
**Tobacco — Determination of nitrate
content — Continuous-flow analysis
method**

*Tabac — Détermination de la teneur en nitrates — Méthode par analyse
en flux continu*

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Foreword

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Tobacco — Determination of nitrate content — Continuous-flow analysis method

1 Scope

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

This method is applicable to manufactured and unmanufactured tobacco.

2 Principle

An aqueous extract (see below) of the tobacco is prepared and the nitrate content of the extract is determined by reduction of the nitrate ions to nitrite ions with hydrazinium sulfate in the presence of a copper catalyst, followed by reaction with sulfanilamide to form the diazo compound. This is coupled with *N*-1-naphthylethylenediamine dihydrochloride to form a coloured complex, which is measured at 520 nm. If there is any nitrite content, it will be detected and included in the nitrate content result.

Collaborative studies have shown that this method gives equivalent results for water and 5 % acetic acid extracts. It is recommended that 5 % acetic acid extracts should be used if nitrate and reducing substances (see ISO 15153) or reducing carbohydrate analyses (see ISO 15154) are to be carried out simultaneously.

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 hydroxide solution (NaOH), p.a.

Dissolve 8,0 g of sodium hydroxide in distilled water. Add 1 ml of Brij 35 solution (3.1) and dilute to 1 litre.

3.3 Copper sulfate stock solution (CuSO₄), p.a.

Dissolve 1,20 g of copper(II) sulfate pentahydrate in distilled water and dilute to 100 ml.

3.4 Hydrazinium sulfate/copper sulfate reagent

Dissolve the optimum amount of hydrazinium sulfate (N₂H₆SO₄, p.a.) in water. Add 1,5 ml of copper sulfate stock solution (3.3) and dilute to 1 litre with water. Store in an amber glass bottle. Prepare a fresh solution every month.

3.5 Sulfanilamide reagent (NH₂C₆H₄SO₂NH₂)

Add 25 ml of concentrated orthophosphoric acid (H₃PO₄, volume fraction 85 %, of grade low in nitrate) to approximately 175 ml of water. Dissolve 2,5 g of sulfanilamide in the solution followed by 0,125 g of *N*-1-naphthylethylenediamine dihydrochloride (C₁₀H₇NHCH₂CH₂NH₂·2HCl).