

CORESTA RECOMMENDED METHOD N° 37

DETERMINATION OF REDUCING SUBSTANCES IN TOBACCO BY CONTINUOUS FLOW ANALYSIS

(August 2010)

0. INTRODUCTION

A CORESTA Task Force studied the various widely-used procedures for the determination of reducing sugars in tobacco in order to adopt one of them as the CORESTA Recommended Method. Two procedures were adopted as CORESTA Recommended Methods N° 37 and N° 38. Studies carried out by the CORESTA Task Force between 1989 and 1993 have shown that the two methods may not produce identical results. For some tobaccos the results obtained with Method N° 37 are higher than those of Method N° 38 because Method N° 37 is sensitive to interferences from reducing substances, other than sugars, present in tobacco. This is reflected in the titles of the two methods. CORESTA has decided to publish both methods since Method N° 37 is easier to implement, while Method N° 38 is more specific. When reporting the results indicate the method used.

1. FIELD OF APPLICATION

This method is applicable to leaf samples and tobacco blends.

2. PRINCIPLE

A tobacco extract in 5 % acetic acid solution is prepared and the content of reducing substances of the extract is determined by reduction of yellow hexacyanoferrate III to colourless hexacyanoferrate II and the decrease in colour is measured at 420 nm.

Note 1 : Collaborative studies have shown that when extracting with distilled water, hydrolysis of sucrose occurs for some tobaccos.

3. REAGENTS

All reagents shall be used according to good laboratory practice and existing national regulations.

3.1 *Brij 35 Solution (Polyoxyethylene Lauryl Ether)*

Add 1 dm³ distilled water to 250 g Brij 35, warm and stir until dissolved.

3.2 *Sodium Chloride Solution (NaCl, 0.9 % m/V)*

Dissolve 9.0 g sodium chloride in distilled water, add 1 cm³ Brij 35 solution (3.1) and dilute to 1 dm³.

3.3 Sodium Hydroxide Solution (NaOH , 1 M)

Prepare 1 dm³ of 1 M sodium hydroxide from ampoules or dissolve 40.0 g sodium hydroxide in 800 cm³ distilled water. Mix and allow to cool. Dilute this solution to 1 dm³ with distilled water.

3.4 Alkaline Potassium Hexacyanoferrate III Solution (Potassium Ferricyanide)

($\text{K}_3\text{Fe}(\text{CN})_6$)

Dissolve 0.15 g of potassium hexacyanoferrate III in 800 cm³ of sodium hydroxide solution (3.3) add 1 cm³ of Brij 35 solution (3.1) and dilute to 1 dm³ with sodium hydroxide solution (3.3).

3.5 Acetic Acid Solution (CH_3COOH , 5 % V/V)

Prepare a 5 % (V/V) solution of acetic acid from "glacial" acetic acid (used in preparation of standards and samples and for wash solution on continuous flow analyzer).

3.6 D-Glucose ($\text{C}_6\text{H}_{12}\text{O}_6$, p.a.) for the Preparation of Standards

Store in a desiccator.

3.7 Standard Glucose Solutions

3.7.1 Stock Solution: Weigh, to the nearest 0.0001 g, approximately 10.0 g of glucose (3.6), dissolve in 800 cm³ of 5 % acetic acid (3.5) and dilute to 1 dm³ in a volumetric flask with 5 % acetic acid (3.5). This solution contains approximately 10 mg glucose per cm³. Store in a refrigerator. Prepare a fresh solution every month.

3.7.2 Working Standards: From the stock solution produce a series of at least five calibration solutions (in 5 % acetic acid) whose concentrations cover the range expected to be found in the samples, e.g. 0.2 - 2.5 mg glucose per cm³. Calculate the exact concentrations for each standard. Store in a refrigerator. Prepare fresh solutions every two weeks.

4. APPARATUS

4.1 The necessary general laboratory equipment, for the preparation of samples, standards and reagents.

4.2 Continuous flow analyzer (see diagram 1) consisting of:

Sampler

Proportioning pump

Dialyser

Heating bath

Delay coils

Colorimeter (or equivalent) with 420 nm filter(s)

Recorder

5. ANALYSIS OF TOBACCO SAMPLES

- 5.1** Prepare the tobacco for analysis by grinding (the sample should totally pass through a 1 mm sieve) and determine the moisture content. If the tobacco is too wet for grinding it can be dried at a temperature not exceeding 40°C.
- 5.2** Weigh, to the nearest 0.0001 g, approximately 250 mg of the tobacco into a 50 cm³ dry conical flask. Add 25 cm³ 5 % acetic acid (3.5), stopper the flask and shake for 30 minutes.
- 5.3** Filter the extract through a Whatman N° 40 (or equivalent) filter paper, reject the first few cm³ of the filtrate, then collect the filtrate in an analyzer cup.
- 5.4** Run the samples and standards through the system in the normal manner (*e.g.* priming with 6 tobacco extracts, calibration standards and samples with 1 intermediate calibration solution after every 6 samples).
If sample concentrations lie outside the range of the standards, the samples shall be diluted and run again.

6. CALCULATION

- 6.1** Plot a graph of peak height against equivalent glucose concentrations for all the standard solutions.
- 6.2** Calculate the percentage reducing substance (expressed as glucose)(dry weight basis) in the tobacco using the formula:

$$\% \text{Reducing Substance (dwb)} = \frac{c \times V \times 100}{m} \times \frac{100}{100 - M}$$

- c** is the reducing substance concentration, expressed in milligrams per millilitre, obtained from the calibration curve (6.1);
- V** is the volume, in millilitres, of extract prepared (5.2) (normally 25 millilitres);
- m** is the mass, in milligrams, of the sample (5.2)
- M** is the moisture content, expressed as percentage by mass, of the tobacco (5.1).
The test result shall be expressed to one decimal place.

Note 2 : If this method is performed simultaneously with CORESTA Recommended Method N° 35 or CORESTA Recommended Method N° 36 combined standards may be prepared.

7. REPEATABILITY AND REPRODUCIBILITY

7.1 An international collaborative study, involving 12 laboratories and 3 samples, conducted in 1993 showed that when single grades of tobacco were analyzed by this method, the following values for repeatability (r) and reproducibility (R) were obtained.

The difference between two single results found on different extractions by one operator using the same apparatus within a short time interval (the time it takes to analyze 40 sample cups) and without recalibration of the equipment during the time of analysis will exceed the repeatability value (r) on average not more than once in 20 cases in the normal and correct operation of the method.

Single results reported by two laboratories will differ by more than the reproducibility value (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 summarised in Table 1

Table 1 (1993 Data)

Tobacco Type	Mean content of Reducing Substances % (dwb)	Repeatability Conditions r	Reproducibility Conditions R
Burley	2.3	0.7	1.7
Oriental	15.1	1.2	2.5
Flue-Cured	19.8	1.8	2.5

For the purpose of calculating the r and R, one test result was defined as the yield obtained from analyzing a single extract once.

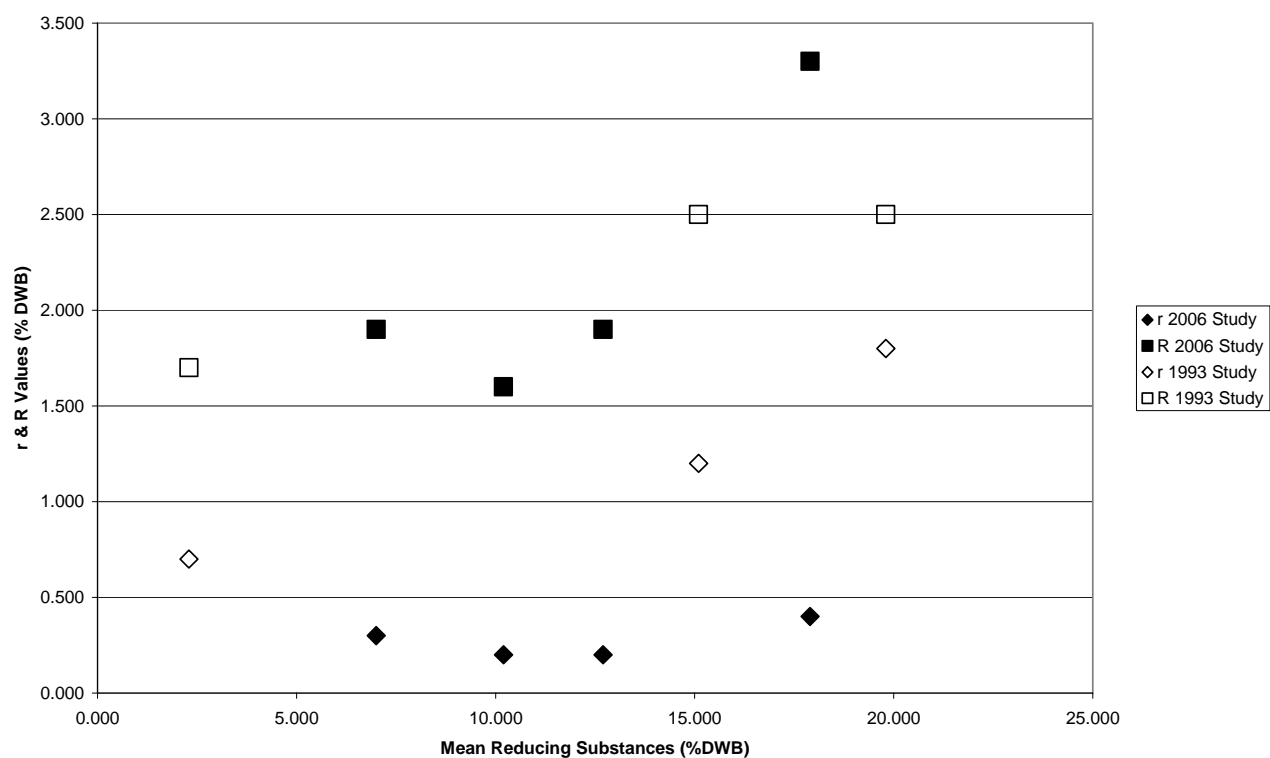
7.2 During 2005 the CORESTA Scientific Commission sanctioned the CORESTA Routine Analytical Chemistry Sub-group to carry out a collaborative study to confirm these r & R values. This international study involved 9 laboratories and 4 samples and was conducted during 2006. The resulting data are to be found in Table 2.

TABLE 2 Results from the 2006 RAC Collaborative Study

Tobacco Type	Mean Content of Reducing Substances % (dwb)	Repeatability conditions r	Repeatability coefficient of variation r CV	Reproducibility conditions R	Reproducibility coefficient of variation R CV
Flue-Cured Sample A	7.0	0.3	4.3	1.9	27.1
Flue-Cured Sample B	17.9	0.4	2.2	3.3	18.4
Flue-Cured Sample C	10.2	0.2	2.0	1.6	15.7
Flue-Cured Sample F	12.7	0.2	1.6	1.9	15.0

A plot comparing this data to that of the original study can be found below:

Comparison of r and R Results from the 1993 and 2006 Studies



Reducing substances

(Technicon part numbers only given for information)
(Wash and sample times only intended as a guide)

