Analyte:	4,6-Dinitro-2-methyl phenol	Method No.:	S166
Matrix:	Air	Range:	0.070-0.62 mg/cu m in a 180-liter sample
OSHA Standard:	0.2 mg/cu m - skin	Precision ($\overline{CV}_T$):	0.075
Procedure:	Filter and bubbler collection, ethylene glycol extraction, HPLC	Validation Date:	12/23/77

1. Synopsis

1.1 A known volume of air is drawn through a mixed cellulose ester membrane filter connected in series to a midget bubbler containing 10 mL of ethylene glycol to collect dinitro-o-cresol.

The filter and bubbler are disconnected. The filter is removed from the cassette holder and added to the bubbler flask. Five milliliters of 2-propanol are added to the flask before analysis.

The resulting sample is analyzed by high pressure liquid chromatography.

2. Working Range, Sensitivity and Detection Limit

2.1 This method was validated over the range of 0.070-0.62 mg/cu m at an atmospheric temperature of 24°C and pressure of 761 mm Hg, using 180-liter samples.

The upper limit of the range of the method is dependent on the capacity of the mixed cellulose ester membrane filter connected in series to the midget bubbler.

3. Interferences

3.1 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.

Any compound that has the same retention time as dinitro-o-cresol 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 0.070-0.62 mg/cu m was 0.075. This value corresponds to a standard deviation of 0.015 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 ±25% of the "true" value on the average 95% of the time over the validation range. The concentrations obtained at 0.5X, 1X, and 2X the OSHA environmental limit were 2% lower than the dynamically generated test concentrations (n = 17). In these experiments the average analytical method recovery was 1.037 for a loading of 17.98-71.9 micrograms of dinitro-o-cresol on the filter. A correction was applied for this recovery. In storage stability studies, the mean of samples analyzed after 7 days were within 5% 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.

A disadvantage of the method is the awkwardness in using midget bubblers for collecting personal samples. If the worker's job performance requires much body movement, loss of the collection solution during sampling may occur.

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

The bubblers are more difficult to ship than adsorption tubes or filters due to possible breakage and leakage of the bubblers during shipping.

6. Apparatus

Filter unit: The filter unit consists of a 37-mm diameter cellulose ester membrane filter (Millipore Type AA or equivalent) with a pore size of 0.80 micrometer, supported by a stainless steel screen on a 37-mm two-piece cassette filter holder. It is important that a stainless steel screen be used since other filter supports may retain part of the vapor.

A glass midget bubbler.

Personal Sampling Pump: A calibrated personal sampling pump whose flow can be determined to an accuracy of 5%. Each personal sampling pump must be calibrated with a representative filter cassette, bubbler,

and trap in the line to minimize errors associated with uncertainties in the volume sampled. The sampling pump is protected from splash-over or solvent condensation by a trap. The trap is a midget bubbler or impinger with the stem broken off which is used to collect spillage. The trap is attached to the pump with a metal holder. The outlet of the trap is connected to the pump by flexible tubing.

6.4 Manometer.

Thermometer.

High pressure liquid chromatograph equipped with a 254-nm fixed wavelength uv detector and a sample injection valve with a 50-microliter external sample loop.

Column (300 mm x 3-mm I.D. stainless steel) packed with μ-Bondapak C-18. This column is available from Waters Associates, Milford, Massachusetts.

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

Tweezers.

6.10 Microliter Syringe: 10-microliter.

6.11 Volumetric Flasks: Convenient sizes for preparing standard solutions.

6.12 Pipets: Convenient sizes for preparing standard solutions and 5-mL and 10-mL pipets for measuring the extraction medium.

6.13 Teflon tubing (15 cm long x 7-mm I.D.) or Teflon plugs for sealing the inlet and outlet of the bubbler stem before shipping.

7. Reagents

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

7.1 4,6-Dinitro-o-cresol.

2 Ethylene glycol.

2-Propanol.

7.4 Methanol, distilled in glass.

7.5 Water, deionized and distilled

7.6 Prepare a 9.00 mg/mL dinitro-o-cresol stock standard solution by dissolving 225 mg dinitro-o-cresol in methanol and diluting to 25 mL in a volumetric flask.

7.7 Glacial acetic acid.

7.8 Toluene.

7.9 Mobile phase for HPLC: 65% methanol/35% water/0.175% acetic acid (% by volume).

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.

Collection and Shipping of Samples

8.2.1 Assemble each filter in the two-piece filter cassette holder and close firmly. The filter is backed up by a stainless steel screen. Secure the cassette holder together with tape or shrinkable band.

8.2.2 Pour 10 mL of ethylene glycol into a midget bubbler, and mark the liquid level before inserting the bubbler frit. Be sure that the bubbler frit is completely immersed in the ethylene glycol. If the ethylene glycol does not cover the frit, do not use the bubbler. Dinitro-o-cresol will not be collected efficiently unless the bubbler frit is completely immersed in the ethylene glycol.

8.2.3 Remove the cassette plugs and attach the outlet of the filter cassette to the inlet arm of the midget bubbler using a short piece of flexible tubing. Connect the outlet of the midget bubbler to either the pump's inlet or the trap's inlet. When a trap is used, it is attached to the pump by tape or a holder The outlet of the trap is connected by tubing to the pump's inlet. Material collected in the trap must never be returned to the midget bubbler. If more than 1 mL of material is collected in the trap after sampling, the sample should be considered invalid, otherwise, it can be discarded. The bubbler must be maintained in a vertical position during sampling.

8.2.4 Air being sampled should not pass through any hose or tubing before entering the filter cassette.

8.2.5 A sample size of 180 liters is recommended. Sample at a flow rate of 1.5 liters per minute. The flow rate should be known with an accuracy of 5%.

8.2.6 Turn the pump on and begin sample collection. 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.

8.2.7 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.

8.2.8 After sampling, disconnect the filter and bubbler. Remove the bubbler stem, and remove the filter from the filter cassette with clean tweezers and add it to the bubbler. Replace the bubbler stem. The inlet and outlet of the bubbler stem should be sealed by connecting a piece of Teflon tubing between them or inserting Teflon plugs in the inlet and outlet. Do not seal with rubber. The standard taper joint of the bubbler should be taped securely to prevent leakage during shipping. It is necessary to place the filter in the bubbler solution at this time, otherwise loss of dinitro-o-cresol from the filter by vaporization may occur.

8.2.9 With each batch or partial batch of ten samples submit one bubbler containing ethylene glycol and a blank filter from the same lot of filters and bubblers used for sample collection. This filter and bubbler must be subjected to exactly the same handling as the samples except that no air is drawn through them. Label this filter and bubbler as the blank.

8.2.10 The bubblers 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.

8.3 Analysis of Samples

8.3.1 Remove the bubbler stem from the bubbler. Allow ethylene glycol to drain from the frit into the bulk of the solution in the bubbler. If the sample volume is less than 10 mL, add ethylene glycol until the volume reaches the 10-mL mark. If the sample volume is more than 10 mL, determine the volume and make an appropriate volume correction in the calculations indicated in Section 10.1. Absorption of water would cause the volume in the bubbler to increase.

8.3.2 Add 5 mL of 2-propanol to each sample and mix the solution by swirling.

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

Column Temperature: Ambient
Column Pressure: 3230 psi
Flow Rate: 1.6 mL/minute
Mobile Phase: 65% methanol/35% water/0.175% acetic acid
Detector: uv photometer at 254 nm
Retention Time: 6 minutes

8.3.4 Injection. The first step in the analysis is to inject the sample into the high pressure liquid chromatograph. The chromatograph is fitted with a sample injection valve and a 50-microliter sample loop. Flush this loop thoroughly with the sample (300 microliters), and inject the 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.

8.4 Determination of Analytical Method Recovery

8.4.1 Need for Determination. To eliminate any bias in the analytical method, it is necessary to determine the recovery of dinitro-o-cresol from the mixed cellulose ester membrane filter. The analytical method recovery should be determined over the concentration range of interest.

8.4.2 Procedure for Determining Analytical Method Recovery. An appropriate aliquot of a 9.00 mg/mL solution of dinitro-o-cresol in toluene is added to a 37-mm MCE filter. The amount added is equal to the amount of dinitro-o-cresol collected in a 180-liter sample at 2X, 1X, and 0.5X the OSHA standard level. Six spiked samples are prepared at each level along with six blanks. The filters are allowed to stand for 2-3 minutes to allow evaporation of toluene. The filter is transferred to a container containing 10 mL of ethylene glycol and 5 mL 2-propanol. The resulting sample is mixed well and allowed to stand for 1 hour before analysis.

Standards are prepared as discussed in Section 9 and analyzed with the samples.

Analytical Method Recovery (A.M.R.) equals the weight in µg found on the filter divided by the weight in µg added to the filter, or

$$\text{A.M.R.} = \frac{\text{Average weight recovered (µg)-Blank (µg)}}{\text{Weight added (µg)}}$$

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 for the sample under study, 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 micrograms/15 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 and variations during the same day of the uv detector response.

9.1 From the stock solution (Section 7.6), appropriate aliquots are withdrawn and added to 10 mL ethylene glycol and 5 mL 2-propanol. Prepare at least 5 working standards to cover the range of 3.6-108 micrograms/15 mL. This range is based on a 180-liter sample. Analyze samples as described in Section 8.3.

9.2 Prepare a standard calibration curve by plotting concentration of dinitro-o-cresol in µg/15 mL versus peak area.

10. Calculations

10.1 Read the weight, in µg, corresponding to each peak area from the appropriate standard curve. No volume correction is needed, because the standard curve is based on µg/15 mL of ethylene glycol/2-propanol 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.

$$\mu g = \mu g \text{ sample} - \mu g \text{ blank}$$

where:

μg sample = µg found in sample

μg blank = µg found in blank

10.3 Corrections for analytical method recovery (A.M.R.) must be made.

$$\text{Corrected } \mu g/\text{sample} = \frac{\mu g \text{ (Section 10.2)}}{\text{A.M.R.}}$$

10.4 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 = flow rate sampled

t = sampling time

P_1 = pressure during calibration of sampling pump (mm Hg)

P_2 = pressure of air sampled (mm Hg)

T_1 = temperature during calibration of sampling pump (°K)

T_2 = temperature of air sampled (°K)

10.5 The concentration of dinitro-o-cresol in the air sample can be expressed in mg/cu m.

$$\text{mg/cu m} = \mu\text{g/L} = \frac{\mu\text{g (Section 10.3)}}{\text{Corr. Air Volume Sampled (L)(Section 10.4)}}$$

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 for Dinitro-o-cresol prepared under NIOSH Contract No. 210-76-0123.