ANILINE AND SELECTED DERIVATIVES BY GAS CHROMATOGRAPHY

1.0 SCOPE AND APPLICATION

1.1 Method 8131 is used to determine (by gas chromatography with a specific detector) the concentration of aniline and certain derivatives of aniline in extracts prepared from environmental samples and RCRA wastes. It has been validated for aqueous matrices. Application to other matrices must be validated by developing spike recovery data. The following compounds can be determined by this method:

Compound	CAS No.[a]
Aniline	62-53-3
4-Bromoaniline	106-40-1
2-Bromo-6-chloro-4-nitroaniline	99-29-6
2-Bromo-4,6-dinitroaniline	1817-73-8
2-Chloroaniline	95-51-2
3-Chloroaniline	108-42-9
4-Chloroaniline	106-47-8
2-Chloro-4,6-dinitroaniline	3531-19-9
2-Chloro-4-nitroaniline	121-87-9
4-Chloro-2-nitroaniline	89-63-4
2,6-Dibromo-4-nitroaniline	827-94-1
3,4-Dichloroaniline	95-76-1
2,6-Dichloro-4-nitroaniline	99-30-9
2,4-Dinitroaniline	97-02-9
2-Nitroaniline	88-74-4
3-Nitroaniline	99-09-2
4-Nitroaniline	100-01-6
2,4,6-Trichloroaniline	634-93-5
2,4,5-Trichloroaniline	636-30-6

[a] Chemical Abstract Service Registry Number.

1.2 When this method is used to analyze unfamiliar samples for any or all of the target analytes, 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. It is highly recommended that gas chromatography/mass spectrometry be utilized for absolute analyte identification when analyzing unfamiliar samples, if concentration permits. See Section 8.6 for guidance. However, the use of the NPD minimizes the possibility of false positives.

1.3 The method detection limit (MDL) for each target analyte is given in Table 1. The MDL for a specific sample may differ from those listed, depending upon the nature of interferences in the

sample matrix. Table 2 provides guidance on the calculation of estimated quantitation limits (EQLs) for various matrices.

1.4 Aniline and many aniline derivatives often result in erratic responses, thereby requiring frequent column maintenance and recalibration.

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Sec. 8.2.

2.0 SUMMARY OF METHOD

2.1 A measured volume of aqueous sample, approximately 1 liter, is solvent extracted at basic pH according to Method 3510 (separatory funnel), Method 3520 (continuous liquid-liquid extraction), or other appropriate technique. Extraction from solid matrices may be performed using Methods 3540, 3541, 3545, or 3550, or other appropriate technique. Both neat and diluted organic liquids may be prepared by Method 3580 (waste dilution) and analyzed by direct injection.

2.2 If interferences are present, the extract may be cleaned up according to Method 3620, Florisil Column Cleanup. Gel Permeation Chromatography Cleanup (Method 3640) has also been validated for aniline and certain derivatives to remove high boiling material that causes chromatography problems.

2.3 The target analytes in the extract are determined by capillary gas chromatography with a nitrogen phosphorus detector (GC/NPD).

3.0 INTERFERENCES

3.1 Refer to Methods 3500, 3600, and 8000.

3.2 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.

3.2.1 Volumetric flasks and glassware used for making up calibration standards must be scrupulously cleaned. Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water and rinses with tap and distilled water. It should then be drained dry, and heated in a muffle furnace at 400°C for 15 to 30 min.

3.2.2 Some thermally stable materials may not be eliminated by this treatment. Solvent rinses with acetone and hexane may be substituted for the muffle furnace heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.3 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from sample to sample. The judicious use of one or more cleanup techniques as listed in Sec. 7.1.2 may be necessary to eliminate or minimize matrix interferences. The use of the NPD will help to minimize many of the interference problems.

3.4 Contamination by carryover can occur whenever high-concentration and low-concentration samples are sequentially analyzed. To reduce carryover, the syringe used for injection must be rinsed out between samples with solvent. Whenever an unusually concentrated sample is encountered, it should be followed by the analysis of a solvent blank to check for cross-contamination. Additional solvent blanks interspersed with the sample extracts should be considered whenever the analysis of a solvent blank indicates cross-contamination problems.

3.5 Retention time data for two capillary columns are found in Table 1. The SE-54 fused silica capillary column does not adequately resolve the following two pairs of compounds: 2-nitroaniline/2,4,6-trichloroaniline, and 4-nitroaniline/4-chloro-2-nitroaniline. Only partial resolution of 3-chloroaniline and 4-chloroaniline is achieved. Unless the purpose for the analysis can be served by reporting the sum of an unresolved pair, the alternate capillary column must be used for these compounds and to verify the absence of either compound in a pair. The alternate fused silica capillary column (SE-30) gives resolution of these compound pairs, but fails to resolve 2,6-dibromo-4-nitroaniline/2,4-dinitroaniline and gives only partial resolution of 3-chloroaniline/4-chloroaniline. Guidelines for selecting alternate capillary columns are given in Sec. 4.1.2.3.

4.0 APPARATUS AND MATERIALS

4.1 Gas chromatograph - an analytical system complete with gas chromatograph suitable for on-column splitless injections and all required accessories, including detector, analytical columns, recorder, gases, and syringes. A data system for measuring peak heights and/or peak areas is recommended.

4.2 Suggested GC columns

4.2.1 Column 1 - 30 m x 0.25 mm fused silica capillary column coated with SE-54 (J&W Scientific or equivalent). The outlet end of the fused silica column should be threaded through the burner tip of the NPD to within 2 to 4 mm from the flame jet in order to minimize losses of anilines.

4.2.2 Column 2 - 30 m x 0.25 mm fused silica capillary column coated with SE-30 (J&W Scientific or equivalent).

4.2.3 The fused silica capillary columns will not resolve certain pairs of aniline compounds, as indicated in Sec. 1.3 and Table 1. Alternate capillary columns may be used if the relative standard deviations of responses for replicate injections meet the requirements of Sec. 8.2. The analyst should be aware of chromatographic peak shape as an indicator of method performance.

4.3 Detector - Nitrogen/Phosphorus (NPD) or equivalent [i.e., Alkali-Flame Detector (AFD) or Thermionic Specific Detector (TSD)].

4.4 Vials - sizes as appropriate, glass with polytetrafluoroethylene (PTFE)-lined screw-caps or crimp tops.

4.5 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.

4.6 Glassware - refer to the applicable 3500 and 3600 series methods.

5.0 REAGENTS

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

5.2 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all 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 chemicals are of sufficiently high purity to permit their use without affecting the accuracy of the determinations.

5.3 Reagents for sample preservation

5.3.1 Sodium hydroxide, NaOH - (1.0 M in organic-free reagent water).

5.3.2 Sulfuric acid, H_2SO_4 - concentrated, specific gravity 1.84.

5.4 Solvents - All solvents must be pesticide quality or equivalent.

5.4.1 Acetone, CH_3COCH_3

5.4.2 Toluene, $C_6H_5CH_3$

5.4.3 Refer to the applicable 3500 and 3600 series methods.

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

5.5.1 Prepare stock standard solutions by accurately weighing about 0.0100 grams of pure materials. Dissolve the material in pesticide quality toluene and dilute to volume in a 10 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.

5.5.2 Transfer the stock standard solutions into PTFE-sealed bottles. 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.

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

5.6 Working standard solutions - Prepare working standards weekly by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with toluene. Prepare at least five different concentrations to cover the expected concentration range of the samples. Aniline and derivatives are not as stable as many of the common semivolatile organics, therefore, their responses must be closely monitored.

6.0 SAMPLE COLLECTION, PRESERVATION AND HANDLING

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

6.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction. For chlorinated waste, immediately add 35 mg sodium thiosulfate per part per million of free chlorine per liter.

6.3 Adjust the pH of the sample to 6 to 8 with sodium hydroxide or sulfuric acid immediately after sampling.

7.0 PROCEDURE

7.1 Extraction and cleanup

7.1.1 Refer to Chapter Two and Method 3500 for guidance on choosing the appropriate extraction procedure. In general, water samples are extracted at a pH > 11 with methylene chloride, using either Methods 3510 or 3520. Solid samples are extracted using either Methods 3540, 3541, 3545, or 3550 with methylene chloride/acetone (1:1) as the extraction solvent. Prepare waste liquids (non-aqueous) by Method 3580 (Waste Dilution).

7.1.2 If necessary, the samples may be cleaned up using Method 3620 (Florisil) and/or Method 3640 (Gel Permeation Chromatography). See Chapter Two, Sec. 2.3 and Method 3600 for general guidance on cleanup and method selection. Method 3660 is used for sulfur removal.

7.1.3 Prior to gas chromatographic analysis by NPD, the extraction solvent must exchanged into toluene by adding 3 - 4 mL of toluene to the vial just prior to the final concentration by nitrogen blowdown.

7.2 Chromatographic conditions (recommended)

Column 1:	SE-54 Fused Silica 30 m x 0.25 mm
Carrier gas:	Helium
Carrier gas flow rate:	28.5 cm/sec at room temperature
Temperature program:	Initial temperature 80°C for 4 min 80°C to 230°C at 4°C/min Hold at 230°C for 4 min
Column 2:	SE-30 Fused Silica 30 m x 0.25 mm
Carrier gas:	Helium
Flow rate:	30 cm/sec at room temperature
Temperature program:	Initial temperature 80°C for 4 min

80°C to 230°C at 4°C/min
Hold at 230°C for 4 min

Chromatographic conditions should be optimized to give separation equivalent to that shown in Table 1.

7.3 Calibration

7.3.1 Prepare calibration standards using the procedures in Section 5.0. Refer to Method 8000 for proper calibration procedures. The procedure for internal or external calibration may be used. Aniline and many aniline derivatives often result in erratic responses, thereby requiring frequent column maintenance and recalibration.

7.3.2 Refer to Method 8000 for the establishment of retention time windows.

7.4 Gas chromatographic analysis of samples

7.4.1 Method 8000 provides instructions on the analysis sequence, appropriate dilutions, establishing daily retention time windows, and identification criteria.

7.4.2 Automatic injections of 1 µL are recommended. Manual injections of no more than 2 µL may be used if the analyst demonstrates quantitation precision of ≤ 10 percent relative standard deviation. The solvent flush technique may be used if the amount of solvent is kept at a minimum. If the internal standard calibration technique is used, add 10 µL of the internal standard to each 1 mL of sample extract prior to injection.

7.4.3 Tentative identification of an analyte occurs when a peak from a sample extract falls within the daily retention time window.

7.4.4 Record the volume injected to the nearest 0.05 µL and the resulting peak size in peak height or area units. Using either the internal or the external calibration procedure (Method 8000), determine the identity and the quantity of each component peak in the sample chromatogram which corresponds to the compounds used for calibration purposes. See Method 8000 for calculation equations.

7.4.5 If the responses exceed the linear range of the system, dilute the extract and reanalyze. Peak height measurements are recommended over peak area integration when overlapping peaks cause errors in area integration.

7.4.6 If partially overlapping or coeluting peaks are found, change columns or employ a GC/MS technique (see Sec. 8.6 and Method 8270). Interferences that prevent analyte identification and/or quantitation may be removed by the cleanup techniques mentioned above.

7.4.7 If the peak response is less than 2.5 times the baseline noise level, the validity of the quantitative result may be questionable. The analyst should consult with the source of the sample to determine whether further concentration of the sample is warranted.

7.5 Instrument maintenance

7.5.1 Injection of sample extracts from waste sites often leaves a high boiling residue in the injection port area, splitters, when used, and the injection port end of the chromatographic column. This residue affects chromatography in many ways (i.e., peak tailing, retention time shifts, analyte degradation, etc.) and, therefore, instrument maintenance is very important. Residue buildup in a splitter may limit flow through one leg and therefore change the split ratios. If this occurs during an analytical run, the quantitative data may be incorrect. Proper cleanup techniques will minimize the problem and instrument QC will indicate when instrument maintenance is required.

7.5.2 Suggested chromatograph maintenance - See Sec. 7.0 of Method 8000 for guidance on corrective action for capillary columns and the injection port.

7.6 GC/MS confirmation

7.6.1 GC/MS techniques should be judiciously employed to support qualitative identifications made with this method. Follow the GC/MS operating requirements specified in Method 8270. Ensure that there is sufficient concentration of the analyte(s) to be confirmed, in the extract for GC/MS analysis.

7.6.2 When available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.

7.6.3 To confirm an identification of a compound, the background corrected mass spectrum of the compound must be obtained from the sample extract and must be compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. At least 20 ng of material should be injected into the GC/MS. The identification criteria specified in Method 8270 must be met for qualitative confirmation.

7.6.4 Should the MS procedure fail to provide satisfactory results, additional steps may be taken before reanalysis. These steps may include the use of alternate GC columns or additional sample cleanup.

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 also 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 include 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 Before processing any samples, the analyst should demonstrate, through the analysis of a method blank, that interferences from the analytical system, glassware, and reagents are under control. Each time a set of samples is analyzed or there is a change in reagents, a method blank should be analyzed as a safeguard against chronic laboratory contamination. The blanks should be carried through all stages of sample preparation and measurement.

8.4.2 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.

8.4.3 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.

8.4.4 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.

8.7 Data from systems that automatically identify target analytes on the basis of retention time or retention time indices should be reviewed by an experienced analyst before they are reported.

9.0 METHOD PERFORMANCE

9.1 Table 1 provides MDLs calculated from the analysis of spiked water samples. Table 2 presents EQLs for various matrices.

9.2 The average recoveries presented in Table 3 were obtained in a single laboratory, using spiked wastewater samples. The standard deviation of the percent recovery is also included in Table 3.

9.3 This method has also been tested for linearity of recovery from spiked organic-free reagent water and has been demonstrated to be applicable over the concentration range from 3 x MDL to 300 x MDL with the following exceptions: 4-chloroaniline recovery was linear over the range 40-400 µg/L (40-400 x MDL). Aniline recovery was linear over the range 40-800 µg/L (16-320 x MDL).

10.0 REFERENCES

1. U.S. EPA Method 650, Aniline and Selected Substituted Derivatives.

2. Analytical Procedures for Aniline and Selected Derivatives in Wastewater and Sludge. Report for U.S. Environmental Protection Agency, Contract Number 68-03-2952.

3. Interagency Testing Committee: Receipt of Fourth Report and Request for Comments, Federal Register, June 1, 1979, V. 44, p. 31866-31889.

4. Burke, J.A., "Gas Chromatography for Pesticide Residue Analysis: Some Practical Aspects", Journal of the Association of Official Analytical Chemists, 48, 1037 (1965).

TABLE 1

RETENTION TIMES AND METHOD DETECTION LIMITS

Analyte	Retention Time (min) Column 1	Retention Time (min) Column 2	Method[a] Detection Limit (µg/L)
Aniline	7.5	6.3	2.3
2-Chloroaniline	12.1	7.1	1.4
3-Chloroaniline	14.6	9.0	1.8
4-Chloroaniline	14.7	9.1	0.66
4-Chloroaniline	18.0	12.1	4.6
2-Nitroaniline	21.9	15.6	1.0
2,4,6-Trichloroaniline	21.9	16.3	5.8
3,4-Dichloroaniline	22.7	16.6	3.2
3-Nitroaniline	24.5	18.0	3.3
2,4,5-Trichloroaniline	26.3	20.4	3.0
4-Nitroaniline	28.3	21.7	11.0
4-Chloro-2-nitroaniline	28.3	22.0	2.7
2-Chloro-4-nitroaniline	31.2	24.8	3.2
2,6-Dichloro-4-nitroaniline	31.9	26.0	2.9
2-Bromo-6-chloro-4-nitroaniline	34.8	28.8	3.4
2-Chloro-4,6-dinitroaniline	37.1	30.1	3.6
2,6-Dibromo-4-nitroaniline	37.6	31.6	3.8
2,4-Dinitroaniline	38.4	31.6	8.9
2-Bromo-4,6-dinitroaniline	39.8	33.4	3.7

[a] MDL based upon seven replicate determinations in organic-free reagent water.

TABLE 2

ESTIMATED QUANTITATION LIMITS (EQL) FOR VARIOUS MATRICES[a]

Matrix	Factor[b]
Ground water	10
Low-concentration soil by sonication with GPC cleanup	670
High-concentration soil and sludges by sonication	10,000
Non-water miscible waste	100,000

[a] Sample EQLs are highly matrix-dependent. The EQLs listed herein are provided for guidance and may not always be achievable.

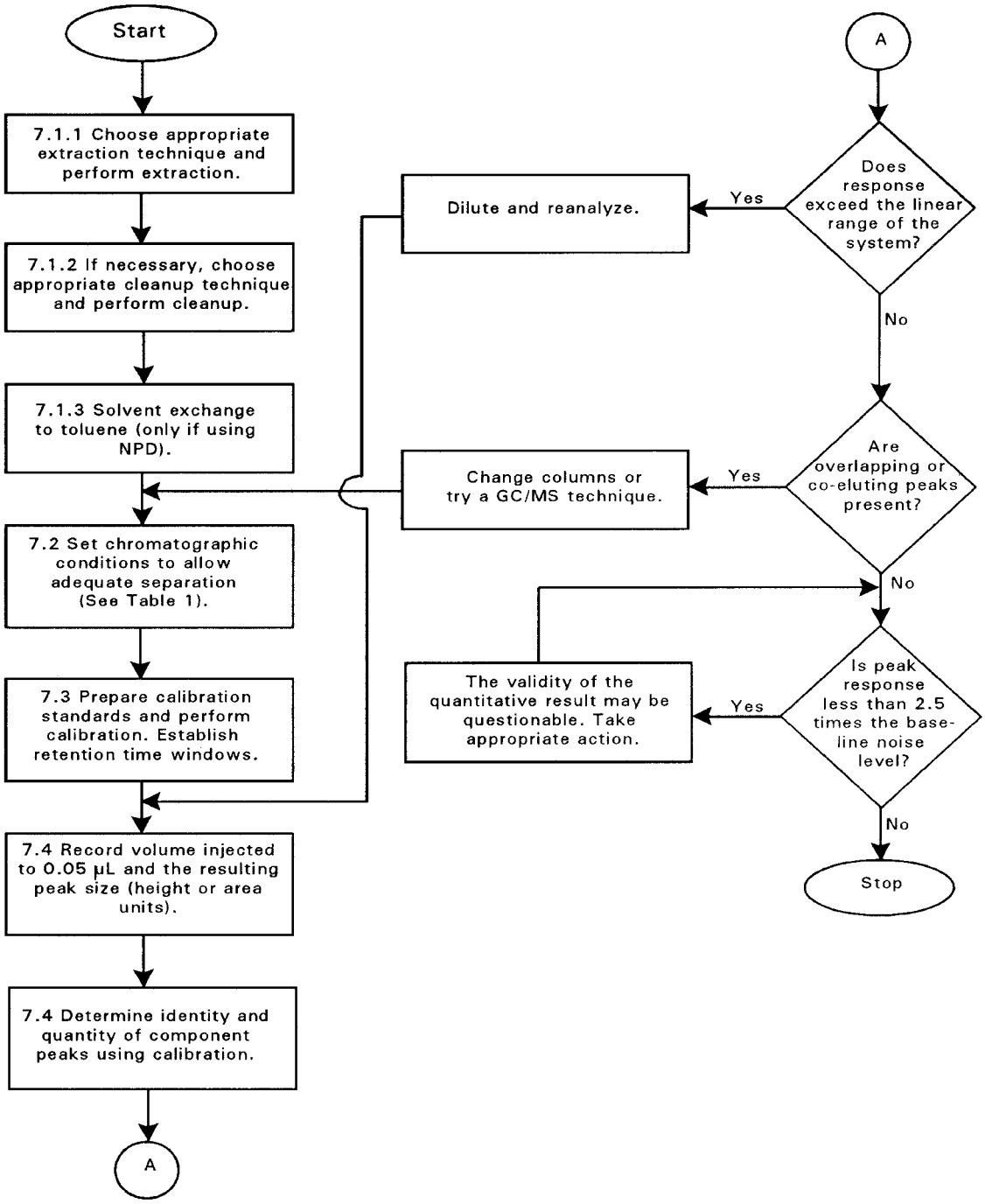
[b] EQL = [MDL for water (Table 1)] times [Factor (Table 3)].
For nonaqueous samples, the factor is on a wet-weight basis.

TABLE 3

SINGLE OPERATOR ACCURACY AND PRECISION

Compounds	Average % Recovery	Standard Deviation %	Spike Range (µg/L)
Aniline	70	8.7	8.0 - 100
2-Chloroaniline	88	9.1	4.0 - 100
3-Chloroaniline	75	10	4.0 - 100
4-Chloroaniline	64	7.5	4.0 - 100
4-Bromoaniline	78	10	16.0 - 160
3,4-Dichloroaniline	79	13	8.0 - 100
2,4,6-Trichloroaniline	93	15	8.0 - 100
2,4,5-Trichloroaniline	85	12	8.0 - 100
2-Nitroaniline	92	14	4.0 - 100
3-Nitroaniline	80	11	8.0 - 100
4-Nitroaniline	94	21	8.0 - 100
2,4-Dinitroaniline	93	20	16.0 - 160
4-Chloro-2-nitroaniline	94	16	8.0 - 100
2-Chloro-4-nitroaniline	96	16	8.0 - 100
2,6-Dichloro-4-nitroaniline	92	12	8.0 - 100
2,6-Dibromo-4-nitroaniline	89	13	8.0 - 100
2-Bromo-6-chloro-4-nitroaniline	110	16	8.0 - 100
2-Chloro-4,6-dinitroaniline	92	17	8.0 - 100
2-Bromo-4,6-dinitroaniline	81	14	8.0 - 100

A total of forty-eight (48) samples comprising four (4) different matrix types were used for this study.


Start
7.1.1 Choose appropriate extraction technique and perform extraction.
7.1.2 If necessary, choose appropriate cleanup technique and perform cleanup.
7.1.3 Solvent exchange to toluene (only if using NPD).
7.2 Set chromatographic conditions to allow adequate separation (See Table 1).
7.3 Prepare calibration standards and perform calibration. Establish retention time windows.
7.4 Record volume injected to 0.05 µL and the resulting peak size (height or area units).
7.4 Determine identity and quantity of component peaks using calibration.
A
A
Does response exceed the linear range of the system?
Yes
Dilute and reanalyze.
No
Are overlapping or co-eluting peaks present?
Yes
Change columns or try a GC/MS technique.
No
Is peak response less than 2.5 times the base-line noise level?
Yes
The validity of the quantitative result may be questionable. Take appropriate action.
No
Stop