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STUDY OF THE DETECTABILITY OF CONTROLLED SUBSTANCES ON BREATH

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16. Abstract

The University of Missouri used high pressure liquid chromatography plus mass spectrometry for a quantitative analysis of marihuana metabolites in blood and breath. A breath collector was developed for road-side sampling of human breath and subsequent laboratory analysis. The group also detected a previously unreported marihuana metabolite which is present in blood and breath for at least five days after smoking.

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SUMMARY

Technology based upon high pressure liquid chromatography - mass spectrometry (HPLC-MS) was developed for detecting and quantitating one nanogram of the major biologically active agent of marijuana, Δ^9-THC, in human breath. Three different studies were conducted using young male volunteers who smoked marijuana containing approximately 20 mg of Δ^9-THC. In two of the studies breath samples were taken at varying time intervals following marijuana smoking using a newly designed breath collector. The breath collector used is applicable to roadside sampling of drivers for marijunaa use and the level of intoxication.

Results obtained in the human marijuana smoking studies have revealed the presence of a previously unreported marijuana metabolite. This metabolite can be detected in the breath samples for five days after marijuana smoking. The exact length of time during which this metabolite can be detected is unknown since breath sampling ceased after five days. The levels of this metabolite in breath are apparently much higher than Δ^9-THC since its presence can be detected by the spectrophotometer used as a detector for the HPLC. Since this metabolite apparently appears only on the breath of marijuana smokers, its presence in the chromatogram unequivocally identifies a marijuana user. Furthermore, the results tend to indicate that a combined analysis of this metabolite and Δ^9-THC would give information about the amount of time which had elapsed between the marijuana smoking and actual breath sampling.

Initial correlations between blood and breath levels of Δ^9-THC as well as the marijuana metabolite demonstrated that the lungs are acting as one excretory route for these drugs. Such a finding means that other more volatile and smaller molecular weight drugs may be detected using technology developed during this study. Likewise a preliminary finding that saliva contains large amounts of Δ^9-THC holds promise for future work with other drugs using the type of sampling technique and analysis technology.

ACKNOWLEDGMENTS

This work was made possible by funds from the United States Department of Transportation, National Highway Traffic Safety Administration and the National Institute of Drug Abuse. The authors wish to thank Drs. Fred Benjamin and Robert Willette of these respective institutions for their advise during the course of this work. A special acknowledgment is also made to Dr. Kurt Duboski who encouraged us to vigorously pursue this breath analysis problem.

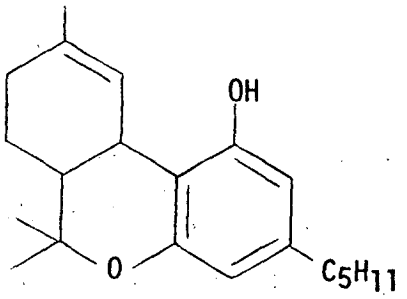
The splendid cooperation afforded us by the Marijuana Research Institute at the University of California - Los Angeles School of Medicine is also gratefully acknowledged. Project coordinators, Ms. Phyllis Lessin and Mr. Robert Nawlon, of UCLA were most cooperative and without their assistance this project could not have been successfully completed.

C O N T E N T S

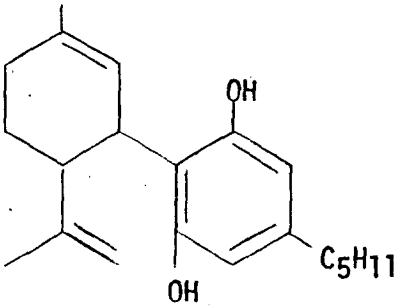
INTRODUCTION

Breath analysis has been used successfully in determining the presence of alcohol in humans. For a drug such as alcohol, breath analysis is feasible because of its high volitility and low molecular weight. Prior to the present study very little research had been done on detecting large drug molecules on breath.

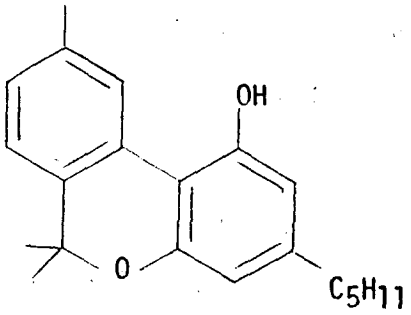
The major emphasis in the present study was to develop a method capable of detecting Δ^9-tetrahydrocannabinol (Δ^9-THC) on human breath and then to validate the method in marijuana smokers. Δ^9-THC was chosen for analysis since it is known to be one of the major psychomimetic agents in marijuana (1). Choosing Δ^9-THC as the modality of interest presented several very difficult experimental problems. First, several other research groups (2-4) had accumulated evidence pointing to the fact that Δ^9-THC occurs in relatively low amounts (less than thirty nanograms) in blood plasma following smoking of marijuana cigarette. Furthermore this peak level was reached only fifteen minutes after smoking and quickly dissipated to less than five nanograms after only two hours. Therefore, it was anticipated that levels of Δ^9-THC on the breath would likewise be exceedingly small and the method developed would have to be capable of detecting at least five nanograms or less of Δ^9-THC. The second major experimental difficulty was to develop maximum selectivity for Δ^9-THC. Marijuana commonly has four major components present (5), viz. Δ^9-THC (I), cannabidiol (CBD,II), cannabinol (CBN,III) and cannabichromene (CBC,IV). Inspection of the



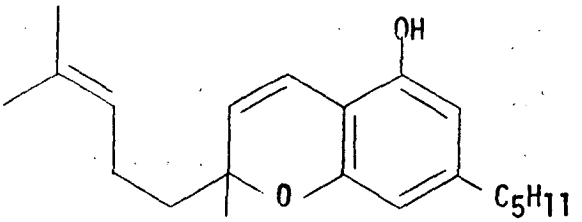
Δ^9-THC
(314)
I



CBD
(314)
II



CBN
(312)
III



CBC
(314)
IV

structural formulas readily reveals that Δ^9-THC, CBD, and CBC all have the same mass number, viz., 314 and when these are compared to CBN one observes many structural similarities. Of the four compounds only Δ^9-THC is psychomimetic (1). Therefore the technology developed had to be capable of separating Δ^9-THC from the other marijuana constituents.

EXPERIMENTAL METHODS

A. High Pressure Liquid Chromatography

High Pressure Liquid Chromatography (HPLC) analyses were conducted using a Varian 8520 liquid chromatograph utilizing a Varian 635M recording spectrophotometer as a detector. The column chosen for this work was a 10 micron silica gel, 25 cm in length and 2 mm i.d. (Varian Si-10). To adequately separate the major constituents of marijuana, a gradient elution program was developed using heptane and methylene chloride. As work progressed, it became quite obvious that purity of solvents was absolutely essential to obtain reproducible separations of the cannabinoids as well as to assure accurate MS quantitation discussed in the following section. The most direct method to insure solvent purity is to routinely take a UV spectrum of all heptane and methylene chloride purchased. Figures 1 and 2 are typical UV spectra obtained with methylene chloride and heptane, respectively. In each figure the lower curve illustrates the purity desired, whereas the upper curve is typical of unacceptable material. Thus this routine check of solvent purity revealed any impurities which might have interferred with determination of the cannabinoids.

The gradient elution program developed for the separation of the cannabinoids was optimized so as to allow a clear distinction between Δ^9-THC, CBD, CBN AND CBC. This gradient elution program requires a total of 18 minutes to complete. Initially the program begins with a 95:5 percent, heptane:methylene chloride mixture and proceeds to 95:5 percent methylene

chloride:heptane mixture over a 9 minute period. Once the 95:5 percent methylene chloride:heptane composition has been reached, the program is reversed to the initial 95:5 percent heptane:methylene chloride mixture, thereby regenerating the column. A solvent flow rate of 120 ml per hour was used for all determinations. Figure 3 illustrates the gradient program by the dark line which is superimposed upon the HPLC chromatogram of a mixture of Δ^9-THC, CBN, CBD and CBC. For example, Δ^9-THC appears at a retention time of 4.3 minutes or at a gradient elution mixture (GEM) of 52:48 percent, methylene chloride:heptane. Likewise, the retention time and GEM of the other cannabinoids can be ascertained from this figure.

Using the above described program and column, it was found that maximum resolution of cannabinoids was achieved if the sample to be injected onto the column was in heptane of the quality used in the GEM. The amount of heptane used to reconstitute the sample was not critical since as much as 500 µl has been injected without loss of resolution. In contrast, if methanol was used for reconstitution of samples, resolution of the cannabinoids was affected as evidenced by broad peaks on the chromatograms.

As discussed earlier, a spectrophotometer was used as the detector for the effluent emanating from the HPLC column. Since the spectrophotometer used had scanning capabilities, it was determined by stop-flow techniques that the maximum UV absorbance for Δ^9-THC in the GEM was 273.7 mµ. Thus all chromatograms were recorded at this wavelength, e.g., Figure 3. Using this wavelength it is possible to detect 100 ng of Δ^9-THC, but as pointed out in an earlier section the blood plasma levels were shown to be one-fifth of this amount.

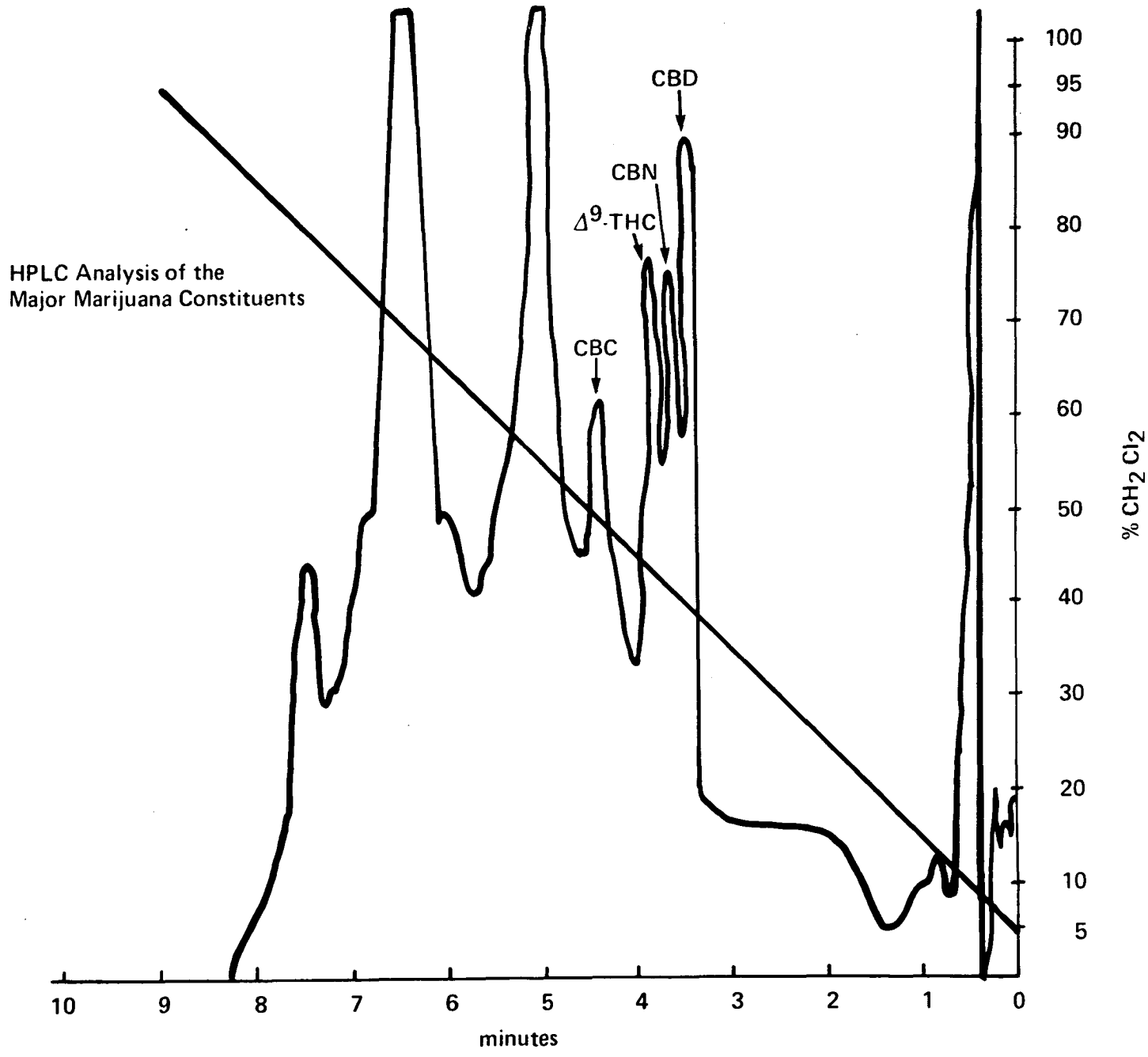

HPLC Analysis of the
Major Marijuana Constituents
CBD
CBN
Δ9-THC
CBC
100
95
90
80
70
60
50
40
30
20
10
5
% CH2 Cl2
10
9
8
7
6
5
4
3
2
1
0
minutes

Figure 1

Therefore in the present work a small amount of deuterated Δ^9-THC (d_3-Δ^9-THC) was used to aid in UV detection of Δ^9-THC. Also as will be pointed out in the following section, this allowed quantitative measurements to be made with the MS. A 10 cm "zero-dead-volume" stainless steel tube was attached to the flow cell of the spectrophotometer to facilitate collection of the effluent droplets almost instantaneously after they had passed through the flow cell.

B. Mass Spectrometer Quantification.

Mass spectrometry (MS) work was accomplished using a Varian MAT SMI-B, high resolution, double-focus mass spectrometer. A new ion-counting technique was developed in conjunction with the peak matching accessory which provided for a rapid (essentially continuous) comparison between data from the internal standard (d_3-Δ^9-THC, mass 317) and the assayed compound (Δ^9-THC, mass 314). As was noted earlier, a known amount of d_3-Δ^9-THC is added to each sample to be analyzed for Δ^9-THC content prior to HPLC analysis. Each sample coming from the HPLC is introduced into the mass spectrometer and the instrument is initially focused exactly on the 317 (d_3-Δ^9-THC) mass signal, then through the action of the peak matching unit, and with the high resolution capability alternately focused to the 314 (Δ^9-THC) mass signal, cf., Figure 4. As this alternation from one signal to another occurs the exact number of ion counts for each compound is recorded and stored in two channels of a dedicated computer. The ion counter-integrator (computer) used was a Princeton Applied Research model -SSR 1110. This unit performs a summation of the number of ion events occurring in both mass peaks (314 and 317) and stores these in two registers. Thus a running total of ions detected from the 317 internal standard

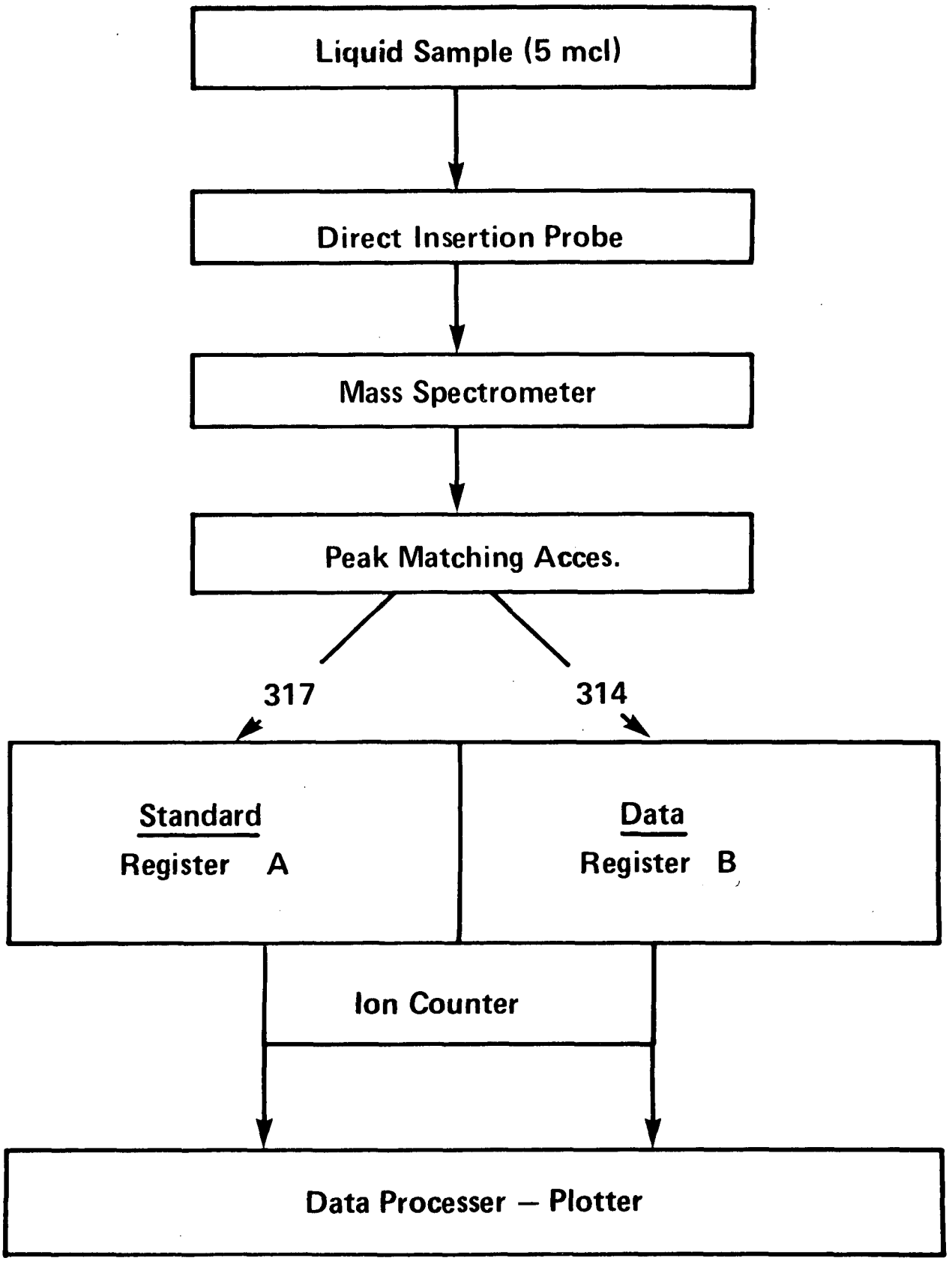

Liquid Sample (5 mcl)
Direct Insertion Probe
Mass Spectrometer
Peak Matching Acces.
317
314
Standard
Register A
Data
Register B
Ion Counter
Data Processer – Plotter

Figure 2

and the unknown amount of the 314 mass are stored separately by the counter as shown schematically in Figure 5. The peak matcher accessory is set to dwell for 67 milliseconds on each mass signal before it alternates to the other mass signal. Repeated experimentation has shown that a total counting time of 67 seconds produces good statistical data values. Thus in 67 seconds the alternating cycle is repeated 500 times yielding 1000 bits of data for comparison and quantification.

The temperature at which the Δ^9-THC sample is run proved to be quite critical. After considerable experimentation, it was found that a probe temperature of 65°C combined with the 67 seconds counting time gave optimum results. Figure 6 illustrates how the 314/317 ratio remains constant with time even as the sample is being depleted. Thus the ratio based upon the internal standard remains linear and provides for dependable quantification. The slope of the line drawn through these data points yields the 314/317 ratio or the percent 314 present.

Using the peak matching and ion counting methods just described, several experiments were conducted with a large number of samples to demonstrate validity and reproducibility of the technique. First a known amount of d_3-Δ^9-THC (1.6 mcg) was introduced into the MS via the direct insertion probe and the 314/317 ratio determined. A determination of this ratio was feasible since the d_3-Δ^9-THC contained a small but constant level of undeuterated Δ^9-THC. The data in Table I summarizes the results of this experiment. As shown, the contribution of Δ^9-THC to the 314/317 ratio is 13.8 ± 0.6 ng. A second experiment was conducted to determine what, if any, contribution was made to the 314/317 ratio when a sample of d_3-Δ^9-THC was placed on the HPLC instrument

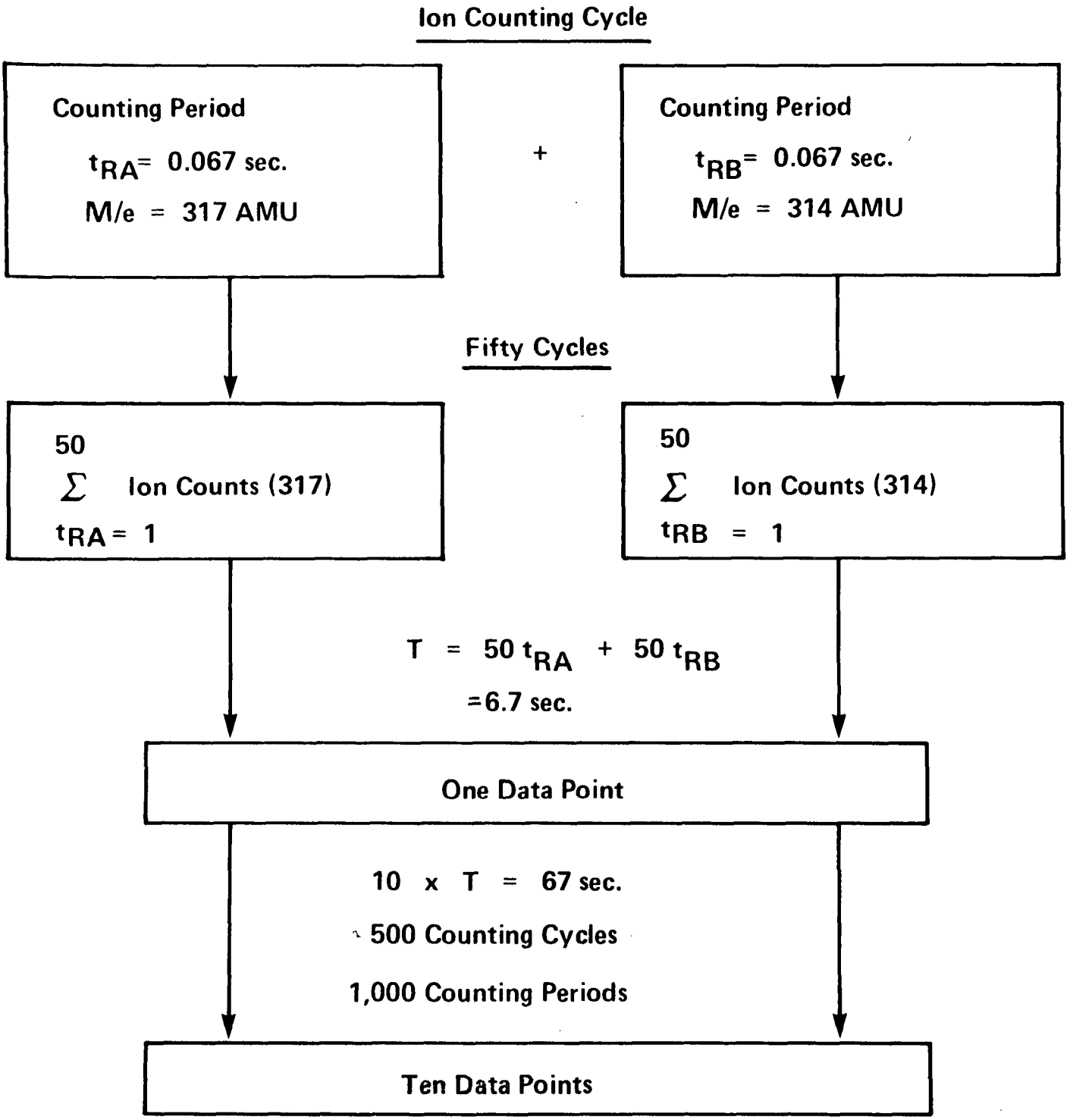

Ion Counting Cycle
Counting Period
t_{RA}= 0.067 sec.
M/e = 317 AMU
+
Counting Period
t_{RB}= 0.067 sec.
M/e = 314 AMU
Fifty Cycles
50
Σ Ion Counts (317)
t_{RA} = 1
50
Σ Ion Counts (314)
t_{RB} = 1
T = 50 t_{RA} + 50 t_{RB}
=6.7 sec.
One Data Point
10 x T = 67 sec.
500 Counting Cycles
1,000 Counting Periods
Ten Data Points

Figure 3

followed by fraction collection and MS analysis. Table II is a summary of this data. As shown, very little contribution is attributable to the HPLC method over and above that found by the direct analysis shown in Table I. Thus the mobile phase collected from the HPLC did not significantly affect the overall results.

Table I. Mass Spectral Analysis of 1600 ng of d_3-Δ^9-THC.

Sample No.	314/317%	ng of Δ^9-THC Found
1	0.865	13.8
2	0.914	14.6
3	0.815	13.0
4	0.848	13.6
5	0.895	14.3
6	0.817	13.1
7	0.865	13.8
Average	0.860	13.8
Standard Deviation	±0.037	±0.6
% Variation	±4.3	±4.3

Table II. Mass Spectral Analysis of 1600 ng of d_3-Δ^9-THC Injected Onto and Collected From the HPLC.

Sample No.	314/317%	ng of Δ^9-THC Found
1	0.90	14.40
2	1.02	16.32
3	0.93	14.88
4	0.81	12.96
5	0.89	14.24
6	0.80	12.80
7	0.86	13.76
8	0.91	14.56
Average	0.89	14.24
Standard Deviation	±0.07	±0.06
% Variation	±7.8	±1.06

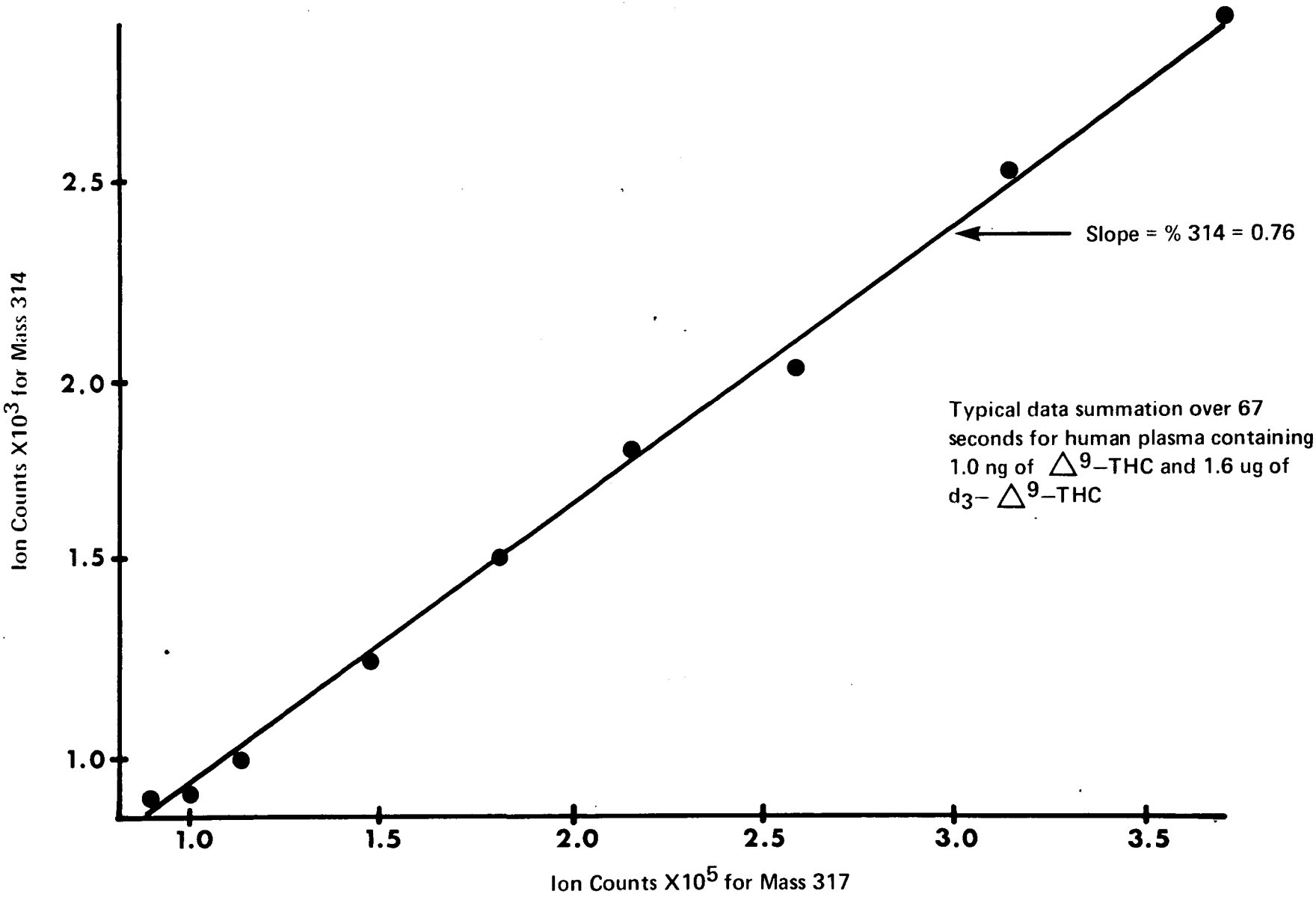

Slope = % 314 = 0.76
Typical data summation over 67 seconds for human plasma containing 1.0 ng of Δ9–THC and 1.6 ug of d3– Δ9–THC
Ion Counts X10^3 for Mass 314
Ion Counts X10^5 for Mass 317
2.5
2.0
1.5
1.0
1.0
1.5
2.0
2.5
3.0
3.5

Figure 4

C. Methods of Breath Analysis.

One objective in the present study was to design and test the applicability of an apparatus capable of collecting Δ^9-THC for subsequent analysis. In a normal human male about 0.5 liters of air is expired in a complete expiration of breath (6). Any Δ^9-THC which might be contained in this volume of expired air would be exceedingly dilute. Thus some device which is capable of concentrating the Δ^9-THC was felt to be absolutely essential. Two concentration methods were evaluated and are discussed below.

1. Polyethylene Foam Wafer

Several years ago the Bendix Corporation in Kansas City developed a process for producing polyethylene which contained varying pore sizes (7). The size pore was adjusted by using sized NaCl crystals during the molding process. Later these salt crystals were removed by allowing the polyethylene to stand in water over approximately a one week period of time. Experimental results indicated that the pore size produced by unsized table salt gave the least resistance to expired air. The polyethylene foam wafers used in the human experiments described later were cut so as to give a disc 3.0 cm in diameter and 0.25 cm thick. Further results from the clinical trials indicated that the hot molded polyethylene wafers were most practical because of their mechanical strength and durability. Figure 7 illustrates a cross-sectional view of a polyethylene foam wafer. In theory this wafer should allow small airborne gases such as oxygen, nitrogen, sulfur dioxide, certain hydrocarbons, etc., to pass through while retaining the larger organic molecules. Collect-

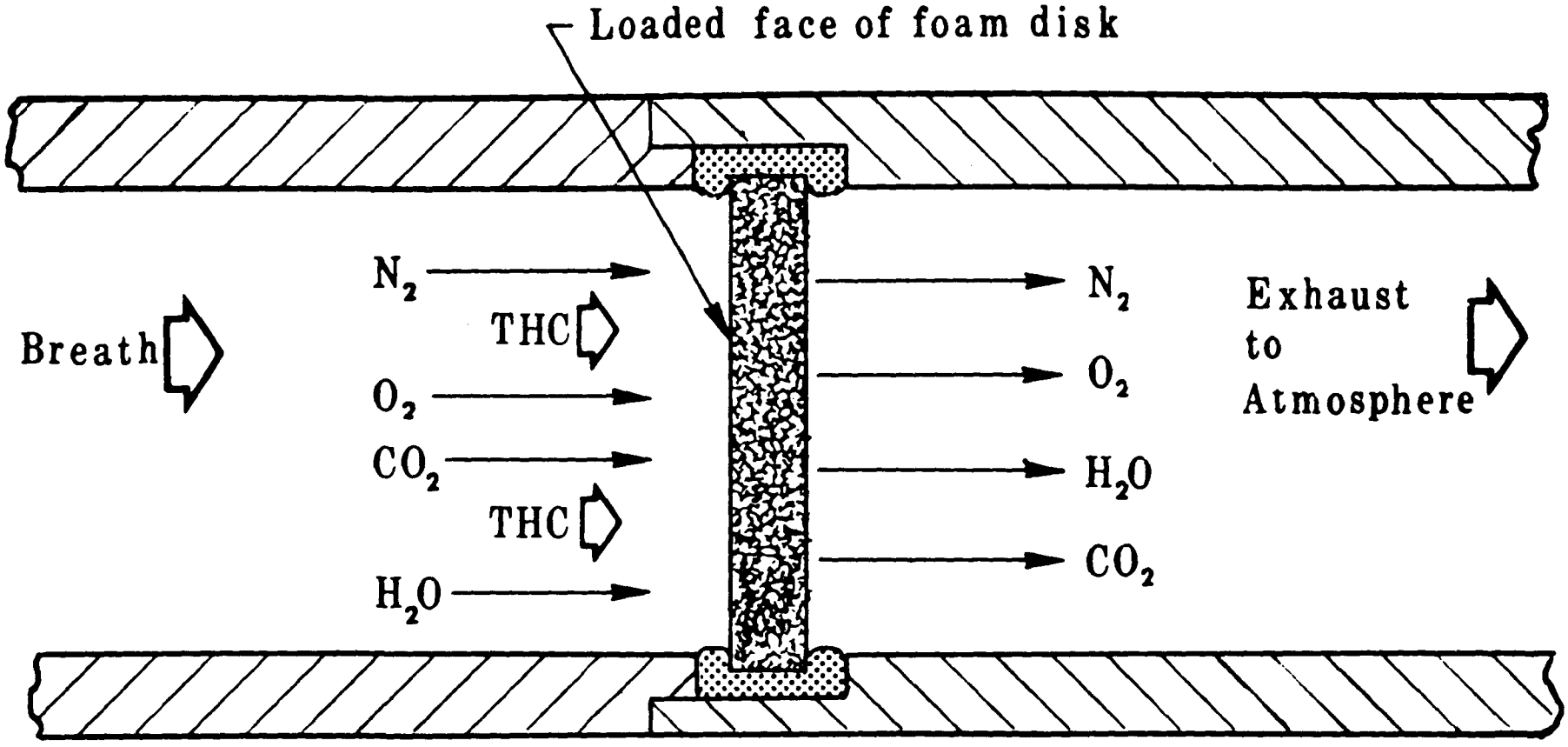

Loaded face of foam disk
Breath
N_2
THC
O_2
CO_2
THC
H_2O
N_2
O_2
H_2O
CO_2
Exhaust to Atmosphere

Figure 5

ing of Δ^9-THC could simply be a physical phenomena due to the tortuous pathway or it might possibly be a chemical (e.g., van der Waals attraction) bonding. The later cases may be the predominant mode since Δ^9-THC has been shown to bind to polyethylene (8).

For the human studies described later, a rather simple device was devised to hold the polyethylene foam wafer. This device consisted of a standard canister respirator mask (Welch model 7500-30G) which was modified by placing a 2.5 cm diameter hole directly in front of the mouth. On the interior of the mask was placed a 4.5 cm (o.d.) by 2.0 cm thick rubber ring of sufficent flexibility to firmly hold the polyethylene foam wafer. Thus the foam wafer was held directly in front of the subject's mouth and approximately 1.5 cm away from the lips. In addition a small flapper valve was placed over the outer hole so as to prevent inhaled air from being drawn back through the wafer. Figure 8 gives a cross sectional view of this modified mask.

2. Ethanol Breath Tube.

An earlier worker (9) in the field of breath analysis had successfully used a "cold trap" to condense airbone breath particles. Since Δ^9-THC was known to be exceedingly soluble in ethyl alcohol (8) a decision was made to combine the two methods and collect breath samples through ethanol which was contained inside a cold trap. After experimenting with a number of prototype devices, the final choice was a "cold-finger" vacuum trap. This device is shown in Figure 9. In the bottom of the vacuum trap is placed 10 ml of ethanol and the entire trap is immersed in a dry ice-acetone bath. Commercially avail-

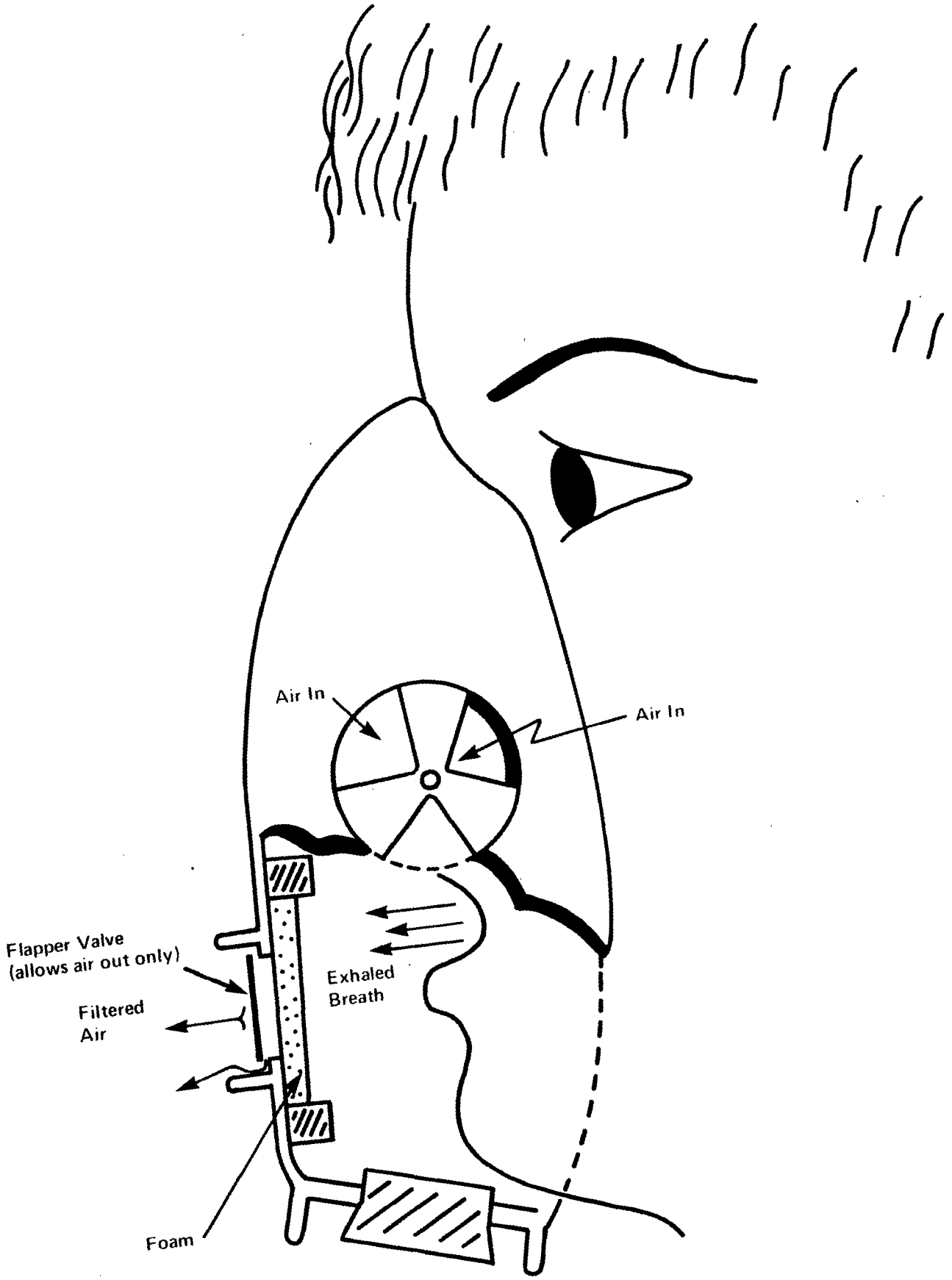
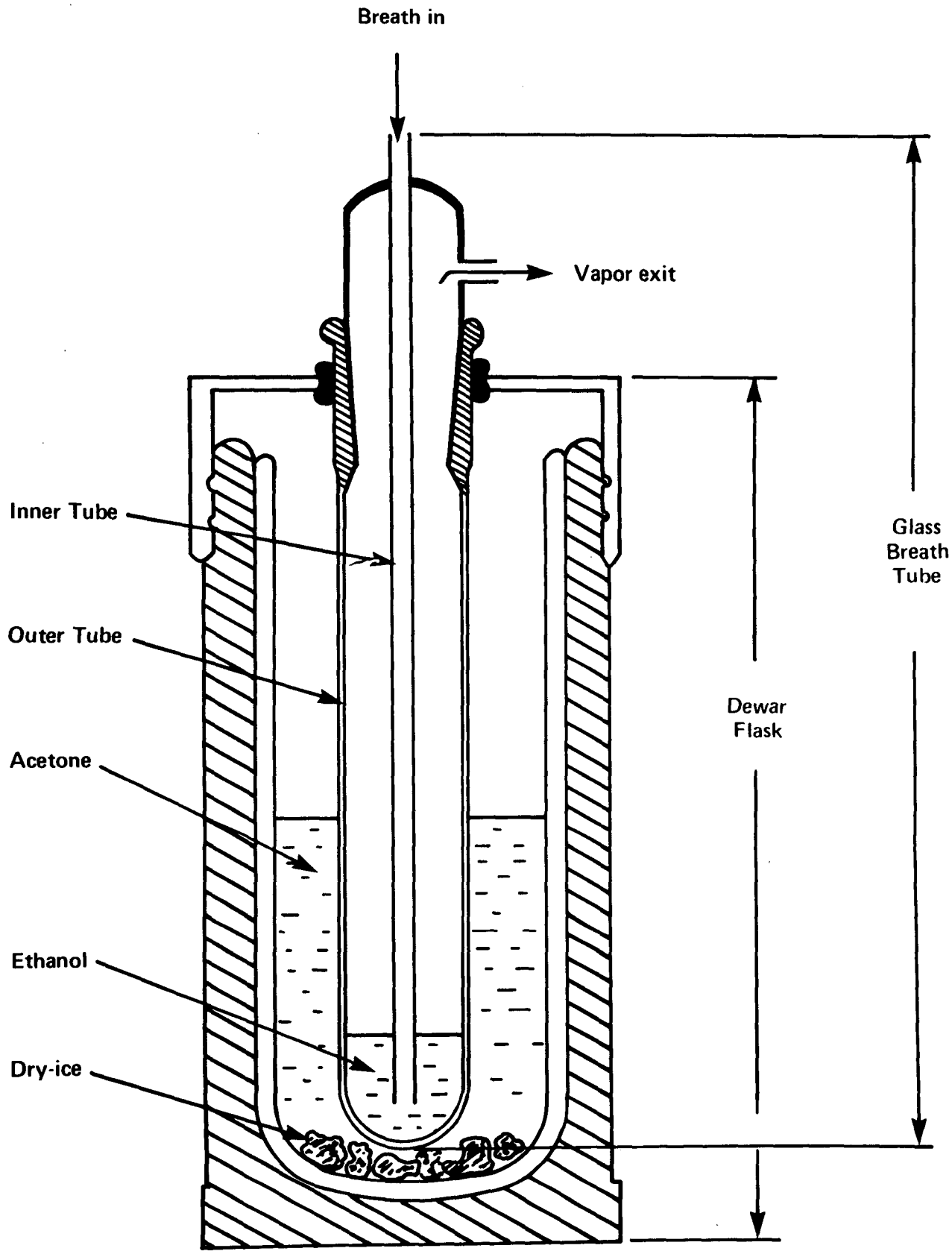

Air In
Air In
Flapper Valve
(allows air out only)
Filtered
Air
Exhaled
Breath
Foam

Figure 6



ETHANOL BREATH APPARATUS

Figure 7

able thermos bottles were modified for use as the bath by placing a hole in the top of the screw cap. This hole was lined with a small piece of rubber and the vacuum trap placed inside the thermos bottle with only the inlet and exit tubes exposed.

D. Human Marijuana Smoking Studies.

Two different studies were conducted with human volunteers who smoked marijuana cigarettes. One of these studies was conducted at the University of California at Los Angeles School of Medicine, while the other was at the Midwest Research Institute in Kansas City. Each study is discussed separately below.

1. Study at University of California - Los Angeles.

The Marijuana Research Institute at UCLA School of Medicine twice invited our research group to participate in marijuana smoking studies being conducted there. On January 23 and April 3, 1975 smoking experiments were performed using six male volunteers for each study. The subjects were brought into the hospital ward 12 hours prior to smoking. The subjects were not allowed any food or drink after 12:00 a.m. prior to smoking. At approximately 8:00 a.m. of the test day, each subject had a heparin-lock "butterfly" placed in a forearm vein. At this time a 5 ml blood sample was drawn and placed in a silinized green top vacutainer. Each blood sample was handled in such a manner that the blood did not contact the rubber stopper. All blood samples were immediately centrifuged at 2600 rpm and the plasma removed and placed in a second silinized tube and frozen for later analysis. The complete analysis of these plasma samples will be detailed in a subsequent report on another project.

After the control blood was drawn from each subject a control breath and saliva sample was taken from each individual. Saliva samples were exudated into silinized glass tubes, stoppered and frozen immediately. Breath samples were collected in two different manners. During the January 23 study samples were collected using the apparatus described in C-1 above. Each subject was asked to breathe for 1 minute with the mask positioned over the nose and mouth using deep inhalations and exhalations through the mouth. The polyethylene foam wafer was placed in, and taken from, the mask using disposable examination gloves. For transportation purposes, each wafer was placed in a small silinized petri dish and transported back to Kansas City for analysis. Once the wafers were returned to Kansas City they were stored under refrigeration until analysis. Using these techniques, breath and saliva were taken at 0, 0.25, 0.5, 1, 2, 3, 4, 8 and 12 hours.

Results from the January 23 study (discussed later) suggested that revisions should be made in sampling techniques and handling of samples once they were collected. Thus during the April 8 study, the breath sampling technique described in C-1 was altered so that breath samples were taken at 0, 0.5, 1, 2, 3, 4, 8, 12, 24, 48, 72, 96 and 120 hours. Also each polyethylene foam wafer was extracted with methanol via methods described in the following section immediately after the sample was procured. In addition, the ethanol breath tube method described in C-2 was used to collect breath samples at 0 and 0.25 hours by asking each subject to exhale through the breath tube twice. In exhaling each subject was asked to completely expire the air in the lungs. Compliance was easy to monitor since the cooled

breath vapors coming out of the exit tube were clearly visible.

2. Study at Midwest Research Institute (MRI).

A total of twenty subjects were used in the marijuana smoking studies at MRI. There were no facilities for quarantining the subjects in this study. Thus the smokers were asked to report at an appointed time and a breath sample was taken prior to and 15 minutes after smoking. Most subjects were released after their 15 minute samples with only a few remaining for 1 hour samples. All breath samples collected in this study were obtained using the ethanol breath tube method described in C-2.

E. Analysis of Samples from Human Studies.

The major objective of the present project was to detect and explore quantitation of Δ^9-THC on human breath following marijuana smoking. Therefore, saliva samples (which were not called for in the work-order) have not been completely processed. However, saliva and blood from one individual have been processed for correlation purposes with breath. The complete procedure for processing these samples will be covered in a subsequent report. Processing of the breath samples was accomplished somewhat differently depending on whether method C-1 or C-2 was used. Both are described below:

1. Analysis of Polyethylene Foam Wafers.

The polyethylene foam wafers were either processed after shipment to Kansas City or immediately after smoking depending on the particular study (cf., F-1 and F-2). No matter which modality was used, the process was the same. Thus, each wafer was subjected to 30 minutes of ultra-sonication while submerged in 10 ml of methanol in a silinized

50 ml beaker. The methanol was decanted into a silinized test tube and stored at -5°C until analysis. To each sample was added 1.6 µg of d_3-Δ^9-THC. After evaporation of the methanol under nitrogen at room temperature the residue was reconstituted immediately in 300 µl of heptane. The solution was injected onto the HPLC followed by 200 µl of heptane used to wash the test tube. The HPLC gradient elution program was run as described earlier and the eluent collected when the recorder tracing of the spectrophotometer indicated the presence of Δ^9-THC in the HPLC effluent. A 3 ml silinized Reacti-Vial^R was used to collect each sample of effluent. These vials which each contained about 1.5 ml of effluent were stored at -5°C until MS analysis. Just prior to MS analysis the sample was concentrated to approximately 10 µl volume under nitrogen. This solution was transfered portionwise to a 5 µl gold cup allowing the solvent to air evaporate after each transfer. The Reacti-Vial^R was washed with 10 µl of methanol and the washings added to the gold cup. After evaporation of the solvents the gold cup was attached to the direct insertion probe of the MS and inserted for analysis as described earlier.

2. Analysis of the Ethanol Breath Tube Contents.

After a breath sample was collected, the inner tube of the breath apparatus was washed with 1 ml of ethanol and this washing added to the ethanol sample. The combined sample was transferred to a silinized tube, followed by a 1 ml ethanol washing and stored at -5°C until analysis. To each tube was added 1.6 µg of d_3-Δ^9-THC and the solution

evaporated to dryness under nitrogen at room temperature. The resultant residue was reconstituted in 300 µl of heptane and then analyzed by HPLC-MS as described above.

RESULTS

Using the developed HPLC-MS analysis techniques, the breath of numerous subjects following marijuana smoking have been evaluated. Analysis of data from these individuals is rather random depending upon which institution the smoking studies were done. Also, the ethanol breath tube method was not time coursed as was the polyethylene foam wafer data. Therefore, results will be subdivided depending upon which breath collecting technique was employed.

A. Polyethylene Foam Wafer Breath Apparatus.

During the January 23, UCLA study, the breath samples of six subjects were collected at 0, 0.25, 0.5, 1, 2, 3, 4 and 12 hours. Since this study was the first done, the sampling and analytical technique were not completely perfected and some errors were introduced inadvertantly. The major difference in the sampling technique used in this study and that of April 8 was in sample handling. Thus each subject was asked to breathe through the breath apparatus for 1 minute. The polyethylene foam wafer was removed from the breath apparatus and depending on the study date handled as follows:

January 23 - The wafer was placed in a silinized glass container and transported back to Kansas City. The glass containers were stored at -5°C for 3 weeks before being processed, as described below for the next study date.

April 8 - Following breath collection, the wafer was immediately placed in 10 ml of methanol and ultra-sonicated as previously described.

Data thus obtained for 8 subjects up to 4 hours is reported in Table III.

Table III. Nanograms of Δ^9-THC Found Per One Minute Breathing Following Marijuana Smoking Using the Polyethylene Foam Wafer.

Subject Number	Study Date	TIME (hours) 0.25	0.5	1.0	2.0	3.0	4.0
1	4-8	--	0.0	27.8	3.8	5.3	6.0
2	4-8	--	4.9	0.4	1.8	2.3	8.7
3	4-8	--	3.9	4.4	6.3	5.9	4.8
4	4-8	--	0.3	4.5	---	---	---
5	4-8	--	1.6	6.2	---	---	---
6	4-8	--	4.1	3.0	---	---	---
7	1-23	--	15.3	---	5.4	12.9	2.7
8	1-23	0.0	0.0	11.8	5.5	4.5	7.1

During the gathering of data such as that shown in Table III, it became quite evident that another compound, i.e., a metabolite of marijuana was also appearing on breath. That is, one additional peak appeared in the chromatograms of marijuana smokers which was not present in non-marijuana smokers. The information on non-marijuana smokers was obtained using the breath samples of 10 laboratory workers. Four of these individuals were cigarette smokers and seven were coffee drinkers. Three of these workers were sampled immediately after eating while the cigarette smokers were

sampled immediately after smoking. In none of the laboratory workers was the peak shown which was present in marijuana smokers. An HPLC recording of a typical non-marijuana smoker is found in Figure 10.

This marijuana metabolite will hereafter be referred to as the t_{R_2}3.1 metabolite. This designation signifies the time in minutes, i.e., the retention time on the column, after injection until the metabolite appears in the effluent. Once the presence of this metabolite became obvious, a systematic study was undertaken to show the relationship between this metabolite and Δ^9-THC in the breath. Figure 11 shows the relationship which exists between the breath levels of Δ^9-THC and the t_{R_2}3.1 metabolite of marijuana following smoking. The t_{R_2}3.1 metabolite is time coursed up to 120 hrs. whereas the Δ^9-THC is followed up to 24 hrs.

When plasma was evaluated for the same three subjects, a second metabolite was discovered which was not apparent if blood bank plasma was used as a control. Thus metabolites were found at t_{R_2}3.1 and t_{R_2}0.6. Figure 12 illustrates the comparison of average levels of Δ^9-THC with these metabolites in plasma.

Figure 13 shows the relationship between plasma and breath levels of Δ^9-THC with time following smoking. Similarly, Figure 14 is a graphic correlation of the t_{R_2}3.1 metabolite in plasma and breath with time.

As was pointed out earlier, saliva samples were taken with each blood and breath sample. Due to the limited objectives of the present study not all saliva samples have been evaluated. However, for illustrative purposes Figure 15 shows the comparison which exists between breath and saliva values of Δ^9-THC up to 12 hrs. for one subject.

In addition to the time coursing results reported above a number of subjects were sampled for breath levels of Δ^9-THC at 0.5 and 1 hour following smoking.

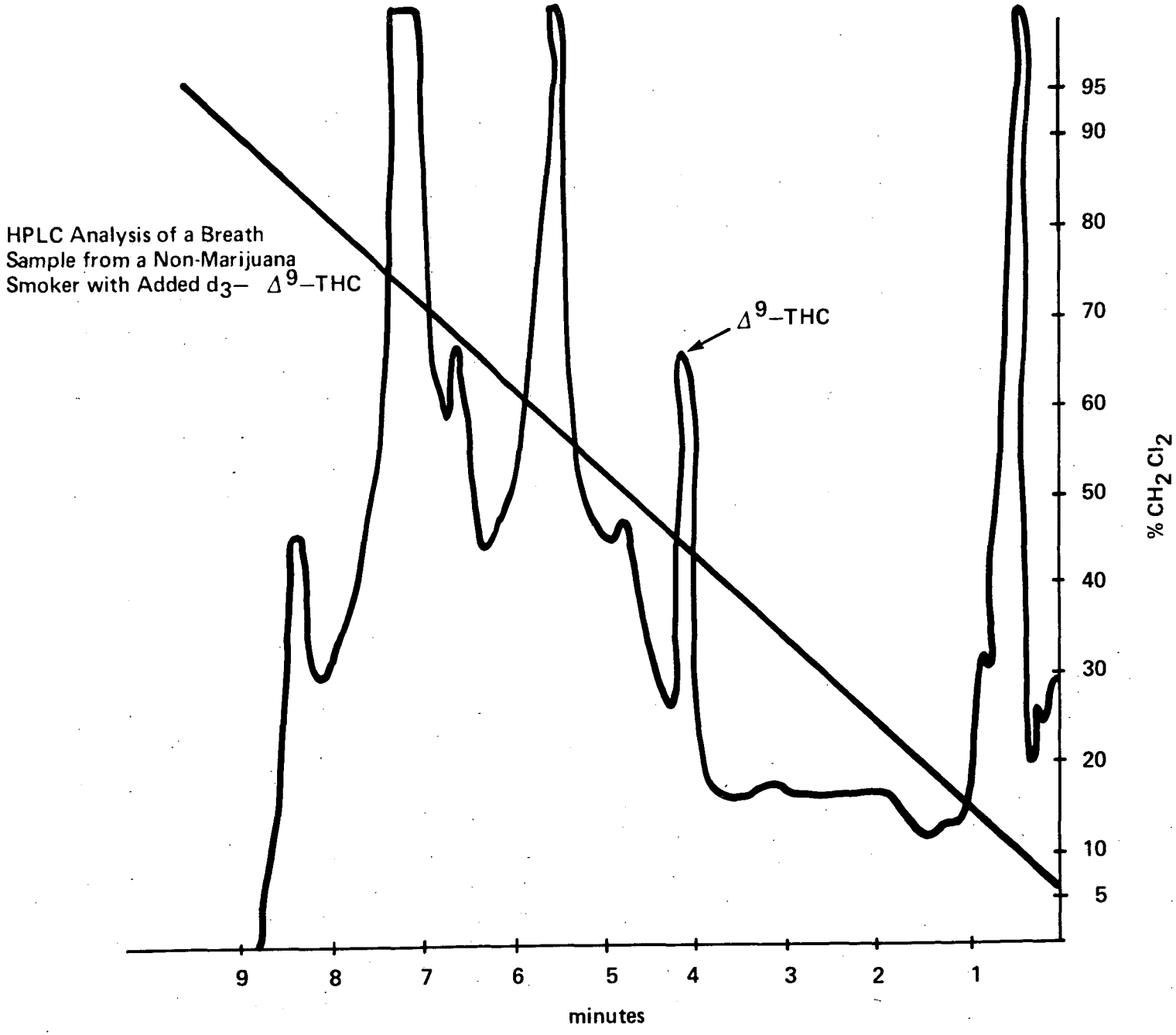

HPLC Analysis of a Breath
Sample from a Non-Marijuana
Smoker with Added d_3– Δ^9–THC
Δ^9–THC
95
90
80
70
60
50
40
30
20
10
5
% CH_2Cl_2
9
8
7
6
5
4
3
2
1
minutes

Figure 8

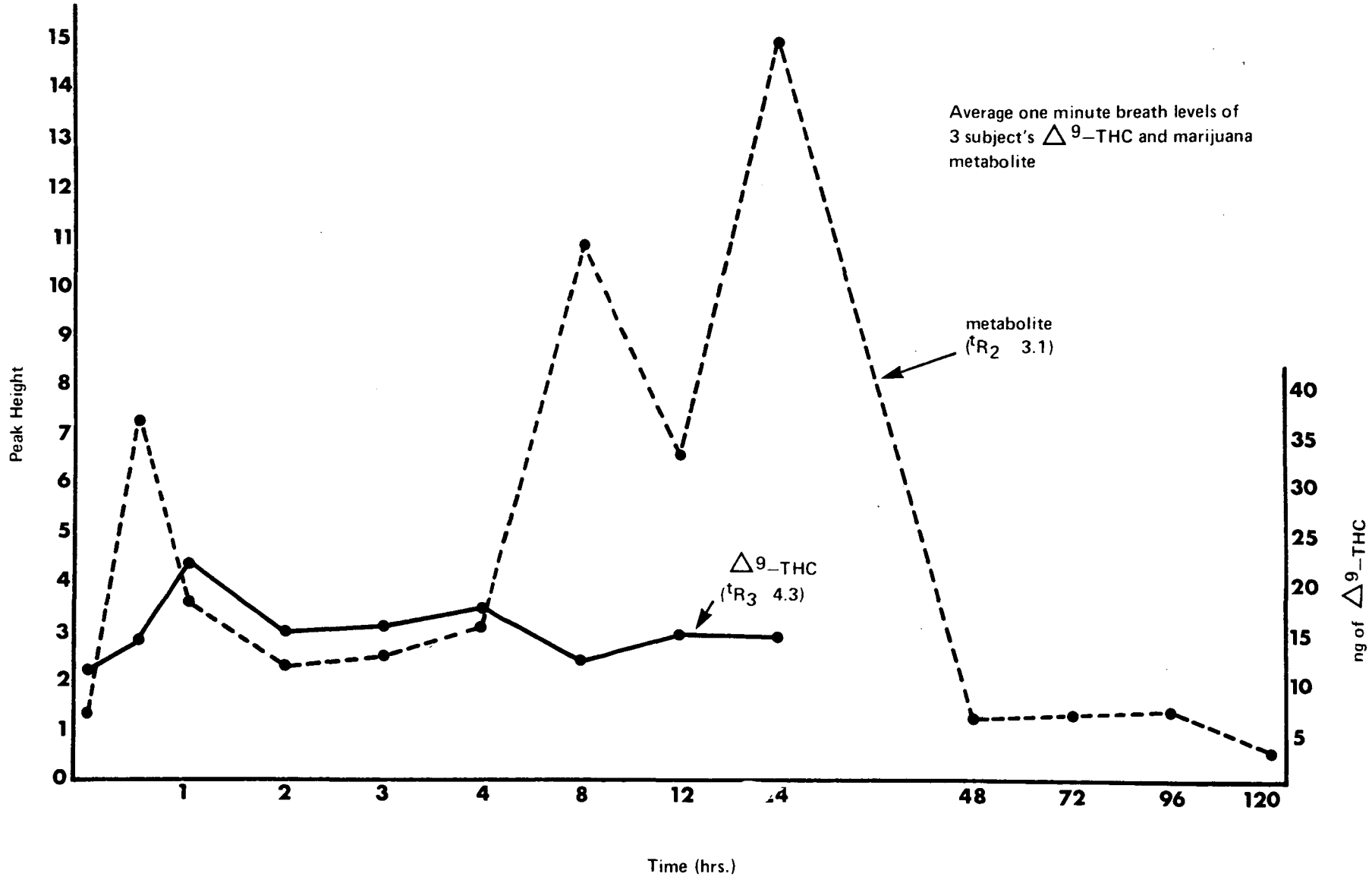

Average one minute breath levels of 3 subject's Δ9–THC and marijuana metabolite
metabolite (tR2 3.1)
Δ9–THC (tR3 4.3)
Peak Height
ng of Δ9–THC
Time (hrs.)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
1
2
3
4
8
12
24
48
72
96
120
5
10
15
20
25
30
35
40

Figure 9

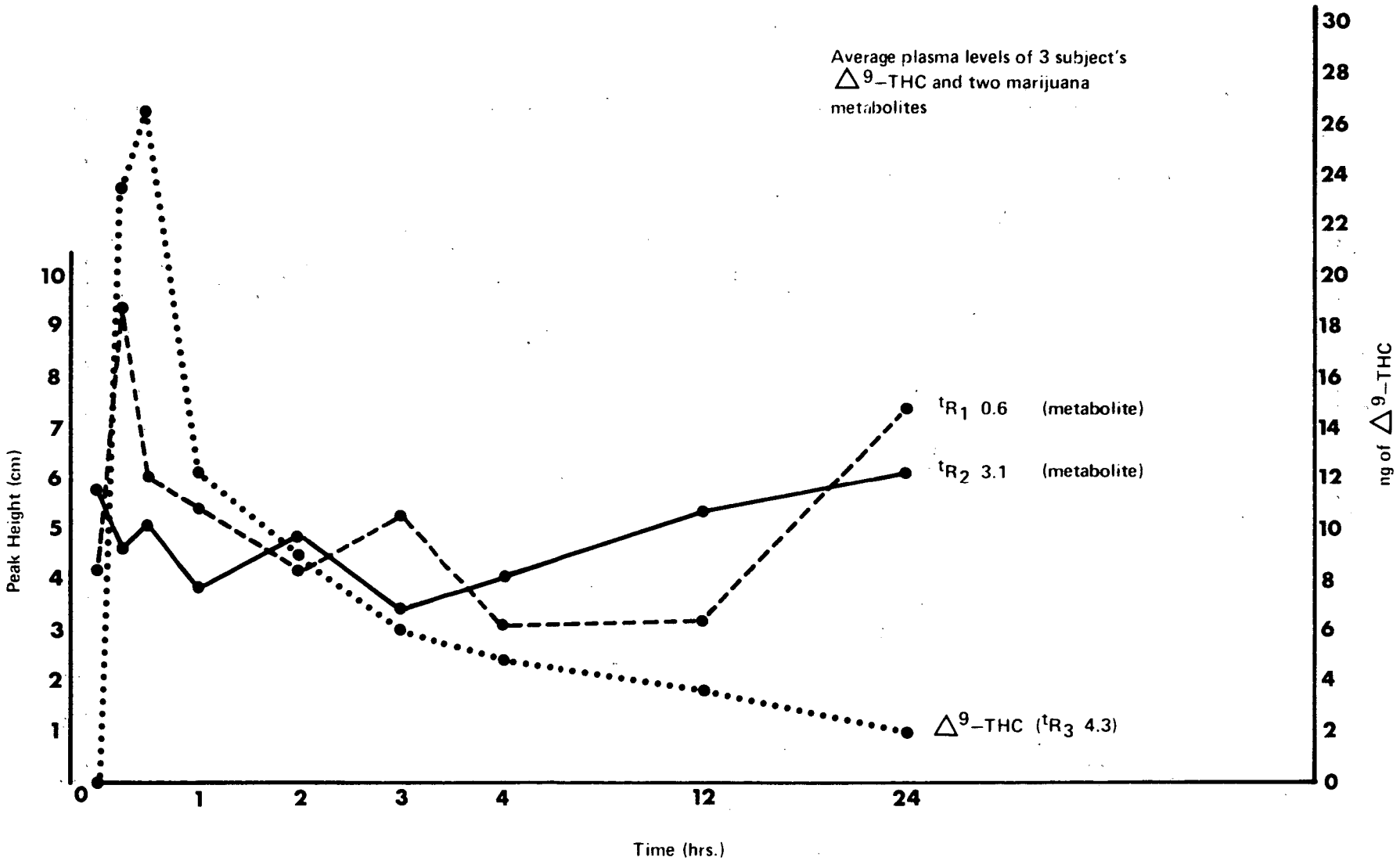

Average plasma levels of 3 subject's
Δ9–THC and two marijuana
metabolites
Peak Height (cm)
ng of Δ9–THC
Time (hrs.)
tR1 0.6 (metabolite)
tR2 3.1 (metabolite)
Δ9–THC (tR3 4.3)
0
1
2
3
4
12
24

Figure 10

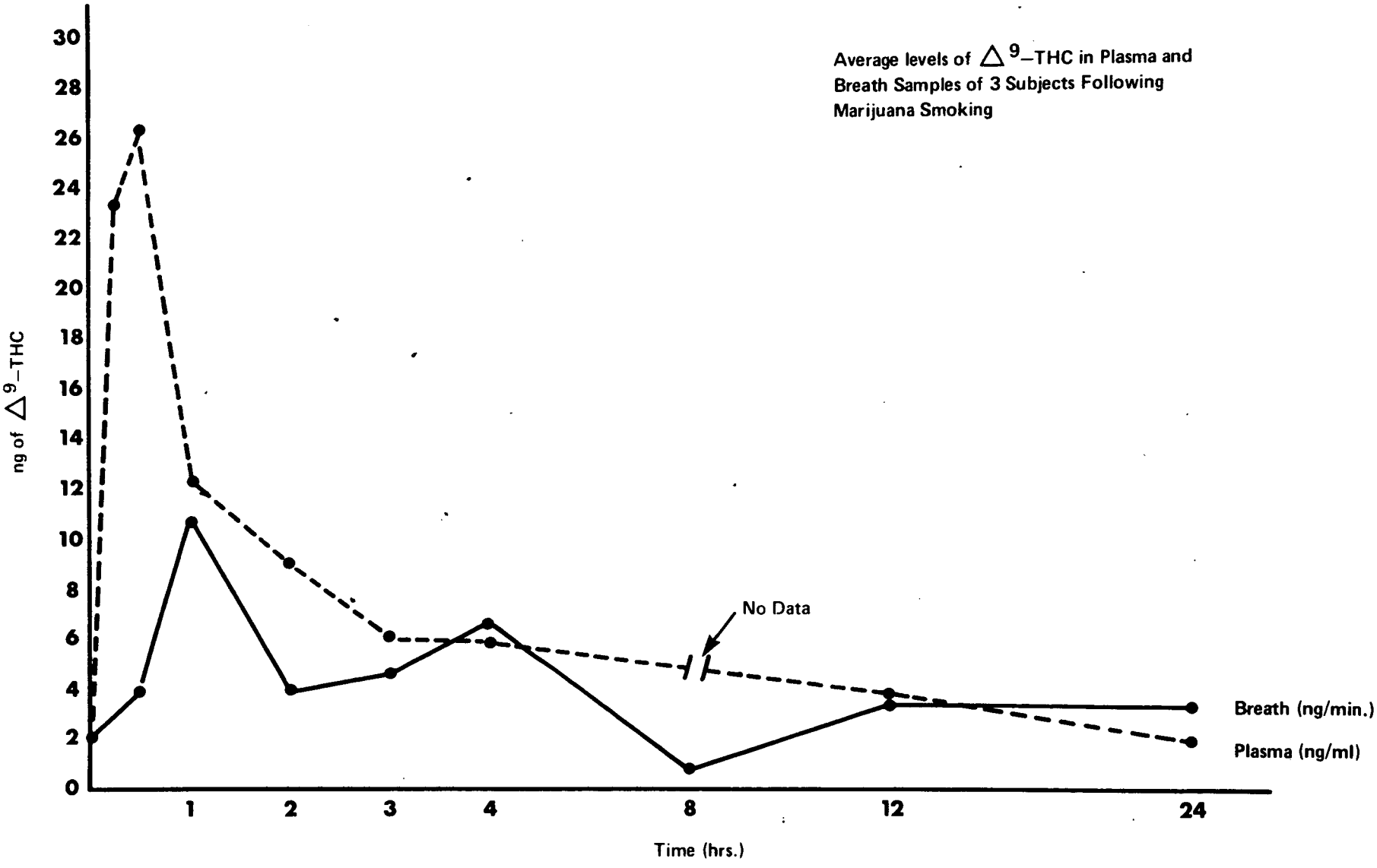

Average levels of Δ9–THC in Plasma and Breath Samples of 3 Subjects Following Marijuana Smoking
ng of Δ9–THC
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
No Data
Breath (ng/min.)
Plasma (ng/ml)
1
2
3
4
8
12
24
Time (hrs.)

Figure 11

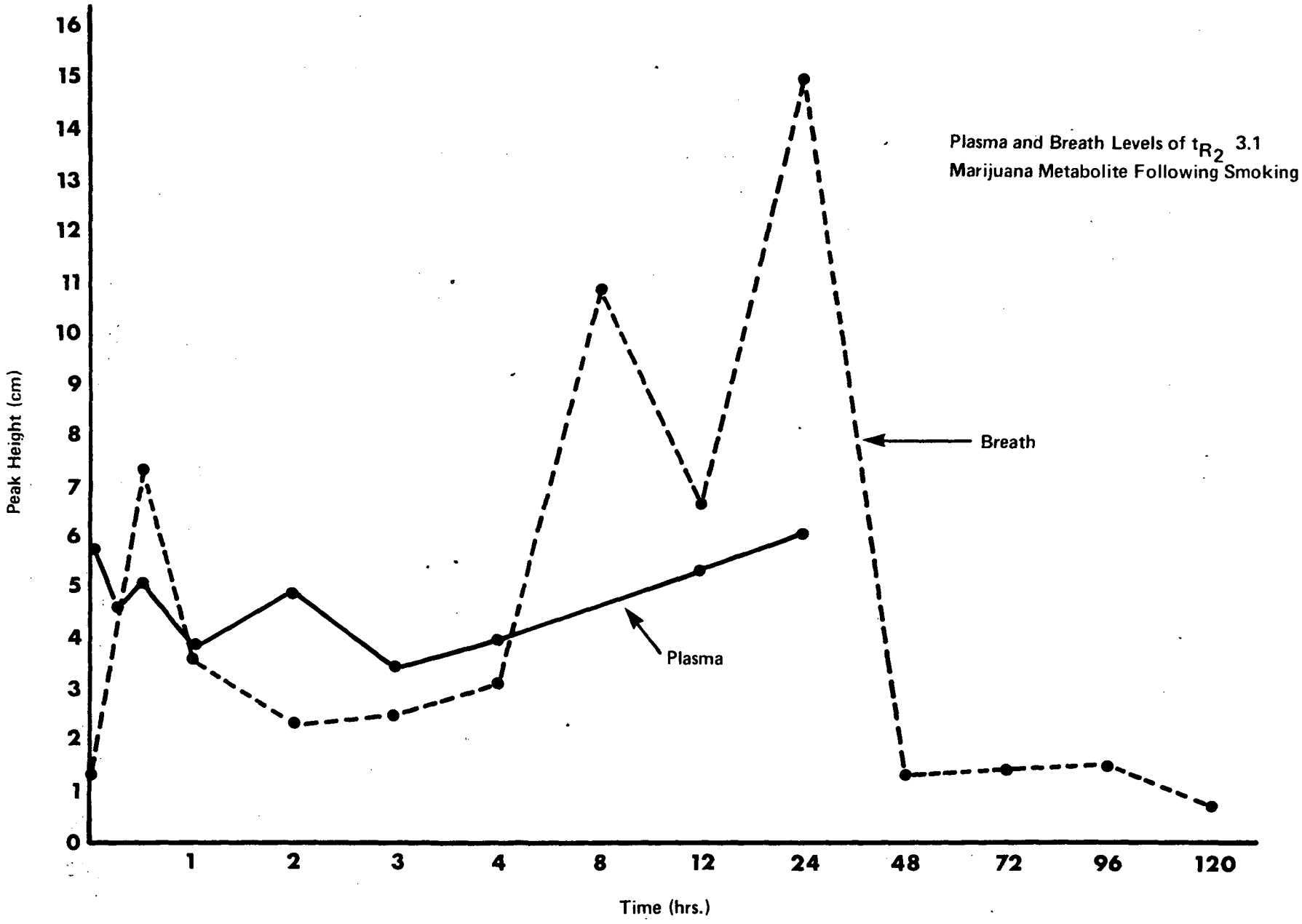

Plasma and Breath Levels of t_{R_2} 3.1
Marijuana Metabolite Following Smoking
Peak Height (cm)
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Breath
Plasma
1
2
3
4
8
12
24
48
72
96
120
Time (hrs.)

Figure 12

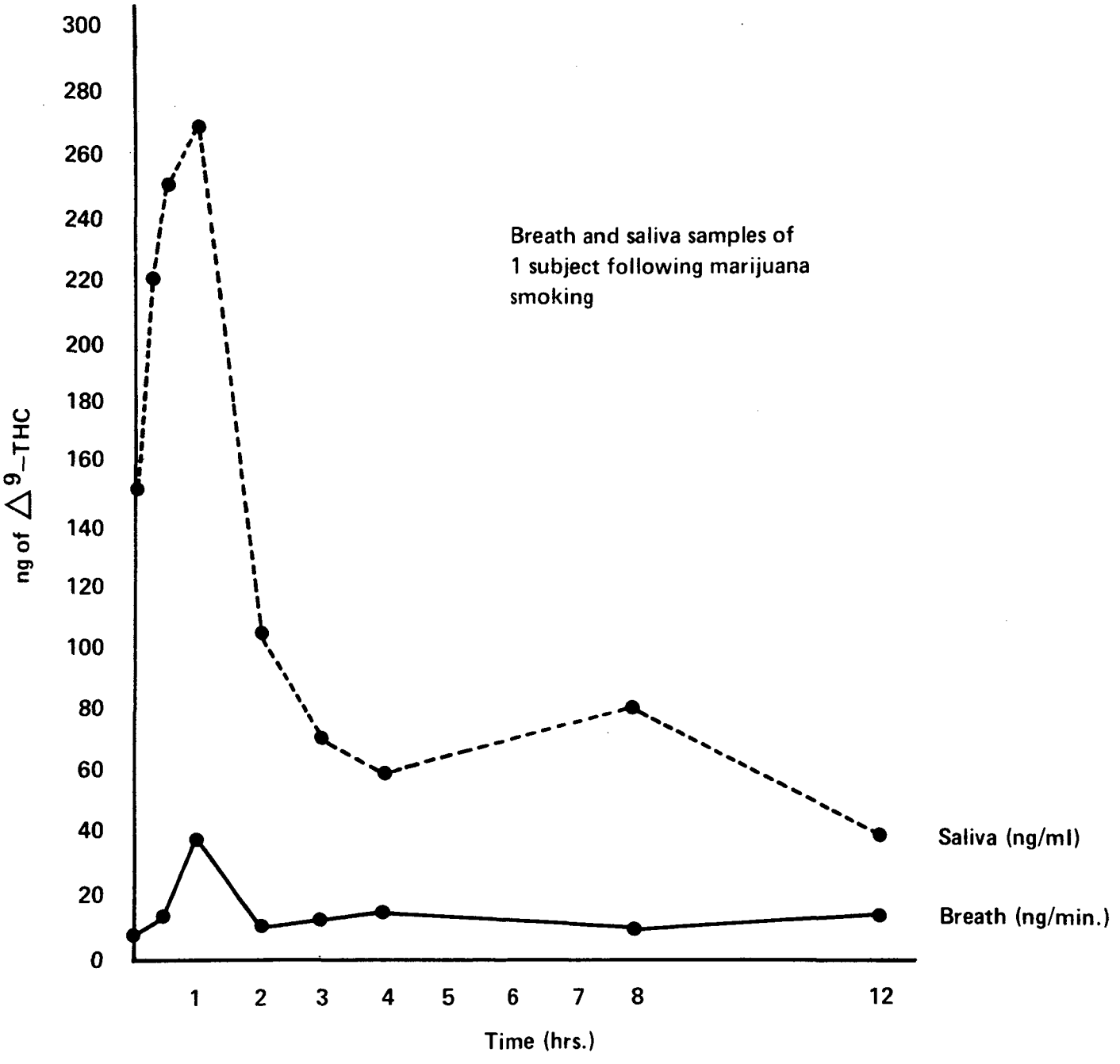

Breath and saliva samples of
1 subject following marijuana
smoking
ng of Δ9-THC
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
12
Time (hrs.)
Saliva (ng/ml)
Breath (ng/min.)

Figure 13

Figure 16 illustrates the Δ^9-THC levels found in six such subjects.

B. Ethanol Breath Tube.

During the April 8 UCLA study the ethanol breath tube apparatus described earlier was used to collect breath samples prior to smoking and at 0.25 hours after smoking. Figure 17 gives a graphic display of the levels of Δ^9-THC found at these time intervals.

The ethanol used in the breath tube was analyzed by HPLC as shown in Figure 18. Subsequent analysis by MS demonstrated that the ethanol impurity was benzene and it chromatographed with the same retention time as the t_{R_2}3.1 metabolite. Quantification of the 314/317 ratio demonstrated that no impurities were present which would interfer with Δ^9-THC analysis.

Figures 19 and 20 represent typical HPLC chromatograms obtained from a participant in the marijuana smoking studies conducted at MRI. As discussed earlier, these samples were all collected with the ethanol breath tube. Of interest to note in Figure 19 is the very large metabolite peak at t_{R_2}3.1 and apparently two other metabolite peaks at t_{R_1}0.6 and t_R 8.6. Also worthy of note are the very small peaks at t_R 3.7 and t_R 4.6 which correspond in retention time to CBN and CBC, respectively. Figure 20 dramatically illustrates how the t_{R_2}3.1 metabolite is surpressed by marijuana smoking also as was shown in Figure 12.

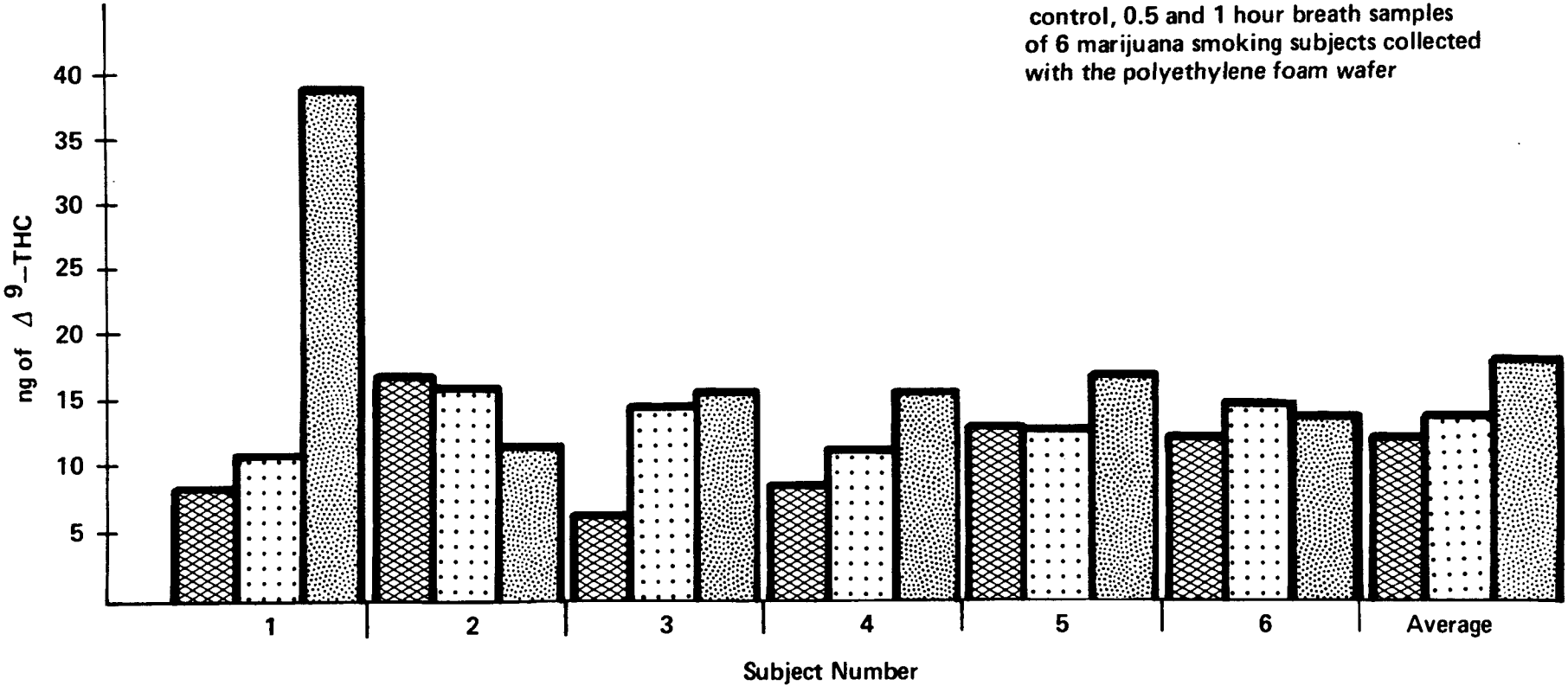

Δ 9–THC levels of the
control, 0.5 and 1 hour breath samples
of 6 marijuana smoking subjects collected
with the polyethylene foam wafer
ng of Δ 9–THC
40
35
30
25
20
15
10
5
1
2
3
4
5
6
Average
Subject Number

Figure 14

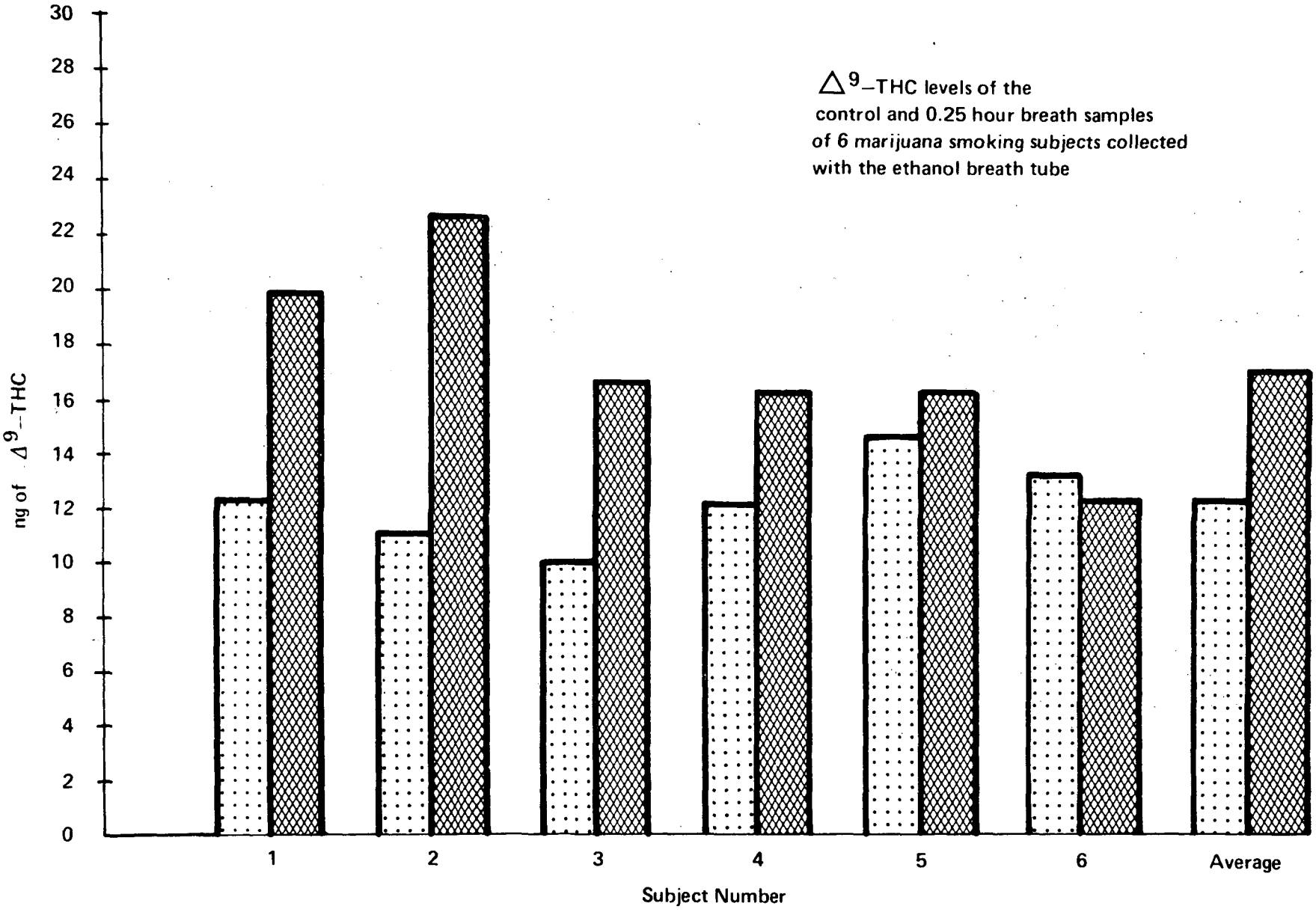

Δ9–THC levels of the
control and 0.25 hour breath samples
of 6 marijuana smoking subjects collected
with the ethanol breath tube
ng of Δ9–THC
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
1
2
3
4
5
6
Average
Subject Number

Figure 15

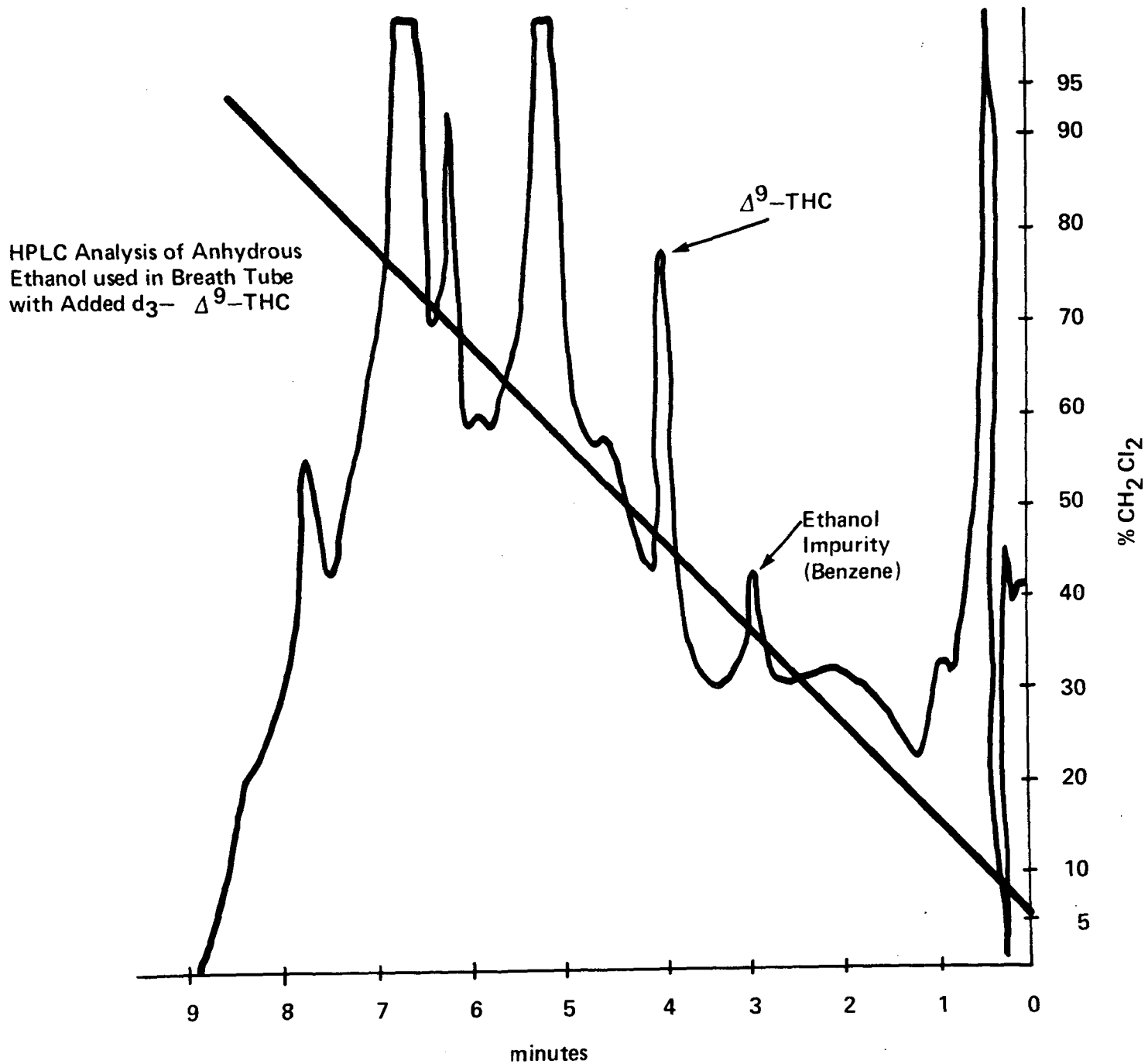

HPLC Analysis of Anhydrous Ethanol used in Breath Tube with Added d_3– Δ^9–THC
Δ^9–THC
Ethanol Impurity (Benzene)
95
90
80
70
60
50
40
30
20
10
5
% CH_2Cl_2
9
8
7
6
5
4
3
2
1
0
minutes

Figure 16

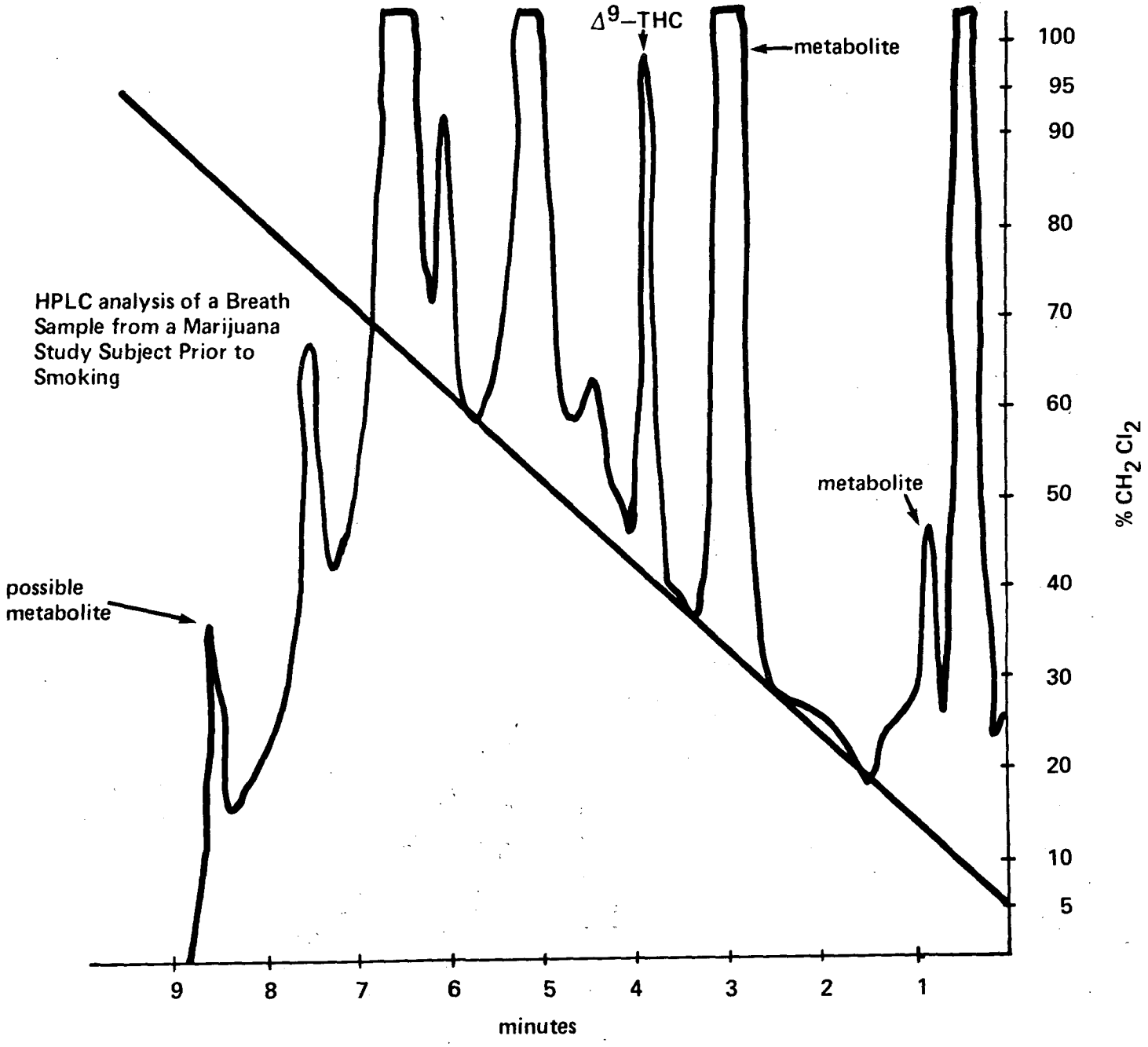

HPLC analysis of a Breath Sample from a Marijuana Study Subject Prior to Smoking
Δ9–THC
metabolite
metabolite
possible metabolite
100
95
90
80
70
60
50
40
30
20
10
5
% CH2 Cl2
9
8
7
6
5
4
3
2
1
minutes

Figure 17

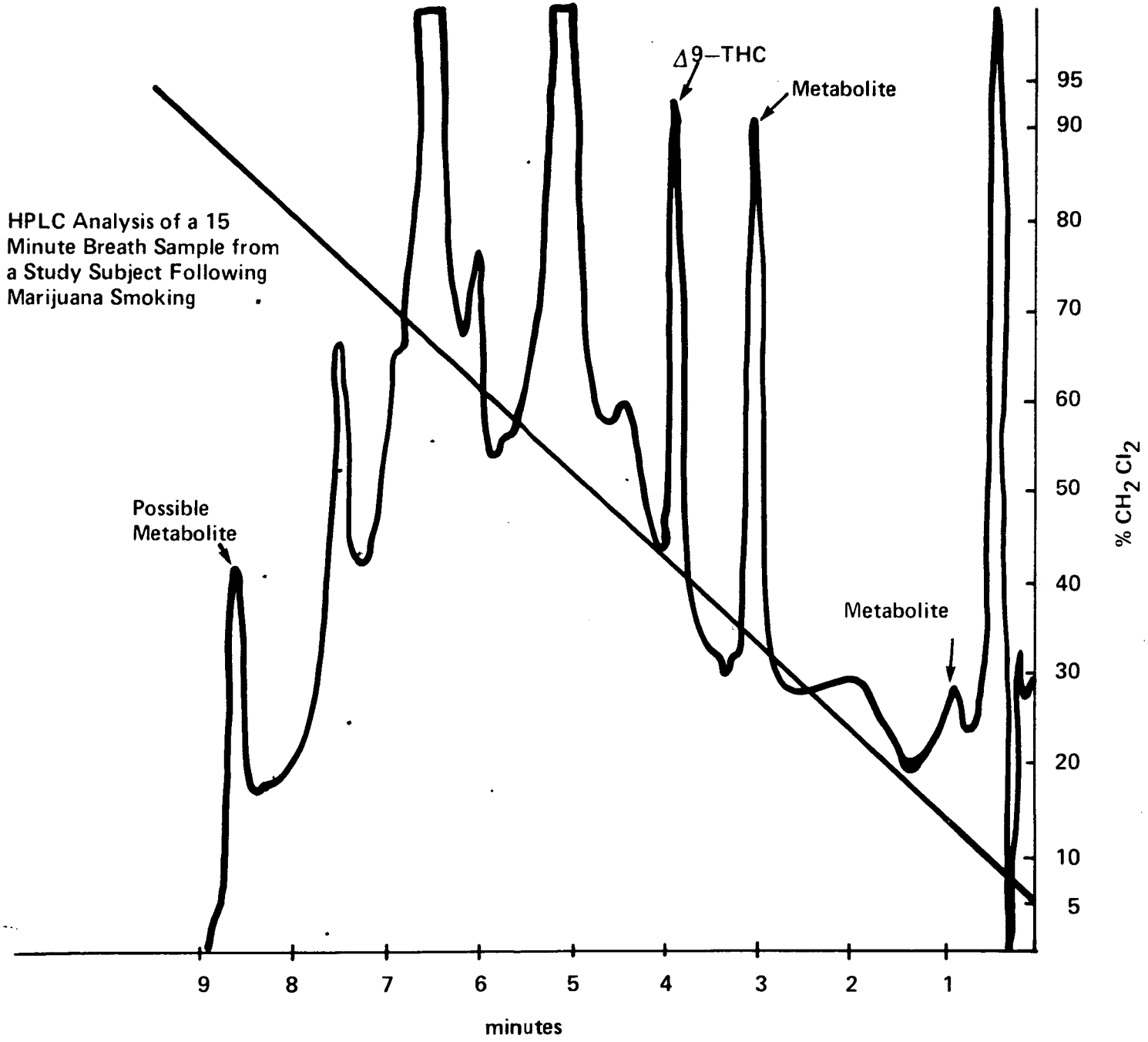

HPLC Analysis of a 15 Minute Breath Sample from a Study Subject Following Marijuana Smoking
Δ9–THC
Metabolite
Possible Metabolite
Metabolite
95
90
80
70
60
50
40
30
20
10
5
% CH_2Cl_2
9
8
7
6
5
4
3
2
1
minutes

Figure 18

CONCLUSIONS

One of the objectives of the present study was to develop technology which would permit an analyst to detect and quantitate Δ^9-THC on human breath. To accomplish this objective a HPLC-MS technique was developed. With this technique Δ^9-THC has been selectively and quantitatively identified on human breath following marijuana smoking. Also two different types of breath collecting apparatus were designed and evaluated during the study. Data obtained with each apparatus clearly demonstrated that each collection method had certain merits. With the polyethylene foam wafer device Δ^9-THC and a marijuana metabolite were concentrated from a subjects expired air stream. This particular device offers the ultimate in simplicity and portability. During one phase of the present study, samples were collected from the breaths of marijuana smokers at UCLA and the polyethylene foam wafers were then either transported or mailed to Kansas City for analysis. The earlier study done at UCLA provided additional information about the portability and mailability of the wafers since the wafers were not processed until three weeks after the breath collection. Results obtained were comparable to those wafers which were processed immediately after breath collection. Experiments with the ethanol breath tube demonstrated several apparent advantages over the polyethylene foam wafer. One advantage was the slightly increased amounts of Δ^9-THC found. This increased level of Δ^9-THC is most likely due to the high solubility of Δ^9-THC in ethanol plus the very cold temperatures which condense water droplets carrying the cannabanoids from the lungs. Another advantage of the ethanol breath tube is that it appears to collect two more metabolites (t_{R_1} 3.1 and t_{R_4} 8.6) than did the polyethylene foam wafer. Thus for metabolite verification on human breath the ethanol breath tube would be the method of choice. However if portability and

mailability were the principal considerations, then the polyethylene foam wafer would be the breath collecting device of choice.

Data collected during this study clearly demonstrated that Δ^9-THC is detectable and can even be quantitated on human breath for up to 24 hours following marijuana smoking. A very exciting finding was the presence of at least one metabolite on breath of all marijuana smokers. The metabolite at t_{R_2} 3.1 remained on the breath for 5 days following smoking of one marijuana cigarette containing 20 mg of Δ^9-THC. The exact half-life of this metabolite is not known since breath sampling was ceased after 5 days. From the data presented earlier (cf., Figures 11, 14 and 19) it is obvious that each subject participating in the marijuana smoking study had smoked prior to reporting for the study. Thus there appears to be no doubt that a marijuana user can be detected simply by the presence of this metabolite. The level of this metabolite on human breath appears to be much higher than that of Δ^9-THC since it can be detected with the UV spectrophotometer attached to the HPLC whereas Δ^9-THC cannot. Thus it would presently be possible to place a small HPLC instrument in a mobile van and perform roadside detection for marijuana use. If the HPLC-MS technology is used for the analysis of a breath sample, two important pieces of information can be obtained. These are level of intoxication, via the Δ^9-THC level, and a cross-check of marijuana use by the presence or absence of the marijuana metabolite. By looking at two parameters instead of one, the possibility of false negative or false positive assays is minimized.

Data curves such as Figure 11 clearly show that for certain time intervals following marijuana smoking a correlation exists between Δ^9-THC and the marijuana metabolite level in breath. For example in the 1-4 hour period following smok-

ing the levels of Δ^9-THC and t_{R_2} 3.1 are parallel. Thus if a driver were sampled during this time interval, an unequivocal determination could be made of the elapsed time since smoking. However, insufficient data points were taken in the earlier and later time intervals to at present have an unambiguous assignment of smoking time.

When experimentally determined plasma and breath levels of Δ^9-THC were compared, such as in Figure 13, it became very obvious that the lungs are acting as an excretory pathway for Δ^9-THC. For example, at 0.5 hour after smoking the plasma level of Δ^9-THC is at a maximum; whereas the breath level is quite low. Between 0.5 and 1 hour the plasma level has undergone a precipitous drop while the breath level is concurrently increasing and reaches a maximum at 1 hour. As the plasma level of Δ^9-THC continues to decline there is another maximum at 4 hours for Δ^9-THC on the breath. Another observation from the data in Figure 13 is that Δ^9-THC appears to be very well absorbed into the blood from the lungs after smoking with an ultimate excretion of some Δ^9-THC back through the lungs.

Literature reports (10-11) have indicated that Δ^9-THC is metabolized principally to 11-hydroxy-Δ^9-THC. Also the conversion of Δ^9-THC to 11-hydroxy-Δ^9-THC appears to depend upon how many times the liver has been induced. That is, a novice marijuana smoker will not convert Δ^9-THC to 11-hydroxy-Δ^9-THC as efficently as an experienced marijuana smoker. Therefore, an experienced marijuana smoker may not excrete as much Δ^9-THC from his lungs as would a novice smoker since a large part of the Δ^9-THC is converted to a metabolite(s). Such a phenomenon may explain why with certain individuals no

Δ^9-THC is found in breath at early times following marijuana smoking. A case in point would be study subject number 2 (cf., Figures 16 and Table III). At 0.5 and 1 hour breath sampling this individual showed values lower than his control. However at the 2 hour sampling the value of Δ^9-THC had increased sharply (cf., Table III). A similar example is study subject number 6, who gave a 0.25 hour breath sample which contained less Δ^9-THC than his control (cf., Figure 17) whereas his 0.5 and 1.0 hour samples demonstrated positive values of Δ^9-THC. However, in the case of both of these individuals, their t_{R_2} 3.1 metabolite would have identified them as marijuana users even though Δ^9-THC was not present on the breath at these samplings.

Saliva was not fully investigated in the present study, but preliminary data (cf., Figure 15) indicates that this sampling method offers great promise since the levels found were quite high when compared to those found in breath. Further correlations need to be made between blood, breath and saliva levels to ascertain whether or not saliva is also acting as an excretory route for Δ^9-THC. One major disadvantage to using saliva as a sampling technique in humans is the inability of certain individuals to provide adequate amounts of exudate for analysis. Especially is this true for short periods of time following smoking or during emotional distress, e.g. being stopped by an officer for a possible traffic violation. The technology developed during this study can provide data on Δ^9-THC level if only 100 µl of a saliva sample is obtained. An alternate method could be an alcohol wash of the mouth which would stimulate salivation. As reported earlier, ethyl alcohol is completely compatible with the developed technology.

The present study has, therefore, been quite successful in determining the presence and quantities of Δ^9-THC on the breath. HPLC-MS technology was developed which can specifically and reproducibly detect Δ^9-THC on the breath of human marijuana smokers. If Δ^9-THC is not present due to individual biological variations, then a marijuana metabolite can identify marijuana use. In addition, a feasible apparatus for roadside collection of breath samples has been developed. Perhaps the most interesting scientific finding is that a large organic drug molecule such as Δ^9-THC can be detected, intact, on human breath. Such a finding gives increased hope that other abused drugs can likewise be determined on human breath. For example, drugs such as the amphetamines and barbiturates are much more volatile than Δ^9-THC and would be expected to be ejected from the body via the lungs as was Δ^9-THC. Similarly, morphine the metabolite of heroin, has a molecular weight 30 mass units less than Δ^9-THC and might also be expected to appear on human breath. The difficulties involved in determining any of these drugs on the breath are twofold. First a method must exist for concentrating the drugs found in the large volume of expired air from the lungs and secondly to have the technology for assaying these concentrated, yet small amount of drugs. With the completion of this project both of these difficulties have been resolved.

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