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 37 of Part II of Schedule I to the Hazardous Products Act.

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 nitrosamine working solutions or concentrates.

2.2 *Waste disposal:* All *N*-nitrosamine 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.

3 APPLICABLE DOCUMENTS

3.1 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)

3.2 M.Lanouette, “ *Revision of Method C-24. Determination of Volatile N-nitrosamines in Infant Feeding Bottle Nipples, Pacifiers, and Other Similar Consumer Products*”. Health Canada, Product Safety Bureau, PSL, Project report no.98-0454. (1999)

3.3 N.P. Sen *et al.*, "*Improved Method for Determination of Volatile Nitrosamines in Baby Bottle Rubber Nipples and Pacifiers*", J. Assoc. Off. Anal. Chem., vol. 70, no. 3, p. 434 (1987).

3.4 N.P. Sen *et al.*, "*Prevention of Artifactual Formation of Nitrosamines During the Analysis of Baby Bottle Rubber Nipples*", Analyst, vol. 111, p. 139 (1986).

3.5 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).

3.6 N.P. Sen, S. Seaman, S. Clarkson, F. Garrod and P. Lalonde, "*Volatile N-nitrosamines in Baby Bottle Rubber Nipples and Pacifiers, Analysis, Occurrence and Migration*", World Health Organization, IARC, I.K. O'Neill *et al.* eds., p. 51 (1984).

3.7 ASTM D4210, volume 11.01 (1998).

4 REAGENTS

4.1 Barium hydroxide octahydrate ($Ba(OH)_2 \cdot 8H_2O$).
4.2 Dichloromethane (CH_2Cl_2, DCM), nitrosamine-free,Burdick & Jackson (Catalogue #300-4) or equivalent *(Note 1)*.
4.3 Distilled deionized water, nitrosamine-free *(Note 2)* .
4.4 Morpholine (C_4H_9NO) solution in DCM, 1 mg/mL: The analysis of a 6 µL aliquot by GLC-TEA should show the absence of *N*-nitrosomorpholine. Store at 4°C.
4.5 *N*-Nitrosamine certified reference standard solution, mixture of 7 *N*-nitrosamines: Solution of 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 *(Note 3)* .
4.6 *N*-Nitrosodi-*n*-propylamine (NDPA) working standard solution, 100 ng/mL: Dilute 1 mL of a 10 µg/mL NDPA certified reference standard solution in ethanol with DCM in a 100 mL volumetric flask. Store at -20°C.
4.7 *n*-Propyl gallate ($C_6H_2(OH)_3COOC_3H_7$).
4.8 Sodium hydroxide (NaOH), 5N.
4.9 Sodium sulphate (Na_2SO_4), anhydrous.

5 APPARATUS

5.1 Boiling chips, 1 - 2 mm particle size.
5.2 Chromatography glass column with PTFE stopcock, 400 x 30 mm I.D., 250 mL.
5.3 Erlenmeyer flask, glass-stoppered, 250 mL.

Note 1: *Test the DCM for volatile N-nitrosamines as follows: Concentrate a 250 mL portion of DCM to 1 mL using a K-D evaporative concentrator and Snyder column in a water bath at 55°C. Inject a 6 µL aliquot of the concentrate into a GLC-TEA operated according to the instrument manufacturer's instructions. The analysis should show the absence of N-nitrosamines.*

*Test the DCM for nitrosation agents or catalysts of N-nitrosation as follows: Add 1 mL of a 1 mg/mL morpholine solution in DCM (q.v.) to 250 mL of DCM in a 500 mL glass stoppered round bottom flask. Cover the flask with aluminum foil and let stand overnight. Start the experimental procedure from **6.5** to the end. The analysis of a 6 µL aliquot of the concentrate by GLC-TEA should not show the presence of more than 10 ng of N-nitrosomorpholine per 100 mL of DCM. This test should be conducted for every new lot of DCM.*

Note 2: *Test the distilled deionized water for volatile N-nitrosamines as follows: Transfer 100 mL of 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. The analysis of a 6 µL aliquot of the concentrate by GLC-TEA should show the absence of N-nitrosamines. This test should be conducted at the beginning of every survey.*

Note 3 *Available from: Chem Service, Inc., 660 Tower Lane, West Chester, PA 19380, USA, phone: 610-692-8729, www.chemservice.com*

5.4 Gas-Liquid Chromatograph - Thermal Energy Analyzer (GLC-TEA)
- 5.4.1 Gas-Liquid Chromatograph conditions (Varian Chrompack, CP-3800):
  - 5.4.1.1 Method: nitroA.mth
  - 5.4.1.2 Carrier gas: Argon, constant column flow: 5.0 mL/min (split 60:1)
  - 5.4.1.3 Column: Supelcowax from Supelco, 30 m, 0.53 mm I.D., 0.5 µm film thickness, part# 25325 or equivalent.
  - 5.4.1.4 Splitless injection mode: Closed 0.75min, 150˚C, 2mm injector liner
  - 5.4.1.5 Oven temp. program: Initial: 40˚C for 1.00 min. Ramping: 20.0˚C/min to 150˚C Hold time: 8 min.
- 5.4.2 Thermal Energy Analyzer detector conditions (Thermo Electron Corp., model 502):
  - 5.4.2.1 Attenuation: 4
  - 5.4.2.2 GC operate mode pressure: 0.3 - 0.4 mm Hg.
  - 5.4.2.3 Interface temperature: 200°C.
  - 5.4.2.4 Oxygen flow: 20 mL/min.
  - 5.4.2.5 Pyrolyser temperature: 500°C.
  - 5.4.2.6 CTR™ gas stream filter.
  - 5.4.2.7 Vent mode pressure: 0.3 mm Hg.

5.5 Graham condenser, 200 mm jacket length.

5.6 Hemispherical Mantle, Series O, Glass-Col, for 1000 mL flask, 380 Watts, 115V.

5.7 Support jack, jumbo, platform size 12" X 12".

5.8 Powerstat variable transformer, output: 0-120/140V(10A), input: 120V 50/60HZ.

5.9 Kontes Brand PTFE-Plugged chromatography column, part # 420530-0275 or equivalent.

5.10 Distilling adapter, Kontes, part # 169500-2400 or equivalent.

5.11 Kuderna - Danish (K-D) evaporative concentrator, 250 mL:
- 5.11.1 Concentrator tube, 4 mL, ±0.1 mL graduations from 0 to 2 mL.
- 5.11.2 Micro Snyder distilling column, 3 chambers, 165 mm.
- 5.11.3 Snyder distilling column, 3 chambers, 305 mm.

5.12 Round bottom flask, 500 mL.

5.13 Separatory funnel, 250 mL.

5.14 Graduate cylinder, 100 mL.

5.15 Glass stoppered Erlenmeyer flask, 250 mL.

5.16 Light Shields (T-12 Polycarbonate Tubeguards-4')# PLA 1165 from Buchann Lighting 129 Loretta Avenue North, Ottawa, Ontario K1Y 2J7

5.17 Standard plastic taper clamps, joint #19 and #24, Wheaton or equivalent.

6 EXPERIMENTAL PROCEDURE

6.1 *Precaution:*

6.1.1 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.

6.1.2 All the DCM used in the method should be distilled before use.
A solvent distillation apparatus should be set-up which include:
- Large volume capacity (4L)
- The addition of a Snyder distillation column with floating ball valves in order to improve vapour-liquid contact

6.1.3 Before each analysis, the glassware must be rinsed with acetone and distilled dichloromethane (DCM). See *Note 1*

6.2 Cut the test sample into small pieces ($\leq$0.2 g). Accurately weigh a 5 g portion to the nearest 0.1 mg and transfer into a 250 mL glass stoppered Erlenmeyer flask. 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 4)*). Stopper (secure the caps with parafin) and cover the flask with aluminum foil. Place the flask on a wrist-action shaker, and gently extract the sample overnight at 22 ± 2°C.

6.3 Transfer the DCM extract solution and sample pieces into a prewashed (with 25 mL DCM) glass chromatography column containing a silanized glass wool plug placed loosely at the bottom. Drain the DCM and collect the filtrate into a 500 mL round bottom flask.

6.4 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.

6.5 Add 100 mL of 5N NaOH, 2 g of $Ba(OH)_2 \cdot 8H_2O$, one or two small boiling chips, 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 distillation flask and connecting adapter. Using low heat, slowly distill the DCM and collect into a clean 250 mL glass-stoppered Erlenmeyer flask. Distill DCM until re-condensation rate slowdown considerably - do not discard. Stopper the flask and replace the collecting vessel with a 100 mL graduate cylinder. Increase the heating rate and continue the distillation until the remaining DCM (approximately 10 mL), and a minimum of 70 mL of water have been collected.

Note 4: The use of NDPA as an internal standard has been found satisfactory for the analysis of the large majority of polymer formulations. However, for any new formulation not previously tested, an analysis of the test sample should be conducted without the addition of an internal standard. Should NDPA be detected, a different N-nitrosamine internal standard must be chosen to serve this purpose.

6.6 Transfer the DCM/water distillate to a 250 mL separatory funnel. Add 5 mL of 5N NaOH and 50 mL of previously distilled DCM 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, collected in par. 6.5, and pool the filtered extracts in the K-D evaporator.

6.7 Wash the Na_2SO_4 filter with 25 mL of fresh DCM and add the washing to the K-D evaporator. Add one or two small boiling chips (1 - 2 mm particle size, prewashed with DCM), and concentrate the combined extracts to approximately 4 mL using a Snyder column in a water bath at 55°C *(Note 5)* . Remove the K-D evaporator from the water bath and cool to ambient temperature.

6.8 Disconnect the concentrator tube from the K-D flask, add a fresh boiling chip, and continue concentration of the extract to 1 mL using a micro Snyder column - do not concentrate to less than 0.8 mL *(Note 6)* . 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 volume to 1.0 - 1.2 mL and stopper. Analyse immediately; otherwise, cover the tube with aluminum foil and store in the freezer. (Temperature less than -20˚C)

7 DETERMINATION

7.1 Prepare a series of at least 4 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 DCM. Protect from light and store in the freezer.

7.2 Prepare a blank solution consisting of a 1 mL DCM concentrate, as obtained (without sample) under identical experimental conditions to that of the test sample concentrate.

Note 5: 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 6: 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.

7.3 Inject, in duplicate, a 6 µL aliquot of the test sample concentrate, as obtained in par. 6.8, into the GLC-TEA using the analytical conditions specified in par. 5.4, and operated according to the instrument manufacturer's instructions. If necessary, dilute the sample concentrate with DCM by an appropriate factor in order to ensure that the chromatographic peak measurements are taken within the instrument's linear dynamic range. 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, and determine the concentration, in ng/mL, of volatile *N*-nitrosamines in the test sample concentrate. A typical chromatogram of a 7 *N*-nitrosamines standard solution in DCM is presented in Figure 1. Determine the nitrosamine content of the blank solution and if present, subtract from the nitrosamine content of the sample.

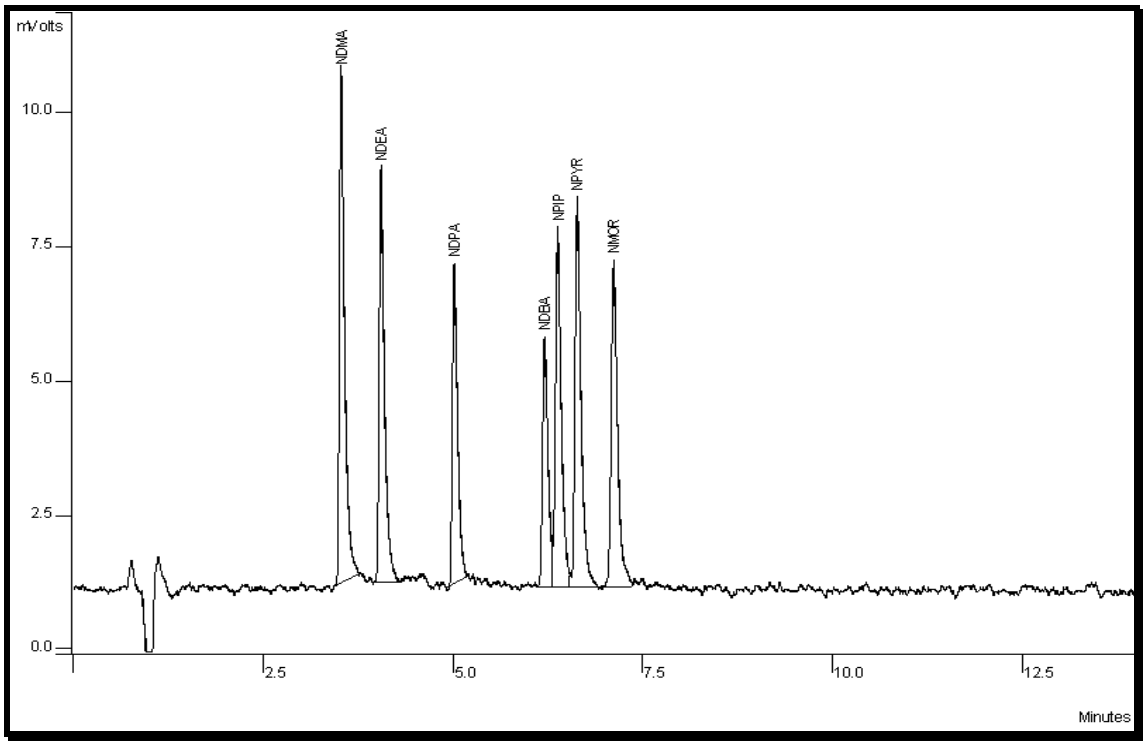

Figure 1: Typical GLC-TEA chromatogram of a 6 µL injection of a 100 ng/mL mixture of 7 *N*-nitrosamines working standard solution in DCM. Conditions as mentioned in the apparatus section of this method.

8 CALCULATIONS AND REPORTING

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

$$\textit{Volatile N-nitrosamine, µg/kg} = \frac{C_c \times V_c \times Df}{Wt}$$

where:

C_c = *Concentration of the N-nitrosamine measured in the sample concentrate (ng/mL),*
V_c = *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:

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

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 sample available for testing is sufficient and where practical, the result of analysis shall be reported as the average of a minimum of two replicate determinations having a precision which meets or exceeds the specifications defined in Section 10.

8.4 When no signals are detected, the result of analysis shall be reported as smaller or equal to the detection limit of the instrumentation used (Section 11.1). When *N*-nitrosamines are detected and higher or equal to the quantification limit of the method the result should be reported as such (Section 11.2). When result falls between these two values, the sample should be retested.

8.5 Where applicable, the mean deviation of the duplicate determinations or the standard deviation of replicate determinations (s for n > 2) shall be calculated *(Note 7)*, and the result of analysis reported in the following format:

Sample no.	*Specimen no.*	*Volatile N-nitrosamine*	*Concentration (µg/kg)*
1	*A*	*NDMA*	*xx.x ± 2s*
		NDEA	*xx.x ± 2s*
		TOTAL:	*xx.x ± 2s*
		% Recovery:	*xx.x*

9 QUALITY CONTROL PROCEDURE

9.1 In order to ensure the proper operation of the available instrumentation 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 GLC-TEA shall be verified according to the following guidelines:

9.2.1 Inject a 6 µL aliquot of the 100 ng/mL mixture of 7 *N*-nitrosamines working standard solution into the GLC-TEA.

9.2.2 Record the peak area of all 7 nitrosamines detector response in the analytical instrument's QC logbook, and verify that the area measurement is within the tolerance limits of the expected value (a 600 pg NDMA on-column injection should give a peak area of 8500 to 11500 on a Varian Star at a detector attenuation of 4. If this control measurement falls within acceptable limits, a note shall be entered in the test sample file to the effect that the instrument calibration was found to be "within control". Should the instrument be found in a state of disrepair or out of specifications, the GLC-TEA shall immediately be repaired and/or reconditioned to meet the prescribed operating conditions prior to proceeding with the analysis. A significant decrease in area counts of NPYR and NMOR means a decrease in efficiency in the column. (The column should be changed.)

Note 7: *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.*

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

9.3 The normal and correct operation of the test method shall be verified according to the following guidelines:

9.3.1 Conduct the analysis of 1.0 mL of a 100 ng/mL NDPA standard solution, as prescribed in par. 6.2, under identical experimental conditions to that of the test sample. Record the percent recovery of the NDPA internal standard in the analytical instrument's QC logbook, and 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 this control measurement result falls within acceptable limits, a note shall be entered in the test sample file to the effect that the test method was found to be "within control". Should the percentage recovery of the internal standard be found to fall outside the specifications of the method, the entire analytical procedure shall be repeated.

10 PRECISION AND ACCURACY

10.1 *Repeatability:* The difference between replicate test results, as obtained by the same analyst with the same instrument under constant operating conditions on identical test material, should, in the normal and correct operation of the test method, not differ more than a 45% (2.8X %CV) repeatability limit at a 95% probability level.
This evaluation was done using a blank sample spiked with NDPA at a 5 µg/kg level. (n=7).
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:* This section of the method is under development and will be added in a revised issue when completed.

10.3 *Recovery:* The recovery range varies form 85.9% to 103.8% for all 7 *N*-nitrosamines for a test range between 5 to 20 µg/kg.

11 LIMITS OF DETECTION AND LIMITS OF 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

11.3 The Limits were determined according to ASTM procedure D4210 and are calculated as follows:

LOD = 2 x 1.645 x s.d. where s.d. is obtained from replicate analyses (n=7 or more)
LOQ = 10 x s.d. where s.d. is obtained from replicate analyses (n=7 or more)

. . . . . END . . . . .