GLUTARALDEHYDE

Method number:	64
Matrix:	Air
Target concentration:	200 ppb (820 µg/m^3)
ACGIH TLV-Ceiling:	200 ppb (820 µg/m^3)
OSHA PEL:	None
(Additional data, 1997)	
Target concentrations:	10 ppb (41µg/m^3) (for short-term samples, (STS))
	2 ppb (8.2 µg/m^3) (for long-term samples, (LTS))
Procedure:	An air sample is collected by drawing a known volume of air through an open-face air monitoring cassette containing 2 glass fiber filters, each of which is coated with 2,4-dinitrophenylhydrazine and phosphoric acid. The sample filters are extracted separately with acetonitrile and analyzed by HPLC using a UV detector.
Recommended air volume and sampling rates:	
200-ppb ACGIH Ceiling:	15 L at 1 L/min
(Additional data, 1997)	
10-ppb STS:	30 L at 2 L/min
2-ppb LTS:	480 L at 2 L/min
Reliable quantitation limits:	
200-ppb ACGIH TLV-Ceiling:	4.4 ppb (18 µg/m^3)
(Additional data, 1997)	
10-ppb STS:	0.44 ppb (1.8 µg/m^3)
2-ppb LTS:	0.027 ppb (0.11 µg/m^3)
Standard errors of estimate at the target concentration:	
200-ppb ACGIH TLV-Ceiling:	6.2%
(Additional data, 1997)	
10-ppb STS:	6.6%
2-ppb LTS:	6.7%
Special requirements:	Ship samples suspected of containing low levels of glutaraldehyde (such as 10-ppb STS samples) in an insulated container using Blue Ice (or
(Additional data, 1997)	equivalent) by overnight delivery service (FedEx, or equivalent). Use an ozone-scavenging filter for LTS, or reduce sample air volume, if ozone in the sampled air is greater than 10 ppb (Sections 2.1.3 and 2.6.4). Store all glutaraldehyde samples in a refrigerator until analysis.
Status of method:	Evaluated method. This method has been subjected to the established evaluation procedures of the Organic Methods Evaluation Branch. Additional evaluation data were collected in 1997 because of increased interest in monitoring lower levels.

Date: June 1987 Chemist: Warren Hendricks

Additional data: January 1998

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1. General Discussion

1.1 Background

1.1.1 History

This work was performed because there was no fully evaluated OSHA method for the sampling and analysis of glutaraldehyde. This method requires the collection of glutaraldehyde on glass-fiber filters which have been coated with 2,4-dinitrophenylhydrazine (DNPH) and phosphoric acid. The sampling method is similar to a procedure found in the literature which was developed for formaldehyde (Ref. 5.1). DNPH is a widely used derivatizing reagent for the determination of aldehydes and ketones (Ref. 5.2). The reaction between glutaraldehyde and DNPH is presented below:

$$\underset{\text{glutaraldehyde}}{HOC(CH_2)_3COH} + 2\ \underset{\text{DNPH}}{(O_2N)_2\ C_6H_3NHNH_2} + \text{acid} \rightarrow$$

$$\underset{\text{glutaraldehyde-bis-DNPH derivative}}{(O_2N)_2C_6H_3NHN{=}CH(CH_2)_3HC{=}NHNC_6H_3(NO_2)_2} + 2\ \underset{\text{water}}{H_2O}$$

The analysis is performed by HPLC using UV detection.

Prior to the development of the coated-filter procedure, it was found that glutaraldehyde could be collected directly on XAD-4 adsorbent. Recoveries near 100% were obtained when samples were analyzed immediately after generation but samples were not stable following storage at ambient temperature. Similar storage instability problems were encountered when glutaraldehyde was collected on XAD-2 adsorbent which had been coated with DNPH and phosphoric acid. Since initial sample recoveries were near 100% and the glutaraldehyde-bis-DNPH derivative is very stable, the most likely explanation for the observed sample instability is that the reagent on the head of the tube was consumed and the glutaraldehyde was collected but not derivatized.

An effort was also made to extend the sampling method used by OSHA for the collection of acrolein and formaldehyde (Ref. 5.3) to include glutaraldehyde. The basis of the method is the reaction of 2-(hydroxymethyl)piperidine (2-HMP) with the aldehyde. The 2-HMP derivative of glutaraldehyde was not detected by gas chromatography using a nitrogen selective detector when a wide variety of GC packing materials and analytical conditions were used. The derivative was also not detected by gas chromatography/mass spectrometry.

Additional data, 1997

Additional evaluation data were collected in 1997 in support of research performed by OSHA's Directorate of Policy. The research was prompted because glutaraldehyde was identified as one of a number of chemicals for which OSHA intends to publish a proposal to update PELs (Ref. 7.1). The target levels, 10-ppb for short-term samples (STS) and 2-ppb for long-term samples (LTS), were selected to meet monitoring requirements for OSHA site visits at selected facilities in which glutaraldehyde was believed to be present. These levels should not be taken as basis for projecting future OSHA rulemaking concerning glutaraldehyde.

ACGIH has published a "Notice of Intended Changes (for 1996)" to change the TWA-Ceiling from 200 ppb to 50 ppb (Ref. 7.2). Therefore, this additional data could be of interest to those wishing to monitor glutaraldehyde at very low levels.

The overall appearance of this method was revised so that it would be more consistent with OME methods written according to 1993 Method Evaluation Guidelines (Ref. 7.3). The original data are intact, and new data are identified by the phrase: "(Additional data, 1997)" and use of "Modern" font. The different font is used to delineate the 1997 data from the original data. New data were collected

in accordance with 1993 OME Guidelines. The original backup data and literature references sections are intact, and new backup data and literature references sections for the additional data are included. Some OME definitions and test criteria for the limit defining parameters were revised in 1993 and it may not be possible to directly compare original and new data because of the revisions. The 1987 detection and reliable quantitation limits have been superseded by the new limits.

Preliminary testing showed that, with modification, Method 64 for glutaraldehyde was capable of monitoring the selected lower target levels. Some instability was observed for STS stored at ambient temperature. The recovery was 105% of theoretical at the beginning of a 19-day storage test, and it was 84% at the end of the test. Only minor instability was observed for refrigerated STS. The sample storage instability seems related to the mass of derivative present on the sampler. LTS were more stable than STS. All glutaraldehyde samples should, however, be stored under refrigeration, and samples suspected of containing low levels of glutaraldehyde (such as 10-ppb STS) should be shipped in an insulated container using Blue Ice (or equivalent) by overnight delivery service (FedEx™, or equivalent). Changes to Method 64 include use of a new LC analytical column specially designed by the manufacturer to separate DNPH derivatives of aldehydes and ketones, and increasing the air sampling rate from 1 to 2 L/min. The 10-ppb STS is monitored with 15-min samples, but the sampling time can be reduced to 5 min if necessary. The 2-ppb LTS is normally assessed with 4-hour samples. The sampling time for LTS may have to be reduced, or an ozone-scavenging filter (OSF) incorporated into the air sampler, if ozone in the sampled air is suspected to be more than 10 ppb.

Ozone has been reported to be a significant sampling interference in some methods which use DNPH-treated sampling media (Ref. 7.4). It was confirmed to be a sampling interference for 2-ppb LTS, but was not severe for 10-ppb STS. The extent of the interference depends both on the amount of ozone in the sampled air and the length of time that the sample is collected. The effects of the interference were reduced by the use of an OSF consisting of a glass fiber filter coated with N-(1,3-dimethybutyl)-N'-phenyl-*p*-phenylenediamine. (Section 6.9.2.d)

The design of the sampler was not altered to routinely incorporate an OSF because it is anticipated that its required inclusion will be more the exception than the rule. Most glutaraldehyde exposures are likely short term, and STS do not require an OSF. Most LTS will be collected in hospitals, and ozone levels at such facilities should be low. The industrial hygienist has the option of reducing the sample air volume size for LTS, or using an OSF, if ozone levels are sufficiently high.

This sampling and analytical method provides adequate sensitivity to work at very low levels. Working at these levels is demanding for both the industrial hygienist and the analyst because of the potential for positive, as well as negative, sampling interferences. The industrial hygienist must determine if sampling interferences are present, and then take corrective action. This action may consist simply of reporting the presence of interferences to the analytical laboratory. The analyst can better qualify sampling results with this knowledge, and perhaps suggest alternative sampling procedures.

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

Glutaraldehyde is a strong respiratory irritant and a less severe skin and eye irritant. It can also cause allergic contact dermatitis from occasional exposure (skin sensitization). The oral LD_{500} for rats has been reported to be as low as 250 mg/kg. The 4-h LC_{50} for rats is 5000 ppm. (Ref. 5.4)

Activated glutaraldehyde, which is an aqueous solution buffered to an alkaline pH of 7.5-8.0, is an effective cold sterilizer with potent antimicrobial properties. Activated glutaraldehyde retains the skin sensitizing properties of non-alkaline glutaraldehyde and its irritation effects are somewhat enhanced. (Ref. 5.4)

The odor threshold for glutaraldehyde is about 0.04 ppm and the irritation response level is about 0.3 ppm. The ACGIH TLV-Ceiling for glutaraldehyde is 0.2 ppm because of its irritation properties, whether from activated or unactivated solutions. (Ref. 5.4)

1.1.3 Workplace exposure

Glutaraldehyde is used in water solutions of varying concentrations as a chemical intermediate in the drug and polymer industries, a fixative for tissues, a cross linking agent for polyhydroxy materials and proteins, a tanning agent in the leather industry, and a cold sterilizer in hospital-medical applications (Ref. 5.4). No data was found regarding the size of the worker population potentially exposed to glutaraldehyde.

1.1.4 Physical properties (Ref. 5.4)

CAS no.:	111-30-8
molecular weight:	100.12
appearance:	colorless liquid often encountered in 2% and 50% aqueous solutions which have no flash points and are not flammable
vapor pressure	
2% solution:	0.16 Pa (0.0012 mm Hg) at 20 °C
50% solution:	2.03 Pa (0.0152 mm Hg) at 20 °C
structural formula:	$HOC(CH_2)_3COH$
synonym:	1,5-pentanedial

The analyte air concentrations listed throughout this method are based on the recommended sampling and analytical procedures. Air concentrations listed in ppb are referenced to 25 °C and 101.3 kPa (760 mm Hg). The analyte concentrations are listed as glutaraldehyde even though the derivative is the actual species analyzed.

1.2 Limit defining parameters

1.2.1 Detection limit of the analytical procedure

The detection limit of the analytical procedure is 1.31 ng per injection. This is the amount of analyte which will give a peak sufficiently large to permit its visual detection in the presence of interfering peaks in a sample chromatogram. (Section 4.1)

(Additional data, 1997). The detection limit of the analytical procedure is 19.1 pg. This is the amount of analyte that will give a response that is significantly different from the background response of a reagent blank. This amount supersedes the previous detection limit of the analytical procedure. (Sections 6.1 and 6.2)

1.2.2 Detection limit of the overall procedure

The detection limit of the overall procedure is 0.268 µg per sample (4.4 ppb or 18 µg/m^3). This is the amount of glutaraldehyde spiked on the sampling device which allows recovery of an amount of analyte equivalent to the detection limit of the analytical procedure. (Section 4.2)

(Additional data, 1997). The detection limit of the overall procedure is 16.5 ng per sample (STS: 0.13 ppb or 0.55 µg/m^3; LTS: 0.0083 ppb or 0.034 µg/m^3). This is the amount of analyte spiked on a sampler that will give a response that is significantly different from the background response of a sampler blank. This amount supersedes the previous detection limit of the overall procedure. (Sections 6.1 and 6.3)

1.2.3 Reliable quantitation limit

The reliable quantitation limit is 0.268 µg per sample (4.4 ppb or 18 µg/m^3). This is the smallest amount of analyte which can be quantitated within the requirements of a recovery of at least 75% and a precision (±1.96 SD) of ±25% or better. (Section 4.2)

(Additional data, 1997). The reliable quantitation limit is 55.0 ng per sample (STS: 0.44 ppb or 1.8 µg/m^3; LTS: 0.027 ppb or 0.11 µg/m^3). This is the amount of analyte spiked on a sampler that will give a signal that is considered the lower limit for precise quantitative measurements. This amount supersedes the previous reliable quantitation limit. (Section 6.4)

1.2.4 Instrument response to the analyte

The instrument response over the concentration range of 0.5 to 2 times the target concentration is linear. (Section 4.4)

1.2.5 Recovery

The recovery of glutaraldehyde from samples used in a 17-day storage test was essentially 100% when the samples were stored at about 23 °C. (Section 4.7) The recovery of the analyte from the collection medium during storage must be 75% or greater.

(Additional data, 1997). The recoveries of glutaraldehyde from samples used in 19-day ambient storage tests remained above 84% for 10-ppb STS, and above 98% for 2-ppb LTS. The ambient storage test for STS revealed a greater than 10% decrease in recovery. An unsuccessful attempt was made to develop a convenient alternative sampler which alleviated the storage loss. Samples suspected of containing low levels of glutaraldehyde (such as 10-ppb STS) should be shipped in an insulated container using Blue Ice (or equivalent) by overnight delivery service (FedEx, or equivalent). LTS exhibited adequate storage stability. (Section 6.7)

1.2.6 Precision (analytical procedure)

The pooled coefficient of variation obtained from replicate determinations of analytical standards at 0.5, 1, and 2 times the target concentration is 0.024. (Section 4.3)

(Additional data, 1997). The precision of the analytical procedure, measured as the pooled relative standard deviation, over a concentration range equivalent to 0.5 to 2 times the target concentration is 0.69% for 10-ppb STS. The precision of the analytical procedure, measured as the pooled relative standard deviation, over a concentration range equivalent to 0.5 to 2 times the target concentration is 0.83% for 2-ppb LTS. (Section 6.5)

1.2.7 Precision (overall procedure)

The precision at the 95% confidence level for the 17-day ambient temperature storage test is ±12%. (Section 4.7) This includes an additional ±5% for sampling error. The overall procedure must provide results at the target concentration that are ±25% or better at the 95% confidence level.

(Additional data, 1997). The precessions of the overall procedure at the 95% confidence level for the 19-day refrigerated storage tests were ±12.9% for 10-ppb STS and ±13.4% for 2-ppb LTS. These each include an additional 5% for sampling error. (Section 6.7)

1.2.8 Reproducibility (sampling)

Six samples, collected from a controlled test atmosphere, and a draft copy of this procedure were given to a chemist unassociated with this evaluation. The samples were analyzed immediately after generation. No individual sample deviated from its theoretical value by more than the ±12% precision reported in Section 1.2.7 (Section 4.8.)

(Additional data, 1997). Twelve samples (6-STS and 6-LTS) were collected from test atmospheres and were submitted for analysis by SLIC. The samples were analyzed according to instructions in a draft copy of this procedure following 10 and 3 days (respective) of storage at about 4 °C. No individual sample result differed from its theoretical value by more than the respective precessions reported in Section 12.7. (Section 6.8)

1.3 Advantage

This sampling and analytical procedure provides a simple, convenient, and precise means to monitor occupational exposure to glutaraldehyde vapors and aerosols.

1.4 Disadvantage

The coated filters are currently not commercially available.

(Additional data, 1997). The coated filters are now commercially available. The OSFs are not currently commercially available.

2. Sampling Procedure

2.1 Apparatus

2.1.1 Samples are collected by use of a personal sampling pump that can be calibrated to within ±5% of the recommended flow rate with the sampling device attached.

2.1.2 A sample is collected using an open-face air monitoring cassette containing 2 glass-fiber filters. The filters are separated and retained using cassette rings (See Figure 2.1.2). Each filter is coated with DNPH and phosphoric acid. Instructions for the preparation of the coated filters and assembly of the sampler are given in Section 4.11 of this method.

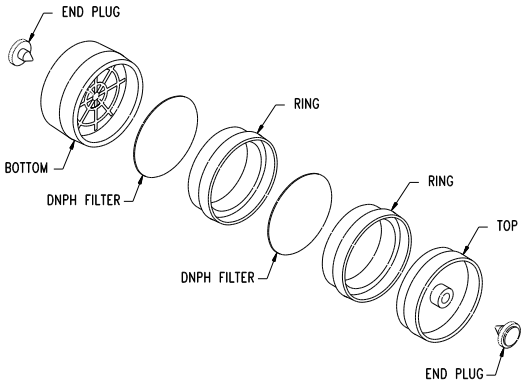

Figure 2.1.2. Glutaraldehyde air sampler.

2.1.3 (Additional data, 1997). Ozone levels greater than 10 ppb may require use of an ozone-scavenging filter (OSF) to prevent a negative sampling interference at the 2-ppb LTS (See Figure 2.1.3). Instructions for preparation of the OSF, and its incorporation into the air sampler are presented in Section 4.11. Detection of low levels of ozone requires the use of an ozone meter, or an ozone detector tube.

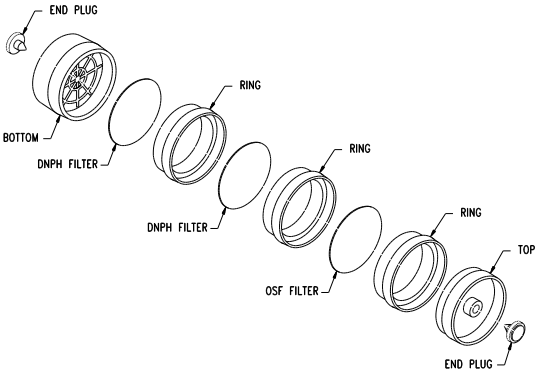

Figure 2.1.3. Glutaraldehyde air sampler with OSF incorporated into the sampler.

2.2 Reagents

No sampling reagents are required.

2.3 Sampling technique

2.3.1 Remove the inlet section (top) and the end plug on the exit section of the air monitoring cassette so that sampling is performed open face.

2.3.2 Attach the sampling device to the sampling pump with flexible, plastic tubing such that the front filter of the sampler is exposed directly to the atmosphere.

2.3.3 Attach the open-face air monitoring cassette vertically (face down) in the worker's breathing zone in such a manner that it does not impede work performance or safety.

2.3.4 Remove the sampling device after sampling for the appropriate time. Replace the inlet section (top) and the end plug on the exit section of the air monitoring cassette. Wrap the sample end-to-end with an official OSHA seal (Form 21).

2.3.5 Keep the collected samples in the dark whenever possible as a precaution against photo-decomposition.

2.3.6 (Additional data, 1997). Ship samples suspected of containing low levels of glutaraldehyde (such as 10-ppb STS) in an insulated container using Blue Ice (or equivalent) by overnight delivery service (FedEx, or equivalent).

2.3.7 Submit at least one blank with each set of samples. The blank should be handled the same as the other samples except that no air is drawn through it.

2.3.8 List any potential interferences on the sample data sheet.

2.4 Sampler capacity

2.4.1 Sampler capacity studies were performed by sampling controlled test atmospheres with the recommended sampling device. The average glutaraldehyde concentration of these controlled test atmospheres was 0.4 ppm and the average relative humidity was 66% at 30 °C. Five-percent breakthrough occurred after sampling for 171 min at 1 L/min. At the end of the sampling time, 171 L of air had been sampled and 256 µg of glutaraldehyde had been collected. (Section 4.5)

2.4.2 An additional sampler capacity experiment was performed at reduced relative humidity to determine if low humidity had an effect on capacity. No breakthrough was observed when a controlled test atmosphere containing 0.2 ppm glutaraldehyde at 33% relative humidity and 30 °C was sampled for 18 min at 1 L/min. The average amount of glutaraldehyde recovered from the samples was 92% of theoretical.

2.4.3 (Additional data, 1997). Sampler capacity studies were performed at 10-ppb glutaraldehyde, 81% relative humidity at 22 °C, and a sampling rate of 2 L/min. Five-percent breakthrough was never attained, even after more than 700 L of air had been sampled. (Section 6.9)

2.4.4 (Additional data, 1997). Other experiments were conducted to test the sampling method. Samples were collected at both high and low humidity, at both 1 and 2 L/min, and for both 5 min and 15 min. The results of these tests were expressed as percent ratios which were calculated by dividing low humidity results by high humidity results, by dividing 1 L/min results by 2 L/min results, and by dividing 5 min results by 15 min results. The respective ratios were 102.1, 97.6, and 105.5%. (Section 6.9)

2.5 Extraction efficiency

2.5.1 The average extraction efficiency for glutaraldehyde from DNPH coated glass-fiber filters at the target concentration was essentially 100%. (Section 4.6)

2.5.2 Extracted samples remain stable for at least 16 h. (Section 4.6)

2.5.3 (Additional data, 1997). The average extraction efficiency over the range of 0.5 to 2 times the 10-ppb STS target concentration was 98.9%. The average extraction efficiency over the range of 0.5 to 2 times the 2-ppb LTS target concentration was 99.7%. (Section 6.10)

2.5.4 (Additional data, 1997). Average extraction efficiencies for 0.05, 0.1 and 0.2 times the 10-ppb STS were 100.5, 92.2, and 95.8% respectively. Average extraction efficiencies for 0.05, 0.1 and 0.2 times the 2-ppb LTS were 95.9, 100.3, and 99.1% respectively. (Section 6.10)

2.5.5 (Additional data, 1997). Extracted samples remain stable for at least 16 hours. (Section 6.10)

2.6 Recommended air volume and sampling rate

2.6.1 The recommended air volume is 15 L and the recommended sampling rate is 1 L/min.

2.6.2 When longer term sampling is necessary, the recommended air volume is 120 L and the recommended sampling rate is 1 L/min. The reliable quantitation limit for a 120-L sample is 0.54 ppb (2.2 µg/m^3).

2.6.3 (Additional data, 1997). Collect 10-ppb STS at 2 L/min for 15 min.

2.6.4 (Additional data, 1997). Collect 2-ppb LTS at 2 L/min for 4 hours if ozone is less than 10 ppb. Ozone present in the sampled air at levels greater than 10 ppb is a negative sampling interference that can cause low results. The severity of the interference depends on the amount of ozone present and on the length of time that the glutaraldehyde derivative is exposed to ozone. Use either an ozone-scavenging filter (Section 4.11.3) when ozone levels are greater than 10 ppb, or a "safe air volume" calculated by dividing 4.6 by the ozone level in ppm. For example: if the ozone level is 0.04 ppm (40 ppb) the "safe air volume" would be 115 L collected at 2 L/min (4.6/0.04=115). (Section 6.9.2.4, Table 6.9.2.4.1, and Figure 6.9.2.4.1)

2.6.5 (Additional data, 1997). The air concentration equivalent to the reliable quantitation limit depends on the air volume sampled.

2.7 Interferences (sampling)

2.7.1 Any substance present in the sampled air and capable of reacting with DNPH or the DNPH derivative of glutaraldehyde is a potential interference. Many aldehydes and ketones are capable of reacting with DNPH.

2.7.2 Suspected interferences should be reported to the laboratory with submitted samples.

2.7.3 (Additional data, 1997). Ozone is a negative sampling interference that can cause sampling results to be low. The severity of the interference depends on the amount of ozone present and on the length of time that the glutaraldehyde derivative is exposed to ozone. Results from STS were about 10% low after sampling a 240-ppb ozone test atmosphere for 15 min, and results from LTS were about 45% low after sampling a 100-ppb ozone test atmosphere for 4 hours. (Section 6.9.2.4).

The effects of ozone can be reduced by use of an ozone-scavenging filter (OSF) consisting of a glass fiber filter coated with N-(1,3-dimethylbutyl)-N'-phenyl-*p*-phenylenediamine (Section 6.9.2.4).

2.8 Safety precautions (sampling)

2.8.1 Attach the sampling equipment to the worker in such a manner that it will not interfere with work performance or safety.

2.8.2 Follow all safety practices that apply to the work area being sampled.

3. Analytical Procedure

3.1 Apparatus

3.1.1 A high performance liquid chromatograph (HPLC) equipped with a UV detector and a manual or automatic sample injector. A Waters Associates Model 6000A HPLC pump, a Waters Associates Model 440 UV detector and a Waters Associates Model U6K manual sample injector were used in this evaluation.

(Additional data, 1997). A Hewlett Packard 1050 Series HPLC consisting of a pumping system, programmable variable wavelength detector, and an autosampler was used to analyze samples for the additional evaluation data.

3.1.2 An HPLC column capable of resolving the glutaraldehyde DNPH derivative from interferences. A 25-cm × 4.6-mm i.d. DuPont Zorbax CN (PN 850952-705) HPLC column was used in this evaluation.

(Additional data, 1997). A Restek Pinnacle TO-11 (5-µm), 25-cm × 4.6-mm i.d., (Catalog no. 9172575) HPLC column was used to analyze samples for the additional evaluation data.

3.1.3 Vials, 4-mL glass with Teflon-lined septum caps.

3.1.4 Volumetric flasks, pipets and syringes for preparing standards, making dilutions and performing injections.

3.1.5 A tube rotator or other suitable means to extract the samples. A Fisher Roto-Rack tube rotator was used for this evaluation.

3.1.6 An electronic integrator or some other suitable means to measure detector response. A Hewlett-Packard Model 3357 Data System was used in this evaluation.

(Additional data, 1997). A Waters Millennium Chromatography Manager system was used to analyze samples for the additional evaluation data.

3.2 Reagents

3.2.1 Acetonitrile, HPLC grade. American Burdick and Jackson acetonitrile UV was used in this evaluation.

3.2.2 Water, HPLC grade. Water from a Millipore Milli-Q water filtration system was used in this evaluation.

3.2.3 Phosphoric acid, reagent grade. "Baker Analyzed" Reagent grade 85% phosphoric acid was used in this evaluation.

3.2.4 Glutaraldehyde. Aldrich Chemical Company, 25% by weight solution in water, glutaraldehyde was used in this evaluation. This solution contained 229.5 mg/mL glutaraldehyde as determined by the procedure which is presented in Section 4.10.

3.2.5 2,4-Dinitrophenylhydrazine (DNPH). DNPH (70%), Lot No. 1707 LJ, obtained from Aldrich Chemical Company was recrystallized from hot acetonitrile for use in this evaluation.

3.2.6 Analytical standard preparation solution. This solution is prepared by diluting 1 g of recrystallized DNPH and 5 mL of phosphoric acid to 1 L with acetonitrile.

3.3 Standard preparation

3.3.1 It is recommended that standards be prepared about 1 h before the air samples are to be analyzed in order to insure the complete reaction between glutaraldehyde and DNPH. Standards should be prepared fresh daily. The actual concentration of the glutaraldehyde solution (Section 3.2.4) should be determined by titration as described in Section 4.10. As a precaution against photo-decomposition, standards and samples should be kept in the dark whenever possible.

3.3.2 Prepare glutaraldehyde standard solutions by diluting known volumes of the nominal 25% glutaraldehyde solution with acetonitrile. A solution containing 0.23 mg/mL glutaraldehyde was prepared by diluting 1.0 mL of the reagent to 1000 mL with acetonitrile.

3.3.3 Place 2.0-mL aliquots of analytical standard preparation solution (Section 3.2.6) into each of several 4-mL glass vials. Seal each vial with a Teflon-lined septum cap.

3.3.4 Prepare standards by injecting appropriate volumes of glutaraldehyde standard solution (Section 3.3.2) into the sealed 4-mL vials. A standard containing 11.5 µg per sample glutaraldehyde was prepared by injecting 50 µL of 0.23 mg/mL glutaraldehyde into a vial containing 2.0 mL of analytical standard preparation solution.

(Additional data, 1997). A standard containing 1.15 µg per sample (approximating the 10-ppb STS) was prepared by injecting 5.0 µL of 0.23 mg/mL glutarabbehyde into a vial containing 2.0 mL of analytical standard preparation solution. A standard containing 3.91 µg per sample (approximating the 2-ppb LTS) was prepared by injecting 17.0 µL of 0.23 mg/mL glutarabbehyde into a vial containing 2.0 mL of analytical standard preparation solution.

3.3.5 Prepare a sufficient number of standards to generate a calibration curve. Analytical standard concentrations should bracket sample concentrations.

3.4 Sample preparation

3.4.1 Open the air monitoring cassette and remove the front coated filter. Fold this filter in half, twice (resulting in a quarter circle) and place it in a 4-mL glass vial. Remove the backup fitter, fold it in a similar manner as the front filter and place it in a separate 4-mL glass vial. Do not wad or crumple the filters.

(Additional data, 1997). Discard the OSF (if present) in a container designated for contaminated waste.

3.4.2 Add 2.0 mL of acetonitrile to each vial.

3.4.3 Seal the vials with Teflon-lined septum caps and place them on the tube rotator. Set the rotation speed to 60 rpm and allow them to extract for 1 h.

3.5 Analysis

3.5.1 HPLC conditions

column:	DuPont Zorbax CN, 25-cm × 4.6-mm i.d. (PN 850952-705)
mobile phase:	55% acetonitrile in water containing 0.1% phosphoric acid (v/v/v)
flow rate:	1 mL/min
injection volume:	10 µL
UV detector:	365 nm
retention time:	5.9 min

(Additional data, 1997). The following alternative conditions were developed. The Restek column provides somewhat better resolution of the glutarabbehyde derivative from the sample matrix than does either the Zorbax, or a Bakerbond CN column.

column:	Restek Pinnacle TO-11 (5-µm), 25-cm × 4.6-mm i.d., (Catalog no. 9172575)
mobile phase:	62% acetonitrile in water containing 0.1% phosphoric acid (v/v/v)
flow rate:	1 mL/min
injection volume:	20 µL
UV detector:	355 nm
retention time:	9.0 min

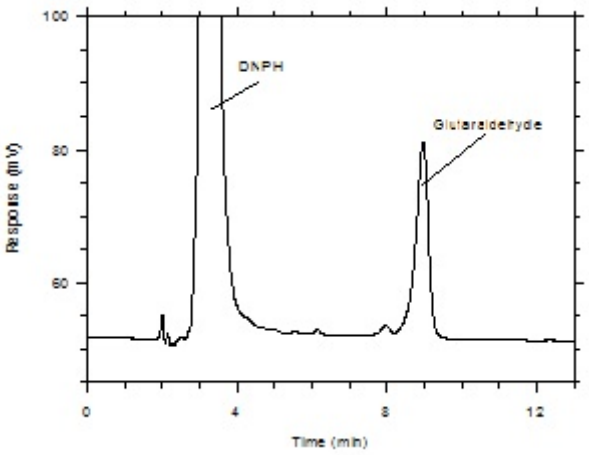

Figure 3.5.1. Glutaraldehyde chromatogram using the alternative conditions.

3.5.2 Use a suitable method such as electronic integration to measure detector response.

3.5.3 Use an external standard procedure to prepare a calibration curve with several standard solutions of different concentrations. Prepare the calibration curve daily. Program the integrator to report results in µg per sample

3.5.4 Make sure that sample concentrations are bracketed with standards as stated in Section 3.3.5.

3.6 Interferences (analytical)

3.6.1 Any compound having a similar retention time as the glutaraldehyde-bis-DNPH derivative is a potential analytical interference.

3.6.2 HPLC parameters (mobile phase composition, column, etc.) may be changed to circumvent interferences.

3.6.3 Retention time on a single column is not proof of chemical identity. Analysis using an alternate HPLC column, detection at another wavelength, comparison of absorbance response ratios and structure determination by mass spectrometry are additional means of identification. (See Figure 6.11 for a UV spectrum of the derivative)

3.7 Calculations

3.7.1 Results are obtained by use of calibration curves. Calibration curves are prepared by plotting detector response against concentration in µg per sample for each standard. The best line through the data points is determined by curve fitting.

3.7.2 The concentration in µg per sample for a particular sample is determined by comparing its detector response to the calibration curve. If glutaraldehyde is found on the backup filter, it is added to the amount found on the front filter. This total amount is then corrected by subtracting the total amount (if any) found on the blank.

3.7.3 The glutaraldehyde air concentration can be expressed using the following equation:

$mg/m^3 = A/B$

where A = µg per sample from Section 3.7.2
B = liters of air sampled

3.7.4 The following equation can be used to convert glutaraldehyde results in mg/m^3 to ppm at 25°C and 760 mm Hg:

$ppm = (mg/m^3)(24.46)/(100.12)$

where mg/m^3 = result from Section 3.7.3
24.46 = molar volume at 760 mm Hg and 25 °C
100.12 = molecular weight of glutaraldehyde

3.8 Safety precautions (analytical)

3.8.1 Avoid skin contact and inhalation of all chemicals.

3.8.2 Restrict the use of all chemicals to a fume hood.

3.8.3 Wear safety glasses and a lab coat in all lab areas.

4. Backup Data

4.1 Detection limit of the analytical procedure

The injection size recommended in the analytical procedure (10 µL) was used to determine the detection limit of the analytical procedure. The detection limit of the analytical procedure was 1.31 ng per injection. This was the amount of glutaraldehyde which gave a peak sufficiently large to permit its visual detection in the presence of potentially interfering peaks in a sample chromatogram. This detection limit was determined by the analysis of a standard containing 0.131 µg/mL glutaraldehyde. Figure 4.1 is a chromatogram of the detection limit of the analytical procedure produced using the Restek TO-11 LC column and the 62% acetonitrile in water containing 0.1% phosphoric acid mobile phase described in Section 3.5.1.

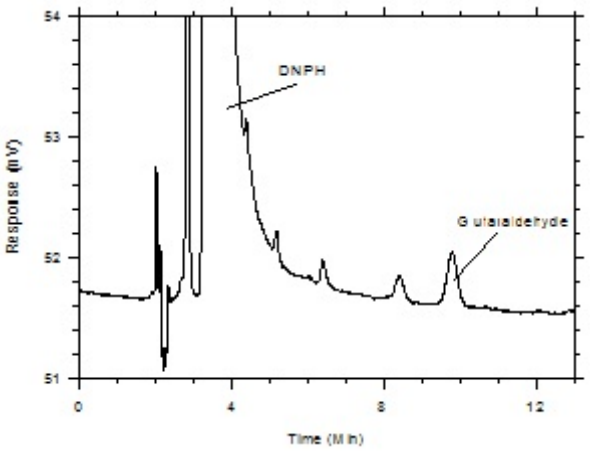

Figure 4.1. The detection limit of the analytical procedure.

4.2 Detection limit of the overall procedure and reliable quantitation limit data

The injection size recommended in the analytical procedure (10 µL) was used in the determination of the detection limit of the overall procedure and in the determination of the reliable quantitation limit. Samples were prepared by injecting 50 µL of a solution containing 5.36 µg/mL glutaraldehyde (50 µL × 5.36 µg/mL = 0.268 µg) onto each of 6 coated glass-fiber filters. This is the amount of analyte that when extracted with 2.0 mL acetonitrile resulted in a solution with a concentration similar to the solution that was used to determine the detection limit of the analytical procedure (0.131 µg/mL). The amount of glutaraldehyde spiked on the coated filters included any amount that was expected to be lost because of incomplete extraction. The spiked filters were placed in separate 4-mL glass vials, stored at room temperature in the dark and then analyzed the next day. Since the glutaraldehyde recoveries were near 100% and the precision was better than ±25%, the detection limit of the overall procedure and the reliable quantitation limit were 0.268 µg per sample (4.4 ppb or 18 µg/m^3).

Table 4.2
Data for Detection Limit of the
Overall Procedure and the Reliable Quantitation Limit

sample number	theo amt (µg)	amt recovered (µg)	recovery (%)
1	0.268	0.269	100.4
2	0.268	0.257	95.9
3	0.268	0.228	85.1
4	0.268	0.284	106.0
5	0.268	0.260	97.0
6	0.268	0.266	99.3
$\overline{X}$		0.261	97.3
SD			6.9
1.96×SD			13.5

4.3 Precision (analytical method only)

The precision of the analytical method was evaluated by performing multiple injections of analytical standards at 0.5, 1, and 2 times the TLV target concentration.

Table 4.3
Glutaraldehyde Precision Data

× target concn (µg per sample)	0.5× 6.0	1× 12.0	2× 24.0
	676428	1249968	2510938
	633559	1241804	2496676
	635204	1268634	2468907
	644284	1213801	2550920
	682320	1250483	2512370
	657713	1301514	2534457
$\bar{X}$	654918	1254367	2512378
SD	20877	29204	28675
CV	0.0319	0.0233	0.0114
pooled CV	0.024		

4.4 Instrument response to the analyte

The experimental data in Table 4.3 are presented graphically in Figure 4.4. This figure is a calibration curve over the concentration range of 0.5 to 2 times the TLV target concentration. The instrument response was linear over this range.

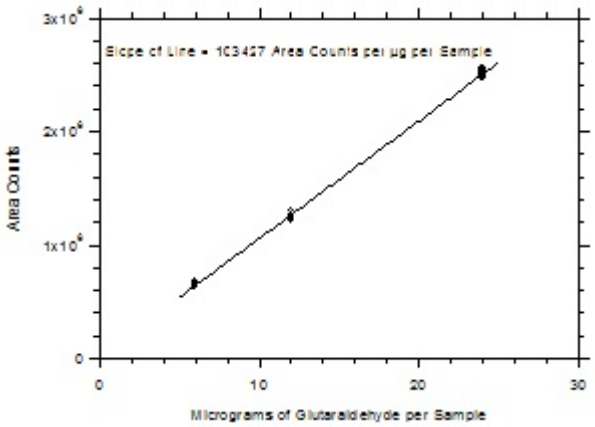

Figure 4.4. Glutaraldehyde calibration curve.

4.5 Breakthrough data

Breakthrough studies were performed with the recommended collection device by sampling controlled test atmospheres containing glutaraldehyde in air. The average glutaraldehyde inlet concentration was 0.4 ppm and the average relative humidity was 66% at 30 °C. The sampling rate was 1 L/min. Five-percent breakthrough occurred after sampling for 171 min. At the end of this time, 171 L of air had been sampled and 256 µg of glutaraldehyde had been collected. The breakthrough concentration for each sample was calculated by dividing the amount of glutaraldehyde found on the backup filter by the volume of air sampled. Percent breakthrough was calculated by dividing the breakthrough concentration by the inlet concentration and multiplying by 100. Five-percent breakthrough was defined as the point at which the amount of glutaraldehyde that was collected on the coated-backup filter was equivalent to 5% of the inlet concentration.

Table 4.5
Glutaraldehyde Breakthrough Data

air volume (L)	breakthrough (%)	air volume (L)	breakthrough (%)
18.1	0.0	105.7	0.0
30.6	0.0	120.0	0.0
51.6	0.0	148.9	1.6
59.6	0.0	155.1	1.2
76.5	0.0	194.0	9.3
98.9	0.0		

4.6 Extraction efficiency and stability of extracted samples

The extraction efficiency of glutaraldehyde from DNPH-coated filters was determined by injecting 55 µL of a solution containing 0.22 mg/mL glutaraldehyde onto each of 6 coated filters. This amount is equivalent to 0.2 ppm for a 15 min air sample. The filters were placed in sealed 4-mL glass vials, stored at room temperature in the dark and then analyzed the next day. Following the initial analysis, the samples were immediately resealed and then reanalyzed about 16 h later using fresh standards. The results of these studies are presented in Table 4.6. The average reanalysis of the extracted samples was 101.6% of the original analysis.

Table 4.6
Extraction Efficiency and Stability Data

	extraction efficiency (%)	reanalysis 16-h later (%)
	98.3	102.0
	103.0	104.0
	101.0	103.0
	105.0	105.0
	96.0	99.3
	97.1	96.0
$\bar{X}$	100.1	101.6

4.7 Storage data

Storage samples were generated by sampling a controlled test atmosphere containing 0.2 ppm glutaraldehyde for 15 min at 1 L/min. The relative humidity of the sampled air was 72% at 31 °C. The samples were stored in the dark either at ambient temperature or at -20°C. The results of the storage test are presented in Table 4.7 and are shown graphically in Figures 4.7.1 and 4.7.2.

Table 4.7
Storage Data

time (days)	ambient recovery (%)			time (days)	refrigerated recovery (%)		
0	103.0	102.0	105.0	0	99.0	95.0	99.6
3	107.0	98.8	103.0	2	99.2	95.2	96.9
6	106.0	98.8	98.3	6	97.3	111.0	98.3
10	105.0	97.9	108.0	9	97.7	99.5	97.3
13	100.0	102.0	102.0	13	102.0	93.1	97.2
17	102.0	105.0	109.0	16	97.3	93.0	98.8

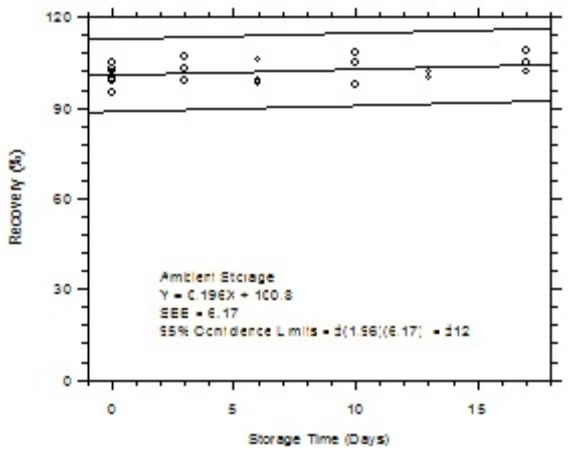

Figure 4.7.1. Ambient temperature storage test.

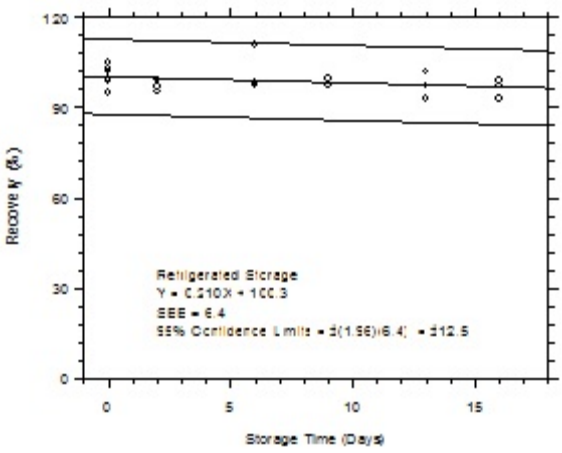

Figure 4.7.2. Refrigerated temperature storage test.

4.8 Reproducibility data

Reproducibility samples were generated by sampling a controlled test atmosphere containing 0.2 ppm glutaraldehyde in air for 15 min at 1 L/min. The relative humidity of the sampled air was 76% at 29 °C. The samples and a draft copy of this evaluation were given to a chemist unassociated with this evaluation. The samples were analyzed immediately after generation. No individual sample deviated from its theoretical value by more than the precision (±12%) at the 95% confidence level for the 17-day storage test. (Section 4.7)

Table 4.8
Reproducibility Results

sample no.	theoretical amount (µg)	analytical result (µg)	recovery (%)
1	11.2	12.1	108.0
2	12.8	13.5	105.5
3	11.6	11.8	101.7
4	11.8	11.9	100.8
5	12.4	12.4	100.0
6	11.6	11.4	98.3

4.9 Generation of controlled test atmospheres

The controlled test atmospheres which were used in this evaluation were generated by pumping a glutaraldehyde/water solution into a heated glass manifold with a Sage Instruments Model 355 Syringe Pump. The glutaraldehyde/water solution was volatilized and then diluted with heated air. The dilution air was metered into the heated glass manifold using a precision, calibrated rotameter. The dilution air was humidified, if desired, by passing it through a water bubbler prior to its entering the heated glass manifold. The water bubbler was contained in a temperature-controlled water bath. The relative humidity of the dilution air could be varied by changing the temperature of the water bath. If dry dilution air was required, the water bubbler was not used. The relative humidity of the test atmosphere was monitored, after mixing, with a YSI Model 91 Dew Point Hygrometer. The test atmosphere passed through a manifold from which samples could be collected.

The glutaraldehyde concentration of the test atmosphere was adjusted to the desired level by varying the aldehyde concentration of the glutaraldehyde/water solution.

The theoretical glutaraldehyde concentrations of the test atmospheres were calculated using the concentration of the glutaraldehyde/water solution, the flow rate of the syringe pump, and the volume of the dilution air. The actual concentration of a controlled test atmosphere, theoretically

containing 0.78 mg/m^3 glutaraldehyde, was determined by sampling the atmosphere using the following sampling and analytical techniques:

I. Direct collection on XAD-4 adsorbent. Immediate desorption and GC analysis using a photoionization detector.

II. Collection using two DNPH impingers connected in series. Analysis by HPLC using a UV detector.

III. Collection on DNPH coated XAD-2 adsorbent. Immediate desorption and analysis by HPLC using a UV detector.

IV. Collection and analysis using the recommended method.

Two samples were collected using each technique and the results of this study are presented in Table 4.9.

Table 4.9
Determination of the Concentration of a Controlled
Test Atmosphere by Comparative Sampling and Analysis

technique	analytical results (mg/m^3)			percent of
	1	2	ave	theoretical
I	0.650	0.642	0.646	82.8
II	0.633	0.656	0.645	82.6
III	0.641	0.632	0.637	81.6
IV	0.704	0.654	0.679	87.1

The average of all of the samples was 83.5% of the calculated theoretical amount. There was no breakthrough observed in any of the samples.

The difference between theoretical and actual concentrations of the test atmospheres may be the result of partial decomposition of glutaraldehyde in the heated volatilization manifold of the generation apparatus.

Actual concentrations of controlled test atmospheres, which were used in this evaluation, were determined by multiplying the theoretical volumetric concentrations by 83.5%.

(Additional data, 1997). Test atmospheres were prepared to collect samples for the additional evaluation data using an all glass vapor generation system. The atmospheres were generated by pumping a solution of glutaraldehyde/methanol with an ISCO Model 100DM syringe pump into a heated glass manifold where it evaporated into a heated dilution air stream. The dilution air was generated using a Miller-Nelson Research, INC Model 401 Flow Temperature Humidity Control System. The relative humidity and temperature of the test atmospheres was monitored using an EG&G Model 911 DEW-ALL Digital Humidity Analyzer.

It was necessary to dilute glutarabldehyde with methanol in order to quantitatively generate atmospheres at the 2-ppb LTS and 10-ppb STS. Use of aqueous solutions of glutarabldehyde to generate test atmospheres gave unacceptably low results.

4.10 Procedure to determine glutaraldehyde by acid titration (Ref. 5.6)

4.10.1 Apparatus

Miscellaneous glassware. Fifty-mL burette, 250-mL Erlenmeyer flasks, 1-L volumetric flasks, pipets, etc.

4.10.2 Reagents

a) Sodium sulfite, anhydrous. Prepare a 0.1 M solution by dissolving 12.6 g of the salt in 1 L of deionized water.

b) Hydrochloric acid, reagent grade. Prepare a 0.1 N solution by diluting 7.9 mL of 38% HCl to 1 L with deionized water.

c) Thymolphthalein indicator. Prepare a 0.1% solution in ethanol.

d) Methyl orange indicator. Prepare a 0.1% solution in ethanol.

e) Sodium carbonate, ACS primary standard grade.

4.10.3 Procedure

Standardize the 0.1 N HCl solution using sodium carbonate and methyl orange indicator. A complete procedure for the standardization is presented in Ref. 5.5.

Place 50 mL of 0.1 M sodium sulfite and three drops of thymolphthalein indicator into a 250-mL Erlenmeyer flask. Titrate the contents of the flask to a colorless end-point with 0.1 N HCl (usually one or two drops is sufficient). Transfer 0.50 mL of the nominal 25% glutaraldehyde/water solution (Section 3.2.4) into the same flask and titrate the mixture with 0.1 N HCl, again, to a colorless endpoint. The glutaraldehyde concentration of the solution may be calculated by the following equation:

Glutaraldehyde, mg/mL = (acid titer × acid normality x 50.06)/mL of sample

This method is based on the quantitative liberation of sodium hydroxide when glutaraldehyde reacts with sodium sulfite to form the glutaraldehyde-bisulfite addition product. The volume of sample may be varied depending on the glutaraldehyde content but the solution to be titrated must contain excess sodium sulfite. Glutaraldehyde solutions containing substantial amounts of acid or base must be neutralized before analysis.

4.11 Procedure to coat glass-fiber filters with DNPH/phosphoric acid and assembly of the sampling device

4.11.1 Apparatus

a) Hotplate

b) Miscellaneous glassware: 250-mL volumetric flask, 30-, 50-, and 150-mL beakers, pipets, etc.

c) Plastic air monitoring cassettes, for 37-mm diameter filters. Unassembled 3-piece cassettes and extra center support sections were obtained from Gelman Sciences for use in this evaluation.

4.11.2 Reagents

a) Acetonitrile and toluene. American Burdick and Jackson HPLC grade acetonitrile and Fisher Scientific Optima grade toluene were used in this evaluation.

b) 2,4-Dinitrophenylhydrazine (DNPH). DNPH (70%) Lot No. 1707 LJ, obtained from Aldrich Chemical Company, was recrystallized from hot acetonitrile for use in this evaluation.

c) Glass-fiber filters, 37-mm diameter Gelman Sciences Type A glass-fiber filters, Lot No. 8318, were used in this evaluation.

d) Phosphoric acid, reagent grade. "Baker analyzed" Reagent grade 85% phosphoric acid was used in this evaluation.

e) DNPH/phosphoric acid solution. Prepare this solution by diluting 1 g of recrystallized DNPH and 5 mL of 85% phosphoric acid to 250 mL with acetonitrile. Allow this solution to stand 2-3 days before use or be certain all the DNPH is in solution. This will help prevent filters with a mottled appearance.

f) (Additional data, 1997). N-(1,3-dimethylbutyl)-N'-phenyl-*p*-phenylenediamine. This reagent was obtained from Flexsys America L.P. (260 Springside Drive, Akron, OH 44333, and should be purified by vacuum distillation before use. Prepare a solution containing 15 mg/mL of vacuum-distilled reagent in toluene.

The following is quoted (with permission) from information provided by Flexsys (Ref. 7.9):

Guidelines for Recrystallizing Santoflex 6PPD (N-(1,3-dimethylbutyl)-N'-phenyl-*p*-phenylenediamine)

The general process for purifying and recrystallizing Santoflex 6PPD is by vacuum distillation. Handling of the recrystallized material should be done under an inert atmosphere to prevent oxidation through contact with oxygen in the atmosphere.

Equipment

- Clean glass distillation equipment is preferred.
- Use Teflon fittings or other air tight fittings.
- DO NOT USE ground glass joints.
- DO NOT USE joint greases, especially silicone greases.
- The distillation column needs only 2 or 3 theoretical plates.
- Need the capability to change out receiving vessels quickly to separate the forecut from the mid cut.

General Procedure

- Pour the sample of Santoflex 6PPD into the distillation flask.
- Connect and secure the distillation column and receiving flasks.
- Flush the system with dry nitrogen to purge any oxygen in the system.
- Close the system, begin heating the sample using a heating mantle. Do not use a flame, as this can create hot spots and degrade the sample.
- Apply a vacuum. Santoflex 6PPD has the following vapor pressures at the temperatures given:

Table 4.11.2
Vapor Pressure of Santoflex 6PPD

temp (°C)	vapor pressure (Torr)
162	0.064
180	0.25
200	1.0
227	4.0

- Once Santoflex 6PPD begins to boil, allow a small portion of material to collect in the receiving flask as a forecut. This will contain some Santoflex 6PPD as well unreacted 4ADPA and ketones among other light materials.
- You should collect no more than 5-10% of the starting material in the forecut.
- Change out the receiving flask after the forecut. If the vacuum seal must be broken continue heating, but purge the system with nitrogen while the flask is being replaced. Be sure the new flask is purged with nitrogen before resealing and reapply the vacuum.

- Continue to collect distilled material in the new flask. Collect about 50-75% of the starting material volume in the receiving flask.
- Discontinue heating. Allow nitrogen to fill the distillation equipment.
- While still warm, Santoflex 6PPD can be transferred to a sample bottle. Keep under nitrogen at all times.
- Distilled Santoflex 6PPD may appear water white or may have a slight pink-purple cast to it. It should be lighter in color than the starting material. Once oxygen comes in contact with distilled material, Santoflex 6PPD quickly discolors to a dark purple to brown/purple. Oxidized 6PPD has an intense color. Even small concentrations (ppb) greatly affect the visual appearance, but does not affect the performance. Oxidization by-products of 6PPD are also antioxidants to some degree.

4.11.3 Procedure

(CAUTION! Evaporation of solvents must be performed in an exhaust hood.)

Place a glass-fiber filter on a 30-mL beaker, or some other suitable support, so that only the outside edge of the filter is supported. Pipet 0.5 mL of the DNPH solution (Section 4.11.2.e) onto the surface of the filter. Make sure that the filter is completely saturated with the solution. Allow the acetonitrile to evaporate for about 20 min. Place the coated filters in a suitable container and allow them to dry overnight. Analyze a blank filter to determine if there are any severe analytical interferences present. If a batch of filters is not suitable, discard the reagents and the filters.

Prepared filters were tested for shelf-life by storing them in a tightly sealed container either at ambient temperature or at -20 °C. Stored filters were used to periodically sample controlled test atmospheres over a month. Sample results did not appear to be dependent on filter storage temperature but prepared filters should be stored at reduced temperature as a precaution against reagent decomposition. Filters prepared and stored as described remain usable for at least a month.

Assemble the sampling device by placing a coated filter in the outlet section of the air monitoring cassette. **DO NOT USE BACK-UP PADS**. Next, place a ring on the first filter. Now, put another coated filter on the ring and another ring on top of that filter. Complete the assembly by placing the inlet section on the ring. Plug the outlet and inlet openings with plastic end plugs. An exploded view of the air sampler is shown in Figure 2.1.2. Put the air sampler on a table top with the outlet section down. Press on the top of the air sampler with sufficient force to seal the cassette. Use tape or shrink bands to further seal the two rings and the outlet sections of the cassette. Store the assembled air sampler at reduced temperature (if possible) when there is an appreciable time before it is to be used for sampling.

(Additional data, 1997). Preparation of ozone-scavenging filter (OSF). Place a glass-fiber filter on a 30-mL beaker, or some other suitable support, so that only the outside edge of the filter is supported. Pipet 0.5 mL of the 15 mg/mL N-(1,3-dimethylbutyl)-N'-phenyl-*p*-phenylenediamine solution (Section 4.11.2.f) onto the surface of the filter. Make sure that the filter is completely saturated with the solution. Allow the toluene to evaporate. Place the coated filters in a suitable container and allow them to dry overnight. These filters remain useable for at least a month when stored in a freezer.

(Additional data, 1997). Incorporation of OSF into air sampler. Refer to Figure 2.1.3. The OSF is positioned before the DNPH filters, and separated from them with a cassette ring, so that sampled air passes through the OSF before passing through the DNPH filters. Remove the cassette top section and place an OSF on the ring. Place another ring on top of the OSF, replace the top section, and seal the sampler. Use tape or shrink bands to further seal the three rings and bottom section. Store the assembled sampler in a freezer.

5. References

5.1 Levin, J.-O.; Andersson, K.; Lindahl, R.; Nilsson, C.-A. J. Anal. Chem. 1985 *57* 1032-1035.

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5.4 "Documentation of the Threshold Limit Values and Biological Indices", 5th ed.; American Conference of Governmental Industrial Hygienists (ACGIH): Cincinnati, ISBN: 0-036712-68-6, 986; p 285.

5.5 Treadwell, F.P.; Hall, W.T. "Analytical Chemistry"; John Wiley and Sons: New York, 1948; Vol. II, pp 481-483.

5.6 Walker, J.F. "Formaldehyde"; Reinhold: New York, 1953; p 382.

6. Backup Data (Additional data, 1997)

6.1 Determination of detection limits

Detection limits, in general, are defined as the amount (or concentration) of analyte that gives a response (Y_{DL}) that is significantly different (three standard deviations (SD_{BR})) from the background response (Y_{BR}).

$$Y_{DL} - Y_{BR} = 3(SD_{BR})$$

The measurement of Y_{BR} and SD_{BR} in chromatographic methods is typically inconvenient and difficult because Y_{BR} is usually extremely low. Estimates of these parameters can be made with data obtained from the analysis of a series of analytical standards or samples whose responses are in the vicinity of the background response. The regression curve obtained for a plot of instrument response versus concentration of analyte will usually be linear. Assuming SD_{BR} and the precision of the data about the curve are similar, the standard error of estimate (SEE) for the regression curve can be substituted for SD_{BR} in the above equation. The following calculations derive a formula for DL:

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

Y_{obs} = observed response
Y_{est} = estimated response from regression curve
n = total number of data points
k = 2 for linear regression curve

At point Y_{DL} on the regression curve

$$\mathbf{Y_{DL} = A(DL) + Y_{BR}}$$

A = analytical sensitivity (slope)

therefore

$$\mathbf{DL} = \frac{(Y_{DL} - Y_{BR})}{A}$$

Substituting 3(SEE) + Y_{BR} for Y_{DL} gives

$$\mathbf{DL} = \frac{3(SEE)}{A}$$

6.2 Detection limit of the analytical procedure (DLAP)

The DIAP is measured as the mass of analyte actually introduced into the chromatographic column. Ten analytical standards were prepared in equal descending increments with the highest standard containing 27.15 ng/mL of glutaraldehyde. This is the concentration that would produce a peak approximately 10 times the background noise of a reagent blank near the elution time of the analyte. These standards, and the reagent blank, were analyzed with the recommended analytical parameters (20-µL injection), and the data obtained were used to determine the required parameters (A and SEE) for the calculation of the DIAP. Values of 9.83 and 62.54 were obtained for A and SEE respectively. DIAP was calculated to be 19.1 pg.

Table 6.2
Detection Limit of the Analytical Procedure

concn (ng/mL)	mass on column (pg)	area counts (µV-s)
0	0	0
2.715	54.3	558
5.430	108.6	1078
8.145	162.9	1638
10.860	217.2	2187
13.575	271.5	2222
16.280	325.6	3365
19.005	380.1	3788
21.720	434.4	4294
24.435	488.7	4688
27.150	543.0	4672

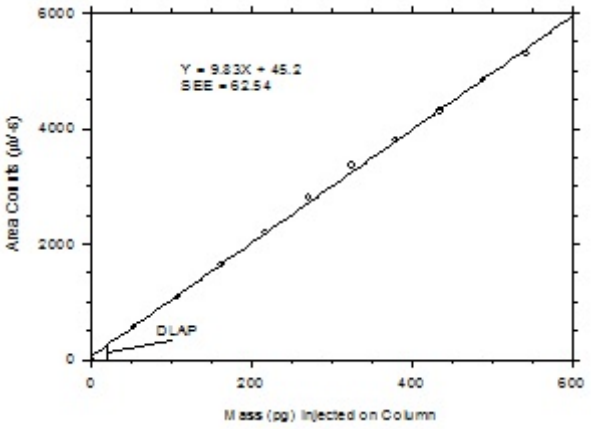

Figure 6.2. Plot of the data in Table 6.2 to determine the DIAP for glutaraldehyde.

6.3 Detection limit of the overall procedure (DLOP)

The DLOP is measured as mass per sample and expressed as equivalent air concentration, based on the recommended sampling parameters. Ten samplers were spiked with equal descending increments of analyte, such that the highest sampler loading was 325.8 ng per sample. This is the amount spiked on a sampler that would produce a peak approximately 10 times the background response for a sample blank. These spiked samplers, and a sample blank, were analyzed with the recommended analytical parameters, and the data obtained used to calculate the required parameters (A and SEE) for the calculation of the DLOP. Values of 90.7 and 499.24 were obtained for A and SEE, respectively. The DLOP was calculated to be 16.5 ng per sample (STS: 0.13 ppb or 0.55 µg/m^3; LTS: 0.0083 ppb or 0.034 µg/m^3).

Table 6.3
Detection Limit of the Overall Procedure

mass per sample (ng)	area counts (µV-s)
0	1411
32.58	4649
65.16	7727
97.74	10163
130.32	12788
162.9	15272
195.48	18258
228.06	22262
260.64	24987
293.22	27984
325.8	31460

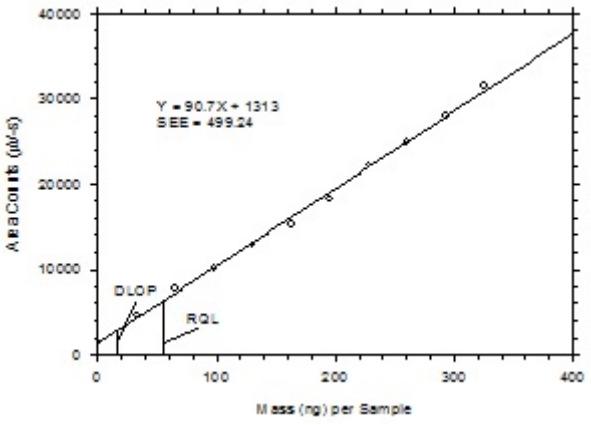

Figure 6.3. Plot of the data in Table 6.3 to determine the DLOP for glutaraldehyde.

6.4 Reliable quantitation limit (RQL)

The RQL is considered the lower limit for precise quantitative measurements. It is determined from the regression line parameters obtained for the calculations of the DLOP (Section 4.3) providing at least 75% of the analyte is recovered. The RQL is defined as the amount of analyte that gives a response (Y_{RQL}) such that

$$Y_{RQL} - Y_{BR} = 10(SD_{BR})$$

therefore

$$\mathbf{RQL} = \frac{\mathbf{10(SEE)}}{\mathbf{A}}$$

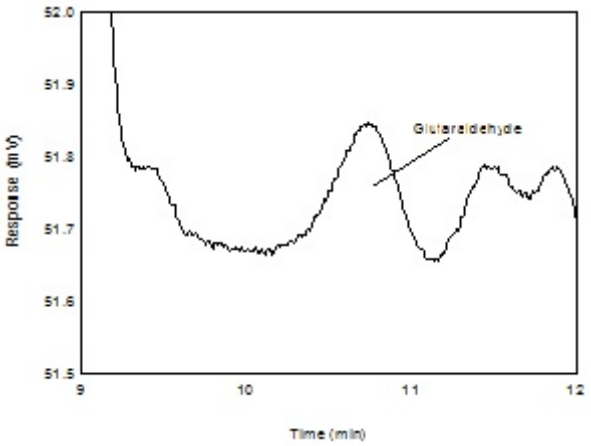

Figure 6.4. Chromatogram of the RQL.

The RQL for glutaraldehyde was calculated to be 55.0 ng per sample (STS: 0.44 ppb or 1.8 µg/m^3); LTS: 0.02 ppb or 0.11 µg/m^3). The recovery at this concentration is essentially 100%.

6.5 Precision (analytical method)

The precision of the analytical procedure is measured as the pooled relative standard deviation (RSD_P). Relative standard deviations are determined from six replicate injections of glutaraldehyde standards at 0.5, 0.75, 1, 1.5 and 2 times the target concentrations. After assuring that the RSDs satisfy the Cochran test for homogeneity at the 95% confidence level, RSD_P was calculated to be 0.68% and 0.83% for the lower and higher target concentration, respectively.

Table 6.5.1
Instrument response to Glutaraldehyde at the 10-ppb STS Concentration

× STS concn	0.5×	0.75×	1×	1.5×	2×
ng per sample	669.06	892.08	1338.12	1784.16	2453.22
area counts	72164	97542	140820	198141	256688
(µV-s)	72959	98516	142396	197413	260312
	72957	98393	142264	198674	261092
	72557	97352	142768	198553	262649
	73213	97366	142666	199091	263156
	72470	96927	140382	199682	257912
$\bar{X}$	72720.00	97682.67	141882.67	198592.33	260301.50
SD	388.47	632.37	1018.59	779.59	2571.03
RSD	0.53	0.65	0.72	0.39	0.99

Table 6.5.2
Instrument response to Glutaraldehyde at the 2-ppb LTS Concentration

× LTS concn	0.5×	0.75×	1×	1.5×	2×
ng per sample	2007.18	2899.26	4014.36	5798.52	7805.7
area counts	215608	320355	432076	650472	885485
(µV-s)	218628	328115	432589	662534	887672
	218996	326240	433613	664159	885390
	218803	327149	438510	656494	879843
	220680	327886	440416	657363	895223
	217201	327600	434058	650118	875522
$\overline{X}$	218319.33	326224.17	435210.33	656856.67	884855.83
SD	1729.85	2950.68	3422.33	5867.16	6757.8
RSD	0.79	0.90	0.79	0.89	0.76

The Cochran test for homogeneity:

$$g = \frac{\text{largest RSD}^2}{RSD_{0.5\times}^2 + RSD_{0.75\times}^2 + RSD_{1\times}^2 + RSD_{1.5\times}^2 + RSD_{2\times}^2}$$

The critical value of the g-statistic, at the 95% confidence level, for five variances, each associated with six observations is 0.5065. The g-statistics are 0.4164 and 0.2363 for the 10-ppb STS and 2-ppb LTS concentrations respectively. Because the g-statistics do not exceed the critical value, the RSDs can be considered homogenous and they can be pooled (RSD_p) to give an estimated

$$RSD_P = \sqrt{\frac{5(RSD_{0.5\times}^2 + RSD_{0.75\times}^2 + RSD_{1\times}^2 + RSD_{1.5\times}^2 + RSD_{2\times}^2)}{5+5+5+5+5}}$$

The (RSD_p)s are 0.69% and 0.83% for the 10-ppb STS and 2-ppb LTS concentrations respectively.

6.6 Precision (overall procedure)

The precision of the overall procedure is determined from the storage data in Section 6.7. The determination of the standard error of estimate (SEE_R) 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_R is similar to the standard deviation, except it is a measure of the dispersion of data about a regression line instead of about a mean. It is determined with the following equation:

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

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

An additional 5% for pump error (SP) is added to the SEE_R by the addition of variances to obtain the total standard error of the estimate.

$$SEE = \sqrt{(SEE_R)^2 + (SP)^2}$$

The precision at the 95% confidence level is obtained by multiplying the standard error of estimate (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 6.7.1.a through 6.7.2.b. The precisions of the overall procedure are 12.9% and 13.4% for 10-ppb STS refrigerated samples and for 2-ppb LTS refrigerated samples respectively.

6.7 Storage tests

6.7.1 Storage test for 10-ppb STS

Storage samples were generated by collecting samples for 15 min at 2 L/min from a 10-ppb glutaraldehyde test atmosphere. The test atmosphere was generated by pumping a solution of glutaraldehyde in methanol into a heated manifold where it evaporated into a heated air stream. The relative humidity was 70% at 23 °C. Thirty-eight storage samples were prepared. Eight samples were analyzed immediately after generation, fifteen samples were stored at reduced temperature (4 °C), and the other fifteen were stored in the dark at ambient temperature (about 22 °C). At three to five day intervals, three samples were selected from each of the two sets and analyzed.

Table 6.7.1
Storage Test for 10-ppb STS

time (days)	ambient storage recovery (%)			refrigerated storage recovery (%)		
0	100.0	109.0	108.5	100.0	109.0	108.5
	108.2	106.9	105.0	108.2	106.9	105.0
	104.9	104.2		104.9	104.2	
5	99.3	97.3	95.3	92.0	100.6	103.8
8	91.6	98.4	94.2	90.3	100.1	97.4
12	88.4	94.6	88.4	100.3	99.2	98.2
15	90.6	88.0	86.3	99.6	100.2	97.9
19	85.8	84.9	86.9	101.9	100.3	101.2

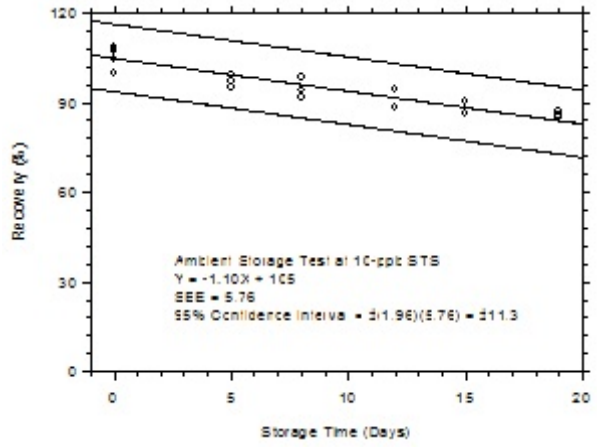

Figure 6.7.1.a. Ambient storage test for 10-ppb STS.

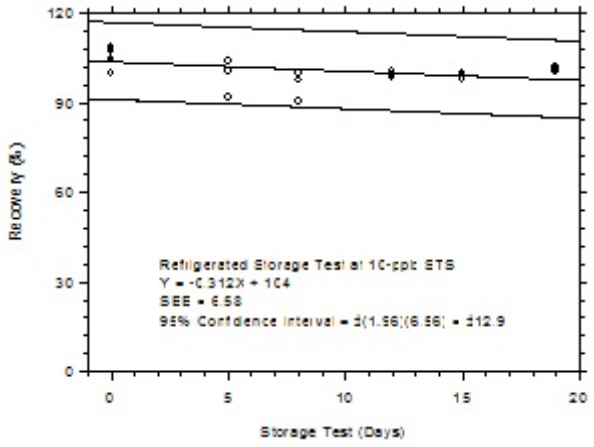

Figure 6.7.1.b. Refrigerated storage test for 10-ppb STS.

Inspection of the ambient storage graph shows that the storage loss was 21% during the 19-day test period. OME Method Evaluation Guidelines require that efforts be made to improve the sampling method if storage loss is greater than 10% so that restrictions do not have to be placed on sample storage time before analysis, or on sample storage temperature. Such attempts were made: sampler treatments (in addition to DNPH and phosphoric acid) with ascorbic acid or with *alpha*-tocopherol (Vitamins C and E); and with diethylphthalate alone, and in combination with 4-*tert*-butylcatechol (TBC). Vitamins C and E were selected because it was thought that the observed instability could be caused by oxidation, TBC was tested because it has been shown to improve storage stability of other analytes, and diethylphthalate was used to retain TBC on the sampling medium. None of these additional treatments improved storage stability, in fact the presence of Vitamins C and E resulted in

even more instability. It was decided, considering that the loss was less than 25%, to continue to utilize the established sampling medium in the interests of method consistency. The storage loss is only 6% when samples are stored at 4 °C therefore, samples suspected of containing low levels of glutaraldehyde (such as 10-ppb STS) should be shipped in an insulated container using Blue Ice™ (or equivalent) by overnight delivery service (FedEx, or equivalent).

6.7.2 Storage test for 2-ppb LTS

The recommended sampling time for LTS is 4 hours. This sampling time is excessive for laboratory use because only five samples can be collected simultaneously with the equipment available. Therefore, samples were collected from a more concentrated test atmosphere for a reduced time in order to provide approximately the same mass that would have been collected had a 2-ppb atmosphere been sampled for 4 hours at 2 L/min. Forty samples were collected by sampling a test atmosphere containing 10.4 ppb glutaraldehyde for 45 min at 2 L/min. The relative humidity was 73% at 22 °C. Ten samples were analyzed immediately after generation, fifteen tubes were stored at reduced temperature (4 °C) and the other fifteen were stored in the dark at ambient temperature (about 22 °C). At 2-5 day intervals, three samples were selected from each of the two sets and analyzed.

Table 6.7.2
Storage Test for 2-ppb LTS

time (days)	ambient storage recovery (%)			refrigerated storage recovery (%)		
0	103.3	99.1	100.6	103.3	99.1	100.6
	100.8	100.0	100.0	100.8	100.0	100.0
	100.0	98.4	99.3	100.0	98.4	99.3
	96.2			96.2		
4	106.9	101.8	102.3	105.9	117.9	101.9
7	105.7	105.2	94.3	100.7	104.7	95.0
11	106.5	101.7	98.5	103.1	105.5	104.0
14	97.9	99.5	88.3	111.2	103.9	98.2
19	105.9	94.6	92.8	109.1	102.4	103.8

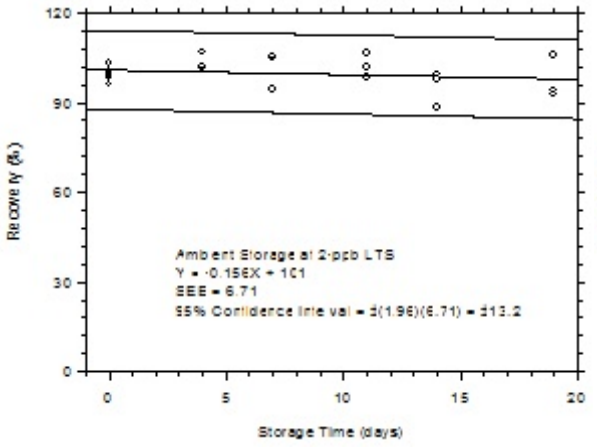

Figure 6.7.2.a. Ambient storage test for 2-ppb LTS.

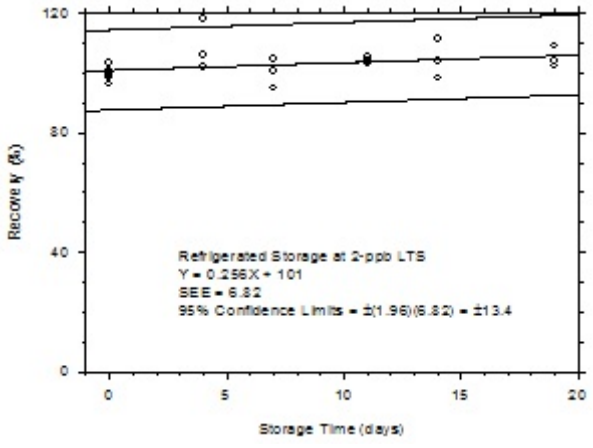

Figure 6.7.2.b. Refrigerated storage test for 2-ppb LTS.

6.7.3 Abbreviated storage test for 2-ppb LTS

An abbreviated storage test was conducted at the 2-ppb LTS by collecting a limited number of samples at 2 L/min from a 1.9-ppb test atmosphere for the full four-hour recommended sampling time. This test was performed to determine if there was a difference in storage stability between LTS

collected for a reduced time and samples collected for the full time. Twenty samples were collected over four consecutive days. The average relative humidity of the test atmospheres was 76% at 24 °C. Eight samples were analyzed on the day they were collected, six were stored at approximately 22 °C and six were stored at 4 °C. Six of the stored samples, three ambient and three refrigerated, were analyzed either eight or ten days following collection and the final six were analyzed either eighteen or twenty days after collection.

Table 6.7.3
Abbreviated Storage Test for 2-ppb LTS

time (days)	ambient storage recovery (%)			time (days)	refrigerated storage recovery (%)		
0	96.6	95.7	95.8	0	96.6	95.7	95.8
	94.2	87.0	94.5		94.2	87.0	94.5
	91.7	89.5			91.7	89.5	
8	86.2	86.2	83.2	10	93.4	90.8	91.3
18	90.9	87.9	82.9	20	96.3	96.6	95.0

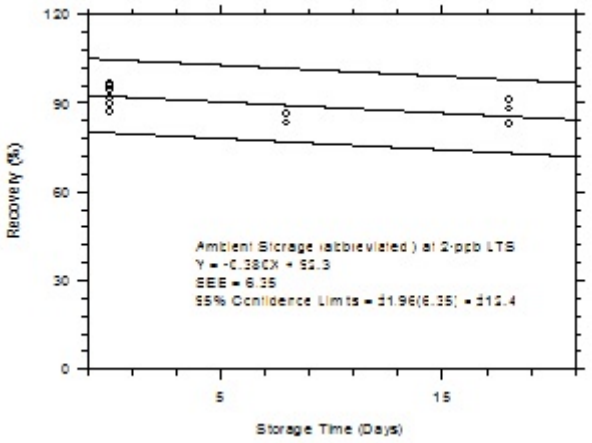

Figure 6.7.3.a. Ambient storage test (abbreviated) for 2-ppb LTS.

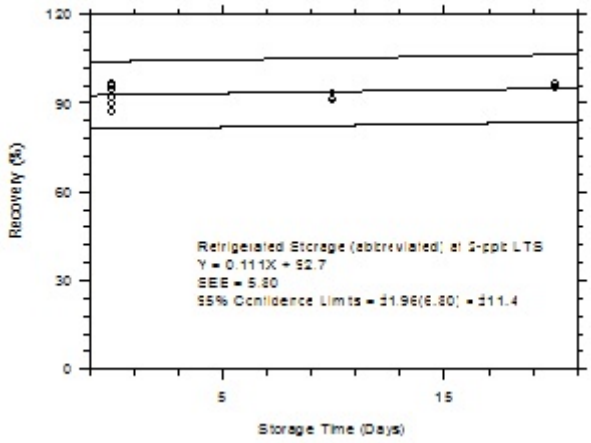

Figure 6.7.3.b. Refrigerated storage test (abbreviated) for 2-ppb LTS.

6.8 Reproducibility

6.8.1 Reproducibility for 10-ppb STS

Six samples were prepared by sampling from a test atmosphere containing 10.4 ppb glutaraldehyde for 15 min at 2 L/min. The relative humidity was 82% at 22 °C. The samples were submitted to SLTC for analysis. The samples were analyzed after being stored for 10 days at 4 °C. Sample results were corrected for extraction efficiency. No sample result had a deviation greater than the precision of the overall procedure determined in Section 6.6, which was ±12.9%.

Table 6.8.1
Reproducibility Data for 10-ppb STS

sample	expected (ppb)	reported (ppb)	recovery (%)	deviation (%)
1	10.4	10.3	99.0	-1.0
2	10.4	10.5	101.0	+1.0
3	10.4	10.2	98.1	-1.9
4	10.4	10.1	97.1	-2.9
5	10.4	10.4	100.0	0.0
6	10.4	9.7	93.3	-6.7

6.8.2 Reproducibility for 2-ppb LTS

The recommended sampling time for LTS is 4 hours. This sampling time is excessive for laboratory use because only five samples can be collected simultaneously with the equipment available. Therefore, reproducibility samples were collected from a more concentrated test atmosphere for a reduced time in order to provide approximately the same mass that would have been collected had a 2-ppb atmosphere been sampled for 4 hours at 2 L/min. Six samples were collected by sampling a test atmosphere containing 9.6 ppb glutaraldehyde for 45 min at 2 L/min. The relative humidity was 71% at 23°C. The samples were submitted to SLTC for analysis. The samples were analyzed after being stored for 3 days at 4°C. Sample results were corrected for extraction efficiency. No sample result had a deviation greater than the precision of the overall procedure determined in Section 6.6, which was ±13.4%.

Table 6.8.2
Reproducibility Data at Mass Equivalent for 2-ppb LTS

sample	expected mass (ng)	reported mass (ng)	recovery (%)	deviation (%)
1	3916	3718	94.9	-5.1
2	3728	3526	94.6	-5.4
3	3547	3280	92.5	-7.5
4	3486	3250	93.2	-6.8
5	3905	3890	99.6	-0.4
6	3517	3350	95.2	-4.8

6.9 Sampler capacity and additional tests

6.9.1 Sampler capacity

The capacity of the sampler for glutaraldehyde was determined at 400 ppb in the original evaluation of Method 64. The breakthrough concentration was calculated by dividing the amount of glutaraldehyde found on the backup filter by the air volume sampled. Percent breakthrough was calculated by dividing the breakthrough concentration by the inlet concentration, and multiplying by 100. These tests were performed at 66% relative humidity at 30°C. Five-percent breakthrough occurred after sampling for 171 min at 1 L/min, and the capacity of the sampler was 256 µg of glutaraldehyde.

Additional sampler capacity tests were performed for this work. Breakthrough (BT) terms were defined as above. These tests were performed at approximately 10-ppb glutaraldehyde, and 81% relative humidity at 22°C. The test atmosphere was sampled at 2 L/min using the recommended two-section samplers. Five-percent breakthrough was never attained. The sample with the largest air volume, 728 L, had about 31 g of glutaraldehyde which is well below the 256-µg capacity determined in the original evaluation. The recommended sampler has more than sufficient capacity to monitor the 2-ppb LTS.

Table 6.9.1
Breakthrough of Glutaraldehyde Collected on Glass Fiber Filters Coated With DNPH and Phosphoric Acid

test no.	air vol (L)	BT concn (ng/L)	inlet concn (ng/L)	BT (%)
1	137.0	0.095	42.82	0.22
	247.5	0.055		0.13
	399.2	0.095		0.22
	489.1	0.26		0.61
	622.8	0.78		1.82
2	395.0	0.0025	42.82	0.01
	484.5	0.00		0.00
	507.4	0.085		0.20
	682.0	0.11		0.26
	675.6	0.068		0.16
3	530.0	0.093	42.75	0.22
	620.5	0.080		0.19
	643.8	0.074		0.17
	722.3	0.082		0.19
	728.1	0.076		0.18

6.9.2 Additional tests

Additional testing of the sampling method was conducted at low relative humidity (Section 6.9.2.a), at 1-L/min sampling rate (Section 6.9.2.b), and at 5-min sampling times (Section 6.9.2.c). The results for the additional testing are presented as the percent ratio of average results for each tested condition. For example, the percent ratio of the average of the samples collected at low humidity to the average of samples collected at high humidity was 102.1. The effects of ozone, a reported negative interference for formaldehyde collected on DNPH-treated silica gel, were tested (Section 6.9.2.d). Sample results obtained using open-face samplers were compared to results from simultaneously collected closed-face samples (Section 6.9.2.e).

a) Humidity effect

The humidity study was performed by collecting samples at a set humidity, changing the humidity, and then collecting additional samples as soon as the humidity stabilized. Two studies were performed: one study at high humidity of 77% and 23°C and at low humidity of 27% at 23°C (run 1); the other study at high humidity of 93% at 22°C and at low humidity of 29% and 22°C (run 2). Both tests were performed at about 10-ppb glutaraldehyde, 2 L/min sampling rate, and 15-min sampling time.

Table 6.9.2.a
Humidity Effect

run no.	results at low humidity (ng/L)	results at high humidity (ng/L)	percent ratio (low RH/high RH)
1	44.93	44.77	100.4
2	38.59	37.17	103.8
$\bar{X}$			102.1

b) Sampling rate effect

The sampling rate study was performed by simultaneously collecting samples at either 2 or at 1 L/min. Five individual tests were performed: 2 tests at about 5-ppb, and 3 at about 9-ppb glutaraldehyde. The average relative humidity was 70% at 24 °C

Table 6.9.2.b
Sampling Rate Effect

run no.	results at 1-L/min (ng/L)	results at 2-L/min (ng/L)	percent ratio (1-L/min/2-L/min)
1	21.84	21.30	102.5
2	20.86	21.58	96.7
3	37.64	38.04	98.9
4	36.40	37.15	98.0
5	42.32	45.93	92.1
$\bar{X}$			97.6

One experiment was performed in which results from samples collected at either 0.5 or at 2-L/min were compared. The percent ratio (0.5/2-L/min) was 37.90 ng/L/39.23 ng/L = 96.6%.

c) Sampling time effect

The sampling time study was performed by collecting a set of samples for 15 min, and another set for 5 min. The sampling rate was 2 L/min, the glutaraldehyde concentration was about 11 ppb, and the relative humidity was 81% at 22 °C.

Table 6.9.2.c
Sampling Time Effect

5-min results (ng/L)	15-min results (ng/L)	percent ratio (5-min/15-min)
48.00	45.50	105.5

d) Ozone interference

Ozone has been reported to be a significant negative interference in formaldehyde methods which utilize DNPH-coated silica gel tubes (Ref 7.4). The interference was caused by the reaction of ozone with the formaldehyde-DNPH derivative. The formaldehyde levels studied were 20, 40, and 140 ppb; and the ozone levels were 0, 120, 300, 500, and 770 ppb. Formaldehyde derivative loss was greater at higher ozone levels, with sampling losses of approximately 60% at 300 ppb ozone. The amount of formaldehyde derivative lost depended more on the ozone level than on the formaldehyde level.

The data in Table 6.9.2.d.i (and in Figure 6.9.2.d.i) shows that ozone can also be a significant negative sampling interference for this method. The interference was not severe for 15-min STS as shown by the data in Table 6.9.2..

LTS experiments were conducted by sampling a 10-ppb glutaraldehyde test atmosphere to collect the mass expected in 2-ppb LTS, and then using the same samplers to sample a separately generated ozone test atmosphere for 4 hours. The relative humidity of the glutaraldehyde atmospheres was about 80% at 23 °C, and about 50% at 23 °C for the ozone atmospheres. These experiments represented the worst case because the full amount of glutaraldehyde derivative was available to react with ozone. Four samples were collected from the glutaraldehyde test atmosphere for each experiment, two samples were used as controls (no ozone), and two were used to sample the ozone test atmosphere (ozone). Glutaraldehyde (glut) results from each set of two samples were averaged, and the percent ratio of glutaraldehyde results from samples which had been exposed to ozone to results from corresponding samples which had not been exposed to ozone was calculated. The ozone dose is a measure of total ozone exposure, and it was calculated by multiplying ppm ozone by L of air sampled. Figure 6.9.2.d.i shows that 95% glutaraldehyde recovery occurs at about 4.6 ppm×L ozone dose. Solution of the equation (4.6=ppm×L) for 0.04 ppm (40 ppb) ozone gives 115 L. This is the air volume that could be sampled if 40 ppb ozone were present and still give 95% glutaraldehyde recovery.

Table 6.9.2.d.i
Ozone Interference

ozone (ppm)	ozone dose (ppm×L)	glut (ng/L) ozone	glut (ng/L) no ozone	ratio (%)
0.0	0	40.77	40.00	101.9
0.016	7.57	34.81	39.08	89.1
0.02	9.46	35.02	39.59	88.5
0.06	28.89	31.28	39.42	79.4
0.10	47.43	26.91	42.32	63.6

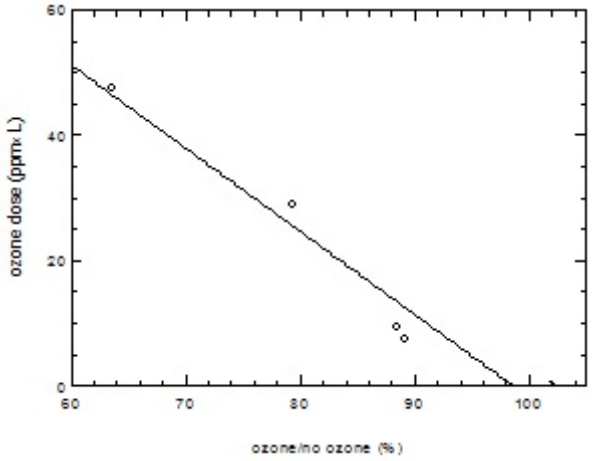

Figure 6.9.2.d.i. Ozone interference.

The experimental results in Table 6.9.2.d.ii were obtained by collecting sets of four samples from glutaraldehyde test atmospheres (either 2 or 10 ppb, and about 80% relative humidity and 23 °C) for 15 min and then using two of the samples to sample ozone test atmospheres for 15 min.

Table 6.9.2.d.ii
Ozone Interference for STS

glut concn (ppb)	ozone dose (ppm×L)	ozone/no ozone (%)
10	7.23	89.8
10	5.46	93.1
2	7.78	91.7
2	1.12	95.1
2	4.43	95.6

Two similar experiments were performed in which the ozone test atmosphere was sampled before sampling the glutaraldehyde atmosphere to determine if ozone deactivated the reagent-coated sampling medium. The percent ratios were 99.6 and 102.0. These results show that the quantity of DNPH reagent coated on the filter is sufficient, and that the interference is primarily caused by ozone reacting with the glutaraldehyde derivative.

Two additional similar experiments were performed by first sampling a 10-ppb glutaraldehyde test atmosphere for either 67 or 46 min, and then sampling ambient indoor SLTC air (during the month of December) for 4 hours with the same samplers. The ambient ozone levels were 8 and 4 ppb, respectively. The percent ratios were 98.2 for 8-ppb ozone, and 91.6 for the 4-ppb ozone tests. These results show that sampling ambient (December) SLTC air had no extreme effect on glutaraldehyde recovery.

The ozone interference manifests itself by reacting with the glutaraldehyde derivative. The product of the interference has not been detected in chromatographic analysis. The severity of the interference depends both on the ozone level and on the length of exposure time. The most expedient approach to solve the problem was to attempt to modify the sampling method in order to reduce or eliminate the interference. One way to accomplish this would be to develop an ozone-scavenging filter which could be placed in front of the sampling filters, and which would remove ozone before it could react with the DNPH derivative. A literature review revealed several reagents which have been used in air sampling to remove ozone. Some of the reagents are mixtures of potassium iodide and glycerol (Ref. 7.5); sodium thiosulfate, potassium carbonate, and glycerol (Ref. 7.6); and sodium nitrite, potassium carbonate, and glycerol (Ref. 7.7) (OSHA's

ozone-sampling reagent). Glycerol is used as a non-volatile substrate, and potassium carbonate provides a chemically basic environment to enhance the reaction with ozone.

Several different combinations of these mixtures were tested by coating them on glass fiber filters and incorporating them into standard glutaraldehyde samplers. The modified samplers consisted of an ozone-scavenging filter placed in the same cassette as the DNPH filters, in front of the glutaraldehyde sampling filters, and separated from the DNPH filters by a cassette ring in the same manner as the two DNPH filters are separated. Modified and standard samplers, used as controls, were used to sample glutaraldehyde test atmospheres. In each case glutaraldehyde results were significantly lower in samples using reagent treated pre-filters than in control samplers without pre-filters. The reducing chemicals coated on the pre-filters apparently reacted with glutaraldehyde before it could reach the DNPH-treated sampling filters.

Goodyear Rubber formulates antiozonants into some of their products to prevent damage from atmospheric ozone. A colleague at Goodyear was contacted and asked to suggest chemicals which might eliminate or reduce the ozone interference in this method. Nickel dibutyl dithiocarbamate and Goodyear's product, Wingstay 300 (N-(1,3-dimethyl-butyl)-N'-phenyl-*p*-phenylenediamine) were identified as possible candidates. A Goodyear employee said that nickel dibutyl dithiocarbamate was the most effective antiozonant they had ever tested, but that it was toxic. Goodyear also supplied a small sample of recrystallized N-(1,3-dimethybutyl)-N'-phenyl-*p*-phenylenediamine for testing purposes. Their assistance is gratefully acknowledged and appreciated. (Ref. 7.8)

Nickel dibutyl dithiocarbamate (NiDBTC) and N-(1,3-dimethybutyl)-N'-phenyl-*p*-phenylenediamine (DMBPPDA) were both tested in the same manner as the inorganic reducing chemicals. Preliminary studies were made in which the reagent levels were varied, and 7.5 mg of reagent per pre-filter was selected as optimal. Experiments were performed in which sets of six samples were collected from a 10-ppb glutaraldehyde test atmosphere (about 80% relative humidity and 23°C) for a sufficient time to collect a similar mass as would be collected in a 4-hour sample at 2-ppb glutaraldehyde. Four samplers were modified by placing a glass fiber filter which had been coated with 7.5 mg of antiozonant in front of the DNPH filters so that sampled air first passed through the ozone-scavenging filter (OSF) and then through the DNPH filters. The OSF was separated from the DNPH filters with a cassette ring in the same manner that the DNPH filters are separated. Two of the four samplers with the OSF were used to sample an 100-ppb ozone test atmosphere (about 50% relative humidity and 23°C) (ozone) at 2 L/min, and the other two samplers were used as controls (no ozone). The remaining two samplers were standard samplers (no OSF), were used to sample only the glutaraldehyde test atmosphere, and were used as the benchmark (BM). Results are expressed as the percent ratio of either ozone or no ozone to BM concentration analytical results. The percent ratios of ozone to no ozone were also calculated to determine the effectiveness of the OSF.

Table 6.9.2.d.iii
Reduction of the Ozone Interference

reagent	ozone dose (ppm×L)	ozone (%)	no ozone (%)	ozone/no ozone (%)
NiDBTC	43.17	91.9	95.2	96.5
NiDBTC	36.85	85.7	91.8	93.4
DMBPPDA	35.62	91.8	95.2	96.4
DMBPPDA	48.08	94.6	97.3	97.2
DMBPPDA	48.25	91.1	95.3	95.6

These results show that both reagents used to prepare OSFs were generally effective. DMBPPDA was selected for use in this method because NiDBTC was identified as a suspect carcinogen on the MSDS that was included with the reagent. The OSF should be used only when ozone levels in sampled air are above 10 ppb, and make its inclusion necessary (Table 6.9.2.d.i). Ozone levels

less than 10 ppb do not require OSF. As an alternative to using OSF, the air sample volume could be reduced. Figure 6.9.2.d.i shows that 95% recovery is attained at an ozone dose of 4.6 ppm×L. A "safe air volume" that would result in 95% recovery could be calculated by dividing 4.6 by the ppm ozone level at the sampling site. For example: if the ozone level were 0.020 ppm (20 ppb), the "safe air volume" would be 230 L. It is unnecessary to use OSF when collecting 15-min STS as shown by the data in Table 6.9.2.d.ii.

e) This method uses open-face sampling so that the full surface of the DNPH-coated filter is available for reaction with incoming glutaraldehyde and that sampler capacity is maximized. Glutaraldehyde samples were collected within a 5 gal glass carboy connected in-line with the OME vapor generation apparatus. Sample results from open-face collection were compared to results from closed-face collection. The DNPH-glutaraldehyde derivative is highly colored, and a colored spot about 1.5 cm diameter was observed on closed-face samples while no such spot was seen on open-face samples. This fact indicates that open-face sampling was having its desired effect. There was no significant difference between open and closed-face sampling.

Table 6.9.2.e
Sample Results

sampling rate (L/min)	sampling time (min)	open face results (ng/L)	closed face results (ng/L)	open face/closed face (%)
2	240	7.43	7.49	99.2
2	48	33.43	33.66	99.3
2	64	35.28	36.14	97.6

6.10 Extraction efficiency and stability of extracted samples

6.10.1 Extraction efficiency at the 10-ppb STS concentration

The extraction efficiencies (EE) of glutaraldehyde were determined by liquid-spiking coated filters with amounts of glutaraldehyde-DNPH approximately equivalent to 0.05 to 2 times the 10-ppb STS concentration. These samples were stored overnight at ambient temperature and then extracted and analyzed. The average extraction efficiency over the working range of 0.5 to 2 times the target concentration was 98.9%.

Table 6.10.1.1
Extraction Efficiency of Glutaraldehyde from Coated Filters at the 10-ppb STS Target Concentration

× STS concn (ng/sample)	0.05× 54	0.1× 108	0.2× 244	0.5× 597	1× 1356	2× 2442
EE (%)	100.8	92.6	94.0	100.4	101.0	99.5
	99.8	90.1	99.6	99.0	99.0	100.2
	104.1	95.7	96.1	96.7	97.8	104.8
	92.5	83.9	95.5	94.1	99.0	98.7
	106.0	94.6	94.5	95.7	101.2	96.3
	99.7	96.2	95.1	96.8	98.7	101.6
$\bar{X}$	100.5	92.2	95.8	97.1	99.4	100.2

The stability of extracted samples was investigated by reanalyzing the 1×STS about 16 h after the initial analysis. After the original analysis was performed, three vials were recapped with new septa while the remaining three retained their punctured septa. The samples were reanalyzed with fresh standards. The average percent change was +1.7% for samples that were resealed with new septa and +1.7% for those that retained their punctured septa.

Table 6.10.1.2
Stability of Extracted Samples at the 10-ppb STS Target Concentration

punctured septa replaced			punctured septa retained		
initial EE (%)	EE after one day (%)	difference (%)	initial EE (%)	EE after one day (%)	difference (%)
101.0	101.6	+0.6	99.0	103.3	+4.3
99.0	102.0	+3.0	101.2	101.8	+0.6
97.8	99.4	+1.6	98.7	98.8	+0.1
	averages			averages	
99.3	101.0	+1.7	99.6	101.3	+1.7

6.10.2 Extraction efficiency at the 2-ppb LTS concentration

The extraction efficiencies (EE) of glutaraldehyde were determined by liquid-spiking coated filters with amounts of glutaraldehyde-DNPH approximately equivalent to 0.05 to 2 times the 2-ppb LTS concentration. These samples were stored overnight at ambient temperature and then extracted and analyzed. The average extraction efficiency over the working range of 0.5 to 2 times the target concentration was 99.7%.

Table 6.10.2.1
Extraction Efficiency of Glutaraldehyde from Coated Filters
at the 2-ppb LTS Target Concentration

× LTS concn (ng/sample)	0.05× 217	0.1× 434	0.2× 841	0.5× 2170	1× 4340	2× 8410
EE (%)	93.3	99.4	99.1	100.0	100.9	98.8
	93.4	99.8	96.3	101.3	111.4	100.2
	94.3	98.6	108.9	101.3	95.4	98.0
	101.4	105.7	96.6	96.0	95.7	101.0
	95.9	100.2	99.3	99.4	95.1	101.5
	97.3	98.2	94.4	99.8	101.1	98.9
$\bar{X}$	95.9	100.3	99.1	99.6	99.9	99.7

The stability of extracted samples was investigated by reanalyzing the 1×LTS about 16 h after the initial analysis. After the original analysis was performed, three vials were recapped with new septa while the remaining three retained their punctured septa. The samples were reanalyzed with fresh standards. The average percent change was -0.7% for samples that were resealed with new septa, and +2.0% for those that retained their punctured septa.

Table 6.10.2.2
Stability of Extracted Samples at the 10-ppb STS Target Concentration

punctured septa replaced			punctured septa retained		
initial EE (%)	EE after one day (%)	difference (%)	initial EE (%)	EE after one day (%)	difference (%)
100.9	101.6	+0.7	95.7	98.1	+2.4
111.4	108.2	-3.2	95.1	98.3	+3.2
95.4	95.9	+0.5	101.1	101.4	+0.3
	averages			averages	
102.6	101.9	-0.7	97.3	99.3	+2.0

6.11 Qualitative analysis

The UV spectrum for the DNPH derivative of glutaraldehyde was obtained with a Hewlett Packard Model 1HP-1090 Liquid Chromatograph equipped with a diode array detector and using a Restek T0-11 LC column.

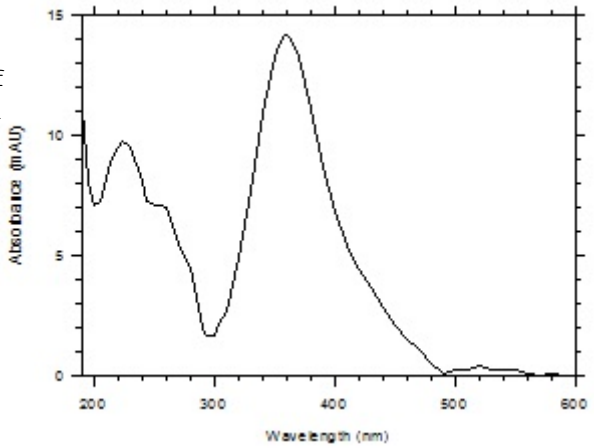

Figure 6.11. UV spectrum of glutaraldehyde derivative.

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