

ANTU\*

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Analyte:	ANTU	Method No.:	S276
Matrix:	Air	Range:	0.128-0.76 mg/cu m
OSHA Standard:	0.3 mg/cu m	Precision ( $\overline{CV}_T$ ):	0.054
Procedure:	Filter collection, methanol extraction, HPLC	Validation Date:	12/22/78

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1. Synopsis

A known volume of air is drawn through a Teflon filter to collect ANTU.

ANTU is extracted from the filter with 10 mL of methanol, and the resulting sample is analyzed by high pressure liquid chromatography.

2. Working Range, Sensitivity, and Detection Limit

This method was validated over the range of 0.128-0.76 mg/cu m at an atmospheric temperature of 22°C and pressure of 761 mm Hg, using 480-liter samples.

The upper limit of the range of the method is dependent on the capacity of the Teflon filter.

Under the instrumental conditions used in the validation study, a 10-microliter injection of a 7.21 mg/mL ANTU solution resulted in a peak whose height was 60% of full scale. The HPLC detector had a range setting of 0.0025 absorbance unit full scale and the electronic integrator had an attenuation of 16.

The detection limit of the method is estimated to be 5 micrograms of ANTU per sample filter.

3. Interferences

When interfering compounds are known or suspected to be present in the air, such information, including their suspected identities, should be transmitted with the sample.

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\*  $\alpha$ -Naphthylthiourea

Any compound that has the same retention time as ANTU at the operating conditions described in this method is an interference. Retention time data on a single column cannot be considered proof of chemical identity.

#### 4. Precision and Accuracy

The Coefficient of Variation ( $\overline{CV}_T$ ) for the total analytical and sampling method in the range of 0.128-0.76 mg/cu m was 0.054. This value corresponds to a standard deviation of 0.016 mg/cu m at the OSHA standard level. Statistical information can be found in Reference 11.1. Details of the test procedures can be found in Reference 11.2.

In validation experiments, this method was found to be capable of coming within  $\pm 25\%$  of the "true value" on the average 95% of the time over the validation range. The analytical method recovery was determined to be 0.984 for a collector loading of 0.072 mg. In storage stability studies, the mean of samples analyzed after 7 days were within 1.3% of the mean of samples analyzed immediately after collection. Experiments performed in the validation study are described in Reference 11.2.

#### 5. Advantages and Disadvantages

Collected samples are analyzed by means of a quick, instrumental method.

The collection is simple and involves no liquids

The precision of the method is limited by the reproducibility of the pressure drop across the filter. This drop will affect the flow rate and cause the volume to be imprecise, because the pump is usually calibrated for one filter only.

#### 6. Apparatus

**Filter Unit.** The filter unit consists of a 37-mm diameter/1-micrometer pore size Teflon filter (Millipore Type FA or equivalent) and a 37-mm polystyrene two-piece cassette filter holder. The filter is held in the two-piece holder, supported by a backup pad. The filter must be free of organic binders. Filter holders made of Tenite should not be used. Secure the cassette holder together with tape or shrinkable band.

**Personal Sampling Pump.** A calibrated personal sampling pump whose flow rate can be determined to an accuracy of 5%. Each personal sampling pump must be calibrated with a representative filter cassette in the line to minimize errors associated with uncertainties in the volume sampled.

**Manometer.**

6.4 Thermometer.

6.5 High pressure liquid chromatograph equipped with a 254 nm fixed wavelength uv detector and a sample injection valve with a 10-microliter external sample loop.

HPLC column packed with Microbondapak C-18 (30 cm x 3.9 mm I.D. stainless steel). This column packing can be obtained from Waters Associates.

Filtration unit for protection of the HPLC from particulate filter fibers: Stainless steel filter holder (13-mm) fitted with 13-mm/5-micrometer pore size Teflon filters.

An electronic integrator or some other suitable method for measuring peak areas.

6.9 Tweezers.

6.10 Syringes: 2-mL with luer-lock fitting.

6.11 Scintillation vials: 20 mL.

6.12 Volumetric flasks: Convenient sizes for preparing standard solutions.

6.13 Pipets: 10-mL and other convenient sizes for preparing standard solutions.

## 7. Reagents

Whenever possible, all reagents used must be ACS reagent grade or better.

$\alpha$ -Naphthylthiourea.

Methanol.

Water, deionized, distilled.

Stock Standard Solution of ANTU. Prepare a 150  $\mu$ g/mL standard of ANTU in methanol by dissolving 150 mg of ANTU in methanol in a 1-liter volumetric flask and make to volume. The ANTU will dissolve very slowly. Mild heating of the methanol will speed the process. This standard should be prepared fresh weekly.

7.5 Compressed air or nitrogen for drying syringes.

## 8. Procedure

Cleaning of Equipment. All glassware used for the laboratory analysis should be detergent washed, thoroughly rinsed with tap water and distilled water, and dried.

## 8.2 Collection and Shipping of Samples

- 8.2.1 Assemble the filter in the two-piece cassette filter holder and close firmly. The filter is supported by a backup pad. Secure the cassette holder together with tape or shrinkable band.
- 8.2.2 Remove the cassette plugs and attach the outlet of the filter cassette to the personal sampling pump inlet with flexible tubing.
- 8.2.3 Air being sampled should not pass through any hose or tubing before entering the filter cassette.
- 8.2.4 A sample size of 480 liters is recommended. Sample at a flow rate of 1.5-2.0 liters/minute. The flow rate should be known with an accuracy of 5%.
- 8.2.5 Set the flow rate as accurately as possible using the manufacturer's directions. Since it is possible for a filter to become plugged by heavy particulate loading or by the presence of oil mists or other liquids in the air, the pump rotameter should be observed frequently, and the sampling should be terminated at any evidence of a problem.
- 8.2.6 Terminate sampling at the predetermined time and record sample flow rate, collection time and ambient temperature and pressure. If pressure reading is not available, record the elevation. Also record the type of sampling pump used.
- 8.2.7 After sampling, disconnect the filter. Cap the inlet and outlet of the filter cassette with plugs. Label the cassette.
- 8.2.8 With each batch or partial batch of ten samples, submit a blank filter from the same lot of filters used for sample collection. This filter must be subjected to exactly the same handling as the samples except that no air is drawn through it. Label this filter as the blank.
- 8.2.9 The cassettes should be shipped in a suitable container, designed to prevent damage in transit. The samples should be shipped to the laboratory as soon as possible.
- 8.2.10 A sample of the bulk material must be submitted to the laboratory in a glass container with a Teflon-lined cap. Never transport, mail, or ship the bulk sample in the same container as the sample or blank filter.

## 8.3 Analysis of Samples

- 8.3.1 Remove the filter from the cassette with clean tweezers and place it into a 20-mL scintillation vial.

8.3.2 Add 10 mL of methanol and mix the solution by swirling. Allow the samples to stand for at least 30 minutes prior to analysis.

8.3.3 HPLC Conditions. The typical operating conditions for the high pressure liquid chromatograph are:

Column Temperature: Ambient  
Flow Rate: 1.2 mL/min  
Mobile Phase: 50% methanol and 50% deionized,  
distilled water  
Detector: uv at 254 nm  
Sample Size: 10 microliters

8.3.4 Injection. The first step in the analysis is to inject the sample into the high pressure liquid chromatograph. The prefilter, a 13-mm filtration unit containing a 13-mm/5-micrometer pore size Teflon filter is fitted to the sample injection valve. Flush the 10-microliter sample loop thoroughly with the sample (500 microliters), and inject the sample. The peak areas for duplicate injections should compare within 3%. The syringe should be rinsed and dried before the injection of another sample.

8.3.5 The area of the sample peak is measured by an electronic integrator or some other suitable form of area measurement, and results are read from a standard curve prepared as discussed below.

## 9. Calibration and Standardization

A series of standards, varying in concentration over the range corresponding to approximately 0.1 to 3 times the OSHA standard, is prepared and analyzed under the same LC conditions and during the same time period as the unknown samples. Curves are established by plotting concentration in mg/10 mL versus peak area. Note: Since no internal standard is used in this method, standard solutions must be analyzed at the same time as the samples. This will minimize the effect of known day-to-day variations.

From the stock standard solution (Section 7.4) appropriate aliquots are withdrawn and dilutions are made in methanol. Prepare at least 5 working standards to cover the range of 1.4-43.2 micrograms/mL. This range is based on a 480-liter sample.

Analyze samples as described in Section 8.3. These samples need not be filtered.

Prepare a standard calibration curve by plotting concentration of ANTU in mg/10 mL versus peak area.

## 10. Calculations

10.1 Read the weight, in mg, corresponding to each peak area from the standard curve. No volume correction is needed, because the standard curve is based on mg/10 mL methanol and the volume of sample injected is identical to the volume of the standards injected.

10.2 A correction for the blank must be made for each sample.

$$\text{mg} = \text{mg sample} - \text{mg blank}$$

where:

$$\text{mg sample} = \text{mg found in sample filter}$$

$$\text{mg blank} = \text{mg found in blank filter}$$

10.3 For personal sampling pumps with rotameters only, the following volume correction should be made.

$$\text{Corrected Volume} = f \times t \left( \sqrt{\frac{P_1}{P_2} \times \frac{T_2}{T_1}} \right)$$

where:

$$f = \text{flow rate sampled (liter/min)}$$

$$t = \text{sampling time (min)}$$

$$P_1 = \text{pressure during calibration of sampling pump (mm Hg)}$$

$$P_2 = \text{pressure of air sampled (mm Hg)}$$

$$T_1 = \text{temperature during calibration of sampling pump (°K)}$$

$$T_2 = \text{temperature of air sampled (°K)}$$

10.4 The concentration of ANTU in the air sample can be expressed in mg/cu m.

$$\text{mg/cu m} = \frac{\text{mg} \times 1000 \text{ (liters/cu m)}}{\text{Corr. Air Volume Sampled (liters)} \text{ (Section 10.3)}}$$

## 11. References

11.1 Documentation of NIOSH Validation Tests, National Institute for Occupational Safety and Health, Cincinnati, Ohio (DHEW-NIOSH Publication #77-185), 1977. Available from Superintendent of Documents, U.S. Government Printing Office, Washington, D.C., Order No. 017-033-00231-2.

11.2 Backup Data Report No. S276 for ANTU prepared under NIOSH Contract No. 210-76-0123.