
Cigarettes — Determination of tobacco specific nitrosamines in mainstream cigarette smoke with an intense smoking regime — Method using LC-MS/MS

Cigarettes — Dosage des nitrosamines spécifiques du tabac dans le courant principal de la fumée de cigarette avec un régime de fumage intense — Méthode par CL-SM/SM



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Foreword

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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*.

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

In 2009 the CORESTA (www.coresta.org) Special Analytes Sub-Group focused on the development of a method for the determination of Tobacco Specific Nitrosamines (TSNAs) in mainstream cigarette smoke. The Sub-Group investigated a liquid chromatography- tandem mass spectrometry (LC-MS/MS) method to complement the gas chromatography with a thermal energy analyser (GC-TEA) technique already available as CORESTA Recommended Method N° 63. Several LC-MS/MS methods have been described in the literature and are referenced herein^{[3][4]}. 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, mainstream cigarette smoke was collected on a glass fibre filter 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 CORESTA collaborative experiment, conducted in 2011, involving 20 laboratories from 12 countries^{[5][6]}. Cigarettes were smoked with the intense smoking regime specified in Health Canada Official Method T-115 (equivalent to ISO 20778) and statistical evaluations were made according to the recommendations provided in ISO 5725^[1].

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 International Standards.

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1 Scope

This document specifies a method for the quantification of four tobacco specific nitrosamines (TSNAs) in the total particulate matter of cigarette mainstream smoke with the intense smoking regime specified in ISO 20778 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 3402, *Tobacco and tobacco products — Atmosphere for conditioning and testing*

ISO 8243, *Cigarettes — Sampling*

ISO 20778, *Cigarettes — Routine analytical cigarette smoking machine — Definitions and standard conditions with an intense smoking regime*

ISO 20779, *Cigarettes — Generation and collection of total particulate matter using a routine analytical smoking machine with an intense smoking regime*

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-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)

[SOURCE: ISO 22303:2008, 3.1]