NITROSAMINES/LOW-TEMPERATURE VACUUM DISTILLATION PROCEDURE

Contents

DETERMINATIVE METHOD

A. INTRODUCTION

1. Theory

This procedure uses a low-temperature distillation technique developed by N.P. Sen (refer to section F.8) to recover the volatile N-nitrosamines from various meat products. Detection and quantitation are accomplished by the use of GC-TEA (refer to method NTR1). An additional column cleanup of the extract is adequate for mass spectral confirmation. USDA Frying Protocol must be strictly followed before weighing the sample for analysis.

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B. EQUIPMENT

1. Apparatus

NOTE: Glassware catalog numbers refer to Kontes catalog TG-50 unless otherwise noted. Equivalent glassware may be substituted.

a. Distillation apparatus—refer to Figure 1 on the facing page.

i. All glass, flash evaporator, vertical, with adjustable heating bath (Buchler PTFE-1 GN, or equivalent.)

ii. 2 L round-bottom flask with 24/40 joint (K-601001).

iii. 250 mL round flask with 24/40 joint (K-601001).

iv. Needle value to adjust air bleed [Pyrex rotoflo (Corning 7740), or equivalent].

v. Ice bath with circulating water pump (Little Giant 501003, or equivalent).

vi. Vacuum pump, minimum 25 L/min capacity (GCA/Precision Scientific, Vac Torr D-25, or equivalent).

vii. Vacuum control cartesian (Gilmont C-2100, or equivalent).

viii. Vacuum gauge capable of monitoring pressure of 20 Torr. A manometer (Dwyer Series 1222 model M-1000, or equivalent) provides satisfactory service. Requires calibration with barometer of MacLeod gauge immediately before use.

b. Extraction and cleanup apparatus.

i. Syringe, all glass, 50 mL capacity.

ii. 250 mL graduated mixing cylinder (K-482000).

iii. 500 mL separatory funnel with Teflon stopcock (K-636030).

iv. Glass filtering funnel, 150 mL with 6 mm coarse porosity fritted disc (K-955000).

c. Evaporative concentrators.

i. 250 mL Kuderna Danish evaporative concentrator (K-570001).

ii. 500 mL Kuderna Danish evaporative concentrator (K-570001).

iii. 4 mL graduated concentrator tube (K-570050-425).

iv. Snyder column, large 3-section with 24/40 joint (K-503000-0121).

v. Snyder column, micro 3 section with 14/20 joint (K-569251).

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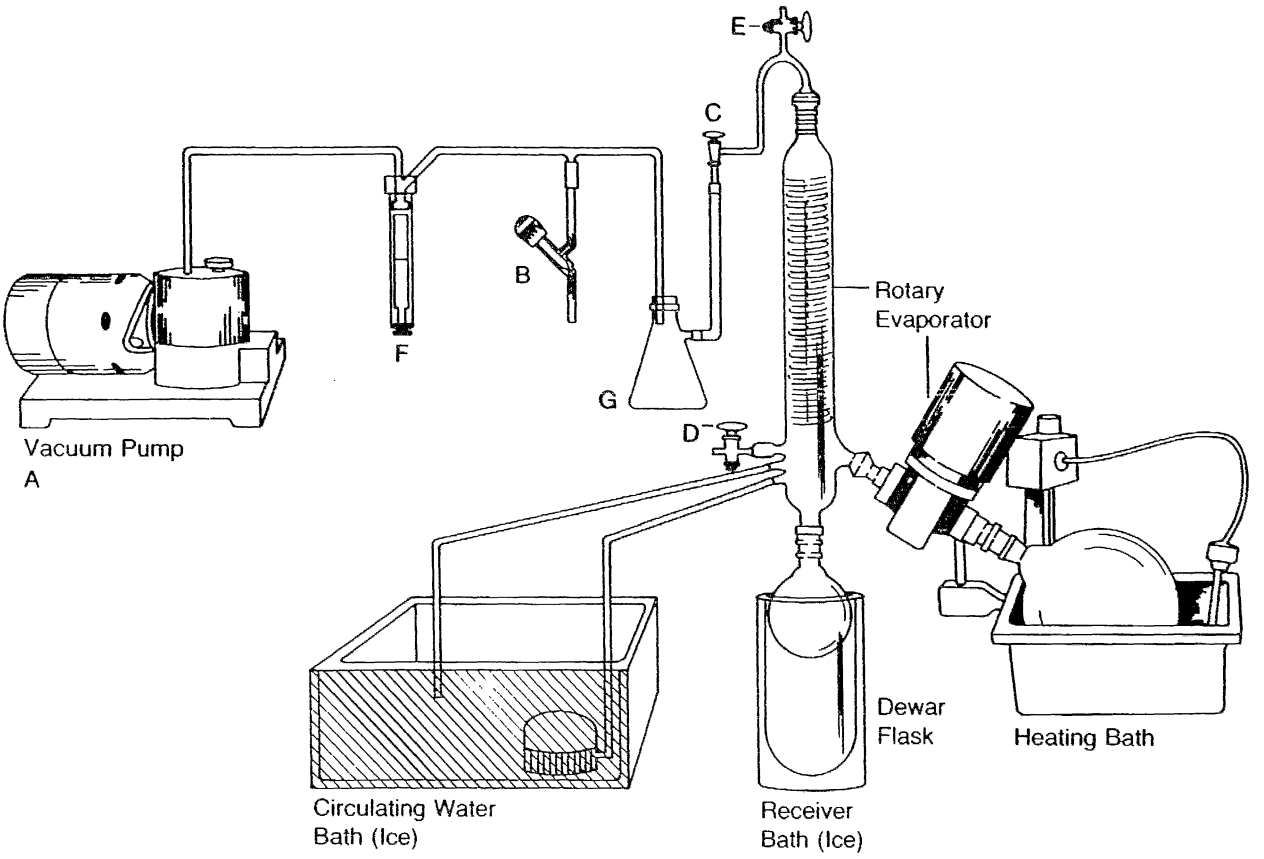
B. EQUIPMENT (Continued)

Figure 1—Distillation Apparatus



A—Vacuum Pump
B—Variable Air Bleed
C—Isolation Valve
D—Vacuum Take-Off (Calibration)
E—Vent
F—Vacuum Regulator (Cartesian)
G—Trap

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B. EQUIPMENT (Continued)

d. Chromatographic apparatus.

i. Chromatographic column, 10.5 mm id × 250 mm length with 200 mL reservoir (K-420280-213).

ii Chromatographic column 22 mm id × 300 mm length (K-420540-233).

iii. Pasteur pipet, disposable, 9 inch (K-88350).

e. Water bath, circulating, with temperature controller, (Precision model 260, or equivalent).

2. Instrumentation

a. GC/TEA Shimadzu 4 CM gas chromatograph, or equivalent, interfaced to Thermo Electron 502 thermal energy analyzer.

b. GC/MS Pye Unicam 104 interfaced to AEI MS30 by all-glass jet separator.

c. GC/MS—Hewlett Packard 5992 with capillary interface and splitless injector.

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C. REAGENTS AND SOLUTIONS

Reagent and Solutions List

a. Dichloromethane (DCM). Concentrate 200 mL of each lot to 0.5 mL and check for interfering peaks. Extract with equal volume 6N HCl prior to use. Discard the HCl layer.

b. Pentane. Concentrate 100 mL of each lot to 1 mL and check for interfering peaks.

c. Methanol, anhydrous.

d. Hexane.

e. Acetone.

f. Water, glass-distilled. Do not pass through ion exchange resins.

g. Potassium hydroxide, reagent grade. Prepare 3N and 1N solutions.

h. Hydrochloric acid, concentrated, reagent grade. Prepare 6N solution.

i. Phosphoric acid, reagent grade. Prepare 6N solution.

j. Alumina, basic activity I (Fisher Scientific Co.). Heat overnight at 300° C. Deactivate 6% (w/w) with distilled water. Store in stoppered container in desiccator. Alumina is suitable for chromatography if cleanup procedure (refer to section F.4), using 1.0 mL mixed nitrosamine std (~.25 μg/mL in DCM), yields at least 85% recovery of NDMA, NPIP, NPYR, and NMOR with no traces of these compounds in the first eluate, and reagent blank shows no interfering peaks.

k. Celite 545, not acid-washed (Fisher Scientific Co.). Activate overnight at 700° C. Store in stoppered container in desiccator. Celite is suitable for chromatography if cleanup procedure (refer to section F.5), using 1.0 mL mixed nitrosamine standard (~.25 μg/mL in DCM), yields at least 85% recovery for NDMA, NPYR, and NMOR, and reagent blank shows no interfering peaks.

l. Silicon carbide granules, 12 mesh (Hengar Granules), or equivalent.

m. Glass wool, fine, for column chromatography.

n. Ice cubes, crushed ice, or dry ice for cooling bath.

o. Sodium sulfate, anhydrous, granular, reagent grade.

NOTE: All solvents listed are glass-distilled (Burdick and Jackson Laboratories, Muskegon, MI).

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D. STANDARDS

1. Source

N-nitrosamine standards to be obtained directly from IITRI, after clearance through FSIS, from: Program Manager, Chemical Respository, Illinois Institute of Technology Research Institute, 10 W 35th Street, Chicago, Illinois 60616.

2. Preparation of Standards

The stock mixed nitrosamine standard consists of an iso-octane solution containing approximately 5μg/mL of N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodipropylamine (NDPA), N-nitrosodibutylamine (NDBA), N-nitrosopiperidine (NPIP), N-nitrosopyrrolidine (NPYR), and N-nitrosomorpholine (NMOR). A separate NPDA standard is provided for use as a surrogate. Prepare working standards for the determinative method at a concentration of about 0.25 μg/mL by diluting the stock standards. GC injection standards are prepared by diluting the stock mixed standard with dichloromethane. Fortification standards are prepared by diluting the stock mixed and NDPA standards with methanol. Standards for GC/MS confirmation are prepared by dilution of the stock standards with hexane to a concentration of 1-2 μg/mL or more.

DETERMINATIVE METHOD

E. EXTRACTION PROCEDURE

1. Adjusting Distillation Procedures

a. Refer to Figure 1, section B. Assemble distillation apparatus as shown. Prior to running samples, adjust the vacuum regulator to maintain the desired pressure. Using a vacuum gauge or manometer, obtain a zero reading by attaching the gauge directly to the vacuum pump (A) and recording the pressure. Re-attach the vacuum pump to the distillation apparatus and attach the vacuum gauge to the vacuum take-off point (D). Turn on the pump and adjust the air bleed (B) until a gauge pressure reading 19-21 torr higher than the zero value is achieved. Adjust the vacuum regulator to maintain this pressure using the instructions provided with the instrument.

b. Fill water bath to a normal operating level and adjust the temperature regulator to maintain a bath temperature in the range of 45°-50° C.

2. Distillation

a. Weigh 25 g sample into a 2 L round-bottom flask. Add 200 mL 3N KOH and 1.0 mL NDPA (0.25 μg/mL in methanol).

b. Attach distilling flask to evaporator and 250 mL round-bottom receiver to condenser.

c. Immerse receiver in an ice bath (0°-3° C) and begin circulation of ice water through condenser.

d. Immerse distilling flask in heating bath at 45°-46° C, apply vacuum, and turn on rotary evaporator. [CRITICAL STEP: Temperature must be maintained at no lower than 45° C.)

e. Adjust air bleed (B), if necessary, to ensure that vapors condense no further than one-third the way up the condenser. If excessive foaming occurs, reduce vacuum with air bleed, as necessary, to prevent mechanical transfer of distillate, until foaming condition diminishes.

f. As distillation proceeds, increase vacuum until full vacuum (20 torr) is reached. Full vacuum should be reached within 10 min of onset of distillation.

g. Continue distillation until drip rate from condenser averages less than one drop per minute. Total distillation time should be about one hour.

h. When distillation is complete, turn off rotary evaporator. Disconnect vacuum, using isolation valve (C), release vacuum, using vent (E), and remove receiver.

i. Transfer contents of receiver to a 250 mL mixing cylinder.

j. Reattach receiver to condenser, and using a large syringe, thoroughly wash down the walls and coils of the condenser with about 160 mL DCM, taking care to wash out all remaining aqueous distillate. Remove receiver and save for following step.

DETERMINATIVE METHOD

E. EXTRACTION PROCEDURE (Continued)

3. Extraction and Cleanup

a. Acidify aqueous distillate with 4 mL concentrated HCl and mix by shaking. Note volume, then transfer to a 500 mL separatory funnel.

b. Fill mixing cylinder with DCM rinse from condenser. Add additional DCM, if necessary, until volume is equal to that of acidified distillate.

c. Mix by shaking and add to aqueous distillate in separatory funnel. Extract distillate by shaking vigorously for 1 min.

d. Allow to stand until layers separate, then drain DCM layer into a second 500 mL separatory funnel.

e. Extract distillate with a second equal volume of DCM as before, and pool extracts. Discard distillate.

f. Wash combined extracts with 50 mL 6N HCl, shaking vigorously for 1 min. After phases separate, drain lower layer into a third 500 mL separatory funnel.

g. Backwash acid twice with 50 mL portions DCM, shaking 1 min each time.

h. Add first backwash to extract in third funnel. Save second backwash to extract KOH solution in step k.

i. Wash DCM in third funnel with 50 mL 1N KOH, shaking 1 min.

j. Allow phases to separate; then drain DCM phase through a 60 mm glass fritted funnel containing 30 g sodium sulfate (prewashed with 30 mL DCM) into a 500 mL Kuderna Danish concentrator fitted with a 4 mL graduated concentrator tube. This is necessary to remove traces of water from the extract.

k. Drain second acid backwash into third funnel now containing 50 mL KOH. Shake 1 min and allow phases to separate. Drain DCM layer through sodium sulfate into concentrator.

l. Add 1 mL hexane and a silicon carbide granule, attach Snyder Column, and evaporate on a circulating water bath at 60°-65° C, to 4-5 mL at a rate not to exceed 3 mL per min. Allow vapor to condense completely. Remove concentrator tube, attach micro Snyder Column, and add a second silicon carbide granule. Immerse tip of tube in 60°-65° C bath and evaporate to about 0.8-0.9 mL (Critical Step—Do Not Evaporate with Nitrogen). Remove from bath and wash down column with a few drops DCM. Cool, and adjust volume to 1.0 mL with DCM. Stopper tube.

DETERMINATIVE METHOD

F. ANALYTICAL QUANTITATION

1. Instrumental Settings and Conditions

a. GC/TEA Shimadzu 4 CM gas chromatograph, or equivalent, interfaced to Thermo Electron 502 thermal energy analyzer.

GC conditions: 2.7 m × 3 mm id glass column packed with 10% Cabowax 20 M + 5% KOH on 100/120 chromosorb PAW; injection port 200° C; interface 280° C isothermal; nitrogen carrier gas 25-30 mL/min TEA conditions: furnance 470° C; liquid nitrogen trap; initial and working vacuum settings and oxygen flow must be determined for particular instrument being used.

Recorder response should be > 10% for 1.5 ng N-nitrospyrrolidine. Resolution (R) between N-nitrosopiperdine and N-nitrosopyrrolidine should not be < 0.8 when calculated as follows:

$$R = \frac{T_2 - T_1}{\frac{1}{2}(W_1 + W_2)}$$

where T_1 and T_2 = retention times (mm) of N-nitrosopiperdine and N-nitrosopyrrolidine; W_1 and W_2 = peak width as base (mm) of N-nitrosopiperdine and N-nitrosopyrrolidine.

b. GC/MS Pye Unicam 104 interfaced to AEI MS 30 by all-glass jet separator.

GC conditions: 9 ft × 3 mm id glass column packed with 5% FFAP on 60/80 chromosorb W HP: injection port 160° C, column temperature 145° C isothermal; jet separator 180° C; helium flow 18 mL/min. MS parameters:

MS 30: Temperature at ion source = 200° C
Resolution of 10% valley definition = 3300
Temperature at jet separator = 180° C

c. GC/MS Hewlett-Packard 5890 gas chromatograph with split/splitless capillary inlet, interfaced via open-split interface to a Hewlett-Packard model 5970 Mass Selective Detector.

GC conditions: Column = HP-1 (bonded methyl silicone) capillary, 0.2 mm × 12 m × 0.33 μm thick. Carrier gas: helium; inlet pressure: 12 psi. Injection mode: Grob splitless; purge delay: 40 sec. Temperatures: inlet 150° C, interface 245° C. Column temperature program: Hold 2 min at 50° C; ramp to 110° C at 5° C/min; ramp to 245° C at 20° C/min; hold 1 min. Elution times: NPYR 6.9, NMOR 7.1, NDPA 7.7, NPIP 8.2, NDBA 13.3.

2. General Operations

Inject a suitable aliquot on GC-TEA and quantitate all nitrosamines. Use standards at ~.25 μg/mL in DCM for quantitation. NDPA recovery must be at least 80%, or distillation should be repeated. If samples require dilution for quantitation, dilute with n-pentane, *not DCM,* or additional cleanup for mass spectral confirmation will be compromised. If samples must be stored overnight prior to further cleanup, add sufficient n-pentane to bring volume to 4-5 mL.

DETERMINATIVE METHOD

F. ANALYTICAL QUANTITATION (Continued)

Samples containing any nitrosamine in concentrations equal to or exceeding 10 ppb are violative, provided that the presence of the nitrosamine can be positively confirmed by mass spectrometry. When a sample requires confirmation, the tissue blank and recovery that have been analyzed concurrently must also be analyzed as confirmation samples.

3. Mass Spectral Confirmation—Additional Cleanup

The nature and amount of interfering substances remaining in the concentrate after initial distillation and cleanup varies markedly from sample to sample. Most bacon samples will require further cleanup prior to successful confirmation by mass spectrometry. Cleanup procedures using alumina and acid-celite column chromatography have been investigated. Acid celite usually provides better overall cleanup for samples containing NPYR and NMOR, while alumina is more effective when NDEA, NDPA, NDBA, or NPIP are present. The column of choice for samples containing NDMA is best determined experimentally by the analyst.

4. Alumina Column Chromatography

a. Add a small glass wool plug to a 10.5 mm × 250 mm chromatographic column. Rinse column with 5 mL DMC, then 5 mL n-pentane, allowing rinses to drain. Close stopcock and add 15 mL pentane to column. Add 5 g alumina (use of a long stem funnel is helpful) and allow to settle. Wash down any alumina that may adhere to column walls with a small amount of n-pentane. Add a layer of sodium sulfate about 1 cm thick to top of column, then drain n-pentane to a level 1 mm above top of sodium sulfate. Add 3 mL n-pentane to column.

b. Using a Pasteur pipet, transfer sample (mixed with 3 mL pentane) to column. Rinse concentrator tube twice with 2 mL n-pentane, adding rinsings to column. If sample contains NDEA, NDPA, or NDBA, place a 250 mL Kuderna- Danish apparatus with 4 mL concentrator tube under column. Drain sample onto column at 60-80 drops/min until level is 1 mm above top of sodium sulfate. Wash down walls of column with 2-3 mL n-pentane and drain to same level.

c. Add 50 mL 20% DCM in n-pentane to column and elute at 60-80 drops/min until level reaches top of column. Momentarily stop flow and replace concentrator, if used, with another Kuderna-Danish apparatus. Add 60 mL DCM to column and drain into concentrator at 60-80 drops/min. If initial fraction was collected (for NDEA, NDPA, NDBA), add 25 mL DCM to that fraction. Add 1 mL hexane and a silicon carbide granule to all fractions collected and concentrate to 4-5 mL on a 55-60° C circulating water bath at a maximum rate of 3 mL/min. Concentrate to approximately 0.5-1.0 using a micro Snyder column as previously described.

5. Acid Celite Chromatography

a. Weigh 5.0 g celite into a 150 mL beaker. Add 7.5 mL 6N H_3PO_4 in approximately 2.5 mL portions, stirring vigorously with a glass rod to assure a uniform mixture that is fluffy in consistency.

b. Pack mixture into a 22 mm id × 300 mm chromatographic column containing a small glass wool plug. Compress celite to 4.5 cm, placing a small glass wool plug on top of absorbent.

c. Add 10 mL n-pentane to column, and drain until liquid layer is 1 mm above top of celite layer.

DETERMINATIVE METHOD

F. ANALYTICAL QUANTITATION (Continued)

d. Using a Pasteur pipet, quantitatively transfer sample onto column using sufficient pentane so that the volume of sample plus washes is approximately 10-12 mL.

e. Drain sample onto column at 60-80 drops/min, stopping when liquid level is 1 mm above celite.

f. Wash column walls with 3-4 mL pentane, draining to previous level.

g. Add 70 mL 2% DCM in pentane to column and drain at 60-80 drops/min.

h. Pass 60 mL DCM through column, collecting eluate in a 250 mL Kuderna-Danish apparatus.

i. Add 1 mL hexane and concentrate as described under alumina column chromatography.

6. Preparing Samples for Mass Spectrometry

Transfer concentrate from sections F.3 or F.4 to a 1 mL reaction vial or centrifuge tube graduated in increments of at least 0.1 mL. Adjust the volume of the sample so that its nitrosamine concentration matches that of the standard to be used for the confirmation. (For example, if the standard injected has a nitrosamine concentration of 5 μg/mL, a sample quantitated at 10 ppb will have to be reduced in volume to 50 μl). Concentrate the sample slowly, using a gentle stream of dry nitrogen.

7. Mass Spectrometry

Successful confirmation by mass spectrometry requires that both the GC retention and the monitored ion fragments of the sample match those of an injected standard. The possibilities of false positives should be compensated for by injections of a tissue blank and a recovery, respectively, in the same run. Both a magnetic sector instrument operating at medium resolution and a quadrupole have been used for confirmation. Parameters for both are listed below.

a. Confirmation parameters—AEI MS 30.

i. Calibration of the mass spectrometer.

The mass spectrometer should be calibrated with perfluorokerosene (PFK) or other suitable calibration compounds for the mass range of interest with the 10% valley definition resolution better than 3000. to separate 29.9980 to 30.0343 and 28.0188 to 28.0061 (H_2^+. ionized from the residual air in vacuum system).

ii. Confirmation of nitrosamines in study sample.

The gas chromatographic retention time of a purported nitrosamine in a study sample should be within $\pm$2% of the nitrosamine in the standard sample or fortified blank.

DETERMINATIVE METHOD

F. ANALYTICAL QUANTIATION (Continued)

The presence of the molecular ion (M^+) (or quasimolecular ion $(M + H)^+$ in case of using chemical ionization mode) and the most significant characteristic fragment ions for nitrosamines at m/z 29.9980 (NO^+) and at least two other fragment ions must be detected with the signal to noise ratio larger than two. These additional two fragment ions are usually of the following ions: m/z 28.0188 (CH_2N^+), 29.0266 (CH_3N^+), 30.0343 (CH_4N^+), 41.0266 ($C^2H_3N^+$), 42.0344 ($C_2H_4N^+$),43.0422 ($C_2H_5N^+$), 44.0500 ($C_2H_6N^+$), and the $(M\text{-}NO)^+$, $(M\text{-}HNO)^+$, and $(M\text{-}H_2\ NO)^+$ ions.

b. Confirmation parameters—HP 5890/5970 MSD.

i. Calibration of mass spectrometer.

Successful autotune calibration must be achieved prior to attempting confirmation. Operate at the highest practical resolution.

ii. The CC retention time in the sample should be within 3% of the standard or recovery. A minimum of 4 characteristic ions, including the molecular ion, must be detected. Ions to be monitored for each compound are: NDBA (158, 116, 141, 84, 57, 30); NPIP (114, 55, 56, 42, 39, 30); NPYR (100, 68, 69, 42, 39, 30); and NMOR (116, 86, 56, 55, 41, 30). Confirmation requires that the ion intensity ratios relative to the molecular ion for at least 3 ions in the sample match those of the standard or recovery within ± 20%.

Table 1. Nitrosamine Confirmation Parameters‡

Compound	*RT*	*Monitored Ions (Relative Ratios)*					
NPYR	6.9	100(1.0)	69(0.1)	68(0.2)	42(0.9)	39(0.3)	30(0.5)
NMOR	7.1	116(1.0)	86(0.9)	56(2.8)	55(0.3)	41(0.5)	30(1.3)
NPIP	8.2	114(1.0)	56(0.5)	55(0.9)	42(2.0)	39(0.4)	30(0.6)
NDBA	13.3	158(0.1)	84(1.0)	116(0.2)	141(0.1)	57(0.9)	30(0.5)

‡Actual retention times and relative ion ratios will vary depending upon the condition of the column used and tune of the mass spectrometer.

8. References

a. Sen, N. P., Private Communication, Canadian Dept. of Health and Welfare, Food Research Division, Ottawa, Ontario.

b. Sen, N. P., et al., *J. Agric. Food Chem.*, Vol. 27, No. 6, 1979, pg. 1354-1357.

DETERMINATIVE METHOD

H. HAZARD ANALYSIS

1. Method Title	Determination of Nitrosamines by the Low-Temperature Vacuum Distillation Procedure.		
2. Required Protective Equipment	Safety glasses, protective gloves (impermeable to dichloromethane), lab coat, shield.		
3. Procedure Steps		*Hazards*	*Recommended Safe Procedures*
	C. Reagents		
	(a) N-nitrosamine standards etc. Dilute with methylene chloride	The nitrosamines are potent carcinogens and the solvent itself is suspected of promoting tumors.	This dilution must be done in a fume hood, being cautious not to contaminate the area.
	D. Standards		
	(b) Distillation	*Explosion:* The Dewar flasks and distillation apparatus operation under vacuum can implode violently. *Cuts:* The distillation apparatus is fragile and is easily broken during assembly.	Check glassware for cracks or flaws. Use protective tape around vacuum glassware where feasible. All work should be performed behind a safety shield and the distillation assembly should not be repositioned once the system is under vacuum.
4. Disposal Procedures	Nitrosamine solution	See above.	Store in a prominently labelled container until disposal by contractor.
	Methylene chloride	See above	Store with chlorinated waste until disposal by contractor or in-house specialist.

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J. QUALITY ASSURANCE PLAN

1. Performance Standard

Compound	*Analtyical Range (ppb)†*	*Acceptable Recovery (%)‡*	*Repeatability CV (%)*
DMNA, NPYR, NMOR	10-20	70-110	8
DBNA	10-20	55-95	15

†For confirmation purposes.

‡With average not less than 80% based on last 10 recoveries. Not less than 80% for DPNA (up through TEA measurement—does not include GC/MS portion of methodology.)

2. Critical Control Points and Specifications

Record	*Acceptable Control*
a. Sample arrival.	
i. Temperature.	25-60° C.
ii. Regular bacon.	18-24 slices to the lb.
iii. Slab.	Cut 10 slices to the inch (about 20 per lb).
iv. Labeled package received.	Thin or thick sliced, do not analyze.
b. Frying protocol.	
i. Weight of bacon used in frying.	2 lb, 1¾ lb if proximate analysis is not run.
ii. Bacon slices.	Randomly selected.
iii. Skillets.	Calibrated daily, to produce a minimum temperature of 340°-345° F (171°-174° C) in the oil. Written records to be kept for each skillet. Readjustments and calibration until required temperature is obtained.
iv. Frying.	3 min on each side for each individual strip. Begin timing when first strip is laid in skillet. At the end of exactly 3 min, each strip is flipped over in the same sequence they were placed in the skillet.
v. Excess fat.	Removed by blotting with paper towels.
vi. Fried sample.	Ground after freezing using an 11/32-inch grinder plate, or Waring blender-type food processor, or equivalent.

DETERMINATIVE METHOD

J. QUALITY ASSURANCE PLAN (Continued)

	Record	*Acceptable Control*
	vii. Temperature.	45-46° C.
	viii. Concentration method.	Do not use any inert gas (N_2) or air to assist in the evaporation. Use Kuderna-Danish evaporators, topped with micro Snyder columns, on a steam bath or water bath.

NOTE: If internal standard recovery is not in the proper range, the sample is to be reanalyzed.

3. Readiness To Perform

a. Familiarization.

i. Phase I: Standards—For this analytical procedure, there is no standard curve preparation. Pure standards are not taken through the method. This phase of familiarization is therefore waived.

ii. Phase II: Fortified samples—Blanks and 4 nominal levels at 7, 10, 15, and 20 ppb over a minimum of 4 days for DMNA, NPYP, and NMOR (20 samples).

iii. Phase III: Check samples for analyst accreditation.

(a) 15 samples from FSIS Science Eastern Laboratory.

(b) Report analytical findings to Chemistry Division.

Notification from Chemistry Division required to commence offical analysis.

b. Acceptability Criteria.

Refer to section J.1 above.

4. Intralaboratory Check Samples

a. System, minimum contents.

i. Frequency: 1 per week per analyst.

ii. Blind samples (Requires "dummy" forms) or random replicates chosen by supervisor after initial analysis.

iii. Records to be maintained by analyst and reviewed by supervisor and Laboratory QA Officer.

(a) All replicate findings.

(b) Control chart on difference between replicates.

(c) All % recoveries.

(d) For all recoveries: the running average, standard deviation, and coefficient of variation.