

8.1.06

AOAC Official Method 986.01
N-Nitrosodibutylamine
in Latex Infant Pacifiers
Gas Chromatographic Method
First Action 1986
Final Action 1992

A. Principle

Volatile *N*-nitrosamines are extracted from chopped latex pacifier nipples with CH_2Cl_2 . Extract is concentrated and subjected to high temperature purge and trap. *N*-nitrosamines are eluted from trap and determined by GC with thermal energy analysis.

B. Reagents

Use all glass-distilled solvents (Burdick & Jackson Laboratories, Inc., or equivalent).

(a) *N*-Nitrosamine stock standard solutions.—(1) *External standard stock solution*.—10 g/mL each of NDMA (*N*-nitrosodimethylamine), NDEA (*N*-nitrosodiethylamine), NDPA (*N*-nitro-sodipropylamine), NDBA (*N*-nitrosodibutylamine), NPIP (*N*-nitrosopiperidine), NPYR (*N*-nitrosopyrrolidine), and NMOR (*N*-nitrosomorpholine) in alcohol.

(*Caution*: Volatile *N*-nitrosamines pose an extreme health hazard. Carry out all manipulations involving handling neat liquids or solutions in adequately ventilated and filtered fume hood or glove box.)

(b) *Mineral oil*.—White, lightweight with Saybolt viscosity 125/135 (No. 6358, Mallinckrodt Chemical Works or equivalent).

(c) *Nitrosation inhibitor*.—10 mg -tocopherol/mL mineral oil.

(d) *Keeper solutions*.—(1) *For Kuderna-Danish evaporation*.—80 mg mineral oil/mL CH_2Cl_2 . (2) *For ThermoSorb/N evaporation*.—20 mg mineral oil/mL isooctane.

C. Apparatus

(a) *ThermoSorb/N™ cartridges*.—Use as received for quantitative trapping of volatile *N*-nitrosamines (Thermedics Detection, Inc., 220 Mill Rd, Chelmsford, MA 01824, USA).

(b) *Variable temperature oil bath*.—Thermostatically controlled, capable of operating at 150–300 °C and of moving vertically with aid of laboratory jack.

(c) *Soxhlet extraction apparatus*.—(Kimble Glass, Inc.) Suitable for an extraction thimble, 25 × 85 mm, borosilicate glass, fitted with coarse porosity frit.

(d) *Kuderna–Danish (K-D) evaporative concentrator*.—(Kontes Glass Co.) 3-ball Snyder column with 24/40 standard taper joints, 250 mL flask with 24/40 standard taper joint and 19/22 standard-taper lower joint, and 4 mL graduated concentrator tube with 19/22 standard taper joint.

(e) *Gas chromatograph*.—Agilent Technologies (Division of Hewlett Packard, 2850 Centerville Rd, Wilmington, DE 19808, USA) 6890, or equivalent, equipped with 1.8 m × 4 mm id glass column packed with 10% Carbowax 20M/2% KOH on 80–100 mesh Chromosorb WAW (Supelco). Condition column overnight at 215 °C. Operate at temperature program mode from 150 to 190 °C at 4 °C/min. Injection port temperature 250 °C. Carrier gas prepurified argon at flow rate 40 mL/min. Interface GC apparatus to thermal energy analyzer, (f), via 3 mm od stainless steel tube connected with Swagelok fittings and heated to 170 °C.

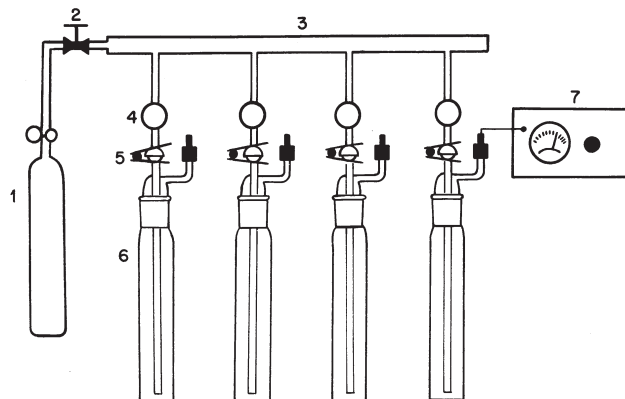


Figure 986.01A. Diagram of purge and trap apparatus equipped with 4 impinger tubes.

(f) *Thermal energy analyzer*.—Model 502, Thermo Electron Corp. (81 Wyman St, Waltham, MA 02454, USA), or equivalent. Operate pyrolysis chamber at 500 °C in GC mode. Oxygen flow to ozonator, 10 mL/min. Keep cold trap at –150 °C using liquid nitrogen/2-methylbutane slush bath. Pressure of reaction chamber, ca 0.9 torr. Record TEA detector response on Hewlett Packard 3380 (replaced by HP-5890 series II) integrator or equivalent.

(g) *Purge and trap apparatus*.—Figure 986.01A contains following parts: (1) Argon gas cylinder and gauge (Air Products Specialty Gas, Tamaqua, PA 18252, USA); (2) metering valve; (3) purge gas manifold, 4-position; (4) Nalgene needle valve type CPE (No. 6400-0125, Nalge Co., 75 Panorama Creek Dr, PO Box 20365, Rochester, NY 14602, USA); (5) 18/7 glass-glass outer joints with pinch clamps (No. 772398, The Wheaton Agency, A Division of Wheaton Industries, 1000 N. Tenth St, Millville, NJ 08332, USA); (6) impingers, 50 mL graduated glass tubes with 24/40 standard taper clearseal, grease-free joints 18/7 glass-glass ball joints, and 1 mm id nozzle ca 5 mm above bottom of impinger (No. 753463, Wheaton Scientific); (7) variable scale flow-meter, calibrated for purge rate in mL argon/min (No. 7083, Alltech Associates, Inc.). Bubble meter for measuring gas flow rates for GC may be substituted. (*Note*: Do not use rubber tubing, gaskets, O-rings, or other items made of rubber in any part of this method.)

D. Description and Use of Purge and Trap Apparatus

Apparatus shown in Figure 986.01A is designed for high temperature purging and trapping of 7 volatile *N*-nitrosamines from concentrated test portion extract/mineral oil mixture on 4 test portions simultaneously. Cylinder containing high purity argon gas equipped with high pressure regulator is used to supply 20 psig to flow-metering valve which regulates final purge flow through extracts. Gas stream is diverted into tubular stainless steel manifold, 25 × 20 mm od, containing 4 exit tubes spaced 50 mm apart and measuring 40 × 10 mm od. Each of these tubes is coupled using 1.0 cm Tygon tubing to Nalgene needle valves which serve dual purposes: as shutoff valve when less than 4 test extracts are analyzed; and for making minor adjustments in purge rate due to slight differences in flow characteristics of impinger and cartridges. An 18/7 glass-glass outer spherical joint is attached to Nalgene valve to permit quick gas-tight connection to 18/7 glass-glass ball joint on impinger inlet, using appropriate pinch clamp.

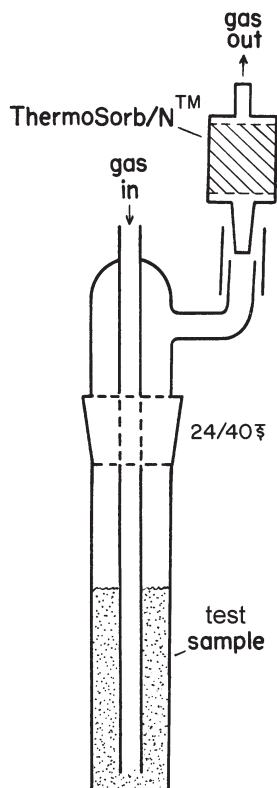


Figure 986.01B. Close-up diagram of impinger tube fitted with ThermoSorb/N cartridge.

As shown in Figure 986.01B, assemble impingers by inserting glass nozzle (1 mm id orifice) into test extract mixture and coupling 24/40 standard taper grease-free male and female joints together to form leak-free seal. Once sealed, allow argon gas to purge through test extract mixture and through outlet tube of impinger (see Figure 986.01B). Use Tygon tubing to connect impinger outlet tube to inlet side marked "AIR IN" of cartridge, which is standard male Luer connector. Collect purged volatile *N*-nitrosamines on sorbent contained in cartridge from Ar effluent exiting female Luer connector. Flow rate of argon is measured directly from cartridge with variable scale flow meter previously calibrated for flow rate of argon gas (mL/min). Control temperature of test sample mixture during purge by immersing impinger up to test extract volume mark (ca 25 mL line) in thermostatically controlled oil bath. Secure gas manifold and impingers by clamps to a support grid and move oil bath vertically into and out of position.

E. Extraction and Cleanup of Pacifier Samples

(*Caution:* Perform entire procedure in a fume hood. Methylene chloride is a suspected carcinogen. Use extreme caution to avoid exposure.)

Accurately weigh 5 g of each chopped test sample into 250 mL round-bottom flask and add 100 mL CH₂Cl₂. Dilute internal standard stock solution to 50 ng/mL with CH₂Cl₂ and spike contents of flask with 2.0 mL diluted standard. Seal flask with glass stopper and let contents stand overnight (16–21 h) at 18 ± 25°C.

Carefully transfer extract and rubber pieces to glass extraction thimble fitted with coarse porosity glass frit in Soxhlet extraction

apparatus. Rinse test sample flask with 25 mL CH₂Cl₂ and transfer rinse to Soxhlet apparatus. Extract rubber pieces for 1 h in apparatus at rate of ca 8 cycles/h.

Let cool and transfer CH₂Cl₂ extract to 250 mL K–D evaporator. Rinse extraction flask with two 10 mL portions of CH₂Cl₂ and add rinses to evaporator. Add 1 mL keeper solution 1 and 2 or 3 boiling chips to extract. Evaporate extract in K–D unit, C(d), with 55°C water bath until volume is reduced to 3–4 mL.

Let K–D unit cool to room temperature, allowing excess solvent in Snyder column to rinse down walls of unit into 4 mL K–D tube (extract volume ca 3–4 mL). Remove 250 mL reservoir and column. Reduce volume of extract to 2 mL in same K–D tube under gentle stream of nitrogen (ca 50 mL/min). Transfer 2 mL extract (using disposable Pasteur pipet) and two 1 mL mineral oil rinses to the 50 mL purge and trap apparatus containing 20 mL mineral oil as nitrosation inhibitor.

Connect cartridges to purge and trap exit tubes with Tygon tubing and adjust argon flow rate to 400 mL/min 5% through cartridge. Flow rate must be maintained at 400 mL/min during initial increase in temperature of test extract. Immerse purge tubes (up to liquid line) or to 25 mL mark in 150 ± 3°C oil bath for 1.5 h. Remove cartridge and tightly cap. (*Note:* Cartridge can be eluted on the following day.)

Elute cartridge using 20 mL glass Luer-Lok syringe connected to female Luer adapter (air exit side) with 20 mL acetone–CH₂Cl₂ (1 + 1, v/v). Collect eluate in 30 mL culture tube. (*Note:* 30 mL tube(s) should be marked with file or tape placed at ca 5 mL volume mark.)

Evaporate extract to ca 5 mL and then transfer (with three 1 mL rinses of CH₂Cl₂) to 10 mL graduated tube. Add 0.5 mL keeper solution 2. Evaporate sample to 2 mL under gentle stream of nitrogen. (*Note:* If 2 mL test extract cannot be analyzed following evaporation, refrigerate test extract at 4–5 mL volume and finish evaporation the next day before analysis by GC-TEA.)

Test extract volumes of 8 L are injected into GC-TEA.

F. Quantitation

Use internal standard technique. Dilute stock external standard solution with CH₂Cl₂ to 50, 100, and 200 ng/mL to be used as working standards for analysis. Inject 8 L into GC-TEA to determine responses (peak heights) of NDPA and other nitrosamines for use in internal standardization calculation. Inject 8 L of each 2 mL test extract into GC-TEA. Determine responses (peak heights) of NDPA and any other *N*-nitrosamines detected for use in internal standardization calculation. Calculate results as follows:

$$\text{ppb } N\text{-Nitrosamine X} = \frac{\text{PH}_x \cdot F_x \cdot W_{\text{NDPA}}}{\text{PH}_{\text{NDPA}} \cdot F_{\text{NDPA}} \cdot \text{g test portion}}$$

where PH_x = peak height in mm of *N*-nitrosamine X in extract; F_x = ng *N*-nitrosamine X/mL in external standard solution divided by peak height in mm of *N*-nitrosamine X in external standard solution; W_{NDPA} = total ng NDPA (internal standard) added to test extract; PH_{NDPA} = peak height in mm of NDPA (internal standard) in test extract; F_{NDPA} = ng NDPA/mL in external standard solution divided by peak height in mm of NDPA in external standard solution; g test portion = g rubber test portion analyzed.

Reference: *JAOAC* 69, 504(1986).