

## Quinone

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Analyte:	Quinone	Method No:	S181
Matrix	Air	Range:	0.17-0.75 mg/cu m
OSHA Standard:	0.4 mg/cu m	Precision: ( $\overline{CV}_T$ ):	0.085
Procedure:	Adsorption on XAD-2, desorption with ethanol/hexane, analysis by HPLC	Validation Date:	9/30/77

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### 1. Principle of the Method

- 1.1 A know volume of air is drawn through a tube containing XAD-2 resin to trap the organic vapors present. The sampling tube consists of a front adsorbing section and a backup section.
- 1.2 The XAD-2 in each tube is transferred to a vial and the quinone is desorbed with a solution of 20% ethanol in hexane and analyzed by high pressure liquid chromatography.

### 2. Range and Sensitivity

- 2.1 This method was validated over the range of 0.17-0.75 mg/cu m at an atmospheric temperature of 25°C and atmospheric pressure of 767 mm Hg using a 24-liter sample volume. This sample volume is less than two-thirds of the 5% breakthrough capacity determined at 81% relative humidity when sampling a test atmosphere at 2 times the OSHA standard. This method is capable of measuring much smaller amounts if the desorption efficiency is adequate. Desorption efficiency must be determined over the range used.
- 2.2 The detection limit of this method is estimated to be at most 0.4 nanograms of quinone based on an injection volume of 20  $\mu$ l.

### 3. Interferences

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

3.3 If the possibility of interference exists, separation conditions (column packing, solvent composition, etc.) must be changed to solve the problem.

#### 4. Precision and Accuracy

4.1 The Coefficient of Variation ( $\overline{CV}_T$ ) for the total analytical and sampling method in the range of 0.17-0.75 mg/cu m was 0.0847. This value corresponds to a 0.0339 mg/cu m standard deviation at the OSHA standard level. Statistical information and details of the validation and experimental test procedures can be found in References 11.1 and 11.2.

4.2 On the average, the concentrations "found" at the OSHA standard level using the overall sampling and analytical method were 1.1% higher than the "true" concentrations found for a limited number of samples analyzed by an alternate method (Reference 11.2). Any difference between the two concentrations does not represent a bias in the sampling and analytical method, but rather a random variation from the experimentally determined "true" concentration. Therefore, no recovery correction should be applied to the final result.

#### 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 can be eliminated by altering chromatographic conditions. The tubes are analyzed by means of a quick, instrumental method.

5.2 All samples must be analyzed within one hour of desorption and interspersed among standard solutions prepared within an hour. This limits the number of samples which can be analyzed at once, but is necessary since the solutions are unstable and may not give reliable results after an hour.

5.3 One disadvantage of the method is that the amount of sample which can be taken is limited by the number of micrograms that the tube will hold before overloading. When an atmosphere at 81% relative humidity containing 0.794 mg/cu m of quinone was sampled at 0.2 liter per minute, 1.6% breakthrough was observed after 240 minutes (capacity is at least 45 liters or 37  $\mu$ g). The sample size recommended is less than the 5% breakthrough capacity at 81% R.H. for a test atmosphere at 2 times the OSHA standard to minimize the probability of overloading the sampling tube.

5.4 When the sample value obtained for the backup section of the sorbent tube exceeds 25% of that found on the front section, the possibility of sample loss exists.

- 5.5 The precision of the method is affected 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.

## 6. Apparatus

### 6.1 Sampling Apparatus

- 6.1.1 A calibrated personal sampling pump whose flow can be determined within  $\pm 5\%$  at the recommended flow rate. (Reference 11.3).
- 6.1.2 Sampling Tube. Glass tube with both ends flame-sealed, 10-cm long with 6-mm O.D. and 4-mm I.D., containing 2 sections of 20/50 mesh XAD-2 resin. The adsorbing section contains 100 mg of resin, the backup section 50 mg. A small wad of silylated glass wool is placed between the front adsorbing section and the backup section; a plug of silylated glass wool is also placed in front of the adsorbing section and at the end of the backup section. Since the pressure drop across the tube must be less than 25 mm of mercury at a flow rate of 1 liter per minute, it is necessary to avoid overpacking with glass wool.
- 6.1.3 Barometer .
- 6.1.4 Thermometer,
- 6.1.5 Stopwatch,
- 6.2 High pressure liquid chromatograph. The unit must be capable of UV detection at 240 nm.
- 6.3 Column, 25-cm x 4.6-mm I.D. x 1/4" stainless steel Partisil<sup>tm</sup> PXS 10/25 ODS or equivalent.
- 6.4 An electronic integrator or some other suitable method for measuring peak areas.
- 6.5 Twelve-milliliter screw cap vials with Teflon-lined caps.
- 6.6 Microliter syringes, 10, 25, 50, 100 and 250-microliter and other convenient sizes for preparing standards.
- 6.7 Pipet, 5-ml, delivery type.
- 6.8 Volumetric flasks, 25-ml or convenient sizes for making standard solutions.

## 7. Reagents

- 7.1 Ethanol, absolute.
- 7.2 Hexane, chromatographic quality, distilled in glass.
- 7.3 Quinone, reagent grade.
- 7.4 Twenty percent ethanol in hexane. Prepare by diluting 200 ml of absolute ethanol to 1000 ml with hexane. This solvent is used for making standard solutions and as a desorption solvent. It is also the mobile phase for the HPLC analysis and is degassed for such use.
- 7.5 Pre-cleaned resin: XAD-2 resin (20-50 mesh) can be obtained from the Rohm and Haas Company. XAD-2 resin is purified by charging an amount into a Soxhlet extractor. Larger batches may be prepared by using a large size Soxhlet extractor. Overnight (24 hours) extractions are then performed successively with water, methanol, diethylether and n-pentane. Finally, several washings with 20% ethanol in hexane are recommended to reduce possible interferences to a minimum when the sorbent is desorbed with this solvent. Distilled-in-glass solvents are used in all cases. Resin has been prepared in this manner using charges of about 700 grams of resin and 1.5 liters of each solvent. The resin is dried in a fluidized bed process using nitrogen gas at room temperature from a liquid nitrogen cylinder. The drying process is terminated when no organics are detected experimentally in the effluent. A final quality control check is performed by desorbing a portion of the resin and analyzing the resulting solution by gas chromatography. Residual organics should be less than 1000 ppm in concentration.

## 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 Sampling Pumps. Each personal sampling pump must be calibrated with a representative sampling tube series in the line; the tube is described in Section 6.1.2. This will minimize the errors associated with uncertainties in the sample volume collected.
- 8.3 Collection and Shipping of Samples
  - 8.3.1 Immediately before sampling, break the two ends of the resin tube to provide an opening at least one-half the internal diameter of the tube (2-mm).
  - 8.3.2 The section containing 50 mg of resin is used as a backup and should be positioned nearest the sampling pump.

- 8.3.3 The resin tube series should be placed in a vertical direction during sampling to minimize channeling through the resin.
  - 8.3.4 Air being sampled should not be passed through any hose or tubing before entering the resin tube.
  - 8.3.5 A sample size of 24 liters is recommended. Sample at a known flow rate between 0.2 and 0.01 liter per minute for 120 minutes. The flow rate should be known with an accuracy of at least  $\pm 5\%$ .
  - 8.3.6 Record the ambient temperature and pressure. If pressure reading is not available, record the elevation.
  - 8.3.7 The resin tube should be labeled appropriately and capped with the supplied plastic caps. Under no circumstances should rubber caps be used.
  - 8.3.8 With each batch of 10 samples, submit one resin tube which has been handled in the same manner as the sample tubes (break, seal and transport), except that no air is sampled through this tube. This tube should be labeled as a blank.
  - 8.3.9 Capped resin tubes should be packed tightly and padded before they are shipped to minimize tube breakage during shipping.
  - 8.3.10 Arrange to have samples analyzed within seven days.
  - 8.3.11 Minimize exposure to light and refrigerate samples.
- 8.4 Analysis of Samples
- 8.4.1 Preparation of Samples. In preparation for analysis, each resin tube is scored with a file in front of the first section of resin and broken open. The glass wool is removed and discarded. The resin in the front 100-mg section is transferred to a 12-ml vial. The separating section of glass wool is removed and discarded. The second 50-mg section is transferred to another vial. These two sections are analyzed separately.
  - 8.4.2 Desorption of Sample. Prior to analysis 5.0 ml of 20% ethanol in hexane is pipetted into each 12-ml vial. The vial is capped immediately after solvent addition and then agitated. Since all samples must be analyzed within an hour of desorption it is extremely important to desorb only as many samples as can be analyzed within that period of time. The number of standards to be analyzed simultaneously must be taken into account as well. All samples may be analyzed as soon as desorbed. Tests have shown this is

a sufficient time for desorption provided the samples are shaken after solvent addition.

8.4.3 HPLC Conditions. The mobile phase is 20% ethanol in hexane. Typical operating conditions for the chromatograph are:

1. 2 ml/min solvent flow
2. Ambient column temperature
3. 200-300 psi system pressure
4. 240 nm UV detection wavelength

A retention time of approximately 2.2 minutes is to be expected for the analyte using these conditions and the column recommended in Section 6.3.

8.4.4 A 20-microliter aliquot of the sample solution is injected into the liquid chromatograph. The sample may be injected directly by syringe or fixed volume sample loop provided that duplicate injections of a solution agree well. No more than a 3% difference in area is to be expected. Desorption is done in a 5-ml sample volume to provide an adequate volume for rinsing the syringe between injections of different solutions.

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 the results are read from a standard curve prepared as discussed in Section 9. Peak heights were found to be an unacceptable method of response measurement.

## 8.5 Determination of Desorption Efficiency

8.5.1 Importance of Determination. The desorption efficiency of a particular compound can vary from one laboratory to another and also from one batch of XAD-2 to another. Thus, it is necessary to determine the percentage of the specific compound that is removed in the desorption process for the particular batch of resin used for sample collection and over the concentration range of interest. The desorption efficiency must be at least 75% at a sample loading equivalent to the OSHA standard level.

8.5.2 Preparation of Analytical Samples for Desorption Efficiency Determination. The desorption efficiency must be determined over the sample concentration range of interest. In order to determine the sample concentration range which should be tested, the samples are analyzed first and then the analytical samples are prepared based on the relative amount of quinone found in the samples. The desorption efficiency must be determined at least in duplicate for each concentration level of quinone found in the samples analyzed.

The analytical samples are prepared as follows: XAD-2, equivalent to the amount in the front section (100 mg), is measured into a 12-ml vial. This resin must be from the same batch as that used in obtaining the samples.

A known amount of a solution of quinone in 20% ethanol in hexane (spiking solution) is injected directly into the resin by means of a microliter syringe. Adjust the concentration of the spiking solution such that no more than a 10- $\mu$ l aliquot is used to prepare the analytical samples.

Note: An ultrasonic cleaner may be needed to break up large particles of quinone.

For the validation studies conducted to determine the precision and accuracy of this method, six analytical samples at each of the three concentration levels (0.5, 1 and 2 times the OSHA standard) were prepared by adding an amount of quinone equivalent to that present in a 24-liter sample at the selected level. A stock solution containing 4.95 milligrams of quinone per milliliter of 20% ethanol in hexane was prepared. One, 2 and 4-microliter aliquots of the solution were added to the XAD-2 resin tubes to produce samples equivalent to 24-liter collections at 0.5, 1 and 2 times the OSHA standard level. The analytical samples were allowed to stand at least overnight to assure complete adsorption of the analyte onto the resin. A parallel blank tube was treated in the same manner except that no sample was added to it.

The procedure described can be used to prepare the analytical samples which are analyzed to determine the desorption efficiency over the concentration range of interest.

- 8.5.3 Desorption and analysis experiments are done on the analytical samples as described in Sections 8.4.2 to 8.4.5. Calibration standards are prepared by adding the appropriate volume of spiking solution to 5.0 ml of 20% ethanol in hexane with the same syringe used in the preparation of the samples. Standards should be prepared at the same time that the sample analysis is done and should be analyzed with the samples.

The desorption efficiency (D.E.) equals the average weight in  $\mu$ g recovered from the tube divided by the weight in  $\mu$ g added to the tube or

$$D.E. = \frac{\text{Average Weight } (\mu\text{g}) \text{ Recovered} - \text{Blank } (\mu\text{g})}{\text{Weight } (\mu\text{g}) \text{ Added}}$$

The desorption efficiency may be dependent on the amount of quinone collected on the resin. Plot the desorption efficiency versus weight of quinone found. This curve is

used in Section 10.3 to correct for adsorption losses.

## 9. Calibration and Standards

- 9.1 Add 5.0 ml of 20% ethanol in hexane to a 12-ml vial. Add aliquots of the same solution as described in Section 8.5.2 to prepare calibration standards or alternatively aliquots of the same solution could be diluted to the appropriate volume. The concentration of standards can be expressed in terms of  $\mu\text{g}$  of quinone per 5 ml of 20% ethanol in hexane.
- 9.2 A series of standards, varying in concentration over the range of interest, is prepared as described above and analyzed under the same chromatographic conditions and during the same time period as the unknown samples. Curves are established by plotting peak area (ordinate) against sample concentration in  $\mu\text{g}/5\text{ ml}$ .

It has been determined that solutions of quinone in 20% ethanol in hexane are unstable. To insure reliable results it is imperative that all solutions be analyzed within an hour of desorption or preparation. In addition, to minimize the effect of variation in detector response, standard solutions should be analyzed at the same time as sample solutions.

## 10. Calculations

- 10.1 Read the weight in units of  $\mu\text{g}$  of quinone corresponding to each peak area from the standard curve. No volume corrections are needed, because the standard curve is based on  $\mu\text{g}$  per 5.0 ml and the volume of sample injected is identical to the volume of the standards injected.

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

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

where:

$$\mu\text{g sample} = \mu\text{g found in front (100-mg) sample section}$$

$$\mu\text{g blank} = \mu\text{g found in front (100-mg) blank section}$$

A similar procedure is followed for the backup (50-mg) section.

- 10.3 Read the desorption efficiency from the curve (see Section 8.5.3) for the amount found in the front section of the tube.

Divide the total weight by this desorption efficiency to obtain the corrected  $\mu\text{g}/\text{sample}$ .

$$\text{Corrected } \mu\text{g/sample} = \frac{\text{Weight (Front Section)}}{\text{D.E.}}$$

- 10.4 Add the amounts present in the front and backup sections for the same sample to determine the total weight in the sample.
- 10.5 Determine the volume of air sampled at ambient conditions in liters based on the appropriate information, such as flow rate in liters per minute multiplied by sampling time. If a pump using a rotameter for flow rate control was used for sample collection, a pressure and temperature correction must be made for the indicated flow rate. The expression for this correction is:

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

where:

f = sample flow rate

t = sampling time

P<sub>1</sub> = atmospheric pressure during calibration of sampling pump (mm Hg)

P<sub>2</sub> = atmospheric pressure of air during sampling (mm Hg)

T<sub>1</sub> = ambient temperature during calibration of sampling pump (°K)

T<sub>2</sub> = ambient temperature of air sampled (°K)

- 10.6 The concentration of quinone in the air sampled can be expressed in mg per cu m which is numerically equal to  $\mu\text{g}$  per liter

$$\text{mg/cu m} = \frac{\text{Corrected } \mu\text{g (see Section 10.4)}}{\text{Sampling Volume (liters)}}$$

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

$$\text{ppm} = \text{mg/cu m} \times \frac{24.45}{108.1} \times \frac{760}{P} \times \frac{(T + 273)}{298}$$

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  
108.1 = molecular weight of quinone  
760 = standard pressure (mm Hg)  
298 = standard temperature (°K)

## 11. References

- 11.1 Memoranda, Kenneth A. Busch, Chief, Statistical Services, DLCD, to Deputy Director, DLCD, dated 1/16/75, 11/8/74, subject: "Statistical Protocol for Analysis of Data from Contract CDC-99-74-45."
- 11.2 Backup Data Report for Quinone, No. S181, prepared under NIOSH Contract No. 210-76-0123.
- 11.3 Final Report, NIOSH Contract HSM-99-71-31, "Personal Sampler Pump for Charcoal Tubes," September 15, 1972.