METHOD 8070

NITROSAMINES BY GAS CHROMATOGRAPHY

## 1.0 SCOPE AND APPLICATION

1.1 This method covers the determination of certain nitrosamines. The following compounds can be determined by this method:

| | | Appropriate Technique | | | | |
|---|---|---|---|---|---|---|
| Compound Name | CAS No.[a] | 3510 | 3520 | 3540 | 3550 | 3580 |
| N-Nitrosodimethylamine | 62-75-9 | X | X | X | X | X |
| N-Nitrosodiphenylamine | 86-30-6 | X | X | X | X | X |
| N-Nitrosodi-n-propylamine | 621-64-7 | X | X | X | X | X |

a Chemical Abstract Services Registry Number.
X Greater than 70 percent recovery by this preparation technique.

1.2 This is a gas chromatographic (GC) method applicable to the determination of the parameters listed above in municipal and industrial discharges. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Method 8270 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for N-nitrosodi-n-propylamine. In order to confirm the presence of N-nitrosodiphenylamine, the cleanup procedure specified in Section 7.3.3 or 7.3.4 must be used. In order to confirm the presence of N-nitrosodimethylamine by GC/MS, chromatographic column 1 of this method must be substituted for the column recommended in Method 8270. Confirmation of these parameters using GC-high resolution mass spectrometry or a Thermal Energy Analyzer is also recommended practice.

1.3 The method detection limit (MDL) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix. Table 2 lists the Estimated Quantitation Limits (EQLs) for various matrices.

1.4 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. However, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible concentration by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also

be made available to all personnel involved in the chemical analysis.

1.5 These nitrosamines are known carcinogens. Therefore, utmost care must be exercised in the handling of these materials. Nitrosamine reference standards and standard solutions should be handled and prepared in a ventilated glove box within a properly ventilated room.

1.6 N-Nitrosodiphenylamine is reported to undergo transnitrosation reactions. Care must be exercised in the heating or concentrating of solutions containing this compound in the presence of reactive amines.

## 2.0 SUMMARY OF METHOD

2.1 A measured volume of aqueous sample, approximately one liter, is solvent extracted with methylene chloride using a separatory funnel. The methylene chloride extract is washed with dilute HCl to remove free amines, dried, and concentrated to a volume of 10 mL or less. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract after it has been exchanged to methanol.

2.2 Method 8070 provides gas chromatographic conditions for the detection of ppb concentrations of nitrosamines. Prior to use of this method, appropriate sample extraction techniques must be used. Both neat and diluted organic liquids (Method 3580, Waste Dilution) may be analyzed by direct injection. A 2 to 5 µL aliquot of the extract is injected into a gas chromatograph (GC) using the solvent flush technique, and compounds in the GC effluent are detected by a nitrogen-phosphorus detector (NPD) or a Thermal Energy Analyzer and the reductive Hall detector.

## 3.0 INTERFERENCES

3.1 Refer to Methods 3500, 3600, and 8000.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedures (Methods 3610 or 3620) can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

3.3 Nitrosamines contaminate many types of products commonly found in the laboratory. The analyst must demonstrate that no nitrosamine residues contaminate the sample or solvent extract under the conditions of analysis. Plastics, in particular, must be avoided because nitrosamines are commonly used as plasticizers and are easily extracted from plastic materials. Serious nitrosamine contamination may result at any time if consistent quality control is not practiced.

3.4 The sensitive and selective Thermal Energy Analyzer and the reductive Hall detector may be used in place of the nitrogen-phosphorus detector when

interferences are encountered. The Thermal Energy Analyzer offers the highest selectivity of the non-mass spectrometric detectors.

3.5 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All these materials must be demonstrated to be free from interferences, under the conditions of the analysis, by analyzing reagent blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.

3.6 Interferences coextracted from samples will vary considerably from source to source, depending upon the waste being sampled. Although general cleanup techniques are recommended as part of this method, unique samples may require additional cleanup.

## 4.0 APPARATUS AND MATERIALS

4.1 Gas chromatograph - An analytical system complete with temperature programmable gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

4.1.1 Column 1 - 1.8 m x 4 mm ID Pyrex glass, packed with Chromosorb W AW, (80/100 mesh) coated with 10% Carbowax 20 M/2% KOH or equivalent. This column was used to develop the method performance statements in Section 9.0. Guidelines for the use of alternate column packings are provided in Section 7.3.2.

4.1.2 Column 2 - 1.8 m x 4 mm ID Pyrex glass, packed with Supelcoport (100/120 mesh) coated with 10% SP-2250, or equivalent.

4.1.3 Detector - Nitrogen-Phosphorus, reductive Hall or Thermal Energy Analyzer. These detectors have proven effective in the analysis of wastewaters for the parameters listed in the scope. A nitrogen-phosphorus detector was used to develop the method performance statements in Section 9.0. Guidelines for the use of alternate detectors are provided in Section 7.3.2.

4.2 Kuderna-Danish (K-D) apparatus

4.2.1 Concentrator tube - 10 mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. A ground glass stopper is used to prevent evaporation of extracts.

4.2.2 Evaporation flask - 500 mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs, clamps, or equivalent.

4.2.3 Snyder column - Three ball macro (Kontes K-503000-0121 or equivalent).

4.2.4 Snyder column - Two ball micro (Kontes K-569001-0219 or equivalent).

4.2.5 Springs - 1/2 inch (Kontes K-662750 or equivalent).

4.3 Boiling chips - Approximately 10/40 mesh. Heat to 400°C for 30 minutes or Soxhlet extract with methylene chloride.

4.4 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

4.5 Balance - Analytical, 0.0001 g.

4.6 Vials - 10 to 15 mL, amber glass with Teflon lined screw-cap or crimp top.

4.7 Volumetric flasks, Class A, Appropriate sizes with ground glass stoppers.

## 5.0 REAGENTS

5.1 Reagent grade inorganic chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all inorganic reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.2 Organic-free reagent water - All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

5.3 Methanol, $CH_3OH$ - Pesticide quality or equivalent.

5.4 Isooctane, $(CH_3)_3CCH_2CH(CH_3)_2$ - Pesticide quality or equivalent.

5.5 Methylene chloride, $CH_2Cl_2$ - Pesticide quality or equivalent.

5.6 Stock standard solutions (1000 mg/L) - Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

5.6.1 Prepare stock standard solutions by accurately weighing 0.1000 $\pm$ 0.0010 g of pure material. Dissolve the material in pesticide quality methanol and dilute to volume in a 100 mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

5.6.2 Transfer the stock standard solutions into bottles with Teflon lined screw-caps or crimp tops. Store at 4°C and protect from light.

Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

5.6.3 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicates a problem.

5.7 Calibration standards - A minimum of five concentrations should be prepared through dilution of the stock standards with isooctane. One of the concentrations should be at a concentration near, but above, the method detection limit. The remaining concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the GC. Calibration solutions must be replaced after six months, or sooner if comparison with check standards indicates a problem.

5.8 Internal standards (if internal standard calibration is used) - To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples.

5.8.1 Prepare calibration standards at a minimum of five concentrations for each analyte of interest, as described in Section 5.7.

5.8.2 To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with isooctane.

5.8.3 Analyze each calibration standard according to Section 7.0.

5.9 Surrogate standards - The analyst should monitor the performance of the extraction, cleanup (when used), and analytical system and the effectiveness of the method in dealing with each sample matrix by spiking each sample, standard, and reagent blank with one or two surrogates (e.g. nitrosamines that are not expected to be in the sample) recommended to encompass the range of the temperature program used in this method. Method 3500 details instructions on the preparation of base/neutral surrogates. Deuterated analogs of analytes should not be used as surrogates for gas chromatographic analysis due to coelution problems.

## 6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See the introductory material to this chapter, Organic Analytes, Section 4.1. Extracts must be stored at 4°C and analyzed within 40 days of extraction.

## 7.0 PROCEDURE

### 7.1 Extraction

7.1.1 Refer to Chapter Two for guidance on choosing the appropriate extraction procedure. In general, water samples are extracted at a neutral, or as is, pH with methylene chloride, using either Method 3510 or 3520. Solid samples are extracted using either Method 3540 or 3550.

7.1.2 Prior to gas chromatographic analysis, the extraction solvent must be exchanged to methanol. The exchange is performed during the K-D procedures listed in all of the extraction methods. The exchange is performed as follows.

7.1.2.1 Following K-D of the methylene chloride extract to 1 mL using the macro-Snyder column, allow the apparatus to cool and drain for at least 10 minutes.

7.1.2.2 Momentarily remove the Snyder column, add 50 mL of methanol, a new boiling chip, and reattach the macro-Snyder column. Concentrate the extract using 1 mL of methanol to prewet the Snyder column. Place the K-D apparatus on the water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature, as required, to complete concentration in 5-10 minutes. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. The extract will be handled differently at this point, depending on whether or not cleanup is needed. If cleanup is not required, proceed to Section 7.1.2.3. If cleanup is needed, proceed to Section 7.1.2.4.

7.1.2.3 If cleanup of the extract is not required, remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1-2 mL of methanol. A 5 mL syringe is recommended for this operation. Adjust the extract volume to 10.0 mL. Stopper the concentrator tube and store refrigerated at 4°C if further processing will not be performed immediately. If the extract will be stored longer than two days, it should be transferred to a vial with a Teflon lined screw-cap or crimp top. Proceed with gas chromatographic analysis.

7.1.2.4 If cleanup of the extract is required, remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with a minimum amount of methylene chloride. A 5 mL syringe is recommended for this operation. Add a clean boiling chip to the concentrator tube and attach a two ball micro-Snyder column. Prewet the column by adding about 0.5 mL of methylene chloride to the top. Place the micro K-D apparatus on the water bath (80°C) so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and

the water temperature, as required, to complete concentration in 5-10 minutes. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

7.1.2.5 Remove the micro-Snyder column and rinse the flask and its lower joint into the concentrator tube with 0.2 mL of methylene chloride. Adjust the extract volume to 2.0 mL and proceed with either Method 3610, 3620, or 3640.

7.1.3 If N-nitrosodiphenylamine is to be measured by gas chromatography, the analyst must first use a cleanup column to eliminate diphenylamine interference (Methods 3610 or 3620). If N-nitrosodiphenylamine is of no interest, the analyst may proceed directly with gas chromatographic analysis (Section 7.3).

7.2 Cleanup

7.2.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 85%. Diphenylamine, if present in the original sample extract must be separate from the nitrosamines if N-nitrosodiphenylamine is to be determined by this method.

7.2.2 Proceed with either Method 3610 or 3620, using the 2 mL methylene chloride extracts obtained from Section 7.1.2.5.

7.2.3 Following cleanup, the extracts should be analyzed by GC, as described in the previous paragraphs and in Method 8000.

7.3 Gas Chromatography

7.3.1 N-nitrosodiphenylamine completely reacts to form diphenylamine at the normal operating temperatures of a GC injection port (200 to 250°C). Thus, N-nitrosodiphenylamine is chromatographed and detected as diphenylamine. Accurate determination depends on removal of diphenylamine that may be present in the original extract prior to GC (see Section 7.1.3).

7.3.2 Table 1 summarizes the recommended operating conditions for the gas chromatograph. This table includes retention times and MDLs that were obtained under these conditions. Examples of the parameter separations achieved by these columns are shown in Figures 1 and 2. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

7.4 Calibration - Refer to Method 8000 for proper calibration techniques. Use Table 1 and especially Table 2 for guidance on selecting the lowest point on the calibration curve.

7.4.1 The procedure for internal or external calibration may be used. Refer to Method 8000 for a description of each of these procedures.

7.4.2 If cleanup is performed on the samples, the analyst should process a series of standards through the cleanup procedure and then analyze the samples by GC. This will confirm elution patterns and the absence of interferents from the reagents.

7.5 Gas chromatographic analysis

7.5.1 Refer to Method 8000. If the internal standard calibration technique is used, add 10 µL of internal standard to the sample prior to injection.

7.5.2 Method 8000 provides instructions on the analysis sequence, appropriate dilutions, establishing daily retention time windows, and identification criteria. Include a mid-concentration check standard after each group of 10 samples in the analysis sequence.

7.5.3 Examples of GC/NPD chromatograms for nitrosamines are shown in Figures 1 and 2.

7.5.4 Record the sample volume injected and the resulting peak sizes (in area units or peak heights).

7.5.5 Using either the internal or external calibration procedure (Method 8000), determine the identity and quantity of each analyte peak in the sample chromatogram. See Method 8000 for calculation equations.

7.5.6 If peak detection and identification are prevented due to interferences, the hexane extract may undergo cleanup using either Method 3610 or 3620.

## 8.0 QUALITY CONTROL

8.1 Refer to Chapter One for specific quality control procedures. Quality control to validate sample extraction is covered in Method 3500 and in the extraction method utilized. If extract cleanup was performed, follow the QC in Method 3600 and in the specific cleanup method.

8.2 Procedures to check the GC system operation are found in Method 8000, Section 8.6.

8.2.1 The quality control (QC) reference sample concentrate (Method 8000, Section 8.6) should contain each analyte of interest at 20 mg/L.

8.2.2 Table 3 indicates the calibration and QC acceptance criteria for this method. Table 4 gives method accuracy and precision as functions

of concentration for the analytes of interest. The contents of both Tables should be used to evaluate a laboratory's ability to perform and generate acceptable data by this method.

8.3 Calculate surrogate standard recovery on all samples, blanks, and spikes. Determine if the recovery is within limits (limits established by performing QC procedures outlined in Method 8000, Section 8.10).

8.3.1 If recovery is not within limits, the following is required.

- Check to be sure that there are no errors in calculations, surrogate solutions and internal standards. Also, check instrument performance.
- Recalculate the data and/or reanalyze the extract if any of the above checks reveal a problem.
- Reextract and reanalyze the sample if none of the above are a problem or flag the data as "estimated concentration.

## 9.0 METHOD PERFORMANCE

9.1 This method has been tested for linearity of recovery from spiked organic-free reagent water and has been demonstrated to be applicable for the concentration range from 4 x MDL to 1000 x MDL.

9.2 In a single laboratory (Southwest Research Institute), using spiked wastewater samples, the average recoveries presented in Table 2 were obtained. Each spiked sample was analyzed in triplicate on three separate occasions. The standard deviation of the percent recovery is also included in Table 2.

## 10.0 REFERENCES


1. Fed. Regist. 1984, 49, 43234; October 26.
2. "Determination of Nitrosamines in Industrial and Municipal Wastewaters"; Report for EPA Contract 68-03-2606, in preparation.
3. Burgess, E.M.; Lavanish, J.M. "Photochemical Decomposition of N-nitrosamines"; Tetrahedron Letters 1964, 1221.
4. Methods for Chemical Analysis of Water and Wastes; U.S. Environmental Protection Agency. Office of Research and Development. Environmental Monitoring and Support Laboratory. ORD Publication Offices of Center for Environmental Research Information: Cincinnati, OH, 1979; EPA-600/4-79-020.
5. "Method Detection Limit and Analytical Curve Studies EPA Methods 606, 607, 608"; U.S. Environmental Protection Agency. Environmental Monitoring and Support Laboratory, Cincinnati, OH, special letter report for EPA Contract 68-03-2606.

TABLE 1.
CHROMATOGRAPHIC CONDITIONS AND METHOD DETECTION LIMITS

| Analyte | Retention Time (minutes) Column 1 | Column 2 | Method Detection Limit (µg/L) |
|---|---|---|---|
| N-Nitrosodimethylamine | 4.1 | 0.88 | 0.15 |
| N-Nitrosodi-n-propylamine | 12.1 | 4.2 | 0.46 |
| N-Nitrosodiphenylamine[a] | 12.8[b] | 6.4[c] | 0.81 |

Column 1 conditions:
Carrier gas (He) flow rate: 40 mL/min
Column temperature: Isothermal, at 110°C, except as otherwise indicated.

Column 2 conditions:
Carrier gas (He) flow rate: 40 mL/min
Column temperature: Isothermal, at 120°C, except as otherwise indicated.

a Measured as diphenylamine.
b Determined isothermally at 220°C.
c Determined isothermally at 210°C.

TABLE 2.
SINGLE OPERATOR ACCURACY AND PRECISION

| Analyte Types | Average Percent Recovery | Standard Deviation % | Spike Range (µg/L) | Number of Analyses | Matrix |
|---|---|---|---|---|---|
| N-Nitrosodimethylamine | 32 | 3.7 | 0.8 | 29 | 5 |
| N-Nitrosodiphenylamine | 79 | 7.1 | 1.2 | 29 | 5 |
| N-Nitrosodi-n-propylamine | 61 | 4.1 | 9.0 | 29 | 5 |

TABLE 3.
QC ACCEPTANCE CRITERIA

| Analyte | Test Conc. (µg/L) | Limit for s (µg/L) | Range for X (µg/L) | Recovery Range (%) |
|---|---|---|---|---|
| N-Nitrosodimethylamine | 20 | 3.4 | 4.6-20.0 | 13-109 |
| N-Nitrosodiphenylamine | 20 | 6.1 | 2.1-24.5 | D-139 |
| N-Nitrosodi-n-propylamine | 20 | 5.7 | 11.5-26.8 | 45-146 |

s = Standard deviation for four recovery measurements, in µg/L.

$\bar{X}$ = Average recovery for four recovery measurements, in µg/L.

D = Detected, result must be greater than zero.

TABLE 4.
METHOD ACCURACY AND PRECISION AS FUNCTIONS OF CONCENTRATION

| Analyte | Accuracy, as recovery, X' (µg/L) | Single analyst precision, $s_r'$ (µg/L) | Overall precision, S' (µg/L) |
|---|---|---|---|
| N-Nitrosodimethylamine | 0.37C+0.06 | $0.25\overline{X}$-0.04 | $0.25\overline{X}$+0.11 |
| N-Nitrosodiphenylamine | 0.64C+0.52 | $0.36\overline{X}$-1.53 | $0.46\overline{X}$-0.47 |
| N-Nitroso-n-propylamine | 0.96C-0.07 | $0.15\overline{X}$+0.13 | $0.21\overline{X}$+0.15 |

X' = Expected recovery for one or more measurements of a sample containing a concentration of C, in µg/L.

$s_r'$ = Expected single analyst standard deviation of measurements at an average concentration found of $\overline{X}$, in µg/L.

C = True value for the concentration, in µg/L.

$\overline{X}$ = Average recovery found for measurements of samples containing a concentration of C, in µg/L.

FIGURE 1.
GAS CHROMATOGRAM OF NITROSAMINES

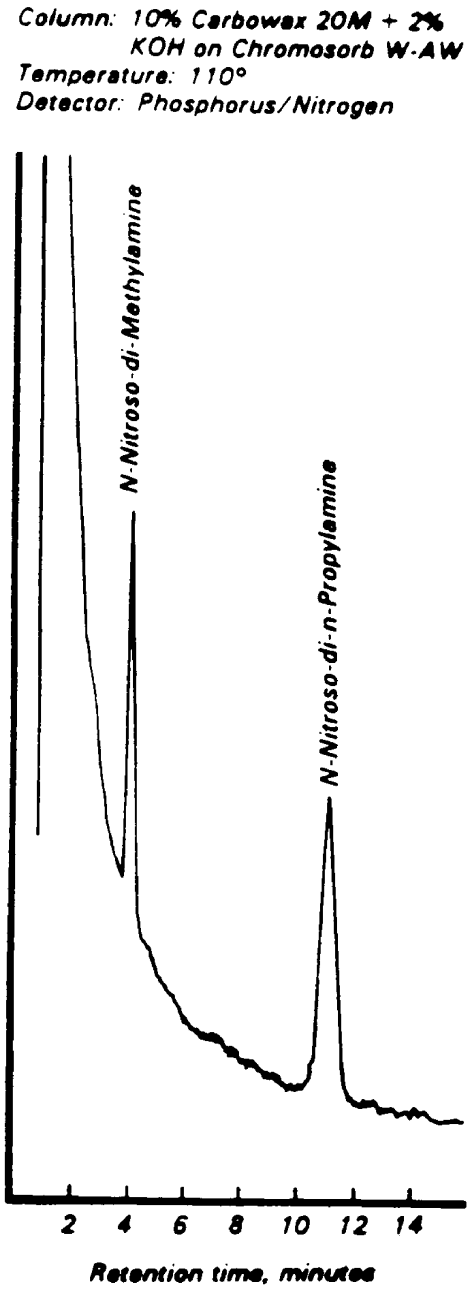
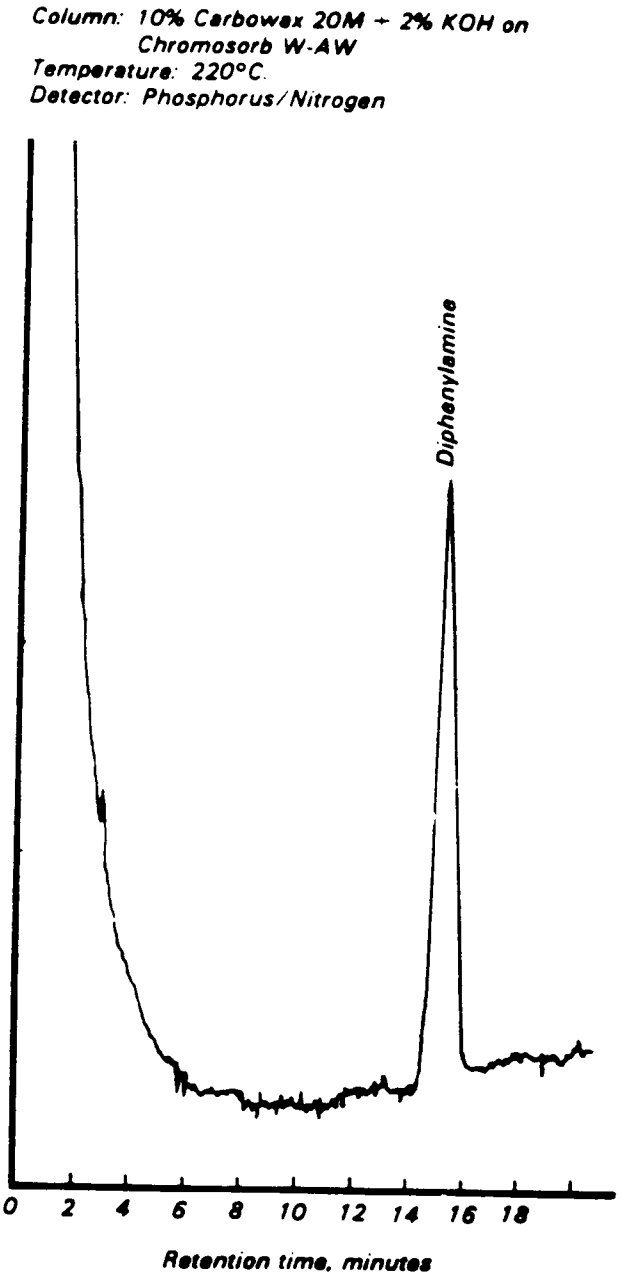
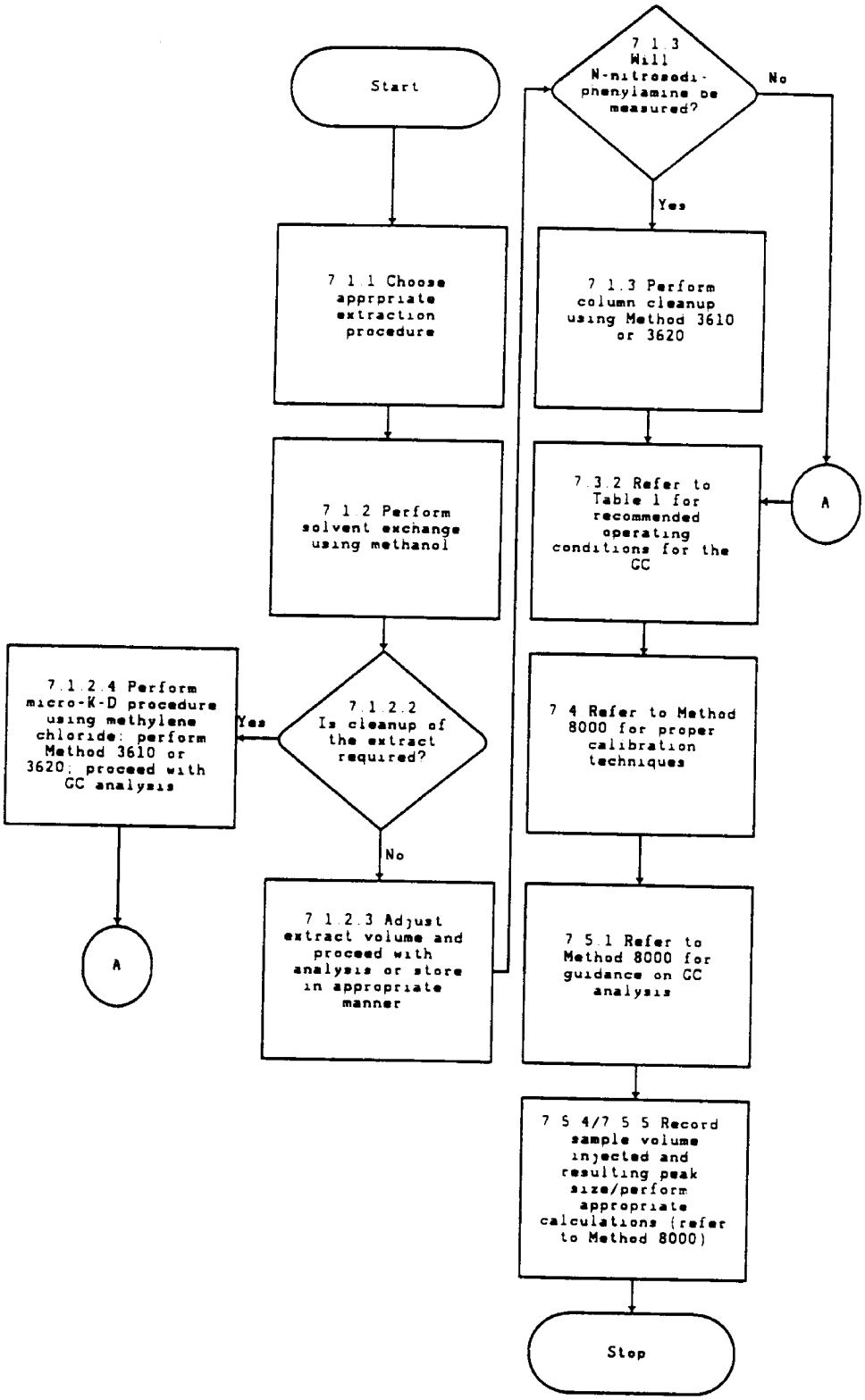


FIGURE 2.
GAS CHROMATOGRAM OF N-NITROSODIPHENYLAMINE AS DIPHENYLAMINE




Start
7.1.1 Choose apprpriate extraction procedure
7.1.2 Perform solvent exchange using methanol
7.1.2.2 Is cleanup of the extract required?
Yes
7.1.2.4 Perform micro-K-D procedure using methylene chloride; perform Method 3610 or 3620; proceed with GC analysis
A
No
7.1.2.3 Adjust extract volume and proceed with analysis or store in appropriate manner
7.1.3 Will N-nitrosodi-phenylamine be measured?
No
Yes
7.1.3 Perform column cleanup using Method 3610 or 3620
7.3.2 Refer to Table 1 for recommended operating conditions for the GC
A
7.4 Refer to Method 8000 for proper calibration techniques
7.5.1 Refer to Method 8000 for guidance on GC analysis
7.5.4/7.5.5 Record sample volume injected and resulting peak size/perform appropriate calculations (refer to Method 8000)
Stop