
**Cigarettes — Determination of
tobacco specific nitrosamines in
mainstream cigarette smoke —
Method using LC-MS/MS**

*Cigarettes — Dosage des nitrosamines spécifiques du tabac dans le
courant principal de fumée de cigarette — 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 on 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 the following URL: www.iso.org/iso/foreword.html.

The committee responsible for this document is ISO/TC 126, *Tobacco and tobacco products*.

Introduction

Between 1999 and 2005, the CORESTA (www.coresta.org) Special Analytes Task Force studied the existing methodologies for the determination of Tobacco Specific Nitrosamines (TSNAs) in the mainstream smoke of cigarettes. Two main types of analytical methodologies had been proposed for this determination: GC-TEA (gas chromatography with a thermal energy analyser) and LC-MS/MS (liquid chromatography- tandem mass spectrometry). The Task Force decided in the first instance to develop a method using GC-TEA, because this methodology was the most widely used in laboratories at that time.

However by 2009, it was ascertained that most laboratories applied an LC-MS/MS technique to measure yields of TSNAs. The Sub-Group (changed from Task Force) then investigated an LC-MS/MS method to complement the GC-TEA technique already available as CORESTA Recommended Method N° 63. Several such methods have been described in the literature and are referenced herein. A joint experiment was carried out in which 14 laboratories participated, using their in-house LC-MS/MS methodologies. The reproducibility data was better for LC-MS/MS than for GC-TEA and methodology was very similar across laboratories. In summary, cigarette mainstream smoke was collected on a Cambridge filter (CF) pad, an internal standard solution was added and, after extraction, an aliquot was separated and quantitatively analysed by LC-MS/MS. A general methodology was agreed, incorporating key learnings from the joint experiment.

This document was produced through a final collaborative experiment involving 20 laboratories from 12 countries. The method includes some notes to inform other laboratories that might wish to adopt it about some of the main features that need to be well controlled to provide data as robust and consistent as the repeatability and reproducibility data provided. Cigarettes were smoked using the smoking regime parameters given in ISO 3308 and statistical evaluations were made according to ISO 5725 recommendations.

No machine smoking regime can represent all human smoking behaviour:

- it is recommended that cigarettes also be tested under conditions of a different intensity of machine smoking than those specified in this document;
- machine smoking testing is useful to characterize cigarette emissions for design and regulatory purposes, but communication of machine measurements to smokers can result in misunderstandings about differences in exposure and risk across brands;
- smoke emission data from machine measurements may be used as inputs for product hazard assessment, but they are not intended to be nor are they valid as measures of human exposure or risks. Communicating differences between products in machine measurements as differences in exposure or risk is a misuse of testing using ISO standards.

Cigarettes — Determination of tobacco specific nitrosamines in mainstream cigarette smoke — Method using LC-MS/MS

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

1 Scope

This document specifies a method for the quantification of four tobacco specific nitrosamines (TSNAs) in the total particulate matter of mainstream cigarette smoke by using reversed phase high performance liquid chromatography with tandem mass spectrometry (LC-MS/MS). The quantified TSNAs are: N-nitrosonornicotine (NNN), N-nitrosoanatabine (NAT), N-nitrosoanabasine (NAB) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK).

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 3308, *Routine analytical cigarette-smoking machine — Definitions and standard conditions*

ISO 3402, *Tobacco and tobacco products — Atmosphere for conditioning and testing*

ISO 4387, *Cigarettes — Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine*

ISO 8243, *Cigarettes — Sampling*

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:

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

3.1

tobacco specific nitrosamines

TSNAs

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

[SOURCE: ISO 22303:2008, 3.1]