

BENZIDINE
3,3'-DICHLOROBENZIDINE
2,4-TOLUENEDIAMINE
2,6-TOLUENEDIAMINE



Method no.: 65

Matrix: Air

Procedure: Samples are collected closed-face by drawing known volumes of air through sampling devices consisting of three-piece cassettes, each containing two sulfuric acid-treated glass fiber filters separated by a spacer. Analysis is performed by quantitating the heptafluorobutyric acid anhydride derivatives of the amines by gas chromatography using an electron capture detector.

Recommended air volume and sampling rate: 100 L at 1 L/min

	Benzidine	3,3'-DCB	2,4-TDA	2,6-TDA
Target conc.: ppb ($\mu\text{g}/\text{m}^3$)	1 (7.5)	1 (10.3)	1 (5)	1 (5)
Reliable quantitation limits: ppt (ng/m^3) (based on 100-L air volume)	4.1 (31)	3.9 (40)	11 (56)	12 (58)
Standard errors of estimate at the target concentration: (Section 4.4)	5.4%	7.2%	6.4%	6.6%

Special requirements: Samples for 3,3'-dichlorobenzidine must be shipped and stored under reduced temperature to help minimize loss of analyte. These samples should be analyzed as soon as possible.

Status of method: Evaluated method. This method has been subjected to the established evaluation procedures of the Organic Methods Evaluation Branch.

Date: August 1987
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1. General Discussion

1.1 Background

1.1.1 History

The previous OSHA-recommended procedures to determine airborne concentrations of benzidine and 3,3'-dichlorobenzidine have never been fully validated. There were no recommended procedures for 2,4- or 2,6-toluenediamine. (Ref. 5.1) In order to have a common air sampler and a more sensitive analysis for aromatic amines, the sulfuric acid-treated glass fiber filter method used for 4,4'-methylenedianiline (MDA) was adapted for these four aromatic amines (OSHA Method 57, Ref. 5.2). Since there are no standards concerning permissible amounts of these analytes in workplace air, an arbitrary target concentration of 1 ppb was chosen for each of these four amines.

The MDA collection procedure involves drawing approximately 100 L of air through Gelman type A/E filters which had previously been treated with sulfuric acid. To improve analyte stability, the sample filters are transferred to separate vials containing 2 mL of deionized water before being shipped to the laboratory for analysis. The analysis involves addition of 1 mL of 0.5 N sodium hydroxide to convert MDA sulfate to free MDA, followed by extraction of the MDA into toluene. The MDA is then derivatized with heptafluorobutyric acid anhydride (HFAA) according to the reaction



The derivative is analyzed by gas chromatography using an electron capture detector.

The analysis scheme of the MDA procedure was slightly modified for these four amines. It was determined that the extraction efficiencies for 2,4- and 2,6-toluenediamine were only about 40% when 0.5 N NaOH was used in the step to convert the amine salts to free amines. The efficiencies are improved to nearly 100% when saturated NaOH is used for these two amines. The extraction efficiency for benzidine was no different when saturated NaOH was used instead of 0.5 N NaOH, but the extraction efficiency for 3,3'-dichlorobenzidine was significantly reduced. Thus, saturated NaOH was used to extract 2,4- and 2,6-toluenediamine and 0.5 N NaOH was used for benzidine and 3,3'-dichlorobenzidine. The analyses in this evaluation were done by using a capillary column rather than a packed column to obtain better peak shapes and resolution.

Note: As a consequence of later evaluation tests done for toluidine, this method has been updated. The sampling device now consists of two acid-treated glass fiber filters assembled in a three-piece cassette instead of a single acid-treated filter with a support pad in a two piece cassette. Not only does this device offer several advantages as listed in the toluidine method, it is now the common sampler for several aromatic amines.

1.1.2 Toxic effects (This section is for information only and should not be taken as the basis of OSHA policy.)

BENZIDINE: IARC reports there is sufficient evidence that benzidine is carcinogenic to man. In numerous cases, the incidence of bladder cancer is strongly associated with occupational exposure to benzidine. (Ref. 5.3) OSHA has strict regulations concerning possible worker exposure to benzidine. (Ref. 5.4)

3,3'-DICHLOROBENZIDINE: Although there is no known case in which 3,3'-dichlorobenzidine has been associated with cancer in man, there is sufficient evidence that it is carcinogenic in mice, rats, hamsters and dogs. (Ref. 5.5) As with benzidine, OSHA has strict regulations to avoid worker contact with 3,3'-dichlorobenzidine. (Ref. 5.6)

2,4- and 2,6-TOLUENEDIAMINE: The toxic effects of 2,4- and 2,6-toluenediamine are reported to be similar to those of aniline. Exposures have been reported to affect the kidney, liver, and blood and to cause convulsions when taken internally. Inhalation can cause respiratory problems and asthma. The most common toxic effect is dermatitis. (Ref. 5.7) Based on studies involving oral administration, 2,4-toluenediamine is carcinogenic in rats. (Ref. 5.9)

The Environmental Protection Agency has recently referred to the Occupational Safety and Health Administration the responsibility for creating any standard for workplace exposure to 2,4-toluenediamine. (Ref. 5.8)

1.1.3 Potential workplace exposure

BENZIDINE: Production of benzidine for sale in the United States was last reported in 1976. The principal commercial use for benzidine is for the manufacture of azo dyes. Over 250 dyes or pigments can be prepared from benzidine. The production and relative importance of benzidine-based dyes in the dye industry have been steadily decreasing. In a 1980 National Occupational Hazard Survey, it was estimated that 700 people were occupationally exposed to benzidine and 79,000 workers in 63 occupations were potentially exposed to benzidine-based dyes. Twenty-six of the dyes produced in the United States were found to contain <1 to 20 mg/kg benzidine and one had 270 mg/kg. Eight dyes from various other countries contained 38 to 1254 mg/kg and 25 had 24 mg/kg or less. Other minor applications for benzidine or its salts include: for the detection of blood in criminal investigations and clinical studies; as a hardener in the rubber, adhesive and plastic industries; for the detection of hydrogen peroxide in milk; in security printing (it reacts with ink erasers to form colored products); and as a laboratory reagent in the determination or detection of a variety of compounds including inorganic ions, nicotine, sugars, metals, chlorinated organic pesticides, bacterial cytochromes, naphthalenesulphonic acids, detergents, chlorine or pyridine in drinking water, and m- and p-cresols. It is not known to what extent benzidine may still be used in these minor applications. (Ref. 5.3)

3,3'-DICHLOROBENZIDINE: The major use for 3,3'-dichlorobenzidine is in the production of organic dyes or pigments. The Society of Dyers and Colourists indicated that fifteen organic dyes or pigments can be made from this chemical. In 1979, it was reported that four of the 3,3'-dichlorobenzidine-based dyes were, but are no longer, commercially produced in the United States. Eight pigments had been manufactured, but Pigment Yellow is no longer produced. 3,3'-Dichlorobenzidine has also been used alone, or with 4,4'-methylenebis(2-chloroaniline) (MOCA), as a curing agent for liquid-castable polyurethane elastomers and also in a color test for the presence of gold. In 1973, it was confirmed that 18 U.S. companies were using 3,3'-dichlorobenzidine and 250 employees were suspected of being exposed. (Ref. 5.5)

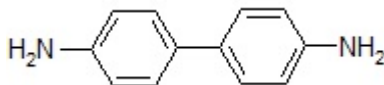
2,4- and 2,6-TOLUENEDIAMINE: A majority of the toluenediamine produced in the United States is used as an 80% 2,4- and 20% 2,6-toluenediamine mixture to make toluene diisocyanate (TDI). A smaller amount of TDI is also made from a mixture of 65% 2,4- and 35% 2,6-toluenediamine. Some isolated 2,4-toluenediamine is used to produce pure 2,4-diisocyanate. 2,4-Toluenediamine is also used to make about 60 dyes, of which 28 are believed to be commercially significant. It is also used as a developer for direct dyes and also in dyeing of furs. The use of 2,4-toluenediamine in hair-dye formulations was banned in the United States in 1971. (Ref. 5.9)

1.1.4 Physical properties

BENZIDINE (Ref. 5.3)

CAS no.:	92-87-5
mol wt:	184.2
mp:	116/129°C (isotropic forms)
bp:	402°C
description:	greyish-yellow, white or reddish-grey crystalline powder
density:	1.250 (20/4°C)
solubility:	practically insoluble in cold water (400 mg/L at 12°C); slightly soluble in hot water (9400 mg/L at 100°C); soluble in diethyl ether; slightly soluble in ethanol
stability:	darkens on exposure to air and light
synonyms:	benzidine base; 4,4'-biphenyldiamine; 4,4'-biphenylenediamine; C.I. 37225; C.I. Azoic Diazo Component 112; 4,4'-bianiline; p,p'-bianiline; 4,4'-diaminobiphenyl; 4,4'-diamino-1,1'-biphenyl; p,p'-diaminobiphenyl; 4,4'-diaminodiphenyl; p-diaminodiphenyl; 4,4'-diphenylenediamine
trade name:	Fast Corinth Base B

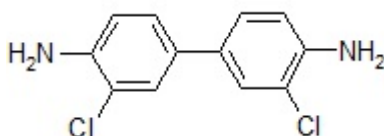
structural formula:



3, 3' -DICHLOROBENZIDINE (Ref. 5.5)

CAS no.: 91-94-1
mol wt: 253.1
mp: 133°C
description: grey to purple crystalline solid
solubility: almost insoluble in water; readily soluble in benzene, diethyl ether, ethanol and glacial acetic acid
synonyms: C.I. 23060; DCB; 4,4'-diamino-3,3'-dichlorobiphenyl; 4,4'-diamino-3,3'-dichlorodiphenyl; dichlorobenzidine; ortho,ortho'- dichlorobenzidine; dichlorobenzidine base; 3,3'-dichlorobenzidine base; 3,3'-dichlorobiphenyl-4,4'-diamine; 3,3'-dichloro-4,4'-biphenyldiamine; 3,3'-dichloro-4,4'-diaminobiphenyl; 3,3'-dichloro-4,4'- diamino(1,1'-biphenyl)

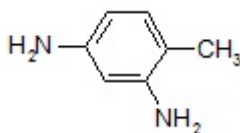
structural formula:



2,4-TOLUENEDIAMINE (Ref. 5.9)

CAS no.: 95-80-7
mol wt: 122.2
mp: 99°C
bp: 292°C
vp: 1 mm (133 Pa) at 106.5°C
10 mm (1330 Pa) at 151.7°C
description: colorless needles
solubility: very soluble in hot water, ethanol, ether and hot benzene
synonyms: 4-methyl-1,3-benzenediamine; 3-amino-paratoluidine; 5-amino-ortho-toluidine; 1,3-diamino-4-methylbenzene; 2,4-diamino-1-methylbenzene; diaminotoluene; 2,4-diaminotoluol; 4-methyl-metaphenylenediamine; MTD; 2,4-diaminotoluene; toluene 2,4-diamine; meta-toluenediamine; 2,4-toluylenediamine; meta-toluylenediamine; tolylene-2,4-diamine; 2,4-tolylenediamine; 4-meta-tolylenediamine; meta-tolylenediamine
trade names: Azogen Developer H; Benzofur MT; Developer B; Developer DB; Developer DBJ; Developer H; Developer MC; Developer MT; Developer MT-CF; Developer MTD; Developer T; Eucanine GB; Fouramine; Fouramine J; Fournine 94; Fournine M; Nako TMT; Pelagol Grey J; Pelagol J; Pontamine Developer TN; Renal MD; Tertral G; Zoba GKE

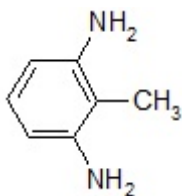
structural formula:



2,6-TOLUENEDIAMINE (Ref. 5.7)

CAS no.: 823-40-5
mol wt: 122.2
mp: 105°C

structural formula:



- 1.2 Limit defining parameters (The analyte air concentrations listed throughout this method are based on an air volume of 100 L and a toluene extraction volume of 2.0 mL. Air concentrations listed in ppb and ppt are referenced to 25°C and 760 mm Hg. Although the derivatives of the amines are analyzed, the equivalent masses of the amines are listed throughout the method.)

1.2.1 Detection limit of the analytical procedure

The detection limits of the analytical procedure are 0.01, 0.02, 0.03, and 0.03 pg per injection for benzidine, 3,3'-dichlorobenzidine, 2,4-toluenediamine, and 2,6-toluene-diamine respectively. These are the amounts of analytes which produce peaks with heights that are approximately 5 times the baseline noise. (Section 4.1.)

1.2.2 Detection limit of the overall procedure

The detection limits of the overall procedure are 4.0, 5.6, and 5.8 ng per sample for benzidine, 3,3'-dichlorobenzidine, 2,4-toluenediamine, and 2,6-toluenediamine respectively. These are the amounts of analytes spiked on a sample filter which allow recoveries of amounts of analyte equivalent to the detection limits of the analytical procedure. These detection limits correspond to air concentrations of 4.1 ppt (31 ng/m³), 3.9 ppt (40 ng/m³), 11 ppt (56 ng/m³), and 12 ppt (58 ng/m³) for benzidine, 3,3'-dichlorobenzidine, 2,4-toluenediamine, and 2,6-toluenediamine, respectively. (Section 4.2)

1.2.3 Reliable quantitation limit

The reliable quantitation limits are 3.1, 4.0, 5.6, and 5.8 ng per sample for benzidine, 3,3'-dichlorobenzidine, 2,4-toluenediamine, and 2,6-toluenediamine respectively. These are the smallest amounts of analytes which can be quantitated within the requirements of a recovery of at least 75% and a precision (± 1.96 SD) of $\pm 25\%$ or better. These reliable quantitation limits correspond to air concentrations of 4.1 ppt (31 ng/m³), 3.9 ppt (40 ng/m³), 11 ppt (56 ng/m³), and 12 ppt (58 ng/m³) for benzidine, 3,3'-dichlorobenzidine, 2,4-toluenediamine, and 2,6-toluenediamine, respectively. (Section 4.2)

The reliable quantitation limits and detection limits reported in this method are based upon optimization of the instrument for the smallest possible amount of analyte. When the target concentration of an analyte is exceptionally higher than these limits, they may not be attainable at the routine operating parameters.

1.2.4 Instrument response to the analyte

The instrument response over concentration ranges representing 0.5 to 2 times the target concentrations is linear for all four analytes. (Section 4.3)

1.2.5 Recovery

The recoveries of benzidine, 3,3'-dichlorobenzidine, 2,4-toluenediamine, and 2,6-toluenediamine from samples used in a 15-day storage test remained above 86, 75, 86, and 87% respectively. The 3,3'-dichlorobenzidine samples were stored in a refrigerator at 0°C while the samples for the other three analytes were stored in a closed drawer at ambient temperatures of 20 to 25°C. (Section 4.4) The recovery of analyte from the collection medium during storage must be 75% or greater.

1.2.6 Precision (analytical method only)

The pooled coefficients of variation obtained from replicate injections of analytical standards at 0.5, 1, and 2 times the target concentrations are 0.032, 0.025, 0.038, and 0.040 for benzidine, 3,3'-dichlorobenzidine, 2,4-toluenediamine, and 2,6-toluenediamine respectively. (Section 4.3)

1.2.7 Precision (overall procedure)

The precisions at the 95% confidence level for the 15-day storage tests are ± 10.4 , ± 14.0 , ± 12.4 , and $\pm 12.8\%$ for benzidine, 3,3'-dichlorobenzidine, 2,4-toluenediamine, and 2,6-toluenediamine respectively. (Section 4.4.) These include an additional $\pm 5\%$ for sampling error. The overall procedure must provide results at the target concentration that are $\pm 25\%$ or better at the 95% confidence level.

1.2.8 Reproducibility

Six samples for each analyte, spiked by liquid injection, and a draft copy of this procedure were given to a chemist unassociated with this evaluation. The samples were analyzed after 1 day of storage at 0°C. No individual sample result deviated from its theoretical value by more than the precision of the overall procedure as reported in Section 1.2.7. (Section 4.5)

1.3 Advantages

1.3.1 The acid-treated filter provides a convenient method of sampling for a number of aromatic amines.

1.3.2 The analysis is rapid, sensitive, and precise.

1.4 Disadvantages

1.4.1 Sample filters must be placed in vials containing water before being submitted to the laboratory for analysis.

1.4.2 The corresponding diisocyanates appear to be positive interferences for 2,4-toluenediamine and 2,6-toluenediamine. It was found that when TDI was spiked onto an acid-treated filter, the TDI partially converted to TDA.

1.4.3 Samples for 3,3'-dichlorobenzidine must be shipped and stored under reduced temperatures and should be analyzed as soon as possible.

2. Sampling Procedure

2.1 Apparatus

2.1.1 Samples are collected by use of a personal sampling pump that can be calibrated within $\pm 5\%$ of the recommended flow rate with the sampling filter in line.

2.1.2 Samples are collected closed-face using a sampling device consisting of two sulfuric-acid treated 37-mm Gelman type A/E glass fiber filters contained in a three-piece cassette. The filters are prepared by soaking each filter with 0.5 mL of 0.26 N sulfuric acid. (0.26 N sulfuric acid can be prepared by diluting 1.5 mL of 36 N sulfuric acid to 200 mL with deionized water.) The filters are dried in an oven at 100°C for 1 h and then assembled into three-piece 37-mm polystyrene cassettes without support pads. The front filter is separated from the back filter by a polystyrene spacer. The cassettes are sealed with shrink bands and the ends are plugged with plastic plugs.

2.1.3 Small sealable vials capable of holding at least 7 mL of liquid are needed for sample storage. Small glass scintillation vials with caps containing Teflon liners are recommended.

2.2 Reagents

Deionized water is needed for addition to the vials in 2.1.3.

2.3 Sampling technique

2.3.1 Immediately before sampling, remove the plastic plugs from the filter cassettes.

2.3.2 Attach the cassette to the sampling pump with flexible tubing and place the cassette in the employee's breathing zone.

- 2.3.3 After sampling, seal the cassettes with plastic plugs until the filters are transferred to the vials containing deionized water.
- 2.3.4 At some convenient time within 10 h of sampling, transfer the sample filters to the vials. The filters are carefully removed from the cassettes and individually transferred to separate vials. Approximately 2 mL of deionized water are added to each vial. This can be done before or after the filters are transferred.
- 2.3.5 Ship and store samples for 3,3'-dichlorobenzidine under reduced temperatures.
- 2.3.6 Seal the small vials lengthwise with OSHA Form 21.
- 2.3.7 Submit at least one blank filter with each sample set. Blanks should be handled in the same manner as samples, but no air is drawn through them.
- 2.3.8 Record sample volumes (in liters of air) for each sample, along with any potential interferences.

2.4 Retention efficiency

A retention efficiency study was performed by drawing 30 L of air (80% relative humidity) at 1 L/min through 6 sample filters that had been spiked with 0.763 µg of benzidine. Instead of using backup pads, blank acid-treated filters were used as backups in each cassette. Upon analysis, the top filters were found to contain an average of 96.0% of the spiked amount. There was no benzidine found on the bottom filters. Similar tests were done for 1.02 µg of 3,3'-dichlorobenzidine, 0.499 µg of 2,4-toluenediamine, and 0.502 µg of 2,6-toluenediamine. Upon analysis, the top filters were found to contain the following average amounts of spiked analyte: 3,3'-dichlorobenzidine-94.8%; 2,4-toluenediamine-96.8%; and 2,6-toluenediamine-96.9%. There were no detectable amounts of these three analytes found on the backup filters.

2.5 Extraction efficiency

- 2.5.1 The average extraction efficiencies for six filters for each amine spiked at the target concentrations are 94.0, 98.7, 98.1, and 96.8% for benzidine, 3,3'-dichlorobenzidine, 2,4-toluenediamine, and 2,6-toluenediamine, respectively. (Section 4.7)
- 2.5.2 The stability of extracted and derivatized samples was verified by reanalyzing the above samples the next day using fresh standards. The average extraction efficiencies for the reanalyzed samples are 98.7, 101.6, 96.9, and 94.7% for benzidine, 3,3'-dichlorobenzidine, 2,4-toluenediamine, and 2,6-toluenediamine, respectively. (Section 4.7)

2.6 Recommended air volume and sampling rate

- 2.6.1 The recommended air volume is 100 L.
- 2.6.2 If a smaller air volume is desired, the reliable quantitation limits will be larger. For example, the reliable quantitation limit for benzidine for a 15-L air sample would be 27.3 ppt.
- 2.6.3 The recommended sampling rate is 1 L/min.

2.7 Interferences (sampling)

- 2.7.1 2,4- and 2,6-Toluenediisocyanate appear to be positive interferences for 2,4- and 2,6-toluenediamine respectively. It was found that when TDI was spiked onto an acid-treated filter, the TDI partially converted to TDA.
- 2.7.2 Suspected interferences should be reported to the laboratory with submitted samples.

2.8 Safety precautions (sampling)

- 2.8.1 Attach the sampling equipment to the employees so that it will not interfere with work performance or safety.
- 2.8.2 Follow all safety procedures that apply to the work area being sampled.

3. Analytical Procedure

3.1 Apparatus: The following are required for analysis.

- 3.1.1 A GC equipped with an electron capture detector. For this evaluation a Varian 3400 Gas Chromatograph equipped with a Nickel 63 electron capture detector and an 8035 Autosampler was used.
- 3.1.2 A GC column capable of separating the amine derivatives from the solvent and interferences. A 15-m x 0.32-mm i.d. (1.0- μ m film) SPB-5 fused silica column was used in this evaluation.
- 3.1.3 An electronic integrator or some other suitable means of measuring peak areas or heights. A Spectra-Physics SP4290 integrator was used in this evaluation.
- 3.1.4 Small resealable vials with Teflon-lined caps capable of holding 4 mL.
- 3.1.5 A dispenser or pipet for toluene capable of delivering 2.0 mL.
- 3.1.6 Pipets (or repetitive pipets with plastic or Teflon tips) capable of delivering 1 mL, for dispensing the sodium hydroxide and buffer solutions.
- 3.1.7 A repetitive pipet capable of delivering 25 μ L of HFAA.
- 3.1.8 Disposable pipets to transfer the toluene layers after the samples are extracted.

3.2 Reagents

- 3.2.1 Saturated and 0.5 N NaOH prepared from reagent grade NaOH.
- 3.2.2 Toluene, pesticide grade. Burdick and Jackson "distilled in glass" toluene was used.
- 3.2.3 Heptafluorobutyric acid anhydride (HFAA). HFAA from Pierce Chemical Company was used.
- 3.2.4 Phosphate buffer, prepared from 136 g of potassium dihydrogen phosphate and 1 L of deionized water. The pH is adjusted to 7.0 with saturated sodium hydroxide solution.
- 3.2.5 Benzidine, 3,3'-dichlorobenzidine, 2,4-toluenediamine, 2,6-toluenediamine, reagent grade. The amines used in this evaluation were from CTC Organics, Atlanta, GA.

3.3 Standard preparation

- 3.3.1 **CAUTION. THESE AROMATIC AMINES ARE OR SHOULD BE CONSIDERED CARCINOGENIC TO HUMANS.** Restrict use of pure compounds and concentrated standards to regulated areas. Prepare concentrated stock standards by diluting the pure amines with toluene. Prepare analytical standards by injecting microliter amounts of diluted stock standards into vials that contain 2.0 mL of toluene.
- 3.3.2 Add 25 μ L of HFAA to each vial. Recap and shake the vials for 10 s.
- 3.3.3 After allowing 10 min for the derivatives to form, add 1 mL of buffer to each vial to destroy the excess HFAA and to extract the heptafluorobutyric acid that is formed.
- 3.3.4 Recap and shake the vials for 10 s.
- 3.3.5 After allowing the layers to separate, analyze aliquots of the toluene (upper) layers by GC.
- 3.3.6 Bracket sample concentrations with analytical standard concentrations. If sample concentrations fall out of the range of prepared standards, prepare additional standards to ascertain detector response.

3.4 Sample preparation

- 3.4.1 The sample filters are received in vials containing deionized water.

- 3.4.2 Add 1 mL of 0.5 N NaOH and 2.0 mL of toluene to each vial for samples to be analyzed for benzidine and 3,3'-dichlorobenzidine. Substitute saturated NaOH for 0.5 N NaOH for samples to be analyzed for 2,4- and 2,6-toluenediamine.
- 3.4.3 Recap and shake the vials for 10 min.
- 3.4.4 After allowing the layers to separate, transfer approximately 1-mL aliquots of the toluene (upper) layers to separate vials with clean disposable pipets.
- 3.4.5 Add 25 μ L of HFAA to each vial. Recap and shake the vials for 10 s.
- 3.4.6 After allowing 10 min for the derivatives to form, add 1 mL of buffer to each vial to destroy the excess HFAA and to extract the heptafluorobutyric acid that is formed.
- 3.4.7 Recap and shake the vials for 10 s.
- 3.4.8 After allowing the layers to separate, analyze aliquots of the toluene (upper) layers by GG.

3.5 Analysis

3.5.1 GC conditions and information

zone temperatures: 145°C (column temperature for 2,4- and 2, 6-toluenediamine)
 225°C (column temperature for benzidine and 3,3'-dichlorobenzidine)
 275°C (injector)
 300°C (detector)

gas flows: 2 mL/min hydrogen (column flow, 10 psi head pressure)
 30 mL/min nitrogen (make up)

injection volume: 1.0 μ L

split ratio: 100:1

column: SPB-5, 15-m \times 0.32-mm i.d. fused silica, 1.0- μ m film (Supelco, Inc.)

retention times of derivatives: 4.0 min (benzidine)
 5.4 min (3,3'-dichlorobenzidine)
 6.0 min (2,4-toluenediamine)
 5.5 min (2,6-toluenediamine)

chromatograms: Section 4.8

- 3.5.2 Measure peak areas or heights by use of an integrator or by other suitable means.
- 3.5.3 Construct a calibration curve by plotting response (peak areas or heights) of standard injections versus micrograms of analyte per sample. Bracket sample concentrations with standards.

3.6 Interferences (analytical)

- 3.6.1 Any compound that gives an electron capture detector response and has the same general retention time as the HFAA derivative of the amine of interest is a potential interference. Suspected interferences reported to the laboratory with submitted samples by the industrial hygienist must be considered before samples are derivatized.
- 3.6.2 GC parameters may be changed to possibly circumvent interferences.
- 3.6.3 Retention time on a single column is not considered proof of chemical identity. Analyte identity should be confirmed by GC/MS if possible.

3.7 Calculations

The analyte concentration for samples is obtained from the calibration curve in terms of micrograms of analyte per sample. If any analyte is found on the blank, that amount is subtracted from the sample amounts. The air concentrations are calculated using the following formulas:

$$\mu\text{g}/\text{m}^3 = \frac{1000 \text{ (micrograms of analyte per sample)}}{\text{liters of air sampled}}$$

$$\text{ppb} = \frac{(24.46)(\mu\text{g}/\text{m}^3)}{\text{molecular weight of analyte}}$$

where 24.46 is the molar volume at 25°C and 760 mmHg.

molecular weights:	benzidine	184.2
	3,3'-dichlorobenzidine	253.1
	2,4-toluenediamine	122.2
	2,6-toluenediamine	122.2

3.8 Safety precautions (analytical)

3.8.1 CAUTION. THESE AROMATIC AMINES ARE OR SHOULD BE CONSIDERED CARCINOGENIC TO HUMANS. Restrict use of pure compounds and concentrated standards to regulated areas. Avoid skin contact and inhalation of all chemicals.

3.8.2 Restrict the use of all chemicals to a hood if possible.

3.8.3 Wear safety glasses and a lab coat at all times while in the lab area.

4. Backup Data

4.1 Detection limits of the analytical procedure

The injection volume listed in Section 3.5.1., 1.0 µL with a 1 to 100 split, was used in the determination of the detection limits of the analytical procedure. The detection limits of 0.01, 0.02, 0.03, and 0.03 pg per injection for benzidine, 3,3'-dichlorobenzidine, 2,4-toluenediamine, and 2,6-toluenediamine respectively were determined by analyzing dilute standards equivalent to 2.9, 3.9, 5.3, and 5.4 ng per sample for benzidine, 3,3'-dichlorobenzidine, 2,4-toluenediamine, and 2,6-toluenediamine respectively. (The samples are extracted into 2.0 mL of toluene.) These amounts were judged to give peaks with heights approximately 5 times the baseline noise. Chromatograms of such injections are shown in Figures 4.1.1 and 4.1.2.

4.2 Detection limits of the overall procedure and reliable quantitation limits

The detection limits of the overall procedure and reliable quantitation limits were determined by analyzing filters spiked with loadings equivalent to the detection limits of the analytical procedure plus the amount expected to be lost due to incomplete recovery. Samples were prepared by injecting 3.1 ng of benzidine, 4.0 ng of 3,3'-dichlorobenzidine, 5.6 ng of 2,4-toluenediamine, and 5.8 ng 2,6-toluenediamine onto acid-treated filters. These amounts are equivalent to 4.1 ppt (31 ng/m³), 3.9 ppt (40 ng/m³), 12 ppt (58 ng/m³), and 11 ppt (56 ng/m³) for benzidine, 3,3'-dichlorobenzidine, 2,4-toluenediamine, and 2,6-toluenediamine respectively.

Table 4.2.1
Detection Limit of the Overall
Procedure and Reliable
Quantitation Limit for
Benzidine

sample no.	ng recovered	% recovery
1	2.94	94.8
2	2.92	94.2
3	2.80	90.3
4	3.00	96.8
5	2.86	92.3
6	2.89	93.2
	̄x	93.6
	SD	2.2
	1.96SD	4.3

Table 4.2.2
Detection Limit of the Overall
Procedure and Reliable
Quantitation Limit for 3,3'-
Dichlorobenzidine

sample no.	ng recovered	% recovery
1	4.65	116.2
2	4.57	114.2
3	3.84	96.0
4	3.99	99.8
5	3.99	99.8
6	3.91	97.8
	̄x	104.0
	Sd	8.8
	1.96SD	17.2

Table 4.2.3
Detection Limit of the Overall
Procedure and Reliable
Quantitation Limit for 2,4-
Toluenediamine

sample no.	ng recovered	% recovery
1	5.67	101.2
2	5.07	90.5
3	5.00	89.3
4	5.20	92.9
5	5.40	96.4
6	5.33	95.2
	\bar{x}	94.2
	SD	4.3
	1.96SD	8.4

Table 4.2.4
Detection Limit of the Overall
Procedure and Reliable
Quantitation Limit for 2,6-
Toluenediamine

sample no.	ng recovered	% recovery
1	5.89	101.6
2	5.33	91.9
3	5.12	88.3
4	5.54	95.5
5	5.54	95.5
6	5.75	99.1
	\bar{x}	95.3
	SD	4.8
	1.96SD	9.4

4.3 Instrument response and precision (analytical method only)

The instrument response and precision of the analytical procedure were determined from multiple injections of analytical standards. These data are given in Tables 4.3.1 - 4.3.4 and Figures 4.3.1 -4.3.4. (CVp is pooled CV)

Table 4.3.1
Precision Data for Benzidine

\times target concn	0.5 \times	1.0 \times	2.0 \times
$\mu\text{g/mL}$	0.382	0.763	1.627
ppb	0.51	1.01	2.16
area counts	342909	972742	1691370
	341740	978345	1800010
	377212	990606	1754440
	378580	965210	1725150
	357601	1006820	1691800
	347260	1014650	1733620
\bar{x}	357550	988062	1732732
SD	16728	19579	41090
CV	0.0468	0.0198	0.0237
CVp	0.032		

Table 4.3.2
Precision Data for 3,3'-Dichlorobenzidine

\times target concn	0.5 \times	1.0 \times	2.0 \times
$\mu\text{g/mL}$	0.512	1.024	2.049
ppb	0.49	0.99	1.98
area counts	461285	1256320	2234810
	410574	1254020	2273500
	444215	1257200	2243820
	446268	1265940	2236130
	431375	1283320	2236840
	426225	1294520	2242800
\bar{x}	436657	1268553	2244650
SD	17737	16667	14607
CV	0.0406	0.0131	0.0065
CVp	0.025		

Table 4.3.3
Precision Data for 2,4-Toluenediamine

\times target concn	0.5 \times	1.0 \times	2.0 \times
$\mu\text{g/mL}$	0.250	0.499	0.998
ppb	0.50	1.00	2.00
area counts	274586	832189	1850780
	276581	825460	1859670
	285266	815411	1873070
	299042	906626	1942210
	296407	905285	1913330
	299178	874911	1953090
\bar{x}	288510	859980	1898690
SD	11259	40992	43686
CV	0.039	0.048	0.023
CVp	0.03		

Table 4.3.4
Precision Data for 2,6-Toluenediamine

\times target concn	0.5 \times	1.0 \times	2.0 \times
$\mu\text{g/mL}$	0.251	0.502	1.003
ppb	0.50	1.00	2.01
area counts	245425	715937	1628870
	247689	706030	1638560
	255370	702345	1650650
	268458	785553	1713120
	265848	781217	1693680
	267697	755390	1730200
\bar{x}	258414	741079	1675850
SD	10347	37828	42175
CV	0.040	0.051	0.025
CVp	0.040		

4.4 Recovery data (Storage)

Storage samples were generated by spiking acid-treated filters with amounts of analyte equal to the target concentrations (0.763 μg of benzidine, 1.024 μg of 3,3'-dichlorobenzidine, 0.499 μg of 2,4-toluenediamine, 0.502 μg of 2,6-toluenediamine). Thirty-six samples were prepared for each analyte. Thirty liters of air at 80% relative humidity were then drawn through each filter. Within 1h

after the completion of drawing air through the samplers, the filters were transferred to scintillation vials, each containing 2 mL of deionized water. Six samples for each analyte were analyzed immediately, fifteen were stored in a refrigerator at 0°C, and fifteen were stored in a closed drawer at ambient temperature. Six samples for each analyte, three from refrigerated and three from ambient storage, were analyzed at intervals over a period of fifteen days. The results are given in Tables 4.4.1-4.4.4 and Figures 4.4.1-4.4.8. The percent recovery versus days of storage was fit to the best regression curve for each analyte under both storage conditions. The standard errors of estimate are 5.4, 7.2, 6.4, and 6.6% and the 95% confidence limits (± 1.96 SD) are ± 10.4 , ± 14.0 , ± 12.4 , and $\pm 12.8\%$ for benzidine, 3,3'-dichlorobenzidine, 2,4-toluenediamine, and 2,6-toluenediamine, respectively.

Table 4.4.1
Storage Tests of Benzidine

time (days)	percent recovery (ambient)			percent recovery (refrigerated)		
	0	96.6	97.2	93.4	96.6	97.2
0	95.6	97.5	95.6	95.6	97.5	95.6
2	92.6	92.6	93.8	93.1	94.1	93.4
6	94.9	90.6	94.2	94.0	91.4	92.5
8	89.9	88.3	90.9	92.1	87.0	90.1
13	89.1	82.9	86.5	92.2	92.3	91.7
18	88.1	86.7	87.2	88.8	86.6	87.8

Table 4.4.2
Storage Tests of 3,3'-Dichlorobenzidine

time (days)	percent recovery (ambient)			percent recovery (refrigerated)		
	0	95.9	94.3	92.3	95.9	94.3
0	95.2	94.4	96.7	95.2	94.4	96.7
2	76.8	79.4	80.1	82.1	85.3	77.7
6	78.0	77.7	80.4	78.8	73.8	79.3
8	73.2	61.8	71.0	85.0	83.6	75.4
13	62.9	59.7	65.4	76.9	82.4	78.3
18	55.8	65.1	51.3	70.0	66.8	76.0

Table 4.4.3
Storage Tests of 2,4-Toluenediamine

time (days)	percent recovery (ambient)			percent recovery (refrigerated)		
	0	98.1	98.6	97.5	98.1	98.6
0	97.6	94.3	94.6	97.6	94.3	94.6
2	89.5	91.7	92.4	93.7	93.9	92.6
6	92.8	87.1	89.3	99.9	90.4	98.5
8	96.0	85.6	98.5	83.6	92.5	87.7
13	81.8	83.2	83.3	83.2	91.4	92.2
18	87.5	90.9	85.3	84.8	87.8	85.4

Table 4.4.4
Storage Tests of 2,6-Toluenediamine

time (days)	percent recovery (ambient)			percent recovery (refrigerated)		
	0	98.1	98.9	97.4	98.1	98.9
0	97.6	94.2	94.9	97.6	94.2	94.9
2	89.2	92.2	92.6	93.5	93.4	91.9
6	88.3	81.9	83.5	93.7	86.4	93.2
8	90.3	80.7	92.3	77.8	86.7	81.6
13	83.9	83.1	83.9	84.3	90.8	92.6
18	87.1	91.6	83.9	82.9	85.8	84.6

4.5 Reproducibility data

Six samples for each analyte were prepared by injecting microliter quantities of standards onto acid-treated filters. The samples were analyzed by a chemist unassociated with this evaluation.

Table 4.5.1
Reproducibility Data for Benzidine

µg found	µg expected	percent found
0.644	0.620	103.9
0.473	0.480	98.5
0.332	0.325	102.2
0.479	0.480	99.8
0.659	0.620	106.3
0.324	0.325	99.7

Table 4.5.2
Reproducibility Data for 3,3'-Dichlorobenzidine

µg found	µg expected	percent found
0.813	0.828	98.2
0.594	0.642	92.5
0.412	0.435	94.7
0.612	0.642	95.3
0.858	0.828	103.6
0.394	0.435	90.6

Table 4.5.3
Reproducibility Data for 2,4-Toluenediamine

µg found	µg expected	percent found
0.384	0.409	93.9
0.288	0.319	90.3
0.312	0.319	97.8
0.404	0.409	98.8
0.246	0.262	93.9
0.254	0.262	96.9

Table 4.5.4
Reproducibility Data for 2,6-Toluenediamine

µg found	µg expected	percent found
0.480	0.501	95.8
0.358	0.391	91.6
0.387	0.391	99.0
0.500	0.501	99.8
0.299	0.321	93.1
0.308	0.321	96.0

4.6 Extraction efficiency data

Six sample filters for each amine were spiked with the target concentration amounts by liquid injection (0.763 µg of benzidine, 1.02 µg of 3,3'-dichlorobenzidine, 0.499 µg of 2,4-toluenediamine, and 0.502 µg of 2,6-toluenediamine). These samples were analyzed to determine the extraction efficiencies. To determine the stability of extracted and derivatized samples, these same six samples were reanalyzed with fresh standards after 24 h.

Table 4.7.1
Extraction Efficiency Data for Benzidine

initial recovery (percent)	recovery after 24 h (percent)
93.8	100.8
92.1	96.4
92.8	97.2
93.4	103.2
96.4	96.5
\bar{x} = 94.0	98.0
SD = 1.6	3.2

Table 4.7.2
Extraction Efficiency Data for 3,3'-Dichlorobenzidine

initial recovery (percent)	recovery after 24 h (percent)
99.7	101.2
98.4	102.7
98.7	98.9
96.6	106.2
100.8	99.2
98.2	101.1
\bar{x} = 98.7	101.6
SD = 1.4	2.7

Table 4.7.3
Extraction Efficiency Data for 2,4-Toluenediamine

initial recovery (percent)	recovery after 24 h (percent)
97.6	96.4
99.6	100.2
102.6	101.0
95.0	95.6
97.0	94.4
96.6	93.6
\bar{x} = 98.1	96.9
SD = 2.7	3.1

Table 4.7.4
Extraction Efficiency Data for 2,6-Toluenediamine

initial recovery (percent)	recovery after 24 h (percent)
97.4	95.0
98.8	95.8
100.4	97.8
94.0	94.6
94.8	93.2
95.6	91.6
\bar{x} = 96.8	94.7
SD = 2.5	2.1

4.8. Chromatogram

Chromatograms at the target concentrations are shown in Figures 4.8.1. and 4.8.2. The chromatograms are from 1.0- μ L injections of standards.

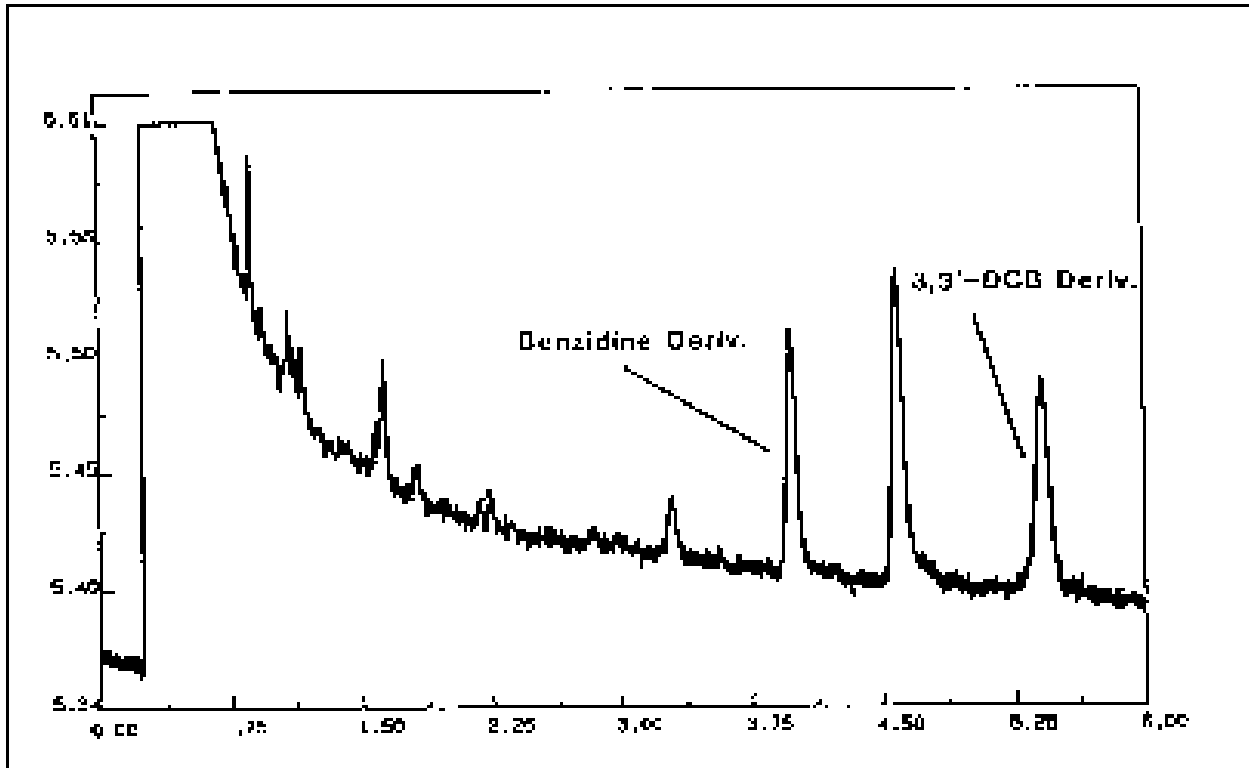


Figure 4.1.1. Detection limit chromatogram for benzidine and 3,3'-DCB.

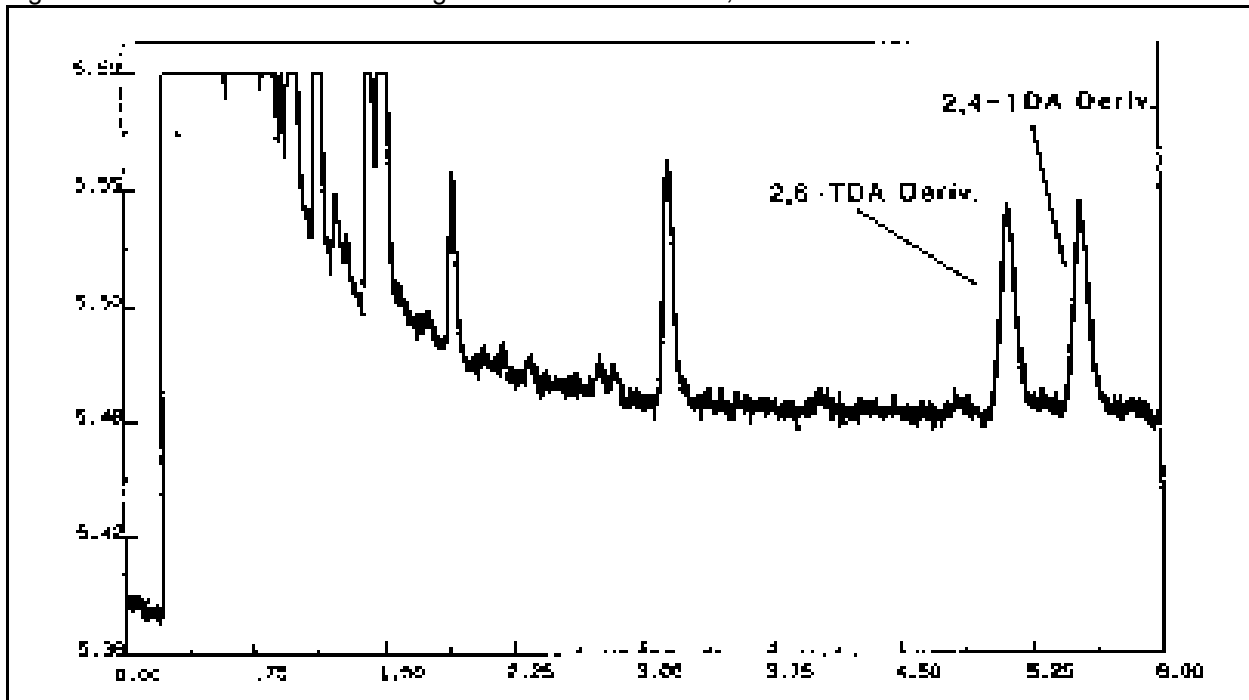


Figure 4.1.2. Detection limit chromatogram for 2,4- and 2,6-TDA.

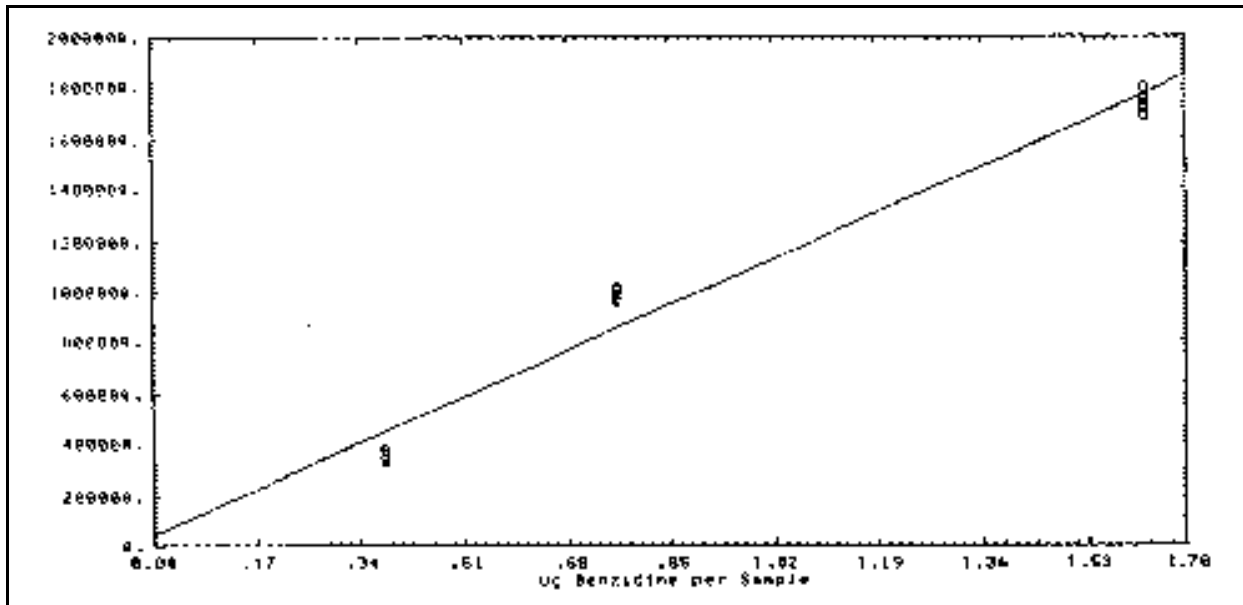


Figure 4.3.1. Benzidine calibration curve (y-axis is peak area).

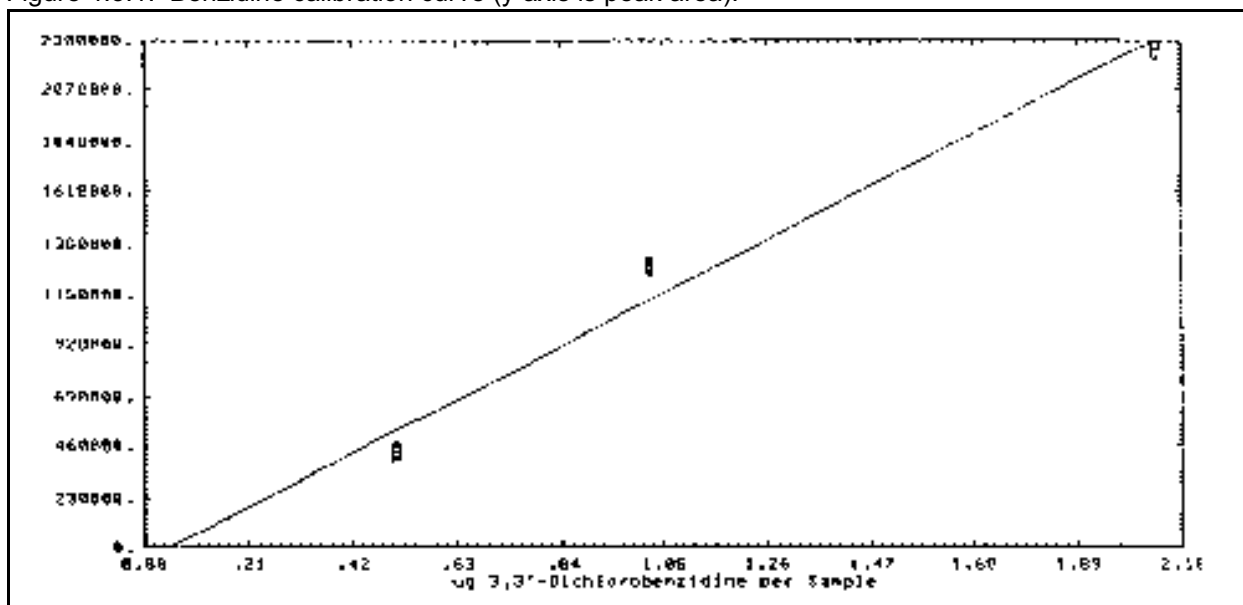


Figure 4.3.2. 3,3'-Dichlorobenzidine calibration curve (y-axis is peak area).

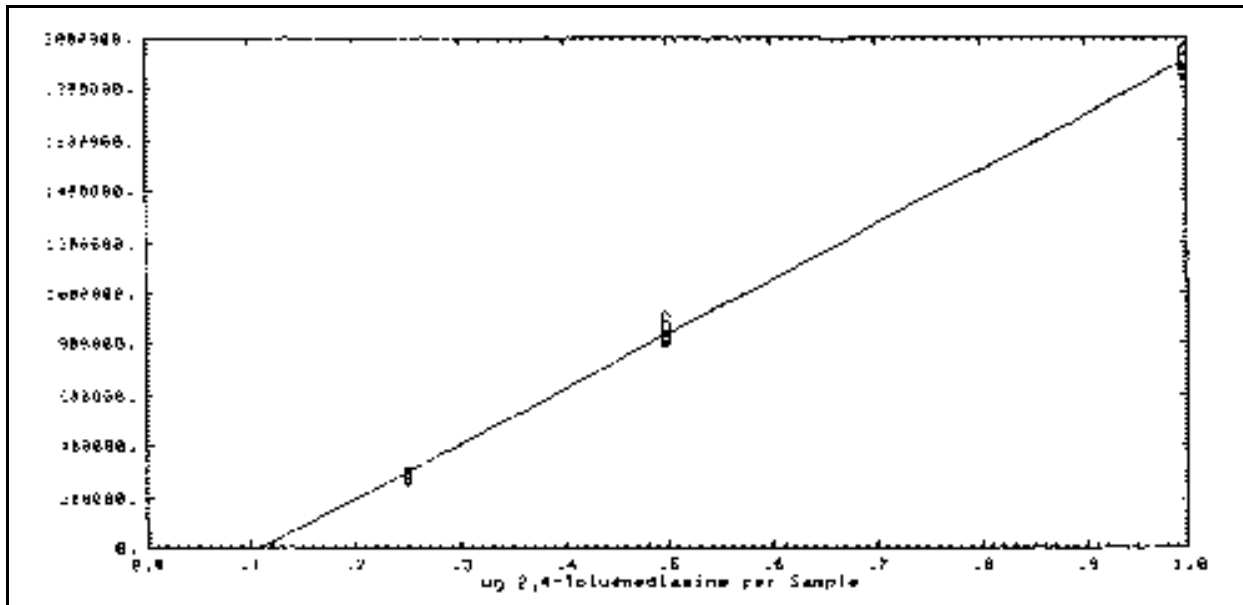


Figure 4.3.3. 2,4-Toluenediamine calibration curve (y-axis is peak area).

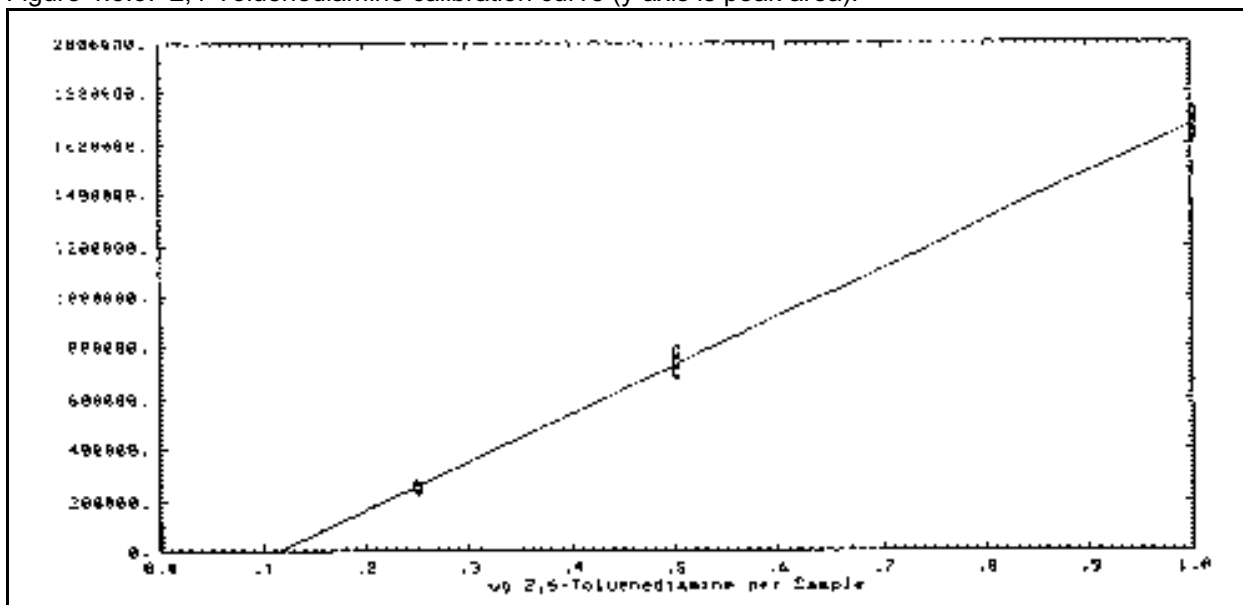


Figure 4.3.4. 2,6-Toluenediamine calibration curve (y-axis is peak area).

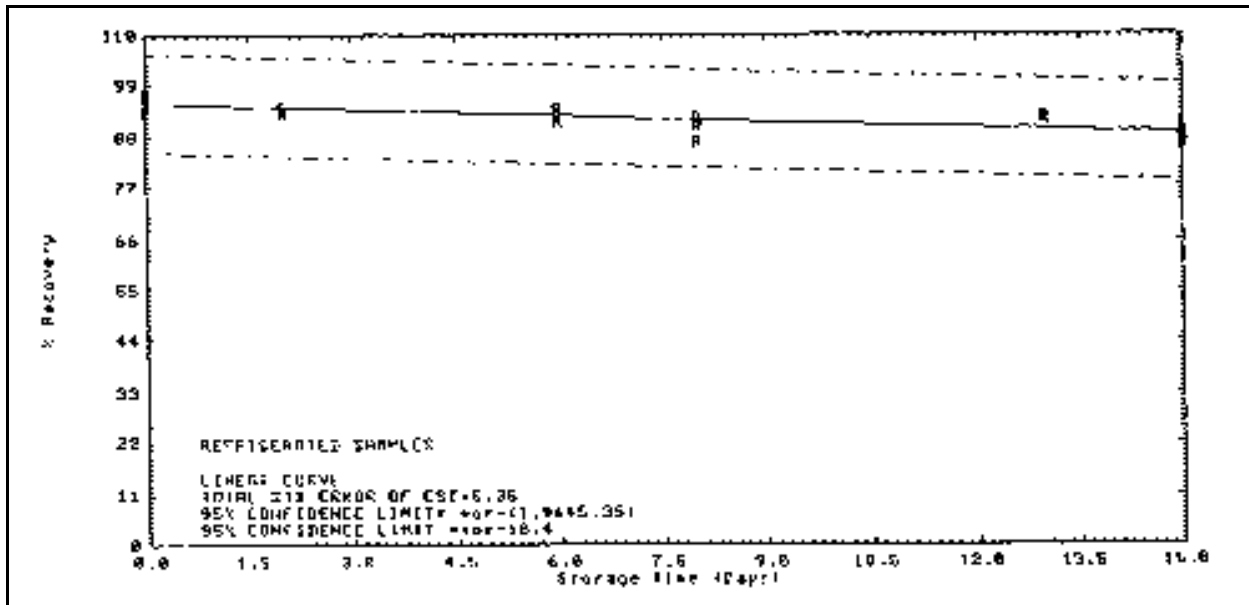


Figure 4.4.1. Benzidine refrigerated storage samples.

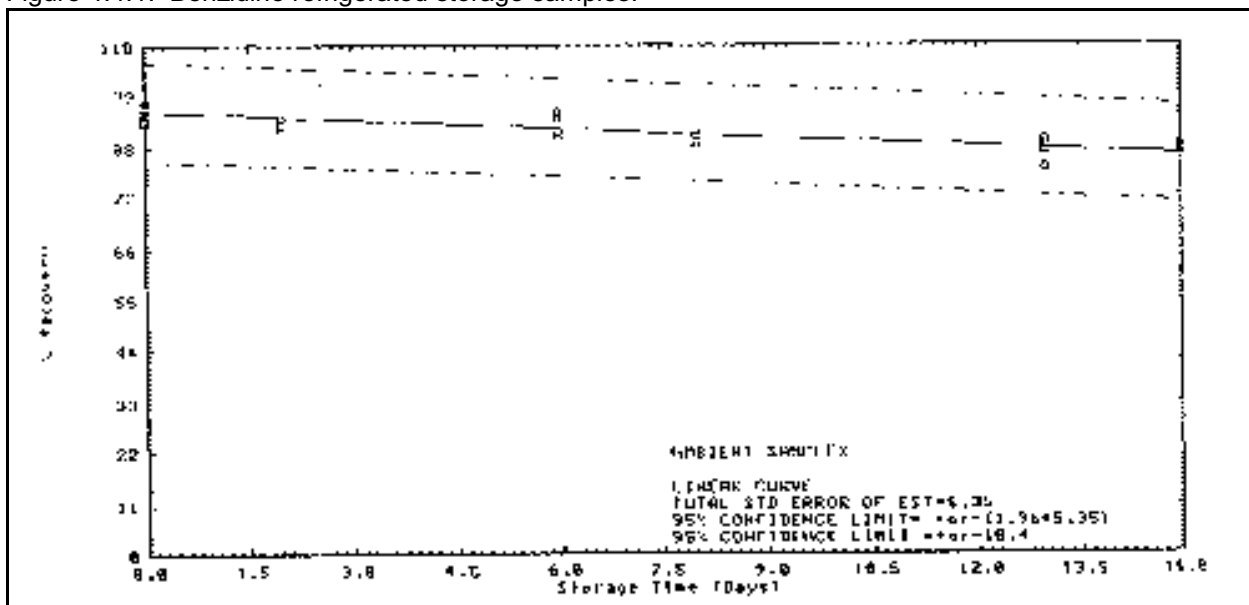


Figure 4.4.2. Benzidine ambient storage samples.

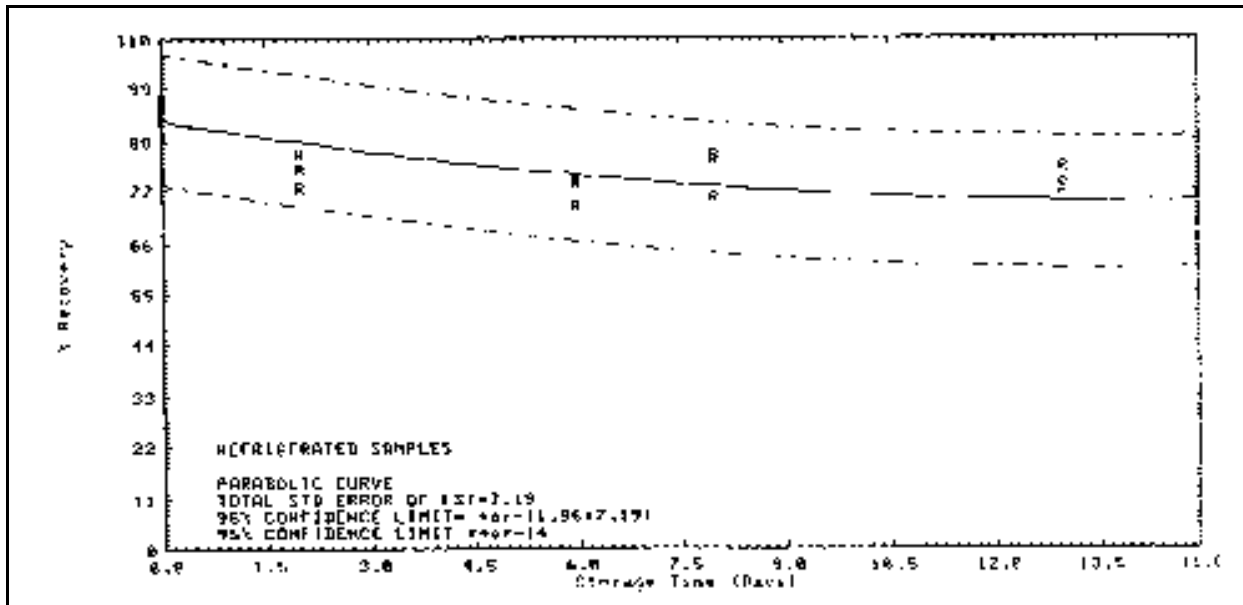


Figure 4.4.3. 3,3'-Dichlorobenzidine refrigerated storage samples.

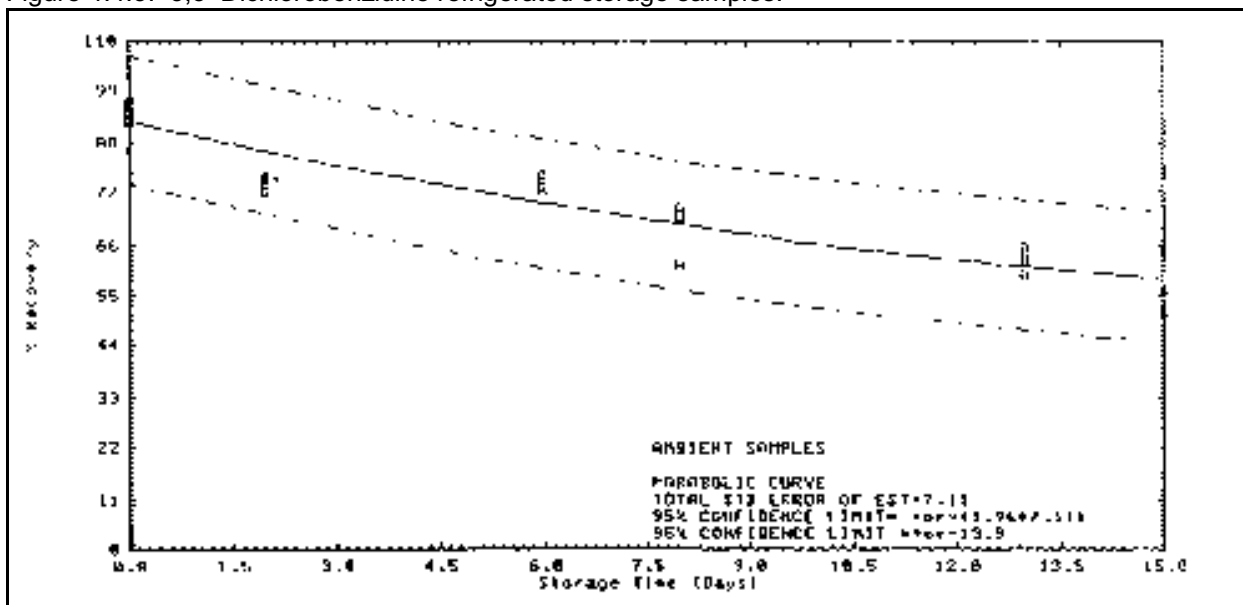


Figure 4.4.4. 3,3'-Dichlorobenzidine ambient storage samples.

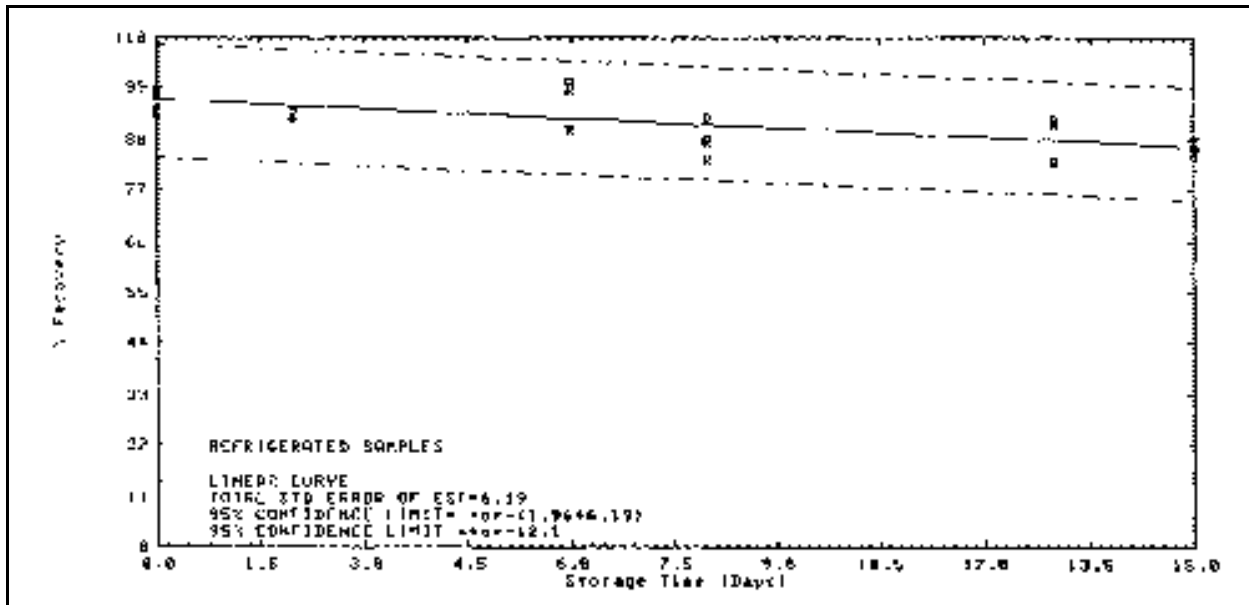


Figure 4.4.5. 2,4-Toluenediamine refrigerated storage samples.

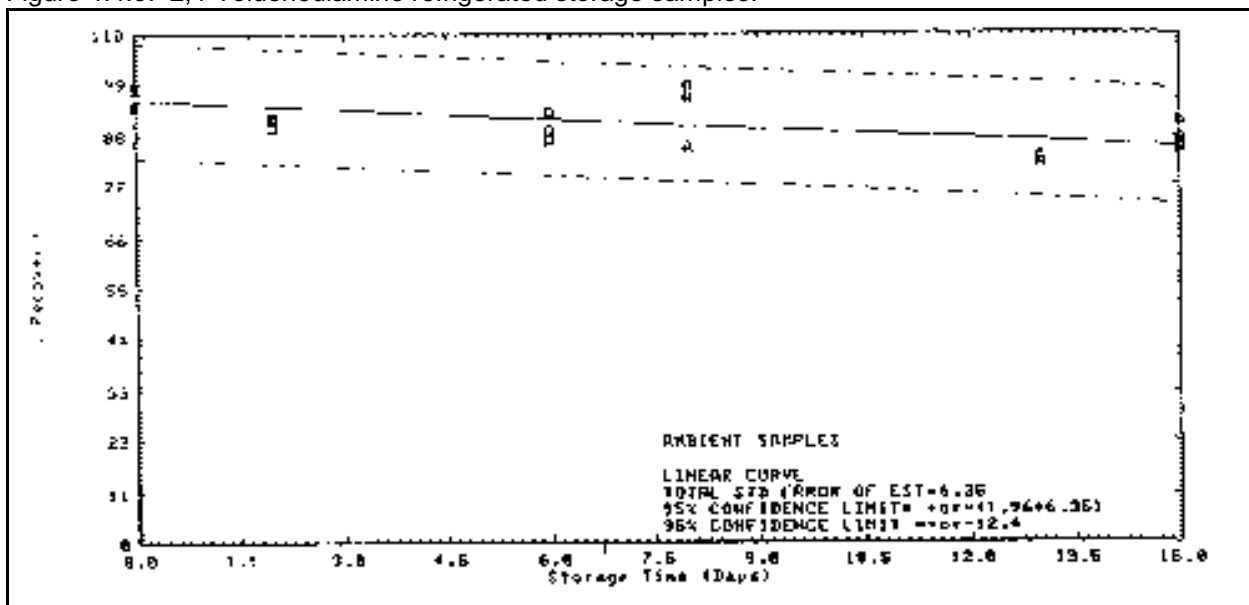


Figure 4.4.6. 2,4-Toluenediamine ambient storage samples.

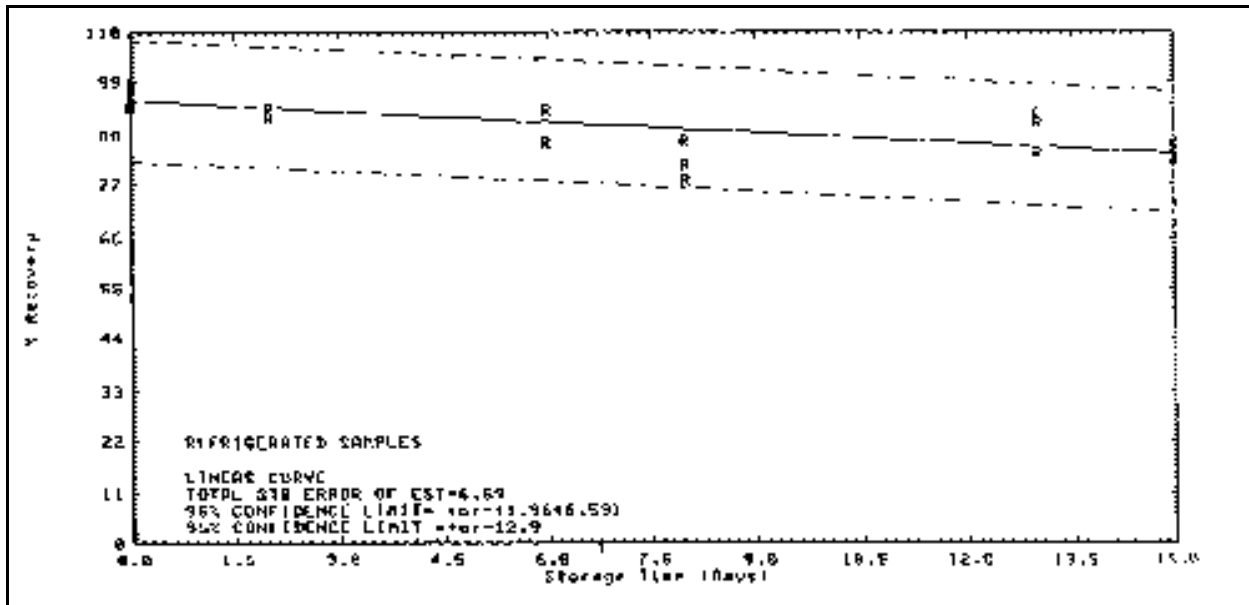


Figure 4.4.7. 2,6-Toluenediamine refrigerated storage samples.

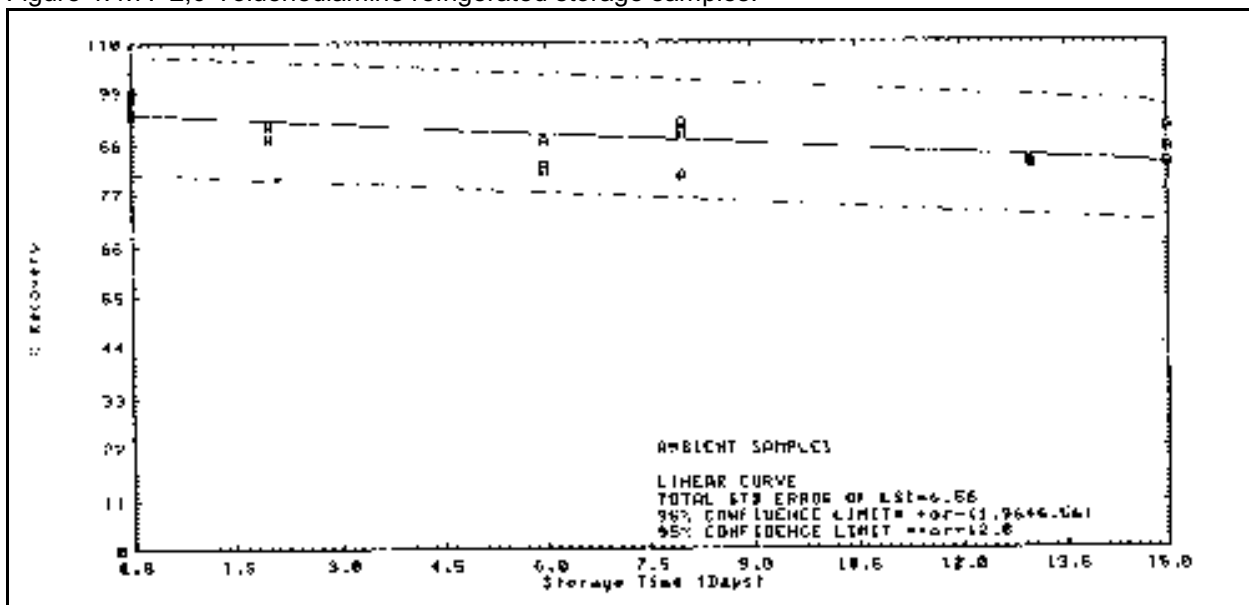


Figure 4.4.8. 2,6-Toluenediamine ambient storage samples.

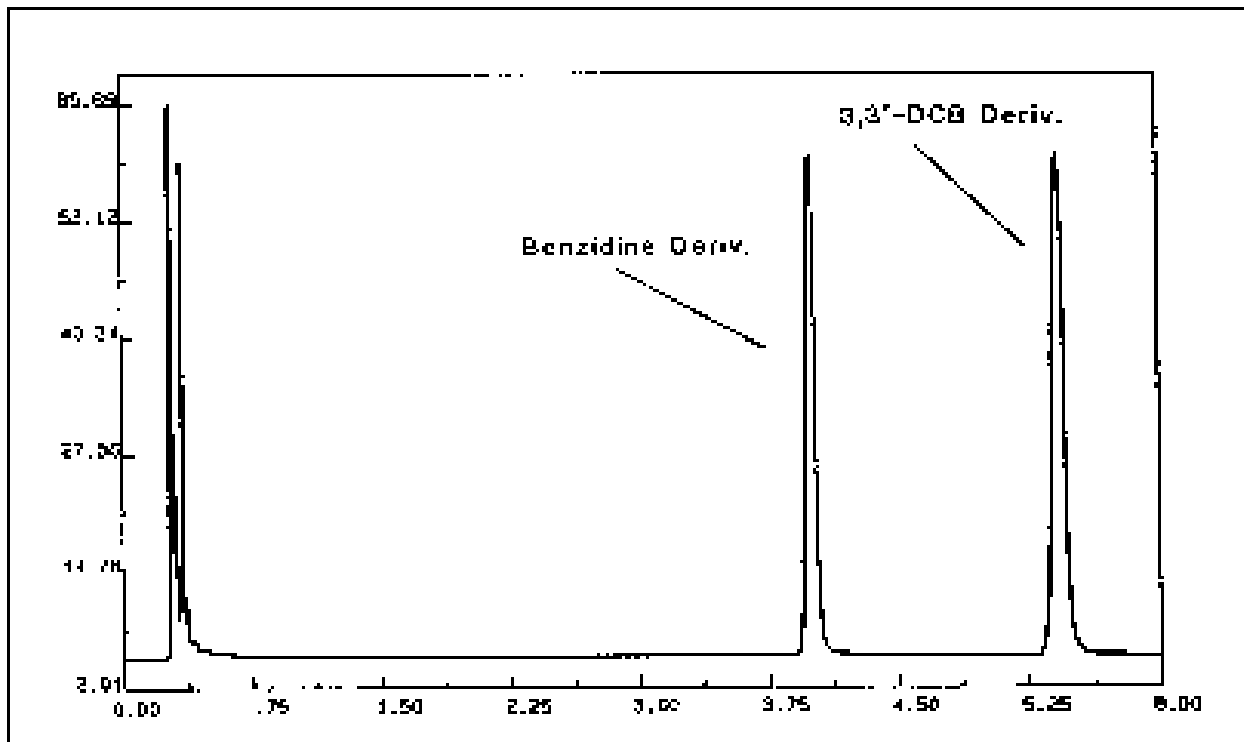


Figure 4.8.1. Benzidine and 3,3'-dichlorobenzidine chromatogram.

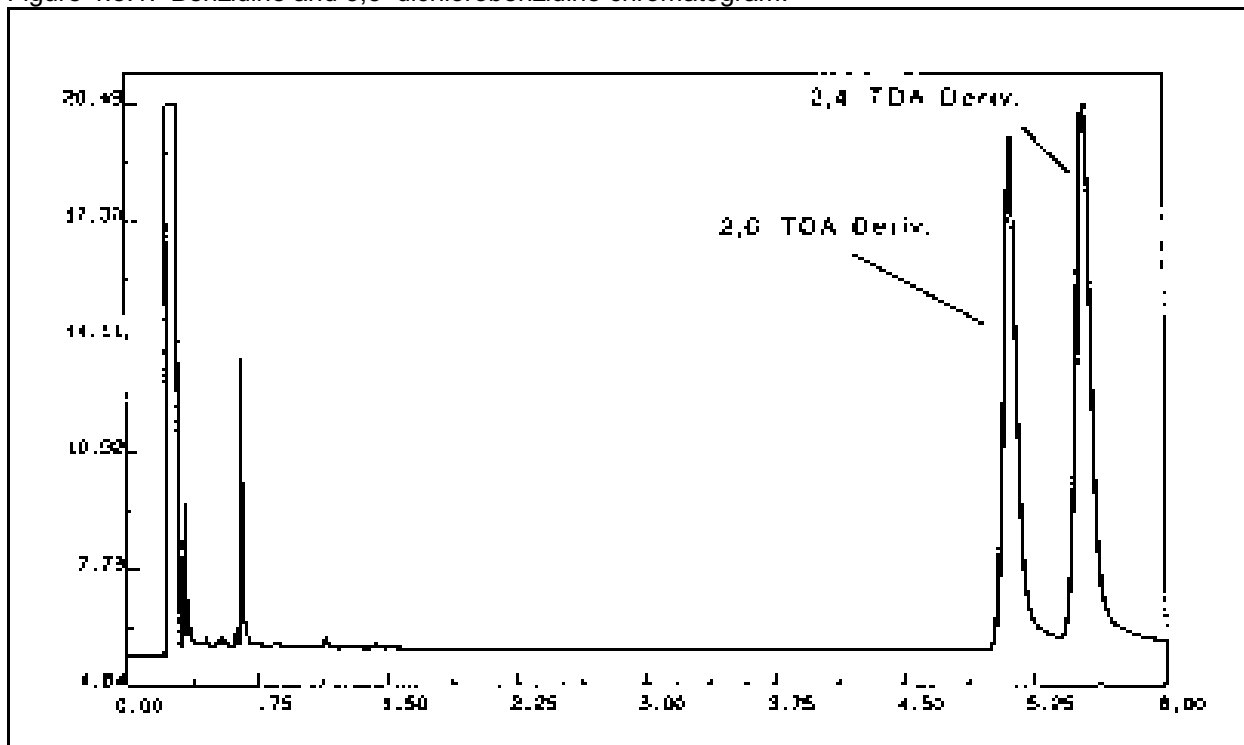


Figure 4.8.2. 2,4-Toluenediamine and 2,6-toluenediamine chromatogram.

5. References

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