

CORESTA RECOMMENDED METHOD N° 60

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

(May 2011)

0. INTRODUCTION

A CORESTA Sub Group studied the various widely-used procedures for the determination of 1,2-propylene glycol (PG) and glycerol (GLY) in tobacco and tobacco products by gas chromatography in order to adopt one of them as the CORESTA Recommended Method. Studies were carried out by the CORESTA Sub Group between 1993 and 1999 to evaluate sample preparation, extraction, and analysis parameters.

1. FIELD OF APPLICATION

The method is applicable to tobacco and tobacco products. The method is applicable to PG and GLY concentrations ranging at least from a mass fraction of 0,3 % to 5,0 %.

2. PRINCIPLE

A methanol extract of the tobacco sample is prepared and PG and GLY are determined in that extract by quantitative gas chromatography. Results are reported as percent (weight/weight).

3. REFERENCES

- 3.1. CORESTA Recommended Method N° 56, Determination of Water in Tobacco and Tobacco Products by Karl Fischer Method.
- 3.2. CORESTA Recommended Method N° 57, Determination of Water in Tobacco and Tobacco Products by Gas Chromatographic Analysis.
- 3.3. CORESTA Technical Report on 2007 Joint Experiments to Update Repeatability and Reproducibility Statistics for:
 - CORESTA Recommended Method 60, Determination of 1,2-Propylene Glycol and Glycerol in Tobacco Products by Gas Chromatography
 - CORESTA Recommended Method 61, Determination of 1,2-Propylene Glycol and Glycerol in Tobacco Products by High Performance Liquid Chromatography

4. APPARATUS

4.1. **Gas chromatograph** with temperature-controlled injection port capable of performing split injections, flame ionization detector, and column oven. A recorder, integrator or chromatographic data system capable of integrating chromatograms to obtain peak area. See Manufacturer's instructions for operation.

4.1.1. *Chromatographic column*: fused silica column, capillary or wide-bore (0,25 mm - 0,53 mm i.d.) with a 1µm film thickness. A list of suitable columns can be found in annex A.1.

- 4.2. **Syringe** suitable for injecting 2 µl aliquots of methanol solutions or an automatic sampler capable of automatic 2 µl injections of methanol solutions with multiple rinsing capability to eliminate carryover.
- 4.3. **Autosampler vials** - 2 ml vials and caps.
- 4.4. **Orbital shaker** capable of about 250 - 275 rpm or a wrist action shaker.
- 4.5. **Pipette**, 50 ml, class A or a calibrated automatic dispenser capable of dispensing 50 ml and chemically inert to methanol.
- 4.6. **Erlenmeyer flask**, 125 ml, glass or disposable 150 ml specimen flask, chemically inert to methanol with caps that prevent solvent loss.
- 4.7. **General laboratory equipment** necessary for the preparation of samples, standards, and reagents.

5. REAGENTS

- 5.1. **Methanol**, minimum purity 99%, used with internal standard to prepare the extraction solution.
- 5.2. **Internal standard, 1,4-butanediol**, analytical grade, minimum purity 99%, used with methanol in the preparation of the extraction solution. Different internal standards may be used after an assessment of purity and determination that the internal standard does not co-elute with PG, GLY or other components found in samples.

Note: Other internal standards utilized are 1,3-butanediol, decanol or heptadecane.
- 5.3. **1,2-Propylene glycol**, analytical grade, minimum purity 99,5%, for the preparation of standard solutions.
- 5.4. **Glycerol**, analytical grade, minimum purity 99,5%, for the preparation of standard solutions (store in desiccator).
- 5.5. **Extraction solution:** Methanol containing approximately 1,5 mg/ml internal standard. Mix the solution well. To ensure adequate equilibration it is recommended to allow the solution to stand overnight before use.

Note 1: A new set of standards must be prepared for each lot of extraction solution.

Note 2: Storage of extraction solution for longer periods of time requires a desiccant tube (indicating drierite - anhydrous calcium sulfate) to dry incoming air in order to prevent the extraction solution from absorbing water.
- 5.6. **Gases:** hydrogen, nitrogen, helium and compressed air necessary for operation of gas chromatograph.

6. STANDARDS

Prepare a stock solution by dissolving PG and GLY in extraction solution containing the internal standard as described in 6.1. Prepare working standards by diluting the stock solution with extraction solution containing the internal standard as described in 6.2 with concentrations covering the range expected to be found in the samples (usually 0,5 % to 5,0 %).

- 6.1. **Stock solution of PG and GLY.** Weigh, to the nearest 0,0001 g, approximately 2 g each of PG and GLY into a clean, dry 100 ml volumetric flask. Dilute to volume with extraction solution and shake well to mix. Calculate the exact concentration of the stock solution and record.

6.2. Working standards. From the stock solution produce a series of at least five working standards to cover the range of expected levels to be found in the samples. For example, when sample humectant concentrations range from 1,0 % to 2,5 %, prepare standards ranging from 0,2 mg/ml to 1,2 mg/ml (0,5 % to 3,0 %) humectants. Transfer the aliquots of the stock standard solution into separate volumetric flasks, dilute to volume with extraction solution and shake well to mix. Calculate the exact concentrations for each standard and record. See Table 1 for suggested dilutions.

Note: A blank with no added PG and GLY may be used in the calibration curve.

Table 1 - Suggested dilutions for working standards (related to 100 ml volumetric flask)

Standard number	Volume of stock solution ml	Concentration of PG, GLY and SOR mg/ml	Concentration 2,5 g sample %
Blank	0	0,0	0,00
1	2	0,02	0,08
2	5	0,05	0,20
3	25	0,25	1,00
4	50	0,50	2,00
5	75	0,75	3,00
6	100	1,00	4,00

7. PROCEDURE

7.1. Gas chromatograph

Set up and operate the gas chromatograph, recorder, integrator, or chromatographic data system, and autosampler (if one is used) according to the Manufacturer's instructions. Ensure that the peaks for solvent, internal standard, PG, GLY and other peaks of interest are well resolved.

7.1.1. Suitable chromatographic conditions

Suitable instrument conditions for a GC equipped with a 0,25 mm i.d. capillary column with a crossbonded stationary phase (35% diphenyl - 65% dimethyl polysiloxane), 1 µm film thickness are:

a. Temperature Set Points

Injection port temperature 250 °C

Injection volume 2 µl

Detector temperature 275 °C

Oven temperature profile:

Equilibration time 1 min.

Initial temperature 110 °C

Initial time 1 min.

Ramp rate 10 °C/min.

Final temperature 150 °C

Final time 3 min.

Secondary ramp rate 30 °C/min.

Secondary final temp. 220 °C

Secondary final time 5 min.

b. Other instrument parameters

Column head pressure of ~ 83 kPa, set to give desired linear velocity

Carrier gas linear velocity of helium between 20 cm/s and 30 cm/s at 220 °C

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

Split Ratio - 50:1

Hydrogen ~ 30 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 4,2 minutes, 1,4-butanediol (internal standard) elutes at 7,3 minutes and GLY elutes at 8,4 minutes (see figure 1 and 2 for chromatograms of a standard solution and sample extract, respectively).

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.

7.2. Calibration of gas chromatograph

Transfer an aliquot of working standards into vials (3.3) and load on autosampler if one is available. Inject an aliquot (2 µl) of each of the calibration solutions (5.2) into the gas chromatograph. Record the peak areas of PG, GLY, and internal standard. Calculate the ratio 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 correlation coefficient, R², should be 0,99. Perform this full calibration procedure when analyses are performed. In addition, inject an aliquot of an intermediate concentration standard after every 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.

7.3. Sample Preparation

Tobacco and tobacco product samples may be analysed as loose cut samples or may be ground.

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.

Note 3: Secondary reference material or monitors (cased tobacco containing PG and GLY, ground to pass a 1 mm sieve) are used in some laboratories as an additional check of the total analysis process. Typically, monitors are prepared according to 7.3 and randomly placed throughout the run. Statistical process control (SPC) is then applied to evaluate these data which may include plotting the mean, range, or standard deviation on control charts, analysing the pattern, and taking action in response to an out of control condition.

Weigh, to the nearest 0,01 g, approximately 2 g of sample into a 125 ml Erlenmeyer flask or 150 ml disposable flask. Add 50 ml of the extraction solution (4.5) from a pipette or calibrated dispenser and cap the flask. Shake two hours (4.4) (see Annex A.2). Remove from shaker and allow extraction mixture to stand 10-15 minutes for particulates to settle. If an autosampler is utilised, transfer an aliquot of the extract into an autosampler vial and cap.

7.4. Measurement and calculation of humectant content of samples

Inject a 2 µl aliquot of the extract into a gas chromatograph using the conditions appropriate for the gas chromatograph and column utilised. Record the peak areas of PG, GLY and the internal standard obtained from the chromatogram. Using the calibration curve produced in 7.2, 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 6.2. Calculate the weight percent of PG and GLY using the following equation:

$$\frac{c * V * 100}{w * 1000}$$

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 50 millilitres)
- w is the weight of tobacco sample, in grams

8. REPEATABILITY AND REPRODUCIBILITY

The precision data were determined from an international collaborative study performed by CORESTA involving 18 laboratories and 4 samples conducted in 1999 and analysed according to ISO 5725 guidelines. The data from the laboratories contained one outlier for the determination of glycerol in cut rag. The outlier was not included in the calculation of the repeatability standard deviations and the reproducibility standard deviations. Data from this collaborative study (with a limited number of participants) showed that when tobacco samples are analysed by this method, the following values for repeatability limit (r) and reproducibility limit (R) are obtained.

The influence of analysing cut tobacco samples instead of ground tobacco samples may be estimated by the corresponding values of “r” and ”R”.

The difference between two average results (of which each is the average of a double analysis) found on matched tobacco samples by one operator using the same apparatus within shortest feasible time interval will exceed the repeatability limit (r) on average not more than once in 20 cases in the normal and correct operation of the method.

Single results (which each is the average of a double analysis) on matched tobacco samples reported by two laboratories will differ by more than the reproducibility limit (R) on average not more than once in 20 cases in the normal and correct operation of the method.

Data analysis gave the estimates as summarized in tables 2 - 5.

Table 2 - 1,2-Propylene glycol – cut tobacco samples

Mean concentration of 1,2 propylene glycol %	Repeatability limit r %	Reproducibility limit R %
0,597	0,046	0,146
1,567	0,079	0,434
2,873	0,120	0,360
4,693	0,244	0,544

Table 3 - Glycerol – cut tobacco samples

Mean concentration of Glycerol %	Repeatability limit r %	Reproducibility limit R %
0,260	0,117	0,325
1,291	0,173	0,401
2,444	0,156	0,522
4,462	0,698	2,668

Table 4 - 1,2-Propylene glycol – ground tobacco samples

Mean concentration of 1,2 propylene glycol %	Repeatability limit r %	Reproducibility limit R %
0,591	0,033	0,090
1,549	0,056	0,170
2,803	0,110	0,334
4,580	0,179	0,514

Table 5 - Glycerol – ground tobacco samples

Mean concentration of glycerol %	Repeatability limit r %	Reproducibility limit R %
0,259	0,056	0,289
1,311	0,100	0,286
2,439	0,143	0,620
4,389	0,173	0,910

During 2007, the CORESTA Scientific Commission requested the Routine Analytical Chemistry (RAC) Sub Group carry out a collaborative study to investigate whether the repeatability (r) & reproducibility (R) values quoted in the methods were still relevant or could be improved.

The r and R statistics published in CRMs 60 and 61 were relatively large. Sample non-homogeneity was suspected to be a key contributor to the observed methods' variability.

Eight tobacco samples were despatched to 19 laboratories. These samples consisted of four pouches of loose cut tobacco and four pouches of finely ground (ground to pass through a 1mm sieve) and homogenized tobacco from the same population as the loose cut tobacco samples. As non-homogeneity was suspected to be a key contributor to the large observed r and big R statistics in the original study, for this study every effort was made to prepare the

samples as homogeneously as possible. Duplicate preparations of each sample pouch were made with two injections per sample preparation, consistent with the original studies. Results were reported on an 'as received' basis.

Table 8 - CORESTA Collaborative Study of 1,2-Propylene Glycol and Glycerol Measurements - Comparison of Previous and Current Study Performance Measures

Propylene Glycol in Cut Loose Tobacco - GC Method								
<i>Previous Study</i>					<i>2007 Study</i>			
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 1	Sample 2	Sample 3	Sample 4
Mean	0,597	1,567	2,873	4,693	0,416	1,227	2,049	3,362
r	0,046	0,079	0,120	0,244	0,016	0,057	0,067	0,135
R	0,146	0,434	0,360	0,544	0,089	0,227	0,248	0,429

Glycerol in Cut Loose Tobacco - GC Method								
<i>Previous Study</i>					<i>2007 Study</i>			
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 1	Sample 2	Sample 3	Sample 4
Mean	0,260	1,291	2,444	4,462	0,585	1,387	2,202	3,386
r	0,117	0,173	0,156	0,698	0,059	0,060	0,132	0,184
R	0,325	0,401	0,522	2,668	0,163	0,236	0,313	0,418

Propylene Glycol in Ground Tobacco - GC Method								
<i>Previous Study</i>					<i>2007 Study</i>			
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 1	Sample 2	Sample 3	Sample 4
Mean	0,591	1,549	2,803	4,580	0,404	1,181	2,011	3,303
r	0,033	0,056	0,110	0,179	0,019	0,029	0,028	0,073
R	0,090	0,170	0,334	0,514	0,058	0,204	0,210	0,360

Glycerol in Ground Tobacco - GC Method								
<i>Previous Study</i>					<i>2007 Study</i>			
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 1	Sample 2	Sample 3	Sample 4
Mean	0,259	1,311	2,439	4,389	0,584	1,365	2,159	3,361
r	0,056	0,100	0,143	0,173	0,032	0,048	0,094	0,145
R	0,289	0,286	0,620	0,910	0,178	0,183	0,276	0,444

The large r and R observed in the original study was hypothesised to result from non-homogeneity of the test samples and not as a result of the analytical methodology or laboratory competence. In the 2007 study therefore, extreme care was taken to prepare

homogeneous test samples and as can be seen a significant improvement r and R was obtained, thus proving sample homogeneity to be a key parameter in result consistency. Details of the sample preparation to improve homogeneity can be found in the technical report (3.3.) available from the CORESTA Secretariat and on the CORESTA web site.

9. 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.

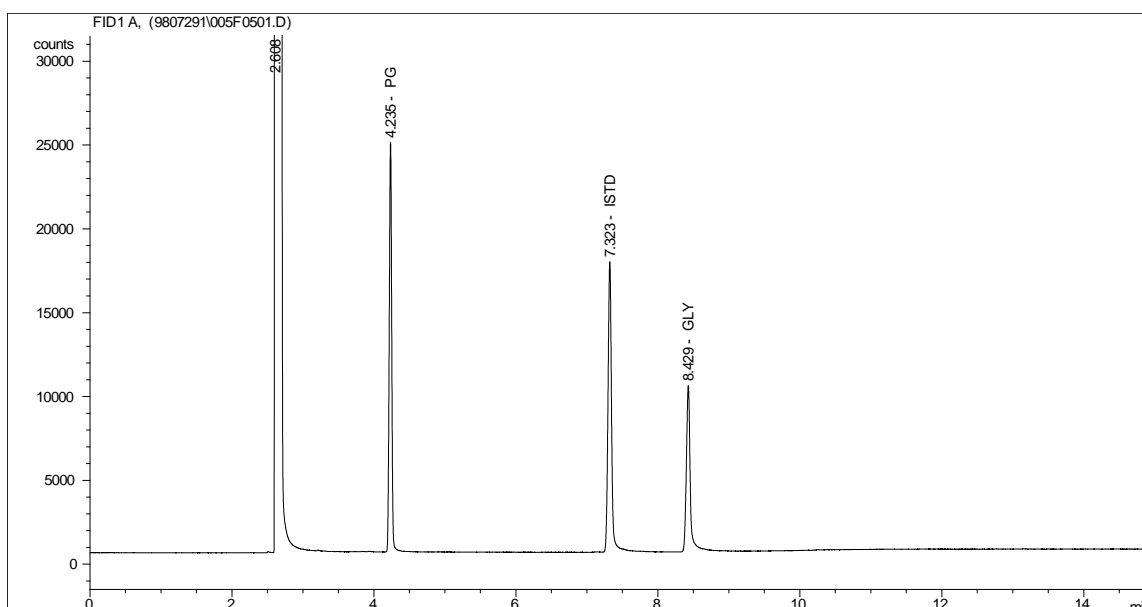


Figure 1 - Example of a chromatogram for a humectant standard

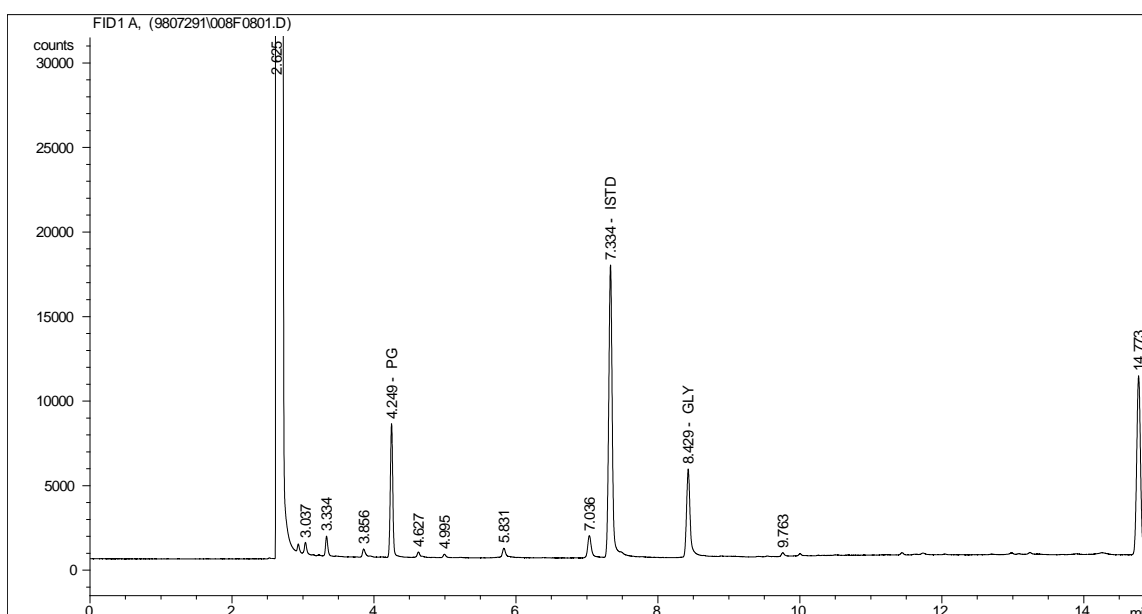


Figure 2 - Example of a chromatogram for a tobacco extract

ANNEX A

(informative)

A.1. Columns

The Restek Rtx-35 (a moderate polarity fused silica capillary column), 30 meter * 0,25 mm i.d. 1 µm film thickness, Part No. 10453, has been shown to give excellent separation capability. The column is 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 1). Other suitable phases are:

Supplier ¹	Column1
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,

A.2. Extraction times

Extraction times of ½, 1, 2, 4, 8, and 16 hours were evaluated and it was determined that there was no significant difference in results obtained due to varying extraction times greater than two hours for the samples evaluated.

¹ 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.