o-DIANISIDINE
4,4' METHYLENEBIS(2-CHLOROANILINE) (MOCA)
o-TOLIDINE

Method no.: 71

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. The sample filters are transferred to separate glass vials containing 2 mL of deionized water within 10 h after sampling. Quantitation is performed by analyzing 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

	o-Dianisidine	MOCA	o-Tolidine
Target conc.: ppb (µg/m^3)	1 (10)	20 (218)	1 (8.7)
Reliable quantitation limits: ppt (ng/m^3)	1.2 (12)	40 (440)	1.3 (11)
Standard errors of estimate at the target concentration: (Section 4.7)	7.8%	5.8%	8.0%

Special requirements: Samples for o-dianisidine must be shipped and stored at 0°C or colder to 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: April 1988
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1. General Discussion

1.1 Background

1.1.1 History

The previous OSHA-recommended procedures to determine airborne concentrations of o-dianisidine, MOCA, and o-tolidine involved collection with an untreated glass fiber filter, a bubbler containing 0.1 N HCl, and a bubbler containing isopropyl alcohol, respectively (Ref. 5.1). The free amines were determined by high-performance liquid chromatography using an ultraviolet detector. The procedures for o-dianisidine and o-tolidine were OSHA laboratory in-house methods which were never fully validated and although the MOCA procedure had been validated, it is not certain how efficient a bubbler is for collection of aerosols. Also bubblers are an inconvenient means for taking personal air samples.

Methodology exists which has previously been validated for benzidine, 3,3'-dichlorobenzidine, 2,4-toluenediamine, 2,6-toluenediamine (Ref. 5.2) and 4,4'-methylenedianiline (Ref. 5.3). The collection of air samples involves sampling on glass fiber filters that had been treated with sulfuric acid. Thus the collected amines are converted to the more stable and less volatile corresponding amine salts on the filter surface. To further enhance the stability of samples, the filters are transferred to small vials containing 2 mL of deionized water within 10 h after sampling. The analysis involves converting the amine salts to free amines by addition of sodium hydroxide, extracting the amines into toluene, removing a portion of the toluene extract, and derivatizing the free amines in the extract with heptafluorobutyric acid anhydride (HFAA) according to the reaction

$$RNH_2 + (C_3F_7C0)_2O \rightarrow RNHCOC_3F_7 + C_3F_7COOH$$

The derivatives are analyzed by capillary gas chromatography using an electron capture detector. This sampling and analytical scheme was used in the validation of the following method for o-dianisidine, MOCA and o-tolidine.

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.)

o-DIANISIDINE: o-Dianisidine has been shown to be carcinogenic to rats and hamsters. There are no conclusive epidemiological studies to show that o-dianisidine alone is carcinogenic to humans. Most workers that are exposed to o-dianisidine are also exposed to other related amines such as benzidine, which is widely believed to be a human bladder carcinogen. (Ref. 5.4) There are no OSHA or ACGIH standards concerning occupational exposure to o-dianisidine.

MOCA: MOCA has been shown to be carcinogenic to rats and mice. There are no conclusive epidemiological studies to show that MOCA is carcinogenic to humans. (Ref. 5.5) Exposure to MOCA results in the same general toxic effects that are characteristic of aromatic amines. These effects include cyanosis (a bluish or purplish discoloration due to deficient oxygenation of the blood) and methemoglobinemia (the presence of methemoglobin in the blood). ACGIH has designated MOCA as a suspect human carcinogen and has assigned it a TLV of 0.02 ppm with a "skin" notation. (Ref. 5.6)

o-T0LIDINE: Rats that had been administered o-tolidine developed cancer in some tissues, but not in the bladder. In another experiment, hamsters given o-tolidine orally did not develop cancer. There is no strong evidence indicating that o-tolidine is or is not carcinogenic to man. As with o-dianisidine, there is very little information on the toxicity of occupational exposure to o-tolidine alone. It is usually used as a mixture with other aromatic amines, such as benzidine. There have been cases where bladder cancers have been found in workers making dyes and also in those exposed to a combination of benzidine and o-tolidine. It is widely thought that benzidine is a human bladder carcinogen. There is some evidence indicating that o-tolidine hastens the formation of cancerous

tumors in rats that had been exposed to benzidine. ACGIH has designated o-tolidine as being a suspected human carcinogen. There is no TLV assigned to o-tolidine. (Ref. 5.7)

1.1.3 Potential workplace exposure

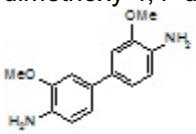
o-DIANISIDINE: The principal use for o-dianisidine is as a chemical intermediate in the production of dyes. It has been reported that 89 dyes are produced with o-dianisidine being an intermediate. probably the next most important use for o-dianisidine is as an intermediate in the production of o-dianisidine diisocyanate. It has also been used as a reagent for the detection of a number of metals, thiocyanates, and nitrites. (Ref. 5.4)

MOCA: MOCA is primarily used as a curing agent for isocyanate containing polymers. It is also widely used for curing liquid-castable polyurethane elastomers. Frequently it is formulated with other aromatic diamines, such as 3,3'-dichlorobenzidine or 4,4'-methylenedianiline, to prepare special curing agents. Small amounts of MOCA are used as curing agents for epoxy and epoxy-urethane resin blends. (Ref. 5.5)

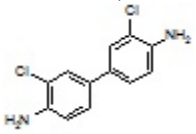
o-TOLIDINE: The most important use for o-tolidine is in the manufacture of dyes. It is also used in some analytical chemistry procedures. (Ref. 5.7)

1.1.4. Physical properties and other descriptive information

o-DIANISIDINE (Ref. 5.4)

CAS no.:	119-90-4
molecular weight:	244.3
melting point:	137-138°C
description:	Colorless crystals which turn violet on standing
solubility:	Almost insoluble in water, soluble in ethanol, ether, acetone, benzene and chloroform; probably soluble in most organic solvents and lipids
chemical reactivity:	A weak base; has the general characteristics of primary aromatic amines
synonyms:	3,3'-Dimethoxybenzidine; bianisidine; 4,4'-diamino-3,3, dimethoxybiphenyl; di-p-amino-di-m-methoxydiphenyl; 3,3'-dimethoxy-4,4'-diaminobiphenyl
structural formula:	

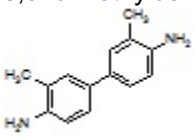
MOCA (Ref. 5.5)

CAS no.:	101-14-4
molecular weight:	267.2
melting point:	110°C
description:	Colorless crystals
solubility:	Almost insoluble in water; soluble in alcohol and ether and probably in most organic solvents and lipids
synonyms:	di-(4-amino-3-chlorophenyl)methane; 3,3, -dichloro-4,4' -diaminodiphenylmethane; bis amine; mboca; methylenebis(ortho-chloroaniline); p,p'-methylenebis(ortho-chloroaniline)
trade names:	Curalin M; Curene 442; DACPM; MOCA; Cyanaset
structural formula:	

o-TOLIDINE (Ref. 5.7)

CAS no.:	119-93-7
molecular weight:	212.3
melting point:	129-131 °C
boiling point:	200 °C
description:	white to reddish crystals or powder

solubility: slightly soluble in water, soluble in alcohol, ether or dilute acids
synonym: 3,3'-dimethylbenzidine
structural formula:



The analyte air concentrations throughout this method are based on the recommended sampling and analytical parameters. 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 Limit defining parameters

1.2.1 Detection limit of the analytical procedure

The detection limits of the analytical procedure are 6.0, 5.5, and 5.4 fg per injection for o-dianisidine, MOCA, and o-tolidine 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 1.2, 44, and 1.1 ng per sample for o-dianisidine, MOCA, and o-tolidine respectively. These are the amounts of analytes spiked on sample filters which allow recoveries of amounts of analytes equivalent to the detection limits of the analytical procedure. These detection limits correspond to air concentrations of 1.2 ppt (12 ng/m^3), 40 ppt (440 ng/m^3), and 1.3 ppt (11 ng/m^2) for o-dianisidine, MOCA, and o-tolidine respectively. (Section 4.2.)

1.2.3 Reliable quantitation limit

The reliable quantitation limits are 1.2, 44, and 1.1 ng per sample for o-dianisidine, MOCA, and o-tolidine 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 ±25% or better. These reliable quantitation limits correspond to air concentrations of 1.2 ppt (12 ng/m^3), 40 ppt (440 ng/m^3), and 1.3 ppt (11 ng/m^3) for o-dianisidine, MOCA, and o-tolidine respectively. (Section 4.3)

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 one-half to two times the target concentrations is linear for all three analytes. (Section 4.4)

1.2.5 Recovery

The recoveries of o-dianisidine, MOCA, and o-tolidine from samples used in a 15-day storage test remained above 79, 100, and 89% respectively. The o-dianisidine samples were stored in a refrigerator at 0°C and the samples for the other two analytes were stored in a closed drawer at ambient temperature (about 21°C). (Section 4.5) 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 0.020, 0.034, and 0.025 for o-dianisidine, MOCA, and o-tolidine respectively. (Section 4.6)

1.2.7 Precision (overall procedure)

The precisions at the 95% confidence level for the 15-day storage tests are ±15.4, ±11.3, and ±15.7% for o-dianisidine, MOCA, and o-tolidine respectively. These include an additional ±5% for sampling error. The o-dianisidine samples were stored in a refrigerator at 0°C and the MOCA and o-tolidine samples were stored in a closed drawer at ambient temperature (about 21°C). (Section 4.7) The overall procedure must provide results at the target concentration that are ±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 o-dianisidine and o-tolidine samples were analyzed after 2 days of storage at 0°C and the MOCA samples were analyzed after 42 days of storage at -35°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 4.8)

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 transferred to vials containing water before being submitted to the laboratory for analysis.

1.4.2 Samples for o-dianisidine 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 ±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 shipment and storage. Glass scintillation vials with caps containing Teflon liners are recommended.

2.2 Reagents

Deionized water is needed for addition to the vials described in Section 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, carefully remove the filters from the cassettes and individually transfer them to separate vials. Add approximately 2 mL of deionized water to each vial. This can be done before or after the filters are transferred.

2.3.5 Seal the vials lengthwise with OSHA Form 21.

2.3.6 Ship and store samples for o-dianisidine at 0°C or colder.

2.3.7 Submit at least one blank filter with each sample set. Handle the blank filters in the same manner as the air samples, but draw no air through them.

2.3.8 Record air volumes (in liters) for each sample, along with any potential interferences.

2.4 Retention efficiency

A retention efficiency study was performed by drawing 100 L of air (76% relative humidity) at 1 L/min through six sample filters that had been spiked with 1.00 µg of o-dianisidine. 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 90.1% (SD = 5.4) of the spiked amount. There was no o-dianisidine found on the bottom filters. Similar tests were done for 21.8 µg of MOCA and 0.868 µg of o-tolidine. Upon analysis, the top filters were found to contain an average of 101.5% (SD = 4.5) and 100.6% (SD = 4.2) of the spiked amounts of MOCA and o-tolidine respectively. There were no detectable amounts of these two analytes found on the backup filters.

2.5 Extraction efficiency

2.5.1 The average extraction efficiencies from six filters for each amine spiked at the target concentrations were 97.2, 95.7, and 99.2% for o-dianisidine, MOCA, and o-tolidine respectively. (Section 4.9)

2.5.2 The stability of extracted and derivatized samples was verified by reanalyzing the above samples 24 h later using fresh standards. The average extraction efficiencies for the reanalyzed samples were 98.9, 93.5, and 100.0% for o-dianisidine, MOCA, and o-tolidine respectively. (Section 4.9)

2.6 Recommended air volume and sampling rate

2.6.1 The recommended air volume is 100 L.

2.6.2 The recommended sampling rate is 1 L/min.

2.6.3 If a smaller air volume is desired, the reliable quantitation limits will be larger. For example, the reliable quantitation limit for o-dianisidine for a 15-L air sample would be 8.0 ppt.

2.7 Interferences (sampling)

2.7.1 Any compound in the sampled air that will react with the sulfuric acid on the treated filters or with the collected analyte is a potential sampling interference.

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

3.1.1 A GC equipped with an electron capture detector. For this evaluation, a Hewlett-Packard 5890A Gas Chromatograph equipped with a Nickel 63 electron capture detector and a 7673A Automatic Sampler was used.

3.1.2 A GC column capable of separating the amine derivatives from the solvent and interferences. A 15-m × 0.32-mm i.d. (1.0-µm film) SPB-5 fused silica column purchased from Supelco, Inc. was used in this evaluation.

3.1.3 An electronic integrator or some other suitable means of measuring peak areas or heights. A Hewlett-Packard 18652A A/D converter interfaced to a Hewlett-Packard 3357 Lab Automation Data System 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 Repetitive pipets, one to deliver 25 µL of HFAA and one to transfer 50-µL aliquots of MOCA samples and standards.

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 Na0H solutions, prepared from reagent grade Na0H.

3.2.2 Toluene. American Burdick and Jackson "High Purity Solvent" brand 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 reagent grade potassium dihydrogen phosphate and 1 L deionized water. The pH is adjusted to 7.0 with saturated sodium hydroxide solution.

3.2.5 o-Dianisidine, MOCA, o-tolidine, reagent grade. The o-dianisidine used in this evaluation was purchased from Aldrich Chemical Company, Inc., Milwaukee WI. The MOCA and o-tolidine were purchased 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. In order to keep the response of MOCA standards which are at or around the target concentration (20 ppb for a 100-L air sample) in the linear range of the electron capture detector used, a further dilution was required. This was accomplished by adding 50-µL aliquots of the MOCA analytical standards to vials containing 2.0 mL of toluene.

3.3.2 Add 25 µL 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 two layers to separate, analyze the toluene (upper) layer of each standard by GC.

3.3.6 Bracket sample concentrations with analytical standard concentrations. If sample concentrations are higher than the upper range of prepared standards, prepare additional standards to ascertain detector response or derivatize a smaller aliquot of the toluene extract of the high samples using toluene as the diluent.

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 Na0H and 2.0 mL of toluene to each vial.

3.4.3 Recap and shake the vials for 10 min.

3.4.4 If the samples are to be analyzed for o-dianisidine or o-tolidine, allow the layers to separate and transfer approximately 1 mL of the toluene (upper) layer of each sample to separate vials with clean disposable pipets. For MOCA samples, allow the layers to separate and transfer a 50-µL aliquot of the toluene layer of each sample to separate vials, each containing 2.0 mL of toluene.

3.4.5 Add 25 µL 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 two layers to separate, analyze the toluene (upper) layer of each sample by GC.

3.5 Analysis

3.5.1 GC conditions and information

zone temperatures:	column, 250°C injector, 225°C detector, 300°C
gas flows:	column 2.3 mL/min hydrogen (35 kPa head pressure) make up 45 mL/min nitrogen
injection volume:	1.0 µL
split ratio:	100:1
column:	SPB-5, 1.0-µm film, 15-m × 0.32-mm i.d. fused silica (Supelco, Inc.)
retention times of derivatives:	o-Dianisidine, 5.5 min MOCA, 4.2 min o-Tolidine, 3.9 min
chromatograms:	Section 4.10

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 elutes in the same general 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 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.

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

where extraction efficiencies are: 97.2% (o-Dianisidine)
95.7% (MOCA)
99.2% (o-Tolidine)

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

where 24.46 is the molar volume (liters) at 25^0C and 760 mm Hg
molecular weights are: 244.3 (o-Dianisidine), 267.2 (MOCA), 212.3 (o-Tolidine)

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.

3.8.2 Avoid skin contact and inhalation of all chemicals. Restrict the use of chemicals to a fume hood if possible. Wear safety glasses and a lab coat while in the lab area.

4. Backup Data

4.1 Detection limit 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 6.0 fg of o-dianisidine, 5.5 fg of MOCA, and 5.4 fg of o-tolidine were determined by analyzing dilute standards equivalent to 1.20 ng of o-dianisidine, 43.8 ng of MOCA, and 1.09 ng of o-tolidine per sample. (The samples are extracted into 2.0 mL of toluene. The MOCA samples are further diluted by transferring a 50-µL aliquot to 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 limit of the overall procedure

The detection limits of the overall procedure were determined by analyzing filters spiked with loadings equivalent to the detection limits of the analytical procedure. Samples were prepared by injecting 1.20 ng of o-dianisidine, 43.8 ng of MOCA, and 1.09 ng of o-tolidine onto acid-treated filters. These amounts are equivalent to 1.2 ppt (12 ng/m^3), 4O ppt (440 ng/m^3), and 1.3 ppt (11 ng/m^3) for o-dianisidine, MOCA, and o-tolidine respectively.

Table 4.2.1
Detection Limit of the Overall Procedure for o-Dianisidine

sample no.	ng spiked	ng recovered
1	1.20	1.07
2	1.20	1.27
3	1.20	1.13
4	1.20	1.01
5	1.20	1.25
6	12.0	1.31

Table 4.2.2
Detection Limit of the Overall Procedure for MOCA

sample no.	ng spiked	ng recovered
1	43.8	37.9
2	43.8	46.3
3	43.8	39.9
4	43.8	47.8
5	43.8	42.7
6	43.8	41.1

Table 4.2.3
Detection Limit of the Overall Procedure for o-Tolidine

sample no.	ng spiked	ng recovered
1	1.09	1.02
2	1.09	1.15
3	1.09	1.01
4	1.09	0.97
5	1.09	1.07
6	1.09	1.06

4.3 Reliable quantitation limit

The reliable quantitation limits were determined by analyzing filters spiked with loadings equivalent to the detection limits of the analytical procedure. Samples were prepared by injecting 1.20 ng of o-dianisidine, 43.8 ng of MOCA, and 1.09 ng of o-tolidine onto acid-treated filters. These amounts are equivalent to 1.2 ppt (12 ng/m^3), 40 ppt (440 ng/m^3), and 1.3 ppt (11 ng/m^3) for o-dianisidine, MOCA, and o-tolidine respectively.

Table 4.3.1
Reliable Quanitation Limit for o-Danisidine
(Based on samples and data of Table 4.2.1)

sample	% recovered	statistics
1	89.2	
2	105.8	$\overline{X}$=97.8%
3	94.2	SD=10.1%
4	84.2	Precision=±(1.96)(10.1%)
5	104.2	=±19.8
6	109.2	

Table 4.3.2
Reliable Quantitation Limit for MOCA
(Based on samples and data of Table 4.2.2)

sample	% recovered	statistics
1	86.5	
2	105.7	$\overline{X}$=97.3%
3	91.1	SD=8.69%
4	109.1	Precision=±(1.96)(8.69%)
5	97.5	=±17.0
6	93.8	

Table 4.3.3
Reliable Quantitation Limit for o-Tolidine
(Based on samples and data of Table 4.2.3

sample	% recovered	statistics
1	93.6	
2	105.5	$\overline{X}$=96.0%
3	92.7	SD=5.70%
4	89.0	Precision=±(1.96)(5.70%)
5	98.2	=±11.2
6	97.2	

4.4 Instrument response to the analyte

The instrument response to the analytes over the range of 0.5 to 2 times the target concentrations was determined from multiple injections of analytical standards. These data are given in Tables 4.4.1 - 4.4.3 and Figures 4.4.1 - 4.4.3. The response is linear for the three analytes with slopes (in area counts per micrograms of analyte per sample) of 150,400 for o-dianisidine, 5080 for MOCA, and 190,100 for o-tolidine.

Table 4.4.1
Instrument Response to o-Dianisidine

× target concn	0.5×	1×	2×
µg/sample	0.50	1.00	2.00
ppb	0.50	1.00	2.00
area counts	106412	195200	333377
	103872	191643	337847
	100769	189055	340122
	106147	195327	335264
	105799	188406	321881
	103801	186838	325757
$\overline{X}$	104467	191078	332375

Table 4.4.2
Instrument Response to MOCA

× target concn	0.5×	1×	2×
µg/sample	10.9	21.8	43.6
ppb	10.0	20.0	39.9
area counts	74749	127250	244357
	75422	122572	255069
	79894	133929	248678
	75636	137936	232331
	77790	132986	235188
	75830	132634	239419
$\overline{X}$	76554	131218	242507

Table 4.4.3
Instrument Response to o-Tolidine

× target concn	0.5×	1×	2×
µg/sample	0.434	0.868	1.736
ppb	0.50	1.00	2.00
area counts	115996	214903	367038
	112884	209958	373575
	110286	205280	371890
	116518	212341	365314
	115563	204580	349354
	110950	201801	354703
$\overline{X}$	113700	208144	363646

4.5 Storage test

Test atmospheres containing these potentially carcinogenic amines could not be safely generated in our laboratory. Storage samples were generated by spiking acid-treated filters with amounts of analyte equal to the target concentrations (1.00 µg of o-dianisidine, 21.8 µg of MOCA, 0.868 µg of o-tolidine). Thirty-six samples were prepared for each analyte. One hundred liters of air at 76% relative humidity were then drawn through each filter. Within 1 h 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 4.5.1 -4.5.3 and in Figures 4.5.1.1, 4.5.1.2, 4.5.2.1, 4.5.2.2, 4.5.3.1 and 4.5.3.2. 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 7.8, 5.8, and 8.0% and the 95% confidence limits (±1.96 SD) are ±15.4, ±11.3, and ±15.7% for o-dianisidine, MOCA, and o-tolidine respectively. These values were obtained from Figures 4.5.1.1, 4.5.2.2 and 4.5.3.2.

Table 4.5.1
Storage Test for o-Dianisidine

storage time (days)	% recovery (ambient)			% recovery (refrigerated)		
0	94.0	97.6	90.6	94.0	97.6	90.6
	102.8	91.2	89.4	102.8	91.2	89.4
3	74.5	70.2	72.0	87.5	92.4	92.5
5	81.2	49.9	63.9	77.0	8.3	84.2
8	62.6	68.8	54.6	86.4	88.6	85.8
12	69.4	34.4	45.9	73.2	72.8	77.0
15	53.7	70.0	67.8	85.6	89.5	84.6

Table 4.5.2
Storage Test or MOCA

storage time (days)	% recovery (ambient)			% recovery (refrigerated)		
0	98.2	95.0	95.4	98.2	95.0	95.4
	104.9	97.0	100.4	104.9	97.0	100.4
3	102.5	100.8	101.9	98.6	103.4	104.7
5	98.4	102.0	99.4	101.4	108.4	99.7
8	102.6	104.8	104.3	101.8	100.6	102.3
12	99.7	97.2	98.1	102.0	99.0	100.9
15	103.2	99.2	100.6	101.3	102.3	104.6

Table 4.5.3
Storage Test for o-Tolidine

storage time (days)	% recovery (ambient)			% recovery (refrigerated)		
0	98.4	102.7	99.0	98.4	102.7	99.0
	109.2	96.8	97.2	109.2	96.8	97.2
3	96.0	87.4	93.6	94.8	101.1	99.3
5	96.2	92.0	91.4	98.1	102.6	101.3
8	96.6	98.2	99.0	103.2	101.2	101.5
12	86.6	76.2	83.6	93.6	98.2	95.
15	92.2	97.2	96.8	100.6	101.0	96.5

4.6 Precision (analytical method only)

The precision of the analytical method for each analyte is the pooled coefficient of variation determined from replicate injections of standards. The standards analyzed for these determinations are described in Section 4.4. The precision of the analytical method for each analyte is given in Tables 4.6.1 - 4.6.3.

Table 4.6.1
Precision of the Analytical Method
for o-Diansidine
(Based on the Data of Table 4.4.1)

× target concn	0.5×	1×	2×
µg/sample	0.50	1.00	2.00
ppb	0.50	1.00	2.00
SD[1]	2138	3594	7117
CV	0.020	0.019	0.021
$\overline{CV}$	0.020		

[1]standard deviation is in area counts

Table 4.6.2
Precision of the Analytical Method
for MOCA
(Based on the Data of Table 4.4.2)

× target concn	0.5×	1×	2×
µg/sample	10.9	21.8	43.6
ppb	10.0	20.0	39.9
SD[1]	1928	5443	8555
CV	0.025	0.041	0.035
$\overline{CV}$	0.034		

[1]standard deviation is in area counts

Table 4.6.3
Precision of the Analytical Method
for o-Tolidine
(Based on the Data of Table 4.4.3)

× target concn	0.5×	1×	2×
µg/sample	0.434	0.868	1.736
ppb	0.50	1.00	2.00
SD[1]	2704	5054	9644
CV	0.024	0.024	0.027
$\overline{CV}$	0.025		

[1]standard deviation is in area counts

4.7 Precision (overall procedure)

The precision of the overall procedure is determined from the storage data. The determination of the standard error of estimate (SEE) for a regression line plotted through the graphed storage data allows the inclusion of storage time as one of the factors affecting overall precision. The SEE is similar to the standard deviation, except it is a measure of dispersion of data about a regression line instead of about a mean. It is determined with the following equation:

$$SEE = \sqrt{\frac{\sum(Y_{obs} - Y_{est})^2}{n - k}}$$

where

n = total number of data points
k = 2 for linear regression
k = 3 for quadratic regression
Y_{obs} = observed % recovery at a given time
Y_{est} = estimated % recovery from the regression line at the same given time

An additional 5% for pump error is added to the SEE by the addition of variances. The precision at the 95% confidence level is obtained by multiplying the SEE (with pump error included) by 1.96 (the z-statistic from the standard normal distribution at the 95% confidence level). The 95% confidence intervals are drawn about their respective regression lines in the storage graphs as shown in Figures 4.5.1.1, 4.5.1.2, 4.5.2.1, 4.5.2.2, 4.5.3.1 and 4.5.3.2. The precisions of the overall procedure of ±15.4 for o-dianisidine, ±11.3 for MOCA, and ±15.7% for o-tolidine were obtained from Figures 4.5.1.1, 4.5.2.2 and 4.5.3.2, respectively.

4.8 Reproducibility

Six samples for each analyte were prepared by injecting microliter quantities of standards onto acid-treated filters. The o-dianisidine and o-tolidine samples were stored at 0°C for 2 days and the MOCA samples were stored at -35°C for 42 days. The samples were analyzed by a chemist unassociated with this evaluation. The results are given in Tables 4.8.1 - 4.8.3. All of the amounts found were corrected for extraction efficiency. The extraction efficiency is 97.2% for o-dianisidine, 95.7% for MOCA, and 99.2% for o-tolidine. The precision of the overall procedure is ±15.4% for o-dianisidine, ±11.3% for MOCA, and ±15.7% for o-tolidine.

Table 4.8.1
Reproducibility Data for o-Diansidine

sample no.	µg found	µg expected	% found	% deviation
1	0.927	0.960	96.6	-3.4
2	0.940	0.960	97.9	-2.1
3	0.963	0.960	100.3	+0.3
4	0.959	0.960	99.9	-0.1
5	0.98	0.960	102.9	+2.9
6	0.960	0.960	100.0	0.0

Table 4.8.2
Reproducibility Data for MOCA

sample no.	µg found	µg expected	% found	% deviation
1	20.80	22.07	94.2	-5.8
2	20.05	22.07	90.8	- 9.2
3	20.42	22.07	92.5	- 7.5
4	19.82	22.07	89.8	-10.2
5	21.90	22.07	99.2	-0.8
6	21.59	22.07	97.8	-2.2

Table 4.8.3
Reproducibility Data for o-Tolidine

sample no.	µg found	µg expected	% found	% deviation
1	0.859	0.872	98.5	-1.5
2	0.861	0.872	98.7	-1.3
3	0.886	0.872	101.6	+1.6
4	0.868	0.872	99.5	-0.5
5	0.871	0.872	99.9	-0.1
6	0.859	0.872	98.5	-1.5

4.9 Extraction efficiency data

Six sample filters for each amine were spiked with the target concentration amounts by liquid injection (1.00 µg of o-dianisidine, 21.8 µg of MOCA, and 0.868 µg of o-tolidine). These samples were analyzed to determine the extraction efficiencies. To determine the stability of extracted and derivatized samples, these same samples were reanalyzed after setting at room temperature for 24 h using fresh standards. The results are given in Tables 4.9.1 - 4.9.3.

Table 4.9.1
Extraction Efficiency for o-Dianisidine

sample no.	% extracted	reanalyzed after 24 h
1	99.2	106.2
2	95.7	97.3
3	94.0	96.2
4	98.2	96.8
5	97.0	96.5
6	99.4	100.3
$\bar{X}$	97.2	98.9

Table 4.9.2
Extraction Efficiency for MOCA

sample no.	% extracted	reanalyzed after 24 h
1	95.4	94.4
2	92.7	94.1
3	99.5	96.6
4	94.0	92.9
5	92.2	90.3
6	100.5	92.8
$\bar{X}$	95.7	93.5

Table 4.9.3
Extraction Efficiency for o-Tolidine

sample no.	% extracted	reanalyzed after 24 h
1	101.7	107.5
2	97.1	95.9
3	96.5	98.9
4	100.5	98.2
5	97.7	95.8
6	101.8	103.7
$\bar{X}$	99.2	100.0

4.10 Chromatogram

Chromatograms at the target concentrations are shown in Figures 4.10.1 and 4.10.2. The chromatograms are from 1.0-µL injections of standards.

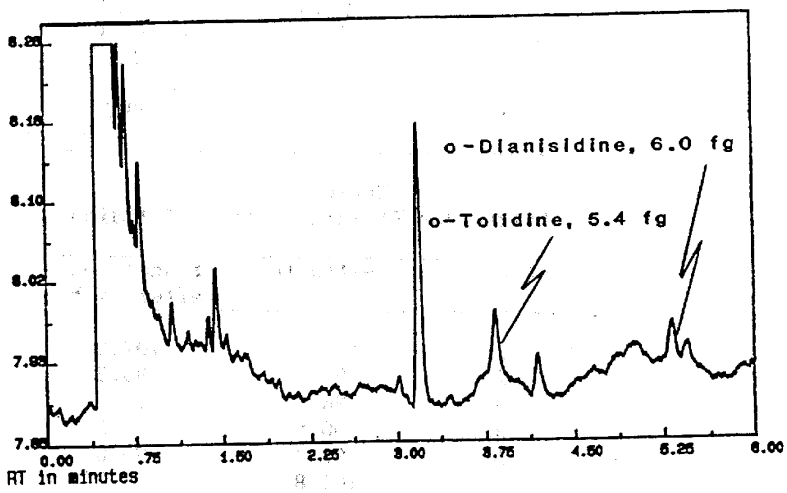


Figure 4.1.1. Detection limit chromatogram for o-dianisidine and o-tolidine.

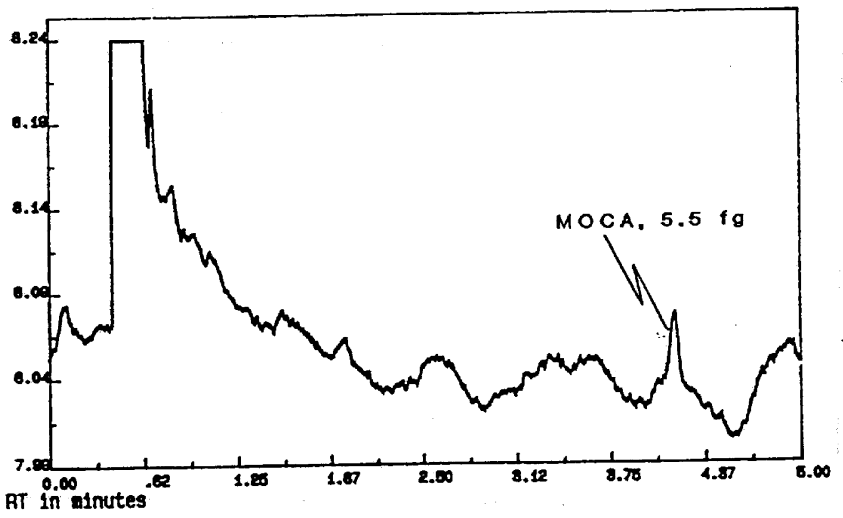


Figure 4.1.2. Detection limit chromatogram for MOCA.

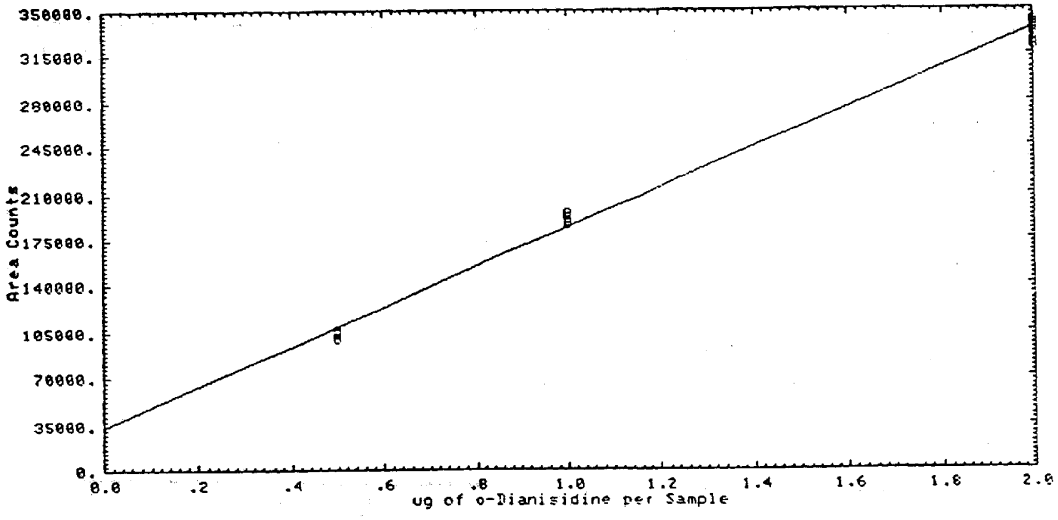


Figure 4.4.1. Instrument response to o-dianisidine.

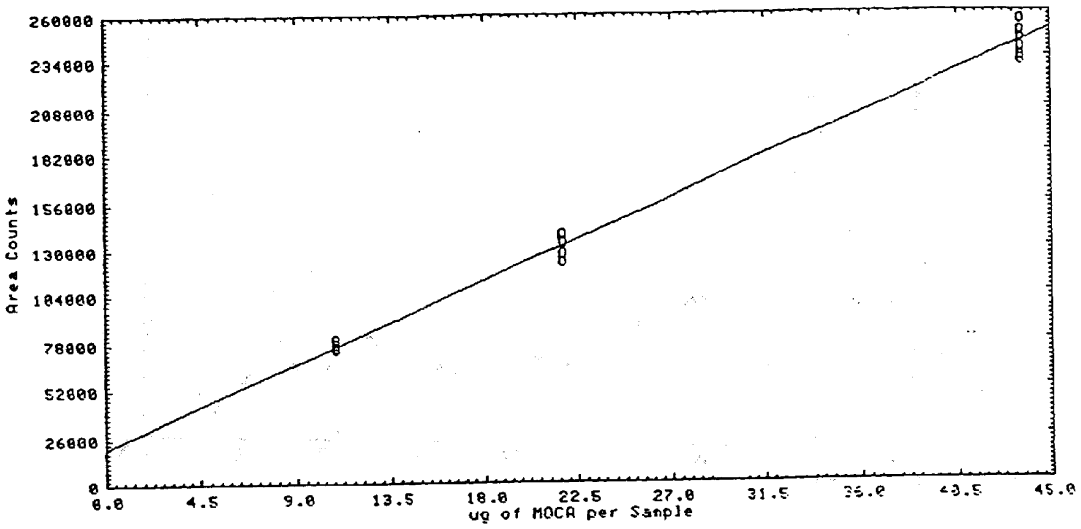


Figure 4.4.2. Instrument response to MOCA.

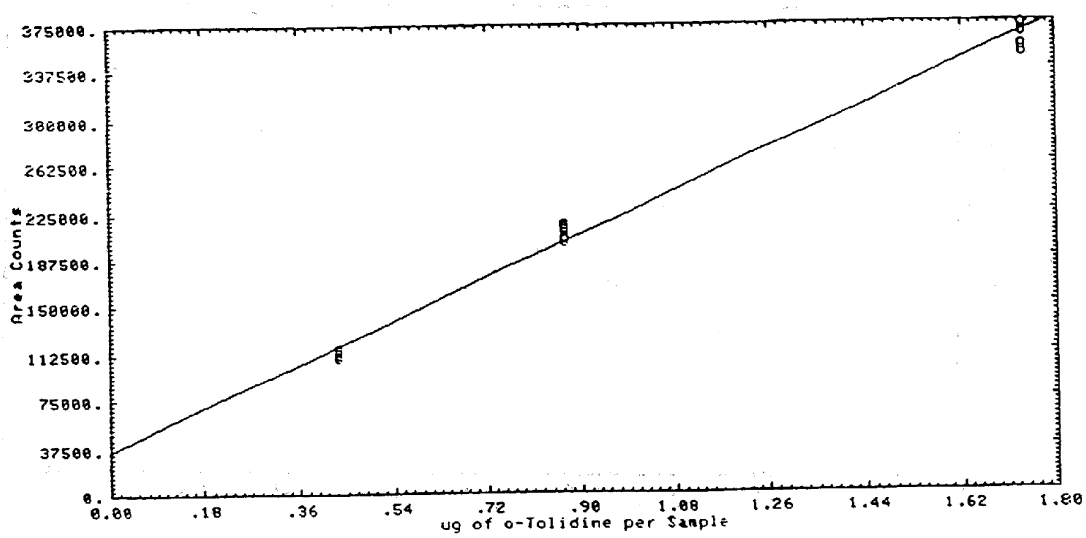


Figure 4.4.3. Instrument response to o-tolidine.

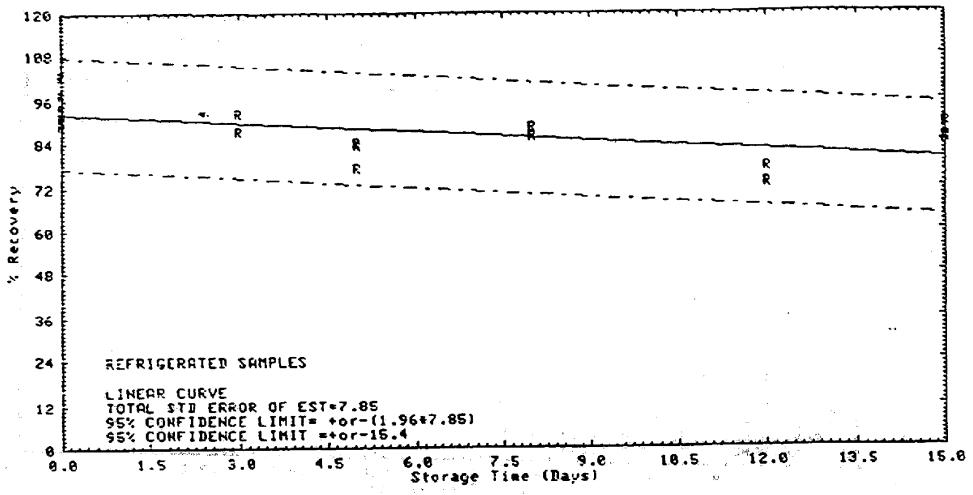


Figure 4.5.1.1. o-Dianisidine refrigerated storage samples.

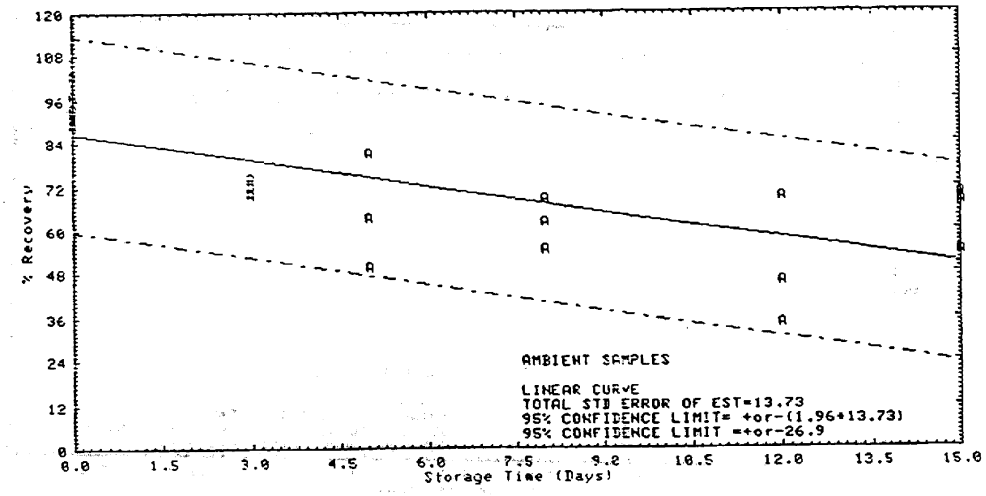


Figure 4.5.1.2. o-Dianisidine ambient storage samples.

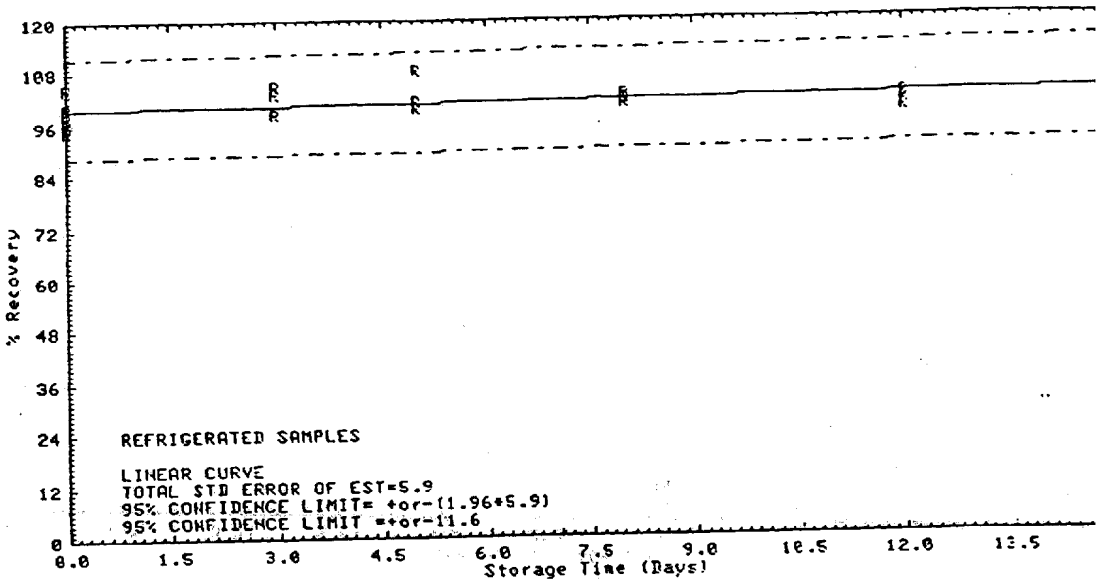


Figure 4.5.2.1. MOCA refrigerated storage samples.

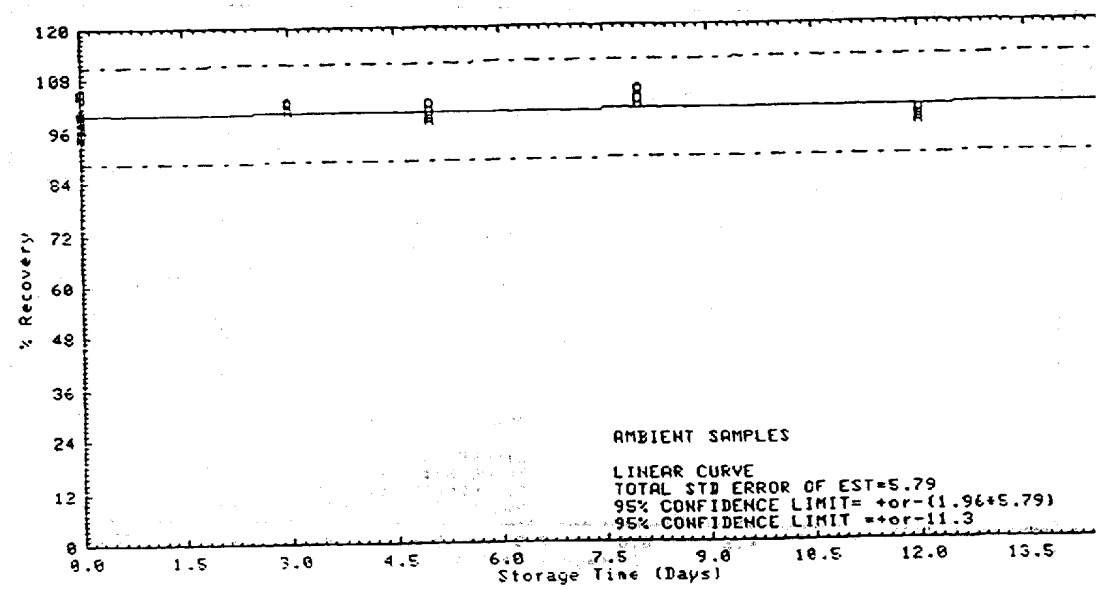


Figure 4.5.2.2. MOCA ambient storage samples.

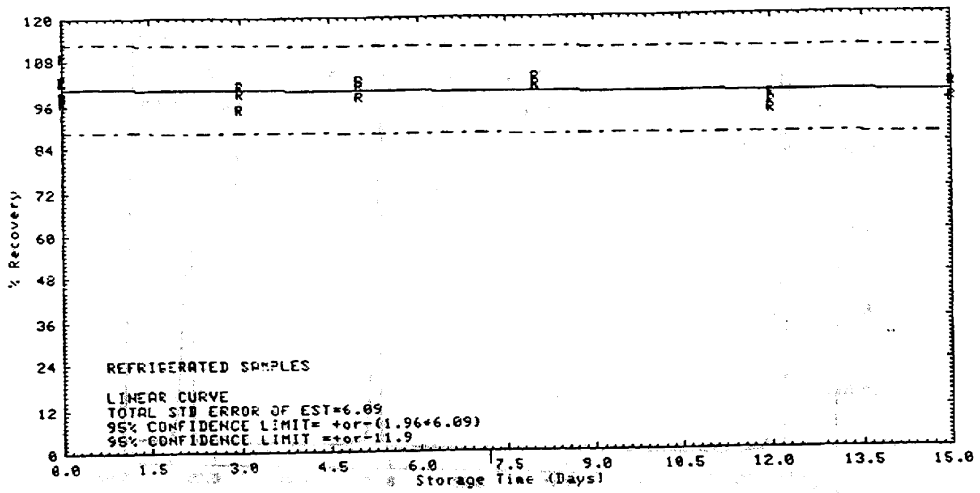


Figure 4.5.3.1. o-Tolidine refrigerated storage samples.

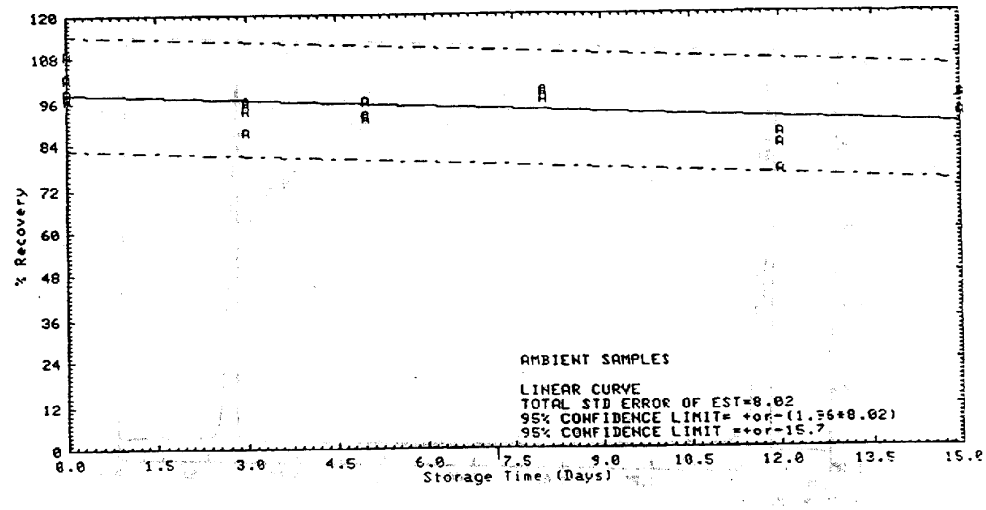


Figure 4.5.3.2. o-Tolidine ambient storage samples.

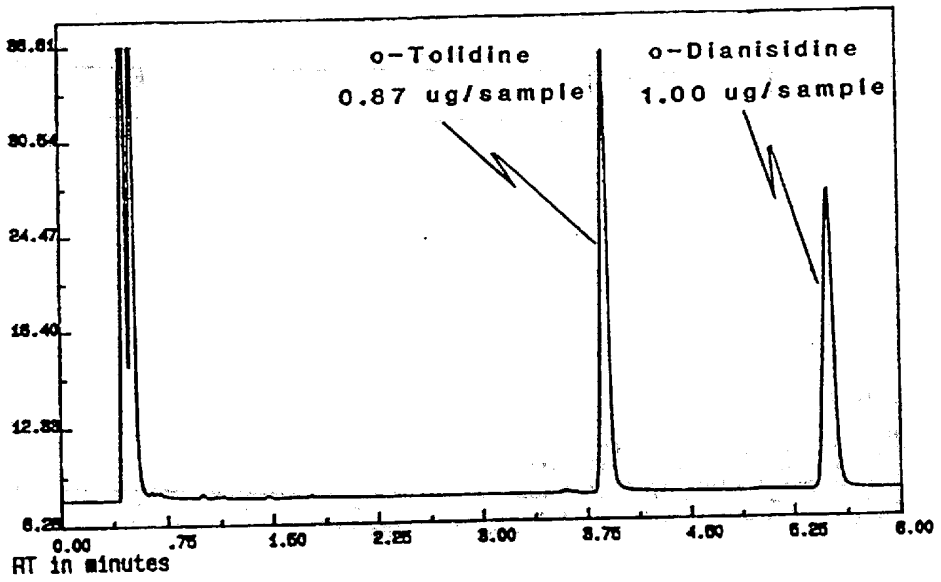


Figure 4.10.1. o-Dianisidine and o-tolidine chromatogram.

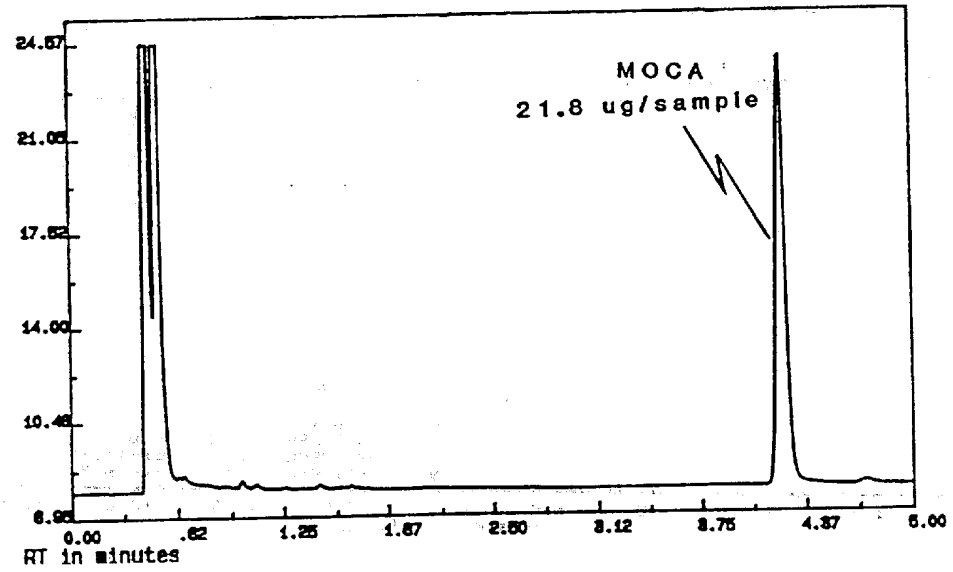


Figure 4.10.2. MOCA chromatogram.

5. References

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