
**Tobacco and tobacco products —
Determination of tobacco-specific
nitrosamines in tobacco products —
Method using LC-MS/MS**

*Tabac et produits du tabac — Dosage des nitrosamines spécifiques du
tabac dans les produits du tabac — Méthode par CL-SM/SM*



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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

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

This second edition cancels and replaces the first edition (ISO 21766:2018), of which it constitutes a minor revision.

The main changes compared to the previous edition are as follows:

- the title and CAS number for NNK-d4 (see [5.12](#)) have been updated;
- the nomenclature of the deuterated nitrosamines in [5.6](#) to [5.13](#) have been harmonized.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

The Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) Smokeless Tobacco Sub-Group studied various widely-used procedures for the determination of tobacco specific nitrosamines (TSNAs) in smokeless tobacco products. A study was conducted in 2009 that evaluated several different methodologies. This study included both liquid chromatography tandem mass spectrometry methods (LC-MS/MS) and gas chromatography combined with nitrogen chemiluminescence detection methods. The results generated with a supplied LC-MS/MS method proved to be the most consistent and was used as the basis for CORESTA Recommended Method N° 72^[2]. Nine laboratories provided data using this method. This study included nine commercial smokeless tobacco products covering eight different product styles. CORESTA Recommended Method N° 72 was updated in 2016 to include repeatability and reproducibility for the four CORESTA reference products.

CORESTA Recommended Method N° 72 was used as the basis for this document. However, the scope of this document was broadened to include ground tobacco, cigarette fillers and cigar fillers in addition to smokeless tobacco products. The respective values for repeatability (r) and reproducibility (R) for ground tobacco, cigarette fillers and cigar fillers have been determined through an international collaborative study that was conducted in 2017 and involved 18 laboratories.

Tobacco and tobacco products — Determination of tobacco-specific nitrosamines in tobacco products — Method using LC-MS/MS

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1 Scope

This document specifies a method for the quantification of four tobacco specific nitrosamines (TSNAs) in tobacco and the following tobacco products: cigarettes, cigars and smokeless tobacco products using reversed phase high performance liquid chromatography with tandem mass spectrometry (LC-MS/MS). The TSNAs determined with this method are: N-nitrosonornicotine (NNN), N-nitrosoanatabine (NAT), N-nitrosoanabasine (NAB) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK).

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

3.1

tobacco specific nitrosamines

TSNAs

four nitrosamines found predominantly in tobacco: N-nitrosonornicotine (NNN), N-nitrosoanatabine (NAT), N-nitrosoanabasine (NAB) and 4-(N-Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)

[SOURCE: ISO 22303:2008, 3.1]

4 Principle

Deuterium-labelled (d4) internal standards are added to the tobacco sample and subsequently extracted with an aqueous buffer. The sample extracts are filtered and then analysed by reversed phase high performance liquid chromatography (HPLC) and quantified by tandem mass spectrometry (MS/MS). The amounts of TSNAs in the tobacco products are reported as ng/g, as is wet mass.

5 Reagents

Use only reagents of recognized analytical grade during the analysis. Solvents shall be of HPLC-grade or better.

5.1 Water, deionized, resistivity $\geq 18,2 \text{ M}\Omega\cdot\text{cm}$ at 25 °C.