METHOD 8070A

NITROSAMINES BY GAS CHROMATOGRAPHY

1.0 SCOPE AND APPLICATION

1.1 Method 8070 is a gas chromatographic (GC) method applicable to the determination of nitrosamine in aqueous matrices such as groundwater and municipal and industrial discharges. It is also applicable to solid matrices such as soils, sediments, and sludges. Specifically, this method covers the determination of the following compounds:

Compound	CAS No.[a]	Appropriate Technique 3510	3520	3540/1	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 Service Registry Number.
X Greater than 70 percent recovery by this preparation technique.

1.2 The method detection limit (MDL) for each analyte of interest 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. 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.

1.3 When this method is used to analyze samples from matrices that are not well characterized, compound identifications should be confirmed by at least one additional qualitative technique. Secondary confirmation can be performed using a dissimilar GC column, specific element detector, or mass spectrometer (MS).

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 safety data 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.

1.7 This method is restricted to use by, or under the supervision of, analysts experienced in the use of gas chromatographs and skilled in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 A measured volume of sample is solvent extracted with methylene chloride using an appropriate sample preparation technique. 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 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 co-extracted 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 co-extracted 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% K0H or equivalent. This column was used to develop the method performance statements in Sec. 9.0. Guidelines for the use of alternate column packings are provided in Sec. 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 Sec. 9.0. Guidelines for the use of alternate detectors are provided in Sec. 7.3.2.

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

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

4.4 Balance - Analytical, capable of accurately weighing 0.0001 g.

4.5 Vials - 10 to 15 mL, amber glass with polytetrafluoroethylene (PTFE)-lined screw-cap or crimp top.

4.6 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 ± 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 PTFE-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 different 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 Sec. 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 Sec. 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.

5.10 Hydrochloric acid (HCl), 1 M.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See the introductory material to this chapter, Organic Analytes, Sec. 4.1.

6.2 The nitrosamines validated for analysis by this procedure are considered semivolatile organic compounds.

6.3 Extracts must be stored at 4°C and protected from light.

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 pH, or as received, with methylene chloride, using an appropriate 3500 series method. Solid samples are extracted using a 3500 series method that is appropriate for such matrices. Both neat and diluted organic liquids (Method 3580, Waste Dilution) may be analyzed by direct injection.

7.1.2 In a separatory funnel, wash the methylene chloride extract with 100 mL of 1 M HCl to remove free amines.

7.1.3 Prior to gas chromatographic analysis, the extraction solvent must be exchanged to methanol. The exchange is performed during the extraction procedures listed in the appropriate 3500 series method.

7.1.4 N-nitrosodiphenylamine measured by gas chromatography requires, the analyst must first use a cleanup column to eliminate diphenylamine interference (Methods 3610 or 3620). If N-nitroso-diphenylamine is of no interest, the analyst may proceed directly with gas chromatographic analysis (Sec. 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 Sec. 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 GC Setup

7.3.1.1 N-nitrosodiphenylamine completely reacts to form diphenylamine at the normal operating temperatures of a GC injection port (200 - 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 Sec. 7.1.3).

7.3.1.2 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.

NOTE: Other columns, chromatographic conditions, or detectors may be used if the requirements of Sec. 8.0 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 Sec. 8.0 are met.

7.3.2 Calibration - Refer to Method 8000 for proper calibration techniques.

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

7.3.2.2 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.

7.3.3 GC Analysis

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

7.3.3.2 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.

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

7.3.3.4 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.

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

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

NOTE: In order to confirm the presence of N-nitrosodiphenylamine an appropriate cleanup procedure must be used.

7.3.4 Secondary confirmation - When this method is used to analyze samples from matrices that are not well characterized, compound identifications should be confirmed by at least one additional qualitative technique. Secondary confirmation can be performed using one of the following techniques:

7.3.4.1 Additional (or alternate) column listed in Sec. 4.1 may be used to document the retention time of the analytes of interest on a dissimilar GC column.

7.3.4.2 Sec. 4.1 also lists three different GC detectors with various compound selectivities that may be used to qualitatively confirm peaks.

7.3.4.3 A GC/MS may also be utilized to confirm compounds identified in the primary analysis. GC/MS Method 8270 is validated for both the qualitative and quantitative confirmation of all the target analytes in Method 8070.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One and Method 8000 for specific quality control (QC) procedures. Quality control procedures to ensure the proper operation of the various sample preparation and/or sample introduction techniques can be found in Methods 3500 and 5000. Each laboratory should maintain a formal quality assurance program. The laboratory should maintain records to document the quality of the data generated.

8.2 Quality control procedures necessary to evaluate the GC system operation are found in Method 8000, Sec. 7.0 and includes evaluation of retention time windows, calibration verification and chromatographic analysis of samples.

8.3 Initial Demonstration of Proficiency - Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the following operations whenever new staff are trained or significant changes in instrumentation are made. See Method 8000, Sec. 8.0 for information on how to accomplish this demonstration.

8.4 Sample Quality Control for Preparation and Analysis - The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, and detection limit). At a minimum, this includes the analysis of QC samples including a method blank, matrix spike, a duplicate, and a laboratory control sample (LCS) in each analytical batch and the addition of surrogates to each field sample and QC sample.

8.4.1 Documenting the effect of the matrix should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch. If samples are expected to contain target analytes, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, laboratories should use a matrix spike and matrix spike duplicate pair.

8.4.2 A Laboratory Control Sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike. When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix.

8.4.3 See Method 8000, Sec. 8.0 for the details on carrying out sample quality control procedures for preparation and analysis.

8.5 Surrogate recoveries - The laboratory must evaluate surrogate recovery data from individual samples versus the surrogate control limits developed by the laboratory. See Method 8000, Sec. 8.0 for information on evaluating surrogate data and developing and updating surrogate limits.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9.0 METHOD PERFORMANCE

9.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero. The MDL concentrations listed in Table 1 were obtained using reagent water. Similar results were achieved using representative wastewaters. The MDL actually achieved in a given analysis will vary depending on instrument sensitivity and matrix effects.

9.2 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.

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

9.4 In a multi-laboratory study, this method was tested by 17 laboratories using reagent water, drinking water, surface water, and three industrial wastewaters spiked at six concentrations over the range 0.8 to 55 µg/L. Results from these analyses have been used to generate accuracy and precision data in Table 4 and the QC acceptance criteria in Table 3.

10.0 REFERENCES

1. "Determination of Nitrosamines in Industrial and Municipal Wastewaters", EPA 600/4-82-016, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH, 45268, May 1982.

2. Burgess, E.M., Lavanish, J.M., "Photochemical Decomposition of N-nitrosamines", Tetrahedron Letters, 1964, 1221.

3. 40 Code of Federal Regulations (CFR): Protection of the Environment, Part 136 Appendix A, Method 607 - Nitrosamines.

4. "Method Detection Limit and Analytical Curve Studies EPA Methods 606, 607, 608", Special letter report for EPA Contract 68-03-2606, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH, 45268.

5. "EPA Method Validation Study 17, Method 607 (Nitrosamines)", Report for EPA Contract 68-03-2606.

TABLE 1

CHROMATOGRAPHIC CONDITIONS AND METHOD DETECTION LIMITS[d]

Compound	Retention Time (minutes) Column 1	Column 2	Method Detection Limit[e] (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.
[d] Reference 3.
[e] MDLs were developed using reagent water.

TABLE 2

SINGLE OPERATOR ACCURACY AND PRECISION

Compound	Average Percent Recovery	Standard Deviation %	Spike Range (µg/L)	Number of Analyses	Matrix Types
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

MULTILABORATORY PERFORMANCE DATA [a]

Analyte	Test Conc. (µg/L)	Limit for s (µg/L)	Range for $\bar{X}$ (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

$\underline{s}$ = 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.
[a] Reference 3.

TABLE 4

METHOD ACCURACY AND PRECISION AS FUNCTIONS OF CONCENTRATION[a]

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	$0.25\bar{X}$ - 0.04	$0.25\bar{X}$ + 0.11
N-Nitrosodiphenylamine	0.64C + 0.52	$0.36\bar{X}$ - 1.53	$0.46\bar{X}$ - 0.47
N-Nitroso-n-propylamine	0.96C - 0.07	$0.15\bar{X}$ + 0.13	$0.21\bar{X}$ + 0.15

X' = Expected recovery for one or more measurements of a sample containing a concentration of C, in µg/L.
C = True value for the concentration, in µg/L.
S' = Expected interlaboratory standard deviation of measurements at an average concentration found of $\bar{X}$, in µg/L.
s_r' = Expected single analyst standard deviation of measurements at an average concentration found of $\bar{X}$, in µg/L.
$\bar{X}$ = Average recovery found for measurements of samples containing a concentration of C, in µg/L.
[a] Reference 3.

FIGURE 1

GAS CHROMATOGRAM OF NITROSAMINES

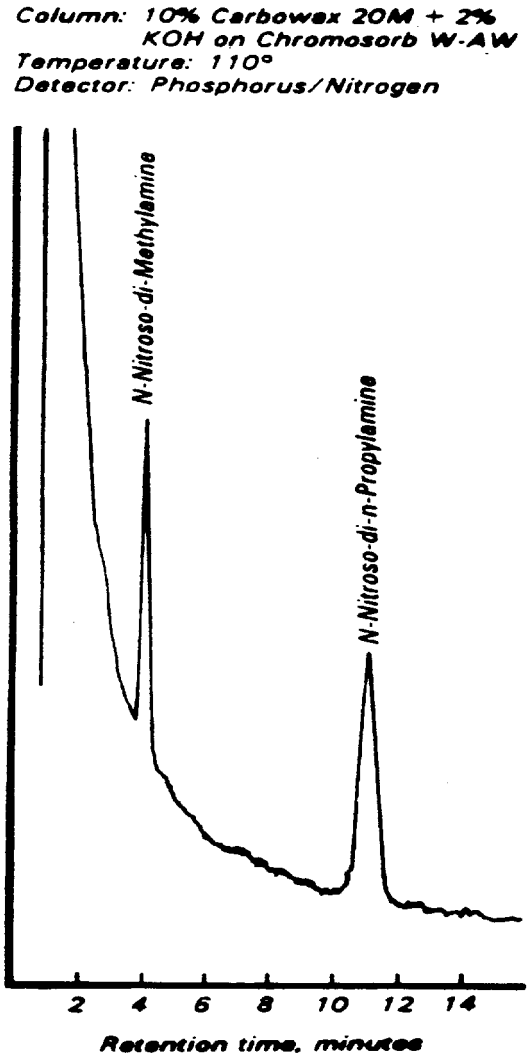
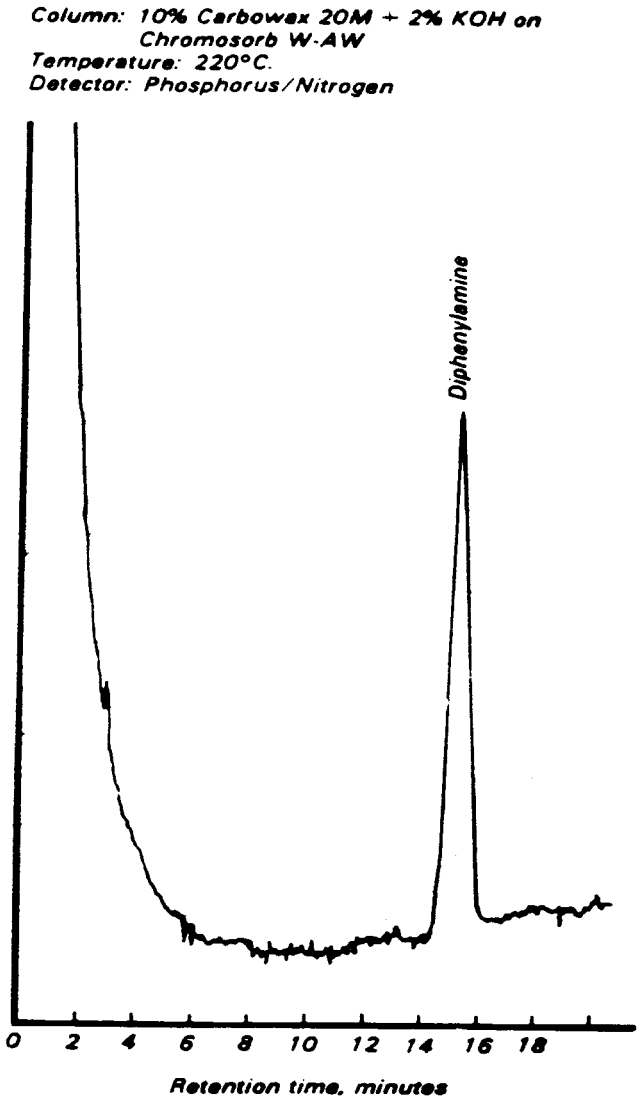
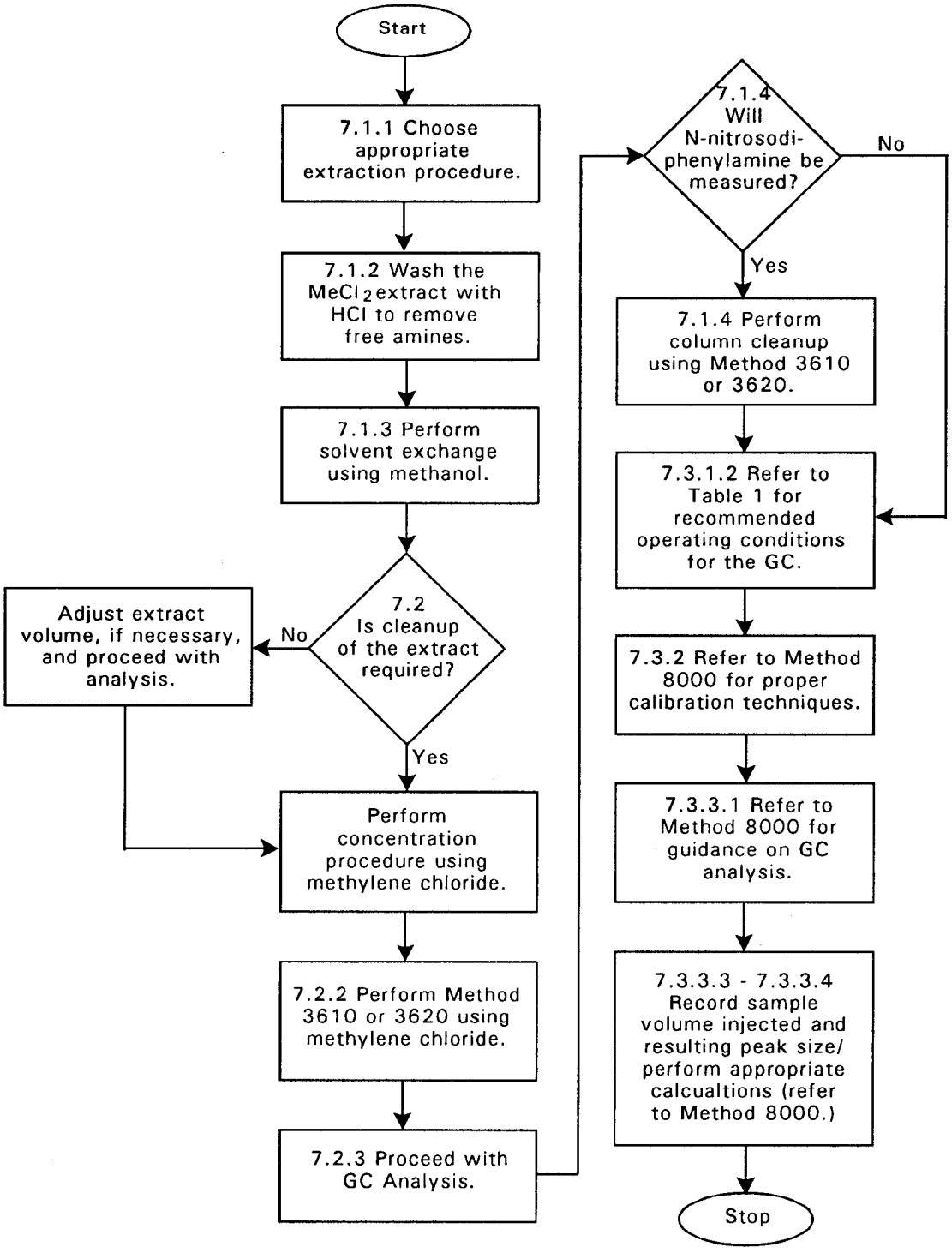


FIGURE 2

GAS CHROMATOGRAM OF N-NITROSODIPHENYLAMINE AS DIPHENYLAMINE




Start
7.1.1 Choose appropriate extraction procedure.
7.1.2 Wash the $MeCl_2$ extract with HCl to remove free amines.
7.1.3 Perform solvent exchange using methanol.
7.2 Is cleanup of the extract required?
No
Adjust extract volume, if necessary, and proceed with analysis.
Yes
Perform concentration procedure using methylene chloride.
7.2.2 Perform Method 3610 or 3620 using methylene chloride.
7.2.3 Proceed with GC Analysis.
7.1.4 Will N-nitrosodi-phenylamine be measured?
No
Yes
7.1.4 Perform column cleanup using Method 3610 or 3620.
7.3.1.2 Refer to Table 1 for recommended operating conditions for the GC.
7.3.2 Refer to Method 8000 for proper calibration techniques.
7.3.3.1 Refer to Method 8000 for guidance on GC analysis.
7.3.3.3 - 7.3.3.4 Record sample volume injected and resulting peak size/ perform appropriate calcualtions (refer to Method 8000.)
Stop