A GAS CHROMATOGRAPHIC METHOD FOR DETERMINING ACETONE CONCENTRATION IN HUMAN BREATH

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Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
July, 1980

A GAS CHROMOTOGRAPHIC METHOD FOR DETERMINING ACETONE CONCENTRATION IN HUMAN BREATH

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ACKNOWLEDGMENTS

The author wishes to express her appreciation to Dr. Donna Payne, major adviser, for her many hours of help, interest, and guidance throughout my graduate work.

The author is also greatful to Dr. R. D. Morrison, professor of statistics, for serving on the advisory committee and for his valuable assistance, and for arranging the statistical design analysis.

Greatful acknowledgment is extended to Dr. B. Kopel, Dr. G. Odell and Dr. G. Waller for serving on the advisory committee and for their assistance in the study.

Special gratitude is expressed to Dr. K. West and Dr. C. Bahr of the Veteran Administration Hospital in Oklahoma City for giving me the opportunity to collect samples from their diabetic patients.

Appreciation is extended to Terry Crone and Marlece Ebessen for their valuable assistance.

The author wishes to express her deepest appreciation to her cousins, Truwork Hulluka and Mekdes Mulat, for their support and encouragement throughout this study.

Finally, the author would like to dedicate this dissertation to her dearest friend, Mounzer Harake, for his sincere concern, support, and encouragement to see her through her graduate work.

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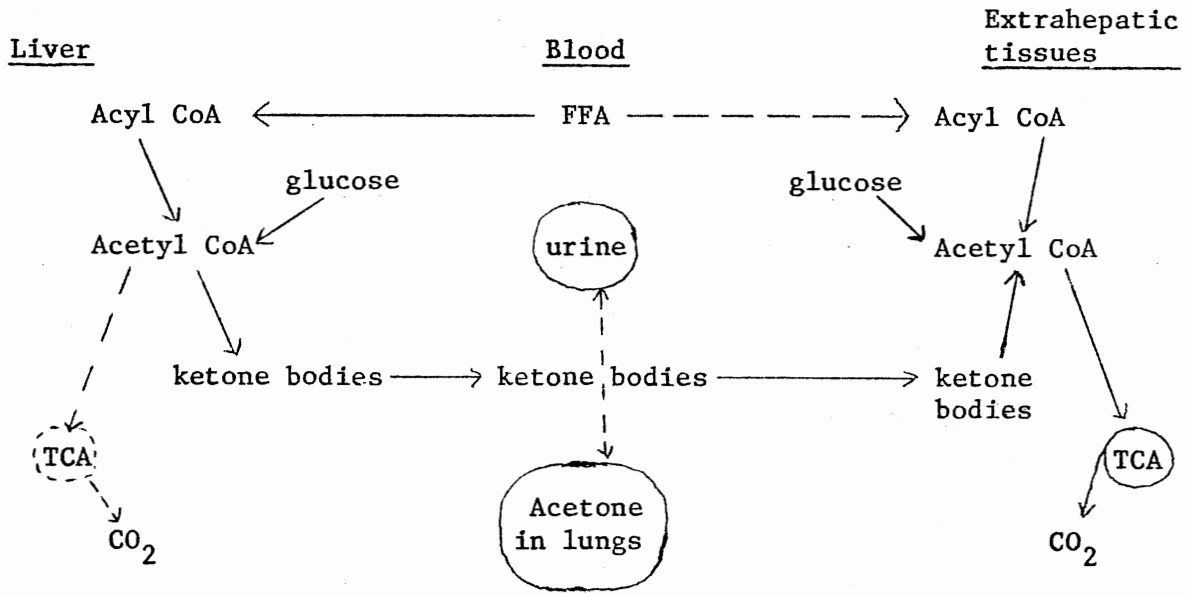
CHAPTER I

INTRODUCTION

The presence of acetone in human breath of diabetic subjects has been known for many years. Breath acetone content of normal human beings was first determined as early as 1920, and it was the first of the three ketone bodies (acetoacetate, β-hydroxybutyrate, acetone) to be identified in uncontrolled diabetics (Sulway and Trotter, 1971). Ketone bodies are found in the blood of normal human beings, but their levels are increased under carbohydrate deficiency, or high fat diet, or severe exercise in the postabsorptive state (Harper, 1973; Lehninger, 1977). When carbohydrate is deficient in the diet, fatty acids which are precursors of ketone bodies, are metabolized to produce energy. The liver appears to be the only organ that adds ketone bodies to the blood. The extrahepatic tissues utilize ketone bodies as respiratory substrates. The formation, utilization, and excretion of ketone bodies is illustrated in Figure 1.

Acetone is not readily utilized by the peripheral tissues and, as such, is eliminated through the lungs and urine. Acetone is present in the blood and is also formed in the lungs by spontaneous decarboxylation of acetoacetate as shown in Figure 2 (Trotter, Sulway, and Trotter, 1971; Harper, 1973; and Sulway, Trotter, Malins, and Trotter, 1971).

Breath acetone can be elevated for reasons other than production by the liver, as in environmental exposure to acetone. Measurements of breath acetone have been used to monitor acetone poisoning. Rooth and



Source: Harper (1973).

Figure 1. Formation, Utilization, and Excretion of Ketone Bodies

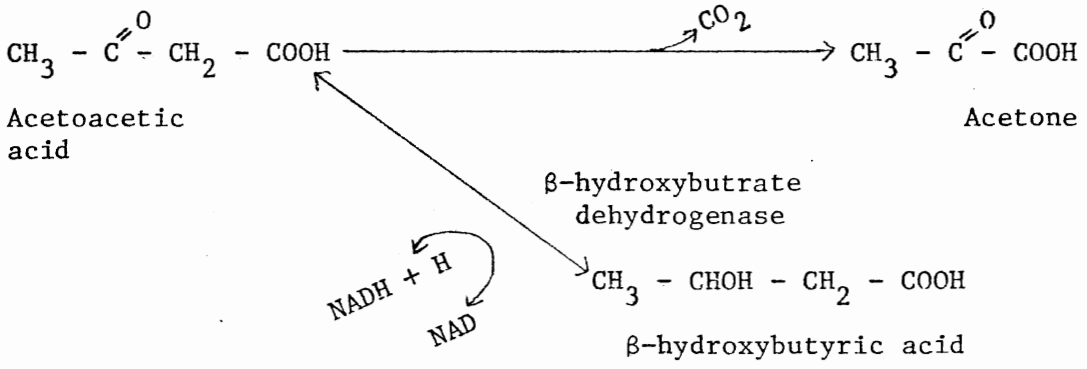


Figure 2. Spontaneous Decarboxylation of Acetoacetate

Ostenson (1966) reported that a man who drank a paint thinner solution containing acetone had about 490 gm of acetone in his body. The patient did recover without incident.

Need for Research

Various gas chromatographic methods to quantitate expired acetone have been reported. None were designed, however, to demonstrate reliability, reproducibility, and validity of the method.

Various methodologies used in this study have not been applied previously to measurements of breath acetone. They include: (1) utilization of an automatic peak integrator to obtain rapid and objective acetone concentration values, (2) use of standard acetone gas mixtures within the acetone concentration range normally found in human breath to construct the standard calibration curve, (3) use of exponential dilution of standard gas mixtures to obtain the standard calibration curve, (4) determination of the lower limits of acetone concentration which can be detected, and (5) utilization of breath sample collection containers which can be used to transport and store samples.

Quantitation of human breath acetone seems to have certain advantages over quantitation of acetone in urine or blood as follows: Expired air is the major route for excretion of acetone (Harper, 1973) and concentration of acetone in the breath is believed to be a reliable index of the degree of ketonemia (Stewart and Boettner, 1964; Krotoszynski, Gabriel and O'Nell, 1977). Elevated breath acetone levels can be detected before elevated ketone levels in urine can be detected by conventional methods (Stewart and Bottner, 1964). Expired air can

be collected without risk and discomfort to the human subject or patient (Dubowski, 1974), and monitoring breath acetone at close intervals in an individual would be much more desirable than obtaining blood samples at close intervals. Thus, its usefulness should be further explored.

Measurements of breath acetone may have use in improving dietary control of diabetes. Through its use, insulin requirements may be decreased. For example, it has been generally practiced to treat hyperglycemic patients with more insulin. However, breath acetone measurements could lead to improvement of the situation without administering more insulin. Crofford, Mallard, Winton, Rogers, Jackson, and Keller, (1977) have classified diabetics with hyperglycemia as being in one of two categories depending on breath acetone concentration as shown in Table I.

Monitoring blood acetone levels of pregnant women may reduce the chances of brain damage to infants which occurs with hyperacetonemia (Worthington, Vermeersh, and Williams, 1977). Insulin requirements are increased in pregnancy so that ketonemia can readily occur in borderline diabetics and even nondiabetics. Breath acetone levels could serve as a monitor for this condition.

Crofford et al. (1977) suggested that breath acetone measurement may be useful as a motivational force in following patients on long-term weight reduction programs, with the patients instructed to restrict caloric intake to such levels that breath acetone is maintained at about 500 nm.

In conclusion, an accurate and practical method of measuring breath acetone may have a number of useful applications to human nutrition.

TABLE I

CLINICAL USE OF BREATH ACETONE MEASUREMENT

Serum Glucose (%)	Breath Acetone (nM)	Dx	Rx
Greatly elevated	Normal (10-50)	Overeating	Less food
Greatly elevated	High (50)	Insulin insufficiency	More insulin
Not greatly elevated	High (50)	Carbohydrate insufficiency	Continue if weight loss is the objective or reapportion diet adding more carbohydrate
Not greatly elevated	Normal (10-50)	Good metabolic control	Continue same Rx

Purpose of Study

The general purposes of the study were to develop a gas chromatographic method to determine human breath acetone concentration and to demonstrate its use. The specific purposes of the study were to:

1. Develop an accurate and practical method to quantitate human breath acetone levels and demonstrate that it is valid, reliable, and reproducible within the range of physiologically realistic concentrations.

2. Demonstrate the limit of the method to quantitate acetone concentration by using an exponential dilution flask.

3. Demonstrate the validity and reproducibility of the sampling procedure and analytical method when using breath samples from relatively normal fasting subjects, fasting and non-fasting diabetic patients, and fasting obese subjects.

CHAPTER II

LITERATURE REVIEW

Acetone in Humans

Elevated levels of acetoacetate in blood and urine and the odor of acetone in breath have long been recognized as hallmarks of uncontrolled diabetes (Henderson, Karger, and Wrenshall, 1952). The odor of "decaying apples" on the breath of several diabetics was reported as early as 1798 (Rolo, 1978). Sulway et al. (1971) showed that there were significant levels of free acetone in the blood of diabetics with hyperketonemia. Ketonuric patients showed increased levels of the three ketone bodies before breakfast and insulin administration. Fasting breath acetone level was increased in both normal and obese subjects and in obese subjects this increase in fasting breath acetone level can be used as a guide in caloric reduction (Rooth and Ostenson, 1966). The various tablet and dip sticks are not sensitive enough to detect the presence of acetoacetate or acetone in the blood, urine, or breath unless the patient is under very poor control (Crofford et al., 1977). Therapeutic decision can be made more rationally if the metabolic state of the diabetic is assessed with both blood and breath acetone measurements.

Crofford et al. (1977) conclude that a normal range of breath acetone concentration in healthy subjects is 10-50 nM. In comparison

with the units used in this dissertation, this is approximately 0.58-2.9 ug/L. Crofford et al. classified patients with breath acetone levels less than 50 nM and greatly elevated blood glucose level as hyperglycemic. This hyperglycemic condition is induced by overeating and is treated by a better dietary management. Crofford et al. also noted that treating these hyperglycemic patients with more insulin cannot help to improve the condition since more insulin requires overeating and as such results in obesity. Hyperglycemia and elevated breath acetone (>50 nM) is a sign of inadequate treatment with insulin and, therefore, insulin dose should be adjusted. An elevated breath acetone with normal blood sugar indicates insufficient carbohydrate intake to meet metabolic need. But on the other hand, if the patient is on a weight loss program this is a good indicator of success. Those patients with blood sugar not greatly elevated and normal breath acetone (10-50 nM) level are under good metabolic control. As such, Crofford et al. further suggest that breath acetone measurements are useful motivational forces in following patients in long-term weight reduction programs. The patient, in this case, restricts his caloric intake to such a level that breath acetone concentration is maintained at about 500 nM. If a proper balance of dietary carbohydrate, fat, and protein is maintained, then the patient can be assured that body fat is being lost at a rate of about one-half pound per week. The patient can readily see that dietary indiscretion results in immediate normalization of breath acetone concentration.

Table II summarizes the breath acetone values reported by other scientists. It should be noted that the analytical methods between researchers differed as well as sampling techniques, such as whole breath samples versus end-expiratory samples. Also, subjects were

TABLE II

SUMMARY OF BREATH ACETONE CONCENTRATIONS REPORTED BY OTHER SCIENTISTS

Desciption of Subjects	Number of Subjects	Breath Acetone Concentration as Reported	Breath Acetone Concentration Converted to µg/L	Reference
Varied	194	5-319 nM (Range)	0.29-18.50	Crofford et al., 1977
Normal	106	1.3-11.8 µg/gm condensate/square meter of body surface (Range)		Henderson et al., 1952
Diabetics	70	1.3-12.7 µg/gm condensate/square meter of body surface (Range)		
Controlled diabetics	10	0.45-9.5 µg/ml		Levy et al., 1964
Uncontrolled diabetics	8	1.6-85.0 µg/ml (Range)		
Controls	67	0.13-4.22 µg/L (Range)		Rooth et al., 1966
Diabetics	151	0.13-25.21 µg/L (Range)		
Nondiabetic	40	1.1 µg/L (Mean)		Stewart et al., 1964
Diabetic	129	30.1 µg/L (Mean)		

sometimes not described as to the presence of diabetic or other disease and whether or not they had fasted. Scientists have reported breath acetone concentration in different units of measures. These have been converted to the units used in this dissertation research, μg/L, for comparison of these results. Where others reported values for blood glucose level versus corresponding breath acetone concentration. Because standardized methodology has not been used by investigators, it should be understood that the figures in Table II serve as only a very general guide for comparison with this dissertation research.

Quantitation, Collection, and Analysis of Breath Acetone Concentration

Breath acetone was first quantified in 1897 by Geelmuyden. Advances were made in the method in 1920; Hummard and Widmark measured the concentration of acetone in normal humans. The methods were insensitive and tedious requiring concentration of the breath sample. Janson and Larson (1969) have investigated methods of sampling and analyzing organic compounds, including acetone in human breath. Their breath sampling procedure involved the use of collection bags made of polyester film (Mylar, DuPont). They used a respiratory mask. The collection bags were conditioned twice with breath before actual sample was taken from the third filling on the bag so that the composition of the samples would not be altered appreciably due to the adsorption of acetone to the walls of the bags. Quantitative analysis of the acetone was made by using gas chromatograph equipped with a flame ionization detector (FID). The flame ionization detector is the best detector for this type of

analysis since it has a low sensitivity for the inorganic compounds of the breath.

Trotter, Sulway, and Trotter (1971) used gas chromatograph to quantitate acetone in breath and plasma. They used aluminum tap assembly obtained from "Douglas" bags and patients were asked to rebreath into the bags five times and the final exhalation was tapped by closing the tap. A 1 ml sample of the breath was directly injected into the column of the gas chromatograph. They compared this gas chromatographic technique with the chemical method and concluded that gas chromatographic technique is favorably comparable to an established chemical method. In addition, they stated that the gas chromatographic technique is rapid and easy to perform.

Stewart and Boettner (1964) also used gas chromatograph to quantitate and analyze expired air acetone in diabetic patients. They collected alveolar air sample in saran bags fitted with corkstopper glass valves. The collection bags were flushed with continuous stream of air for 18 hours to remove volatile contaminants. The acetone concentration of the alveolar samples were analyzed by a gas chromatograph equipped with a flame ionization detector. Their result showed that juvenile diabetics whose urine was negative for ketone bodies had a ten-fold greater alveolar air acetone concentration as compared with adult type whose urine is negative for ketone bodies. In addition, they showed that there is a cyclic variation of the concentration of acetone for those nondiabetic patients during the waking hours. But diabetic patients with poor control did not show cyclic variation, rather showed a steady increase or decrease of acetone concentration. Their findings also demonstrated the highest value recorded for conscious patients in

diabetic acidosis, 1900 µg/L. They concluded that in the case of diabetic acidosis the expired air acetone concentration appeared to be an adequate indicator for clinical response to treatment as any of the standard laboratory tests employed. In addition, they indicated that the acetone value was a more sensitive and accurate reflection of the degree of ketonemia than the reagent-tablet method of semiquantitative estimate of plasma or urinary ketone body concentration. They also indicated that a value of acetone concentration less than 3 µg/L would indicate good metabolic control in the absence of hypoglycemia; a value more than 10 µg/L indicates poor metabolic control, and a value greater than 50 µg/L indicates a state of ketosis.

Stekelenburg and Bruyn (1970) also used gas chromatograph but their technique of sample collection was different, they determined acetone concentration by head space analysis. Their technique involved the transfer of fresh blood into a vial and let it reach equilibrium. One ml of the head space gas was removed and injected directly into the column of the gas chromatograph.

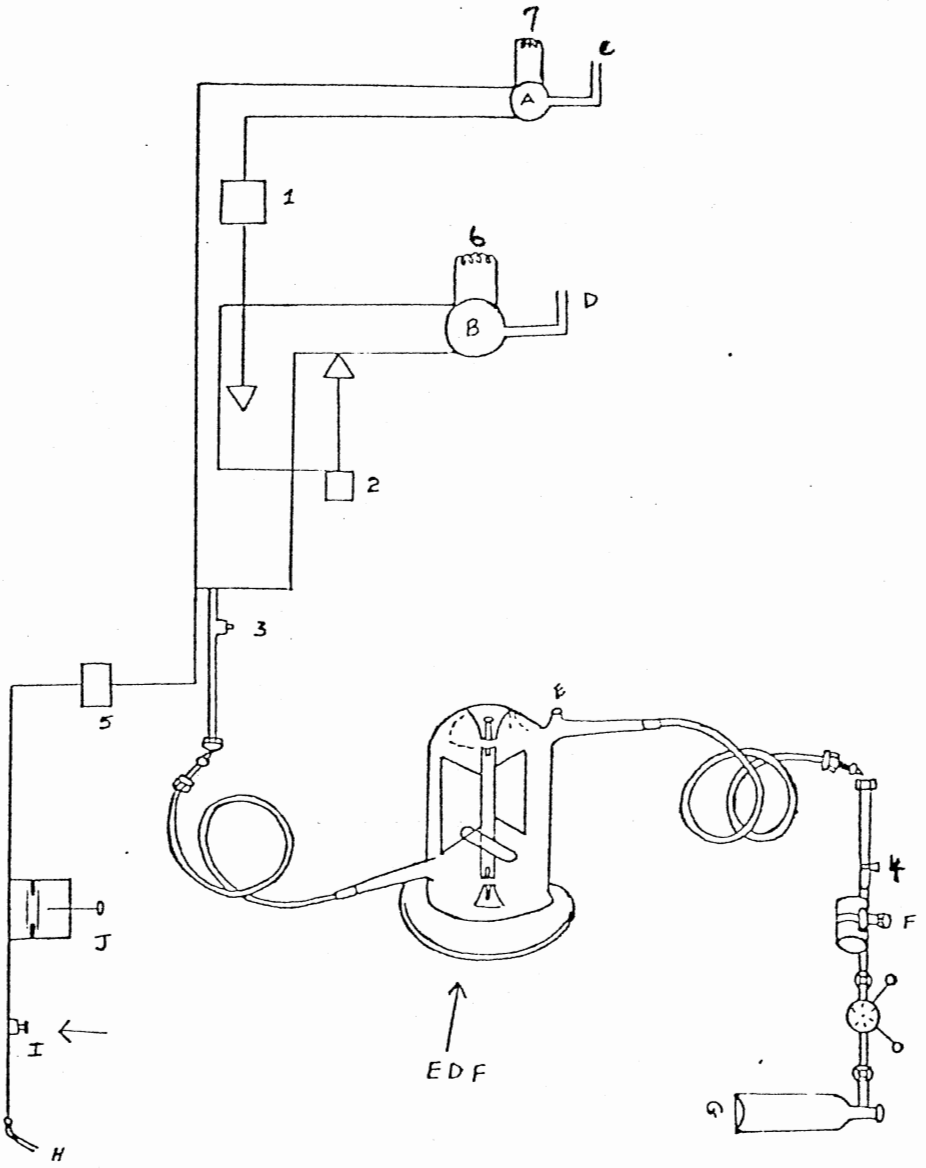
In spite of all the different methods used to analyze breath acetone, several investigators have applied breath acetone concentration to clinical problems (Rooth, 1972; Rooth and Tibbling, 1968; Tassopoulos, Barnett, and Fraser, 1969; Sulway and Mallins, 1970; Sulway et al., 1971; Trotter et al., 1971; Polo and Ilkovo, 1972; Crofford et al., 1977). Since no standardized methodology was employed, information on the precision and reliability of the method of analysis was not given.

CHAPTER III

METHODS AND MATERIALS

A Hewlett-Packard model 5830A gas chromatograph equipped with a flame ionization detector, an automatic 0.5 ml gas sampling valve and utilizing an exponential dilution flask (Figure 3) was used. It has 6 foot stainless steel matched dual columns, 1/8 inch SS packed with 0.1% Sp-1000 on carbopack C, recommended because of tis inertness gives good symmetry with polar compounds. Other operating parameters were: oven temperatures of 120°C, 130°C, 140°C, and 150°C; FID temperature of 350°C; chart speed of 0.8 cm/min.; peak attenuation of 2^{10}, 2^{8}, 2^{4}, 2^{2}; slope sensitivity of 1 for concentrations above 5 µg/L and 0.5 for concentrations below 2.5 µg/L; area rejection of 1; carrier (He) gas flow through the dual columns 34 to 35 ml/min; nitrogen flow rate through the exponential dilution flask of 19.9, 26.2, 30.8, 37.7, 41.7, 42.9, 43.5, and 46.8 ml/min.; hydrogen and air entered the burner at a flow rate of 35 and 250 ml/min., respectively.

The exponential dilution flask (EDF) is a device used for calibration of detectors with gas samples. It consists of a glass vessel with a diluent gas inlet and an outlet. The outlet is connected to a gas sampling valve (GSV) for introduction of the sample into a gas chromatograph. A gas-tight syringe was used to introduce a known volume of the standard gas mixtures to be used for calibration.



A & B = sampling valves, C & D = carrier gas, E = injection port, F = flow regulator, G = N_2 cylinder, H = to pump, I = needle valve, J = pressurizer, 1 = valve 1, 2 = valve 2, 3 = valve 3, 4 = valve 4, 5 = valve 5, 6 & 7 = 0.5 ml sample loop.

Figure 3. Schematic Representation of Sampling and Analysis

The volume of the exponential dilution flask was determined by weighing the flask before and after being filled with water (volume of flask in this study was 165.85 ml). The flow rate of nitrogen through the exponential dilution flask was measured by the use of a soap film flowmeter.

The gases are mixed in the exponential dilution flask by a stirring vane, and an exponential dilution of the sample gas in the diluent is achieved with time (Hammarstrand, 1976).

The concentration of the exponentially diluted acetone (commercial standard gas mixtures and head space above laboratory prepared acetone in ethyl acetate standards) injected into the exponential dilution flask was determined by the following formula (Shatting, 1969; Scolnick, 1970; Ritter and Adams, 1976; Mitchell, 1972):

$$C_O = \left(\frac{(V_A)(D_A)}{M_A}\right) \div \left(\frac{(V_E)(D_N)}{M_N}\right)$$

where:

V_A = volume of acetone injected in µl

D_A = density of acetone (0.790 mg/ml)

M_A = molecular weight of acetone (58.00 mg/ml)

V_E = volume of exponential dilution flask (165.85 ml)

D_N = density of nitrogen (1.252 mg/ml)

M_N = molecular weight of nitrogen (28.00 gm/mole).

The gas chromatograph was programmed for each run. Time for each peak was calculated by using the formula given below, and this formula was used to determine the concentration of the sample (C_i) at any time of the run.

$$T = \frac{(V_E)}{(Q)} \times \frac{\ln(C_o)}{(C_i)}$$

where:

V_E = volume of exponential dilution flask

Q = nitrogen flow rate through the exponential dilution flask in ml/minute

ln = natural logarithm

C_o = initial concentration of acetone at T_o

C_i = concentration of acetone at time T.

Three 1,000 ml volumetric flasks were silylated in order to avoid adsorption of acetone on the walls of the flasks (Grob, Grob, and Grob, 1977; Sandra and Verzele, 1977). The volumetric flasks were filled with 20% HCl (hydrochloric acid) solution and stored for three days. The flasks were washed with distilled water, and 3 ml of 1,1,1,3,3,3-Hexamethyldisilazane was poured into each volumetric flask. The flasks were fitted with stopcocks and evacuated for 3.3 minutes by using a pump. After evacuation the volumetric flasks were placed in an oven at a temperature of 300°C so that the hexamethyldisilazane could react. The silylated flasks were used to prepare and calculate the concentration of acetone in ethyl acetate standards (Hala, Wichterle, Potak, and Boublik, 1968):

$$\log P_1^\circ = \frac{(A_1 - B_1)}{(t + C_1)}$$

where:

P_1° = pure vapor pressure of acetone

t = room temperature in degrees Celsius

A_1, B_1, C_1 = are Antoine's constants for vapor pressure equation given with respective temperature regions in degree Celsius.

$$a_{12} = \frac{y_1 x_2}{y_2 x_1}$$

where:

a_{12} = relative volatility

x_1 = mole fraction of acetone in the liquid phase

x_2 = mole fraction of ethyl acetate in the liquid phase

y_1 = mole fraction of acetone in the vapor phase

y_2 = mole fraction of ethyl acetate in the vapor phase.

When assuming ideal behavior of vapor phase, a_{12} can be defined as:

$$a_{12} = \frac{\gamma_1 P_1^o}{\gamma_2 P_2^o}$$

where:

P_1^o = pure vapor pressure of acetone

P_2^o = pure vapor pressure of ethyl acetate

γ_1 = activity coefficient of acetone

γ_2 = activity coefficient of ethyl acetate.

The equilibrium composition of liquid and vapor phase in a two component system of nonelectrolytes is expressed as:

$$y_1 = \frac{a_{12}(x_1/x_2)}{1 + a_{12}(x_1/x_2)}$$

where:

a_{12} = relative volatility

x_1 = mole fraction of acetone in the liquid phase

x_2 = mole fraction of ethyl acetate in the liquid phase

y_1 = mole fraction of acetone in the vapor phase.

$$P_A = (y_1)(\gamma_1)(P_1^o)$$

where:

P_A = partial pressure of acetone in the flask

y_1 = mole fraction of acetone in the vapor phase

γ_1 = activity coefficient of acetone

P_1^o = pure vapor pressure of acetone.

$$N_1 = \frac{P_A(Vsyr)}{(R)(K)}$$

where:

N_1 = number of moles of acetone

P_A = partial pressure of acetone in the EDF

Vsyr = injection volume in liters

R = gas constant

K = degree Kelvin

Techniques Used for Calibration and Standardization

1. Head space above laboratory prepared acetone in ethyl acetate standards injected by syringe: The concentration of acetone in ethyl acetate standard was calculated by using room temperature, the vapor pressure of acetone, mole fraction of acetone in the liquid phase, activity coefficient of acetone, partial pressure of acetone in the flask, the ideal gas law, and the flame ionization detector (FID) response factor for acetone and ethyl acetate (see equations given on pages 16-18). Some of the prepared acetone in ethyl acetate standard was transferred into a 125 ml evacuated bulb and was allowed to reach equilibrium with its liquid phase. A gas-tight syringe was inserted through the septum that closes the opening of the bulb containing the standard and a 10 µl of the vapor was drawn. The plunger of the syringe was repeatedly moved to and

fro (10 times) and held in the bulb for one minute before the 10 µl sample was drawn. This sample was directly injected into the gas chromatograph through the stainless steel gas valve fitted with an adapter which provides a tight fit between the injection port and the syringe which contained the sample. A Supelco, Inc., Thermogreen LB-1 septum (0.5 mm) was placed in the fitting valve of the injection port. As the sample passed through the column, the different components were differentially retarded by adsorption or absorption by the column filling material. As the carrier gas (He) continually flowed through the column, acetone and ethyl acetate were eluted at different times. The effluent from the column entered the burner base through a millipore filter and was mixed with the hydrogen entering the burner and burned in filtered air. During this combustion, ionic fragments and free electrons are formed. These ions and free electrons produce an electric current proportional to the rate at which the sample enters the flame. Under the conditions stated above, acetone emerged from the column and was detected 56 seconds after injection of the sample (Figure 4).

2. Head space above laboratory prepared acetone in ethyl acetate standards injected by automatic sample loop after exponential dilution: Valves 3 and 4 (Figure 3) were opened to allow the nitrogen to flow through the exponential dilution flask so that any contamination was swept out through vent 1 or 2. Valves 3 and 4 were closed, and 195 µl of the vapor of acetone in ethyl acetate standard was injected into the exponential dilution flask through the injection port of the EDF and mixed for 1 1/2 minutes. Thorough mixing is required for better results (Ritter and Adams, 1976). Valves 3 and 4 were opened (to allow a continuous flow of nitrogen to dilute the sample) at the same time and the

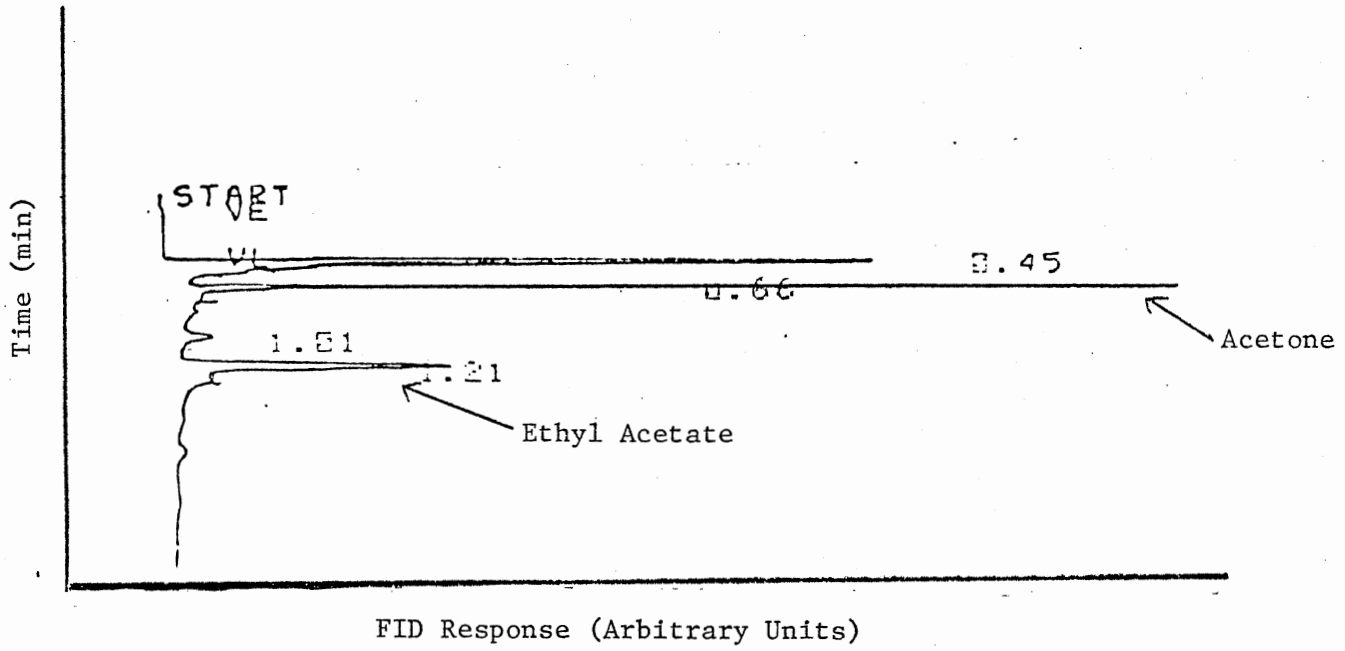


Figure 4. Typical Chromatogram of Acetone Peak, Actual Size

actuator button was pressed. The gas chromatograph was programmed to sample at a given time and time for each concentration was calculated by the formula given on page 16.

3. Commercially obtained acetone standard injected by an automatic sampling loop from sampling bags: The sample bags were filled with commercial standard (acetone) gas mixtures and the acetone was pulled from the bags into the sample loop by the use of a pump (Figure 3).

4. Commercial standard (acetone) gas mixtures injected by an automatic sample loop after exponential dilution: The exponential dilution flask was filled with commercial acetone standard and was diluted by a continuous flow of nitrogen through the exponential dilution flask. The gas chromatograph was programmed to sample at given times and time for each concentration was calculated by the use of the formula given on page 16.

Duplicate samples were collected for each column temperature, flow rate, day, and run chosen. The day-to-day variations and the run-to-run variations were studied. All other conditions were held constant while temperature and flow rates were varied to determine which flow rate temperature combination fitted the standard curve best. Statistical analyses on the different procedures were made to measure the effects of flow rate changes, day-to-day variation, run-to-run variation within a day and between duplicate variations.

Collection of Expired Air

Breath acetone samples from normal, obese and diabetic patients were collected by a 12 by 12 inch Mylar bag (especially constructed sampling bags, Payne-Bose, Tsegaye, Morrison, and Waller, 1978). Two drying

columns, one packed with Drierite (anhydrous $CaSO_4$, to absorb water), and the other with barium hydroxide lime (to absorb CO_2) were connected to the Mylar bag by a tygon tubing. A 3-cm tygon tubing was slipped into the top column to serve as a mouthpiece. Subjects were asked to breath through the mouthpiece thereby inflating the bag. The concents of the bags were introduced into the column of the gas chromatograph through the 0.5 ml automatic sample loop. Duplicate samples were drawn from each bag to demonstrate reproducibility. Samples were stored in the bags for more than three days and analyzed to check if there was variation because of storage or adhesion to the walls of the bags.

Subjects

Breath acetone concentration of 56 diabetic and healthy subjects were determined. Postparandial and fasting breath samples of 42 diabetic out-patients of the Veteran Administration Hospital in Oklahoma City, three well controlled and one brittle diabetic subjects from Stillwater were collected and analyzed. Fasting breath samples of one obese and nine normal (lean) subjects were also collected and analyzed. Twenty-seven of the 46 diabetic subjects were insulin dependent. The concentration of acetone in the sample breath was compared with the blood sugar level. Proper statistical procedures were used to analyze the data.

CHAPTER IV

RESULTS AND DISCUSSION

There were no differences in the peak areas due to the different column temperatures used. The difference on area count due to flow rate (nitrogen flow through the exponential dilution flask) was not significant at $\alpha = 0.05$ (Table III).

There was a significant difference in area count due to test dates at $\alpha = 0.005$ (Table IV and Figure 5). When the curves obtained by using two different procedures (the exponential dilution flask procedure and sampling from bags procedure) for each of the two sources of acetone were compared, it was found that the four fitted straight lines were parallel (Table V). However, it was also found that there was a difference due to the source of acetone and differences due to procedures. A higher degree of polynomial effect was found for each curve and the shape of the curves were not the same. All of these differences were significant at $\alpha \leq 0.05$.

When three concentrations of commercial acetone standards were analyzed by the two different procedures, it was found that there was a difference between procedures ($\alpha < 0.05$). The two fitted straight lines were parallel, but there was a quadratic effect. The curvatures were not the same for both procedures.

When direct injection procedure was compared with the exponential dilution flask (EDF) procedure, it was found that there was a significant

TABLE III

ANALYSIS OF VARIANCE OF ACETONE PEAK AREA FOR THE DIFFERENT FLOW RATES

Source	df	Mean Square	Values for F
Flow Rate (Q)	1	0.05669968	1.483
Time Linear (T_2)	1	7.79896399	204.000
Time Linear in Flow Rate T_1 (Q)	1	0.00017708	0.005
Time Quadratic (T_2)	1	0.00173470	0.046
Time Quadratic in Flow Rate T_2 (Q)	1	0.00035210	0.092
Time Residual in Flow Rate	9	0.00198497	0.052
Run in Flow Rate (= error a)	2	0.03822060	
T_1 * Run in Flow Rate	2	0.0012197	5.529*
T_2 * Run in Flow Rate	2	0.0000255	0.116
Residual Time * Run in Flow Rate (= error b)	9	0.0002206	

*Significant at 0.05 probability level.

TABLE IV

ANALYSIS OF VARIANCE OF ACETONE LOGARITHM PEAK AREA FOR THE DIFFERENT TEST DATES

Source	df	Mean Square	Values for F
Test Dates	2	0.321910245	76.945*
Run in Test Dates (= error a)	3	0.00418366	
Time Linear (T_1)	1	33.01588093	789.069*
Time Quadratic (T_2)	1	0.20246885	48.395*
Time Residual	11	0.000615155	0.147
Time Linear * Test Dates	2	0.034695675	8.293*
Time Quadratic * Test Dates	2	0.00650963	1.556
Time Residual * Test Dates	22	0.00007793	0.186
Time Linear * Run in Test Dates	3	0.00152476	0.346
Time Quadratic * Run in Test Dates	3	0.00006795	0.016
Time Residual * Run in Test Dates (= error b)	33	0.00000837	

*Significant at 0.01 probability level.

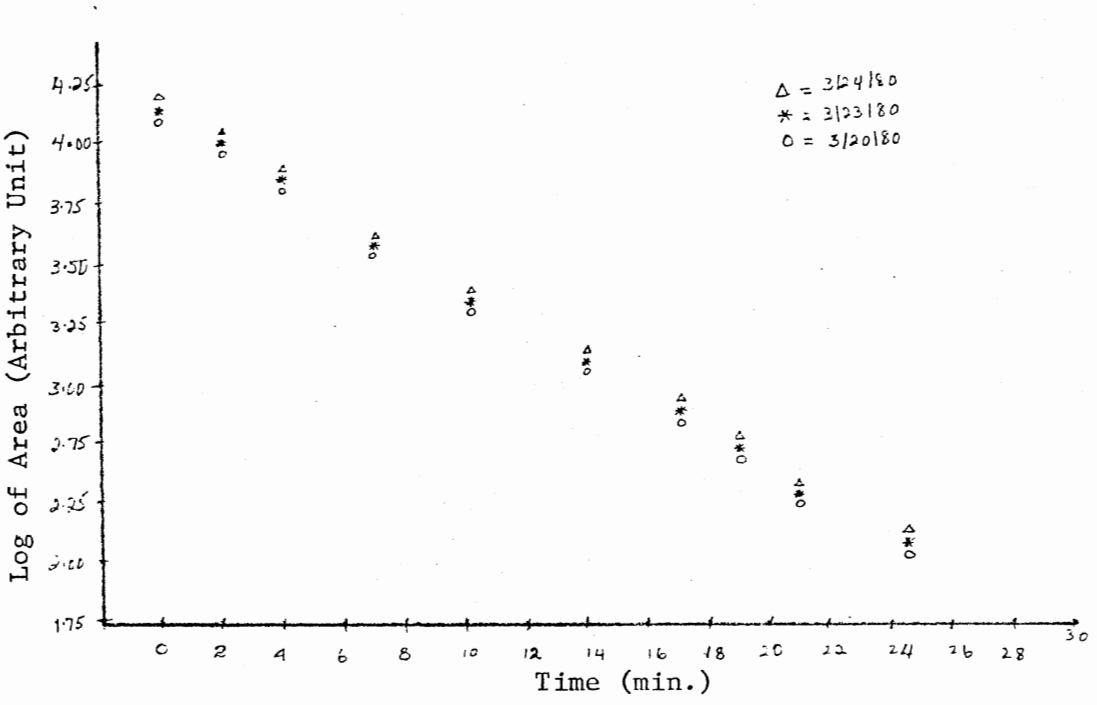


Figure 5. Logarithm of Reported Area (Arbitrary Unit) of Acetone Peak Versus Time

TABLE V

ANALYSIS OF VARIANCE OF ACETONE LOGARITHM PEAK AREA OF THE FOUR PROCEDURES AND THE TWO SOURCES

Source	df	Mean Square	Values for F
Total	120		
Procedures and Sources (P-S)	3	4.34405173	114890.0*
Test Dates in (P-S*Log Concentration) (= error a)	18	0.00378103	99958.0*
Log Concentration Linear (common slope)	1	37.7943977	6.5*
Log Concentration Linear in (P-S) parallelism	3	0.00246159	400.0*
Log Concentration Residual in (P-S) curvature	5	0.15138078	
Runs in (P-S and Test Dates) = error b	90	0.00091552	
Run in Test Dates for Com-EDF[1]	6	0.00199545	
Run in Test Dates for Lab-EDF[2] m	4	0.00196879	
Run in Test Dates for Direct Sampling from Bag	11	0.00000395	
Run in Test Dates for Direct Injection	69	0.00090580	

*Significant at 0.05 probability level.

[1]Commercial standard acetone mixture sampled through exponential dilution flask.

[2]Laboratory prepared acetone standard sampled through the exponential dilution flask.

difference between the responses ($\alpha < 0.005$). However, the slope of the two straight lines relationship between log area and concentration were parallel. There was a quadratic effect in the injection technique. The quadratic effect in the exponential dilution flask could not be obtained since only two concentrations of acetone were used.

When the laboratory prepared acetone standard curve obtained by using the exponential dilution flask procedure was compared with the commercial standard curve obtained by the same procedure, it was found that there was a significant difference ($\alpha < 0.005$) due to the type of acetone standard used. The two linear responses were parallel. It was also found that there was a quadratic effect in the commercial standard curve obtained by utilizing the exponential dilution flask.

When the two different procedures for each of the two different acetone sources were tested for precision (Table VI), it was found that direct sampling of commercial standard acetone from the bags through the sampling loop had a smaller variance and was more precise than the procedure which utilized the exponential dilution flask (EMS = 0.00000395 and 0.00199545 respectively). The error mean square for the laboratory acetone standard curve utilizing the exponential dilution flask was found to be of the same magnitude (0.001968796 vs. 0.00199545) as that of the commercial standard curve utilizing the exponential dilution flask.

The technique (procedure) of direct injection by the use of a syringe using laboratory prepared acetone standard was compared with that which utilized the exponential dilution flask (Table VI). It was found that direct injection technique had a better precision than the exponential dilution flask technique (EMS = 0.0009058 vs. 0.00196879 respectively). Statistical analysis showed that the two procedures gave

TABLE VI

COMPARISON OF LOGARITHM OF PEAK AREAS BETWEEN EXPONENTIAL DILUTION FLASK AND DIRECT INJECTION PROCEDURES USING LABORATORY PREPARED ACETONE

Source	df	Mean Square	Values for F
Total	86		
Procedure	1	3.92287333	1038.00*
Test Dates in Procedure and Concentration (= error a)	7	0.00868842	
Log Concentration Linear	1	25.69790495	6795.00*
Log Concentration Linear in Procedure (parallelism)	1	0.00191634	0.51
Log Concentration Quadratic in Procedure	1	0.28501309	75.40*
Log Concentration Residual in Procedure	2	0.01302183	13.51*
Runs in Procedures and Concentration and Test Dates (= error b)	73	0.00096405	

*Significant at 0.005 probability level.

significantly different responses ($\alpha < 0.005$). Even though the two linear lines were parallel there was a quadratic effect present.

Analysis of the data from application of the procedure on human subjects is shown in Table VII and Figure 6. The concentration of acetone in the unknown breath samples was calculated by using the equation for the standard curve (Figure 7). The value obtained by using this curve gave essentially the same results as that obtained by direct sampling from the bags.

Discussion

The exponential dilution flask was designed to be an accurate procedure to calibrate the detector of the gas chromatograph. A sketch of two runs on the same day of log area peak with respect to times is shown in Figure 8 (Appendix F). Although the two lines essentially coincide, it was found that runs on different days do not give this precision. This study showed that direct sampling from sampling bags through the automatic gas sampling loop has a better precision.

Since there is a significant variation between test dates, it is recommended to calibrate the gas chromatograph every day when unknown samples are run.

This study demonstrates that gas chromatograph equipped with a flame ionization detector is sensitive, rapid, and reliable to measure breath acetone concentration. The concentration of acetone in the sample is given as a peak area (Figure 6) by the instrument.

Nineteen of the 46 diabetic patients studied had breath acetone concentrations ranging from 3.58 µg/L to 13.50 µg/L. Normal subjects have fasting breath acetone concentrations ranging from 0.66 µg/L to 1.10 µg/L

TABLE VII

BREATH ACETONE CONTENT OF DIABETIC PATIENTS

Patients on Insulin Therapy			Patients Without Insulin Therapy		
Initial	Serum Glucose (mg%)	Breath Acetone in µg/L	Initial	Serum Glucose (mg%)	Breath Acetone in µg/L
E.S.	230	4.40	B.G.	220	0.59 (Fasting)
C.H.	290	2.85	J.A.	170	1.16 (Fasting)
T.L.	220	0.54	T.G.	300	0.22 (Fasting)
M.B.	400	5.40	P.M.	300	3.75 (Fasting)
L.H.	220	5.30	J.E.	100	3.75 (Fasting)
M.D.	230	6.13	J.Y.	320	4.26
J.B.	120	1.88	T.H.	220	1.32
J.R.	240	4.95	R.P.	130	2.32
J.G.	240	9.06	E.H.	240	8.17
O.B.	400	0.96	G.P.	220	2.44
B.K.	270	12.73	E.G.	190	1.28
K.F.	210	4.64	C.W.	120	7.57
V.C.	120	0.97	R.H.	360	0.93
W.W.	260	2.55	L.W.	200	1.01
R.J.	240	5.75	G.J.	300	3.99
E.D.	260	13.48	H.W.	260	1.86
M.W.	220	2.00	B.B.	220	1.89
G.S.	300	0.53			
R.L.	180	1.77			
M.L.	240	3.48 (Fasting)			
H.L.	150	3.14 (Fasting)			
J.M.	380	2.36			
F.B.	340	10.18 (Fasting)			

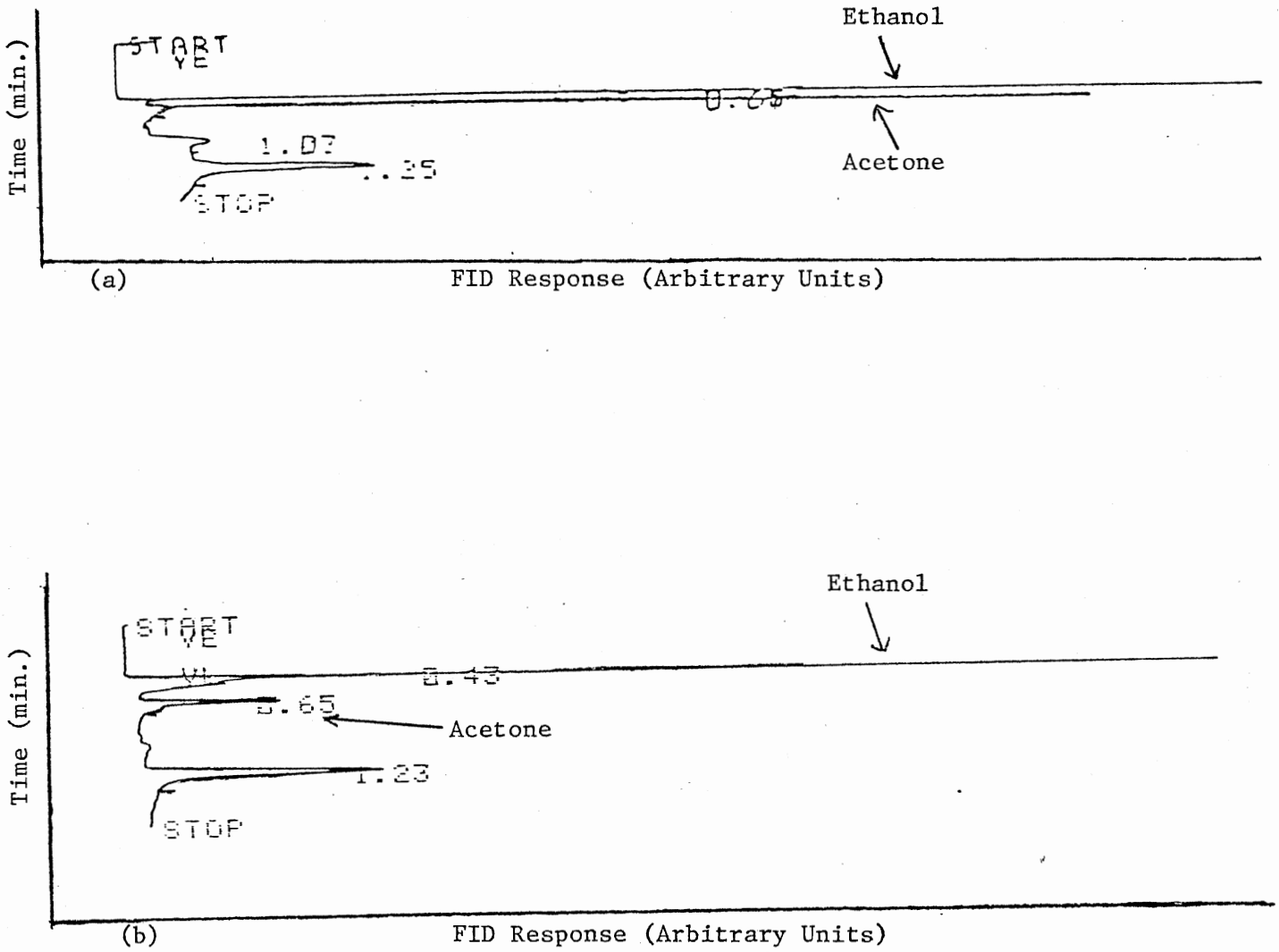
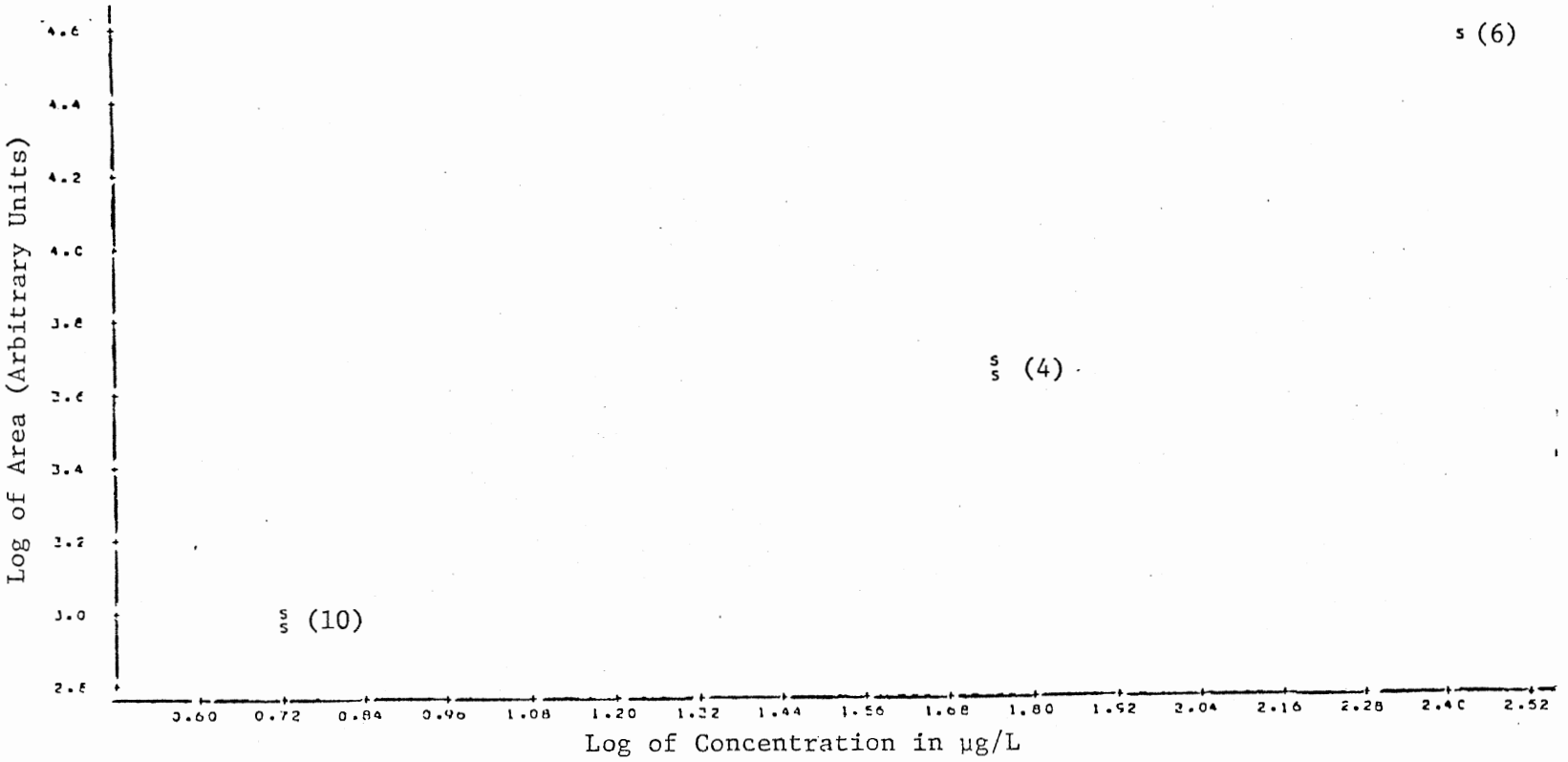


Figure 6. Typical Chromatogram of Breath Acetone Concentration of (a) a Diabetic Patient and (b) a Normal Fasting Subject



Note: Direct sampling through the automatic sampling valve from collection bags. If areas for duplicates at a given time are equal, only one value is printed. There are 17 additional observed values hidden in the above figure. The values in parentheses indicate the number of observations for the corresponding log concentration.

Figure 7. Logarithm of Reported Area of Acetone Peak Versus the Logarithm of Acetone Concentration

(Stewart and Boettner, 1964; Rooth and Ostenson, 1966); thus, these diabetic subjects had elevated breath acetone concentrations. Their serum glucose levels ranged from 100 mg% to 400 mg% (Table VII). These people were under poor metabolic control (Stewart and Boettner, 1964; Crofford et al., 1977). Patients who did not fast but were on insulin therapy showed higher concentrations of breath acetone, 13.48 µg/L (Table VII) as compared to 10.18 µg/L for those patients who fasted and were on insulin therapy. Patients who did not fast and were not on insulin therapy had higher breath concentration (8.17 µg/L) than those patients who fasted and were not on insulin therapy (3.75 µg/L). Using Crofford et al.'s (1977) classification of diabetic control, considering both serum glucose level and breath acetone concentration: 16 were overeating, 17 administered insufficient insulin, 2 consumed insufficient dietary carbohydrates, and 2 were under good metabolic control (Table VIII).

Determination of breath acetone concentrations in addition to determination of blood glucose level allows more accurate control of the diabetic state by better dietary management than determination of blood glucose level alone. In this way, control may be achieved by dietary changes rather than increases in insulin administration.

The procedures developed in this study may also have possible application in veterinary medicine to monitor breath acetone levels in animals such as dairy cattle, as well as other areas of forensic science to determine the alcohol concentration in the blood.

TABLE VIII

INTERPRETATION OF RESULTS OF BREATH ACETONE CONCENTRATION AND BLOOD GLUCOSE ANALYSES

Serum Glucose (mg%)	Breath Acetone (μg/L)	Dx	Rx
Greatly elevated (130-400)	Normal (0.22-2.85)	Overeating	Less food
Greatly elevated (150-400)	High (3.14-13.48)	Insulin insufficiency	More insulin
Not greatly elevated (100-120)	High (3.75-7.57)	Carbohydrate insufficiency	Continue if weight is objective or Reapportion diet adding more carbohydrate
Not greatly elevated (120)	Normal (0.97-1.88)	Good metabolic control	Continue same Rx

CHAPTER V

SUMMARY

A gas chromatographic method to determine human breath acetone concentration has been developed. It has been shown that as little as 0.22 µg/L of breath acetone can be detected.

Method development included four different techniques for obtaining the standard curve. Two different types of standard mixtures were used: commercial standard gas mixtures of acetone in nitrogen and head space above prepared acetone in ethyl acetate standards. The commercial standard gas mixtures were introduced into the gas chromatographic column by an automatic sampling loop both directly from especially constructed sampling bags and after exponential dilution. The head space above laboratory prepared standards were introduced into the column by syringe through an injection port and after exponential dilution through the automatic sample loop.

Statistical analysis showed that sampling directly from laboratory-constructed sample bags through the automatic sampling loop to be the more precise procedure than using the exponential dilution flask (EMS = 0.00000395 vs. 0.00199545 respectively). The head space above laboratory prepared and commercially obtained acetone standards utilizing the exponential dilution flask had the same error mean square (0.001968796 vs. 0.0019545). In addition, it was shown that direct injection by the

use of a syringe is a more precise technique than the exponential dilution flask (EMS = 0.0009058 vs. 0.001968796 respectively).

It has been shown that the four sets (Table V) appear to be quite similar in predicting area in respect to concentration. However, in spite of the parallelism of the lines, they do not coincide. Statistical analysis has shown that direct sampling from bags through the sampling loop is by far the better procedure than the one using the exponential dilution flask. Whatever procedure is chosen, it is important that the researcher is consistent.

In conclusion, the method developed was demonstrated on subjects with breath acetone concentrations ranging from 0.22 μg/L in a healthy nondiabetic to 13.5 μg/L in a poorly controlled diabetic representing about a 60-fold difference in concentration.

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APPENDIXES

APPENDIX A

CALCULATIONS OF DIFFERENT CONCENTRATIONS OF ACETONE IN ETHYL ACETATE STANDARDS

TABLE IX

INDIVIDUAL CALCULATIONS OF THE DIFFERENT CONCENTRATIONS OF ACETONE IN ETHYL ACETATE STANDARD

Acetone in Ethyl Acetate 1:500

$A_1 = 7.117$ $A_2 = 7.102$ Room temperature = 24.5°

$B_1 = 1210.595$ $B_2 = 1244.950$ = 1.18

$C_1 = 229.664$ $C_2 = 217.881$ = 1.13

$R = 0.082$ $x_1/x_2 = 2 \times 10^{-3}$ $x_1 = 0.02$

injection volume = 1×10^{-5} L $x_2 = 0.98$

$x_1/x_2 = 2 \times 10^{-3}$

$$\log P_1^o = 7.117 - \frac{1210.595}{24.5 + 299.664}$$

$$P_1^o = 255.99$$

$$a_{12} = \frac{(1.18)(255.99)}{(1.13)(92.35)}$$

$$y_1 = \frac{(2.555)(2 \times 10^{-3})}{1 + 2.555\ (2 \times 10^{-3})}$$

$$= 0.00508$$

$$P_1 = 0.00508 \times 1.18 \times 225.99$$

$$= 0.533$$

$$N_1 = \frac{(0.533)(1 \times 10^{-5}\ L)}{760 \times 0.082 \times 297.5}$$

$$= 2.873 \times 10^{-10} \text{ moles}$$

$$= 2.873 \times 10^{-1} \text{ nM}$$

TABLE IX (Continued)

Acetone in Ethyl Acetate 1:100

$$\log P_1^o = 7.117 - \frac{1210.595}{24.5 + 229.664}$$

$x_1 = 0.01$

$x_2 = 0.99$

$x_1/x_2 = 0.01$

injection volume = 1×10^{-5} L

$P_1 = 225.992$

$$a_{12} = \frac{(1.18)(225.99)}{(1.13)(92.35)}$$

$= 2.555$

$$y_1 = \frac{(2.555)(0.01)}{1 + (2.555)(0.01)}$$

$= 0.025$

$P_1 = 1.18 \times 0.01 \times 225.99$

$= 2.667$

$$N_1 = \frac{(2.667)(1 \times 10^{-5}\ L)}{760 \times 0.082 \times 297.5}$$

$= 1.438 \times 10^{-9}$ moles

$= 1.438$ nM

Acetone in Ethyl Acetate 1:50

$$\log P_1^o = 7.11714 - \frac{1210.595}{24.5 + 229.664}$$

Room temperature = 24.5°

$x_1 = 0.02$

$x_2 = 0.98$

$\gamma_1 = 1.18$

$\gamma_2 = 1.13$

$P_1 = 225.992$

TABLE IX (Continued)

Acetone in Ethyl Acetate 1:50 (Continued)

$$y_1 = \frac{(2.5554)(0.020408)}{1 + (2.554)(0.020408)}$$

$$= 0.05$$

$$P_1 = (0.02)(1.18)(225.99)$$

$$= 5.33$$

$$N_1 = \frac{(5.33)(1 \times 10^{-5}\ L)}{769 \times 0.08205 \times 297.5}$$

$$= 2.87 \times 10 \text{ moles}$$

$$= 2.87 \text{ nM}$$

Acetone in Ethyl Acetate 1:10,000

$$\log P_1^o = 7.11714 - \frac{1210.595}{22 + 229.664}$$

$$P_1^o = 202.66$$

$$y_1 = \frac{(2.592)(1 \times 10^{-4})}{1 + (2.592 \times 1 \times 10^{-4})}$$

$$= 0.0259$$

$$P_1 = 1.18\ (0.0001)(202.6645)$$

$$= 0.02391$$

$$N_1 = \frac{0.0239\ (1 \times 10^{-5}\ L)}{760 \times 0.08205 \times 295}$$

$$= 1.3 \times 10^{-11} \text{ moles}$$

$$= 1.3 \times 10^{-2} \text{ nM}$$

Room temperature = 22°C

$x_1 = 0.0001$

$x_2 = 0.9999$

$x_1/x_2 = 1 \times 10^{-4}$

Injection volume = 1×10^{-5} L

$\gamma_1 = 1.18$

$\gamma_2 = 1.13$

TABLE IX (Continued)

Acetone in Ethyl Acetate 1:5000

$$\log P_1^o = 7.11714 - \frac{1210.595}{21.5 + 229.664}$$

$$P_1^o = 198.24$$

$$y_1 = \frac{(2.599)(0.0002/0.0002)}{1 + 2.59968(0.0002/0.0002)}$$

$$= 0.0005$$

$$P_1 = 1.18 \times 0.0002 \times 198.244$$

$$= 0.046$$

$$N_1 = \frac{0.4678\ (1 \times 10^{-5}\ L)}{760 \times 0.08205 \times 294.5}$$

$$= 2.54788 \times 10^{-11} \text{ moles}$$

$$= 2.55 \times 10^{-2} \text{ nM}$$

Room temperature = 21.5°C

$x_1 = 0.0002$

$x_2 = 0.9999$

$x_1/x_2 = 0.0002$

$\gamma_1 = 1.18$

$\gamma_2 = 1.13$

Acetone in Ethyl Acetate 1:1000

$$\log P_1^o = 7.11714 - \frac{1210.595}{21.5 + 229.664}$$

$$P_1^o = 198.2444$$

$$y_1 = \frac{(2.59968)(0.001/0.999)}{1 + 2.59968(0.001/0.999)}$$

$$= 0.00259$$

$$N_1 = \frac{0.233\ (1 \times 10^{-5}\ L)}{760 \times 0.8205 \times 294.5}$$

$$= 1.2738 \times 10^{-10} \text{ moles}$$

$$= 1.2738 \times 10^{-1} \text{ nM}$$

Room temperature = 21.5°C

$x_1 = 0.001$

$x_2 = 0.999$

$x_1/x_2 = 1 \times 10^{-3}$

Injection volume = 1×10^{-5} L

$\gamma_1 = 1.18$

$\gamma_2 = 1.13$

APPENDIX B

DILUTION OF THE DIFFERENT SOURCES OF STANDARD ACETONE MIXTURES BY USING THE EXPONENTIAL DILUTION FLASK

TABLE X

DILUTION OF ACETONE STANDARDS BY USING THE EXPONENTIAL DILUTION FLASK

Flow Rate in ml/min.	Retention Time in min.	Expected Concentration (µg/l)	Area (Arbitrary Units)
	Initial Concentration = 97 µg/l		
39.8	0.65	50.0	4965
39.8	2.65	30.0	3656
39.8	4.35	20.0	2569
39.8	7.25	10.0	1430
39.8	10.15	5.0	806
39.8	13.06	2.5	469
39.8	16.86	1.0	238
39.8	19.77	0.5	150
	Initial Concentration = 5.18 µg/l		
39.8	0.65	5.0	726
39.8	2.65	3.0	435
39.8	4.35	2.0	280
39.8	7.25	1.5	211
39.8	10.15	1.0	138
39.8	13.06	0.5	58
	Initial Concentration = 56.98 µg/l		
36.8	1.17	50.0	4533
36.8	2.17	40.0	3621
36.8	5.27	20.0	1801
36.8	8.37	10.0	921
36.8	11.58	5.0	470

TABLE X (Continued)

Flow Rate in ml/min.	Retention Time in min.	Expected Concentration (µg/l)	Area (Arbitrary Units)
36.8	14.68	2.5	253
36.8	16.99	1.5	161
36.8	18.79	1.0	124
36.8	21.89	0.5	77
	Initial Concentration = 195 µg/l		
36.9	0.65	100.0	8284
36.9	2.85	60.0	5555
36.9	4.65	40.0	3794
36.9	7.75	20.0	2162
36.9	10.85	10.0	1565
36.9	14.25	5.0	846
36.9	17.15	2.5	491
36.9	19.45	1.5	378
36.9	21.25	1.0	300
36.9	24.35	0.5	202
	Initial Concentration = 274.54 µg/l		
40.1	0.97	250.0	30490
40.1	1.87	200.0	25210
40.1	3.07	150.0	19790
40.1	4.77	100.0	13850
40.1	7.67	50.0	7454
40.1	8.57	40.0	6152
40.1	9.77	30.0	4874

TABLE X (Continued)

Flow Rate in ml/min.	Retention Time in min.	Expected Concentration (µg/l)	Area (Arbitrary Units)
40.1	11.37	20.0	3559
40.1	14.28	10.0	2064
40.1	17.18	5.0	1249
40.1	20.08	2.5	778
40.1	22.18	1.5	575
40.1	23.88	1.0	465
40.1	26.69	0.5	342

APPENDIX C

DIRECT SAMPLING OF THE DIFFERENT ACETONE STANDARDS AND DETECTOR RESPONSE EXPRESSED AS PEAK AREA

TABLE XI

DIRECT SAMPLING OF COMMERCIALLY PREPARED ACETONE AND REPORTED AREA (ARBITRARY UNITS)

Concentration in µg/l	Run	Area	Date
56.98	1	4638	2-5-80
56.98	2	4667	2-5-80
56.98	1	4283	2-6-80
56.98	2	4262	2-6-80
56.98	1	4413	2-10-80
56.98	2	4461	2-10-80
56.98	1	4578	2-11-80
56.98	2	4528	2-11-80
5.18	1	954	2-5-80
5.18	2	959	2-5-80
5.18	1	954	2-6-80
5.18	2	957	2-6-80
5.18	1	963	2-10-80
5.18	2	963	2-10-80
5.18	1	960	2-11-80
5.18	2	962	2-11-80
274.54	1	36820	4-10-80
274.54	2	36530	4-10-80
274.54	1	36430	4-8-80
274.54	2	36300	4-8-80
274.54	1	36150	4-11-80
274.54	2	36390	4-11-80

TABLE XII

DIRECT INJECTION OF LABORATORY PREPARED ACETONE AND REPORTED AREA (ARBITRARY UNITS)

Concentration in µg/l	Room Temperature in °C	Attenuation	Peak Height in cm	Area
147.9	21.5	2	5.8	292
147.9	21.5	2	6.0	317
147.9	21.5	3	3.5	357
147.9	21.5	3	3.7	386
147.9	21.5	3	3.4	358
147.9	21.5	3	3.7	398
147.9	21.5	3	3.6	365
147.9	21.5	3	4.0	408
147.9	21.5	3	4.1	436
147.9	21.5	3	4.1	428
147.9	21.5	3	4.1	385
147.9	21.5	3	3.8	416
1.392×10^5	21.0	4	15.7	15120
1.392×10^5	21.0	5	15.8	14790
1.392×10^5	21.0	5	15.8	13940
1.392×10^5	21.0	5	15.8	13870
1.392×10^5	21.0	5	15.8	15110
1.392×10^5	21.0	5	15.7	14390
1.392×10^5	21.0	5	15.7	13930
1.392×10^5	21.0	5	15.7	13760
75.1	20.5	3	1.4	151
75.1	20.5	3	1.3	138

TABLE XII (Continued)

Concentration in µg/l	Room Temperature in °C	Attenuation	Peak Height in cm	Area
75.1	20.5	3	1.5	156
75.1	20.5	3	1.3	138
75.1	20.5	3	1.5	158
75.1	20.5	3	1.5	157
75.1	20.5	3	1.3	137
75.1	20.5	3	1.4	147
75.1	20.5	3	1.5	159
1.666×10^3	21.0	5	7.2	3171
1.666×10^3	21.0	4	15.2	3290
1.666×10^3	21.0	4	15.1	3322
1.666×10^3	21.0	4	15.7	3460
1.666×10^3	21.0	4	15.1	3324
1.666×10^3	21.0	4	15.7	3450
1.666×10^3	21.0	4	15.7	3434
1.666×10^3	21.0	4	15.4	3383
1.666×10^3	21.0	4	15.7	3432
1.666×10^3	21.0	4	15.6	3430
7.38×10^2	21.0	4	7.5	1377
7.38×10^2	21.0	4	7.9	1483
7.38×10^2	21.0	4	7.5	1404
7.38×10^2	21.0	4	7.7	1417
7.38×10^2	21.0	4	8.9	1640
7.38×10^2	21.0	4	8.8	1631
7.38×10^2	21.0	4	9.0	1644

TABLE XII (Continued)

Concentration in µg/l	Room Temperature in °C	Attenuation	Peak Height in cm	Area
7.38×10^2	21.0	4	8.1	1502
7.38×10^2	21.0	4	7.8	1461
7.38×10^2	21.0	4	8.4	1541
7.38×10^2	21.0	4	8.7	1585

APPENDIX D

DETECTOR RESPONSE TO BREATH ACETONE CONCENTRATION OF DIABETIC PATIENTS

TABLE XIII

BREATH ACETONE RESPONSE OF DIABETIC SUBJECTS

Patient	Bag No.	Duplicates	Area
E.D.	4	1	1068
E.D.	4	2	1036
B.K.	88	1	900
B.K.	88	2	915
J.E.	9	1	1019
J.E.	9	2	1026
R.J.	3	1	354
R.J.	3	2	401
F.B.	229	1	951
F.B.	229	2	892
R.J.	9	1	480
R.J.	9	2	479
J.E.	2	1	548
J.E.	2	2	552
E.S.	6	1	379
E.S.	6	2	381
C.H.	720	1	247
C.H.	720	2	244
T.H.	112	1	127
T.H.	112	2	164
B.B.	89	1	167
B.B.	89	2	164

TABLE XIII (Continued)

Patient	Bag No.	Duplicate	Area
R.P.	201	1	12
R.P.	200	2	12
M.B.	468	1	502
M.B.	469	2	502
E.H.	708	1	102
E.H.	708	2	102
D.S.	777	1	239
D.S.	777	2	239
L.H.	459	1	503
L.H.	461	2	503
G.P.	211	1	237
G.P.	210	2	237
M.B.A.	531	1	19
M.B.A.	537	2	19
E.C.	111	1	83
E.C.	109	2	83
C.W.	656	1	248
C.W.	660	2	248
J.B.	163	1	250
J.B.	162	2	250

APPENDIX E

BREATH ACETONE CONCENTRATION OF NORMAL FASTING SUBJECTS

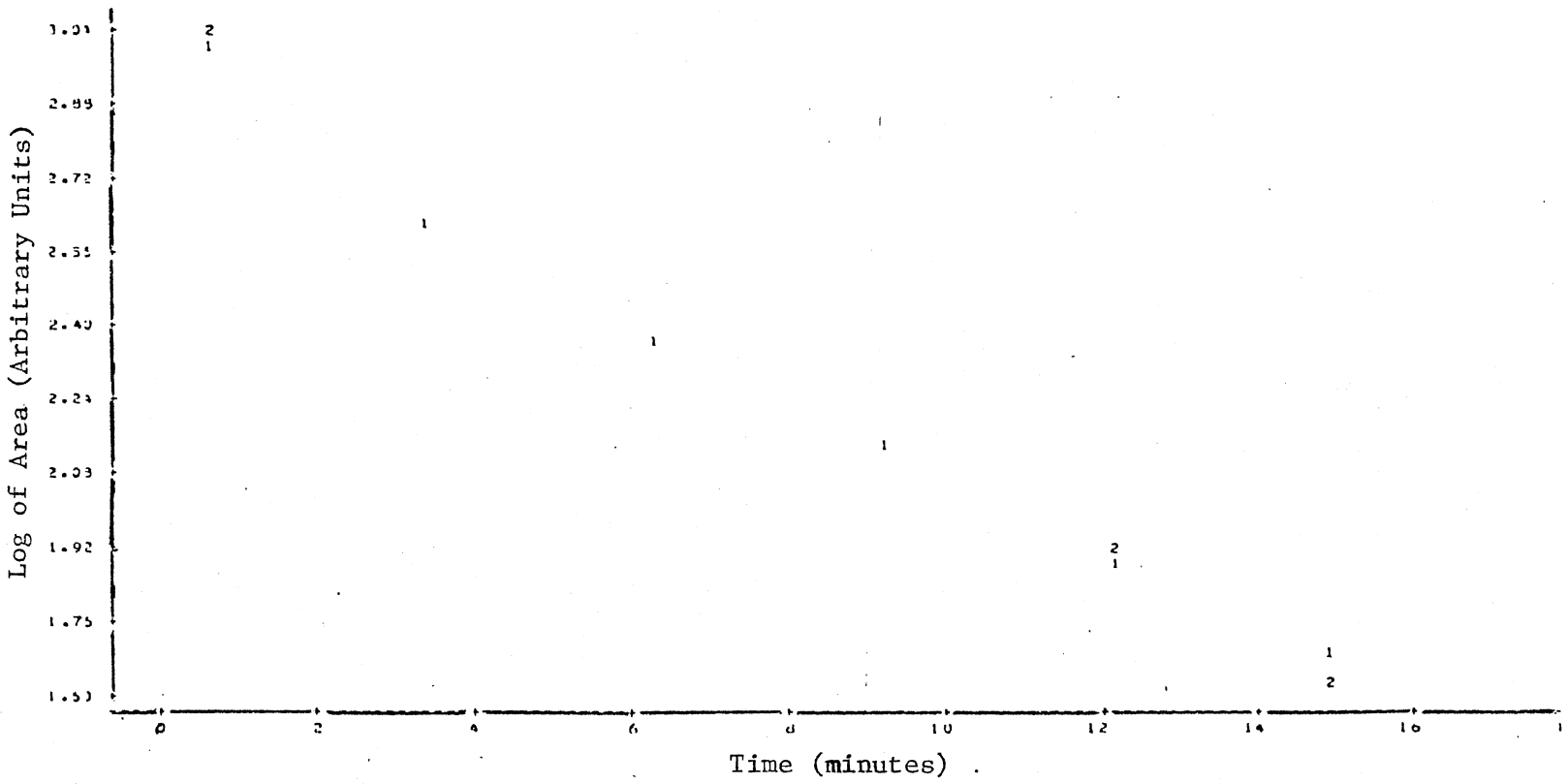
TABLE XIV

BREATH ACETONE CONCENTRATION OF NORMAL FASTING SUBJECTS

Breath Acetone (μg/l)	Initial
1.56	D.G.
1.37	A.D.
4.66	B.S.
0.77	J.M.
0.64	J.J.
0.67	J.D.
0.83	B.S.
5.97	A.T.
1.14	T.C.
0.22	B.C.

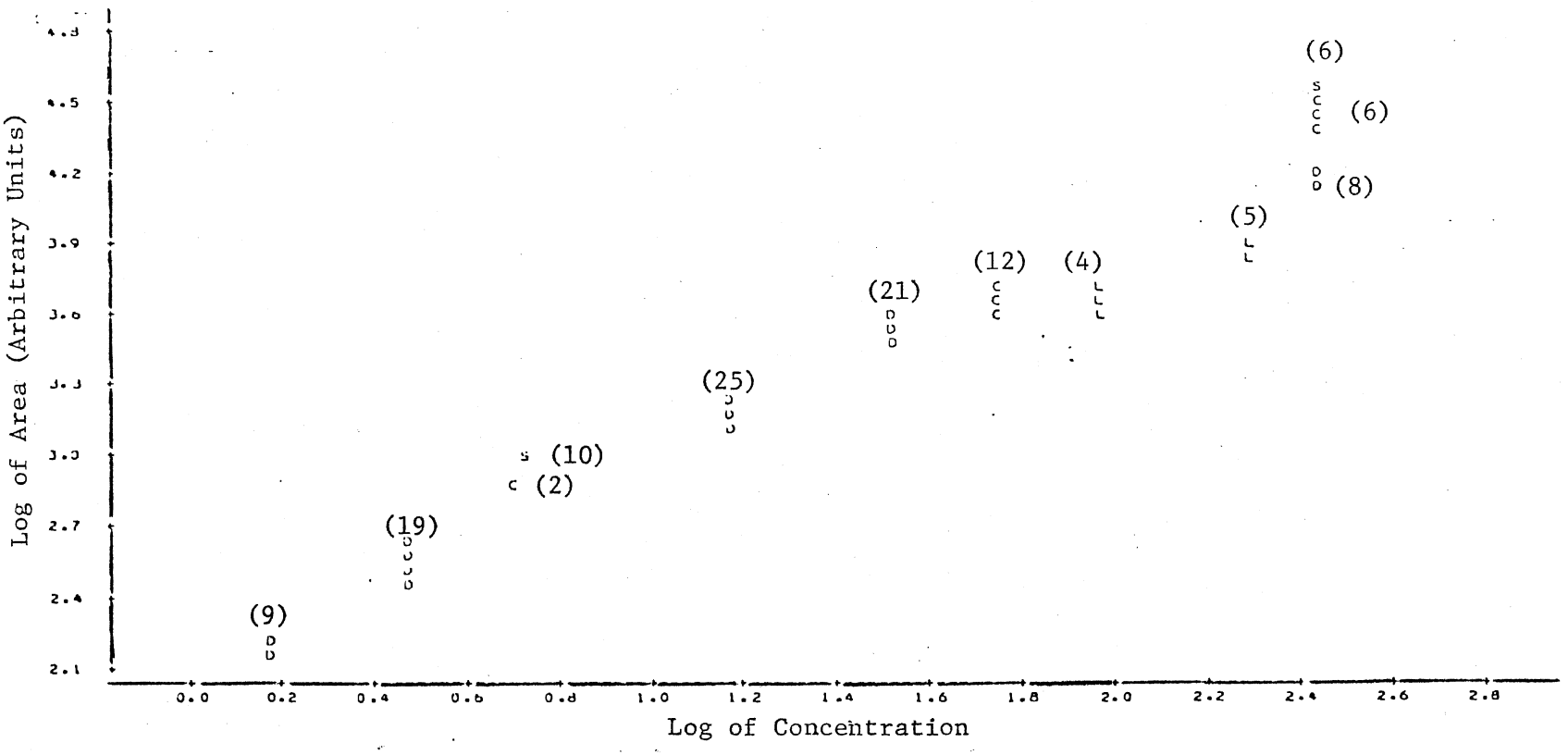
APPENDIX F

FIGURES



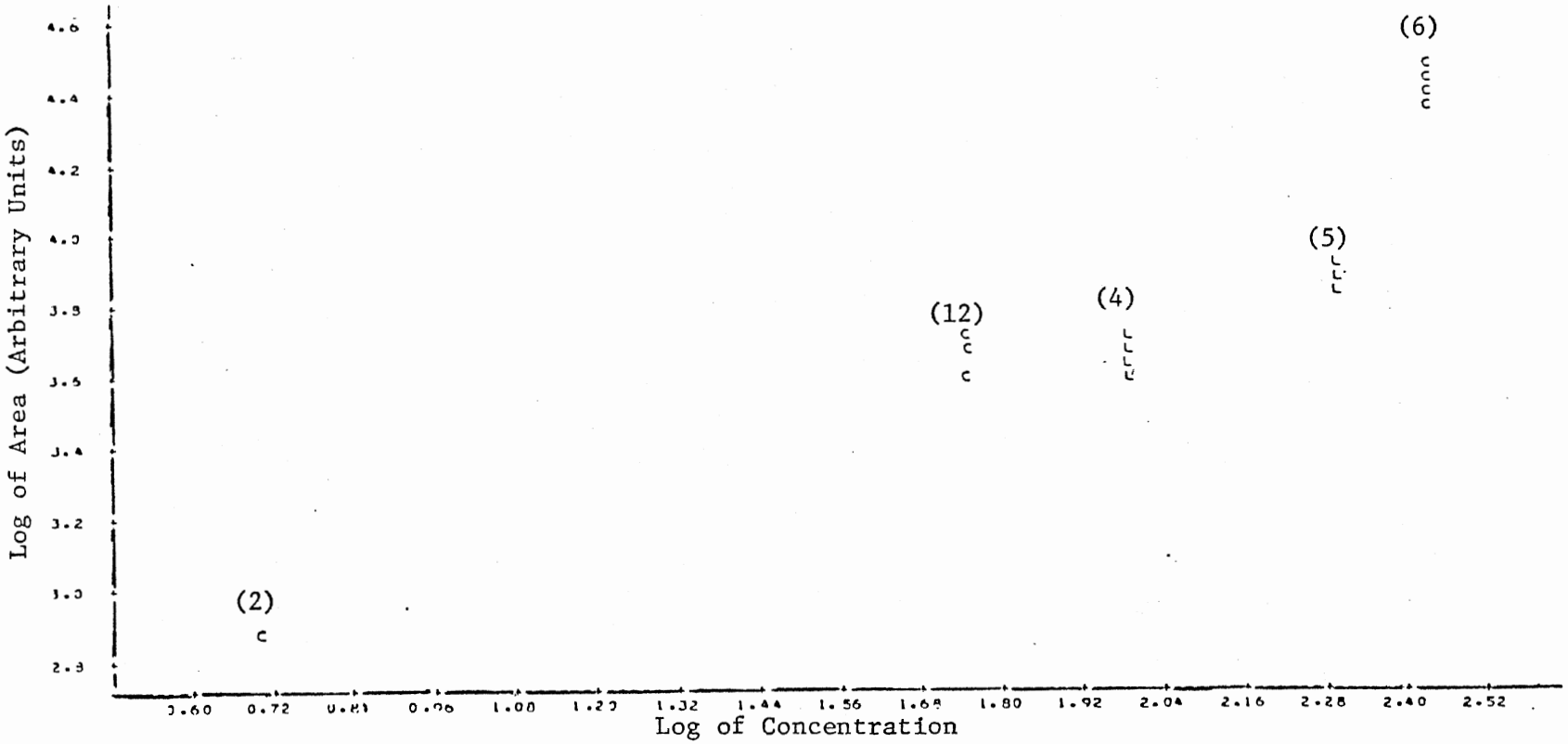
Note: 1 and 2 are duplicate samples. If the areas at a given time are equal, only one value is printed for that area. There are three hidden observed values in the above figure.

Figure 8. Logarithm of Peak Area of Acetone Versus Time of Sampling from the Exponential Dilution Flask



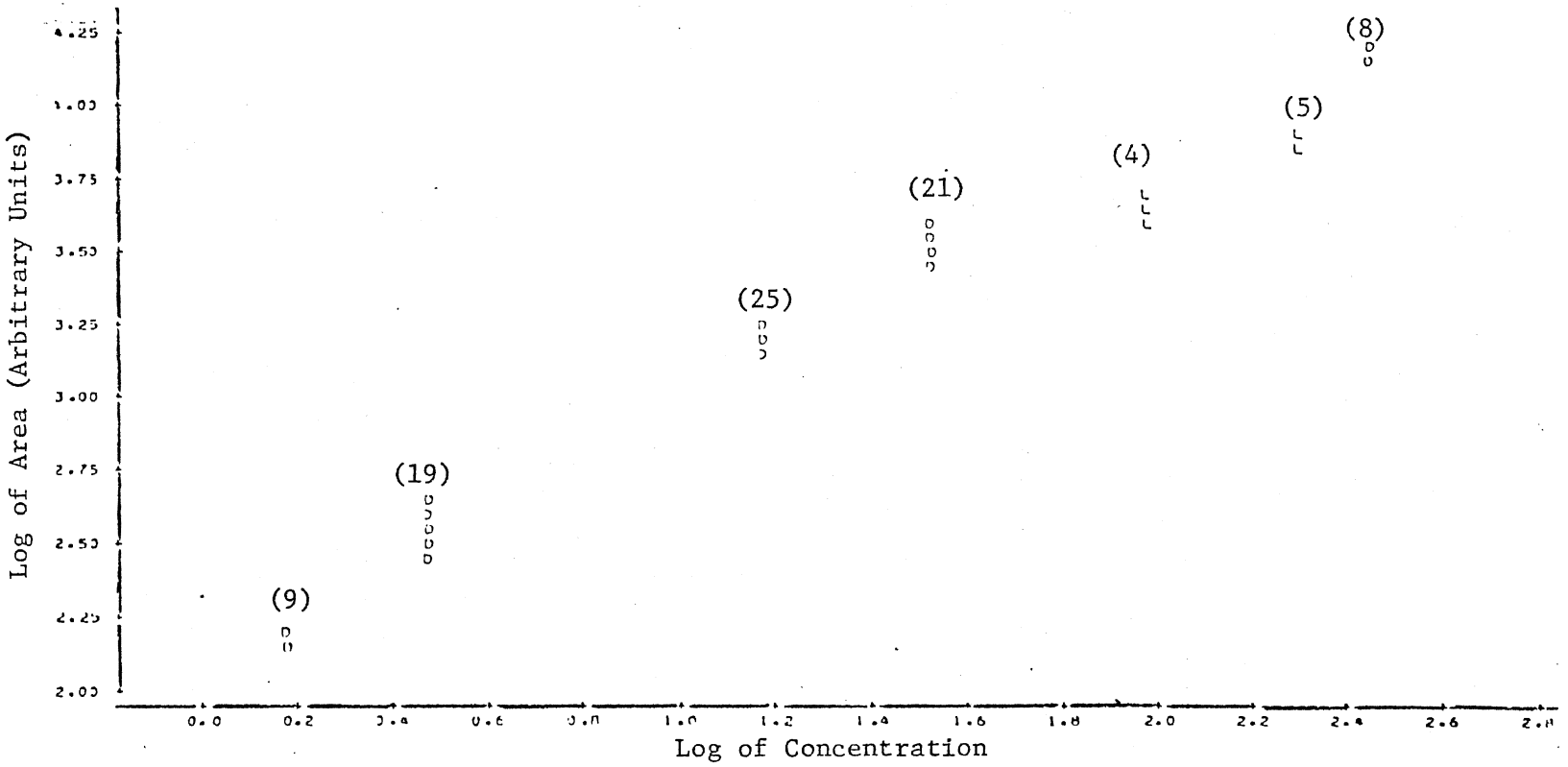
Note: If areas for duplicates at a given time are equal, only one value is printed. There are 93 observed values hidden. C = commercial acetone standard using the exponential dilution flask, D = direct injection of head space acetone in ethyl acetate standard, L = head space acetone in ethyl acetate standard using the exponential dilution flask, S = commercial acetone standard sampled directly from collection bags. The values in parentheses indicate the number of observations for the corresponding concentration.

Figure 9. Logarithm of Reported Peak Area Versus Logarithm of Acetone Concentration Using Different Sampling Procedures and Acetone Sources



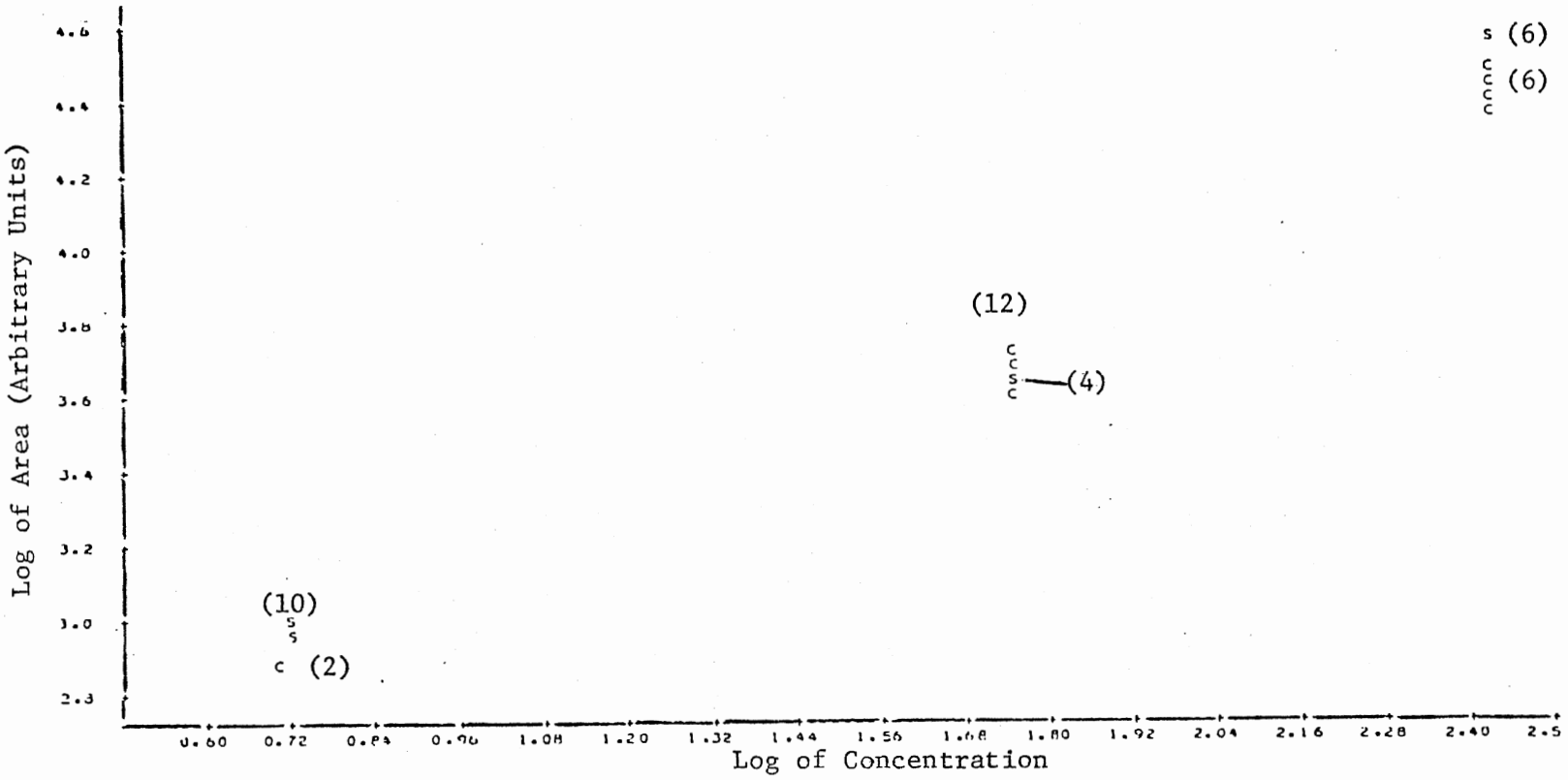
Note: If areas for duplicate samples at a given time are equal, only one value is printed. There are six additional observed values hidden. C = commercial acetone standard, L = head space acetone in ethyl acetate standard. The values in parentheses indicate the number of observations for the corresponding log concentration.

Figure 10. Logarithm of Reported Area of Acetone Peak Versus Logarithm of Acetone Concentration Using Two Types of Acetone Sources and Sampling from the Exponential Dilution Flask



Note: D = direct injection, L = sampling from the exponential dilution flask. If areas for duplicate samples are equal at a given time, only one value is printed. There are 66 additional observed values hidden. The values in parentheses indicate the number of observations for the corresponding log concentration.

Figure 11. Logarithm of Reported Area of Acetone Peak Versus Logarithm of Acetone Concentration--Comparison Between the Two Procedures (Direct Injection Versus Exponential Dilution Flask) Using Head Space Acetone in Ethyl Acetate Standard



Note: C = commercial acetone standard sampled from the exponential dilution flask, S = commercial acetone standard sampled from collection bags. If areas for duplicate samples are equal at a given time, only one value is printed. There are 22 additional observed values hidden. The values in parentheses indicate the number of observations for the corresponding log concentration.

Figure 12. Logarithm of Reported Acetone Peak Area Versus Logarithm of Acetone Concentration Using Commercial Standard Acetone and Sampling from Collection Bags and the Exponential Dilution Flask

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