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E-liquid — Determination of nicotine, propylene glycol and glycerol in liquids used in electronic nicotine delivery devices — Gas chromatographic method

E-liquide — Détermination de la teneur en nicotine, propylène glycol et glycérol dans les liquides utilisés avec les systèmes électroniques de délivrance de nicotine — Méthode par chromatographie en phase gazeuse

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Contents

Page

Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 126, *Tobacco and tobacco products*, Subcommittee SC 3, *Vape and vapour products*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

E-liquid — Determination of nicotine, propylene glycol and glycerol in liquids used in electronic nicotine delivery devices — Gas chromatographic method

1 Scope

This document specifies an analytical method to quantify the nicotine, propylene glycol and glycerol content in e-liquids by gas chromatography.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
e-liquid
liquid or gel consumable which may or may not contain nicotine intended for transformation into an aerosol and then inhaled with an *electronic nicotine delivery device* (3.2)

3.2
electronic nicotine delivery device
device used to transform an *e-liquid* (3.1) into an inhalable aerosol

4 Principle

The e-liquid sample is diluted with a solution of isopropanol containing internal standard(s). The nicotine, propylene glycol and glycerol content of the diluted sample is analysed by capillary gas chromatography with flame ionization detection (GC-FID) and quantified by using an internal standard.

5 Reagents

Use only reagents of recognized analytical grade.

5.1 Carrier gas: helium (CAS: 7440-59-7) or hydrogen (CAS: 1333-74-0) of high purity.

5.2 Auxiliary gases: hydrogen (CAS: 1333-74-0) of high purity and air for the flame ionization detector.

5.3 Isopropanol (CAS: 67-63-0), minimum purity 99 %, used with internal standard(s) to prepare the dilution solution.

5.4 Internal standards of high purity: quinaldine (CAS: 91-63-4), 1,3-butanediol (CAS: 107-88-0), n-heptadecane (CAS: 629-78-7), n-octadecane (CAS: 593-45-3). Anethol and other appropriate internal standards may be used after assessment of their purity and determination that the internal standard does not co-elute with other components in the dilution. The peak area of the internal standard on samples should be monitored for consistency. In cases where inconsistencies are found, analysis of a diluted sample without the internal standard should be performed to confirm the absence of a peak in the extract eluting at the same time as the internal standard.

5.5 Dilution solution: isopropanol (5.3) containing an appropriate concentration of the internal standard (5.4), e.g. 0,2 mg/ml of quinaldine, 1 mg/ml of 1,3-butanediol, 1 mg/ml of n-heptadecane or 1 mg/ml of n-octadecane (5.4).

5.6 Reference substances

5.6.1 Nicotine (CAS: 54-11-5), of known purity not less than 98 %. Nicotine salicylate (CAS: 29790-52-1) of known purity not less than 98 % may also be used.

NOTE The purity of the nicotine or nicotine salicylate can be verified using ISO 13276 or by any other validated method.

5.6.2 Propylene glycol (CAS: 57-55-6), analytical grade, minimum purity 99 %.

5.6.3 Glycerol (CAS: 56-81-5), analytical grade, minimum purity 99 %.

Store these reference substances at a temperature in accordance with the manufacturer's recommendation.

5.7 Calibration solutions

Prepare a series of at least five calibration solutions with concentrations that cover the range of expected levels to be found in the test portion by adding weighed amounts of nicotine (5.6.1), propylene glycol (5.6.2) and glycerol (5.6.3) to the dilution solution (5.5). The following linear ranges were used in the collaborative study: 0,005 mg/ml to 1,5 mg/ml for nicotine, 0,12 mg/ml to 10 mg/ml for propylene glycol and glycerol.

Store these solutions between 2 °C and 8 °C in a dark environment.

Solutions stored at low temperatures shall be allowed to equilibrate to room temperature before use.

6 Apparatus

Usual laboratory apparatus and, in particular, the following items.

6.1 Gas chromatograph, equipped with a flame ionization detector, recorder, integrator or data handling system.

6.2 Capillary column

DB-ALC1[1)] capillary column (30 m length × 0,32 mm ID, 1,8 µm film thickness) has been found to be satisfactory.

Alternative capillary columns (such as a WAX column) may be used provided that the peaks for solvent, internal standards, nicotine, propylene glycol, glycerol and other e-liquids components are well resolved which can require optimization of the instrument conditions.

1) ALC1 is an example of a suitable product commercially available. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

7 Sampling

Carry out sampling according to a suitable procedure.

8 Procedure

8.1 Test portion

Allow sample to equilibrate to room temperature before opening.

Weigh, to the nearest 0,001 g, 0,1 g of the sample into the dilution vessel.

Pipette 10 ml of dilution solution (5.5) into the dilution vessel, immediately seal the vessel and mix well. Transfer the solution into a gas chromatography vial and cap the vial. Analysis should be performed as soon as possible, but if storage is inevitable then store the sample at between 2 °C and 8 °C in a dark environment until gas chromatography analysis.

8.2 Setting up the apparatus

Set up the apparatus and operate the gas chromatograph (6.1) in accordance with the manufacturer's instructions. Ensure that the peaks for isopropanol, internal standard(s), nicotine, propylene glycol, glycerol, and other e-liquid component peaks are well resolved.

Example of suitable operating conditions for gas chromatography with DB-ALC1 are as follows.

Oven temperature profile:

— Initial temperature: 100 °C;

— Initial hold time: 1 min;

— Temperature ramp A: 15 °C/min;

— Final temperature A: 130 °C;

— Temperature ramp B: 40 °C/min;

— Final temperature B: 220 °C;

— Final hold time B: 10 min;

— Injection temperature: 250 °C;

— Detector temperature: 275 °C;

— Injection volume: 1 µl;

— Injection mode: split;

— Split ratio: 50:1;

— Carrier gas: helium, at a flow rate of 1,8 ml/min;

— Air: at a flow rate of 450 ml/min;

— Hydrogen: at a flow rate of 40 ml/min.

Optimize the gas chromatography conditions for analytes separation and sensitivity. Once optimized, the same gas chromatography conditions shall be used for the analysis of all standards and samples.

NOTE Change of the temperature ramp from 40 °C/min to 5 °C/min improves peak separation. Use of DB-Wax as an alternative column also improves peak separation.

8.3 Calibration of the gas chromatograph

Inject an aliquot (1 µl) of each of the calibration solutions (5.7) into the gas chromatograph. Record the peak areas of nicotine, propylene glycol, glycerol, and internal standard(s) (5.4).

Calculate the ratio of the nicotine, propylene glycol and glycerol peak areas to the appropriate internal standard peak area for each of the calibration solutions. Plot the graphs of the nicotine, propylene glycol and glycerol concentrations in accordance with the area ratios, and calculate linear regression equations (concentration of nicotine, propylene glycol and glycerol according to the area ratios) from these data. The graphs should be linear and the regression lines should not be forced through the origin.

A calibration solution should be run as a quality control periodically throughout the analysis sequence to verify that each calibration curve remains valid.

8.4 Determination

Inject an aliquot (1 µl) of the test portion (see 8.1) into the gas chromatograph. Calculate the ratios of each analyte peak area with appropriate internal standard peak area, separately.

9 Expression of results

The nicotine, propylene glycol, and glycerol content, w, of the e-liquid sample, expressed in milligrams per gram, is given by Formula (1):

$$w = \frac{c \times V}{m} \tag{1}$$

where

c is the concentration of target compound (nicotine, propylene glycol, or glycerol) obtained from the calibration curves, in milligrams per millilitre;

V is the volume of dilution solution, in millilitres (normally 10 ml);

m is the mass of e-liquid sample, in grams.

10 Repeatability and reproducibility

An interlaboratory study involving 19 laboratories was conducted. Test samples consist of seven e-liquids with varying percentages of target compounds and different flavours. The following values for the repeatability limits (r) and the reproducibility limits (R) were obtained.

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

Single results on matched e-liquid samples reported by two laboratories will differ by more than the reproducibility limit (R) on average not more than one in 20 cases in the normal and correct operation of the method.

Data analysis gave the estimates as summarized in Table 1.

Table 1 — Repeatability and reproducibility data from the collaborative study of the method

Compound	Sample	1	2	3	4	5	6	7
Nicotine	No. of laboratories	15	18	13	16	16	18	19
	Mean value, mg/g	10,02	9,92	4,97	5,62	2,77	15,12	32,92
	Repeatability limit, *r*, mg/g	0,45	0,68	0,27	0,33	0,27	1,17	2,33
	Reproducibility limit, *R*, mg/g	1,18	2,14	1,36	1,19	0,89	3,18	5,54
Propylene glycol	No. of laboratories	13	15	14	16	13	13	16
	Mean value, mg/g	711,7	473,7	178,4	761,0	461,3	269,1	862,1
	Repeatability limit, *r*, mg/g	23,8	22,3	9,0	36,0	19,9	13,5	46,5
	Reproducibility limit, *R*, mg/g	51,3	61,0	27,0	90,5	35,1	23,5	86,8
Glycerol	No. of laboratories	18	17	17	17	18	15	15
	Mean value, mg/g	239,6	478,7	782,5	200,6	496,9	685,9	98,8
	Repeatability limit, *r*, mg/g	19,6	33,4	64,0	24,1	43,4	41,6	6,7
	Reproducibility limit, *R*, mg/g	40,9	66,0	123,2	35,2	75,8	85,8	17,4

11 Test report

The test report shall contain the following:

a) the nicotine, propylene glycol and glycerol content of the test sample, in milligrams per gram (three significant figures);

b) the sampling method used;

c) the method used;

d) all conditions, which can affect the result.