INTERNATIONAL STANDARD

**ISO 15152**

First edition
2003-04-15

# Tobacco — Determination of the content of total alkaloids as nicotine — Continuous-flow analysis method

*Tabac — Détermination de la teneur en alcaloïdes totaux exprimés en nicotine — Méthode par analyse en flux continu*



Reference number
ISO 15152:2003(E)

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Published in Switzerland

 

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## Foreword

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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 15152 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 total alkaloids in tobacco in order to adopt one of them as the CORESTA Recommended Method. Two procedures were adopted as ISO 2881 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 dark tobaccos or those containing significant levels of alkaloids other than nicotine. The studies have indicated that these differences may be due to the fact that the recoveries and detection sensitivities of the methods for alkaloids other than nicotine are different.



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

# Tobacco — Determination of the content of total alkaloids as nicotine — Continuous-flow analysis method

## 1 Scope

This International Standard specifies a method for the determination of the content of total alkaloids as nicotine in tobacco by continuous-flow analysis.

This method is applicable to manufactured and unmanufactured tobacco.

## 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 13276, *Tobacco and tobacco products — Determination of nicotine purity — Gravimetric method using tungstosilicic acid*



## 3 Principle



An aqueous extract (see below) of the tobacco is prepared and the total alkaloids content (as nicotine) of the extract is determined by reaction with sulfanilic acid and cyanogen chloride. Cyanogen chloride is generated *in situ* by the reaction of potassium cyanide and chloramine T (see Annex A). The developed colour is measured at 460 nm.

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 total alkaloids (as nicotine) and reducing substances (see ISO 15153) or reducing carbohydrate analyses (see ISO 15154) are to be carried out simultaneously.

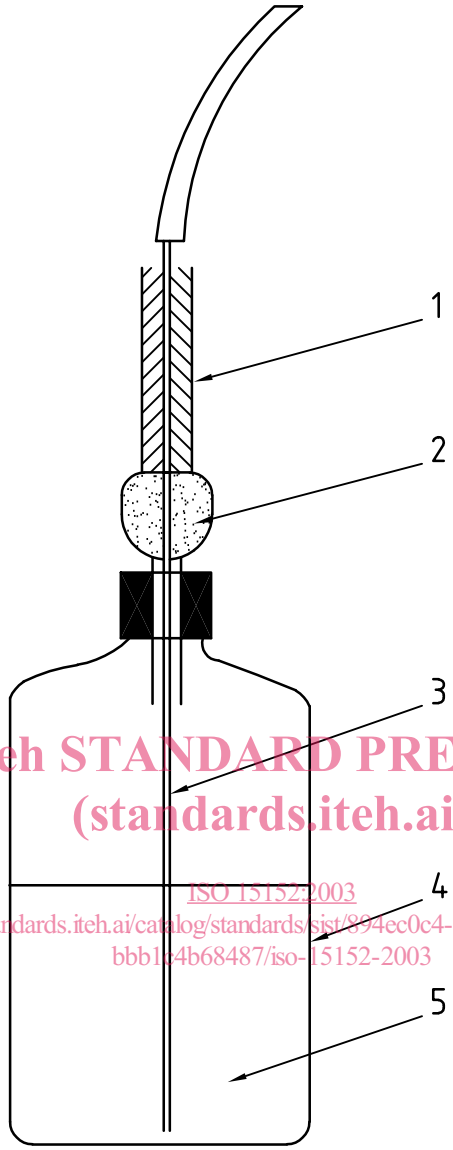
## 4 Safety precautions

**WARNING — Potassium cyanide is poisonous and an irritant, thus all safety precautions shall be observed when handling this material.**

Solutions shall be prepared by a designated responsible person. Gloves and safety glasses shall always be used when making up solutions. Bottles of the made-up reagent shall always be carried in a suitable safety carrier. To prevent the escape of vapour into the laboratory, reagent pick-up tubes shall pass through a soda-lime trap into the reagent bottle (see Figure 1).

The cyanide neutralizing solutions A and B (5.5 and 5.6) shall be pumped as shown in the flow diagram (see Figure B.1) and mixed in a 2 litre Büchner flask with magnetic stirring (see Figure 2). All waste solutions containing cyanogen chloride shall be run into this flask where conversion to the “Prussian Blue” complex occurs. The contents of the Büchner flask shall be allowed to overflow into a storage flask, the contents of which shall be stored overnight in a fume cupboard and then disposed of as waste.

Suitable cyanide poisoning treatment kits are available from laboratory equipment suppliers and shall be located in the vicinity of the analyser to be used by a competent person.

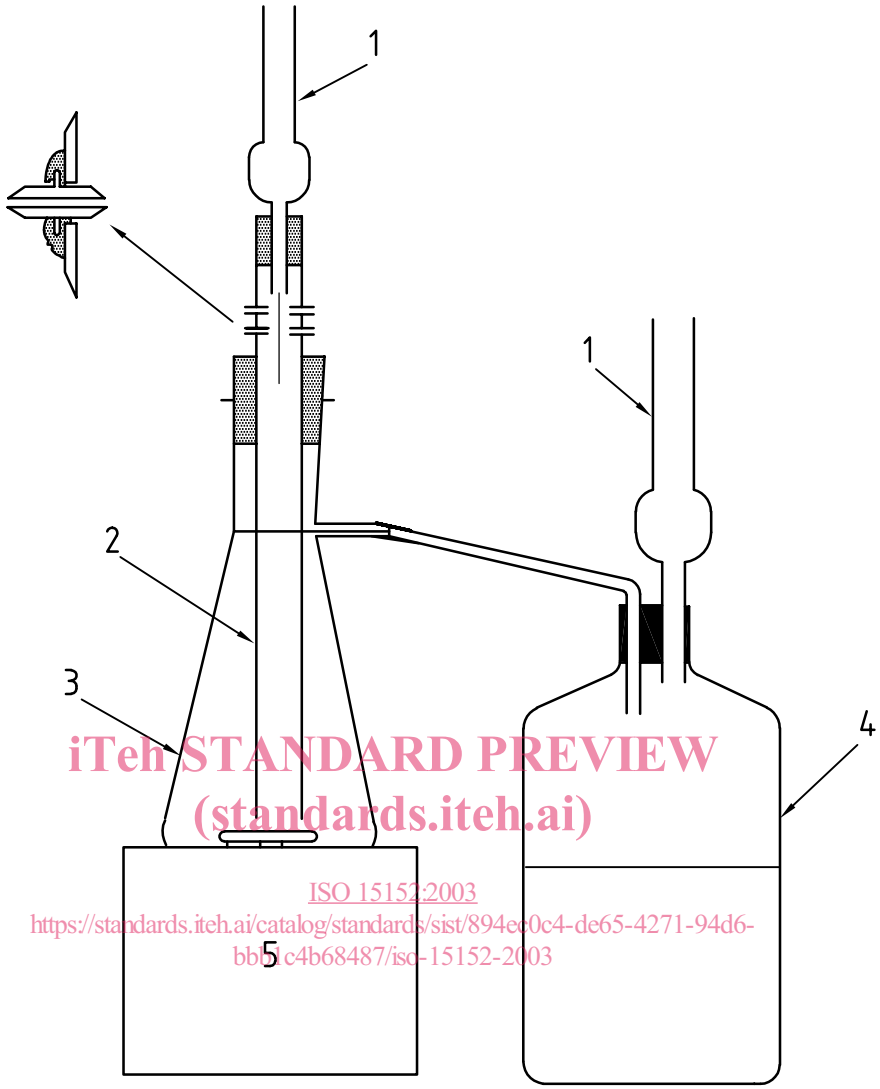





**Key**

1 soda lime
2 cotton wool
3 reagent pick-up tube
4 storage flask
5 reagent

**Figure 1 — Soda-lime trap**






**Key**

1 soda lime traps
2 PVC tube, 2 cm diameter
3 Büchner flask
4 Winchester bottle
5 magnetic stirrer

NOTE The apparatus consists of a 2 litre Büchner flask on a magnetic stirrer, with a 2 cm diameter PVC tube inserted into it, through a rubber bung, such that the tube is just above the magnetic follower in the flask. Four holes are drilled in the tube and nipples attached by gluing into position. The pullback line and the debubble line containing the cyanogen chloride are attached to the nipples, together with the two neutralizing agents. This arrangement ensures that the cyanogen chloride has to pass down the tube and through the bulk of the flask before overflowing to waste, thus ensuring complete neutralization.

**Figure 2 — On-line apparatus for destruction of cyanogen chloride**