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Material used for producing wrappings for cigarette filters, cigarettes and other tobacco products — Determination of citrate content

Matériaux utilisés pour la fabrication des enveloppes pour les filtres de cigarette, pour les cigarettes et pour les autres produits du tabac — Dosage du citrate

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Foreword

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ISO 20369 was prepared by Technical Committee ISO/TC 126, *Tobacco and tobacco products*.

Material used for producing wrappings for cigarette filters, cigarettes and other tobacco products — Determination of citrate content

WARNING — The use of this International Standard can involve hazardous materials, operations and equipment. This International Standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this International Standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

1 Scope

This International Standard specifies a method for the determination of the citrate content of material used to produce wrappings for cigarette filters, cigarettes and other tobacco products.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 187, *Paper, boards and pulps — Standard atmosphere for conditioning and testing and procedure for monitoring the atmosphere and conditioning of samples*

ISO 287, *Paper and board — Determination of moisture content of a lot — Oven-drying method*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
citrate content
⟨materials for producing wrappings for cigarette filters, cigarettes and other tobacco products⟩ anhydrous citric acid content determined by the enzymatic method

NOTE Citrate is generally added to wrapping materials, in particular cigarette paper, as trisodium citrate and tripotassium citrate or mixtures thereof to influence the burning rate of the cigarette and, consequently, the puff number.

4 Principle

The citrate content is determined by an enzymatic method in which (citrate) citric acid, catalyzed by the enzyme citrate lyase (CL), is first converted to oxaloacetate and acetate in accordance with the following reaction:

$$\text{citrate} \xrightarrow{\text{CL}} \text{oxaloacetate} + \text{acetate} \quad (1)$$

In the presence of the enzymes L-malate dehydrogenase (L-MDH) and L-lactate dehydrogenase (L-LDH), oxaloacetate and its decarboxylation product, pyruvate, are converted by reduced nicotinamide adenine dinucleotide (NADH) to L-malate and L-lactate, respectively, according to the following reactions:

$$\text{oxaloacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{L-MDH}} \text{L-malate} + \text{NAD}^+ \quad (2)$$

$$\text{pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{L-LDH}} \text{L-lactate} + \text{NAD}^+ \quad (3)$$

The amount of oxidized NADH is proportional to the amount of citrate. The residual NADH is determined from its absorbance at 340 nm by spectrophotometry.

5 Reagents

5.1 General

All reagents used shall be of recognized analytical grade. Water used shall be in accordance with at least grade 3 of ISO 3696.

5.2 Test kit for enzymatic citrate determination

5.2.1 General

Commercially available test kits shall be used that generally contain two reagent mixtures [Roche-Biopharm 10.139.076.035, or equivalent[1)]].

Optionally, the determination may be performed using individual reagents. In that case, the procedure is to be found in the literature or commercial information documents.

5.2.2 Reagent mixture 1

Reagent mixture 1 shall be diluted with water in accordance with the manufacturer's instructions to produce solution 1. The ready-to-use solution 1, which is buffered to a pH of 7,8 using glycylglycine buffer, contains the following:

— L-malate dehydrogenase (L-MDH), about 12 IU[2)]/ml;

— L-lactate dehydrogenase (L-LDH), about 23 IU/ml;

1) Roche-Biopharm 10.139.076.035 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.

2) IU (international unit) is the amount of enzyme (activity) that catalyses the conversion of 1 µmol of substrate per minute under standard conditions.

— reduced nicotinamide adenine dinucleotide (NADH), 0,5 mg/ml;

— stabilizers.

Solution 1 may be stored for two weeks at +4 °C or for four weeks at −20 °C.

5.2.3 Reagent mixture 2

Reagent mixture 2 shall be diluted with water in accordance with the manufacturer's instructions to produce solution 2. The ready-to-use solution 2 contains about 40 IU/ml of citrate lyase.

Solution 2 may be stored for two weeks at +4 °C or for four weeks at −20 °C.

The activity of the enzyme system shall be (100 ± 5) %.

5.3 Citric acid monohydrate.

6 Apparatus

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

6.1 Conical flasks, of nominal capacity 250 ml.

6.2 Funnel, of diameter 80 mm.

6.3 Filter paper, of diameter 125 mm [Whatman No. 40, or equivalent[3)]].

6.4 Pipettes, with graduations suitable for nominal capacities of 1 ml, 2 ml, 5 ml and 10 ml; enzyme assay pipettes might be used as well [2].

6.5 Piston-operated pipette, of nominal capacity 20 µl.

6.6 Double-beam spectrophotometer, suitable for a wavelength of 340 nm.

6.7 Glass or **plastic cuvets**, of light path 10 mm and capacity 5 ml.

6.8 Ultrasonic bath or **magnetic stirrer**.

6.9 Analytical balance, suitable for measuring to the nearest 0,001 g.

7 Procedure

7.1 Sample preparation

Extract approximately 1,0 g, to the nearest 0,001 g, of cut wrapping material previously conditioned as specified in ISO 187, in 100 ml of water in a 250 ml conical flask (6.1), by the aid of an ultrasonic bath or magnetic stirrer (6.8) for 30 min. Then filter the extract through a filter paper (6.3).

7.2 Determination

Perform the determination at a constant temperature of between 20 °C and 25 °C. The following pipetting procedure (see Table 1) has proved satisfactory for the blank solution (water) and the test solution (sample extract as prepared in 7.1).

3) 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.

The absorbance shall be determined using a double-beam spectrophotometer (6.6) at a wavelength of 340 nm with air (no cuvet in the beam path) or water as reference. The total volume, V, of test solution in the cuvet shall be 3,02 ml.

To calibrate the method, replace the sample extract by standard solutions of citric acid monohydrate (5.3) having mass concentrations of 50 mg/l, 25 mg/l and 12,5 mg/l and proceed as described in this clause.

Table 1 — Pipetting procedure

Pipette into cuvets	Blank cuvet ml	Test cuvet ml
Solution 1 according to 5.2.2	1,00	1,00
Water	2,00	1,80
Sample extract	—	0,20
Mix, read off the absorbance of the solutions (A_1) after about 5 min, and start the second reaction by addition of:		
Solution 2 according to 5.2.3	0,02	0,02
Mix, and on completion of the reaction (after about 5 min), read off the absorbance of the solutions (A_2).		

8 Calculation

In the reactions on which this determination is based, there is a linear proportionality between the amount of NADH consumed — and, consequently, the absorbance difference, ΔA — and the concentration by mass of citric acid (see Clause 4). Calculate the absorbance difference using the following equation:

$$\Delta A = (A_1 - A_2)_{\text{sample}} - (A_1 - A_2)_{\text{blank}} \tag{4}$$

Occasionally a negative value is obtained for the absorbance difference of the blank solution, $(A_1 - A_2)_{\text{blank}}$. In such cases, Equation (4) is still used, and $|(A_1 - A_2)_{\text{blank}}|$ is added to $(A_1 - A_2)_{\text{sample}}$.

To obtain reliable results, the absorbance difference of the sample extract should be at least 0,1. If the absorbance difference of the sample extract is higher than 0,850, the concentration by mass of citric acid in the sample extract is too high. In this case, the sample extract should be diluted until the concentration by mass of citric acid in the cuvet is less than 80 µg.

Calculate the citric acid mass content, ρ_C, in grams per litre of the sample extract, using the following equation:

$$\rho_C = \frac{V \times M \times F}{\varepsilon \times \delta \times V_P \times 1\,000} \times \Delta A \tag{5}$$

where

V is the total volume of test solution in the cuvet, in millilitres (generally 3,02 ml);

M is the molar mass of the substance to be determined;

F is the dilution factor of the sample solution;

ε is the absorption coefficient of NADH at 340 nm: 6,30 l·mmol^{-1}·cm^{-1};

δ is the light path of the cuvet, in centimetres;

V_P is the volume of sample solution used for the preparation of the test solution, in millilitres;

ΔA is the absorbance difference.

If the volumes are the same as in 7.2 and it is unnecessary to dilute the sample extract, calculate the citrate content, ρ_C, given as a concentration by mass in grams per litre of sample extract, as anhydrous citric acid (M = 192,1 g/mol) using the following equation:

$$\rho_C = 0{,}461 \times \Delta A \quad (6)$$

Calculate the content of anhydrous citric acid as a mass fraction, ω_C, in the wrapping material, as a percentage by mass, using the following equation:

$$\omega_C = \frac{\rho_C}{\rho_P} \times 100\ \% \quad (7)$$

where ρ_P is the mass concentration of the wrapping material sample, in grams per litre of sample extract.

If 1 g of wrapping material is extracted with 100 ml of water, ρ_P is equal to 10 g/l and the content of anhydrous citric acid, ω_C, is given by the following equation:

$$\omega_C = \rho_C \times 10\ \% \quad (8)$$

Report the citrate content (mass fraction) as a percentage by mass of anhydrous citric acid in the wrapping material.

9 Precision

Table 2 shows mean standard deviations of repeatability, s_r, and reproducibility, s_R, obtained from a collaborative study. The study, involving nine laboratories, was conducted in 2005, using three cigarette paper samples with citrate levels between 0,6 % and 2,5 %. Nine laboratories reported results obtained by this method.

Table 2 — Data analysis of collaborative study

Sample	n data considered	Mean value		
		m_N	s_r	s_R
		Results expressed as mass fraction in % citric acid monohydrate		
C1	8	0,68	0,025	0,038
C2	6	2,44	0,046	0,060
C3	8	0,60	0,012	0,022

10 Test report

The test report shall include the following:

a) all information required for the complete identification of the sample (type of sample, its origin and its designation);

b) reference to this International Standard;

c) date of sampling and method;