

METHOD TO-8

METHOD FOR THE DETERMINATION OF PHENOL  
AND METHYLPHENOLS (CRESOLS) IN AMBIENT AIR USING  
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

1. Scope

- 1.1 This document describes a method for determination of phenol and methylphenols (cresols) in ambient air. With careful attention to reagent purity and other factors, the method can detect these compounds at the 1-5 ppbv level.
- 1.2 The method as written has not been rigorously evaluated. The approach is a composite of several existing methods (1-3). The choice of HPLC detection system will be dependent on the requirements of the individual user. However, the UV detection procedure is considered to be most generally useful for relatively clean samples.

2. Applicable Documents

- 2.1 ASTM Standards  
D1356 - Definitions of Terms Related to Atmospheric Sampling and Analysis(4).
- 2.2 Other Documents  
U.S. EPA Technical Assistance Document (5).

3. Summary of Method

- 3.1 Ambient air is drawn through two midget impingers, each containing 15 mL of 0.1 N NaOH. The phenols are trapped as phenolates.
- 3.2 The impinger solutions are placed in a vial with a Teflon<sup>®</sup>- lined screw cap and returned to the laboratory for analysis. The solution is cooled in an ice bath and adjusted to pH <4 by addition of 1 mL of 5% v/v sulfuric acid. The sample is adjusted to a final volume of 25 mL with distilled water.
- 3.3 The phenols are determined using reverse-phase HPLC with either ultraviolet (UV) absorption detection at 274 nm, electrochemical detection, or fluorescence detection. In general, the UV detection approach should be used for relatively clean samples.

4. Significance

4.1 Phenols are emitted into the atmosphere from chemical operations and various combustion sources. Many of these compounds are acutely toxic, and their determination in ambient air is required in order to assess human health impacts.

4.2 Conventional methods for phenols have generally employed colorimetric or gas chromatographic techniques with relatively large detection limits. The method described here reduces the detection limit through use of HPLC.

5. Definitions

Definitions used in this document and in any user-prepared Standard Operating Procedures (SOPs) should be consistent with ASTM D1356 (5). All abbreviations and symbols are defined within this document at the point of use.

6. Interferences

6.1 Compounds having the same retention times as the compounds of interest will interfere in the method. Such interferences can often be overcome by altering the separation conditions (e.g., using alternative HPLC columns or mobile phase compositions) or detectors. Additionally, the phenolic compounds of interest in this method may be oxidized during sampling. Validation experiments may be required to show that the four target compounds are not substantially degraded.

6.2 If interferences are suspected in a "dirty" sample, preliminary cleanup steps may be required to identify interfering compounds by recording infrared spectrophotometry followed by specific ion-exchange column chromatography. Likewise, overlapping HPLC peaks may be resolved by increasing/decreasing component concentration of the mobile phase.

6.3 All reagents must be checked for contamination before use.

7. Apparatus

7.1 Isocratic HPLC system consisting of a mobile-phase reservoir, a high-pressure pump, an injection valve, a Zorbax ODS or C-18 reverse-phase column, or equivalent (25 cm x 4.6 mm ID), a variable-wavelength UV detector operating at 274 nm, and a data

- system or strip-chart recorder (Figure 1). Amperometric (electrochemical) or fluorescence detectors may also be employed.
- 7.2 Sampling system - capable of accurately and precisely sampling 100-1000 mL/minute of ambient air (Figure 2).
  - 7.3 Stopwatch.
  - 7.4 Friction-top metal can, e.g., one-gallon (paint can) - to hold samples.
  - 7.5 Thermometer - to record ambient temperature.
  - 7.6 Barometer (optional).
  - 7.7 Analytical balance - 0.1 mg sensitivity.
  - 7.8 Midget impingers - jet inlet type, 25-mL.
  - 7.9 Suction filtration apparatus - for filtering HPLC mobile phase.
  - 7.10 Volumetric flasks - 100 mL and 500 mL.
  - 7.11 Pipettes - various sizes, 1-10 mL.
  - 7.12 Helium purge line (optional) - for degassing HPLC mobile phase.
  - 7.13 Erlenmeyer flask, 1 L - for preparing HPLC mobile phase.
  - 7.14 Graduated cylinder, 1 L - for preparing HPLC mobile phase.
  - 7.15 Microliter syringe, 100-250 uL - for HPLC injection.
8. Reagents and Materials
- 8.1 Bottles, 10 oz, glass, with Teflon<sup>®</sup>-lined screw cap - for storing sampling reagent.
  - 8.2 Vials, 25 mL, with Teflon<sup>®</sup>-lined screw cap - for holding samples.
  - 8.3 Disposable pipettes and bulbs.
  - 8.4 Granular charcoal.
  - 8.5 Methanol - distilled in glass or pesticide grade.
  - 8.6 Sodium hydroxide - analytical reagent grade.
  - 8.7 Sulfuric acid - analytical reagent grade.
  - 8.8 Reagent water - purified by ion exchange and carbon filtration, or distillation.
  - 8.9 Polyester filters, 0.22 um - Nuclepore, or equivalent.
  - 8.10 Phenol, 2-methyl-, 3-methyl-, and 4-methylphenol - neat standards (99+ % purity) for instrument calibration.
  - 8.11 Sampling reagent, 0.1 N NaOH. Dissolve 4.0 grams of NaOH in reagent water and dilute to a final volume of 1L. Store in a glass bottle with Teflon<sup>®</sup>-lined cap.
  - 8.12 Sulfuric acid, 5% v/v. Slowly add 5 mL of concentrated sulfuric acid to 95 mL of reagent water.

- 8.13 Acetate buffer, 0.1M, pH 4.8 - Dissolve 5.8 mL of glacial acetic acid and 13.6 grams of sodium acetate trihydrate in 1 L of reagent water.
- 8.14 Acetonitrile - spectroscopic grade.
- 8.15 Glacial acetic acid - analytical reagent grade.
- 8.16 Sodium acetate trihydrate - analytical reagent grade.

9. Sampling

- 9.1 The sampling apparatus is assembled and should be similar to that shown in Figure 2. EPA Federal Reference Method 6 uses essentially the same sampling system (6). All glassware (e.g., impingers, sampling bottles, etc.) must be thoroughly rinsed with methanol and oven-dried before use.
- 9.2 Before sample collection, the entire assembly (including empty sample impingers) is installed and the flow rate checked at a value near the desired rate. In general, flow rates of 100-1000 mL/minute are useful. Flow rates greater than 1000 mL/minute should not be used because impinger collection efficiency may decrease. Generally, calibration is accomplished using a soap bubble flow meter or calibrated wet test meter connected to the flow exit, assuming the entire system is sealed. ASTM Method D3686 describes an appropriate calibration scheme that does not require a sealed-flow system downstream of the pump (4).
- 9.3 Ideally, a dry gas meter is included in the system to record total flow, if the flow rate is sufficient for its use. If a dry gas meter is not available, the operator must measure and record the sampling flow rate at the beginning and end of the sampling period to determine sample volume. If the sampling time exceeds two hours, the flow rate should be measured at intermediate points during the sampling period. Ideally, a rotameter should be included to allow observation of the flow rate without interruption of the sampling process.
- 9.4 To collect an air sample, two clean midjet impingers are loaded with 15 mL of 0.1 N NaOH each and sample flow is started. The following parameters are recorded on the data sheet (see Figure 3 for an example): date, sampling location, time, ambient temperature, barometric pressure (if available), relative humidity (if available), dry gas meter reading (if appropriate), flow rate, rotameter setting, 0.1 N NaOH reagent batch number, and dry gas meter and pump identification numbers.

- 9.5 The sampler is allowed to operate for the desired period, with periodic recording of the variables listed above. The total volume should not exceed 80 L. The operator must ensure that at least 5 mL of reagent remains in the impinger at the end of the sampling interval. (Note: for high ambient temperatures, lower sampling volumes may be required.)
- 9.6 At the end of the sampling period, the parameters listed in Section 9.4 are recorded and the sample flow is stopped. If a dry gas meter is not used, the flow rate must be checked at the end of the sampling interval. If the flow rates at the beginning and end of the sampling period differ by more than 15%, the sample should be discarded.
- 9.7 Immediately after sampling, the impinger is removed from the sampling system. The contents of the impinger are emptied into a clean 25-mL glass vial with a Teflon<sup>®</sup>-lined screw-cap. The impinger is then rinsed with 5 mL of reagent water and the rinse solution is added to the vial. The vial is then capped, sealed with Teflon<sup>®</sup> tape, and placed in a friction-top can containing 1-2 inches of granular charcoal. The samples are stored in the can and refrigerated until analysis. No degradation has been observed if the time between refrigeration and analysis is less than 48 hours.
- 9.8 If a dry gas meter or equivalent total flow indicator is not used, the average sample flow rate must be calculated according to the following equation:

$$Q_A = \frac{Q_1 + Q_2 + \dots + Q_N}{N}$$

where

$Q_A$	=	average flow rate (mL/minute).
$Q_1, Q_2, \dots, Q_N$	=	flow rates determined at beginning, end, and intermediate points during sampling.
$N$	=	number of points averaged.

9.9 The total flow is then calculated using the following equation:

$$V_m = \frac{(T_2 - T_1) \times Q_A}{1000}$$

where

$V_m$	=	total volume (L) sampled at measured temperature and pressure.
$T_2$	=	stop time.
$T_1$	=	start time.
$T_2 - T_1$	=	total sampling time (minutes).
$Q_A$	=	average flow rate (ml/minute).

9.10 The volume of air sampled is often reported uncorrected for atmospheric conditions (i.e., under ambient conditions). However, the value should be adjusted to standard conditions (25°C and 760 mm pressure) using the following equation:

$$V_s = V_m \times \frac{P_A}{760} \times \frac{298}{273 + T_A}$$

where

$V_s$	=	total sample volume (L) at 25°C and 760 mm Hg pressure.
$V_m$	=	total sample volume (L) under ambient conditions. Calculated as in Section 9.9 or from dry gas meter reading.
$P_A$	=	ambient pressure (mm Hg).
$T_A$	=	ambient temperature (°C).

## 10. Sample Analysis

### 10.1 Sample Preparation

10.1.1 The samples are returned to the laboratory in 25-mL screw-capped vials. The contents of each vial are transferred to a 25-mL volumetric flask. A 1-mL volume of 5% sulfuric acid is added and the final volume is adjusted to 25 mL with reagent water.

10.1.2 The solution is thoroughly mixed and then placed in a 25-ml screw-capped vial for storage (refrigerated) until HPLC analysis.

10.2 HPLC Analysis

10.2.1 The HPLC system is assembled and calibrated as described in Section 11. The operating parameters are as follows:

Column: C-18 RP  
Mobile Phase: 30% acetonitrile/70% acetate buffer solution  
Detector: ultraviolet, operating at 274 nm  
Flow Rate: 0.3 mL/minute

phenol - 9.4 minutes  
o-cresol - 12.5 minutes  
m-cresol - 11.5 minutes  
p-cresol - 11.9 minutes

} Individual

Retention Time:

phenol - 9.4 minutes  
o-cresol - 12.8 minutes  
m/p-cresol - 11.9 minutes

} Combined

Before each analysis, the detector baseline is checked to ensure stable operation.

10.2.2 A 100-uL aliquot of the sample is drawn into a clean HPLC injection syringe. The sample injection loop (50 uL) is loaded and an injection is made. The data system, if available, is activated simultaneously with the injection and the point of injection is marked on the strip-chart recorder.

10.2.3 After approximately one minute, the injection valve is returned to the "load" position and the syringe and valve are flushed with water in preparation for the next sample analysis.

10.2.4 After elusion of the last component of interest, data acquisition is terminated and the component concentrations are calculated as described in Section 12.

10.2.5 Phenols have been successfully separated from cresols utilizing HPLC with the above operating parameters. However, meta- and para-cresols have not been successfully separated. Figure 4 illustrates a typical chromatogram.

10.2.6 After a stable baseline is achieved, the system can be used for further sample analyses as described above.

- 10.2.7 If the concentration of analyte exceeds the linear range of the instrument, the sample should be diluted with mobile phase, or a smaller volume can be injected into the HPLC.
- 10.2.8 If the retention time is not duplicated, as determined by the calibration curve, you may increase or decrease the acetonitrile/water ratio to obtain the correct elution time, as specified in Figure 4. If the elution time is long, increase the ratio; if it is too short, decrease the ratio.

## 11.0 HPLC Assembly and Calibration

- 11.1 The HPLC system is assembled and operated according to Section 10.2.1.
- 11.2 The HPLC mobile phase is prepared by mixing 300 mL of acetonitrile and 750 mL of acetate buffer, pH 4.8. This mixture is filtered through a 0.22- $\mu$ m polyester membrane filter in an all-glass and Teflon<sup>®</sup> suction filtration apparatus. The filtered mobile phase is degassed by purging with helium for 10-15 minutes (100 mL/minute) or by heating to 60°C for 5-10 minutes in an Erlenmeyer flask covered with a watch glass. A constant back pressure restrictor (50 psi) or short length (6-12 inches) of 0.01-inch I.D. Teflon<sup>®</sup> tubing should be placed after the detector to eliminate further mobile phase outgassing.
- 11.3 The mobile phase is placed in the HPLC solvent reservoir and the pump is set at a flow rate of 0.3 mL/minute and allowed to pump for 20-30 minutes before the first analysis. The detector is switched on at least 30 minutes before the first analysis and the detector output is displayed on a strip-chart recorder or similar output device. UV detection at 274 nm is generally preferred. Alternatively, fluorescence detection with 274-nm excitation at 298-nm emission (2), or electrochemical detection at 0.9 volts (glassy carbon electrode versus Ag/AgCl) (3) may be used. Once a stable baseline is achieved, the system is ready for calibration.
- 11.4 Calibration standards are prepared in HPLC mobile phase from the neat materials. Individual stock solutions of 100 mg/L are prepared by dissolving 10 mg of solid derivative in 100 mL of mobile phase. These individual solutions are used to prepare calibration standards containing all of the phenols and cresols of interest at concentrations spanning the range of interest.



- 11.5 Each calibration standard (at least five levels) is analyzed three times and area response is tabulated against mass injected. Figures 5a through 5e illustrate HPLC response to various phenol concentrations (1 mL/minute flow rate). All calibration runs are performed as described for sample analyses in Section 10. Using the UV detector, a linear response range of approximately 0.05 to 10 mg/L should be achieved for 50-uL injection volumes. The results may be used to prepare a calibration curve, as illustrated in Figure 6 for phenols. Linear response is indicated where a correlation coefficient of at least 0.999 for a linear least-squares fit of the data (concentration versus area response) is obtained. The retention times for each analyze should agree within 2%.
- 11.6 Once linear response has been documented, an intermediate concentration standard near the anticipated levels for each component, but at least 10 times the detection limit, should be chosen for daily calibration. The response for the various components should be within 10% day to day. If greater variability is observed, recalibration may be required or a new calibration curve must be developed from fresh standards.
- 11.7 The response for each component in the daily calibration standard is used to calculate a response factor according to the following equation:

$$RF_c = \frac{C_c \times V_I}{R_c}$$

where

- RF<sub>c</sub> = response factor (usually area counts) for the component of interest in nanograms injected/response unit.
- C<sub>c</sub> = concentration (mg/L) of analyte in the daily calibration standard.
- V<sub>I</sub> = volume (uL) of calibration standard injected.
- R<sub>c</sub> = response (area counts) for analyte in the calibration standard.

12. Calculations

12.1 The concentration of each compound is calculated for each sample using the following equation:

$$W_d = RF_c \times R_d \times \frac{V_E}{V_I} \times \frac{V_D}{V_A}$$

where

- $W_d$  = total quantity of analyte (ug) in the sample.
- $RF_c$  = response factor calculated in Section 11.6.
- $R_d$  = response (area counts or other response units) for analyte in sample extract.
- $V_E$  = final volume (ml) of sample extract.
- $V_I$  = volume of extract (uL) injected onto the HPLC system.
- $V_D$  = redilution volume (if sample was rediluted).
- $V_A$  = aliquot used for redilution (if sample was rediluted).

12.2 The concentration of analyte in the original sample is calculated from the following equation:

$$C_A = \frac{W_d}{V_m \text{ (or } V_s)} \times 1000$$

where

- $C_A$  = concentration of analyte (ng/L) in the original sample.
- $W_d$  = total quantity of analyte (ug) in sample.
- $V_m$  = total sample volume (L) under ambient conditions.
- $V_s$  = total sample volume (L) at 25°C and 760 mm Hg.

12.3 The analyte concentrations can be converted to ppbv using the following equation:

$$C_A \text{ (ppbv)} = C_A \text{ (ng/L)} \times \frac{24.4}{MW_A}$$

where

$C_A$  (ng/L) is calculated using  $V_s$ .

$MW_A$  = molecular weight of analyte.

### 13. Performance Criteria and Quality Assurance

This section summarizes required quality assurance (QA) measures and provides guidance concerning performance criteria that should be achieved within each laboratory.

#### 13.1 Standard Operating Procedures (SOPs).

- 13.1.1 Users should generate SOPs describing the following activities in their laboratory: (1) assembly, calibration, and operation of the sampling system, with make and model of equipment used; (2) preparation, purification, storage, and handling of sampling reagent and samples; (3) assembly, calibration, and operation of the HPLC system, with make and model of equipment used; and (4) all aspects of data recording and processing, including lists of computer hardware and software used.
- 13.1.2 SOPs should provide specific stepwise instructions and should be readily available to and understood by the laboratory personnel conducting the work.

#### 13.2 HPLC System Performance

- 13.2.1 The general appearance of the HPLC chromatogram should be similar to that illustrated in Figure 4.
- 13.2.2 The HPLC system efficiency and peak asymmetry factor should be determined in the following manner: A solution of phenol corresponding to at least 20 times the detection limit should be injected with the recorder chart sensitivity and speed set to yield a peak approximately 75% of full scale and 1 cm wide at half height. The peak asymmetry factor is determined as shown in Figure 7, and should be between 0.8 and 1.8.
- 13.2.3 HPLC system efficiency is calculated according to the following equation:

$$N = 5.54 \left( \frac{t_r}{W_{1/2}} \right)^2$$

where

- N = column efficiency (theoretical plates).  
 $t_r$  = retention time (seconds) of analyte.  
 $W_{1/2}$  = width of component peak at half height (seconds).

A column efficiency of >5,000 theoretical plates should be obtained.

13.2.4 Precision of response for replicate HPLC injections should be  $\pm 10\%$  or less, day to day, for calibration standards. Precision of retention times should be  $\pm 2\%$ , on a given day.

### 13.3 Process Blanks

13.3.1 Before use, a 15-mL aliquot of each batch of 0.1 N NaOH reagent should be analyzed as described in Section 10. In general, analyte levels equivalent to  $< 5$  ng/L in an 80-L sample should be achieved.

13.3.2 At least one field blank, or 10% of the field samples, whichever is larger, should be shipped and analyzed with each group of samples. The number of samples within a group and/or time frame should be recorded so that a specified percentage of blanks is obtained for a given number of field samples. The field blank is treated identically to the samples except that no air is drawn through the reagent. The same performance criteria described in Section 13.3.1 should be met for process blanks.

### 13.4 Method Precision and Accuracy

13.4.1 At least one duplicate sample, or 10% of the field samples, whichever is larger, should be collected during each sampling episode. Precision for field replication should be  $\pm 20\%$  or better.

13.4.2 Precision for replicate HPLC injections should be  $\pm 10\%$  or better, day to day, for calibration standards.

13.4.3 At least one spiked sample, or 10% of the field samples, whichever is larger, should be collected. The impinger solution is spiked with a known quantity of the compound of interest, prepared as a dilute water solution. A recovery of  $> 80\%$  should be achieved routinely.

13.4.4 Before initial use of the method, each laboratory should generate triplicate spiked samples at a minimum of three concentration levels, bracketing the range of interest for each compound. Triplicate nonspiked samples must also be processed. Spike recoveries of  $> 80 \pm 10\%$  and blank levels of  $< 5$  ng/L (using an 80-L sampling volume) should be achieved.

REFERENCES

- (1) NIOSH P & CAM Method S330-1, "Phenol," National Institute of Occupational Safety and Health, Methods Manual, Vol. 3, 1978.
- (2) Ogan, K. and, Katz, E., "Liquid Chromatographic Separation of Alkylphenols with Fluorescence and Ultraviolet Detection," Anal. Chem., 53, 160-163 (1981).
- (3) Shoup, R. E., and Mayer, G. S., "Determination of Environmental Phenols by Liquid Chromatography Electrochemistry," Anal. Chem., 54, 1164-1169 (1982).
- (4) Annual Book of ASTM Standards, Part 11.03, "Atmospheric Analysis," American Society for Testing and Materials, Philadelphia, Pennsylvania, 1983.
- (5) Riggins, R. M., "Technical Assistance Document for Sampling and Analysis of Toxic Organic Compounds in Ambient Air," EPA-600/4-83-027, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, 1983.
- (6) "Method 6 Determination of SO<sub>2</sub> Emissions from Stationary Sources," Federal Register, Vol. 42., No. 160, August, 1977.

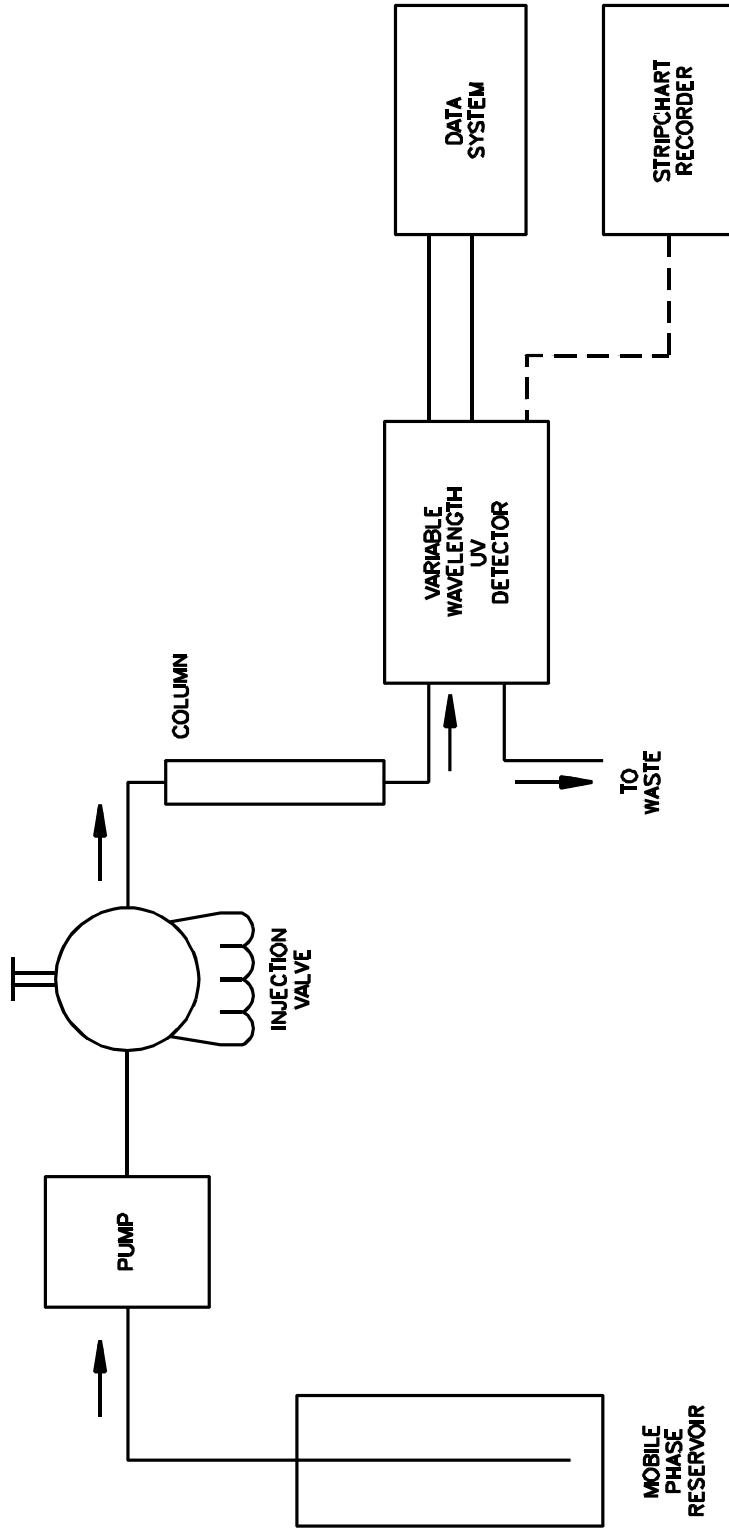


FIGURE 1. TYPICAL HPLC SYSTEM

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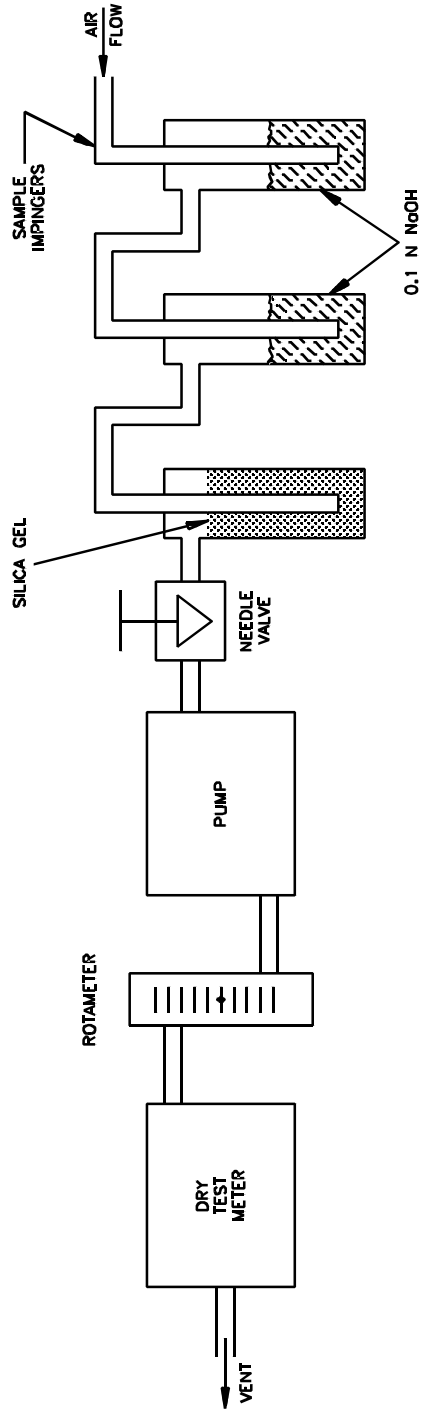


FIGURE 2. TYPICAL SAMPLING SYSTEM FOR MONITORING PHENOLS/CRESOLS IN AMBIENT AIR



SAMPLING DATA SHEET  
(One Sample per Data Sheet)

PROJECT: \_\_\_\_\_ DATES(S) SAMPLED: \_\_\_\_\_  
 SITE: \_\_\_\_\_ TIME PERIOD SAMPLED: \_\_\_\_\_  
 LOCATION: \_\_\_\_\_ OPERATOR: \_\_\_\_\_  
 INSTRUMENT MODEL NO: \_\_\_\_\_ CALIBRATED BY: \_\_\_\_\_  
 PUMP SERIAL NO: \_\_\_\_\_

SAMPLING DATA

Sample Number: \_\_\_\_\_

Start Time: \_\_\_\_\_

Stop Time: \_\_\_\_\_

Time	Dry Gas Meter Reading	Rotameter Reading	Flow Rate, *Q mL/min	Ambient Temperature °C	Barometric Pressure, mm Hg	Relative Humidity, %	Comments
1.							
2.							
3.							
4.							
N.							

Total Volume Data\*\*

$$V_m = (\text{Final} - \text{Initial}) \text{ Dry Gas Meter Reading, or} = \text{_____ L}$$

$$= \frac{Q_1 + Q_2 + Q_3 \dots Q_N}{N} \times \frac{1}{1000 \times (\text{Sampling Time in Minutes})} = \text{_____ L}$$

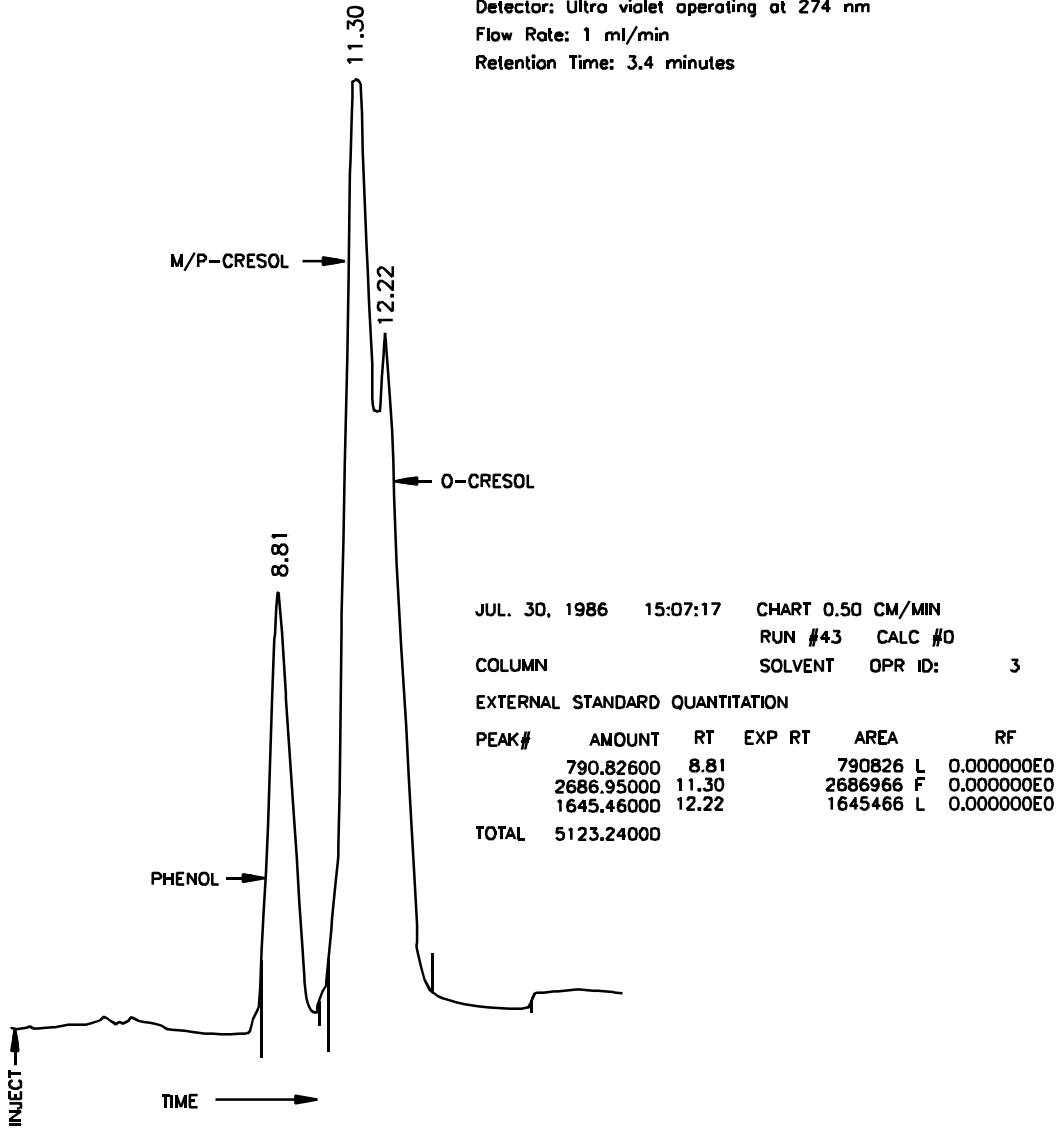
\* Flow rate from rotameter or soap bubble calibrator (specify which).

\*\* Use data from dry gas meter if available.

**FIGURE 3. EXAMPLE SAMPLING DATA SHEET**

**OPERATING PARAMETERS  
HPLC**

Column: C-18 RP  
 Mobile Phase: 30% Acetonitrile/70% Acetate Buffer  
 Detector: Ultra violet operating at 274 nm  
 Flow Rate: 1 ml/min  
 Retention Time: 3.4 minutes



**FIGURE 4. TYPICAL CHROMATOGRAM ILLUSTRATING SEPARATION OF PHENOLS/CRESOLS BY HPLC**

T08-19

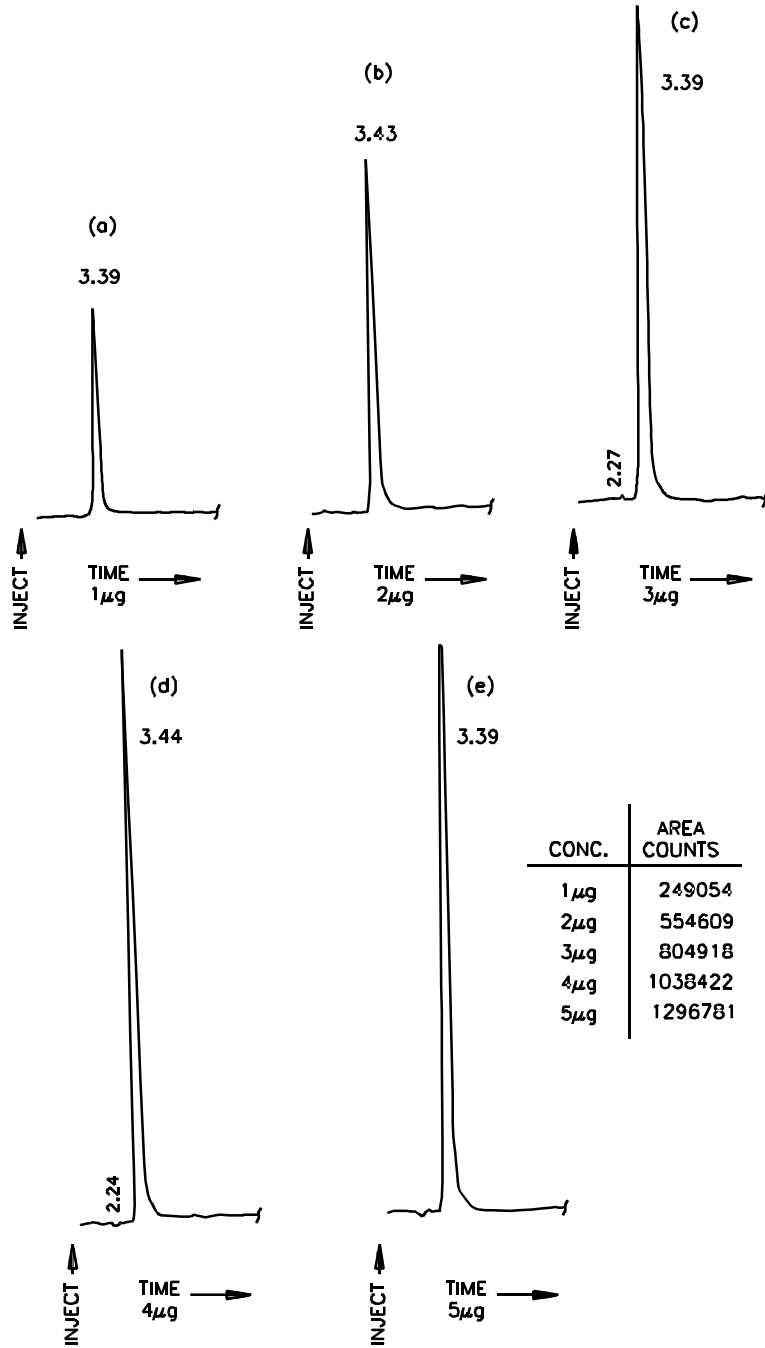


FIGURE 5a-5e. HPLC CHROMATOGRAM OF VARYING PHENOL CONCENTRATIONS

TO8-21

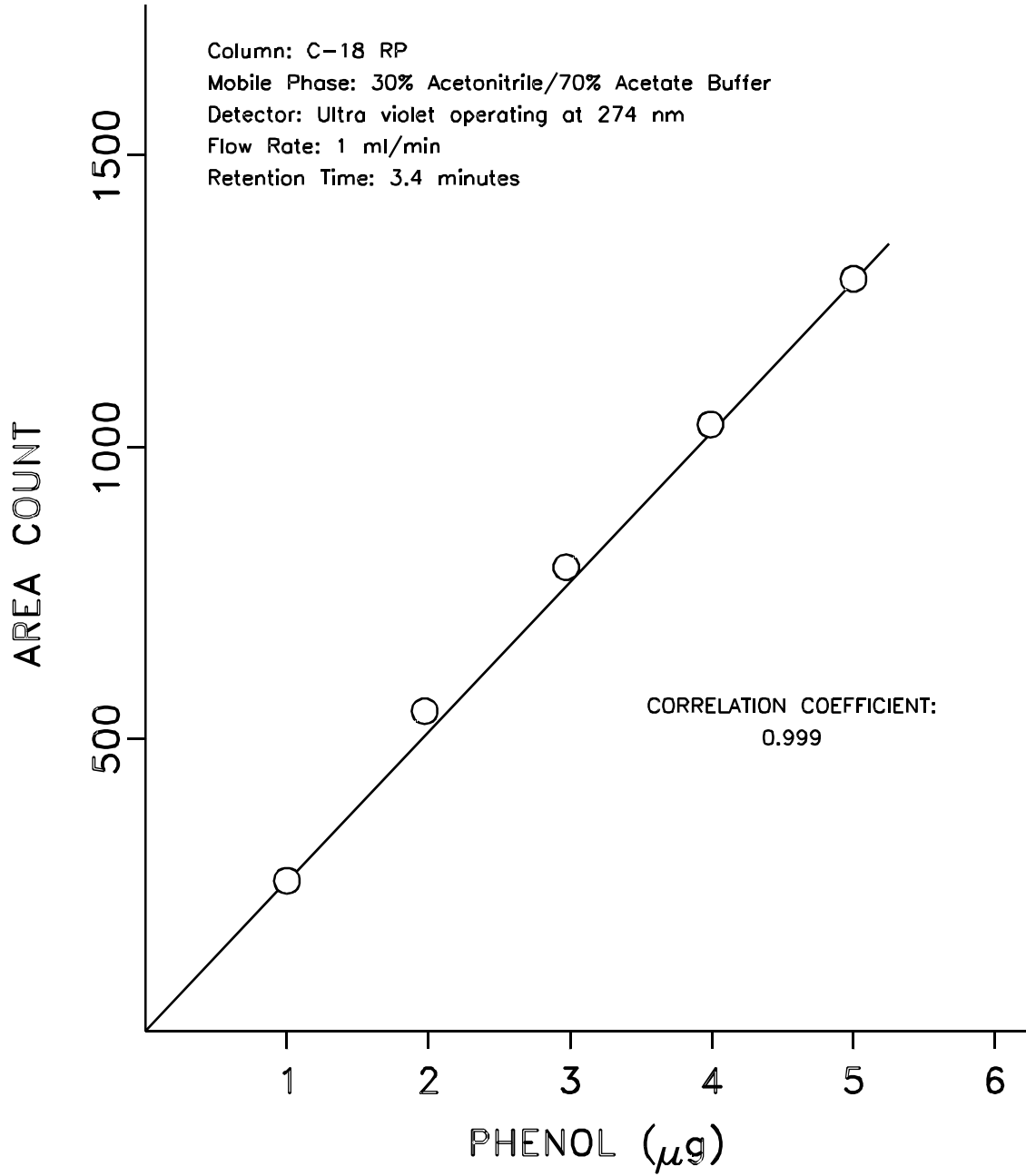
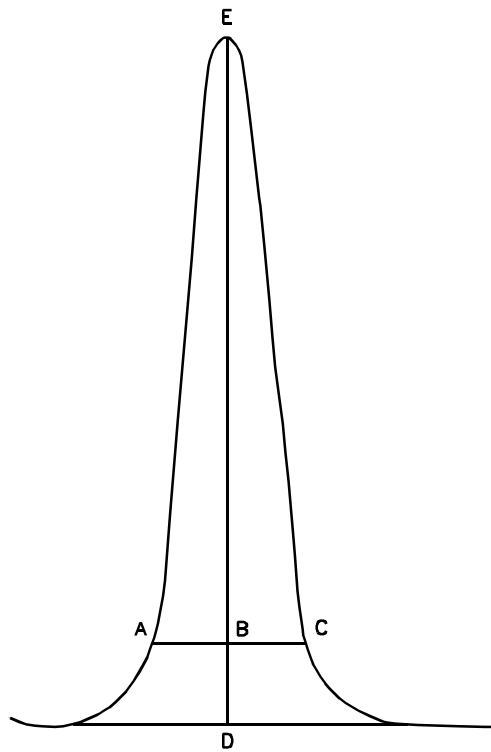


FIGURE 6. CALIBRATION CURVE FOR PHENOL

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$$\text{Asymmetry Factor} = \frac{BC}{AB}$$

Example Calculation:

Peak Height = DE = 100mm

10% Peak Height = BD = 10mm

Peak Width at 10% Peak Height = AC = 23mm

AB = 11mm

BC = 12mm

$$\text{Therefore: Asymmetry Factor} = \frac{12}{11} = 1.1$$

FIGURE 7. PEAK ASYMMETRY CALCULATION



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