specified in Table 2.

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

Store the standard solutions, protected from light, at 4–8 °C (maximum storage of one week).

10.3.3 Determine the final nicotine, glycerol and propylene glycol concentrations in the calibration standard solutions from:

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

where *x* is the original weight (in mg) of the analyte as weighed in **10.1** or **10.2**, and y is the volume of the standard stock solution (in mL) as pipetted in **10.3.1** and **10.3.2**.

The nominal concentrations in the nicotine calibration standard solutions are shown in Table 1, the glycerol and propylene glycol concentrations are shown in Table 2.

Table 1. Concentrations of nicotine in calibration standard solutions

Standard	Volume of nicotine standard stock solution (5 g/L) (mL)	Volume of internal standard solution (µL)	Total volume (mL)	Approximate nicotine concentration in final mixed standard solution (mg/mL)	Corresponding nicotine concentration in e-liquid (mg/mL)
1	0.2	Not applicable, included in diluent solution	100	0.01	1.0
2	1.0		100	0.05	5.0
3	2.0		100	0.10	10.0
4	4.0		100	0.20	20.0
5	6.0		100	0.30	30.0

Table 2. Concentrations of glycerol and propylene glycol in calibration standard solutions

Standard	Volume of glycerol / propylene glycol standard solution (50 g/L) (mL)	Volume of internal standard solution (µL)	Total volume (mL)	Approximate glycerol / propylene glycol concentration in final mixed standard solution (mg/mL)	Corresponding glycerol / propylene glycol concentration in e-liquid (mg/mL)
1	4.0	Not applicable, included in diluent solution	100	2.0	200
2	8.0		100	4.0	400
3	12.0		100	6.0	600
4	16.0		100	8.0	800
5	20.0		100	10.0	1000

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

All solvents and solutions shall be allowed to equilibrate to room temperature (22 ± 5 °C) before use.

11 SAMPLING

11.1 Sample collection

Sampling of e-liquids to obtain a representative sample shall be performed according to applicable legislation or international standards and shall take the availability of samples into account.

11.2 Constitution of test sample

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

For each test sample, take a representative number of products from the laboratory sample.

Each test portion shall consist of at least one unit of the product to be tested.

12 SAMPLE PREPARATION

Due to the high viscosity of e-liquids, special care must be taken when these types of liquids are to be pipetted. For pipetting of high-viscosity liquids, only positive displacement pipettes can be used. Internal laboratory validation showed great effect of pipetting procedures on the results, for this reason a dedicated pipetting procedure is included in the SOP.

If no positive displacement pipettes are available, weighing of the e-liquid can be used as an alternative. However, to be able to report the results in mg/mL units, the density of the e-liquid needs to be determined and the mass of the e-liquid has to be converted into volumetric units (mL).

The specific procedure for pipetting e-liquids is described in **12.1**, extraction of e-liquids from prefilled cartridges is described in **12.2**.

12.1 Refill bottles

12.1.1 Homogenize the e-liquid prior to opening the container. Formation of air bubbles needs to be avoided. If air bubbles are present in the e-liquid, do not use the e-liquid until all air bubbles have disappeared (sonication of the e-liquid will help to remove the air bubbles, avoid increasing temperature caused by sonication).

12.1.2 Aspirate 1 mL of e-liquid, without air bubbles, by using a positive displacement pipette with the possibility of 10-fold repetitive dispensation, set to 100 µL dispensation.

12.1.3 Strike the outer side of the pipette tip against the sample container edge to remove excess liquid.

12.1.4 Dispense twice 100 µL into a waste bin, without touching the waste bin with the pipette tip.

12.1.5 Check if the outside of the pipette tip contains e-liquid remains, if so, repeat step **12.1.3**.

12.1.6 Again, dispense twice 100 µL into a waste bin, without touching the waste bin with the pipette tip.

12.1.7 Check if the outside of the pipette tip contains e-liquid remains.

12.1.8 Pipette 100 µL e-liquid ($V_{e\text{-}liquid}$) into a 20 mL sample vial [**6.3**].

12.1.9 Pipette the remaining e-liquid into a waste bin.

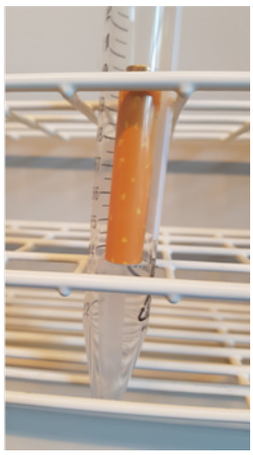
12.1.10 Close the e-liquid container as soon as possible to prevent evaporation or contamination of the sample.

12.2 Prefilled cartridges

To be able to analyse the e-liquid in prefilled cartridges, the liquid needs to be extracted from the cartridge. To avoid contamination of the e-liquid by parts of the cartridge, or tools used for opening the cartridge, a procedure for extracting the total amount of e-liquid from cartridges using a centrifuge is described.

Alternative methods can also be used taking into account that the e-liquid extracted from the cartridge needs to be a representative sample of the cartridge.

12.2.1 Cut off a piece of a disposable pipette tip (100—1000 µL) and place the piece into a suitable centrifuge tube [**6.8**].



12.2.2 Remove the upper cap of the e-liquid cartridge.

12.2.3 Place the cartridge top down into the centrifuge tube, on top of the pipette tip.

12.2.4 Close the centrifuge tube, using the screw cap.

12.2.5 Centrifuge the centrifuge tube for at least five minutes at 1500 rpm (relative centrifugal force (RCF) ≈ 315).

12.2.6 Remove the cartridge and pipette tip from the centrifuge tube (use a pair of tweezers if needed, avoid contamination).

12.2.7 Pipette the e-liquid according to **12.1**.

Note: When less than 1 mL of e-liquid is available for pipetting, an alternative pipetting method needs to be executed.

If for instance only 600 µL e-liquid is available, aspirate 400 µL (instead of 1 mL) and pipette 100 µL e-liquid into the sample vial after the first dispensation into the waste bin.

If less than 500 µL e-liquid is available, aspirate as much e-liquid as possible and pipette 100 µL e-liquid into the sample vial without dispensing e-liquid into the waste bin. If less than 100 µL e-liquid is available, pipette the highest volume possible.

Detail the pipetting procedure in the analysis report if a divergent method (less than 1 mL available) is used.

13 PREPARATION OF THE AEROSOL GENERATING MACHINE

Not applicable for this method.

14 SAMPLE GENERATION

Not applicable for this method.

15 SAMPLE PREPARATION

15.1 Add 9.9 mL diluent solution [**9.1**] to the 20 mL sample vial containing the 100 µL e-liquid by using the positive displacement pipette [**6.2**] and close the sample vial.

15.2 Homogenize the liquids in the sample vial by using the vortex mixer [**6.5**] for at least 30 seconds. Set the speed of the vortex mixer at such a level that a vortex is created during homogenization of the liquid.

15.3 Transfer an aliquot of the homogenized liquid (test solution) into an autosampler vial compatible with the GC-FID instrument.

15.4 If the sample is to be stored, keep it protected from light at 4–8 °C (maximum storage of one week).

Note: Make sure no visual inhomogeneity is present after homogenizing the liquid. If inhomogeneity is still visible, homogenization accordingly [**15.2**] needs to be repeated until no inhomogeneity can be noticed.

16 SAMPLE ANALYSIS

GC with a flame ionization detector is used to quantify nicotine, glycerol and propylene glycol in e-liquids. The analytes are separated from other potential interferences on the column used. Analyte concentrations of test samples are derived by comparison of the area ratio of analyte and internal standard peaks of the test solutions with the area ratio of the analytes in calibration standards with known analyte concentrations.

16.1 GC operating conditions: example

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

Column program:	
Initial temperature:	140 °C
Hold time:	5:00 min
Temperature rate:	40 °C/min
End temperature:	250°C
Hold time:	4:00 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 (ratio 1:50)

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

Annex 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

For the conditions described here, the expected sequence of elution will be propylene glycol, 1,3-Butanediol, glycerol, nicotine and n-heptadecane.

Note 1: Differences in e.g. temperature, gas flow rate and age of the column may alter retention times.

Note 2: The elution order and retention times must be verified before analysis is begun.

Note 3: Under the above conditions, the expected total analysis time will be about 11 min. (The analysis time may be extended to optimize performance.)

16.3 DETERMINATION OF NICOTINE, GLYCEROL AND PROPYLENE GLYCOL

The sequence of measurement of test samples shall be designed in agreement with the applicable laboratory quality system. This section provides an example of a sequence of measurements performed for the determination of nicotine, glycerol and propylene glycol in e-liquids.

Inject aliquots of the calibration standard solutions and test solutions under identical conditions.

16.3.1 Condition the system just before running the sequence by injecting two 1-µL aliquots of a sample test solution as primer.

16.3.2 Inject 1 µL diluent solution [**9.1**] and a test calibration standard solution under the same conditions as the samples to verify the

performance of the GC system and component contamination of reagents used.

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

16.3.4 Assess the retention times and responses (area counts) of the standards. 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 retention times and/or responses are outside specifications, seek corrective action according to your laboratory policy.

16.3.5 Record the peak areas of nicotine, glycerol and propylene glycol and the internal standard components.

16.3.6 Calculate the relative response ratios (RF) of the analyte peaks to the internal standard peaks ($RF = A_{analyte} / A_{IS}$) for each analyte of the calibration standard solutions, including the solvent blanks.

16.3.7 Plot the graph of the relative response ratios (Y axis) against the concentration of the analytes (X axis).

16.3.8 Linearity of the calibration curve should cover the entire calibration range.

16.3.9 Perform linear regression ($Y = a + bx$) on these data and use both the slope (b) and the intercept (a) of the linear regression equation for calculation of the results. If the coefficient of determination R^2 is less than 0.99, the calibration should be repeated. Check for individual outliers according to laboratory procedures.

16.3.10 Inject 1 µL of each of the quality control samples and the test samples, and determine the peak areas with the appropriate software.

16.3.11 The signal (peak area) of the analytes obtained for all test solutions must fall within the working range of the calibration curve; otherwise solutions should be diluted with diluent solution (**9.1**) as necessary.

Note: See **Annex 1** for representative chromatograms.

17 DATA ANALYSIS AND CALCULATIONS

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

17.2 Calculate the analyte concentration (C_{TP} in mg/mL) for each test solution using the coefficients of the linear regression:

$$C_{TP} = \frac{Y_t - a}{b}$$

17.3 Calculate the analyte concentration ($C_{e\text{-}liquid}$) of the e-liquid sample expressed in milligrams per millilitre using the following equation:

$$C_{e\text{-}liquid} = C_{TP} \times \frac{V_{tot}}{V_{e\text{-}liquid}}$$

Where:

$C_{e\text{-}liquid}$ is the concentration of the analyte in the e-liquid, in mg/mL

C_{TP} is the concentration of the analyte in the test solution, in mg/mL

V_{tot} is the total volume of the test solution after dilution of the e-liquid, in mL (default value is 10 mL)

$V_{e\text{-}liquid}$ is the volume of the e-liquid used (**12.1.8**), in mL.

18 SPECIAL PRECAUTIONS

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

18.2 It is recommended to purge high-boiling-point components from the GC column after each sample set (series) by raising the column temperature to 220 °C for 30 min.

18.3 When the peak areas (or heights) for the internal standard(s) are significantly higher than expected, it is recommended to dilute the e-liquid sample without internal standard in the diluent solution and analyse this sample as described in this procedure. This makes it possible to determine whether any component co-elutes with the internal standard, which would cause artificially lower results for the specific analyte(s).

19 DATA REPORTING

19.1 Report individual measurements for each of the samples evaluated.

19.2 Report results as specified by method specifications.

Note: For more information, see WHO TobLabNet SOP 02 [**2.4**].

20 QUALITY CONTROL

20.1 Control parameters

Note 1: If the quality control measurements are outside the tolerance limits of the expected values, appropriate investigation and action must be taken according to laboratory quality procedures.

Note 2: 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 procedural blank determination of diluent solution (**9.1**), as described in **16.3.2**. The results should be less than the limit of detection of the respective analyte.

20.3 Quality control sample

To verify the consistency of the entire analytical process, analyse a reference or quality control e-liquid in accordance with the practices of the individual laboratory.

21 METHOD PERFORMANCE SPECIFICATIONS

21.1 Limit of detection (LOD) and limit of quantification (LOQ)

The LOD is defined as the lowest level of the component for which a higher signal is measured than three times the noise of the instrument used and for which the software identifies the component. The LOQ is set to twice the LOD. These are shown in Table 3 (data provided by internal laboratory validation).

Table 3. LOD and LOQ nicotine, glycerol and propylene glycol in e-liquids

Component	LOD (mg/mL)	LOQ (mg/mL)
Nicotine	0.1	0.2
Glycerol	1.0	2.0
Propylene glycol	0.5	1.0

21.2 Laboratory-fortified matrix recovery

The recovery of the analyte(s) spiked into the matrix was used as a surrogate measure of trueness. The recovery of the components was determined by internal laboratory validation as well as in an international collaborative study.

The recovery was determined in an internal laboratory validation by preparing recovery samples by weighing different amounts of nicotine, glycerol and propylene glycol into 1L flasks. After homogenizing the recovery samples for two hours using a 3D rotating mixer [**6.4**], the recovery samples were transferred into 2 mL vials. Nicotine, glycerol and propylene glycol were determined in each of the recovery samples, analysing the individual vials in five-fold on one day. The native (not spiked) e-liquid was also analysed. The recovery is calculated from the following formula and is summarized for nicotine in Table 4 and for glycerol and propylene glycol in Table 5.

$$\textit{Recovery (\%)} = 100 \times \frac{\textit{(concentration analyte recovery sample} - \textit{concentration analyte native sample)}}{\textit{nominal analyte concentration recovery sample}}$$

Table 4. Mean and recovery of nicotine by internal laboratory validation

Spiked amount (mg/mL)	Nicotine	
	Mean (mg/mL)	Recovery (%)
0.195	0.235	120.6
3.098	3.117	96.5
12.388	12.410	101.1
20.209	20.232	98.9

Table 5. Mean and recovery of glycerol and propylene glycol by internal laboratory validation

Glycerol			Propylene glycol		
Spiked amount (mg/mL)	Mean (mg/mL)	Recovery (%)	Spiked amount (mg/mL)	Mean (mg/mL)	Recovery (%)
378.7	377.7	99.7	727.4	723.1	99.4
630.5	636.5	101.0	519.6	515.1	99.1
883.0	898.2	101.7	311.2	307.3	98.7

The recovery was determined in an international collaborative study by weighing four different amounts of nicotine, glycerol and propylene glycol into flasks. After homogenizing the flasks using a 3D rotating mixer [**6.4**], the recovery samples are transferred into 10 mL flasks. For each of the four spiked samples nicotine, glycerol and propylene glycol are determined by analysing the individual flasks in two-fold in an international collaborative study (conducted in 2020). The recovery is calculated from the following formula and is summarized for nicotine in Table 6 and for glycerol and propylene glycol in Table 7.

$$Recovery\ (\%) = 100 \times \frac{(concentration\ analyte\ recovery\ sample - concentration\ analyte\ native\ sample)}{nominal\ analyte\ concentration\ recovery\ sample}$$

Table 6. Mean and recovery of nicotine obtained in an international collaborative study

Sample	Nicotine		
	Theoretical concentration (mg/mL)	Mean value study (mg/mL)	Recovery (%)
A	0.25	0.326	130.4%
B	5.08	5.08	100.0%
C	8.03	7.91	98.5%
D	21.27	22.06	103.7%

Table 7. Mean and recovery of glycerol and propylene glycol obtained in an international collaborative study

Sample	Glycerol (mg/mL)			Propylene glycol (mg/mL)		
	Theoretical concentration (mg/mL)	Mean value study (mg/mL)	Recovery (%)	Theoretical concentration (mg/mL)	Mean value study (mg/mL)	Recovery (%)
A	568.0	567.5	99.9%	568.7	563.1	99.0%
B	213.9	211.5	98.9%	855.0	843.2	98.6%
C	772.5	738.0	95.5%	278.3	268.4	96.4%
D	321.5	316.7	98.5%	749.9	736.8	98.2%

21.3 Analytical specificity

The retention time of the analyte of interest is used to verify the analytical specificity. An established range of ratios of the response of the analyte to that of the internal standard of quality control e-liquid is used to verify the specificity of the results for an unknown sample.

21.4 Linearity

The established nicotine calibration curves are linear over the standard concentration range of 0.01—0.30 mg/mL (1.0—30 mg/mL e-liquid).

The propylene glycol and glycerol calibration curves established are linear over the standard concentration range of 2.0—10.0 mg/mL (200—1000 mg/mL e-liquid).

21.5 Possible interference

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

22 REPEATABILITY AND REPRODUCIBILITY

An international collaborative study [**23.4**] conducted in 2020, involving 23 laboratories and five samples (four spiked e-liquids and one commercial e-liquid) gave the following values for this method.

The difference between two single results found on matched e-liquid samples by the same operator using the same apparatus within the shortest feasible time will exceed the repeatability limit, r, on average not more than once in 20 cases with normal, correct application of the method.

Single results for matched e-liquid samples reported by two laboratories will differ by more than the reproducibility limit, R, on average no more than once in 20 cases with normal, correct application of the method.

The test results were analysed statistically in accordance with ISO 5725-1 [**23.1**] and ISO 5725-2 [**2.3**] to give the precision data shown in Tables 8–10.

Table 8. Precision limits for determination of nicotine (mg/mL) in e-liquids

E-liquid	n	$\hat{m}$	$Repeatability_{limit}$	$Reproducibility_{limit}$
Sample A	15	0.326	0.079	0.277
Sample B	22	5.08	0.52	1.06
Sample C	21	7.91	0.26	1.49
Sample D	21	22.06	0.66	3.74
Sample E	22	11.38	1.30	1.93

n is the number of laboratories that participated

$\hat{m}$ is the mean value of nicotine content in e-liquid

$Repeatability_{limit}$ is the repeatability limit of nicotine content in e-liquid

$Reproducibility_{limit}$ is the reproducibility limit of nicotine content in e-liquid

Table 9. Precision limits for determination of glycerol (mg/mL) in e-liquids

E-liquid	n	$\hat{m}$	Repeatability limit	Reproducibility limit
Sample A	20	567.5	13.5	48.1
Sample B	21	211.5	8.5	43.6
Sample C	21	738.0	49.3	69.9
Sample D	20	316.7	17.2	32.1
Sample E	21	365.5	16.1	40.7

n is the number of laboratories that participated

$\hat{m}$ is the mean value of glycerol content in e-liquid

$Repeatability_{limit}$ is the repeatability limit of glycerol content in e-liquid

$Reproducibility_{limit}$ is the reproducibility limit of glycerol content in e-liquid

Table 10. Precision limits for determination of propylene glycol (mg/mL) in e-liquids

E-liquid	n	$\hat{m}$	Repeatability limit	Reproducibility limit
Sample A	22	563.1	13.1	23.2
Sample B	22	843.2	37.6	60.7
Sample C	22	268.4	15.5	18.3
Sample D	22	736.8	21.5	38.6
Sample E	23	570.1	58.3	58.3

n is the number of laboratories that participated

$\hat{m}$ is the mean value of propylene glycol content in e-liquid

$Repeatability_{limit}$ is the repeatability limit of propylene glycol content in e-liquid

$Reproducibility_{limit}$ is the reproducibility limit of propylene glycol content in e-liquid

23 BIBLIOGRAPHY

23.1 ISO5725-1. Accuracy (trueness and precision) of measurement methods and results – Part 1: General principles and definitions.

23.2 ISO 5725-4. Accuracy (trueness and precision) of measurement methods and results – Part 4: Basic methods for the determination of the trueness of a standard measurement method.

23.3 ISO Standards – Products by TC: ISO/TC 126 (http://www.iso.org/iso/home/store/catalogue_tc/catalogue_tc_browse.htm?commid=52158).

23.4 Report of a collaborative study for the validation of an analytical method for the determination of nicotine, glycerol and propylene glycol in e-liquids (in press).

ANNEX 1

Typical chromatograms obtained in the analysis of e-liquid for nicotine, propylene glycol and glycerol content

Fig. A1.1. Example of a chromatogram of a standard solution with a nicotine concentration of 0.30 mg/mL and a propylene glycol and glycerol concentration of 10.0 mg/mL

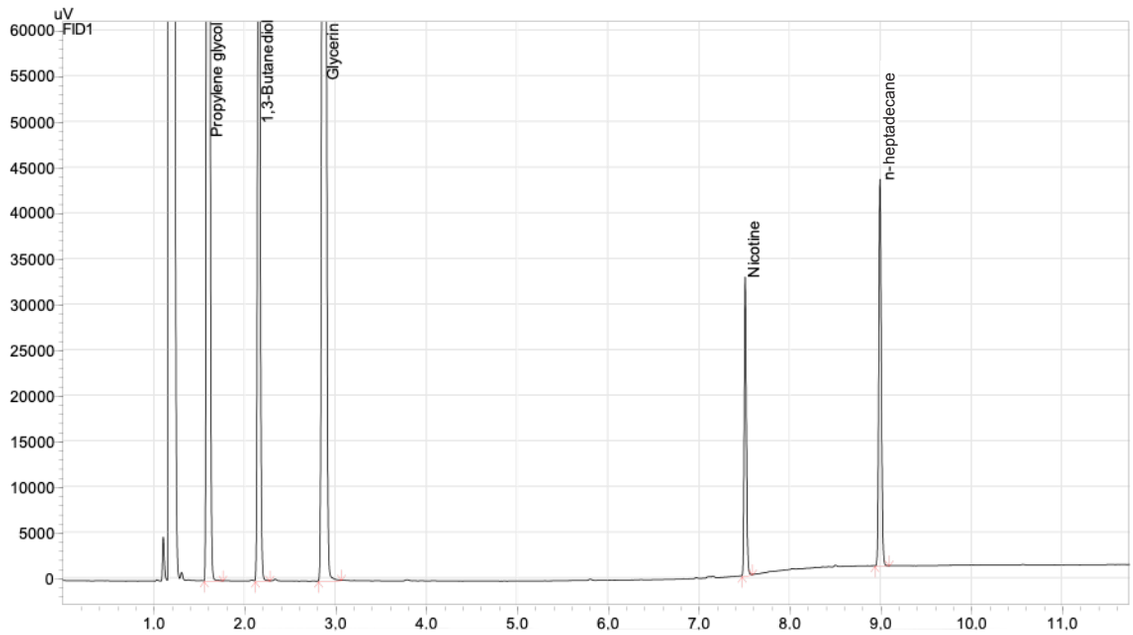
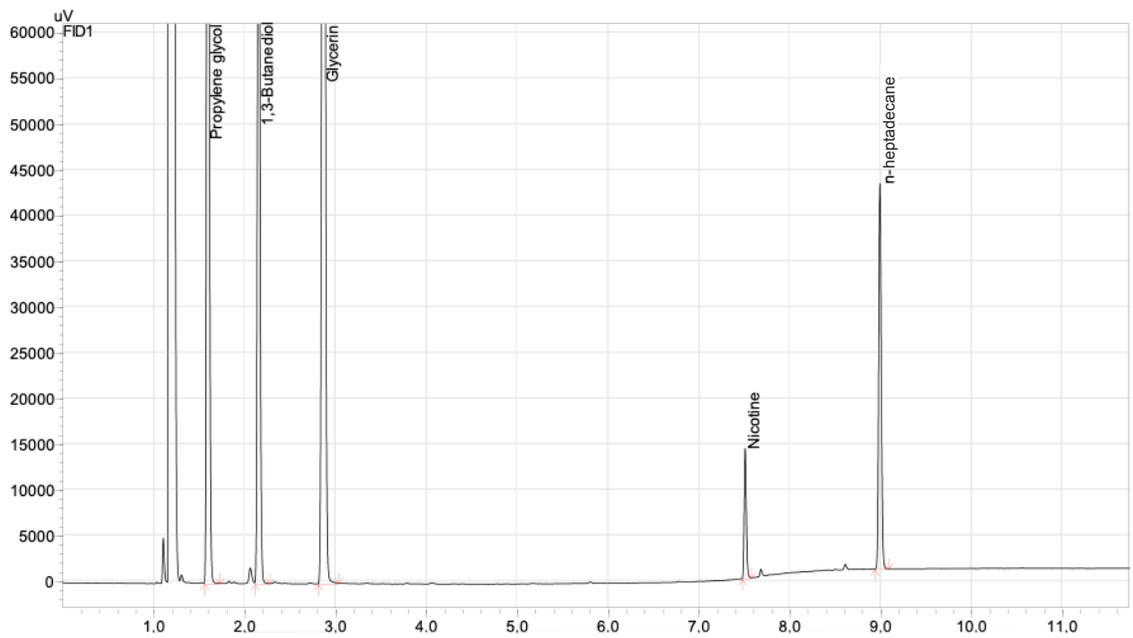


Fig. A2.2. Example of a chromatogram of a sample solution



ANNEX 2

GC-MS settings for alternative measurement technique

Mass spectrometry (MS) operating conditions

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

Transfer line temperature: >= 180 °C

Dwell time:	50 msec
Ionization mode:	Electron Ionization (electron energy 70 eV)
Detection:	propylene glycol: m/z 61 (quantifier) 45 (qualifier) glycerol: m/z 61 (quantifier ion) 43 (confirmation ion) nicotine: m/z 162 (quantifier ion) 133 (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.

This document was prepared by the No Tobacco Unit of the Health Promotion Department of the World Health Organization (WHO) and members of the WHO Tobacco Laboratory Network (TobLabNet), in cooperation with member laboratories of the European Joint Action on Tobacco Control (JATC) as an analytical method standard operating procedure (SOP) for measuring nicotine, glycerol and propylene glycol in e-liquids.

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