ETHYLENE THIOUREA

Method no.:	95
Matrix:	Air
Target concentration:	60 µg/m^3
Procedure:	Samples are collected open-face by drawing air through a four-piece sampling cassette containing two glass fiber filters. Samples are extracted with water and analyzed by HPLC using a UV detector.
Recommended air volume and sampling rate:	480 L at 2.0 L/min
Reliable quantitation limit:	1.39 µg/m^3
Standard error of estimate at the target concentration: (Section 4.7)	6.8%
Special requirement:	Samples should be stored in a refrigerator when not in transit.
Status of method:	Evaluated method. This method has been subjected to the established evaluation procedures of the Organic Methods Evaluation Branch.

Date: August 1992 Chemist: Yihlin Chan

Organic Methods Evaluation Branch
OSHA Salt Lake Technical Center
Salt Lake City, UT 84165-0200

1. General Discussion

1.1 Background

1.1.1 History

Airborne ethylene thiourea has been determined by collection on PVC or mixed-cellulose esters membrane filters using colorimetric analysis. (Ref. 5.1) NIOSH Method 5011 is based on this earlier work (Ref. 5.2). One of the potential disadvantages of colorimetric analyses is that they are liable to interference. In the case of ethylene thiourea, thione (C=S) compounds will complex with the colorizing reagent and may interfere. Ethylene thiourea has also been analyzed by HPLC/UV and GC/FID. (Refs. 5.3-5.7)

In this method, airborne ethylene thiourea is collected on glass fiber filters and analyzed by HPLC. Compared to colorimetirc analysis, HPLC is specific, speedy, and sensitive. Glass fiber filters were selected because they are better suited for HPLC analysis than the membrane filters.

Although ethylene thiourea has been shown to be an animal carcinogen and NIOSH recommends that it be handled as if it were a human carcinogen and teratogen (Ref. 5.8), there is no PEL or TLV. The target concentration used in this evaluation was arbitrarily set at 60 $\mu g/m^3$, approximately 40 times the reliable quantitation limit.

1.1.2 Toxic effects (This section is for information only and should not be taken as the basis of OSHA policy.) (Refs. 5.9-5.11)

Ethylene thiourea is an antithyroid substance and animal carcinogen. In groups of rats fed 125 or 625 ppm for up to 90 days, marked increases in serum thyroid stimulating hormone were found. Ethylene thiourea produced a high incidence of follicular carcinoma of the thyroid in rats after oral administration. Ethylene thiourea is believed to induce thyroid tumors through the suppression of thyroxine syntheses, leading to hyperplasia of the thyroid gland. Ethylene thiourea was a potent teratogen in rats at doses that produced no maternal toxicity or fetal deaths. Ethylene thiourea is a skin, eye, and mucous membrane irritant to laboratory animals.

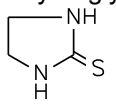
Human toxicity data is limited. One reported a clinical examination and thyroid function tests carried out over a period of 3 years on 13 exposed workers showed one subgroup (the mixers) to have significantly lower levels of total thyroxine than other workers (Ref. 5.9). In another incidence study, approximately 2,000 workers were identified as having worked at some time with ethylene thiourea in one of several rubber manufacturing companies and in one firm producing ethylene thiourea. No case of thyroid cancer was reported in this group (Ref. 5.10). The IARC has determined that there is sufficient evidence for carcinogenicity of ethylene thiourea to animals but inadequate evidence for humans (Ref.5.11).

1.1.3 Workplace exposure

Workers in the rubber industry face potential occupational exposure to ethylene thiourea because it is used extensively as an accelerator in the curing of polychloroprene (Neoprene) and other elastomers. NIOSH estimated that approximately 3500 workers in the rubber industry have potential occupational exposure to ethylene thiourea. This estimate was based on the NIOSH National Occupational Hazard Survey conducted between 1972 and 1974 (Ref. 5.8). No recent data was found. Although ethylene thiourea has been proposed for a variety of uses and although numerous patents have been issued, no evidence was found that it is being used commercially for other than rubber-curing purposes (Ref. 5.11). Exposure to ethylene thiourea may also result from the very widely used ethylene bisdithiocarbamate fungicides. Ethylene thiourea may be present as a contaminant in these fungicides and can also be formed when food containing the fungicides is cooked. (Ref. 5.8)

1.1.4 Physical properties and other descriptive information (Refs. 5.12-13)

CAS no.:	96-45-7
synonyms:	2-imidazolidinethione; 4,5-dihydroimidazole-2(3H)-thione; ethylenethiourea; N,N'-ethylenethiourea; 1,3-ethylene-2-thiourea; ETU; 4,5-dihydroimidazole-2(3H)-thione; 2-mercaptoimidazoline;

	NA-22; NA-22-D; NCI-C03372; Pennac CRA; RCRA Waste Number U116; Sodium-22 Neoprene Accelerator; 2-thiol-dihydroglyoxaline; USAF EL-62; Vulkacit NPV/C2; Warecure C
structural formula:	
molecular wt:	102.17
melting point:	203-204°C
odor:	faint amine odor
appearance:	white crystalline solid
solubility:	solubility in 100 mL water: 2 g at 30°C, 9 g at 60°C, 44 g at 90°C. Moderately soluble in methanol, ethanol, ethylene glycol, and pyridine; insoluble in acetone, ether, chloroform, benzene, ligroin.

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

1.2 Limit defining parameters

1.2.1 Detection limit of the analytical procedure

The detection limit of the analytical procedure is 2.1 ng per injection (10-µL injection of a 0.21 µg/mL solution). This is the amount of analyte that will produce a peak with a height that is approximately 5 times the baseline noise. (Section 4.1)

1.2.2 Detection limit of the overall procedure

The detection limit of the overall procedure is 0.666 µg per sample (1.39 µg/m^3). This is the amount of analyte spiked on the sampling device that, upon analysis, produces a peak similar in size to that of the detection limit of the analytical procedure. (Section 4.2)

1.2.3 Reliable quantitation limit

The reliable quantitation limit is 0.666 µg per sample (1.39 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 4.3)

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

1.2.4 Instrument response to the analyte

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

1.2.5 Recovery

The recovery of ethylene thiourea from samples used in a 15-day ambient storage test remained above 88.3%. (Section 4.5)

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 0.016. (Section 4.6)

1.2.7 Precision (overall procedure)

The precision at the 95% confidence level for the 15-day ambient temperature storage test is ±13.3%. (Section 4.7) This includes an additional ±5% for sampling error.

1.2.8 Reproducibility

Six samples, collected from a test atmosphere of ethylene thiourea, and a draft copy of this procedure were given to a chemist unassociated with this evaluation. The samples were

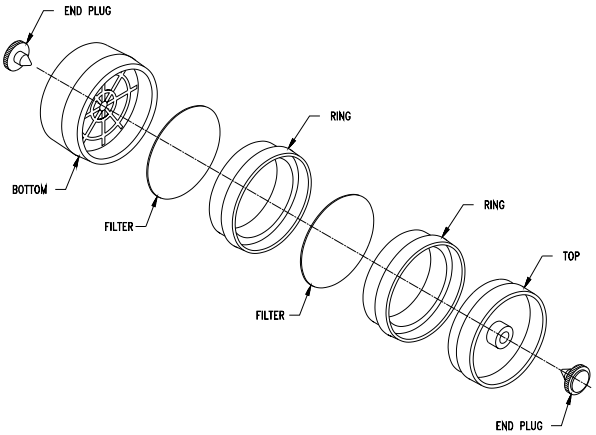
analyzed after 11 days of storage at 5°C. No individual sample result deviated from its theoretical value by more than the precision reported in Section 1.2.7. (Section 4.8.)

2. Sampling Procedure

2.1 Apparatus

2.1.1 A personal sampling pump that can be calibrated within ±5% of the recommended flow rate with the sampling device in line.

2.1.2 A four-piece polystyrene cassette containing two glass fiber filters assembled as shown.



2.2 Reagents

No reagents are required for sampling.

2.3 Technique

2.3.1 Prepare the sampler for open-face sampling by removing the top piece and the end plug from the bottom piece. Attach the sampler to the sampling pump with a piece of flexible tubing and place it in the worker's breathing zone with the open face of the cassette facing down.

2.3.2 Replace the top piece and the two end plugs after sampling. Seal each sample with an official Form OSHA-21.

2.3.3 Submit at least one blank with each set of samples. Handle the blank the same as the other samples except draw no air through it.

2.3.4 List any potential interferences on the sample data sheet.

2.4 Sampler capacity

Sampling capacity exceeds 276 µg, or 720 L at 6.4 times the target concentration and at a sampling rate of 2.0 L/min. This loading is equivalent to 2300 L at 2 times the target concentration. (Section 4.9)

2.5 Extraction efficiency (Section 4.10)

2.5.1 The average extraction efficiency of ethylene thiourea from glass fiber filters is 99.9%.

2.5.2 Extracted samples remain stable for at least 24 h.

2.6 Recommended air volume and sampling rate

2.6.1 For TWA samples the recommended air volume is 480 L collected at 2.0 L/min.

2.6.2 For short term samples the recommended air volume is 30 L collected at 2.0 L/min (15-min samples).

2.6.3 When short term samples are required, the reliable quantitation limit becomes larger. For example, the reliable quantitation limit is 22 µg/m^3 when 30 L of air is collected.

2.7 Safety precautions (sampling)

Attach the sampling equipment to the worker in such a manner that it will not interfere with work performance or safety. Follow all safety practices applicable to the work area.

3. Analytical Procedure

3.1 Apparatus

3.1.1 An HPLC equipped with a UV detector. A BAS 200 HPLC (Bioanalytical Systems) and Waters WISP auto-sampler were used in this evaluation.

3.1.2 An HPLC column capable of separating ethylene thiourea from any interferences. An Alltech C18 column (4.6 × 250 mm) was used in this evaluation.

3.1.3 An electronic integrator or other suitable means of measuring detector response. A Waters 860 Networking Computer System was used in this evaluation.

3.1.4 Sample vials, 4-mL glass, with polytetrafluoroethylene-lined caps.

3.1.6 A mechanical shaker.

3.2 Reagents

3.2.1 Ethylene thiourea. Ethylene thiourea, 98%, was obtained from Aldrich and recrystallized from methanol.

3.2.2 Water, HPLC grade. Water was obtained from a Millipore Milli-Q water purification system.

3.2.3 Methanol, HPLC grade. Methanol, Optima grade, was obtained from Fisher Scientific.

3.3 Standard preparation

3.3.1 Prepare stock standards by weighing 10-20 mg of ethylene thiourea in 10-mL volumetric flasks and diluting to volume with methanol. (Water may be used to prepare the stock standard, but ethylene thiourea crystals tend to float on top of the water initially.) Prepare analytical standards by diluting the stock standards with water. A 9.6 µg/mL standard solution corresponds to the target concentration.

3.3.2 Prepare a sufficient number of analytical standards to generate a calibration curve. Analytical standard concentrations must bracket sample concentrations.

3.4 Sample preparation

3.4.1 Transfer the two filters to separate 4-mL glass vials.

3.4.2 Add 3.0 mL of water to each vial.

3.4.3 Cap the vials and shake them on a mechanical shaker for 30 min.

3.5 Analysis

3.5.1. HPLC conditions

column:	Alltech C18
eluent:	methanol/water 10:90 (v/v)
flow rate:	1.0 mL/min
injection vol:	10 µL
retention time:	5.3 min
UV detector:	234 nm

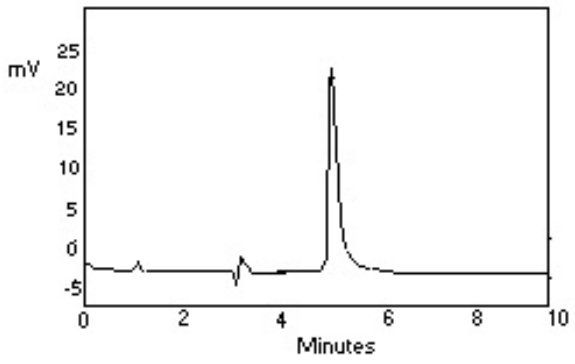


Figure 3.5 Chromatogram of ethylene thiourea at target concentration.

3.5.2 Construct a calibration curve using an external standard method by plotting micrograms per milliliter versus detector response of standards.

3.6 Interferences (analytical)

3.6.1 Any compound that absorbs at 234 nm and has a similar retention time as the analyte is a potential interference. Generally, chromatographic conditions can be altered to separate an interference from the analyte.

3.6.2 Retention time on a single column is not considered proof of chemical identity. Additional means of identification include: GC/MS, analysis using an alternate HPLC column, detection at another wavelength (peak ratioing), and analysis by GC/FID.

3.7 Calculations

The analyte amount for a sample is obtained from the calibration curve in terms of micrograms per milliliter uncorrected for extraction efficiency. The back filter is analyzed primarily to determine if there was any breakthrough from the front filter during sampling. If a significant amount of analyte is found on the back filter (e.g., greater than 25% of the amount found on the front filter), this fact should be reported with sample results. If any analyte is found on the back filter, it is added to the amount on the front filter. The analyte amount is then corrected by subtracting the total amount found in the blank. The air concentration is obtained by using the following equation.

$$mg/m^3 = \frac{A \times B}{C \times D}$$

where A = micrograms of analyte per milliliter
B = extraction volume
C = liters of air sampled
D = extraction efficiency

3.8 Safety precautions (analytical)

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

3.8.2 Avoid skin contact and inhalation of all chemicals.

3.8.3 Wear safety glasses, gloves and a lab coat at all times while working with chemicals.

4. Backup Data

4.1 Detection limit of the analytical procedure

The injection size recommended in the analytical procedure (10-µL) was used in the determination of the detection limit of the analytical procedure. The detection limit of 2.1 ng on-column was determined by analyzing a dilute standard of ethylene thiourea (0.21 µg/mL). This amount gave a peak with a height about 5 times the height of the baseline noise.

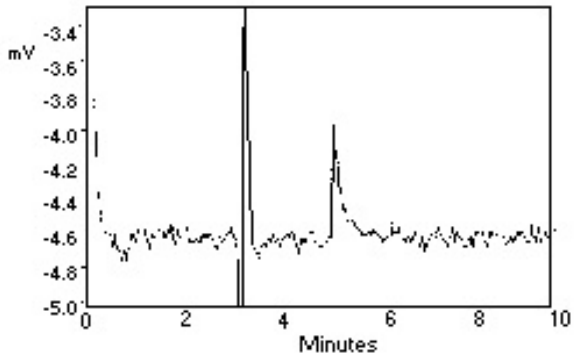


Figure 4.1 Chromatogram of the analytical detection limit.

4.2 Detection limit of the overall procedure

The detection limit of the overall procedure was determined by analyzing filters liquid spiked with 0.666 µg of ethylene thiourea. This amount corresponds to an air concentration of 1.39 µg/m^3. The injection size listed in the analytical procedure (10 µL) was used in the determination of the detection limit of the overall procedure.

Table 4.2.
Detection Limit of the
Overall Procedure

sample no.	µg spiked	µg recovered
1	0.666	0.690
2	0.666	0.670
3	0.666	0.658
4	0.666	0.652
5	0.666	0.688
6	0.666	0.621

4.3 Reliable quantitation limit

The reliable quantitation limit was determined by analyzing filters liquid spiked with 0.666 µg of ethylene thiourea. This amount corresponds to an air concentration of 1.39 µg/m^3. Because the recovery of the analyte from the spiked samples was greater than 75% with a precision of ±25% or better, the detection limit of the overall procedure and reliable quantitation limit are the same.

Table 4.3.
Reliable Quantitation Limit
(Based on samples and data of Table 4.2.)

percent recovered	statistics
103.6	
100.6	mean = 99.6%
98.8	SD = 3.9%
97.9	Precision = (1.96)(±3.9%)
103.3	= ±7.6
93.2	

4.4 Instrument response to the analyte

The instrument response to ethylene thiourea over the range of 0.5 to 2 times the target concentration was determined from multiple injections of analytical standards. The response is linear with a slope of 2.43×10^5 area counts per microgram per milliliter.

Table 4.4.
Instrument Response

× target concn µg/mL	0.5× 4.76	1.0× 9.52	2.0× 19.04
area counts	1036261	2207258	4517505
	1010263	2193614	4512732
	1051344	2262707	4497015
	1023608	2307770	4493679
	1022236	2214339	4492322
	1024264	2164339	4502761
mean	1027996	2225005	4502669

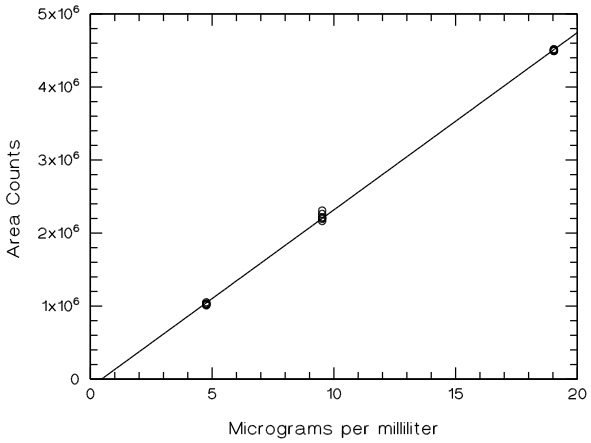


Figure 4.4. Calibration curve for ethylene thiourea.

4.5. Storage data

Storage samples were prepared from a test atmosphere of ethylene thiourea aerosol generated by pumping an isopropanol solution of ethylene thiourea (0.4 mg/mL) at a rate of 0.4 mL/min through a TSI Model 3076 atomizer (TSI Incorporated, St. Paul, MN), where it was dispersed with an air stream of 3.5 L/min. The aerosol passed through an electrostatic charge neutralizer and was mixed with a dilution air stream of 97 L/min (25°C, 80% RH). The diluted aerosol flowed into a chamber fitted with 18 sampling ports. Thirty-six samples were collected. Six samples were analyzed immediately. The rest were divided into two groups: 15 were stored in a refrigerator at 5°C, and the other 15 were stored in a closed drawer at about 22°C. Six samples, three from each group, were analyzed at intervals over a period of fifteen days. The recovery of ethylene thiourea from samples stored at ambient temperature remained above 88.3%.

Table 4.5.
Storage Test

days of storage	% recovery (ambient)			% recovery (refrigerated)		
0	104.0	94.3	96.1	104.0	94.3	96.1
0	100.4	100.7	104.5	100.4	100.7	104.5
2	96.3	101.2	92.0	95.4	99.0	104.1
5	99.6	97.9	97.7	100.4	106.5	96.6
8	96.2	93.1	94.7	103.2	97.5	99.5
12	87.6	94.1	100.7	97.3	105.9	98.4
15	89.7	77.9	88.1	100.2	99.0	99.8

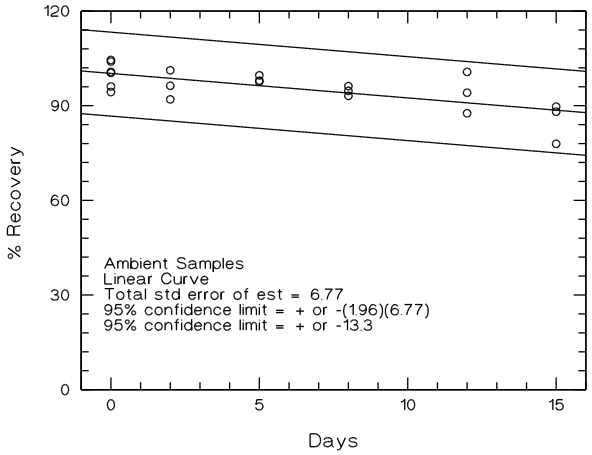


Figure 4.5.1. Storage test at ambient temperature.

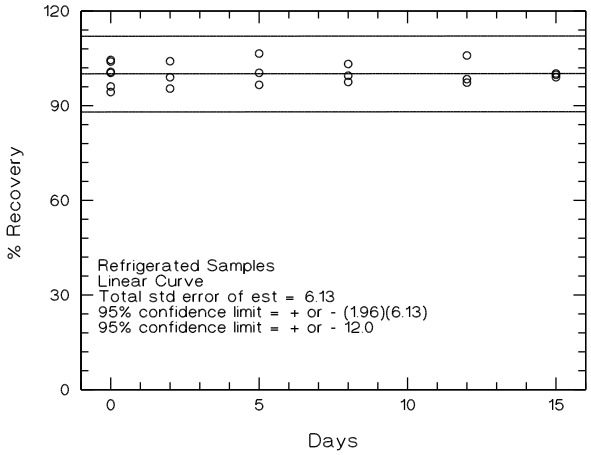


Figure 4.5.2. Storage test at reduced temperature (5°C).

4.6 Precision (analytical method)

The precision of the analytical procedure is defined as the pooled coefficient of variation determined from replicate injections of analytical standards representing 0.5, 1, and 2 times the target concentration. The coefficients of variation are calculated from the data in Table 4.4. The pooled coefficient of variation is 0.016.

Table 4.6.
Precision of the Analytical Method
(Based on the Data of Table 4.4.)

× target concn	0.5×	1.0×	2.0×
µg/mL	4.76	9.52	19.04
SD[1]	14101	51703	10404
CV	0.014	0.023	0.002

[1] - 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 percent recovery at a given time
Y_{est} = estimated percent 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 line in the storage graph as shown in Figure 4.5.1. The data for Figure 4.5.1 were used to determine the SEE of ±6.77% and the precision of the overall procedure of ±13.3%.

4.8 Reproducibility data

Six samples were collected from a test atmosphere of ethylene thiourea aerosol at 25°C and 80% RH. A draft copy of this method and the samples were submitted to a chemist unassociated with this evaluation for analysis. All of the sample results were within the range of ±13.3%, the precision of the overall procedure.

Table 4.8.
Reproducibility Data

µg expected	µg recovered	percent recovered	percent deviation
80.8	87.8	108.7	+8.7
80.6	82.7	102.6	+2.6
78.3	77.8	99.4	-0.6
79.1	88.0	111.3	+11.3
79.9	86.2	107.9	+7.9
80.3	84.5	105.2	+5.2

4.9 Sampler capacity

The sampler capacity was assessed by sampling from a dynamically generated test atmosphere of ethylene thiourea (25°C, 80% RH) at 2.0 L/min and for various sampling times analyzing the back filter. At 2 times the target concentration and a sampling time of 6 h, no ethylene thiourea was detected on the back filter. At 0.384 mg/m^3 (6.4 times the target concentration) and 6 h, the average amount collected on the front filter was 278 µg and on the back filter, 2.4 µg. This sample loading is equivalent to 2300 L at 2 times the target concentration. Separately, two glass fiber filters were each spiked with 63 µg of ethylene thiourea and humid air (80% RH) was pulled through them at 2 L/min for 226 min. The recovery was 99.5%.

4.10 Extraction efficiency and stability of extracted samples

4.10.1 Extraction efficiency

To determine the extraction efficiency, six glass fiber filters were liquid spiked with ethylene thiourea at the target concentration. These samples were stored overnight at ambient temperature and then extracted with water and analyzed. The average recovery was 99.9%.

Table 4.10.1.
Extraction Efficiency

sample no.	µg spiked	µg recovered	% recovery
1	28.81	28.64	99.4
2	28.81	28.38	98.5
3	28.81	28.81	100.0
4	28.81	29.93	103.9
5	28.81	28.88	100.2
6	28.81	28.03	97.3
mean			99.9

4.10.2 Stability of extracted samples

The stability of extracted samples was ascertained by reanalyzing the above samples 24 h later with fresh standards. The samples were stored at room temperature and were not recapped. The average recovery was 100.3%.

Table 4.10.2.
Stability of Extracted Samples

initial recovery (%)	recovery after 24 h (%)	percent change
99.4	99.6	+0.2
98.5	99.8	+1.3
100.0	98.9	-1.1
103.9	104.5	+0.6
100.2	99.5	-0.7
97.3	99.3	+2.0

5. References

5.1 Palassis, J., "Sampling and Analytical Determination of Airborne Tetramethyl and Ethylene Thiourea", *Am. Ind. Hyg. Assoc. J.*, **1980**, **41**(2), 91-98.

5.2 "NIOSH Manual of Analytical Methods", 3rd ed.; U.S. Department of Health and Human Services, Center for Disease Control, National Institute of Occupational Safety and Health; Cincinnati, OH, 1984, Method 5011, DHHS (NIOSH) Publ. No. 84-100.

5.3 Prince, J.L., "Analysis of Ethylenethiourea in Urine by High Performance Liquid Chromatography", *J. Agric. Food Chem.,* **1985**, **33**(1), 93-94.

5.4 Kurttio, P., T. Vartiainen, and K. Savolainen, "A High Performance Liquid Chromatographic Method for the Determination of Ethylenethiourea in Urine and on Filters", *Anal. Chim. Acta,* **1988**, **212**(1/2), 297-301.

5.5 Onley, J.H., and G. Yip, "Determination of Ethylene Thiourea Residues in Foods, Using Thin ZLayer and Gas Chromatography", *J. Assoc. Off. Anal. Chem.,* **1971**, **54**, 165-169.

5.6 Farrington, D.S., and R.G. Hopkins, "Determination of Ethylenethiourea in Ethylenebisdithiocarbamate Fungicides: Comparison of High-Performance Liquid Chromatography and Gas-Liquid Chromatography", *Analyst,* **1979**, **104**, 111-116.

5.7 Lehotay, J., E. Brandsteterova, and D. Oktavec, "High-Performance Liquid Chromatographic Determination of Ethylenethiourea in Food", *J. Liq. Chromatogr.,* **1992**, **15**(3), 525-534.

5.8 "Current Intelligence Bulletin 22: Ethylene Thiourea", U.S. Department of Health, Education, and Welfare, Center for Disease Control, National Institute of Occupational Safety and Health, DHEW (NIOSH) Publication No. 78-144, 1978.

5.9 Hathaway, G.J., et al., Ed., "Proctor and Hughes' Chemical Hazards of the Workplace", 3rd ed., Van Nostrand Reinhold, New York, 1991.

5.10 "IARC Monographs on the Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42", International Agency for Research on Cancer, Lyon, 1987; Supplement 7, pp 207-208.

5.11 "IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man: Some Anti-thyroid and Related Substances, Nitrofurans and Industrial Chemicals", International Agency for Research on Cancer, Lyon, 1974; Vol. 7, pp 45-52.

5.12 Budavari, S., Ed., "Merck Index", 11th ed., Merck & Co., Rahway, NJ, 1989

5.13 Sweet, D.V., Ed., "Registry of Toxic Effects of Chemical Substances", 1985-86 edition, U.S. Department of Health and Human Services, Government Printing Office, DHHS (NIOSH) Publication No. 87-114.