CORESTA RECOMMENDED METHOD N° 75

DETERMINATION OF TOBACCO SPECIFIC NITROSAMINES IN MAINSTREAM CIGARETTE SMOKE BY LC-MS/MS

(July 2014)

0. INTRODUCTION

Between 1999 and 2005, the CORESTA Special Analytes Task Force studied the existing methodologies for the determination of Tobacco Specific Nitrosamines (TSNAs) in the mainstream smoke of cigarettes. Two types of analytical methodologies had been mainly 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 general, 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 and a general methodology was agreed, incorporating key learnings from the joint experiment.

This Recommended Method 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. Statistical evaluations were made according to ISO recommendations and are included.

1. FIELD OF APPLICATION

This method is applicable to 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-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK).

The use of these machine smoking parameters reflects their inclusion in the reporting requirements of various national regulations rather than an endorsement of their appropriateness by CORESTA.

2. NORMATIVE REFERENCES

2.1. *ISO 3308:2000/Amd 1:2009*
Routine analytical cigarette-smoking machine – Definitions and standard conditions

2.2. *ISO 8243:2006*
Cigarettes – Sampling

2.3. *ISO 3402:1999*
Tobacco and tobacco products – Atmosphere for conditioning and testing

2.4. *ISO 4387:2000/Amd 1:2008*
Cigarettes – Determination of Total and Nicotine-free Dry Particulate Matter Using a Routine Analytical Smoking Machine

2.5. *Health Canada Official Method T–115: December 1999*
Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke

3. METHOD SUMMARY

3.1. Cigarettes are smoked on a standard smoking machine. Mainstream smoke is trapped on a glass fiber filter pad.

3.2. After addition of an internal standard, the total particulate matter collected on the glass fiber filter pad is extracted into 20 mL of 0.1M ammonium acetate solution using a shaker for 60 minutes.

3.3. 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).

4. APPARATUS AND EQUIPMENT

Usual laboratory apparatus and equipment and in particular the following items:

- Equipment needed to perform conditioning of cigarettes.
- Equipment needed to perform marking for butt length of cigarettes.
- Equipment needed to perform smoking of cigarettes complying with ISO 3308.

The necessary general laboratory equipment for the preparation of samples, standards, and reagents (examples):

- Analytical balance, capable of measuring to at least four decimal places
- Erlenmeyer flask: 100 mL
- Centrifuge tube: 50 mL
- Dispenser (20 mL for extracting solutions)
- Gas-tight syringes: 250 µL
- Volumetric pipettes: 0.5, 1, 2, 4, 5, 10, 20 and 50 mL
- Automated volumetric pipette
- Volumetric flasks: 10, 100, 200, 500 and 2000 mL
- Shaker

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

- Binary pump
- Autosampler
- Tandem mass spectrometer
- Data collection system
- LC Column: XTerra MS C18, 2.5 µm, 2.1 x 50 mm or equivalent

5. REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade in quality.

- N-Nitrosonornicotine (NNN) (min. 98 %)
- N-Nitrosoanatabine (NAT) (min. 98 %)
- N-Nitrosoanabasine (NAB) (min. 96 %)
- 4-(N-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) (min. 97 %)
- N-Nitrosonornicotine-2,4,5,6-d4 (NNN-d4) (min. 98 %)
- N-Nitrosoanatabine-2,4,5,6-d4 (NAT-d4) (min. 98 %)
- N-Nitrosoanabasine-2,4,5,6-d4 (NAB-d4) (min. 98%)
- 4-(N-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone-2,4,5,6-d4 (NNK-d4) (min. 98 %)
- Ammonium acetate (min. 97 %)
- Acetonitrile – HPLC Grade
- Methanol – HPLC Grade
- Acetic Acid (min. 99.7 %)
- De-ionized water > 18.2 MΩ
- Syringe Filter – 0.45 µm PTFE or equivalent
- Disposable syringes – 5 mL
- Autosampler vials (amber), caps and Teflon faced septa

Warning notice: The solvents and chemicals to be used for this method are classified as toxic, highly toxic, harmful, carcinogenic, mutagenic, sensitising, teratogenic, irritant, corrosive, easily flammable and dangerous for the environment. The instructions specified in the individual material safety data sheets concerning safe handling, storage and waste disposal as well as protective equipment must be followed.

6. PREPARATION OF GLASSWARE

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

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

7. PREPARATION OF SOLUTIONS

7.1. Extraction Solution (100 mM ammonium acetate solution)

- Weigh 15.4 g ± 0.05 g of ammonium acetate. Put into a 2000 mL volumetric flask and dilute to the mark with de-ionized water.

7.2. HPLC Mobile Phase A (0.1% acetic acid solution in water)

- Add 1 mL of acetic acid into a 1000 mL volumetric flask and dilute to the mark with de-ionized water.

7.3. HPLC Mobile Phase B (0.1% acetic acid solution in methanol)

- Add 1 mL of acetic acid into a 1000 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.

8. PREPARATION OF STANDARDS

8.1. Preparation of Internal Standard Solutions

8.1.1. Primary Solution

- Weigh 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 1000 µg/mL.

8.1.2. 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.

8.1.3. 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.

8.2. Preparation of Calibration standard solutions

8.2.1. Primary Single TSNA Solutions

- Weigh 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 1000 µg/mL.

8.2.2. 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.

8.2.3. 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.

8.2.4. 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 to 80 ng/mL of NNN, NAT and NNK and from 0 to 20 ng/mL of NAB (Table 2).

Note: 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

	Working standard solutions for calibration						
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**
Solutions	mL	mL	mL	mL	mL	mL	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

	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**
Concentrations	**ng/mL**	**ng/mL**	**ng/mL**	**ng/mL**	**ng/mL**	**ng/mL**	**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	8	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

8.2.5. Storage

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

9. SAMPLING

Sampling is done in accordance with ISO 8243:2006.

10. TOBACCO PRODUCT PREPARATION

Conditioning of the cigarettes is done in accordance with ISO 3402:1999.

11. SAMPLE GENERATION – SMOKING OF CIGARETTES

Cigarettes are smoked according to ISO 4387 and/or under a more intense smoking regime according to Health Canada Method T -115 §16.1 with the following modifications:

11.1. Linear Smoking

- Check the puff volume of each port and adjust accordingly.
- Typically 5 cigarettes are smoked per trap under ISO smoking regime.
- Typically 2 or 3 cigarettes are smoked per trap under intense smoking regime.

11.2. Rotary Smoking

- Check the puff volume of the rotary smoking machine and adjust accordingly.
- Typically 5 or 10 cigarettes are smoked under ISO smoking regime.
- Typically 3 or 5 cigarettes are smoked under intense smoking regime.

Note: The number of cigarettes is reduced under intense smoking condition to avoid total particulate matter (TPM) breakthrough.

12. SAMPLE ANALYSIS

12.1. Sample preparation

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.

12.1.1. 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.

12.1.2. 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.

Note: 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.

12.1.3. Final sample preparation

- Perform extractions by using a shaker and agitate for 60 minutes at 210 rpm.
- Filter the pad extract directly into vials through a syringe filter (0.45 µm PTFE).

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

12.2. Reversed phase high performance liquid chromatography

Note: The choice / adjustment to the chromatographic conditions may be required depending on the different instrument configuration and columns applied for separation.

12.2.1. HPLC Set-up Parameters (Example)

- Column Temperature: 65 °C
- Autosampler Tray Temperature: 5 °C
- Injection Volume: 5 µL
- Flow Rate: 250 µL/min

12.2.2. Mobile phase (Example)

- A: 0.1 % Acetic acid in water
- B: 0.1 % Acetic acid in methanol

12.2.3. Mobile phase: Gradient (Example)

Table 3: Gradient Program

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

12.2.4. MS/MS Set-up Parameters (Example)

Note: The following conditions are suitable for the analysis:

- The instrument is operated in electrospray ionization (ESI) mode.
- Gas 1 (GS1): N2, 50 psi
- Gas 2 (GS2): N2, 60 psi
- Turbo Ion Spray Temperature: 700 °C
- Interface Temperature: off
- Curtain Gas (CUR): N2, 40 psi
- Collision Gas (CAD): N2, 3 psi
- Ion Spray Voltage (IS): 4500 V
- Inject 5 µL of each sample onto the HPLC column and analyze as per the chromatographic conditions listed above.

Note: The retention time in the chromatogram may be different depending on the choice of column.

Note: According to the described chromatographic system, peak splitting and peak fronting might be observed in particular for the early eluting compounds (e.g. NNN, NNN-d4).

Note: 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.

Table 4: Mass spectrometric parameters

Compounds	Precursor ion (m/z)	Quantifier (m/z)	Qualifier (m/z)	DP* (V)	CE* (V)	CXP* (V)	Dwell time (m sec)
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

* DP: Declustering potential, CE: Collision energy, CXP: Collision cell exit potential

12.3. Calculations

12.3.1. 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.

Note: When laboratories either have problems obtaining 4 internal standards or have checked / validated that using 2 internal standards gives comparable data then the method can 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.

12.3.2. 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.

12.3.3. 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 Figure 1 and 2.
- TSNA concentrations are reported in (ng/mL) by the chromatography software.
- Determination of Mainstream Smoke TSNA Deliveries, M in [ng/cigarette]

$$M = C*Ws / N$$

where

C: the ratio by weight obtained from the calibration curve
Ws: the amount in ng of the internal standard added to the sample
N: the number of cigarettes smoked

13. REPEATABILITY AND REPRODUCIBILITY

- These were determined from an international study involving 20 laboratories and ten cigarette samples including the reference cigarettes KR 1R5F and KR 3R4F and the CORESTA Monitor CM6. The seven commercially available cigarettes and the references covered a wide range of blends and constructions. The collaborative study was carried out in 2011.
- The statistical evaluation was performed according to ISO 5725-2:1994 "Accuracy (trueness and precision) of measurement methods and results – Part 2: Basic method for the determination of repeatability (r) and reproducibility (R) of a standard measurement method."

13.1. Determination of Outliers

- Individual data points reported as non-numeric (i.e. below the LOQ) were removed prior to evaluation of numeric data for outliers.
- Raw data was checked for consistency both within labs and among labs using Mandel's k and Mandel's h statistics, respectively. These statistics are represented graphically as plots across all labs for each cigarette sample and analytical parameter (not shown).
- Actual removal of data prior to the estimation of the repeatability (r) and reproducibility (R) limits were made based on the Cochran's C and Grubbs numerical outlier tests, with outliers (α = 1%) being removed and stragglers (α = 5%) retained.

13.2. Determination of General Mean, Repeatability and Reproducibility Variance

- The general mean was determined as per ISO 5725-2 section 7.4.4 across the participating laboratories whose data remained following the removal of outliers.
- Repeatability variance (sr) was determined as per ISO 5725-2 section 7.4.5.1 and reproducibility variance (sR) was determined as per ISO 5725-2 section 7.4.5.5.
- Repeatability (r) and reproducibility (R) figures calculated for 95 % confidence level were also indicated for the individual TSNAs and cigarette samples.

13.3. Results

Calculated statistical data are indicated in the tables below.

13.3.1. ISO smoking regime

	NNN [ng/cigarette]					
Sample	**# laboratories**	**Mean**	***sr***	***sR***	***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**	***sr***	***sR***	***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**	***sr***	***sR***	***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**	***sr***	***sR***	***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
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

13.3.2. Intense smoking regime

	NNN [ng/cigarette]					
Sample	**# laboratories**	**Mean**	***sr***	***sR***	***r***	***R***
1	18	603	43	80	122	225
2	18	87.5	9.9	12.7	27.9	35.9
3	18	68.6	7.0	10.6	19.7	29.9
4	18	34.9	6.0	10.1	16.8	28.6
5	16	51.2	5.0	15.0	14.0	42.4
6	18	48.0	8.3	11.6	23.5	32.8
7	16	63.6	6.2	8.2	17.6	23.3
CM 6	18	37.9	5.1	7.5	14.4	21.3
KR 1R5F	18	237	17	26	49	72
KR 3R4F	19	297	26	31	73	88

	NAT [ng/cigarette]					
Sample	**# laboratories**	**Mean**	***sr***	***sR***	***r***	***R***
1	17	322	23	76	64	214
2	15	91.2	7.2	20.8	20.5	58.9
3	15	76.8	7.0	16.9	19.7	47.7
4	16	39.4	3.5	8.9	9.9	25.2
5	16	54.0	5.6	15.8	15.8	44.7
6	15	54.0	5.9	11.7	16.7	33.0
7	16	83.0	5.9	17.4	16.7	49.1
CM 6	17	64.8	6.1	14.2	17.1	40.3
KR 1R5F	16	230	13	49	37	138
KR 3R4F	17	279	22	47	63	132

	NAB [ng/cigarette]					
Sample	**# laboratories**	**Mean**	***sr***	***sR***	***r***	***R***
1	17	42.9	3.8	7.6	10.8	21.4
2	16	11.8	1.3	2.6	3.6	7.3
3	16	10.1	1.4	2.5	4.1	7.2
4	14	5.5	1.0	2.2	2.9	6.2
5	15	6.7	0.9	1.8	2.5	5.0
6	14	7.5	1.0	2.2	2.7	6.2
7	15	8.7	0.7	1.6	1.9	4.7
CM 6	15	7.5	0.8	2.3	2.2	6.4
KR 1R5F	16	27.8	1.9	4.3	5.3	12.2
KR 3R4F	17	31.2	2.7	4.7	7.7	13.2

	NNK [ng/cigarette]					
Sample	**# laboratories**	**Mean**	***sr***	***sR***	***r***	***R***
1	18	297	25	51	71	144
2	17	55.5	5.6	7.5	15.7	21.2
3	18	49.9	5.8	6.9	16.3	19.5
4	13	12.1	1.1	3.3	3.2	9.3
5	17	15.0	2.6	4.2	7.2	11.9
6	15	14.3	1.5	3.0	4.2	8.4
7	18	46.6	4.9	8.2	13.9	23.2
CM 6	19	50.8	5.8	9.7	16.5	27.4
KR 1R5F	19	121	7	19	20	52
KR 3R4F	19	252	20	33	58	92

14. RESULTS

14.1. Test results

- 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 ng/cig should be rounded to the nearest 0.1 ng.

15. REFERENCES

15.1. Related Publications

- Wagner, K.A., Finkel, N.H., Fossett, J.E., Gillman, I.G., 2005: Development of a quantitative method for the analysis of tobacco-specific nitrosamines in mainstream cigarette smoke using isotope dilution liquid chromatography/electrospray ionization tandem mass spectrometry; Analytical Chemistry Volume 77, Issue 4, p. 1001-1006.
- Wu, J., Joza, P., Sharifi, M., Rickert, W.S., Lauterbach, J.H., 2008: Quantitative method for the analysis of tobacco-specific nitrosamines in cigarette tobacco and mainstream cigarette smoke by use of isotope dilution liquid chromatography tandem mass spectrometry; Analytical Chemistry Volume 80, Issue 4, p. 1341-1345.
- Xiong, W., Hou, H., Jiang, X., Tang, G., Hu, Q., 2010: Simultaneous determination of four tobacco-specific N-nitrosamines in mainstream smoke for Chinese Virginia cigarettes by liquid chromatography-tandem mass spectrometry and validation under ISO and "Canadian intense" machine smoking regimes; Analytica Chimica Acta Volume 674, Issue 1, p. 71-78.

EXAMPLE CHROMATOGRAMS

Figure 1: Chromatograms of a typical TSNAs calibration standard

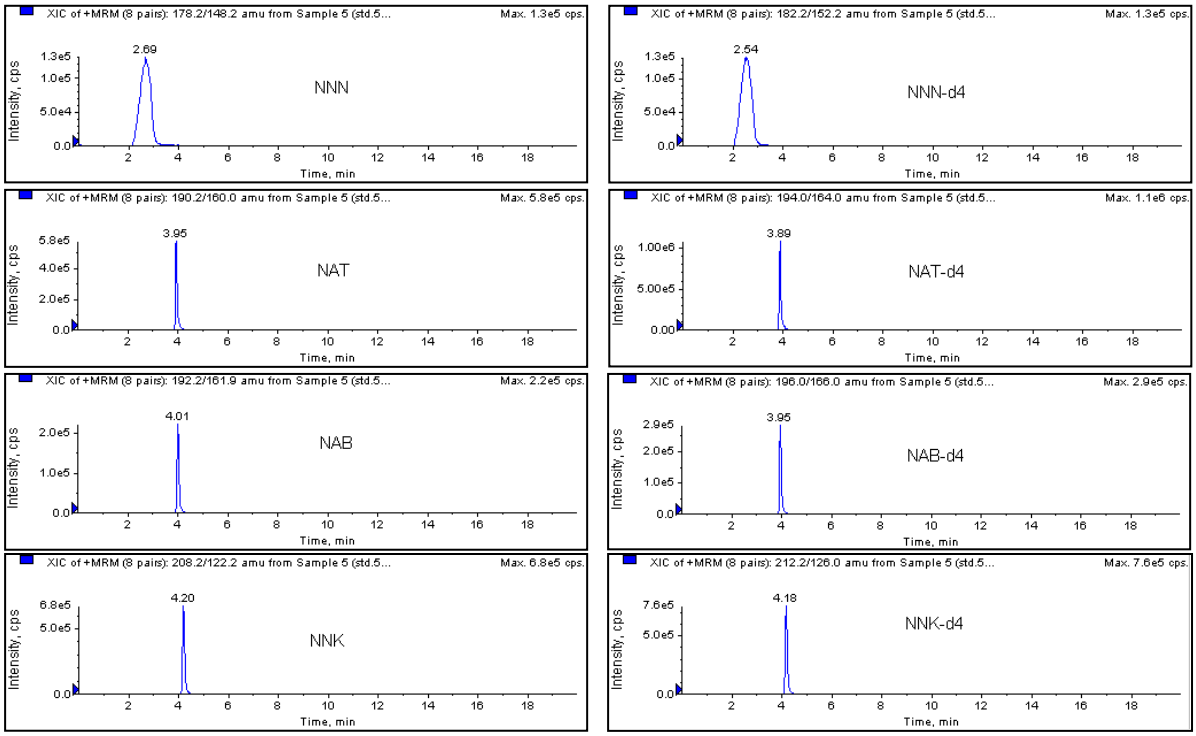

Figure 2: Chromatograms of TSNAs in mainstream cigarette smoke extract (KR 3R4F)