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

bsi.

National foreword

This British Standard is the UK implementation of ISO 19290:2016.

The UK participation in its preparation was entrusted to Technical Committee AW/40, Tobacco and tobacco products.

A list of organizations represented on this committee can be obtained on request to its secretary.

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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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Contents

Page

Foreword

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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 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]

4 Principle

Cigarettes are smoked on a standard smoking machine. The mainstream smoke is trapped on a glass-fibre filter pad. After addition of an internal standard, the total particulate matter collected on the glass-fibre filter pad is extracted with 100 mM ammonium acetate solution using a shaker.

The extract is syringe filtered through a 0,45 µm PTFE column directly into an auto sampler vial.

The samples are subjected to reversed phase high performance liquid chromatography (HPLC) and quantified via tandem mass spectrometry (MS/MS).

5 Apparatus

Usual laboratory apparatus and equipment for use in preparation of samples and standards and in particular the following items. All glassware shall be cleaned before use to avoid any contamination.

5.1 Equipment needed to perform conditioning of cigarettes, in accordance with ISO 3402.

5.2 Equipment needed to perform marking for butt length of cigarettes.

5.3 Equipment needed to perform smoking of cigarettes, complying with ISO 3308.

5.4 Analytical balance, capable of measuring to at least four decimal places.

5.5 Centrifuge tubes, 50 ml.

5.6 Dispenser, of capacity 20 ml for extracting solutions.

5.7 Gas-tight syringes, of capacity 250 µl.

5.8 Automated volumetric pipette.

5.9 Shaker.

5.10 High performance liquid chromatograph coupled to tandem mass spectrometer (LC-MS/MS), consisting of:

5.10.1 Binary pump.

5.10.2 Autosampler.

5.10.3 Tandem mass spectrometer.

5.10.4 Data collection system.

5.10.5 LC column: XTerra MS C18®[1)], 2,5 µm, 2,1 mm × 50 mm or equivalent.

6 Reagents

Use only reagents of recognized analytical reagent grade.

1) XTerra MS C18 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

6.1 N-Nitrosonornicotine, (NNN) CAS-No: 80508-23-2, $w \geq 98$ % (mass fraction).

6.2 N-Nitrosoanatabine, (NAT) CAS-No: 71267-22-6, $w \geq 98$ %.

6.3 N-Nitrosoanabasine, (NAB) CAS-No: 1133-64-8, $w \geq 96$ %.

6.4 4-(N-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone, (NNK) CAS-No: 64091-91-4, $w \geq 97$ %.

6.5 N-Nitrosonornicotine-2,4,5,6-d4, (NNN-d4) CAS-No: 66148-19-4, $w \geq 98$ %.

6.6 N-Nitrosoanatabine-2,4,5,6-d4, (NAT-d4) CAS-No: 1426174-82-4, $w \geq 98$ %.

6.7 N-Nitrosoanabasine-2,4,5,6-d4, (NAB-d4) CAS-No: 1020719-68-9, $w \geq 98$ %.

6.8 4-(N-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone-2,4,5,6-d4,(NNK-d4), CAS-No: 76661-24-7, $w \geq 98$ %.

6.9 Ammonium acetate, $w \geq 97$ %.

6.10 Acetonitrile, HPLC grade.

6.11 Methanol, HPLC grade.

6.12 Acetic acid, $w \geq 99{,}77$ %.

6.13 De-ionized water, >18,8 MΩ.

6.14 Syringe filter, 0,45 µm polytetrafluoroethylene (PTFE) or equivalent.

6.15 Disposable syringes, 5 ml.

6.16 Autosampler vials (amber), caps and PTFE faced septa.

7 Preparation

7.1 Preparation of glassware

Glassware shall be cleaned and dried in such a manner to ensure that contamination does not occur.

It is important that all possible sources of contamination which could interfere with the analytical process are removed from the work area.

7.2 Preparation of solutions

7.2.1 Extraction solution, 100 mM ammonium acetate solution.

Weigh 15,4 g ± 0,05 g of ammonium acetate. Put into a 2 000 ml volumetric flask and dilute to the mark with de-ionized water.

7.2.2 HPLC mobile phase A, 0,1 % acetic acid solution in water.

Add 1 ml of acetic acid into a 1 000 ml volumetric flask and dilute to the mark with de-ionized water.

7.2.3 HPLC Mobile Phase B, 0,1 % acetic acid solution in methanol.

Add 1 ml of acetic acid into a 1 000 ml volumetric flask and dilute to the mark with methanol.

NOTE Extraction solution and mobile phases are stable for up to three months at room temperature.

7.3 Preparation of standards

7.3.1 General

For the preparation of standard solutions volumetric pipettes should be used.

7.3.2 Preparation of internal standard solutions

7.3.2.1 Primary solution

Weigh, to the nearest 0,1 mg, approximately 10 mg each of NNN-d4, NAT-d4, NAB-d4 and NNK-d4.

Put into individual 10 ml volumetric flasks and dilute each flask to the mark with acetonitrile and mix well.

The concentration in each solution is approximately 1 000 µg/ml.

7.3.2.2 Combined secondary solution

Transfer 5 ml of each primary solution of NNN-d4, NAT-d4 and NNK-d4 and 1 ml of NAB-d4 into a 100 ml volumetric flask. Dilute to the mark with acetonitrile and mix well.

The concentration in this solution is approximately 50 µg/ml of NNN-d4, NAT-d4 and NNK-d4 and 10 µg/ml of NAB-d4.

7.3.2.3 Working solution

Transfer 50 ml of the combined secondary solution into a 500 ml volumetric flask. Dilute to the mark with acetonitrile and mix well.

The concentration in this solution is approximately 5 µg/ml of NNN-d4, NAT-d4 and NNK-d4 and 1 µg/ml of NAB-d4.

7.3.3 Preparation of calibration standard solutions

7.3.3.1 Primary single TSNA solutions

Weigh, to the nearest 0,1 mg, approximately 10 mg each of NNN, NAT, NAB and NNK.

Put into individual 10 ml volumetric flasks and dilute each flask to the mark with acetonitrile and mix well.

The concentration in each solution is approximately 1 000 µg/ml.

7.3.3.2 Mixed TSNAs stock solution (I)

Transfer 4 ml of the primary single TSNA solutions of NNN, NAT and NNK and 1 ml of the primary single TSNA solution of NAB into a 100 ml volumetric flask. Dilute to the mark with acetonitrile and mix well.

The concentration in this solution is approximately 40 µg/ml of NNN, NAT and NNK and 10 µg/ml of NAB.

7.3.3.3 Mixed TSNAs stock solution (II)

Transfer 2 ml of the mixed TSNAs stock solution (I) into a 200 ml volumetric flask. Dilute to the mark with acetonitrile and de-ionized water mixed solution (30:70 volume fraction) and mix well.

The concentration in this solution is approximately 400 ng/ml of NNN, NAT and NNK and 100 ng/ml of NAB.

7.3.3.4 Working standard solutions

Prepare 7 working standard solutions that cover the concentration range of interest.

Add selected volumes of solutions listed in Table 1 in a 100 ml volumetric flask and dilute to the mark with de-ionized water.

These solutions have concentrations of approximately 50 ng/ml of NNN-d4, NAT-d4 and NNK-d4, 10 ng/ml of NAB-d4, from 0 ng/ml to 80 ng/ml of NNN, NAT and NNK and from 0 ng/ml to 20 ng/ml of NAB (see Table 2).

Each laboratory should establish the most suitable calibration range depending on the equipment used and the type of samples to be analysed. The standard preparation procedure is given as an example and is applicable for the range of the products in a collaborative study.

Table 1 — Preparation of working standard solutions for calibration

Solutions	**S0** ml	**S1** ml	**S2** ml	**S3** ml	**S4** ml	**S5** ml	**S6** ml
Internal standard solution	1	1	1	1	1	1	1
Mixed TSNAs stock solution (II)	0	0,5	1	2	5	10	20
Ammonium acetate (100 mM)	10	10	10	10	10	10	10
Acetonitrile	10	10	10	10	8	7	4
Final volume	100	100	100	100	100	100	100

Table 2 — Concentration of each calibration standard

Concentrations	**S0** ng/ml	**S1** ng/ml	**S2** ng/ml	**S3** ng/ml	**S4** ng/ml	**S5** ng/ml	**S6** ng/ml
NNN	0	2	4	8	20	40	80
NAT	0	2	4	8	20	40	80
NAB	0	0,5	1	2	5	10	20
NNK	0	2	4	4	20	40	80
NNN-d4	50	50	50	50	50	50	50
NAT-d4	50	50	50	50	50	50	50
NAB-d4	10	10	10	10	10	10	10
NNK-d4	50	50	50	50	50	50	50

7.3.3.5 Storage

The above standard solutions are stable for up to six months if refrigerated below 5 °C.

8 Sampling

Carry out sampling in accordance with ISO 8243.

9 Tobacco product preparation

Conditioning of the cigarettes is done in accordance with ISO 3402.

10 Sample generation — Smoking of cigarettes

10.1 General

Cigarettes are smoked in accordance with ISO 4387.

10.2 Linear smoking

Typically 5 cigarettes are smoked per filter pad constituting one replicate.

10.3 Rotary smoking

Typically 5 or 10 cigarettes are smoked per filter pad constituting one replicate.

11 Sample analysis

11.1 Preparation of sample

11.1.1 General

Remove the filter pad and place it into an Erlenmeyer flask. Wipe the inside of the holder with two quarter sections of a pad, and add the quarter pads to the flask.

11.1.2 Extraction for linear smoking (44 mm pad)

After adding 200 µl of internal standard solution to the pad, add 20 ml of 100 mM ammonium acetate solution to each Erlenmeyer flask containing a pad from the analytical run and cap.

11.1.3 Extraction for rotary smoking (92 mm pad)

After adding 400 µl of internal standard solution to the pad, add 40 ml of 100 mM ammonium acetate solution to each Erlenmeyer flask containing a pad from the analytical run and cap.

The extraction volume can be adjusted in each laboratory.

NOTE It is acceptable to extract the filter pads with 100 mM ammonium acetate solutions containing the internal standards instead of spiking internal standard solution directly to the filter pads.

11.1.4 Final sample preparation

Perform extractions by using a shaker and agitate for 60 min at 210 r/min.

Filter the pad extract directly into vials through a syringe filter (0,45 µm PTFE).

NOTE 1 The above sample extracts are stable for up to six days if refrigerated below 5 °C.

NOTE 2 Depending on manufacturer and model of MS-MS a dilution of the sample extract and the concentration of the calibration could be required to ensure operating the instrument within the manufacturer advised ranges (Detector saturation area).

11.2 Reversed phase high performance liquid chromatography

11.2.1 General

An adjustment to the chromatographic conditions can be required depending on the different instrument configuration and columns chosen for separation.

11.2.2 HPLC set-up parameters (Example)

— Column temperature: 65 °C

— Autosampler tray temperature: 5 °C

— Injection volume: 5 µl

— Flow rate: 250 µl/min

11.2.3 Mobile phase (Example)

— A: 0,1 % acetic acid in water

— B: 0,1 % acetic acid in methanol

11.2.4 Mobile phase: Gradient (Example)

See Table 3 for an example of a gradient programme.

Table 3 — Gradient programme

Time min	Mobile phase A %	Mobile phase B %
0	98	2
4	2	98
7	2	98
8	98	2
20	98	2

11.2.5 MS/MS Set-up parameters (Example)

The following conditions are suitable for the analysis.

The instrument is operated in electrospray ionization (ESI), positive mode.

— Gas 1 (Nebulizer gas): N_2, 50 psi

— Gas 2 (Drying/Evaporation gas): N_2, 60 psi

— Temperature heater gas 2: 700 °C

— Interface temperature: on

— Curtain gas (CUR): N_2, 40 psi

— Collision activated dissociation (CAD): N_2, 3 psi (medium)

— Ion spray voltage (IS): 4 500 V

Other suitable instruments may be used as well. Based on the instrument make and model, the optimal MS/MS parameters can be different and may be used too.

Inject 5 µl of each sample onto the HPLC column and analyse as per the chromatographic conditions listed above.

The retention time in the chromatogram can be different depending on the choice of column.

NOTE 1 Depending on the chromatographic system used, peak splitting and peak fronting can be observed in particular for the early eluting compounds (e.g. NNN, NNN-d4).

NOTE 2 Some laboratories reported that NNN-d4 peak could not be found. A reduction of the acetic acid in the mobile phases did not improve the sensitivity. Another mobile phase (A: 2 mM ammonium acetate / B: Methanol and 0,01 % formic acid) improved sensitivity.

The mass spectrometric parameters are given in Table 4.

Table 4 — Mass spectrometric parameters

Compounds	Precursor ion	Quantifier	Qualifier	DP[a]	CE[b]	CXP[c]	Dwell time
	m/z	m/z	m/z	V	V	V	ms
NNN	178	148	120	41	15	10	150
NAT	190	160	106	41	15	10	150
NAB	192	162	133	36	17	10	150
NNK	208	122	79	41	17	8	150
NNN-d4	182	152	124	41	15	8	150
NAT-d4	194	164	110	41	15	10	150
NAB-d4	196	166	137	36	17	10	150
NNK-d4	212	126	83	41	17	8	150

[a] DP: Declustering potential.

[b] CE: Collision energy.

[c] CXP : Collision cell exit potential.

11.3 Calculation

11.3.1 Calibration curve

A calibration curve is generated by calculating a linear regression of the area ratios of each TSNA to corresponding internal standard peak as a function of the concentration ratios of each TSNA to corresponding internal standard.

When laboratories either have problems obtaining 4 internal standards or have checked/validated that using 2 internal standards gives comparable data then the method may be run using NNN-d4 as substitute for the deuterated NAT/NAB standards. There is low NAT in some blends and it is recommended using 4 internal standards for cigarettes containing such blends.

11.3.2 Determination of the TSNAs concentrations

Inject the sample, calculate the area ratio of each TSNA to corresponding internal standard peak and obtain the concentration ratio by comparing the area ratio with the calibration curve.

11.3.3 Sample quantification

The amount of the various TSNA compounds in smoke samples is quantified by the internal standard method. Examples of chromatograms are shown in Annex A, Figures A.1 and A.2.

TSNA concentrations are reported in ng/ml by the chromatography software.

11.3.4 Determination of mainstream smoke TSNA deliveries

The mainstream smoke TSNA deliveries, M, expressed in nanograms per cigarette, are given by Formula (1):

$$M = \frac{C \times W_s}{N} \quad (1)$$

where

C is the ratio by mass obtained from the calibration curve;

W_S is the amount, in nanograms, of the internal standard added to the sample;

N is the number of cigarettes smoked.

The expression of the laboratory data depends on the purpose for which the data are required, and the level of laboratory precision. Confidence limits should be calculated and expressed on the basis of the laboratory data before any rounding has taken place.

TSNA yields in the mainstream smoke of cigarette, in nanograms per cigarette, should be rounded to the nearest 0,1 ng.

12 Repeatability and reproducibility

A major international collaborative study was conducted in 2011 involving 20 laboratories and 10 cigarette samples including the reference cigarettes KR 1R5F and KR 3R4F and the CORESTA Monitor CM6 and covering a wide range of blends and constructions. The values for repeatability limit, r, and reproducibility limit, R, given in Table 5 were obtained for this method.

The difference between two single results found on matched cigarette samples by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability limit, r, on average not more than once in 20 cases in the normal and correct operation of this method.

Single results on matched cigarette samples reported by two laboratories will differ by more than the reproducibility limit, R, on average not more than once in 20 cases in the normal and correct operation of the method.

Data analysis for the 10 cigarette samples gave the estimates as summarised in Table 5.

The statistical evaluation was performed according to ISO 5725-2.

Table 5 — Results overview

NNN (ng/cigarette)						
Sample	**# laboratories**	**Mean**	s_r	s_R	r	R
1	16	277	17	25	47	70
2	18	37,3	3,1	4,4	8,8	12,4
3	17	24,0	2,4	2,8	6,7	7,8
4	18	9,6	1,5	2,4	4,2	5,8
5	18	12,1	1,3	2,0	3,6	5,6
6	16	22,7	3,8	4,2	10,8	11,9
7	17	10,5	1,1	1,7	3,0	4,8
CM 6	18	20,0	1,8	2,9	5,1	8,1
KR 1R5F	19	44,4	3,1	5,9	8,6	16,7
KR 3R4F	18	115	6	12	18	34
NAT (ng/cigarette)						
Sample	**# laboratories**	**Mean**	s_r	s_R	r	R
1	16,	145	10	26	27	74
2	16	40,5	2,9	8,0	8,2	22,5
3	16	27,9	2,4	5,7	6,7	16,2
4	15	11,0	1,2	2,2	3,4	6,2
5	16	12,8	1,2	2,5	3,5	7,2
6	16	27,9	3,0	5,7	8,5	16,1
7	16	14,4	1,2	2,4	3,5	6,8
CM 6	17	33,7	3,0	6,6	8,5	18,6
KR 1R5F	17	45,8	3,3	8,3	9,2	23,4
KR 3R4F	16	113	5	20	14	55
NAB (ng/cigarette)						
Sample	**# laboratories**	**Mean**	s_r	s_R	r	R
1	17	20,0	1,8	3,2	5,2	9,0
2	14	5,3	0,5	1,0	1,4	2,7
3	14	3,7	0,5	0,7	1,5	2,1
4	13	1,5	0,2	0,3	0,6	0,9
5	11	1,8	0,2	0,3	0,5	0,9
6	13	3,6	0,5	1,3	1,3	3,5
7	14	1,8	0,2	0,3	0,6	0,9
CM 6	13	3,7	0,3	1,0	0,9	2,7
KR 1R5F	16	6,5	0,5	1,0	1,5	2,9
KR 3R4F	16	13,0	0,8	1,9	2,3	5,2
NNK (ng/cigarette)						
Sample	**# laboratories**	**Mean**	s_r	s_R	r	R
1	18	133	12	14	33	41
2	17	24,5	2,8	3,6	7,8	10,2
3	18	17,9	1,9	3,2	5,3	9,0
4	14	3,6	0,6	1,1	1,7	3,0
5	13	3,3	0,6	0,7	1,8	2,1
6	15	7,2	0,9	2,0	2,7	5,7
7	15	7,4	0,7	1,1	1,9	3,1

Table 5 *(continued)*

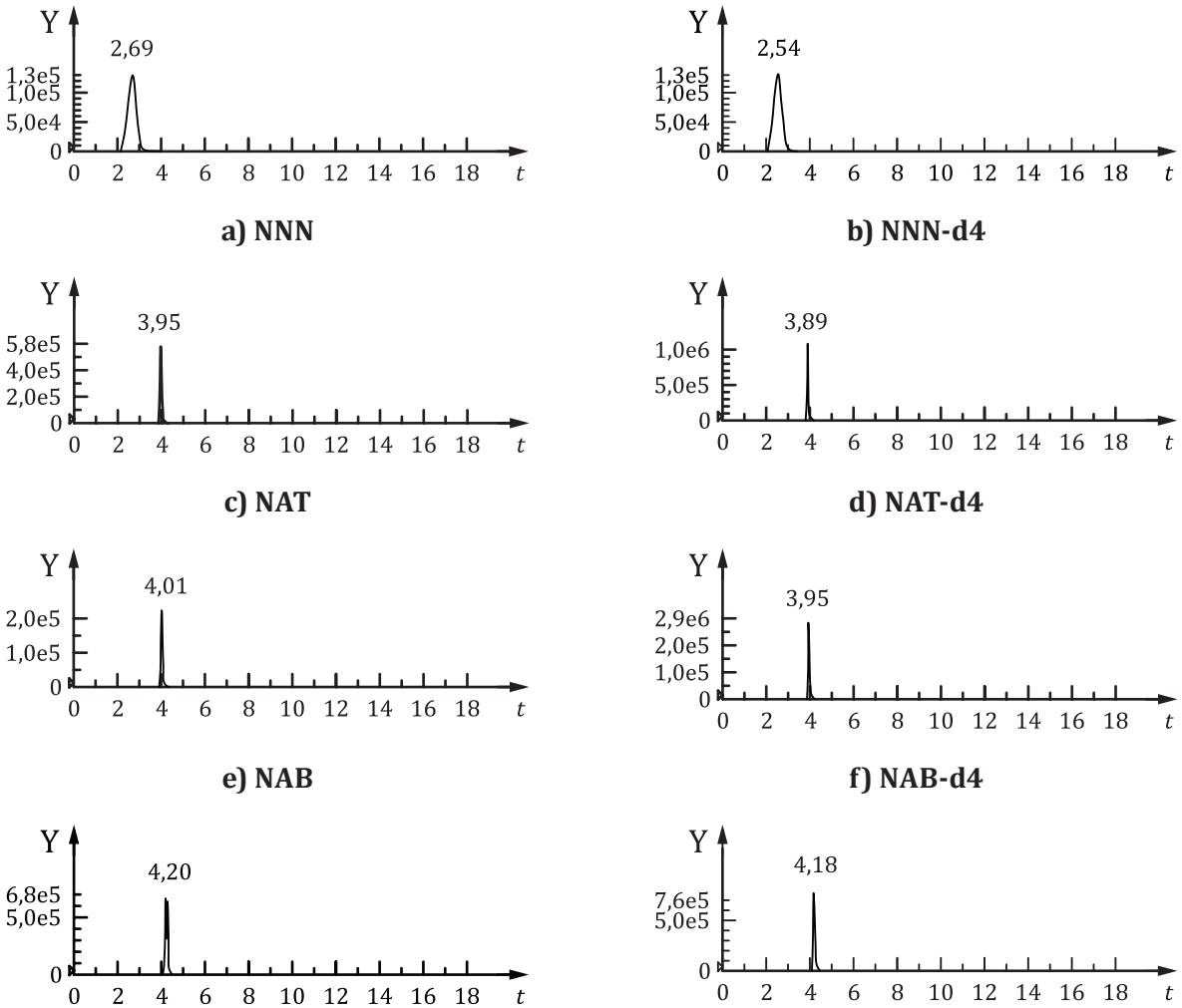
CM 6	17	26,5	2,3	3,0	6,5	8,6
KR 1R5F	19	21,8	1,4	2,8	3,9	8,0
KR 3R4F	19	97,1	5,2	10,8	14,6	30,5

13 Test report

The test report shall state the yield of TSNAs in nanograms per cigarette smoked and the method used and shall include all conditions which can affect the result. It shall also give all details necessary for the identification of the cigarettes smoked.

Annex A
(informative)

Example of typical chromatograms

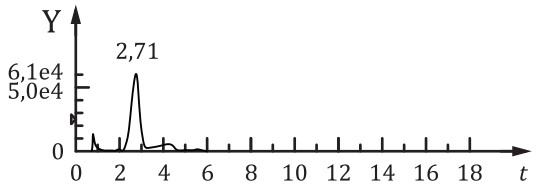


Key

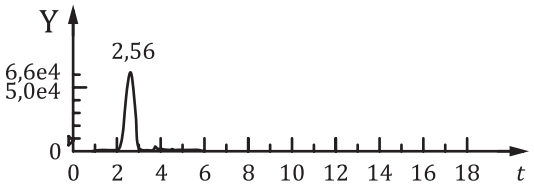
t time, min

Y intensity, cps

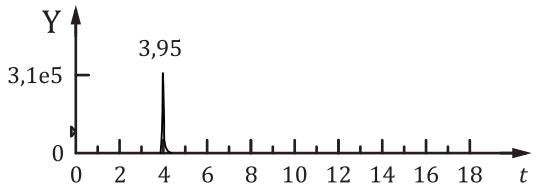
Figure A.1 — Chromatograms of a typical TSNAs calibration standard



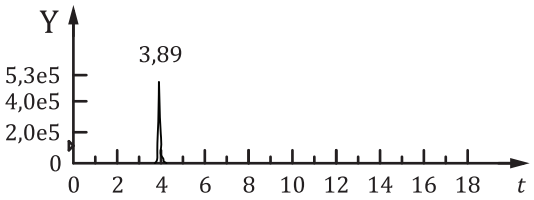
a) NNN



b) NNN-d4



c) NAT



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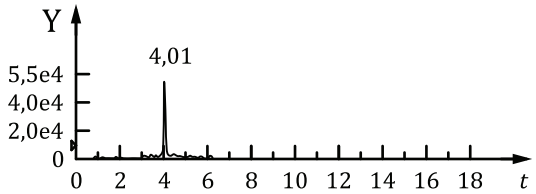

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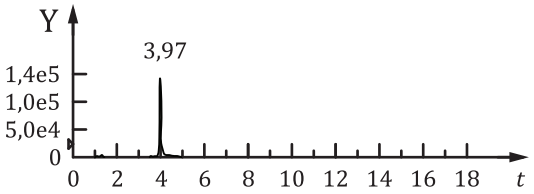

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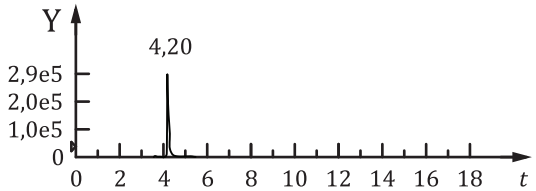

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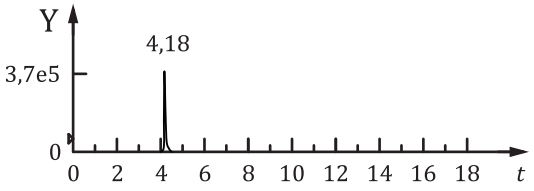

h) NNK-d4

Key

t time, min

Y intensity, cps

Figure A.2 — Chromatograms of TSNAs in mainstream cigarette smoke extract (KR 3R4F)

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