

# CORESTA RECOMMENDED METHOD N° 61

## DETERMINATION OF 1,2-PROPYLENE GLYCOL, GLYCEROL AND SORBITOL IN TOBACCO AND TOBACCO PRODUCTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

(July 2015)

### 0. INTRODUCTION

The CORESTA Routine Analytical Chemistry (RAC) Sub-Group studied various widely-used procedures for the determination of 1,2-propylene glycol (PG), glycerol (GLY) and sorbitol (SOR) by high performance liquid chromatography (HPLC) in order to adopt one of them as the CORESTA Recommended Method (CRM). Studies were carried out by the RAC Sub-Group between 1993 and 1999 to evaluate sample preparation, extraction and analysis parameters. This CRM was originally developed with tobacco and did not include smokeless tobacco products. Therefore, 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.

### 1. FIELD OF APPLICATION

This method is applicable to 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. PRINCIPLE

The sample is extracted into water and filtered. The filtrate is analysed by HPLC with refractive index detection. Results are reported as percent (weight/weight).

**Note:** During the joint experiments equivalent results were obtained for the extraction with 0,01M NaOH which inhibited the inversion of sucrose. This can be useful for a possible simultaneous determination of sugars.

### 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 N° 60, Determination of 1,2-Propylene Glycol and Glycerol in Tobacco Products by Gas Chromatography.
  - CORESTA Recommended Method N° 61, Determination of 1,2-Propylene Glycol and Glycerol in Tobacco Products by High Performance Liquid Chromatography.

### 3.4 CORESTA Smokeless Tobacco Sub-Group Technical Report: 2012 Collaborative Study on Humectants.

## 4. APPARATUS

It is essential to clean all glassware very thoroughly before use. All volumetric flasks and pipettes shall comply with class A.

### 4.1. HPLC-equipment

Liquid chromatograph with pump, autosampler, column oven and refractive index detector with thermostatic cell. A recorder, integrator or chromatographic data system capable of integrating chromatograms to obtain peak area. See Manufacturer's instructions for operation.

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

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

## 5. REAGENTS

5.1. Water, double distilled or of other equivalent quality (conductivity less than 0,2  $\mu\text{S}/\text{cm}$ ).

5.2. Ethylene diamine tetra acetic acid, calcium-disodium salt (Ca,Na-EDTA).

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 a desiccator).

5.5. D(-)-sorbitol, minimum purity 99,5 %, for the preparation of standard solutions (store in a desiccator).

5.6. Celite Hyflo Super Cel (silica, particle size less than 5  $\mu\text{m}$ ).

## 6. STANDARDS

Prepare a stock solution by dissolving PG, GLY and SOR in water. Prepare working standards by diluting the stock solution with water as described in 6.2 with concentrations covering the range expected to be found in the samples (usually 0,3 % to 5,0 %).

6.1. **Stock solution** of PG, GLY and SOR. Weigh, to the nearest 0,0001 g, approximately 0,2 g of each humectant in a 200 ml volumetric flask. Dilute to volume with water 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 results to be found in the samples. For example, when sample humectant concentration range from 1,0 % to 2,5 %, prepare standards ranging from 0,5 % to 3,0 % humectants. Transfer the aliquots of the stock solution into separate volumetric flasks, dilute to volume with water 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, GLY and SOR 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 %
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

All solutions shall be stored in a cool and dark place (nominally 5 °C). Under these conditions the solutions are stable for 12 days.

## **7. PROCEDURE**

### **7.1. HPLC analysis**

Set up and operate the liquid chromatograph according to the Manufacturer's instructions. Ensure that the peaks for PG, GLY, SOR and other peaks of interest (*e.g.* sugars) are well resolved.

#### 7.1.1. Suitable HPLC conditions

Mobile phase: water + 50 mg/l calcium-disodium EDTA  
Flow rate: 0,5 ml/min  
Oven temperature: 80 °C  
Injection volume: 20 µl  
Column: calcium salt of sulfonated Styrenedivinylbenzene Copolymer *e.g.* Sugar Pak I™ (Waters)<sup>1</sup> 30 cm x 6,5 mm i.d. (see Annex A for details of column care); a guard column with the same packing is recommended  
Detector cell: cell temperature 40 °C

The performance of the column should be sufficient to achieve a satisfactory separation of all components similar to that given in the specimen chromatogram (see figure 2).

See figure 1 and 2 for chromatograms of a standard and a tobacco extract respectively.

### **7.2. Calibration of the HPLC**

Inject 20 µl of each working standard into the HPLC. Record peak heights of PG, GLY and SOR. Plot the PG, GLY and SOR peak heights versus the concentrations on a graph and calculate a linear regression equation from these data. The graph should be linear and the regression equation line should not be forced through the origin. The minimum correlation coefficient,  $R^2$ , should be 0,99. Perform this full calibration procedure when analyses are performed. In addition, inject an aliquot of an

---

<sup>1</sup> Sugar Pak I is a trade name of a product supplied by Waters. This information is given for the convenience of users of this Recommended Method and does not constitute an endorsement by CORESTA of this product. Equivalent products may be used if they can be shown to lead to the same result.

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, reanalyse 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 and SOR do 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, GLY and SOR 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 2,5 g of sample into a 250 ml Erlenmeyer flask. Add 100 ml water and stopper the flask. Shake vigorously at high speed (see 4.2) for 30 minutes. Filter extract in small portions under vacuum through a bed of hyflo supercel (3-4 g) supported on a filter paper in a Buchner funnel. Discard the first two or three volumes of filtrate and collect the remaining filtrate. Transfer an aliquot of the extract into an autosampler vial and cap.

### 7.4. Measurement and calculation of humectant content of samples

Inject a 20 µl aliquot of the extract into a HPLC using the conditions appropriate for the HPLC and column utilised. Record the peak heights of PG, GLY and SOR obtained from the chromatogram. The amount of humectants is determined by the external standard method using the calibration curve produced in 7.2. Ensure that the values of the sample extract lie within the ranges of the standards prepared in section 6.2.

The content of humectant (PG, GLY or SOR), in the tobacco sample, expressed as a percentage by weight, is given by the equation:

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

where

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

V is the volume of extraction solution, in millilitres (normally 100 millilitres)

w is the weight of tobacco sample, in grams

## 8. REPEATABILITY AND REPRODUCIBILITY

The data presented below are from three large international studies. The first two studies conducted in 1999 and 2007 included tobacco while the third study conducted in 2012 included smokeless tobacco products.

An international collaborative study performed by CORESTA involving 8 laboratories and 4 samples was conducted in 1999 and analysed according to ISO 5725 guidelines. Only 6 of the participating laboratories provided sorbitol data. The data from the laboratories contained 3 outliers for the determination of glycerol in cut rag and 2 outliers for the determination of sorbitol in cut rag. The outliers were 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 for the 1999 study gave the estimates as summarized in tables 2 - 7

**Table 2 – 1999 Study: 1,2-Propylene glycol – cut tobacco samples**

Mean concentration of 1,2 propylene glycol %	Repeatability limit r %	Reproducibility limit R %
0,585	0,120	0,530
1,568	0,062	0,315
2,724	0,208	0,825
4,723	0,153	0,480

**Table 3 – 1999 Study: Glycerol – cut tobacco samples**

Mean concentration of Glycerol %	Repeatability limit r %	Reproducibility limit R %
0,223	0,048	0,448
1,244	0,053	0,311
2,442	0,106	0,862
4,396	0,181	1,369

**Table 4 – 1999 Study: Sorbitol – cut tobacco samples**

Mean concentration of Sorbitol %	Repeatability limit r %	Reproducibility limit R %
0,079	0,051	0,179
1,003	0,113	0,294
1,971	0,167	0,195
2,709	0,193	0,452

**Table 5 – 1999 Study: 1,2-Propylene glycol – ground tobacco samples**

Mean concentration of 1,2 propylene glycol %	Repeatability limit r %	Reproducibility limit R %
0,574	0,054	0,555
1,596	0,067	0,276
2,669	0,102	0,666
4,554	0,096	0,450

**Table 6 – 1999 Study: Glycerol – ground tobacco samples**

Mean concentration of Glycerol %	Repeatability limit r %	Reproducibility limit R %
0,167	0,039	0,326
1,211	0,090	0,214
2,367	0,095	0,810
4,281	0,108	0,572

**Table 7 – 1999 Study: Sorbitol – ground tobacco samples**

Mean concentration of Sorbitol %	Repeatability limit r %	Reproducibility limit R %
0,097	0,022	0,207
1,028	0,080	0,236
1,943	0,118	0,323
2,620	0,169	0,206

During 2007, the CORESTA Scientific Commission requested the RAC Sub-Group carry out a second 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 dispatched to 19 laboratories for the second collaborative study. 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 1999 and 2007 Study Performance Measures**

	<b>Propylene Glycol in Cut Loose Tobacco - HPLC Method*</b>							
	<b>1999 Study</b>				<b>2007 Study</b>			
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 1	Sample 2	Sample 3	Sample 4
<b>Mean (%)</b>	0,585	1,568	2,724	4,723	0,439	1,251	2,103	3,573
<b>r</b>	0,120	0,062	0,208	0,153	0,077	0,071	0,090	0,174
<b>R</b>	0,530	0,315	0,825	0,48	0,358	0,408	0,503	0,307

	<b>Glycerol in Cut Loose Tobacco - HPLC Method*</b>							
	<b>1999 Study</b>				<b>2007 Study</b>			
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 1	Sample 2	Sample 3	Sample 4
<b>Mean (%)</b>	0,223	1,244	2,442	4,396	0,560	1,413	2,324	3,707
<b>r</b>	0,048	0,053	0,106	0,181	0,035	0,051	0,064	0,208
<b>R</b>	0,448	0,311	0,862	1,369	0,129	0,196	0,217	0,450

	<b>Propylene Glycol in Ground Tobacco - HPLC Method</b>							
	<b>1999 Study</b>				<b>2007 Study</b>			
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 1	Sample 2	Sample 3	Sample 4
<b>Mean (%)</b>	0,574	1,596	2,669	4,554	0,424	1,236	2,072	3,435
<b>r</b>	0,054	0,067	0,102	0,096	0,050	0,054	0,093	0,075
<b>R</b>	0,555	0,276	0,666	0,450	0,230	0,390	0,469	0,204

	<b>Glycerol in Ground Tobacco - HPLC Method*</b>							
	<b>1999 Study</b>				<b>2007 Study</b>			
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 1	Sample 2	Sample 3	Sample 4
<b>Mean (%)</b>	0,167	1,211	2,367	4,281	0,558	1,425	2,307	3,555
<b>r</b>	0,039	0,090	0,095	0,108	0,037	0,059	0,023	0,070
<b>R</b>	0,328	0,214	0,810	0,572	0,131	0,155	0,245	0,369

**\*Note:** r and R statistics for the HPLC methods are based on a very small number of laboratories, eight in the original study and four in this study with Lab 15 excluded.

The large r and R observed in the original 1999 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.

In 2012, the CORESTA Smokeless Tobacco Sub-Group conducted the third collaborative study for the determination of propylene glycol and glycerol in smokeless tobacco products using CRM N° 61. The five products included in this study are described in the table below. Not all samples contained both humectants,

**Table 9 – Sample Descriptions for 2012 CORESTA Collaborative Study**

Sample	Sample Description	Contains Propylene Glycol	Contains Glycerol
Sample A	American style moist snuff	yes	no
Sample B	American style loose leaf chewing tobacco	yes	yes
Sample C	Swedish style snus - loose tobacco	yes	yes
CRP 1	Swedish style snus pouch	yes	no
CRP 4	American style loose leaf chewing tobacco	no	yes

The overall level of variability for smokeless tobacco products seen in this study was similar to the 2007 RAC study for ground tobacco samples. The results for the 2012 study showed similar intra-laboratory and inter-laboratory variability for both tobacco and smokeless tobacco products which indicates that this CRM is appropriate for the analysis of both tobacco and smokeless tobacco products. No evidence was found for any smokeless tobacco related method interferences. The r and R results for the 2012 study are presented below:



**Table 10 – 2012 CORESTA Collaborative Study for the determination of Propylene Glycol and Glycerol in Smokeless Tobacco Products**

	Sample	Number of Labs <sup>1</sup>	Mean (%)	r	R	%r	%R
Propylene Glycol	Sample A	7	1.064	0.0640	0.117	6.0	11.0
	Sample B	7	0.809	0.0350	0.130	4.3	16.1
	Sample C	7	3.489	0.0560	0.286	1.6	8.2
	CRP 1	7	2.751	0.2780	0.475	10.1	17.3
	CRP 4	NA <sup>2</sup>	NA <sup>2</sup>	--	--	--	--
Glycerol	Sample A	NA <sup>2</sup>	NA <sup>2</sup>	--	--	--	--
	Sample B	7	2.504	0.0510	0.563	2.0	22.5
	Sample C	6	2.855	0.0260	0.656	0.9	23.0
	CRP 1	NA <sup>2</sup>	NA <sup>2</sup>	--	--	--	--
	CRP 4	7	4.004	0.2650	0.980	6.6	24.5

1. The number of laboratory data sets remaining after removal of outliers (Grubbs' test, Cochran's test).
2. The sample did not contain the humectant listed.

## 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.

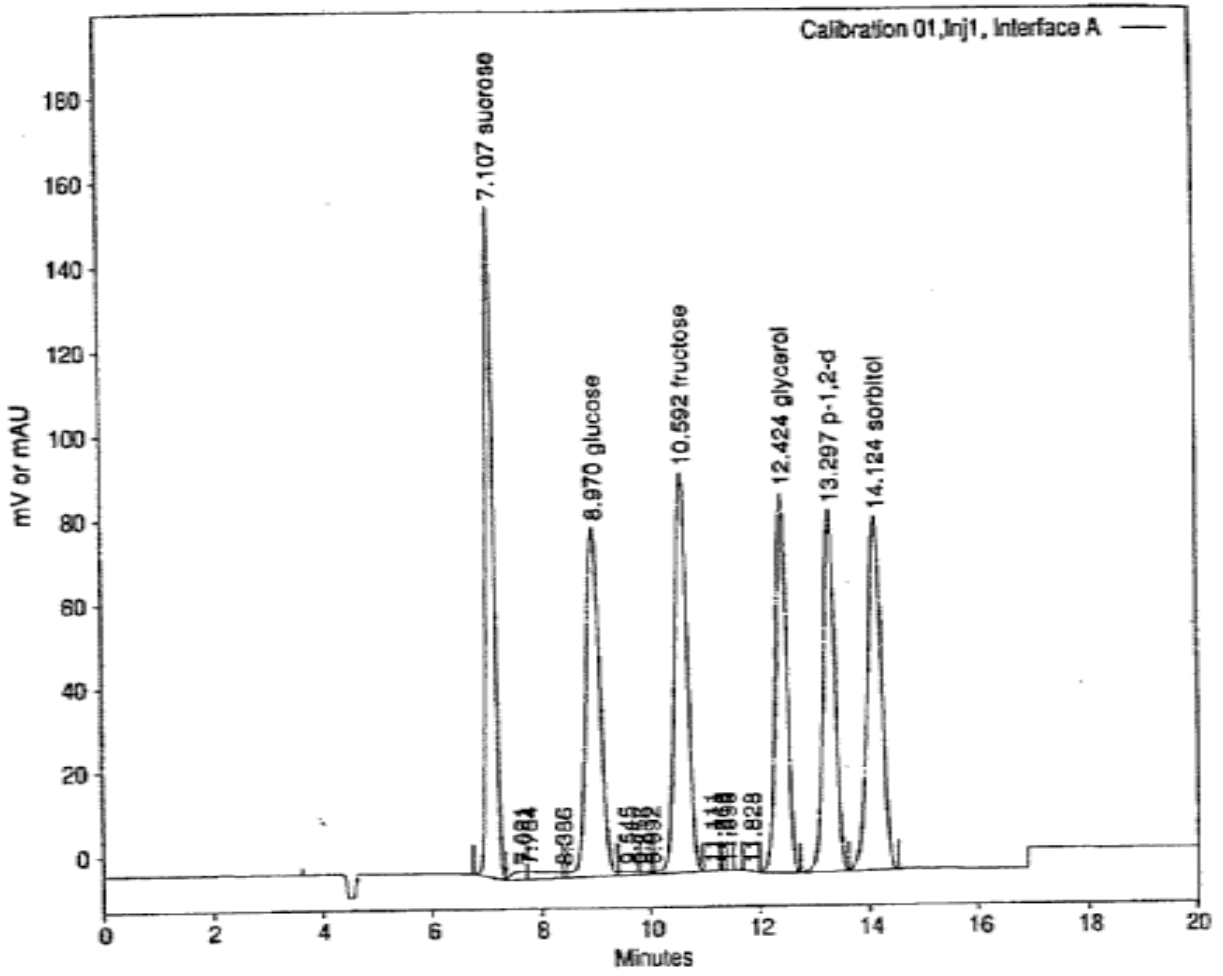


Figure 1 – Example of a chromatogram for a humectant standard

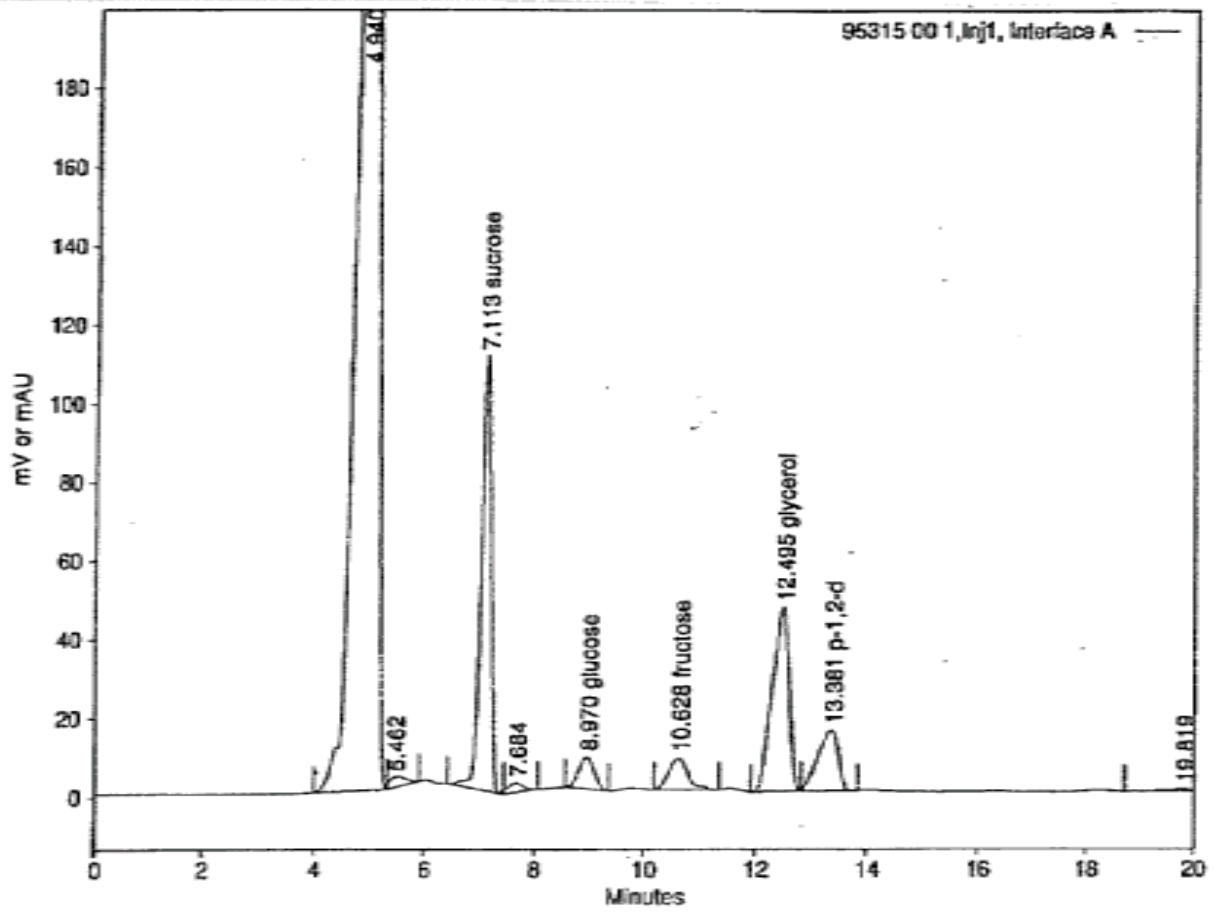


Figure 2 – Example of a chromatogram for a tobacco extract

## ANNEX A

(informative)

### Column care

Experience has shown that the tobacco matrix can lead to a contamination of some parts of the HPLC system. To ensure good resolution and chromatographic conditions a regeneration of the HPLC column and a cleaning of the injection system are recommended. Pre-column exchange is recommended once a week to avoid a contamination of the HPLC column which could lead to a loss in column life time (see figure A 1).

#### A.1. Regeneration of the column

Regeneration of the HPLC-column should be done according to the manufacturer's instructions.

**Note:** There are newer column types that have become available since the completion of the CORESTA studies for the development of this method that may not require regeneration (e.g. Varian MetaCarb 67C)<sup>2</sup>

#### A.2. Cleaning of the injection system

The autosampler is disconnected and the injection system is flushed with distilled water at a flow rate of 1,5 ml/min followed by injection of 30 µl of distilled water (3 times), 50 µl 6M HNO<sub>3</sub> (3 times) and 70 µl distilled water (3 times)

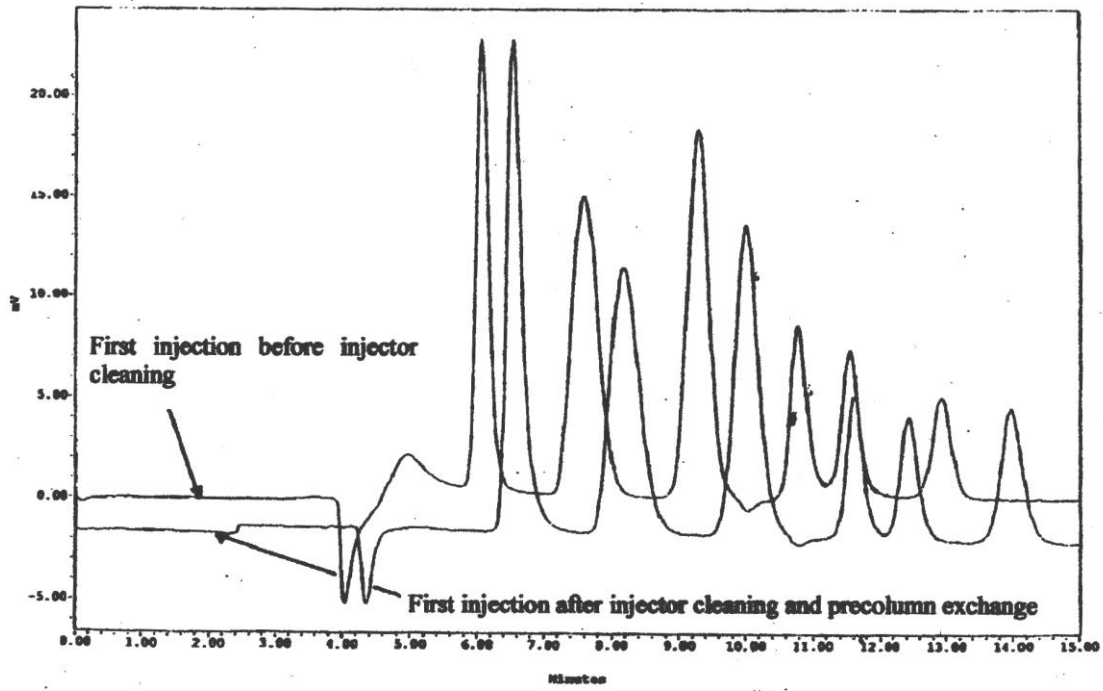
#### A.3. Lifetime of columns

Examples of lifetime and number of samples for HPLC-Sugarpak columns used for routine analysis are documented in the following table

Column	Number of samples	Runtime (hours)	Period of use
T 61571 A 28	2243	1226	07.01.97 – 31.10.97
T 61292 A 14	2304	1250	07.01.97 – 31.10.97
T 62991 A 15	2979	1484	10.02.97 – 23.04.98
T 71201 A 14	200 (complaint)	99	31.10.97 – 03.12.97
T 63461 A 04	3905	2108	05.06.97 – 18.12.98
T 72651 A 30	373 (complaint)	211	31.08.98 – 30. 11.98

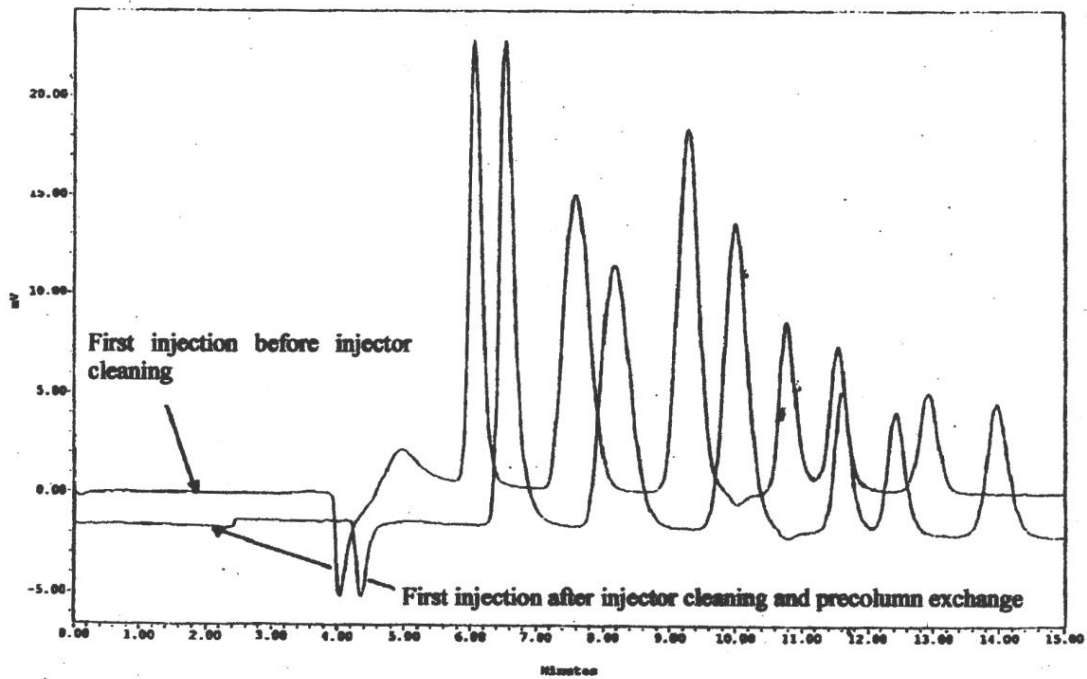
---

<sup>2</sup> MetaCarb 67C is a trade name of a product supplied by Varian. This information is given for the convenience of users of this Recommended Method and does not constitute an endorsement by CORESTA of this product.



Analysenummer: Kalib. 1:5 Wial: 45 Inj: 1 Ch: SATIN Type: Standard

Figure A.1 – Example of column regeneration



Analysenummer: Kalib. 1:5 Wial: 45 Inj: 1 Ch: SATIN Type: Standard

Figure A.2 – Example of injector cleaning and precolumn exchange