
**Cigarettes — Determination of
selected volatile organic compounds
in the mainstream smoke of cigarettes
— Method using GC/MS**

*Cigarettes — Dosage de composés organiques volatils sélectionnés dans
le courant principal de la fumée de cigarette — Méthode par CG-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).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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For an explanation on 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 the following URL: www.iso.org/iso/foreword.html. (standards.iteh.ai)

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

The CORESTA (www.coresta.org) Special Analytes Sub-Group (since 2017 the Sub-Group changed its name to Smoke Analytes) carried out a collaborative study in 2005 to compare smoke analyte yield data obtained from different laboratories using their own preferred methodologies. This study have shown significant and unacceptable differences in volatiles yields, especially for 1,3-butadiene and acrylonitrile and suggested that further work was required to understand factors influencing the yield variability. Key parameters of existing methodologies have been reviewed and further studies have been carried out on selected volatiles between 2008[1] and 2009[2]. These studies investigated critical method steps that required optimization before incorporation into a CORESTA Recommended Method (CRM).

These studies have shown that similar yields were obtained when comparing data from Tedlar bag trapping with those from cooled impinger traps, the latter method being used by the majority of laboratories. It has been decided that the CRM would be based on collecting the selected volatiles from mainstream cigarette smoke in cryogenically cooled impinger traps containing methanol. The impinger solutions were fortified with benzene-D₆ and analysed by gas chromatography/mass spectrometry (GC-MS).

This document was produced after a 2009 collaborative study involving 20 laboratories from 12 countries using the ISO 3308 smoking regime[2]. Further data were provided for the same selected volatile substances from 10 samples with different tar yields from a 2012 collaborative study, which involved 16 laboratories from 11 countries[3]. The method includes recommendations about critical steps that should be controlled to provide data as robust and consistent as the repeatability and reproducibility data provided in the ISO standard. Statistical evaluations carried out according to ISO 5725-1 and ISO 5725-2 are included.

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.

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Cigarettes — Determination of selected volatile organic compounds in the mainstream smoke of cigarettes — Method using GC/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 any other restrictions prior to use.

1 Scope

This document specifies a method for the quantification of selected volatile organic compounds (VOCs: 1,3-butadiene, isoprene, acrylonitrile, benzene and toluene) by GC-MS in mainstream cigarette smoke using ISO 3308 smoking parameters.

This method is applicable to cigarettes with nicotine-free dry particulate matter (NFDPM) yields between 1 mg/cigarette and 15 mg/cigarette.

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 8243, *Cigarettes — Sampling*

3 Terms and definitions

No terms and definitions are listed in this document.

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/>

4 Principle

Selected volatiles are collected by passing the mainstream smoke of cigarettes through a glass fibre filter pad as specified in ISO 3308 (e.g. Cambridge filter pad, CFP) into cryogenic traps containing methanol.

The impinger solutions are fortified with internal standard and analysed by GC-MS.

5 Apparatus

The usual laboratory apparatus for use in preparation of samples, solutions and standards and, in particular, the following:

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5.1 Smoking machine, complying with ISO 3308.

5.2 Impinger trapping system, capable of being connected in series, a cryogenically cooled liquid impinger to efficiently trap volatile organic compounds present in the vapour phase of mainstream smoke.

5.3 GC-MS system, to obtain chromatographic data to quantify specific ion (Selected Ion Monitoring mode, SIM or equivalent).

The GC shall be configured to perform split injections on a capillary column.

It is recommended to equip the GC with an auto sampler for sample injection.

5.4 Gas tight syringes, of appropriate volumes.

5.5 Fused silica capillary column, for example DB-624, length 60 m with internal diameter of 0,25 mm and 1,4 µm film thickness, or equivalent.

5.6 Spectrophotometer, to estimate 1,3-butadiene concentration in secondary stock solution.

6 Reagents

6.1 Dry ice.

6.2 Isopropanol, for Dewar flasks.

6.3 Methanol, HPLC grade or better.

The methanol should be checked to ensure the background levels of the analytes will not negatively affect the analysis.

6.4 Ethanol, reagent grade or equivalent.

6.5 Benzene-D₆, min. 99 % D; checked for the absence of not-labelled analogue.

6.6 Toluene-D₈, min. 99 % D; checked for the absence of not-labelled analogue.

6.7 1,3-Butadiene, min. 99 %.

6.8 Isoprene, min. 99 %.

6.9 Acrylonitrile, min. 99 %.

6.10 Benzene, min. 99 %.

6.11 Toluene, min. 99 %.

7 Preparation

7.1 Preparation of glassware

Glassware shall be cleaned and dried in such a manner which ensures that contamination from glassware does not occur.

It is important that all possible sources of contamination are removed from the work area.

7.2 Preparation of standards

7.2.1 General

Where available, certified reference solutions of the required standards and internal standards can be used.

7.2.2 Preparation of internal standard spiking solution

7.2.2.1 Internal standard stock solution

Transfer the contents of a 1 g ampoule of benzene-D₆ into a 10 ml amber volumetric flask. Dilute to volume with methanol.

The inclusion of other internal standards, such as toluene-D₈, may also be suitable. Laboratories shall demonstrate the suitability of the inclusion of additional internal standards.

7.2.2.2 Internal standard spiking solution

Using a volumetric pipette, transfer 4 ml of the stock solution ([7.2.2.1](#)) into a 100 ml volumetric flask and dilute to volume with methanol. This solution has a concentration of 4 000 µg/ml.

7.2.2.3 Storage

Store the diluted solutions in 25 ml vials with PTFE-lined caps in freezer.

7.2.3 Preparation of working standards for isoprene, acrylonitrile, benzene, and toluene

7.2.3.1 Primary isoprene, acrylonitrile, benzene and toluene stock solutions

Using gas tight syringes, weigh accurately 100 mg of isoprene, acrylonitrile, benzene, and toluene into separate 10 ml amber volumetric flasks that are half filled with methanol. Dilute each compound to volume with methanol. Each solution has a nominal concentration of 10 mg/ml.

NOTE Approximate volumes corresponding to 100 mg are: isoprene = 150 µl, acrylonitrile = 140 µl, benzene = 130 µl, toluene = 120 µl.

7.2.3.2 Secondary stock solution (mixture of isoprene, acrylonitrile, benzene and toluene primary stock solutions)

A combined secondary stock solution is prepared by transferring appropriate amounts ([Table 1](#)) of isoprene, acrylonitrile, benzene, and toluene primary stock solutions ([7.2.3.1](#)) into a 50 ml volumetric flask that is a third full with methanol. Dilute to volume with methanol.

Table 1 — Preparation of secondary stock solution

Analyte	Volume of primary stock (ml)	Concentration (µg/ml)
Isoprene	3,0	600
Acrylonitrile	1,0	200
Benzene	1,0	200
Toluene	1,0	200

7.2.3.3 Calibration standard solutions (for isoprene, acrylonitrile, benzene and toluene)

Prepare seven working standard solutions by mixing appropriate volumes of secondary stock solution (7.2.3.2) and internal standard spiking solution (7.2.2.2) to cover the concentration range of interest, i.e. (12 – 600) µg/ml (isoprene); (4 – 200) µg/ml (acrylonitrile); (4 – 200) µg/ml (benzene); (4 – 200) µg/ml (toluene) and 40 µg/ml of internal standard (e.g. benzene-D₆).

The concentration change of the highest calibration standard solution after adding internal standard solution should not be significant.

Transfer aliquots of each calibration standard solution into amber GC vials and fill each vial up to the shoulder of the vial to minimize headspace.

Adjust standard concentrations accordingly to reflect levels of volatiles found in smoke samples.

PTFE lined GC vial caps are recommended, although other materials may also be suitable.

7.2.3.4 Storage

Store all calibration standard solutions in freezer.

7.2.4 Preparation of working standards for 1,3-butadiene

7.2.4.1 Primary 1,3-butadiene stock solution

Attach a piece of chemically resistant polymer tubing to the valve of a cylinder containing 1,3-butadiene. Place a Pasteur pipette on the other end of the tubing and immerse the tip of the pipette into a 100 ml amber glass volumetric flask containing methanol up to the base of the neck of the flask. Open the valve and gently bubble the 1,3-butadiene into the methanol for approximately 5 min. Dilute to volume using methanol and mix well.

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7.2.4.2 Secondary 1,3-butadiene stock solution

Pipette 1 ml of the primary 1,3-butadiene stock solution (7.2.4.1) into a 100 ml volumetric flask and dilute to volume with methanol. Mix well.

7.2.4.3 Determination of secondary 1,3-butadiene stock concentration

Pipette 1 ml of the secondary 1,3-butadiene stock solution (7.2.4.2) into a 100 ml volumetric flask and dilute to volume using ethanol. This solution is used only to check the concentration of the secondary stock solution and shall not be used to prepare the working standards.

Measure the absorbance of the solution against an ethanol blank on a spectrophotometer (use 1 cm long cuvettes). Conduct a wave scan from 200 nm to 250 nm to determine the wavelength of maximum absorbance. 1,3-butadiene in hexane absorbs at 217 nm whereas 1,3-butadiene in ethanol can have a peak shift. Measure the absorbance at the peak maximum.

Repeat the above measurement three more times and calculate the mean absorbance, *A* (at least three significant figures). The absorbance should be between 0,2 and 0,6 extinction units. If it is higher, make a new secondary stock solution using a smaller volume of the primary stock solution and repeat the spectrophotometer measurements to determine the concentration of the secondary stock. If the absorbance is lower, make a new secondary stock solution using a larger volume of the primary stock solution and repeat the spectrometer measurements to determine the concentration of the secondary stock.

The concentration of the secondary stock solution, in micrograms per millilitre, c_{2S} , is calculated by [Formula \(1\)](#):

$$c_{2S} = \frac{A}{20\,893} \times 54 \times 100 \times 1\,000 \quad (1)$$

where

A	is the mean absorbance;
$20\,893 \text{ l mol}^{-1} \text{ cm}^{-1}$	is the molar absorption coefficient of 1,3-butadiene;
54 g/mol	is the molar mass of 1,3-butadiene;
100 ml	is the volume of the solution;
$1\,000$	is the units conversion factor.

7.2.4.4 1,3-butadiene calibration standard solutions

Prepare five working standard solutions by mixing 1,3-butadiene secondary stock solution ([7.2.4.2](#)) and internal standard spiking solution ([7.2.2.2](#)) that cover the concentration range of interest, i.e. (5 to 50) $\mu\text{g/ml}$ for 1,3-butadiene and 40 $\mu\text{g/ml}$ for internal standard.

Transfer aliquots of each calibration standard solution into amber GC vials and fill each vial up to the shoulder of the vial to minimize headspace.

NOTE Certified concentrations of 1,3-butadiene in methanol can be purchased and used to prepare the standards.

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7.2.4.5 Storage

Store all calibration standard solutions in freezer until use.

Stability of all standard solutions for all components should be assessed by laboratories under their own in-house conditions before use as stability is dependent on the actual storage conditions of each laboratory.

8 Sampling

Carry out sampling in accordance with ISO 8243.

9 Tobacco product preparation

Condition the cigarettes in accordance with ISO 3402.

10 Sample generation — Smoking of cigarettes

10.1 General

The smoking parameters for which the method has been studied are defined in ISO 3308 (see [Table 2](#)).