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Tobacco — Determination of the content of reducing substances — Continuous-flow analysis method

Tabac — Détermination de la teneur en substances réductrices — Méthode par analyse en flux continu

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Contents

Page

1 Scope 1
2 Principle 1
3 Reagents 1
4 Apparatus 2
5 Procedure 2
6 Calculation 3
7 Repeatability and reproducibility 3
8 Test report 3
Annex A (informative) Example of a continuous-flow analyser 5
Bibliography 6

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 15153 was prepared by Technical Committee ISO/TC 126, *Tobacco and tobacco products*, Subcommittee SC 2, *Leaf tobacco*.

Introduction

A CORESTA[1)] 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 ISO 15154 and this International Standard. 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 the method given in this International Standard are higher than those of the method given in ISO 15154, because the former is sensitive to interferences from reducing substances, other than sugars, present in tobacco. Collaborative studies have shown that when extracting with distilled water, hydrolysis of sucrose occurs with some tobaccos.

1) CORESTA: Cooperation Centre for Scientific Research Relative to Tobacco.

Tobacco — Determination of the content of reducing substances — Continuous-flow analysis method

1 Scope

This International Standard specifies a method for the determination of the content of reducing substances in tobacco by continuous-flow analysis.

This method is applicable to manufactured and unmanufactured tobacco.

2 Principle

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

3 Reagents

All reagents shall be used according to good laboratory practice and existing national regulations. Use distilled water or water of at least equivalent purity.

3.1 Polyoxyethylene lauryl ether (Brij 35 solution).

Add 1 litre of water to 250 g of Brij 35. Warm and stir until dissolved.

3.2 Sodium chloride solution (NaCl).

Dissolve 9,0 g sodium chloride in water, add 1 ml Brij 35 solution (3.1) and dilute to 1 litre.

3.3 Sodium hydroxide solution, c(NaOH) = 1 mol/l.

Prepare 1 litre of 1 mol/l sodium hydroxide from ampoules or dissolve 40,0 g of sodium hydroxide in 800 ml of water. Mix and allow to cool. Dilute this solution to 1 litre with water.

3.4 Alkaline potassium hexacyanoiron(III) solution (potassium ferricyanide) [$K_3Fe(CN)_6$].

Dissolve 0,15 g of potassium hexacyanoiron(III) in 800 ml of sodium hydroxide solution (3.3). Add 1 ml of Brij 35 solution (3.1) and dilute to 1 litre with sodium hydroxide solution (3.3).

3.5 Acetic acid solution (CH_3COOH), volume fraction of 5 %.

Prepare a 5 % (volume fraction) solution of acetic acid from glacial acetic acid. (This is used in the preparation of standards and samples and for the wash solution for the continuous-flow analyser.)

3.6 D-Glucose ($C_6H_{12}O_6$).

Store in a desiccator.

3.7 Standard glucose solutions

3.7.1 Stock solution

Weigh, to the nearest 0,1 mg, approximately 10,0 g of glucose (3.6). Dissolve in 800 ml of the acetic acid (3.5) and dilute to 1 litre in a volumetric flask with acetic acid (3.5). This solution contains approximately 10 mg/ml of glucose. Store in a refrigerator. Prepare a fresh solution every month.

3.7.2 Working standards

From the stock solution (3.7.1), prepare by dilution with the acetic acid (3.5) a series of at least five calibration solutions, the glucose concentrations of which cover the range expected to be found in the test samples (e.g. 0,2 mg/ml to 2,5 mg/ml). Calculate the exact concentrations for each standard. Store in a refrigerator. Prepare fresh solutions every 2 weeks.

4 Apparatus

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

4.1 Continuous flow analyser, consisting of

— sampler,

— proportioning pump,

— dialyser,

— heating bath,

— delay coils,

— colorimeter (or equivalent) with 420 nm filter(s), and

— recorder.

See Annex A for an example of a suitable layout.

5 Procedure

5.1 Preparation of samples for analysis

Prepare the tobacco samples 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 may be dried at a temperature not exceeding 40 °C.

5.2 Test portion

Weigh, to the nearest 0,1 mg, approximately 250 mg of the tobacco into a 50 ml dry conical flask. Add 25 ml of the acetic acid (3.5), stopper the flask and shake for 30 min.

5.3 Preparation of test extract

Filter the extract through a Whatman No. 40[2)] (or equivalent) filter paper. Reject the first few millilitres of the filtrate, then collect the filtrate in an analyser cup.

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2) Whatman No. 40 is an example of a suitable product available commercially. This information is given for the convenience of the users of this International Standard and does not constitute an endorsement by ISO of this product.

Run the samples and standards through the system in the normal manner (e.g. priming with six tobacco extracts, calibration standards and samples with one intermediate calibration solution after every six samples). If the sample concentrations lie outside the range of the standards, the samples shall be diluted with the acetic acid solution (3.5) and run again.

When using 5 % acetic acid extracts, the wash solution shall be 5 % acetic acid.

NOTE If this method is performed simultaneously with the method described in ISO 15152 or ISO 15517, combined standards may be prepared.

6 Calculation

6.1 Plot a graph of peak height against equivalent glucose concentrations for all the standard solutions.

6.2 Calculate the percentage of reducing substances, w (expressed as glucose), on a dry weight basis, in the tobacco using the formula

$$w = \frac{c \times V \times 100}{m} \times \frac{100}{100 - M}$$

where

c is the reducing substances concentration, expressed in milligrams per millilitre, obtained from the calibration curve (see 6.1);

V is the volume, in millilitres, of extract prepared (see 5.2) (normally 25 ml);

m is the mass, in milligrams, of the sample (see 5.2);

M is the moisture content, expressed as a percentage by mass, of the tobacco (see 5.1).

The test result shall be expressed to one decimal place.

7 Repeatability and reproducibility

An international collaborative study, involving 12 laboratories and 3 samples, conducted in 1993, showed that when single grades of tobacco were analysed by this method, the following values for repeatability limit (r) and reproducibility limit (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 analyse 40 sample cups) and without recalibration of the equipment during the time of analysis, 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 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 summarised in Table 1. For the purpose of calculating r and R, one test result was defined as the yield obtained from analysing a single extract once.

8 Test report

When reporting results, the method used shall be specified.