Analyte:	Phthalic Anhydride	Method No.:	S179
Matrix:	Air	Range:	5.5-28 mg/cu m
OSHA Standard:	2 ppm (12 mg/cu m)	Precision ($\overline{CV}_T$):	0.089
Procedure:	Filter collection, extraction and hydrolysis with aqueous ammonia, HPLC	Validation Date:	8/29/75

1. Principle of the Method

1.1 A known volume of air is drawn through a cellulose membrane filter to trap the organic aerosol present.

1.2 The filter in the cassette is transferred into a jar and treated with aqueous ammonia; the anhydride is hydrolyzed to the acid and extracted in the acid form.

1.3 An aliquot of the sample is injected into a high performance liquid chromatograph (HPLC) equipped with a 254 nm UV detector.

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

2. Range and Sensitivity

2.1 This method was validated over the range of 5.5-28 mg/cu m at an atmospheric temperature and pressure of 22°C and 765 mm Hg, using a 100-liter sample. Under the conditions of sample size (100 liters), the probable useful range of this method is 1-36 mg/cu m at a detector sensitivity that gives nearly full deflection on the strip chart recorder for a 3.6 mg sample based on the dilution and sample aliquots specified in this method. (Refer to Section 8.4.)

2.2 The upper limit of the range of the method is dependent on the collection efficiency of the cellulose membrane filter. If higher concentrations than those tested are to be sampled, smaller sample volumes should be used. The average collection efficiency for phthalic anhydride aerosol is 96% when sampled for 70 minutes at 1.5 liters per minute from test atmospheres containing 17-50 mg/cu m.

3. Interference

3.1 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.

3.2 It must be emphasized that phthalic acid or any other compound which has the same retention time as the analyte at the operating conditions described in this method is an interference. Retention time data based on a single set of conditions cannot be considered as 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 5.5-28 mg/cu m was 0.089. This value corresponds to a standard deviation of 1.1 mg/cu m at the OSHA standard level. Statistical information and details of the validation and experimental test procedures can be found in Reference 11.1.

4.2 A collection efficiency of 95.6% was determined for the collecting medium; thus, no significant bias was introduced in the sample collection step. Likewise, there was no bias in the analytical method - the average recovery for the filters was 98.9%. Thus, $\overline{CV_T}$ is a satisfactory measure of both accuracy and precision of the sampling and analytical method.

5. Advantages and Disadvantages of the Method

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

6. Apparatus

6.1 Sampling equipment - The sampling unit for the collection of personal air samples for the determination of organic aerosol has the following components:

6.1.1 The filter unit consisting of the filter media (Section 6.2) and appropriate 37 mm 3-piece cassette filter holder.

6.1.2 Personal Sampling Pump: A calibrated personal sampling pump whose flow can be determined to an accuracy of $\pm$5% at the recommended flow rate. The pump must be calibrated with a representative filter holder and filter in the line.

6.2 Mixed cellulose ester membrane filter, 0.8 micrometer pore size and 37 mm diameter. The filter is held in the three piece cassette by a cellulose backup pad.

6.3 High Pressure Liquid Chromatograph equipped with a 254 nm UV detector.

6.4 Column (25 cm x 4.6 mm I.D. stainless steel) packed with Partisil^{tm} 10.

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

6.6 Jars for sample extraction: 2 oz. ointment jars, squat form with aluminum-lined screw caps.

6.7 Microliter syringes: 10-microliter and other convenient sizes for making standard solution.

6.8 Volumetric flasks: 10-milliliter and other convenient sizes for making standard solutions and sample dilutions.

6.9 Pipets: 1,5-milliliter and other convenient sizes for making standard solutions and sample dilutions.

7. Reagents

7.1 Phthalic anhydride, reagent grade.

7.2 Ethylacetate, Spectroquality.

7.3 Methanol, Spectroquality.

7.4 Glacial Acetic acid.

7.5 Acetic acid in methanol, 10%. Prepare by diluting 10 ml of glacial acetic acid to 100 ml with methanol.

7.6 Ammonium hydroxide, 0.2 N. Add 14 ml of concentrated ammonia (15 N) to distilled water and dilute to 1.0 liter.

7.7 Chromatographic Solvent System. Mix together glacial acetic acid, methanol, and methylene chloride in the proportion - 10 ml, 20 ml and 220 ml, respectively.

7.8 Acetone, Spectroquality.

7.9 Isopropyl alcohol, Spectroquality.

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.

8.2 Calibration of personal pumps. Each personal pump must be calibrated with a representative filter cassette in the line. This will minimize errors associated with uncertainties in the sample volume collected.

8.3 Collection and shipping of samples.

8.3.1 Assemble the filter in the three-piece filter cassette holder and close firmly to insure that the center ring seals the edge of the filter. The cellulose membrane filter is held in place by a cellulose backup pad and the filter holder is held together by plastic tape or a shrinkable cellulose band. If the middle piece of the filter holder does not fit snugly into the bottom piece of the filter holder, sample leakage will occur around the filter. A piece of flexible tubing is used to connect the filter holder to the pump.

8.3.2 Clip the cassette to the worker's lapel. Air being sampled should not be passed through any hose or tubing before entering the filter cassette.

8.3.3 A sample size of 100 liters is recommended. Sample at a flow rate of 1.5 liter per minute. The flow rate should be known with an accuracy of at least $\pm$5%.

8.3.4 Turn the pump on and begin sample collection. 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 observed frequently, and the sampling should be terminated at any evidence of a problem.

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

8.3.6 Collected sample cassette should be firmly sealed with the plugs in both the inlet and outlet ends.

8.3.7 Carefully record sample identity and all relevant sample data.

8.3.8 Blank. With each batch of ten samples submit one filter from the same lot of filters which was used for sample collection and which is subjected to exactly the same handling as for the samples except that no air is drawn through it. Label this as a blank.

8.3.9 Shipping. The filter cassettes should be shipped in a suitable container, designed to prevent damage in transit.

8.4 Analysis of Samples

8.4.1 Preparation and Extraction of Samples

1. Open the cassette filter holder. Carefully remove the cellulose membrane filter from the holder and cellulose backup pad with the aid of Millipore filter tweezers and transfer filter to the jar.

2. Pipet 5-ml of 0.2 N ammonium hydroxide into the jar and cap unit. Gently swirl the jar to ensure that the filter is thoroughly wetted. Let stand for at least one hour, with occassional agitation, to effect complete hydrolysis and extraction.

3. Transfer, with a pipet, 1.0 ml of the above extract into a 10-ml volumetric flask. Acidify with 2 ml of the 10% acetic acid/methanol solution and dilute to volume with ethylacetate. It should be noted that this solution of phthalic acid is not stable beyond a normal working day and should accordingly be prepared as needed.

8.4.2 Analysis by high pressure liquid chromatograph. The chromatographic solvent system used is described in Section 7.7. The typical operating conditions for the liquid chromatograph are:

1. 0.8 ml/min solvent flow rate

2. Ambient column temperature

3. 500-800 psi system pressure

8.4.3 Injection. The first step in the analysis is the injection of the sample into the liquid chromatograph. A 20 µl-sample aliquot is recommended for this analysis. The sample may be injected either by using a high pressure 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.

8.4.4 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 Sample 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 should be determined in duplicate and should cover the concentration ranges of interest. If the recovery is less than 95%, the appropriate correction factor should be used to calculate the "true" value.

8.5.2 Procedure for determining recovery. A known amount of the analyte, preferably equivalent to the sample concentration expected, is added to a representative cellulose membrane filter and air-dried. The analyte is then extracted from the filter and analyzed as described in Section 8.4. Duplicate determinations should agree within ±5%.

The spiking solution* is prepared as follows. Transfer 0.3400 g of phthalic anhydride into a 25 ml volumetric. Add 2.5 ml of acetone to dissolve analyte and gradually add isopropyl alcohol. Phthalic anhydride might initially crystallize out during the isopropyl alcohol addition but the crystals will readily go into solution upon addition of more alcohol and ultrasonic agitation. Dilute to volume with isopropyl alcohol. An amount of the analyte equivalent to that present in a 100-liter sample at the selected level should be used for the extraction studies. Six filters at each of the three levels (0.5X, 1X and 2X the OSHA standard) are spiked. A parallel blank filter is also prepared except that no sample is added to it. All filters are then extracted and analyzed as described in Section 8.4. The recovery values obtained in validating this method were at least 99% and as such no correction factor has been used in the determination of the "true" values.

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

$$\text{Recovery} = \frac{\text{Average Weight (µg) recovered}}{\text{Weight (µg) added}}$$

*The spiking solution is not stable. The phthalic anhydride is gradually converted to the half-acid/half-ester by the isopropyl alcohol. Unfortunately, no other solvent system was found which satisfies the requirements for phthalic anhydride solubility and cellulose membrane filter compatibility. Limited studies indicate that the anhydride content of this solution decreases to 95% within the first hour and 90% by the second hour and must therefore be used within the first hour to minimize errors.

9. Calibration and Standards

9.1 Phthalic anhydride stock solution*, 10 µg/µl. Dissolve 1.000 g of phthalic anhydride in 100 ml of ethylacetate. This solution is stable for at least one week.

9.2 From the 10 µg/µl stock standard solution, prepare at least 6 working standards to cover the concentration range of 100-600 µg/10 ml. Transfer 10 to 60 µl aliquots of the stock standard into 10-ml volumetric flasks and treat with 1.0 ml of 0.2 N ammonium hydroxide solution. Let stand for at least 15 minutes, acidify with 2 ml of 10% acetic acid/methanol, and dilute to volume with ethylacetate.

9.3 These series of standards are analyzed under the same HPLC conditions and during the same time period as the unknown samples. Curves are established by plotting concentration in micrograms per 10.0 ml versus peak area.

NOTE: To minimize effect of variations in LC conditions and detector response due to sample cell conditions, frequent standardization should be practiced.

10. Calculations

10.1 Read the concentration, in µg/10 ml, corresponding to the peak area from the standard curve. No volume corrections for sample aliquots analyzed are needed, because the standard curve is based on µg per 10.0 ml and the volume of sample injected is identical to the volume of the standards injected. However, this concentration found must be multiplied by the dilution factor 5 to obtain the total µg found.

10.2 Corrections 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 filter
µg blank = µg found in blank filter

10.3 Divide the total weight by the recovery to obtain the corrected µg/sample

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

*This solution should not be used for spiking because the ethylacetate attacks the filter leading to very low recoveries of anhydride.

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

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

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

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

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
MW = molecular weight
760 = standard pressure (mm Hg)
298 = standard temperature (°K)

11. Reference

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