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METHOD FOR THE DETERMINATION OF ALDEHYDES AND KETONES IN AMBIENT AIR USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

1. Scope

1.1 This document describes a method for determination of individual aldehydes and ketones in ambient air. With careful attention to reagent purity and other factors the method can detect most monofunctional aldehydes and ketones at the 1-2 ppbv level.

1.2 Specific compounds for which the method has been employed are listed in Table 1. Several studies have used the same basic method, with minor procedural differences, for analysis of ambient air (1-3).

2. Applicable Documents

2.1 ASTM Standards:
D 1356 Definitions of Terms Related to Atmospheric Sampling and Analysis(s)

2.2 Other Documents
Ambient air studies (1-3)
U.S. EPA Technical Assistance Document (4)

3. Summary of Method

3.1 Ambient air is drawn through a midget impinger containing 10 mL of 2N HC1/0.05% 2,4-dinitrophenylhydrazine (DNPH reagent) and 10 mL of isooctane. Aldehydes and ketones readily form stable 2,4-dinitrophenylhydrazones (DNPH derivatives).

3.2 The impinger solution is placed in a screw-capped vial having a teflon-lined cap and returned to the laboratory for analysis. The DNPH derivatives are recovered by removing the isooctane layer, extracting the aqueous layer with 10 mL of 70/30 hexane/methylene chloride, and combining the organic layers.

3.3 The combined organic layers are evaporated to dryness under a stream of nitrogen and the residue dissolved in methanol.

3.4 The DNPH derivatives are determined using reversed phase HPLC with an ultraviolet (UV) adsorption detector operated at 370 nm.

4. Significance

4.1 Aldehydes and ketones are emitted into the atmosphere from chemical operations and various combustion sources. In addition, several of these compounds (e.g., formaldehyde and acetaldehyde) are produced by photochemical degradation of other organic compounds. Many of these compounds are acutely toxic and/or carcinogenic, thus requiring their determination in ambient air in order to assess human health impacts.

4.2 Conventional methods for aldehydes and ketones have generally employed colorimetric techniques wherein only one or two compounds are detected, or the sum of numerous compounds is determined. The method described herein provides a means for specifically determining a wide variety of aldehydes and ketones at typical ambient concentrations.

5. Definitions

Definitions used in this document and any user prepared 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 The only significant interferences in the method are certain isomeric aldehydes or ketones which may be unresolved by the HPLC system. Such interferences can often by overcome by altering the separation conditions (e.g., using alternate HPLC columns or mobile phase compositions).

6.2 Formaldehyde contamination of the DNPH reagent is a frequently encountered problem. The reagent must be prepared within 48 hours before use and must be stored in an uncontaminated environment before and after sampling to minimize blank problems. Acetone contamination is apparently unavoidable.

Consequently, the method cannot be used to accurately measure acetone levels except in highly contaminated environments.

7. Apparatus

7.1 Isocratic HPLC system-consisting of high pressure pump, injection valve, Zorbax ODS column (25 cm x 4.6 mm ID), variable wavelength UV detector, and data system or stripchart recorded. See Figure 3.

7.2 Sampling system-capable of accurately and precisely sampling 100-1000 mL/minute of ambient air. See Figure 1.

7.3 Stopwatch

7.4 Friction top metal can, e.g., one-gallon (paint can) - to hold DNPH reagent and samples

7.5 Thermometer - to record ambient temperature

7.6 Barometer (optional)

7.7 Analytical balance - 0.1 mg sensitivity

7.8 Reciprocating shaker

7.9 Midget impingers - jet inlet type - 25 mL volume

7.10 Ice bath - for cooling impingers during sampling

7.11 Nitrogen evaporator with heating block - for concentrating samples

7.12 Suction filtration apparatus - for filtering HPLC mobile phase.

7.13 Volumetric flasks - 100 mL and 500 mL.

7.14 Pipettes - various sizes, 1-10 mL.

7.15 Helium purge line (optional) - for degassing HPLC mobile phase.

7.16 Erlenmeyer flask, 1-liter - for preparing HPLC mobile phase.

7.17 Graduated cylinder, 1 liter - for preparing HPLC mobile phase.

7.18 Microliter syringe, 10-25 μL - for HPLC injector.

8. Reagents and Materials

8.1 Bottles, 10 oz. glass, with teflon-lined screw cap - for storing DNPH reagent.

8.2 Vials, 50 mL, with teflon-lined screw cap - for holding samples and extracts.

8.3 Disposable pipettes and bulbs.

8.4 Granular charcoal.

8.5 Methanol, hexane, methylene chloride, isooctane - distilled in glass or pesticide grade.

8.6 2,4-Dinitrophenylhydrazine - highest purity available (20% moisture).

8.7 Nitrogen, compressed gas cylinder - 99.99% purity for sample evaporation.

8.8 Polyester filters, 0.22 μm - Nuclepore or equiv.

8.9 DNPH derivatives of the components of interest - synthesized from DNPH and neat aldehydes according to reference (7). Recrystalized from ethanol before use.

9. Preparation of DNPH Reagent

9.1 Each batch of DNPH reagent should be prepared and purified within 48 hours of sampling, according to the procedure described in this section.

9.2 Two hundred and fifty milligrams of solid 2,4-dinitrophenylhydrazine and 90 mL of concentrated hydrochloric acid are placed into a 500 mL volumetric flask and the flask is filled to the mark with reagent water. The flask is then inverted several times or sonified until all of the solid material has dissolved.

9.3 Approximately 400 mL of the DNPH reagent is placed in a 16 ounce glass screw-capped bottle having a teflon-lined cap. Approximately 50 mL of a 70/30 (V/V) hexane/methylene chloride mixture is added to the bottle and the capped bottle is shaken for 15 minutes on a reciprocating shaker. The organic

layer is then removed and discarded by decanting as much as possible and using a disposable pipette to remove the remaining organic layer.

9.4 The DNPH reagent is extracted two more times as described in 9.3. The bottle is then tightly capped, sealed with teflon tape, and placed in a friction top can (paint can) containing a 1-2 inch layer of granulated charcoal. The bottle is kept in the sealed can prior to use.

9.5 A portion of the DNPH reagent is analyzed using the procedure described in Section 11 prior to use in order to ensure that adequate background levels are maintained.

10. Sampling

10.1 The sampling apparatus is assembled and should be similar to that shown in Figure 1. EPA Method 6 uses essentially the same sampling system (8). All glassware (e.g., impingers, sampling bottles, etc.) must be thoroughly rinsed with methanol and oven dried before use.

10.2 Prior to 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 not requiring a sealed flow system downstream of the pump.

10.3 Ideally a dry gas meter is included in the system to record total flow. 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 period 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.

10.4 To collect an air sample two clean midget impingers are loaded with 10 mL of purified DNPH reagent and 10 mL of isooctane. The impingers are connected in series to the sampling system 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, DNPH reagent batch number, and dry gas meter and pump identification numbers.

10.5 The sampler is allowed to operate for the desired period, with periodic recording of the variables listed above. The total flow should not exceed ~80 liters. The operator must ensure that at least 2-3 mL of isooctane remains in the first impinger at the end of the sampling interval (i.e., for high ambient temperatures lower sampling volumes may be required).

10.6 At the end of the sampling period the parameters listed in 10.4 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 rate at the beginning and end of the sampling period differ by more than 15% the sample should be marked as suspect.

10.7 Immediately after sampling the impingers are removed from the sampling system. The contents of the first impinger are emptied into a clean 50 mL glass vial having a teflon-lined screw cap. The first impinger is then rinsed with the contents of the second (backup) impinger and the rinse solution is added to the vial. The vial is then capped, sealed with teflon tape and placed in a friction top can containing 1-2 inches of granular charcoal. The samples are stored in the can, refrigerated until analysis.

10.8 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:

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

where

Q_A = Average flow rate in mL/minute.
Q_1, Q_2....Q_N = Flow rate determined at the beginning, end, and intermediate points during sampling.
N = Number of points averaged.

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

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

V_m = Total volume sampled in liters at measured temperature and pressure
T_2 = Stop time
T_1 = Start time (T_2 - T_1 given in minutes)

11. Sample Analysis

11.1 Sample Preparation

11.1.1 The samples are returned to the laboratory in 50 mL screw-capped glass vials. To recover the DNPH derivatives the following procedure is employed.

11.1.2 The vials are shaken in a horizontal position on a reciprocating shaker for 10 minutes. The vials are then removed from the shaker and the isooctane layer is removed and placed in a second clean 50 mL screw-capped glass vial using a disposable pipette.

11.1.3 The remaining aqueous layer is extracted with 10 mL of 70/30 (V/V) hexane/methylene chloride in the same manner as described in 11.1.2. The organic layer is removed and combined with the isooctane extract.

11.1.4 The combined organic extracts are then concentrated to dryness at 40°C under a stream of pure nitrogen. When the sample just reaches dryness the vial is removed from the nitrogen stream and a measured volume (2-5 mL) of methanol is added to the vial. The vial is tightly capped and stored refrigerated until analysis.

11.2 HPLC Analysis

11.2.1 The instrument is assembled and calibrated as described in Section 12. Prior to each analysis the detector baseline is checked to ensure stable operation.

11.2.2 A 5-25 μL aliquot of the sample, dissolved in methanol, is drawn into a clean HPLC injection syringe. The sample injection loop 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 stripchart recorder.

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

11.2.4 After elution of the last component of interest the acquisition is terminated and the component concentrations are calculated as described in Section 13.

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

11.2.6 If the concentration of a component exceeds the linear range of the instrument the sample should be diluted with methanol, or a smaller volume can be injected onto the HPLC.

12. HPLC Assembly and Calibration

12.1 The HPLC system is assembled as shown in Figure 3. The typical chromatographic performance and operating parameters are shown in Figure 4.

12.2 Mobile phase is prepared by mixing 800 mL of methanol and 200 mL of reagent water. This mixture is filtered through a 0.22 μm polyester membrane filter in all glass and teflon suction filtration apparatus. The filtered mobile phase is degassed by purging with helium gas 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 tubing should be placed after the detector to further eliminate mobile phase outgassing.

12.3 The mobile phase is placed in the HPLC solvent reservoir and the pump flow is set at 1 mL/minute and allowed to pump for 20-30 minutes prior to the first analysis. The detector is switched on at least 30 minutes prior to the first analysis and the detector output is displayed on a stripchart recorder or similar output device at a sensitivity of .008 absorbance units full scale (AUFS). Once a stable baseline is achieved the system is ready for calibration.

12.4 Calibration standards are prepared in methanol from the solid DNPH derivatives. Individual stock solutions of ~100 mg/L are prepared by dissolving 10 mg of the solid derivative in 100 mL of methanol. These individual solutions are used to prepare calibration standards containing all of the derivatives of interest at concentrations of 0.1 - 10 mg/L, which spans the concentration of interest for most ambient air work.

12.5 All calibration runs are performed as described for sample analyses in Section 11. Before initial use the operator should inject a series of calibration standards (at least three levels) spanning the concentration range of interest. Using the UV detector, a linear response range of approximately 0.1 to 10 mg/L should be achieved, for ~10 μL injection volumes. Linear response is indicated where a correlation coefficient of a least 0.999 for a linear least squares fit of the data (concentration versus area response) is obtained.

12.6 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 DNPH components should be within 10% day to day. If greater variability is observed more frequent calibration may be required to ensure that valid results are obtained.

12.7 The response for each component in the daily calibration standard is used to calculate a response factor according to the following equation:

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

where

RF_c = response factor for the component of interest in nanograms injected/response unit (usually area counts).
C_c = concentration of component in the daily calibration standard (mg/L).
V_I = volume of calibration standard injected (μL).
R_c = response for component of interest in calibration standard (area counts).

13. Calculations

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

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

where

V_s = total sample volume at 25°C and 760 mm Hg pressure (liters).
V_m = total sample volume under ambient conditions (liters). Calculated in 10.9 or from dry gas meter reading.
P_A = ambient pressure (mmHg).
T_A = ambient temperature (°C).

13.2 The concentration of each aldehyde (as the DNPH derivative) is calculated for each sample using the following equation:

$$W_d = RF_c x R_d x \frac{V_E}{V_I}$$

where

W_d = total quantity of derivative in the sample (μg).
RF_c = response factor calculated in 12.7
R_d = response for component in sample extract (area counts or other response units).
V_E = final volume of sample extract (mL).
V_I = volume of extract injected onto the HPLC system (μL).

13.3 The concentration of aldehyde in the original sample is calculated from the following equation:

$$C_A = \frac{W_d}{V_m (or V_s)} x \frac{MW_A}{MW_d} x 1000$$

where

C_A = concentration of aldehyde in the original sample (ng/L).
V_m or V_s are as specified in Section 13.1.
MW_A and MW_d are the molecular weights (g/mole) of the aldehyde and its corresponding DNPH derivative, respectively.

13.4 The aldehyde concentrations can be converted to ppbv using the following equation:

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

where

C_A (ng/L) is calculated using V_s.

14. Performance Criteria and Quality Assurance

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

14.1 Standard Operating Procedures (SOPs)

14.1.1 Each user should generate SOPs describing the following activities as accomplished in their laboratory: 1) assembly, calibration and operation of the sampling system, 2) preparation, purification, storage and handling of DNPH reagent and samples, 3) assembly, calibration and operation of the HPLC system, and 4) all aspects of data recording and processing.

14.1.2 SOPs should provide specific stepwise instructions and should be readily available to, and understood by, the laboratory personnel conducting the work.

14.2 HPLC System Performance

14.2.1 The general appearance of the HPLC chromatograph should be similar to that shown in Figure 4.

14.2.2 The HPLC system efficiency and peak asymmetry factor should be determined in the following manner. A solution of the formaldehyde DNPH derivative 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 5, and should be between 0.8 and 1.8.

14.2.3 HPLC system efficiency is calculated according to the following equation:

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

where

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

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

14.2.4 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.

14.3 Process Blanks

14.3.1 Prior to use a 10 mL aliquot of each batch of DNPH reagent should be analyzed as described in Section 11. In general, formaldehyde levels equivalent to >5 ng/L in a 60 liter sample should be achieved and other aldehyde levels should be <1 ng/L.

14.3.2 At least one field blank should be shipped and analyzed with each group of 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 14.3.1 should be met for process blanks.

14.4 Method Precision and Accuracy

14.4.1 Analysis of replicate samples indicates a precision of $\pm$ 15-20% relative standard deviation can be readily achieved. Each laboratory should collect parallel samples periodically (at least one for each batch of samples) to document their precision in conducting the method.

14.4.2 Precision for replicate HPLC injections should be ± 10% or better, day to day, for calibration standards.

14.4.3 Method accuracy is difficult to assess because of the difficulty in generating accurate gaseous standards. Literature results indicate (1-3) recoveries of 75% or greater are achieved for a broad range of aldehydes. Each laboratory should periodically collect field samples wherein the impinger solution is spiked with a known quantity of the compound of interest, prepared as a dilute methanol solution. Formaldehyde cannot be spiked in this manner and therefore a solution of the DNPH derivative should be used for spiking purposes.

14.4.4 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. Recoveries of >70 ± 20% and blank levels of <5 ng/L for formaldehyde and 1 ng/L for the other compounds (assuming a 60 liter air sample) should be achieved.

References

(1) Grosjean, D., Fung, K., and Atkinson, R., "Measurements of Aldehydes in the Air Environment", Proc. Air Poll. Cont. Assoc., Paper 80-50.4, 1980.

(2) Grosjean, D. and Fung, K., "Collection Efficiencies of Cartridges and Micro-Impingers for Sampling of Aldehydes in Air as 2,4-Dinitrophenylhydrazones", Anal. Chem. 54, 1221-1224, 1982.

(3) Grosjean, D., "Formaldehyde and Other Carbonyls in Los Angeles Ambient Air", Environ. Sci. Technol. 16, 254-262, 1982.

(4) Riggin, 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.

(5) Annual Book of ASTM Standards, Part 11.03, "Atmospheric Analysis", American Society for Testing and Materials, Philadelphia, Pennsylvania, 1983.

(6) Berry, D. A., Holdren, M. W., Lyon, T. F., Riggin, R. M., and Spicer, C. W., "Turbine Engine Exhaust Hydrocarbon Analysis-Interim Report on Task 1 and 2", Report on Contract No. F-08635-82-C-0131, Air Force Engineering and Services Center, Tyndall AFB, Florida, 1983.

(7) Shiner, R., Fuson, R., and Curtin, D., "The Systematic Identification of Organic Compounds", John Wiley and Sons, Inc., 5th ed., New York, 1964.

(8) "Method 6 Determination of SO_2 Emissions from Stationary Sources", Federal Register, Vol. 42, No. 160, August 1977.

TABLE 1. ALDEHYDES AND KETONES FOR WHICH THE METHOD HAS BEEN EVALUATED

Compound	Molecular Weight Derivative	Molecular Weight Compound	Typical Relative Retention Time[a]
Formaldehyde	210	30	1.0
Acetaldehyde	224	44	1.3
Acrolein	236	56	1.6
Propanal	238	58	1.7
Acetone	238	58	1.9[b]
Crotonaldehyde	250	70	2.3
Isobutyraldehyde	252	72	2.4
Methyl Ethyl Ketone	252	72	2.8
Benzaldehyde	286	106	3.2
Pentanal	266	86	3.7
o-Tolualdehyde	300	120	4.8
m-Tolualdehyde	300	120	5.1
p-Tolualdehyde	300	120	5.3
Hexanal	280	100	5.7

[a] Using HPLC conditions shown in Figure 4. Formaldehyde = 1.0

[b] Acetone background levels in the reagent prevent its determination in most cases.

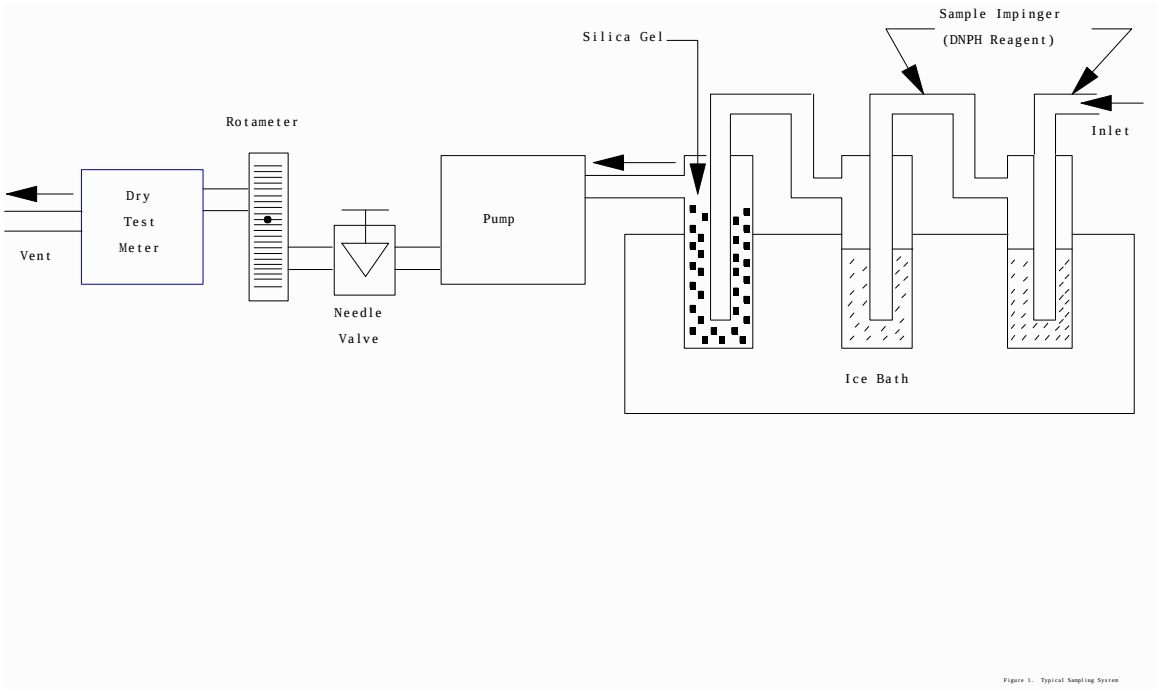


FIGURE 1. TYPICAL SAMPLING SYSTEM

SAMPLING DATA SHEET
(One Sample Per Data Sheet)

PROJECT: _____ DATE(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 Flow Rate, Q* ml/Min	Ambient Temp. °C	Barometric Pressure, mmHg	Relative Humidity,%	Comments
1.						
2.						
3.						
4.						
N.						

Total Volume Data**

V_m = (Final - Initial) Dry Gas Meter Reading, or = _____ Liters

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

* Flowrate from rotameter or soap bubble calibrator (specify which).
** Use data from dry gas meter if available.

FIGURE 2. EXAMPLE SAMPLING DATA SHEET

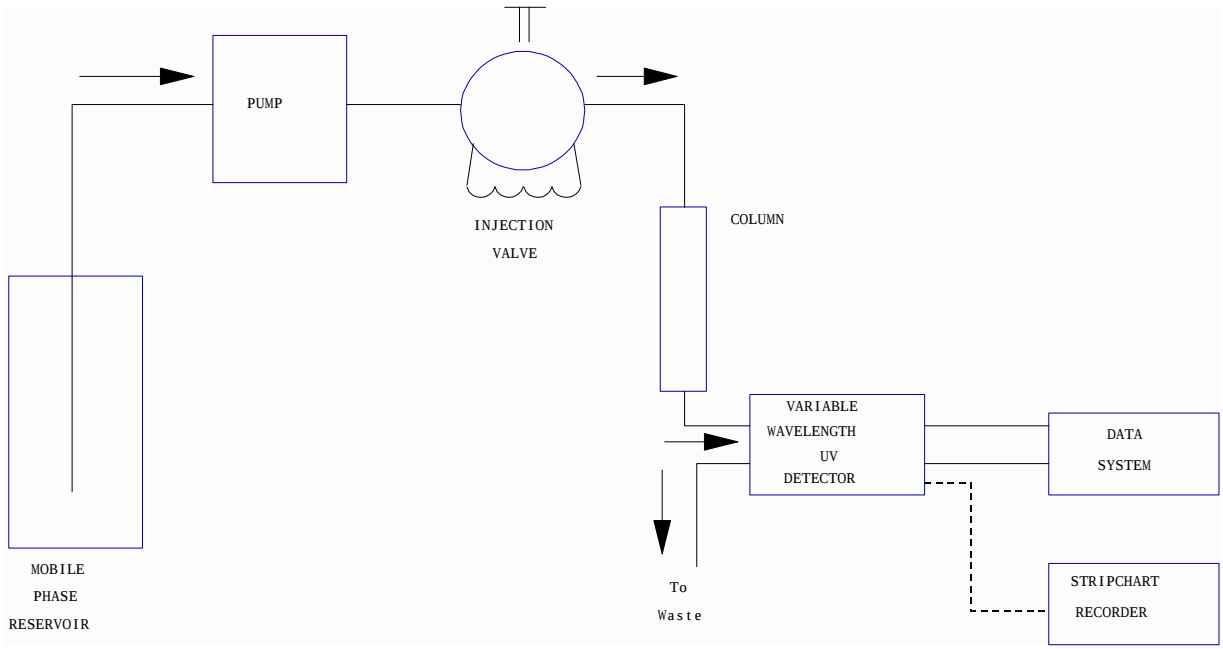
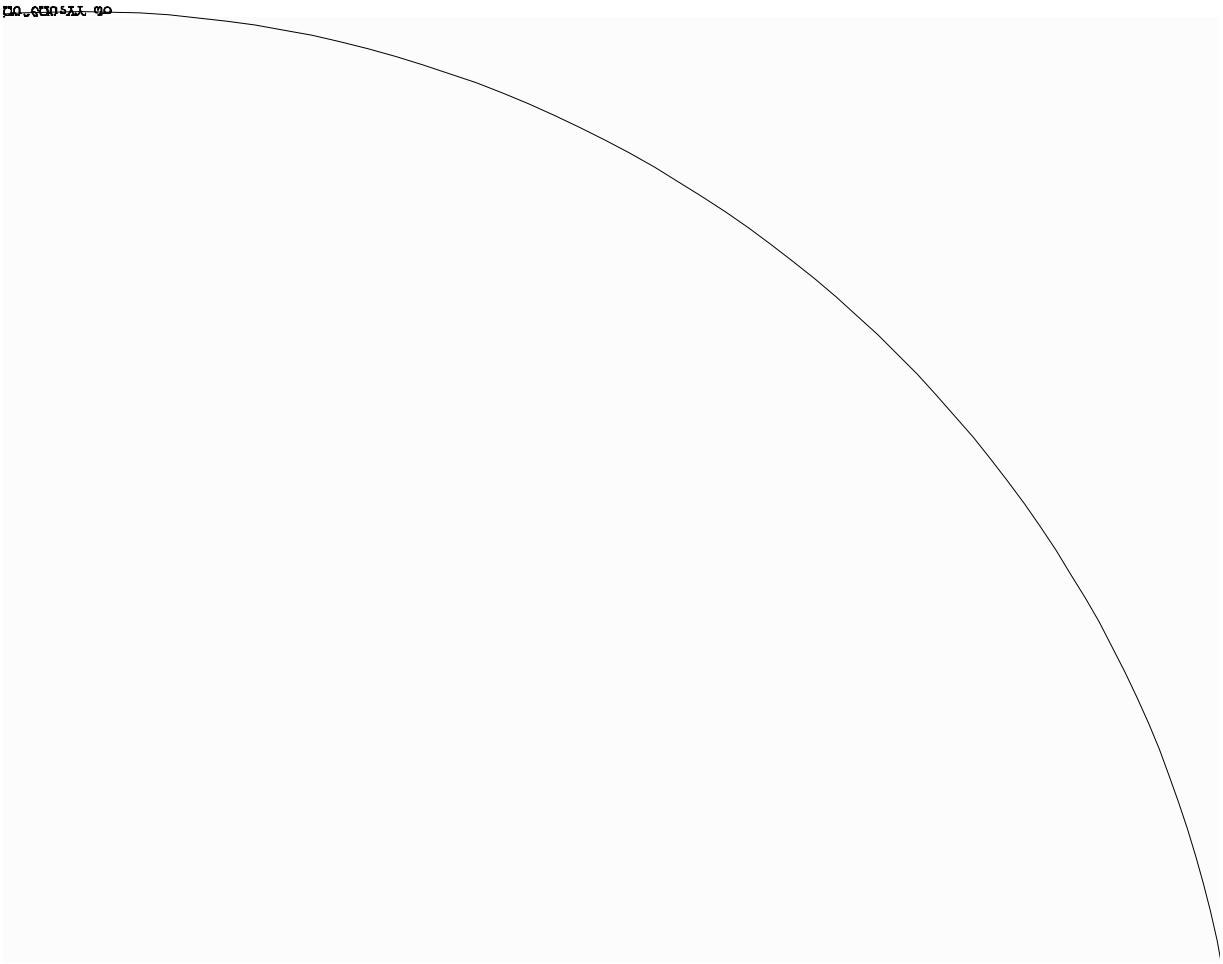
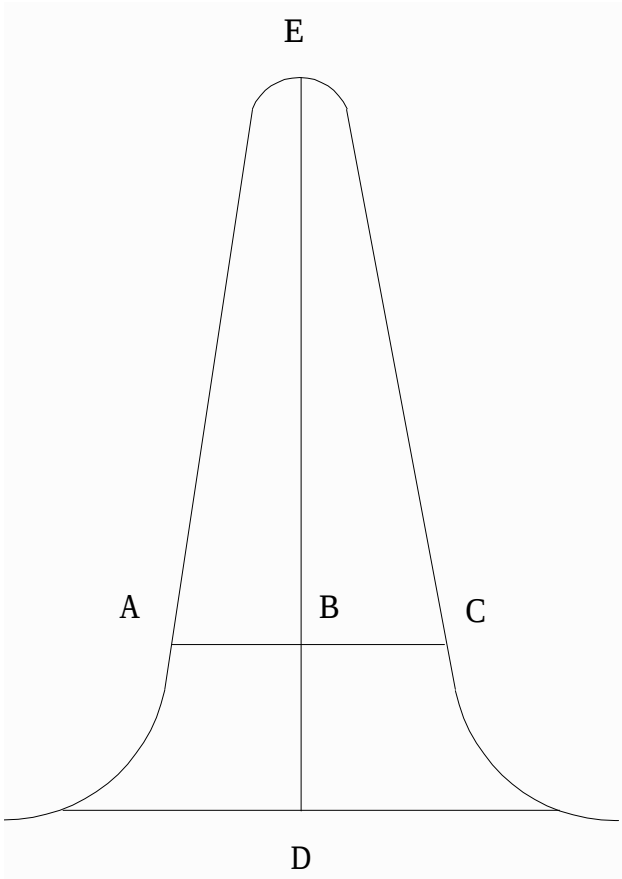


FIGURE 3. TYPICAL HPLC SYSTEM



Column - Zorbax ODS, 250 x 4.6 mm
Mobile Phase - 80/20 Methanol/H_2O
Flow Rate - 1 mL/Minute
Detector - UV at 370 nm

FIGURE 4. TYPICAL HPLC CHROMATOGRAM



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

Example Calculation:

Peak Height = DE = 100 mm

10% Peak Height = BD = 10 mm

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

AB = 11 mm

BC = 12 mm

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

FIGURE 5. PEAK ASYMMETRY CALCULATION