1 SCOPE

1.1 This method describes a general procedure for the determination of volatile N-nitrosamines in infant feeding bottle nipples, pacifiers, or other similar consumer products by dichloromethane extraction, as applicable to item 27 and 37 of Part II of Schedule I to the Hazardous Products Act (SOR/91-260 and SOR/2004-65) and as included in the Canadian Environmental Protection Act (SOR/2005-38).

2 WARNING

2.1 Hazards:
Volatile N-nitrosamines are reported to be potent carcinogens. Adequate precautions must be taken to avoid undue exposure. Wherever possible, all steps of the analytical process must be conducted in a well ventilated fume hood. The analyst is also directed to wear protective gloves, and use mechanical pipetting devices while handling all nitrosamines working solutions or concentrates.

2.2 Waste disposal:
All N-nitrosamines containing solutions must be properly stored and disposed of according to the applicable procedures of the Environmental Protection Act, Hazardous Chemical Waste Removal Regulations. All N-nitrosamines containing waste should be decontaminated using overnight exposure to UV light or well established procedure involving boiling with HCl, KI, and sulfamic acid. (Williams, D.L.H., Food Cosmet. Toxicol.,13,302, 1975) or (IARC Sci. Pub. No.43, Lyon, France, 1982). Decontaminated waste should be checked for any residual N-nitrosamines before disposal.

2.3 Volatile N-nitrosamines are highly photo labile. The analytical process should be conducted under subdued light wherever possible or Light Shields (clear) which filters 99.9% UV light.

3 APPLICABLE DOCUMENTS

3.1 S. Kushwaha, "*HWC-CCA Protocol for the Determination of Volatile N-nitrosamines in Latex Rubber Nipples and Pacifiers*", Health Canada, Product Safety Bureau, SLSD, Project report no. 85-0268A (1985).

3.2 ASTM D4210, volume 11.01 (1998)

3.3 A. Soucy," *Revision of Method C-24. Determination of Volatile N-nitrosamines in Infant Feeding Bottle Nipples, Pacifiers, and Other Similar Consumer Products*". Health Canada, PSL, Project report no.2000-0568. (2001-2002)

3.4 M. Charette, *Determination of Volatile N-nitrosamines in Infant Feeding Bottle Nipples, Pacifiers, and Other Similar Consumer Products*, Project # 2006-0944.

4 REAGENTS AND APPARATUS

4.1 Reagents

4.1.1 Barium hydroxide octahydrate ($Ba(OH)_2 \cdot 8H_2O$).

4.1.2 Dichloromethane (CH_2Cl_2, DCM), nitrosamine-free,Burdick & Jackson (Catalogue #300-4) or equivalent *(Note 1)*

4.1.3 Distilled/deionized water, nitrosamine-free

4.1.4 Morpholine (C_4H_9NO) solution, 1 mg/mL

4.1.5 Mix of 7 analytes, 10 µg/mL nominal concentration of each of N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodi-n-propylamine (NDPA), N-nitrosodi-n-butylamine (NDBA), N-nitrosopiperidine (NPIP), N-nitrosopyrrolidine (NPYR), and N-nitrosomorpholine (NMOR) in ethanol.

4.1.6 Nitrosodi-n-propylamine (NDPA) (or F63) solution, 100 ng/mL (Internal standard)

4.1.7 n-Propyl gallate ($C_6H_2(OH)_3COOC_3H_7$).

Note 1: Where applicable, suitable equivalent reagents and materials may be used.

4.1.8 Sodium hydroxide (NaOH), 5N.
4.1.9 Sodium sulphate (Na_2SO_4), anhydrous.
4.1.10 Acetone
4.1.11 Contrad 70®, 5% in aqueous solution or equivalent *(Note 1)*

4.2 Apparatus

4.2.1 Glassballs, 1 - 2 mm particle size, and Boileezers, Alumina Granules or equivalent (*Note 1*).
4.2.2 Erlenmeyer flask, glass-stoppered, 250 mL.
4.2.3 Graham condenser, 200 mm jacket length.
4.2.4 Hemispherical Mantle, Series O, Glass-Col, for 1000 mL flask, 380 Watts, 110V
4.2.5 Support jack, jumbo, platform size 12" X 12".
4.2.6 Powerstat variable transformer, output: 0-120/140V(10A), input: 120V 50/60HZ
4.2.7 KONTES Brand PTFE - Plugged chromatography column, part # 420530-0275 or equivalent *(Note 1)*.
4.2.8 KONTES Brand PTFE - Distilling adapter, part # 169500-2400 or equivalent *(Note 1)*.
4.2.9 Kuderna - Danish (K-D) evaporative concentrator, 250 mL
4.2.10 Concentrator tube, 4 mL, ±0.1 mL graduations from 0 to 2 mL.
4.2.11 Micro Snyder distilling column, 3 chambers, 165 mm.
4.2.12 Snyder distilling column, 3 chambers, 305 mm.
4.2.13 Round bottom flask with grounded joint 24/40, 500 mL.
4.2.14 Separatory funnel, 250 mL.
4.2.15 Graduate cylinder, 100 mL.
4.2.16 Glass stoppered Erlenmeyer flask with grounded joint 24/40, 250 mL
4.2.17 Light Shields (T-12 Polycarbonate Tubeguards-4')# PLA 1165 from Buchann Lighting 129 Loretta Avenue North, Ottawa, Ontario K1Y 2J7 or equivalent *(Note 1)*
4.2.18 Standard plastic taper clamps, joint #19 and #24, Wheaton or equivalent *(Note 1)*.
4.2.19 Wrist-Action shaker
4.2.20 Silanized glass wool
4.2.21 Chromatography glass column with PTFE stopcock, 400 x 30 mm I.D., 250 mL.

4.2.22 Gas Chromatograph (GC) with split-splitless injector, Varian Chrompack CP-3800
4.2.23 Thermal Energy Analyzer (TEA™), model 502B, Thermedics Inc.
4.2.24 Column: Supelcowax from Supelco, 30 m, 0.53 mm I.D., 0.5 µm film thickness, part# 25325 or equivalent. *(Note 1)*
4.2.25 Analytical balance
4.2.26 Semi-analytical balance
4.2.27 Amber vials 2.0 mL with Teflon faced septum
4.2.28 Thermometer 0 - 250˚C
4.2.29 Freezer maintain at approximately -15˚C

5 EXPERIMENTAL PROCEDURE

5.1 Distillation of Dichloromethane (DCM):

5.1.1 All the DCM used in the method should be distilled before use. A solvent distillation apparatus should be set-up which include:

5.1.1.1 Large volume capacity (4L)
5.1.1.2 The addition of a Snyder distillation column with floating ball valves in order to improve vapour-liquid contact.
5.1.1.3 Adjust the powerstat at approximately 50 to maintain about 50˚C
5.1.1.4 Label the distilled lot with the date

5.1.2 Test the distilled DCM for volatile N-nitrosamines as follows: (This test should be conducted for every new lot of DCM)

5.1.2.1 Concentrate a 250 mL portion of distilled DCM to 1 mL using a K-D evaporative concentrator and Snyder column in a water bath at 55°C.
5.1.2.2 Keep this solution for analysis.

5.1.3 Test the DCM for nitrosation agents or catalysts of N-nitrosation as follows:
(This test should be conducted for every new lot of distilled DCM)

5.1.3.1 Add 1 mL of a morpholine solution to 250 mL of distilled DCM in a 500 mL glass stoppered round bottom flask. Cover the flask with aluminum foil and let stand overnight. Resume the experimental procedure from 5.4.8 to the end.

5.1.3.2 Keep this solution for analysis.

5.2 Distilled/Deionized Water:
(This test should be conducted at the beginning of any new survey)

5.2.1 Test the distilled/deionized water for volatile N-nitrosamines as follows:
Transfer 100 mL of distilled/deionized water in a 250 mL separatory funnel, add 2 mL of 5N NaOH, 2 g $Ba(OH)_2.8H_2O$ and extract the solution with three 75 mL portions of DCM. Draw off the DCM through 30 g of granular anhydrous Na_2SO_4 contained in a 60 mL coarse sintered-glass funnel prewashed with 25 mL DCM, and collect the filtrates into a 250 mL K-D evaporative concentrator. Concentrate the pooled extracts to 1 mL using a Snyder column in a water bath at 55°C.

5.2.2 Keep this solution for analysis.

5.3 Preparation of equipment:

5.3.1 All glassware should be soaked overnight in a solution of Contrad 70® 5%, and rinse with distilled water.

5.3.2 Before each analysis, the glassware must be rinsed with acetone then with distilled dichloromethane (DCM) (section 5.1.1)

5.3.3 The clean glassware are dried in an oven maintain at 110˚C.

5.4 Samples preparation:

5.4.1 Cut the test sample into small pieces ($\leq$0.2 g).

5.4.2 Accurately weigh two 5 g portion to the nearest 0.1 mg and transfer into a 250 mL glass stoppered Erlenmeyer flask.

5.4.3 To the first portion, add 100 mL of DCM and 100 mg n-propyl gallate.

5.4.4 To the second portion, add 100 mL of DCM, 100 mg n-propyl gallate and 1.0 mL of the 100 ng/mL NDPA working standard solution (added as internal standard *(Note 2)*).

5.4.5 Secure both erlenmeyer flasks with glass grounded stopper and cover with paraffin film. Wrap the flask with aluminum foil. Place the flasks on a wrist-action shaker (setting at 4), and gently extract the samples overnight at 22 ± 2°C.

5.4.6 For each portion, prewashed a glass chromatography column (burette) containing a silanized glass wool plug placed loosely at the bottom with 25 mL DCM. Closed the stopcock at the bottom of the burette. Transfer the DCM extract solution and sample pieces into the burette. Drain and collect the DCM into a 500 mL round bottom flask.

5.4.7 Rinse the extraction flask with 25 mL DCM and pour the rinse solution into the glass column. Add DCM, as required, to ensure that the sample pieces are well immersed and allow the sample pieces to soak for 10 minutes. Drain and pool the DCM rinse into the round bottom flask. Repeat the procedure with a fresh 25 mL portion of DCM for another 10 minutes and pool the DCM in the same flask.

5.4.8 Add 100 mL of 5N NaOH, 2 g of $Ba(OH)_2 \cdot 8H_2O$, one or two small glassballs (prewashed with DCM), and connect the round bottom flask to a distillation apparatus fitted with a vertically mounted Graham condenser. Insulate the apparatus with glass wool loosely wrapped around the round bottom flask and connecting adapter. Slowly distill the DCM, by adjusting the voltage at 40, and collect into a clean 250 mL glass-stoppered erlenmeyer flask. Distill DCM until re-condensation rate slowdown considerably - keep the distilled fraction. Installed the grounded stopper on the flask and replace the collecting vessel with a clean 100 mL graduate cylinder. Increase the heating rate, by adjusting the voltage at 70, and continue the distillation until the remaining DCM

Note 2: *- The use of NDPA as an internal standard has been found satisfactory for the analysis of the large majority of polymer formulations.*
- Should NDPA be detected, a different N-nitrosamine internal standard must be chosen to serve this purpose

(approximately 10 mL), and a minimum of 70 mL of water have been collected.

5.4.9 Transfer the DCM/water distillate to a 250 mL separatory funnel. Add 5 mL of 5N NaOH and 50 mL of previously distilled DCM (section 5.4.8) to the DCM/water distillate, stopper the separatory funnel and shake vigorously for 1 minute. Allow the layers to separate. Draw off the lower DCM layer through 30 g of granular anhydrous Na_2SO_4 contained in a 60 mL coarse sintered-glass funnel prewashed with 25 mL DCM, and collect into a 250 mL K-D evaporative concentrator. Repeat the extraction with two additional 50 mL portions of the previously distilled DCM (section 5.4.8) and pool the filtered extracts in the K-D evaporator.

5.4.10 Wash the Na_2SO_4 filter with 25 mL of fresh DCM and transfer it to the K-D evaporator. Add one or two small alumina granules (prewashed with DCM). Concentrate the combined extracts to approximately 4 mL using a Snyder column in a water bath at 55°C *(Note 3)*. Remove the K-D evaporator from the water bath and cool to ambient temperature.

5.4.11 Disconnect the concentrator tube from the K-D flask, add fresh alumina granules, and continue concentration of the extract to 1 mL using a micro Snyder column - **do not concentrate to less than 0.8 mL** *(Note 4)*. Remove the apparatus from the water bath and cool to ambient temperature. Rinse the column with a few drops of fresh DCM and allow the rinse to drain into the concentrator tube. Disconnect the tube, adjust the final

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Note 3: *Avoid excessive accumulation of DCM in the column chambers. The boiling rate should be adjusted by lowering or raising (without completely lifting out) the K-D flask in the water bath in order to obtain an evaporation rate of approximately 1 mL/min.*

Note 4: *The final concentration must be conducted slowly over a period of at least 30 min. When the extract volume approaches 0.8 mL, raise the tube (without completely lifting out of the water bath) and let the distilled DCM drain down. Should the final extract volume be more than 1 mL, carefully continue the distillation. The concentration should not be conducted under N_2 stream in any circumstances.*
For extracts exhibiting excessive foaming, replace the micro Snyder column with a 3-chamber modified micro distilling column without floating balls, available from the KONTES Glass Co.

volume to 1.0 - 1.2 mL and transfer in an amber vial *(Note 5)* . Analyse both concentrates immediately; otherwise, cover the tubes with aluminum foil and store in the freezer.

5.5 Method blank:

Prepare a blank sample by using 1 mL of DCM, as obtained from the distillation (section 5.1). Resume sample blank preparation as per section 5.4.3.

5.6 Internal Standard: NDPA, 100 ng/mL

Dilute 1 mL of a 10 µg/mL NDPA certified reference standard solution in ethanol with distilled DCM in a 100 mL volumetric flask. Store in the freezer.

5.7 Certified Reference Standard Solution: Mixture of 7 N-nitrosamines, 100 ng/mL

Dilute 1 mL of a 10 µg/mL mixture of 7 N-nitrosamines certified reference standard solution with distilled DCM in a 100 mL volumetric flask. Store in the freezer

5.8 Control samples:
(This test should be conducted on a daily basis)

5.8.1 Prepare an aliquot (1 mL) of the 100 ng/mL NDPA standard solution for each batch of analysis.

5.8.2 Prepare an aliquot (1 mL) of the 100 ng/mL mixture of 7 N-nitrosamines standard solution for each batch of analysis.

5.9 Morpholine:
(This test should be conducted at the beginning of any new survey)

Prepare an aliquot (1 mL) of the morpholine solution. Store at 4˚C.

Note 5: *Volatile N-nitrosamines are photo labile and should not be exposed to natural or unprotected fluorescent light before analysis.*

6 CALIBRATION

6.1 Prepare a series of at least four working standard solutions of 25, 50, 75 and 100 ng/mL N-nitrosamine nominal concentration by making appropriate dilutions of the 10 µg/mL mixture of 7 N-nitrosamines certified reference standard solution in dichloromethane as prepared in section 5.1.1. Protect from light and store in the freezer.

6.2 For each N-nitrosamine detected (including the NDPA internal standard), prepare a calibration curve of concentration versus peak area using the mixture of 7 N-nitrosamines working standard solutions.

7 DETERMINATION

7.1 Gas Chromatograph conditions:

7.1.1	Method:	nitroA.mth
7.1.2	Carrier Gas: Argon, constant column flow: 5.0 mL/min	
7.1.3	Split Ratio:	60 : 1
7.1.4	Injection Mode:	Splitless: Closed 0.75min, 150˚C, 2mm injector liner
7.1.5	Oven Temperature Program:	Initial: 30˚C for 1.00 min.
7.1.6	Ramping:	20.0˚C/min to 150˚C
7.1.7	Hold time:	8 min.
7.1.8	GC column:	Supelcowax, 30 m, 0.53 mm I.D., 0.5 µm film thickness
7.1.9	Injection volume:	6 µl

7.2 Thermal Energy Analyzer detector conditions (Thermo Electron Corp., model 502):

7.2.1	Attenuation:	4
7.2.2	GC operate mode pressure:	0.3 - 0.4 mm Hg.
7.2.3	Interface temperature:	200°C.
7.2.4	Oxygen flow:	20 mL/min.
7.2.5	Pyrolyser temperature:	500°C.
7.2.6	CTR™ gas stream filter.	
7.2.7	Vent mode pressure:	0.3 mm Hg.

7.3 Determine the presence of volatile N-nitrosamines, nitrosation agents or catalysts of N-nitrosation (section 5.1.2 and 5.1.3) of the distilled DCM. The results should show the absence of N-nitrosamines and not more than 10 ng of N-nitrosomorpholine (NMOR) per 100 mL of DCM for the nitrosation agents.

7.4 Determine the presence of N-nitrosamines in distilled/deionized water (section 5.2). The results should show the absence of N-nitrosamines.

7.5 Determine the N-nitrosamines content of the blank solution (section 5.5) before and after the analysis.

7.6 Determine the concentration of the 100 ng/mL of the mixture of 7 N-nitrosamines standard solution (section 5.7).

A typical chromatogram of a 7 N-nitrosamines standard solution in DCM is presented in Figure 1.

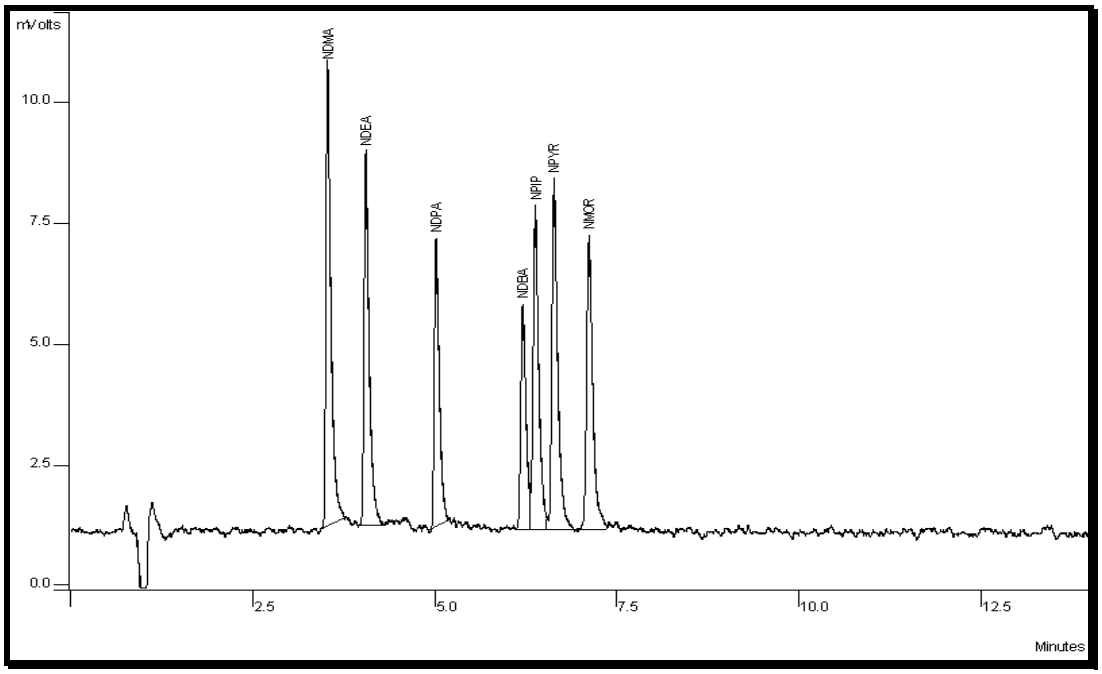

Figure 1: Typical GLC-TEA chromatogram of a 6 µL injection of a 100 ng/mL mixture of 7 N-nitrosamines working standard solution in DCM. Conditions as mentioned in the apparatus section of this method.

7.7 Determine the concentration of the 100 ng/mL of NDPA standard solution (section 5.6).

7.8 Determine the concentration of morpholine (section 5.9) . The results should show the absence of N-nitrosomorpholine.

7.9 Determine the volatile N-nitrosamines, in ng/mL, for both concentrates of each sample (section 5.4) using the GC-TEA according to the instrument manufacturer's instructions. If necessary, dilute the sample concentrate with DCM, as prepared in section 5.1.1 by an appropriate factor in order to ensure the chromatographic peak measurements are taken within the range of the calibration curve.

8 CALCULATIONS AND REPORTING

8.1 Calculate the concentration, in µg/kg, of each detected volatile N-nitrosamine for each test sample solution according to the following equation:

$$\textit{Volatile N-Nitrosamine, µg/kg} = \frac{C_i \times V_i \times Df}{Wt}$$

where:

C_i = Concentration of the N-nitrosamine measured by the instrument in the sample concentrate (ng/mL),
V_i = Volume of the test sample concentrate (mL),
Df = Dilution factor,
Wt = Weight of test sample used (g).

8.2 Calculate the percentage recovery of the NDPA internal standard according to the following equation:

$$\textit{Recovery, \%} = \frac{C_c \times V_c \times Df \times 100}{C_{is} \times V_{is}}$$

where:

C_c = Concentration of the internal standard measured in the sample concentrate(ng/mL),
V_c = Volume of the test sample concentrate (mL),
Df = Dilution factor,
C_{is} = Concentration of the internal standard solution used (100 ng/mL).
V_{is} = Volume of the internal standard solution added (1 mL),

8.3 Where the quantity of a sample available for testing is sufficient and where practical, the result of analysis will be reported as the average of a minimum of three replicate determinations of the same test sample solution having a precision which meets or exceeds the specifications defined in Section 10.1.

8.4 Where applicable, the average (x.x) of replicate determinations and the standard deviation (s) of replicate determinations (s for n > 2) will be calculated *(Note 6)*, and the result of analysis reported in the following format:

Sample no.	Specimen no.	Volatile N-nitrosamine	Concentration (µg/kg)
S-1001234	A1	NDMA	xx.x ± 2s
		NDEA	xx.x ± 2s
		TOTAL:	xx.x ± 2s

8.5 All results less than the limit of detection of the instrument $(LOD)_i$ shall be reported as per the limit of detection in section 11.1. Should the value be larger than LOD_i, the results should be reported as such.

9 QUALITY CONTROL PROCEDURE

9.1 In order to ensure the proper operation of the analytical instrument and that the precision and accuracy of the analytical measurements meet the specifications of the method, the following quality control procedures shall be conducted concurrently with the analysis of the test sample.

9.2 The normal and correct operation of the GC-TEA shall be verified according to the following guidelines:

9.1.1 Record the concentration of the 100 ng/mL of NDPA standard solution in the analytical instrument's QC logbook. If the instrument is found in a state of disrepair or out of calibration, the GC-TEA, shall immediately be repaired and/or re-calibrated to meet the prescribed operating conditions prior to proceeding with the analysis.

Note 6: *The standard deviation (s) of the test results may be calculated according to the following equation, where: x_i is the result of each individual determination, $\bar{x}$ is the average of the replicate determinations and n is the total number of replicates.*

$$\left[s = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n - 1}} \right]$$

9.1.2 Record the peak area of the 100 ng/mL of the mixture for each 7 N-nitrosamines of the standard solution in the analytical instrument's QC logbook for monitoring the purpose of the efficiency of the column *(Note 7)*. Verify that the area measurement is within the warning limits (±2s) and does not exceed the control limits (±3s).

9.3 Record the percent recovery of the NDPA internal standard in the analytical instrument's QC logbook. Verify that the result is within the tolerance limits of the accepted value (recoveries outside the range of 75 to 110% should be considered suspect). If the control sample results are outside of the control limits, the entire analytical procedure shall be repeated.

10 PRECISION AND BIAS

10.1 Repeatability:
The deviation between replicate test results, as obtained using a blank sample spiked with NDPA at a 5 µg/kg level. (n=7) by the same analyst with the same instrument under constant operating conditions, should, in the normal and correct operation of the test method, not differ by more than a 45% (2.8 X %CV) repeatability limit at a 95% probability level.

This section is still under development and a more representative value will be obtained using a positive sample or a blank sample spiked at a value higher than the limit of quantification of the method. This value will be added in a revised issue when completed.

10.2 Reproducibility:
The reproducibility will be presented in a revised copy of this method.

10.3 Bias: There is no bias observed for this method.

Note 7: *A significant decrease in area counts of NPYR and NMOR means a decrease in efficiency in the column. The column should be changed.*

11 LIMIT OF DETECTION AND QUANTIFICATION

11.1 Limits of detection (LOD) and quantification (LOQ) established from statistical evaluation of instrumentation performance only. (Results based on 5g test sample):

Volatile N-nitrosamine	Detection limits (µg/kg)	Quantification limits (µg/kg)
N-nitrosodimethylamine (NDMA)	1.1	3.4
N-nitrosodiethylamine (NDEA)	0.5	1.5
N-nitrosodi-n-propylamine (NDPA)	1.1	3.2
N-nitrosodi-n-butylamine (NDBA)	0.8	2.5
N-nitrosopiperidine (NPIP)	0.9	2.8
N-nitrosopyrrolidine (NPYR)	1.0	3.1
N-nitrosomorpholine (NMOR)	0.9	2.6
Total Volatile N-nitrosamine	6.3	19.1

11.2 Limits of detection (LOD) and quantification (LOQ) established from statistical evaluation of the entire C24 method performance. (Results based on 5g test sample):

Volatile N-nitrosamine	Detection limits (µg/kg)	Quantification limits (µg/kg)
N-nitrosodimethylamine (NDMA)	1.5	4.6
N-nitrosodiethylamine (NDEA)	1.2	3.7
N-nitrosodi-n-propylamine (NDPA)	2.3	7.0
N-nitrosodi-n-butylamine (NDBA)	2.8	8.6
N-nitrosopiperidine (NPIP)	2.2	6.6
N-nitrosopyrrolidine (NPYR)	2.3	6.9
N-nitrosomorpholine (NMOR)	1.4	4.1
Total Volatile N-nitrosamine	13.7	41.5

----------END----------