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

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



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

acetate content

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

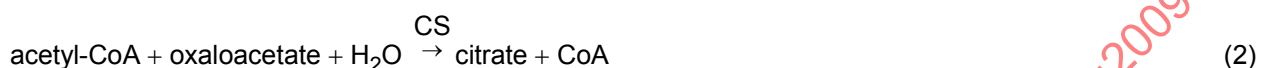
NOTE Acetate is generally added to wrapping materials, in particular cigarette paper, as sodium acetate and potassium acetate to influence the burning rate of the cigarette and, consequently, the puff number.

4 Principle

The acetate content is determined by an enzymatic method in which acetate (acetic acid), catalyzed by the enzyme acetyl coenzyme A synthase (ACS) with adenosine 5'-triphosphate (ATP) and coenzyme A (CoA), is first converted to acetyl coenzyme A, adenosine 5'-monophosphate (AMP) and pyrophosphate in accordance with the following reaction:



Catalyzed by citrate synthase (CS), acetyl coenzyme A reacts with oxaloacetate to form citrate in accordance with the following reaction:



The oxaloacetate required for the reaction (2) is formed from malate and nicotinamide-adenine dinucleotide (NAD⁺) by catalysis using L-malate dehydrogenase (L-MDH) in accordance with the following reaction:



The determination is based on the formation of NADH, which is measured by the increase in absorbance at 340 nm. Since the determination reaction is preceded by an indicator reaction, the amount of NADH formed is not linearly proportional to the acetate content.

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 acetate determination

5.2.1 General

Commercially available test kits shall be used that generally contain two reagent mixtures [Roche-Biopharm 10.148.261.035, or equivalent¹⁾].

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

The ready-to-use solution 1, which is buffered to a pH of 8,4 using triethanolamine buffer, contains the following:

- L-malic acid, about 4,2 mg/ml;
- magnesium chloride, about 2,1 mg/ml.

Solution 1 will be stable for one year at +4 °C.

1) Roche-Biopharm 10.148.261.035 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.

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 the following:

- adenosine 5'-triphosphate (ATP), about 25,0 mg/ml;
- coenzyme A (CoA), about 2,6 mg/ml;
- nicotinamide adenine dinucleotide (NAD), about 12,3 mg/ml.

Solution 2 will be stable for four weeks at +4 °C.

5.2.4 Reagent mixture 3

The ready-to-use solution 3 contains the following:

- L-malate dehydrogenase (L-MDH), about 2 750 IU²/ml;
- citrate synthase, about 675 IU/ml.

Solution 3 will be stable for one year at +4 °C.

The activity of the enzyme system shall be $(100 \pm 5) \%$.

5.2.5 Reagent mixture 4

Reagent mixture 4 shall be diluted with water in accordance with the manufacturer's instructions to produce solution 4. The ready-to-use solution 4 contains about 20 IU/ml of acetyl coenzyme A synthase (ACS).

Solution 4 will be stable for five days at +4 °C.

5.3 Sodium acetate trihydrate.

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 equivalent³].
- 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 pipettes**, of nominal capacities 10 µl and 20 µl.
- 6.6 Double-beam spectrophotometer**, suitable for a wavelength of 340 nm.

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

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.

6.7 Glass or plastic cuvetts, 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).

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 the test solution in the cuvet shall be 3,23 ml.

To calibrate the method, replace the sample extract by standard solutions of sodium acetate trihydrate (5.3) having mass concentrations of 300 mg/l, 100 mg/l and 50 mg/l and proceed as described in this clause.

Table 1 — Pipetting procedure

Pipette into cuvetts	Blank cuvet ml	Test cuvet ml
Solution 1 according to 5.2.2	1,00	1,00
Solution 2 according to 5.2.3	0,20	0,20
Water	2,00	1,90
Sample extract	—	0,10
Mix, read off the absorbance of the solutions, A_0 , and then add the following.		
Solution 3 according to 5.2.4	0,01	0,01
Mix, and on completion of the reaction (after about 3 min), read off the absorbance of the solutions, A_1 . Then start the second reaction by adding the following.		
Solution 4 according to 5.2.5	0,02	0,02
Mix, and on completion of the reaction (after about 10 min to 15 min), read off the absorbances of the solutions, A_2 . If the reaction has not stopped after 15 min, read off the absorbance at 2 min intervals until the absorbance increases constantly for 2 min. If the absorbance increases at a constant rate, extrapolate it to the time when the solution was added.		

8 Calculation

In the reactions on which this determination is based, there is not a linear proportionality between the amount of NADH consumed — and, consequently, the absorbance difference, ΔA — and the acetic acid concentration (see Clause 4). Calculate the absorbance difference using the following equation:

$$\Delta A = \left[(A_2 - A_0)_{\text{sample}} - \frac{(A_1 - A_0)_{\text{sample}}^2}{(A_2 - A_0)_{\text{sample}}} \right] - \left[(A_2 - A_0)_{\text{blank}} - \frac{(A_1 - A_0)_{\text{blank}}^2}{(A_2 - A_0)_{\text{blank}}} \right] \quad (4)$$

To obtain reliable results, the absorbance difference of the sample extract should be at least 0,1. The acetic acid concentration in the cuvet shall be between 1 µg and 15 µg, and the sample shall therefore be diluted beforehand so that the acetic acid content is 0,01 g/l to 0,15 g/l. The absorbance difference should be between 0,2 and 0,4.

Calculate the acetic acid mass concentration, ρ_A , in grams per litre of the sample extract, using the following equation:

$$\rho_A = \frac{V \times M \times F}{\varepsilon \times \delta \times V_P \times 1000} \times \Delta A \quad (5)$$

where

V is the total volume of test solution in the cuvet, in millilitres (generally 3,23 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 acetate content, ρ_A , given as a concentration by mass in grams per litre of sample extract, as anhydrous acetic acid ($M = 60,05$ g/mol) using the following equation:

$$\rho_A = 2,566 \times \Delta A \quad (6)$$

Calculate the content of acetate as mass fraction, ω_A , in the wrapping material given as a percentage by mass as anhydrous acetic acid, using the following equation:

$$\omega_A = \frac{\rho_A}{\rho_P} \times 100 \% \quad (7)$$

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. In this case, the content of acetate, ω_A , is given by the following equation:

$$\omega_A = \rho_A \times 10 \% \quad (8)$$