Dinitrobenzene
(all isomers)

Analyte:	Dinitrobenzene	Method No.:	S214
Matrix:	Air	Range:	0.42-2.4 mg/cu m
OSHA Standard:	1 mg/cu m - skin	Precision ($\overline{CV}_T$):	0.091
Procedure:	Filter and bubbler collection, ethylene glycol extraction, HPLC	Validation Date:	4/15/77

1. Principle of the Method

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 dinitrobenzene.

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

1.3 The resulting sample is analyzed by high pressure liquid chromatography.

2. Range and Sensitivity

2.1 This method was validated over the range of 0.418-2.386 mg/cu m at an atmospheric temperature of 23°C and pressure of 758 mm Hg, using 90-liter samples.

2.2 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 and the capacity of 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.

3.2 Any compound that has the same retention time as dinitrobenzene 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

4.1 The Coefficient of Variation ($\overline{CV}_T$) for the total analytical and sampling method in the range of 0.418-2.386 mg/cu m was 0.091. This value corresponds to a standard deviation of 0.09 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.

4.2 On the average the concentrations obtained in the laboratory validation study at 0.5X, 1X, and 2X the OSHA standard level were 2.4% lower than the "true" concentrations for 18 samples. Any difference between the "found" and "true" concentrations may not represent a bias in the sampling and analytical method, but rather a random variation from the experimentally determined "true" concentration. The Coefficient of Variation is a good measure of the accuracy of the method since the recoveries, storage stability, and collection efficiency were good and would not contribute to a bias in a determined concentration. Storage stability studies on samples collected from a test atmosphere at a concentration of 1.191 mg/cu m indicate that collected samples are stable for at least 7 days.

5. Advantages and Disadvantages of the Method

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

5.2 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.

5.4 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

6.1 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.

6.2 A glass midget bubbler containing 10 ml of ethylene glycol.

6.3 Personal Sampling Pump: A calibrated personal sampling pump whose flow can be determined to an accuracy of 5%. The sampling pump is protected from splashover or solvent condensation by a trap consisting of a second bubbler or impinger downstream from the midget bubbler.

6.4 Manometer.

6.5 Thermometer.

6.6 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. The injection valve is fitted with a syringe filter to remove filter fibers which would eventually block the flow to the LC column.

6.7 Column (250 mm x 3-mm I.D. stainless steel) packed with Spherisorb ODS. The superficially porous packing material consists of spherical silica particles with a 5% bonded coating of octadecyl groups. This packing can be obtained from Spectra-Physics in Santa Clara, California.

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

6.9 Tweezers.

6.10 Microliter Syringes: 50 and 100-microliter.

6.11 Volumetric Flasks: Convenient sizes for preparing standard solutions.

6.12 Pipets: Convenient sizes for preparing standard solutions and 5- 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

7.1 o-Dinitrobenzene, m-dinitrobenzene, and p-dinitrobenzene, reagent grade.

7.2 Ethylene glycol, reagent grade.

7.3 Methanol, distilled in glass.

7.4 2-Propanol, reagent grade.

7.5 Water, deionized and distilled.

8. Procedure

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

8.2 Calibration of Personal Sampling Pumps. Each personal sampling pump must be calibrated with a representative filter cassette and bubbler in the line to minimize errors associated with uncertainties in the volume sampled.

8.3 Collection and Shipping of Samples

8.3.1 Assemble the 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.

8.3.2 Pour 10 ml of ethylene glycol into each midget bubbler. Be sure that the bubbler frit is completely immersed in the ethylene glycol. If necessary adjust liquid level to cover the frit.

8.3.3 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. After sampling, discard the material collected in the trap. The bubbler must be maintained in a vertical position during sampling.

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

8.3.5 A sample size of 90 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%.

8.3.6 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.

8.3.7 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.

8.3.8 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 dinitrobenzene from the filter by vaporization may occur.

8.3.9 With each 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.

8.3.10 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.

8.4 Analysis of Samples

8.4.1 If the sample volume is less than 10 ml, add ethylene glycol until the volume reaches the 10-ml mark.

8.4.2 Add 5 ml of methanol to each sample and mix the solution by swirling. If the volume is not exactly 15.0 ml, make an appropriate correction in the calculation in Section 10.1. Allow the samples to stand for 2 hours before analysis.

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

Column Temperature: Ambient
Column Pressure: 2500 psi
Flow Rate: 1.9 ml/min
Mobile Phase: 20% methanol/80% water (V/V)
Detector: uv photometer at 254 nm
Capacity Ratio: 8.6

There are three isomers of dinitrobenzene. This method was validated using only the meta isomer, which is the predominant isomer in a mixture of isomers of dinitrobenzene (approximately 93% of the total as given in Reference 3).

Using the HPLC operating conditions above, the isomers can be eluted separately from the column. If more than one isomer is present in the sample, it is important that the HPLC conditions allow adequate separation of the isomers, since they must be quantitated independently. The response for each isomer should be determined.

8.4.4 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.

8.4.5 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 Standards

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 mg/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 Prepare a 2.25 mg/ml dinitrobenzene stock standard solution (for the isomer of interest) by dissolving 56.25 mg dinitrobenzene in 2-propanol and diluting to 25 ml in a volumetric flask.

9.2 From the above stock solution, appropriate aliquots are withdrawn and added to 10 ml ethylene glycol and 5 ml methanol. Prepare at least 5 working standards to cover the range of 0.013-0.40 mg/15 ml. This range is based on a 90-liter sample. Analyze samples as per Section 8.4.

9.3 Prepare a standard calibration curve by plotting concentration of dinitrobenzene in mg/15 ml versus peak area. A calibration curve should be prepared for each isomer of interest.

10. Calculations

10.1 Read the weight, in mg, corresponding to each peak area from the appropriate standard curve. No volume correction is needed, because the standard curve is based on mg/15 ml of ethylene glycol/methanol and the volume of sample injected is identical to the volume of the standards injected. If more than one isomer is present, combine the mg found for each isomer to give the total weight of dinitrobenzene in the sample.

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

$$mg = mg\ sample - mg\ blank$$

where:

mg sample = mg found in sample filter

mg blank = 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 = 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)

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

$$\text{mg/cu m} = \frac{\text{mg (Section 10.2) x 1000 (liters/cu m)}}{\text{Corr. Air Volume Sampled (liters) (Section 10.3)}}$$

11. References

11.1 Documentation of NIOSH Validation Tests, Contract No. CDC-99-74-45.

11.2 Backup Data Report for Dinitrobenzene, prepared under NIOSH Contract No. 210-76-0123.

11.3 March, J. Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, McGraw-Hill, New York (1968), 382.