Cooperation Centre for Scientific Research Relative to Tobacco

Routine Analytical Chemistry
&
Tobacco and Tobacco Products Analytes Sub-Groups

CORESTA Recommended Method No. 60

DETERMINATION OF 1,2-PROPYLENE GLYCOL AND GLYCEROL IN TOBACCO PRODUCTS BY GAS CHROMATOGRAPHY

June 2019

CORESTA RECOMMENDED METHOD Nº 60

Title:

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Status: Valid

Note: This document will be periodically reviewed by CORESTA

Document history:

Date	**Information**
Feb 2005	Version 1
May 2011	Version 2 - Revised r and R from homogeneity study
July 2015	Version 3 - Scope expanded to include smokeless products
June 2019	Version 4 - Revised method including r and R values from 2016 collaborative study

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DETERMINATION OF 1,2-PROPYLENE GLYCOL AND GLYCEROL IN TOBACCO PRODUCTS BY GAS CHROMATOGRAPHY

(May 2019)

0. INTRODUCTION

Between 1993 and 1999, the CORESTA Routine Analytical Chemistry (RAC) Sub-Group studied the various widely-used procedures for the determination of 1,2-propylene glycol (PG) and glycerol (GLY) in tobacco by gas chromatography in order to adopt one of them as the CORESTA Recommended Method (CRM) for tobacco. After the first version was published (February 2005), further work was done by the RAC Sub-Group to evaluate the effects of sample homogeneity on the repeatability (r) and reproducibility (R) values. A second collaborative study was conducted in 2007 and the improved r and R values were added to the revised CRM (May 2011). In 2012, the CORESTA Smokeless Tobacco Sub-Group (STS) conducted a collaborative study for the determination of PG and GLY in smokeless tobacco products in order to expand the scope of this CRM (July 2015). In 2016, an improved extraction procedure was evaluated by a joint collaborative study undertaken with the RAC and STS Sub-Groups. The tobacco sample is pre-wetted with water then extracted with methanol. PG and GLY are determined in that extract by quantitative gas chromatography with flame-ionisation detection (FID). The addition of water to the tobacco prior to the addition of the solvent resulted in more complete extraction in a shorter time (1 hour versus 2 hours).

1. FIELD OF APPLICATION

The method is applicable to the determination of propylene glycol and glycerol in tobacco and tobacco products, including smokeless tobacco and cigarette filler. The method is applicable to PG and GLY concentrations ranging at least from a mass fraction of 0,3 % to 5,0 %.

2. NORMATIVE REFERENCES

2.1 *CORESTA Guide N° 11*
Technical Guideline for Sample Handling of Smokeless Tobacco and Smokeless Tobacco Products

2.2 ISO 4874:2000
Tobacco -- Sampling of batches of raw material --General principles

2.3 ISO 8243:2013
Cigarettes – Sampling

2.4 ISO 3696:1987
Water for analytical laboratory use – Specification and test methods

3. TERMS AND DEFINITIONS

No terms and definitions are listed in this document.

4. APPARATUS

4.1 Analytical balance (0,0001 g accuracy)

4.2 Gas chromatograph equipped with flame ionisation detector and data acquisition system. See manufacturer's instructions for operation.

4.3 GC Column, moderate polarity fused silica, 30 m capillary or wide-bore (0,25 mm – 0,53 mm i.d.) with a 1 µm film thickness[1].

4.4 Autosampler vials - 2 ml vials and caps.

4.5 Orbital shaker capable of about 250 rpm – 275 rpm or a wrist action shaker.

4.6 Pipette, 25 ml, class A or a calibrated automatic dispenser capable of dispensing 25 ml and chemically inert to methanol.

4.7 Erlenmeyer flask, 125 ml, glass or disposable 50 ml specimen flask, chemically inert to methanol with caps that prevent solvent loss.

4.8 Syringe and syringe filters (0,45 µm, nylon), for filtering extract if needed.

4.9 General laboratory equipment necessary for the preparation of samples, standards, and reagents.

5. REAGENTS

5.1 Water, complying with grade 2 of ISO 3696, or better.

5.2 Methanol, minimum purity 99 %.

5.3 Internal standard, 1,3-butanediol, analytical grade, minimum purity 99 %. Different internal standards may also be acceptable; however, an assessment shall be performed that the internal standard does not co-elute with PG, GLY or other components found in the samples.

5.4 1,2-Propylene glycol, analytical grade, minimum purity 99,5 %, for the preparation of standard solutions.

5.5 Glycerol, analytical grade, minimum purity 99,5 %, for the preparation of standard solutions (store in desiccator).

5.6 Gases: hydrogen, nitrogen, helium and compressed air necessary for operation of gas chromatograph.

[1] See Annex B.

6. STANDARDS

6.1 Internal Standard Solution (50 mg/ml). This solution is used for calibration standards and samples. Prepare an Internal Standard solution by dissolving 5,00 g ± 0,05 g of 1,3-butanediol in methanol in a 100-ml volumetric flask. Make to volume with methanol and mix well.

6.2 Stock solution of PG and GLY (30 mg/ml). Weigh, to the nearest 0,0001 g, approximately 1,5 g each of PG and GLY into a clean, dry 50 ml volumetric flask. Dilute to volume with methanol and shake well to mix. Calculate the exact concentration of the stock solution and record.

6.3 Working standards. From the stock solution produce a series of at least five non-zero working standards to cover the range of expected levels to be found in the samples. Transfer the aliquots of the stock standard solution into separate 50 ml volumetric flasks, add 500 µl of the Internal Standard Solution (6.1) to each flask, dilute to volume with methanol and shake well to mix. Calculate the exact concentrations for each standard and record. See Table 1 for suggested dilutions.

Table 1 – Suggested Dilutions for Working Standards (related to 50-ml volumetric flask)

Standard number	Volume of stock solution (ml)	Nominal Concentration of PG, GLY (mg/ml)
Blank	0	0,0
1	0,20	0,12
2	0,50	0,3
3	1,0	0,6
4	2,0	1,2
5	4,0	2,4
6	6,0	3,6

6.4 Storage. The standard solutions should be stored refrigerated at approximately 4 °C and have been shown to be stable for 2 months at these conditions. Users should determine stability in their laboratory.

7. SAMPLE PROCEDURE

7.1 Sample Handling

Refer to *CORESTA Guide N^{o} 11, Technical Guideline for Sample Handling of Smokeless Tobacco and Smokeless Tobacco Products* for sample handling guidelines.

Note 1: Drying and/or grinding of samples that contain PG will cause a significant loss of this compound due to its volatility.

Note 2: GLY does not distribute homogeneously in tobacco. This should be taken into account when determining the number of replicates per sample.

7.2 Sample Preparation

7.2.1 Weigh, to the nearest 0,01 g, approximately 2 g of sample into a 125 ml Erlenmeyer flask or 50 ml disposable flask. For portioned products, analyse unit portions (pouches) by cutting the pouch in half and adding both parts of the tobacco and pouch material directly to the extraction vessel. Record the exact weight to the nearest 0,0001 g

7.2.2 Add 5 ml of water (5.1) and allow sample to stand for at least 5 minutes.

7.2.3 Add 300 µl of Internal Standard Solution (6.1) and 25 ml of methanol and cap the flask.

7.2.4 Shake 60 minutes (4.4). Remove from shaker and allow extraction mixture to stand 10 minutes to15 minutes for particulates to settle.

7.2.5 If necessary, filter an aliquot of the extract through a 0, 45 µm syringe filter into an autosampler vial and cap.

8. SAMPLE ANALYSIS

8.1 Gas chromatograph

Set up and operate the gas chromatograph, and data system according to the manufacturer's instructions. Ensure that the peaks for solvent, internal standard, PG, GLY and other peaks of interest are well resolved.

Suitable instrument conditions for a GC equipped with a 0,32 mm i.d. capillary column with a crossbonded stationary phase, 1 µm film thickness are:

8.1.1. Temperature Set Points

Injection port temperature	250 °C
Detector temperature	250 °C – 275 °C

Oven temperature profile:

Equilibration time	1 min.
Initial temperature	110 °C
Initial time	1 min.
Ramp rate	20 °C/min.
Final temperature	220 °C
Final time	5 min.
Run time	11,5 min.

8.1.2. Other Instrument Parameters

Carrier gas linear velocity of helium between 20 cm/s and 30 cm/s

Column flow (if constant flow capable):

0,25 mm column	~ 2 ml/min.
0,32 mm column	~ 6 ml/min.

Injection volume 1 µl

Split Ratio - 20:1

Detector Makeup Gas: helium or nitrogen at 30 ml/min,

Hydrogen ~ 30 ml/min – 40 ml/min.

Air ~ 400 ml/min.

Note 1: Adjust hydrogen and air flows for maximum sensitivity

Note 2: Retention times: Under the conditions specified, PG elutes at approximately 5 minutes, 1,3-butanediol (internal standard) elutes at approximately 6 minutes and GLY elutes at approximately 9 minutes (see Annex A for chromatograms of a standard solution and sample extract).

Note 3: Prior to generating the calibration curve, condition the system by injecting several high-level standards and sample extracts. This will deactivate active sites which can be occupied by polar compounds injected, such as GLY.

8.2 Calibration of gas chromatograph

Create an internal standard calibration method in the instrument operating software. Generate calibration curves for PG and GLY using a linear regression of the area ratios of each analyte to the internal standard as a function of the concentration ratios.

Inject an aliquot (1 µl) of each of the calibration solutions (6.3) into the gas chromatograph. Record the peak areas of PG, GLY, and internal standard. Calculate the ratios of the PG peak area and GLY peak area to the internal standard peak area for each of the calibration solutions. Plot the PG and GLY peak area ratios versus concentration on a graph or calculate a linear regression equation (concentration of PG and GLY to the area ratios) from these data. The graph should be linear and the regression line should not be forced through the origin. The minimum Coefficient of Determination, R^2, should be 0,99. Perform this full calibration procedure when analyses are performed. In addition, inject an aliquot of an intermediate concentration standard after about 20 sample determinations. If the calculated concentration for this solution differs more than 5 % from the original value, repeat the full calibration procedure and, as appropriate, re-analyse samples associated with that calibration.

8.3 Measurement and calculation of humectant content of samples

Inject a 1 µl aliquot of the sample extract into a gas chromatograph. Record the peak areas of PG, GLY and the internal standard obtained from the chromatogram. Using the calibration curve produced in 8.2, determine the concentration in mg/ml of PG and GLY in the sample extract by comparing respective ratios to corresponding standard calibration curves. Ensure that the values lie within the ranges of the standards prepared in section 6.3. Calculate the weight percent (%) of PG and GLY using the following equation:

$$\text{PG or GLY (\%)} = \frac{c * V * 100}{w * 1000} \qquad (1)$$

where

c is the concentration of humectant (PG or GLY) obtained from the calibration curve, in milligrams per millilitre

V is the volume of extraction solution, in millilitres (normally 30 millilitres)

w is the weight of tobacco sample, in grams

9. REPEATABILITY AND REPRODUCIBILITY

In 2016, the CORESTA Smokeless Tobacco and Routine Analytical Sub-Groups conducted a joint collaborative study for the determination of propylene glycol and glycerol in tobacco products using a proposed modification to CRM Nº 60. The eight products included in this study are described in the table below. Test samples A to F included six loose cut tobacco samples that were carefully blended to improve homogeneity and distributed by R.J. Reynolds; and two smokeless CRPs available from the North Carolina State University[2].

Table 2 – Sample Descriptions for 2016 CORESTA Collaborative Study

Sample	Target Glycerol Nominal Concentration (%)	Target 1,2-Propylene Glycol Nominal Concentration (%)
A	None[1]	None[1]
B	0,4	0,4
C	1	1
D	2	2
E	3	3
F	5	5
H CRP 1 Swedish style snus	None added[2]	2,7
I CRP 4 American style loose leaf chewing tobacco	3,8	None added[3]

The following samples were excluded from the statistical evaluation for glycerol and/or 1,2-propylene glycol because participants reported contents below or close to the limit of quantification of the corresponding CRM:

[1] Sample A - Glycerol and 1,2-propylene glycol

[2] Sample H - Glycerol

[3] Sample I - 1,2-propylene glycol.

[2] RAC-051-1-CTR 2016 Collaborative Study on Humectants, May 2019

The overall level of variability for smokeless tobacco products seen in this study was similar to the 2007 and 2012 studies. The r and R results for the 2016 study are presented below:

Table 3 – Repeatability and Reproducibility for 2016 CORESTA Collaborative Study

Parameter	Sample	Number of labs	Mean (%w/w)	Repeatability		Reproducibility	
				r	r (%)	R	R (%)
Glycerol	B	11	0,461	0,0522	11,3	0,1499	32,5
	C	11	1,029	0,1521	14,8	0,2429	23,6
	D	11	1,946	0,2075	10,7	0,4333	22,3
	E	11	2,847	0,3178	11,2	0,6231	21,9
	F	10	4,889	0,3726	7,6	1,2948	26,5
	I - CRP 4	8	4,035	0,5039	12,5	1,0022	24,8
1,2-Propylene glycol	B	11	0,365	0,0300	8,2	0,1559	42,7
	C	10	0,964	0,0705	7,3	0,2129	22,1
	D	10	1,956	0,1105	5,6	0,2458	12,6
	E	10	2,887	0,1854	6,4	0,2912	10,1
	F	11	4,997	0,2858	5,7	0,4851	9,7
	H - CRP 1	8	2,954	0,2511	8,5	0,5137	17,4

10. TEST REPORT

The test report shall give concentration of humectant in % (weight/weight) and shall include all conditions, which may affect the result (e.g. grinding, drying and, if corrected to dry weight basis, method for determination of moisture content). It shall also give all details necessary for the identification of the sample.

11. BIBLIOGRAPHY

[1] RAC-051-1- CTR 2016 Collaborative Study of Humectants – May 2019

ANNEX A Example Chromatograms

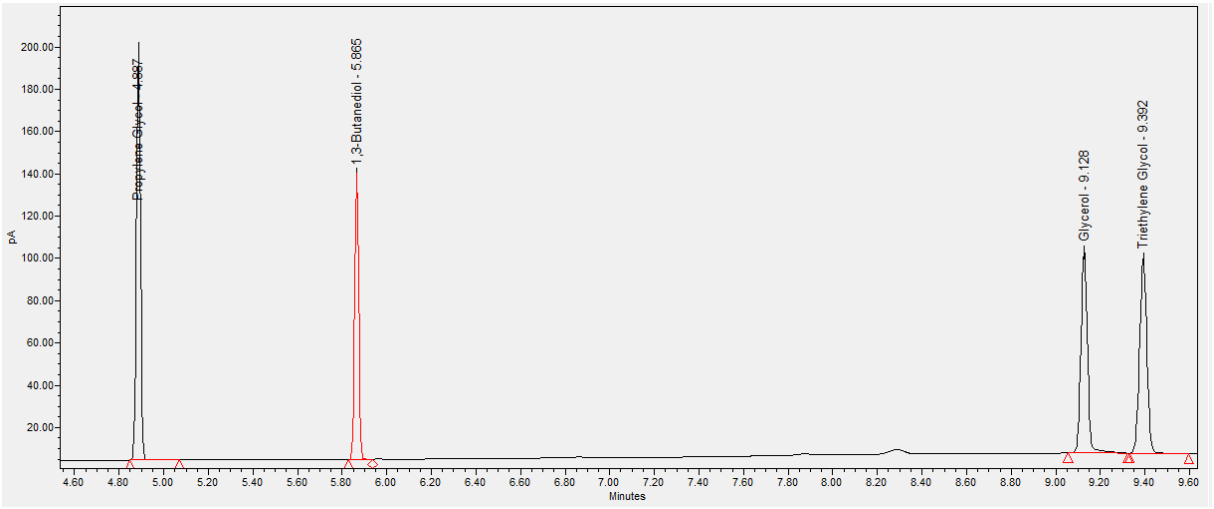

Figure 1 – Example of a chromatogram for a humectant standard (PG 0.8 mg/ml and GLY 0,8 mg/ml)

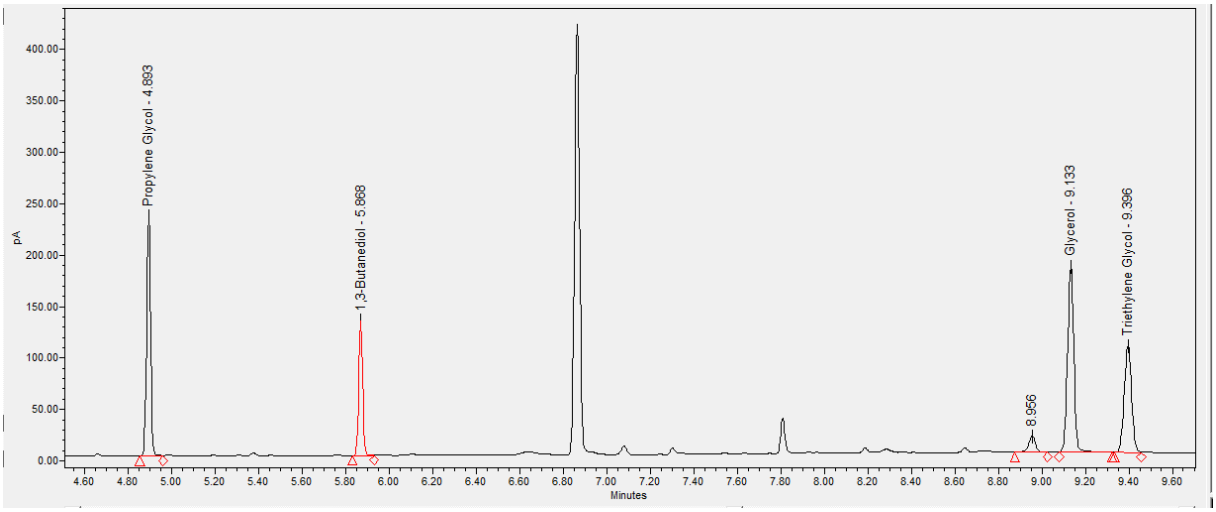

Figure 2 – Example of a chromatogram for a tobacco extract (KY3R4F extract spiked with humectants spiking solution mix)

ANNEX B

(*informative*)

B.1 Columns

The current method was developed and validated using a wax column (30 m * 0,32 mm i.d., 1 µm film thickness). Other columns have been found to be suitable, for example, Restek Rtx-35 (30 meter * 0,25 mm i.d., 1 µm film thickness) coated with crossbonded 35 % diphenyl/65 % dimethyl polysiloxane stationary phase which is stable up to 300 °C. Condition column as directed by the manufacturer after installation. Other suitable phases are:

Supplier[3]	Column[3]
J&W Scientific	DB-35, DB Wax
Restek	Rtx-35, Stabilwax
Hewlett Packard	HP-35, HP INNO Wax
Supelco	SPB-35, Supelcowax 10, Carbowax 20M
Chrompack	CP-Wax 52 CB

[3] These are trade names of examples of suitable products available commercially. This information is given for the convenience of the user of this Recommended Method and does not constitute an endorsement by CORESTA of these products.