o-Chlorobenzylidene Malononitrile (OCBM)

Measurements Research Branch
Analytical Method

Analyte:	o-Chlorobenzylidene Malononitrile	Method No.:	P&CAM 304
Matrix	Air	Range:	0.147 - 0.82 mg/cu m
Procedure:	Filter/sorbent collection, extraction with 20% methylene chloride in hexane, HPLC	Precision ($\overline{CV}_T$):	0.102
		Classification:	E (proposed)
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1. Synopsis

A known volume of air is drawn through a polytetrafluoroethylene (PTFE) membrane filter followed by a Tenax-GC sorbent tube to trap the particulate and vapors present.

The filter and sorbent are transferred to a jar or other suitable container and OCBM is extracted with 20% methylene chloride in hexane (HPLC mobile phase).

An aliquot of the sample is injected into a high performance liquid chromatograph equipped with a UV detector capable of detection at 305 nm.

The area of resulting sample peak is used as a measure of analyte concentration by comparison with corresponding areas obtained from the injection of standards.

2. Working Range, Sensitivity and Detection Limit

The method was tested over the range of 0.1472-0.819 mg/cu m at an atmospheric temperature and pressure of 18°C and 764 mm Hg, using a 90-liter sample. The method may be capable of measuring smaller amounts if the desorption efficiency is adequate. Desorption efficiency must be determined over the range used.

The upper limit of the method depends upon the adsorptive capacity of the Tenax-GC. This capacity may vary with the concentration of OCBM in the air. Breakthrough is defined as the time that the effluent concentration from the collection tube (containing 70 mg of Tenax-GC) reaches 5% of the concentration in the test gas mixture. Five percent breakthrough occurred after

120 minutes at a sampling rate of 1.5 liters per minute and relative humidity of 85% and temperature of 24°C. The breakthrough test was conducted at an average concentration of 1.3 mg/cu m.

The detection limit of the analytical method was not rigorously determined but is estimated to be at least 0.3 µg/sample.

3. Interferences

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

It must be emphasized that any other compound which has the same retention time as the analyte at the operating conditions described in this method and has UV absorption at 305 nm is an interference. Retention time data based on a single set of conditions 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 0.1472-0.819 mg/cu m was 0.1024. This value corresponds to a 0.041 mg/cu m standard deviation at the OSHA standard level. Statistical information can be found in Reference 11.1. Details of the test procedure are found in Reference 11.2.

In validation experiments, this method was not found to be capable of coming within ±25% of the "true value" on the average of 95% of the time over the validation range. The concentrations measured at 0.5, 1 and 2 times the OSHA standard were identical to the dynamically generated concentrations (n = 18). The analytical recovery was determined to be 95.2% for a collector loading of 18.52 µg. In storage stability studies, the mean of samples analyzed after seven days was within 1.0% of the mean of samples analyzed the day after collection. Experiments performed in these studies are described in Reference 11.2.

5. Advantages and Disadvantages

5.1 The sampling device is small, portable and involves no liquids. Interferences are minimal and most of those which do occur may be eliminated by altering chromatographic conditions. The filters are analyzed by means of a quick, instrumental method.

5.2. The amount of sample that can be taken is limited by the number of micrograms that the tube will hold before overloading. When the amount of OCBM found on the backup section exceeds 25% of that found on the front section, the probability of sample loss exists.

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

The sampling train places a heavy load on the sampling pump since it has a high pressure drop.

The method does not measure exposures to within ±25%

6. Apparatus

Sampling Equipment. The sampling unit for the collection of personal air samples for the determination of mixed vapor/particulate samples has the following components:

6.1.1 Filter. The filter unit consists of a polytetrafluoroethylene (PTFE) membrane filter, 1.0 micrometer pore size and 37-mm diameter, supported by a stainless-steel screen (Mine Safety Appliances Co., catalog number 456224), and a 37-mm, two-piece filter holder held together by tape or a shrinkable band.

6.1.2 Personal Sampling Pump. A calibrated personal sampling pump is needed whose flow can be determined to an accuracy of ±5% at the recommended flow rate. The pump must be calibrated with a representative sampling train in the line.

6.1.3 Tenax-GC Tubes. The tubes are constructed of glass tubing with both ends unsealed. The tubes are approximately 10-cm long with a 10-mm O.D., and an 8-mm I.D. The front section contains 70-mg of 35/60 mesh Tenax-GC, and the backup section contains 35 mg. Tenax-GC is held in place in the tube with 3-mm plugs of silanized glass wool. A 3-mm plug also separates the two sections. To facilitate handling of the Tenax-GC resin, the tubes should be acetone rinsed and dried. This reduces the problem of the resin adhering to the walls of the tube.

6.1.4 Thermometer.

6.1.5 Barometer.

6.1.6 Stopwatch.

Connection of Filter Holder and Sorbent Tube. The Tenax-GC tube is connected to the outlet of the two-piece filter holder using a modified Luer-lock to 1/4-in I.D., tubing adapter (Millipore Corp., catalog number XX30025 64) and 1/4-in I.D., Tygon tubing.

High performance liquid chromatograph equipped with an ultraviolet detector, capable of detection at 305 nm.

Column (25-cm x 4.6-mm x 6.4-mm stainless steel) packed with μBondapak CN (Waters Assoc., Milford, Mass.) or equivalent.

6.5 A syringe or fixed volume sample loop for HPLC injection. A 20-μL sample volume was used for these studies.

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

Microliter syringes in convenient sizes for making standard solutions.

6.8 Volumetric flasks in convenient sizes for making standard solutions.

12-mL vials with Teflon-lined screw caps or squat form ointment jars with Teflon film gaskets and screw caps for desorption of sample.

6.10 Filtration device for samples, Swinney 13-mm (Millipore Corp.) or equivalent with Teflon filters.

6.11 A 5-mL pipette.

6.12 Tweezers.

7. Reagents

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

o-Chlorobenzylidene malononitrile (OCBM).

n-Hexane, distilled in glass.

Methylene chloride, distilled in glass.

7.4 OCBM, 8 mg/mL stock solution. Prepare by adding 8 milligrams of o-chlorobenzylidene malononitrile to a tared 2-mL septum capped vial. Add 1 mL of 20% methylene chloride in hexane to dissolve the OCBM.

NOTE: OCBM is an extremely strong lachrymator. Keep under cover whenever possible. Store and use only in a hood

8. Procedure

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

Calibration of Personal Pumps. Each personal pump must be calibrated with a representative sampling train in the line. This will minimize errors associated with uncertainties in the sample volume collected.

Collection and Shipping of Samples

8.3.1 Assemble the filter in the two-piece filter holder and close firmly to insure that the center ring seals the edge of the filter. The PTFE membrane filter is held in place by a stainless steel screen and the filter holder is held together by plastic tape or a shrinkable cellulose band. If the top piece of the filter holder does not fit snugly into the bottom piece of the filter holder, sample leakage will occur around the filter.

8.3.2 Remove the filter holder plugs and sorbent tube caps and assemble the sampling train. Attach the outlet of the sorbent tube to the personal sampling pump tubing. Clip the sampler to the worker's lapel.

8.3.3 Air being sampled should not be passed through any hose or tubing before entering the filter holder.

8.3.4 A sample size of 90 liters is recommended. Sample at a flow rate of 1.5 liters per minute for sixty minutes.

8.3.5 Turn the pump on and begin collection. Set the flow rate as accurately as possible using the manufacturer's directions. Since it is possible for filters 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 checked frequently and readjusted as needed. If the rotameter cannot be readjusted, terminate sampling.

8.3.6 Terminate sampling at the predetermined time and note sample flow rate, collection time and ambient temperature and pressure. If pressure reading is not available, record the elevation.

8.3.7 After sampling, remove the sorbent tube and Luer-lock adapter from the outlet of the filter holder and connect to the inlet side. Cap the open end of the sorbent tube and plug the outlet of the filter holder with the

plugs supplied. Under no circumstances should rubber caps be used.

8.3.8 Carefully record sample identity and all relevant sample data.

8.3.9 With each batch of samples, submit one sampling train which is subjected to exactly the same handling as the samples except that no air is drawn through it. Label this as a blank. Submit one blank for every ten samples.

8.3.10 The sampling train should be shipped in a suitable container designed to prevent damage in transit.

8.3.11 A bulk sample of the suspected material should be submitted to the laboratory in a glass container lined with Teflon cap. Label of the bulk sample should match air samples for identification purposes. This sample should not be transported in the same container as the samples.

8.4 Analysis of Samples

8.4.1 Preparation of Samples

1. Open the filter holder. Carefully remove the Teflon filter from the holder with the aid of appropriate tweezers and transfer to a 2-oz ointment jar or 12-mL vial.

2. Remove the glass wool plug from the sorbent tube and add only the front section of Tenax-GC to the same container as the Teflon filter.

3. Add 5 mL of 20% methylene chloride in hexane to the jar and properly cap unit. Gently swirl the jar to ensure that the filter is thoroughly wetted.

4. The backup Tenax-GC section is added to a different container and analyzed separately.

8.4.2 Filtration of Samples. Prior to injection, the sample should be filtered through a Teflon filter (1.0 µm pore size) using a 5-mL syringe fitted with a Swinney filter holder or equivalent. The filtrate should be placed in the 12-mL Teflon-capped vials.

8.4.3 Analysis by High Pressure Liquid Chromatography. The mobile phase is 20% methylene chloride in hexane. The typical operating conditions for the liquid chromatograph are:

1. 1.0 mL/min solvent flow rate.

2. Ambient column temperature.

3. 305 nm UV detection wavelength.

8.4.4 Injection. A 20 µL-sample aliquot is recommended for this analysis. The sample may be injected either by using an appropriate syringe or by filling a fixed volume sample loop, provided that reproducibility requirements are satisfied. Duplicate injections of each sample and standard should be made. No more than a 3% difference in area is to be expected.

8.4.5 Measurement of Area. 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 in Section 9.

8.5 Determination of Analytical Method Recovery

8.5.1 Need for Determination. To eliminate any bias in the analytical method, it is necessary to determine the recovery of the compound. The sample recovery determinations should cover the concentration range of interest.

8.5.2 Procedure for Determining Recovery. A known amount of OCBM, equivalent to that present in a 90-liter sample at the selected level, is added to a PTFE filter and Tenax-GC in a container. A stock solution containing 8 milligrams of OCBM per milliliter of 20% methylene chloride in hexane is prepared. Add 25, 50 and 100-microliter aliquots of the solution to the filter and sorbent to produce samples equivalent to 90-liter collections at 0.5, 1 and 2 times the OSHA standard. Six samples at each of the three levels are prepared and allowed to stand overnight. A parallel blank sample is also prepared except no sample is added to it. All samples are then extracted and analyzed as described in Section 8.4.

The sample recovery equals the average weight in µg recovered divided by the weight in µg added, or

$$\text{Recovery} = \frac{\text{Average Weight (µg) Recovered - Blank (µg)}}{\text{Weight (µg) Added}}$$

9. Calibration and Standardization

A series of standards, varying in the concentration range corresponding to approximately 0.1 to 3 times the OSHA standard for the samples under study, is prepared and analyzed under the same HPLC conditions and during the same time period as the unknown samples.

From the stock solution listed in Section 7.4, prepare at least six standards to cover the concentration range of 3.2 - 112 µg/5 mL. This is done by adding from 2 to 70-microliter aliquots of the stock solution to 25 mL of 20% methylene chloride in hexane (HPLC mobile phase) in volumetric flasks. Analysis is done as described in Section 8.4.

The series of standards is analyzed under the same HPLC conditions and during the same time period as the unknown samples. It is convenient to express concentration of standards in µg/5 mL, because samples are extracted in this amount of solvent. Curves are established by plotting concentrations in µg/5 mL versus peak area.

10. Calculations

10.1 Read the concentration in µg/5 mL corresponding to the sample peak area from the standard curve. No volume corrections are needed because the standard curve is based on µg/5 mL and the volume of the sample injected is identical to the volume of standards injected.

10.2 Corrections for the blank must be made for each sample.

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

where:

$$\mu g \text{ sample} = \mu g \text{ found in sample}$$
$$\mu g \text{ blank} = \mu g \text{ found in blank}$$

10.3 Divide the total weight by the recovery (Section 8.5.2) to obtain the corrected µg/sample.

$$\text{Corrected } \mu g/\text{sample} = \frac{\text{Total Weight}}{\text{Recovery}}$$

For personal sampling pumps with rotameters only, the following corrections 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 = sampling flow rate

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)

The concentration of the analyte in the air sampled can be expressed in mg per cu m (µg per liter = mg per cu m).

$$\text{mg/cu m} = \frac{\text{Corrected µg (Section 10.3)}}{\text{Volume of Air Sampled in Liters}}$$

Another method of expressing concentration is ppm (corrected to standard conditions of 25°C and 760 mm Hg).

$$\text{ppm} = \text{mg/cu m} \times \frac{24.45}{188.5} \times \frac{760}{P} \times \frac{(T + 273)}{298}$$

where:

P = pressure (mm Hg) of air sampled

T = temperature (°C) of air sampled

24.45 = molar volume (liter/mole) at 25°C and 760 mm Hg.

188.5 = molecular weight of OCBM

760 = standard pressure (mm Hg)

298 = standard temperature (°K)

11. References

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

11.2 Backup Data Report for o-Chlorobenzylidene Malononitrile, No. 304, prepared by Richard H. Smith, Arthur D. Little, Inc., under NIOSH Contract No. 210-76-0123, February 16, 1979.