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Title: Elimination of 11-Nor-Delta-9-Tetrahydrocannabinol-9-Carboxylic Acid When Normalized To Urinary Creatinine.

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John Mark Christensen

Gas chromatography mass/spectrometry quantitative analysis of 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THCCOOH), the major metabolite of delta-9-tetrahydrocannabinol (THC) found in urine following marijuana use, was performed on serial specimens collected from an inpatient adolescent population of marijuana users. Creatinine normalization of THCCOOH was used to compensate for dilute or concentrated urine specimens. The urinary terminal elimination rate constant and terminal half-life were calculated for each subject using data fit to a biexponential equation. The mean urinary elimination rate constant for THCCOOH normalized to creatinine was 0.08433 $days^{-1}$ (range 0.05408 - 0.16544) reflecting a 8.22 day terminal half-life. Data was also collected on subjects who reused marijuana. The creatinine normalized THCCOOH level was a better indicator for predicting reuse of marijuana than urinary concentrations of THCCOOH in these subjects.

Elimination of 11-Nor-Delta-9-Tetrahydrocannabinol-9-Carboxylic Acid When Normalized To Urinary Creatinine

by

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INTRODUCTION

Marijuana contains a psychoactive compound, delta-9-tetrahydrocannabinol (THC), which has been extensively abused. THC has been shown to be responsible for the perceptual, cognitive and affective changes produced in the user of cannabis products (1). The effects of marijuana have been primarily attributed to THC activity. The 11-hydroxy-delta-9-tetrahydrocannabinol (11-hydroxy THC) metabolite contributes effects that are similar to THC and, in addition, may have anticonvulsant activity (2,3). Other pharmacological effects of THC and its metabolites that have been described include inhibition of protein and nucleic acid synthesis (4), reduction of intraocular pressure (5, 6), suppression of testosterone production (6,7), increased heart rate (6, 8), development of hypothermia (9), suppression of emesis (8,10) and subjective and behavioral effects (2).

Following the administration of THC to humans, rapid and extensive metabolism occurs by the liver cytochrome P-450 microsomal enzyme systems (11). The mean elimination phase half-life of THC from plasma has been found to be 50 hours for renal and nonrenal plasma clearance (12). The initial biotransformation conversion of THC is to 11-hydroxy THC (13). 11-hydroxy THC is largely eliminated in the feces,

the major excretory route of all THC metabolites (13), and just trace amounts appear in urine. Further metabolism of 11-hydroxy THC by liver alcohol dehydrogenase enzymes produces 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THCCOOH), and is eliminated as mostly the glucuronic acid ester which represents about 27% of all urinary metabolites found after THC administration (14). Eighteen acidic urinary metabolites have been characterized, fourteen account for less than 1% of the total THC administered (14). Less than 1% of unchanged or parent THC is excreted in urine (14,15). Urinary excretion is a minor route of cannabinoid elimination.

The structures of THC and the two primary metabolites produced in man are shown in Figures 1, 2 and 3 below using the formal numbering system for pyran compounds (14).

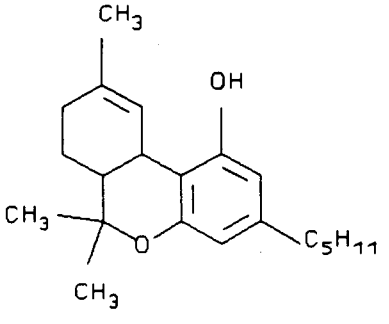

Figure 1. Delta-9 THC

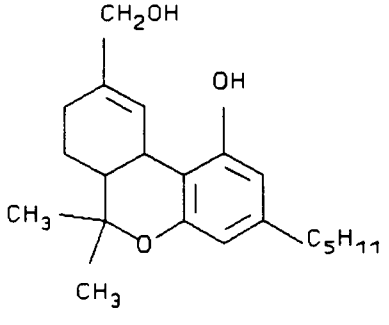

Figure 2. 11-hydroxy THC

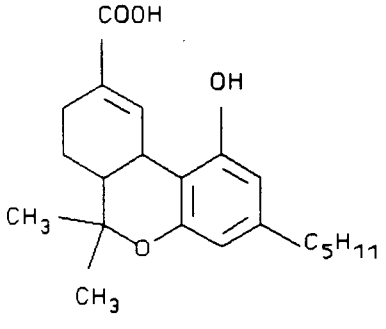

Figure 3.
11-nor-9-carboxy-delta-9 THC

A number of analytical methods have been used to determine the concentration of THCCOOH in various body fluids. These include high performance liquid chromatography, gas chromatography, and gas chromatography/mass spectrometry (16). For urinary THCCOOH screening purposes, the total amount of immunologically reacting cannabinoids is commonly performed by a qualitative enzyme immunoassay (EIA) or radioimmunoassay (RIA) technique. However, absolute specificity of the immunoassay is lessened in that some false positive results may be produced from cross-reacting substances that presumably have a similar chemical structure to THCCOOH that exist in the urine specimen (17).

The degree of hydration or dehydration in an individual is expected to vary during a given time period, causing the concentration of urinary contents to fluctuate in randomly or sequentially collected urine specimens. This variation of urinary concentration due to water content in a urine specimen may be closely monitored by determining the creatinine concentration (18). Greater than usual intake of fluids can dilute the amount of creatinine in the urine by increasing output volume and conversely, a limited state of hydration can lead to the formation of more concentrated creatinine by decreasing urine output volume, yet the amount of creatinine excreted over a given period of time is relatively constant (18,19). Similarly, cannabinoid metabolites in excessively dilute urine may not be detected by conventional testing

methods, consequently resulting in a false negative interpretation of the test.

Creatinine is a nonprotein nitrogen compound produced in vivo from creatine and phosphocreatine during muscle catabolism. The principal route of creatinine elimination from the body is by the kidney, where it is freely filtered without appreciable reabsorption or tubular secretion. Creatinine production varies within individuals depending on age, weight and sex. However, within a given subject the urinary elimination of creatinine has been shown to be relatively constant within a 24 hour interval and when 24 hour urine volumes were collected at intervals over a six to ten month period (18,20,21). The production of creatinine in a normal individual is essentially equal to the rate of renal creatinine elimination and is dependent on lean muscle mass, which is modified slowly (18,22).

The purpose of this study was to improve methods of verifying abstinence of marijuana use by characterizing the urinary elimination of THCCOOH when normalized to urinary creatinine. Without normalization to urinary creatinine, quantitative levels of THCCOOH in serial urinalysis can be misleading due to the concentration and dilution effects of urine formation. In this study the urinary excretion pattern of the primary cannabinoid metabolite, THCCOOH, was investigated in an adolescent population during marijuana abstinence in a secure treatment center. Two additional subjects

acknowledged reusing marijuana during the elimination phase of previous usage were also included in this study.

EXPERIMENTAL

Instrumentation and Analytical Methods:

The qualitative EMIT d.a.u. cannabinoid assay (Syva Co.) was used to screen for the total amount of cannabinoids in the untreated urine samples (23). A Technicon RAXT random access spectrophotometric analyzer was used to perform the functions of automated sample dilution, timed addition of reagents, and monitoring of absorbance change in the reaction mixture. The EMIT immunoassay system for cannabinoid testing has been shown to detect a number of different urine metabolites of THC, especially those being metabolized at the C-11 position (23), which account for most of the urinary metabolites so far identified (14). A qualitative estimation of the amount of total cross reacting metabolites in the urine was obtained with the 20 ng/mL of THCCOOH calibrator (supplied by Syva) which represents the threshold cutoff for distinguishing positive from negative specimens. Urine specimens having an absorbance response less than the 20 ng/mL THCCOOH calibrator where identified as negative for cannabinoids. Those urine specimens having an equivalent or greater absorbance response relative to the 20 ng/mL THCCOOH calibrator were identified as those specimens requiring confirmation testing for the identification and quantitation of THCCOOH.

Gas chromatography/mass spectrometry (GC/MS) was utilized in this study to detect and quantify THCCOOH after the alkaline hydrolysis of the glucuronide conjugated THCCOOH, solvent extraction and derivatization steps (24, 25). The procedure was modified to include alkaline hydrolysis of conjugates using 11.8 Mol/L KOH for thirty minutes at room temperature followed by the addition of maleic acid for adjusting to pH 3. Extraction of the hydrolyzed acidic THCCOOH metabolite was accomplished using 5% ethyl acetate in hexane and mixing for twenty minutes on a rocker. The solvent phase was then separated, transferred to another tube and dried under a gentle stream of nitrogen at 56° C. The procedure included methyl derivatization of the carboxylic and phenolic hydroxy groups by iodomethane in the presence of tetramethylammonium hydroxide to facilitate gas chromatography separation. A Hewlett-Packard (HP) Model 5890 Gas Chromatograph equipped with a 5970A Mass Selective Detector was used for the analysis of the derivatized product, methyl-1-dehydroxy-1-methoxy-11-nor-delta-9-tetrahydrocannabinol-9-carboxylate and the methylated deuterium internal standard, methyl 5'-$^{2}H_{3}$-1-dehydroxy-1-methoxy-11-nor-delta-9-tetrahydrocannabinol-9-carboxylate. Separation was accomplished using an 12 m X 0.2 mm i.d. HP-Ultra 2 capillary column with a 0.33um film thickness consisting of cross-linked 5% phenylmethylsilicone gum phase. Helium was the carrier gas with a column flow rate of 1 mL/min, using a

1:10 split configuration. The injection port temperature was 265° C and the oven temperature conditions were as follows: initial temperature 200° C then immediately increased at 16° C/min to 290° C and held for 3 minutes. The transfer line temperature was 275° C. The mass spectrometer was operated in the electron impact mode at 70 eV and was autotuned every 24 hours with perfluorotributylamine calibration standard. For sample analysis, the electron multiplier was set at 200V above the autotune voltage. Data acquisition and processing were performed using the standard software supplied by Hewlett Packard and by ThruPut Systems (Orlando, Florida). 11-Nor-delta-9-tetrahydrocannabinol-9-carboxylic acid and deuterium labeled THCCOOH internal standard, 5'-$^{2}H_{3}$-11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid, were purchased from Research Triangle Institute. Criteria for the identification of THCCOOH in a subject's specimen by GC/MS require that the target analyte demonstrate characteristic retention time and mass spectral ion ratios that correspond to a THCCOOH standard. The ions monitored for THCCOOH were m/z 313, 357 and 372. Quantitation was performed using the integrated area ratio of the m/z 313 base peak for derivatized THCCOOH and the m/z 360 for the derivatized deuterium labeled THCCOOH internal standard. The limit of detection was 3 ng/mL using 10 mL of urine, with a chromatographic signal to noise ratio of 6 at the m/z 313 base peak ion. Assay linearity was observed from the limit of quantitation

(8 ng/mL) to 1400 ng/mL. Values for THCCOOH below 8 ng/mL by GC/MS were reported as negative.

Creatinine was measured in duplicate then averaged for each immunoassay positive urine specimen using the Jaffe' alkaline picrate spectrophotometric assay modification for the Technicon RAXT random access analyzer (18,26). This method produces a colored adduct when creatinine reacts with picric acid in an alkaline leading to a spectrophotometric change in absorbance. This method provides appropriate analytical sensitivity (2 mg/dL) and specificity in urine where noncreatinine chromagenic materials are very low relative to urinary creatinine unless muscle trauma, myopathies, infections, starvation, liver disease, impaired carbohydrate metabolism, or hyperthyroidism exist in the subject (18,27).

Kinetic analysis to determine the urinary excretion parameters including half-life and excretion rate constants were estimated by nonlinear regression analysis of the data points in using RSTRIP (Micromath Corp., Inc., Salt Lake City, Utah).

Subjects and Sample Collection:

Three males and five females, ages thirteen to eighteen years old (mean = 16.1 years), volunteered to take part as study subjects A through H. Each subject was entered into the study protocol while being admitted to a hospital based

inpatient drug and alcohol recovery program in western Oregon. Subjects were enrolled with prior consent of their parent(s) and the admitting physician. Upon admission, each study subject submitted to a urine drug screen test which indicated a positive cannabinoid result (time day 0). Each subject remained at the treatment facility for the duration of the collecting period during which continuous supervision was provided. They were not allowed to use marijuana or any other illicit drugs during the inpatient treatment phase. Urine specimen collection occurred approximately every other day for each subject (range 1.1 - 2.7 days) until two sequential negative cannabinoid test results occurred. The urine collections were obtained by nursing staff at the inpatient unit, however direct observation collection methods were not used in the study protocol. All urine specimens were refrigerated immediately after collection and tested by enzyme immunoassay within 12 hours. Subsequent GC/MS analysis was performed within 48 hours after collection.

Two additional individuals, subjects I and J, were included into the study because of suspected then admitted intermittent marijuana reuse. Subject I, a sixteen year old male, was an outpatient in the hospital recovery program and was submitting random urine specimens for drug testing at periodic intervals. Subject J, an adult female (age not provided) was an inpatient during a portion of this study at

an adult alcohol and drug treatment program in southern Oregon. During the study period she was released from in-patient care but continued to provide urine specimens at periodic intervals when requested by her drug counselor.

Statistical Methods:

The EPISTAT statistical analysis software program (T.L. Gustafson, M.D., Round Rock, Texas, 1984) was used to calculate linear regressions. The probability (p) to assess the likelihood that an event will occur and Pearson's correlation coefficient (r) to judge how two independent variables with normal distribution tend to agree (28) were also calculated using EPISTAT.

RESULTS

The excretion patterns of cannabinoid metabolite and normalized cannabinoid metabolite for subjects A through H showed substantial variations in form, slope and length of time that the elimination of cannabinoids were detected. Urine concentrations of creatinine, THCCOOH and the THCCOOH normalized to creatinine ratio (THCCOOH/Cr) are presented in Table 1 for subjects A through H. The subject's age, sex and date of admission to the recovery treatment program are also listed. The cannabinoid concentrations declined after admission during treatment, with greater day-to-day fluctuations of THCCOOH while the decline of THCCOOH/Cr was less erratic in most subjects. The length of time that urinary THCCOOH was detected after admission ranged from 8.4 to 27.5 days (mean = 20.8 days). Urinary creatinine levels demonstrated significant variation in each subject between collection intervals as expected due to varying states of hydration. As the cannabinoid concentration declined in a subject, the concentration of creatinine in a given urine specimen was found to fluctuate randomly. To verify that the strength of linear association was negligible between the THCCOOH concentration and the paired creatinine concentration in a specimen for each subject A through H, Pearson's correlation coefficient was calculated and found not to be significantly

different than zero [$-0.50 < r < +0.60$] (28).

While the trend of decreasing concentrations of THCCOOH as a function of time was apparent in subjects A through H, noted increases of as much as 125% above a previous THCCOOH level did occur in many subjects. Subject A for example, had a 62% increase (from 45 to 73 ng/mL) on day 5.7, and a 16% increase (from 24 to 28 ng/mL) on day 10.6. Subject C was noted to have fluctuating concentrations of THCCOOH from day 7.6 through the duration of the monitoring period. Fluctuating THCCOOH concentrations were also apparent in subject D throughout the 25 day period of cannabinoid metabolite elimination. Similarly, on days 3.5, 10.5 and 18.5, subject E had increases in THCCOOH of 57%, 78% and 74%, respectively, above the previous measurement. Subject F had a 125% increase in THCCOOH (from 16 to 36 ng/mL) occur on day 22.4. Subject G also had increases in THCCOOH on day 15.7 of 30% (from 67 to 87 ng/mL) and on day 20.0 of 25% (from 26 to 35 ng/mL). An increase of 10% in Subject H was noted on day 5.4.

All of the above mentioned subjects, with the noted exception of subject B, had normalized THCCOOH/Cr ratio values which decreased more consistently over time than were the observed corresponding THCCOOH concentrations. However, periodic THCCOOH/Cr values did increase in some subjects by as much as 38% from the previously normalized value (subject D between days 11.2 and 12.8). Urine collected from subject

B on day 13.0 was found to have a THCCOOH concentration of 36 ng/mL that subsequently increased (69%) to 61 ng/mL on day 16.0. The THCCOOH/Cr ratio also increased (125%) in the same three day interval from a level of 12 ng/mg to 27 ng/mg. This individual was involved with a supervised outdoor support-group event during the time interval. Efforts made to determine whether subject B used marijuana between collection periods were investigated and did not reveal the likelihood or opportunity to indulge.

Table 1. Urinary THCCOOH:Creatinine Ratio in Marijuana Using Adolescent Subjects A - H

Subject: A (18yr. Male)
(Admit 04/25/89 = Day 0)

Days	Creatinine (mg/dL)	THCCOOH (ng/mL)	THCCOOH/Cr (ng/mg)
0.0	138	703	509
2.7	87	97	111
3.9	98	80	81
4.9	92	45	48
5.7	170	73	42
6.7	169	57	34
7.7	164	51	31
8.6	133	21	16
9.6	150	24	16
10.6	208	28	13
11.6	127	20	15
12.5	119	18	15
13.5	171	18	10
14.5	141	17	12

Subject: B (18yr. Male)
(Admit 02/26/88 = Day 0)

Days	Creatinine (mg/dL)	THCCOOH (ng/mL)	THCCOOH/Cr (ng/mg)
0.0	295	358	121
3.0	319	104	33
5.0	345	106	31
7.2	283	88	31
9.0	312	69	22
11.0	380	59	16
13.0	290	36	12
16.0	229	61	27
18.0	326	46	14
20.0	340	38	11
24.0	243	26	11
26.0	317	23	7

(Table 1 continued)

Subject: C (13yr. Female)
(Admit 03/28/89 = Day 0)

Days	Creatinine (mg/dL)	THCCOOH (ng/mL)	THCCOOH/Cr (ng/mg)
0.0	258	294	114
2.3	245	170	69
3.4	459	135	29
4.9	214	69	32
5.6	162	51	31
6.5	133	39	29
7.6	174	30	17
8.4	249	47	18
9.7	48	8	16
11.0	102	17	16
11.5	136	20	14
16.7	230	23	10

Subject: D (17yr. Female)
(Admit 01/20/89 = Day 0)

Days	Creatinine (mg/dL)	THCCOOH (ng/mL)	THCCOOH/Cr (ng/mg)
0.0	303	557	183
3.3	111	42	37
4.6	202	62	30
5.6	111	43	38
6.9	178	50	28
7.3	268	60	22
9.2	360	50	13
10.2	217	29	13
11.2	274	37	13
12.8	118	22	18
13.5	76	15	19
14.1	201	33	16
15.7	152	23	15
16.0	236	31	13
17.0	276	27	10
20.0	281	30	10
25.0	245	15	6

(Table 1 continued)

Subject: E (18yr. Female)
(Admit 02/17/88 = Day 0)

Days	Creatinine (mg/dL)	THCCOOH (ng/mL)	THCCOOH/Cr (ng/mg)
0.0	143	344	239
1.5	144	230	160
3.5	229	361	158
6.5	252	309	123
9.2	150	120	81
10.5	276	213	77
13.5	229	220	96
17.2	163	99	61
18.5	213	172	81
20.5	170	79	46
22.5	183	47	26
24.2	176	40	23
25.5	129	34	27
27.5	136	33	25

Subject: F (17yr. Female)
(Admit 01/26/88 = Day 0)

Days	Creatinine (mg/dL)	THCCOOH (ng/mL)	THCCOOH/Cr (ng/mg)
0.0	97	601	620
2.4	127	182	144
9.4	207	74	36
11.4	264	66	25
13.4	300	58	19
15.4	216	38	18
17.8	139	25	18
19.4	149	26	17
21.4	137	16	12
22.4	221	36	16
23.4	263	36	14
24.8	202	28	14

(Table 1 continued)

Subject: G (15yr. Female)
(Admit 12/21/87 = Day 0)

Days	Creatinine (mg/dL)	THCCOOH (ng/mL)	THCCOOH/Cr (ng/mg)
0.0	128	877	685
2.7	203	520	256
5.7	191	202	106
7.9	153	103	68
9.7	115	76	66
12.7	116	67	59
15.7	180	87	49
18.9	82	26	33
20.0	126	35	28
23.6	89	17	19

Subject: H (13yr. Male)
(Admit 11/19/87 = Day 0)

Days	Creatinine (mg/dL)	THCCOOH (ng/mL)	THCCOOH/Cr (ng/mg)
0.0	116	162	140
2.6	277	123	45
4.6	165	42	26
5.4	194	46	24
7.4	234	40	17
8.4	137	18	13

The data from Table 1 are shown graphically using semilog plots of THCCOOH/Cr (left y-axis) and THCCOOH (right y-axis) concentrations versus time in Figures 4-11 for subjects A through H, respectively. Inspection of the excretion graphs revealed the decline followed a biphasic pattern in most subjects. After a steep drop lasting 3 to 6 days, the levels of THCCOOH often fluctuated moderately in the terminal phase while THCCOOH/Cr fluctuations were much less prominent in most subjects after the initial declining phase. No obvious large peaks in the THCCOOH/Cr excretion profiles from subjects A, and C through H were observed that would suggested continued use of cannabinoids during the study period. The profile for subject B demonstrated a relatively large peak on day 16 for both THCCOOH and THCCOOH/Cr.

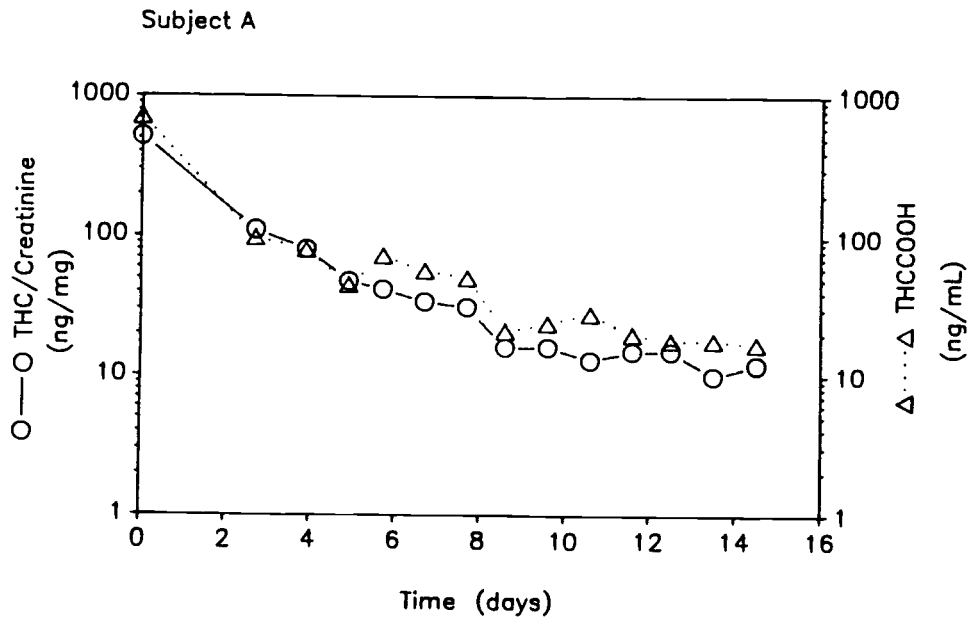

Figure 4. Subject A

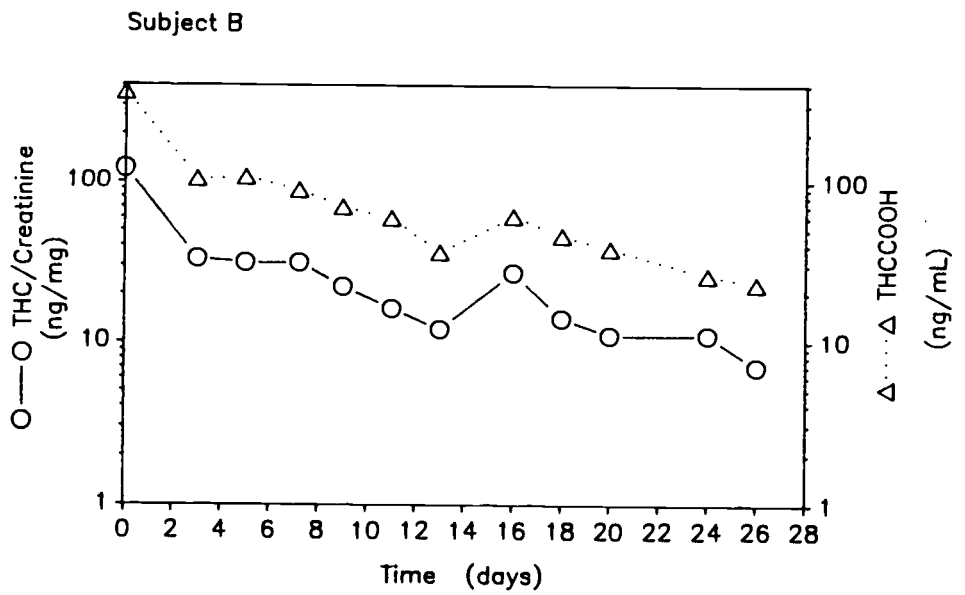

Figure 5. Subject B

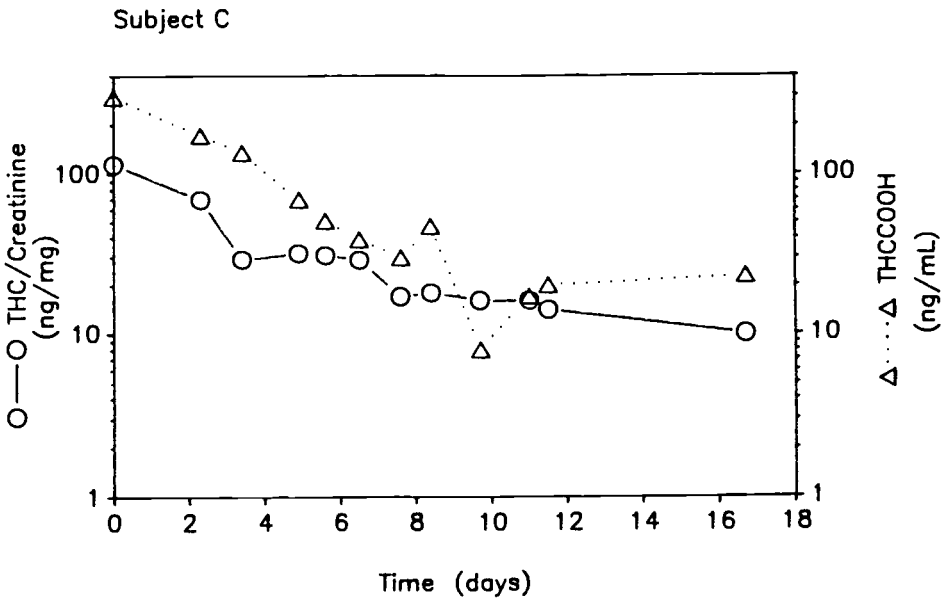

Figure 6. Subject C

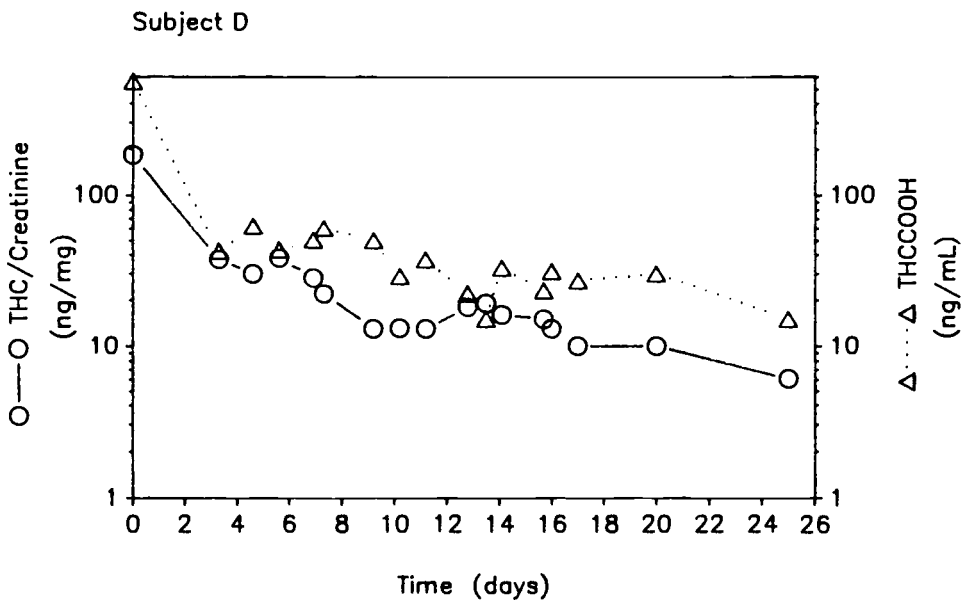

Figure 7. Subject D

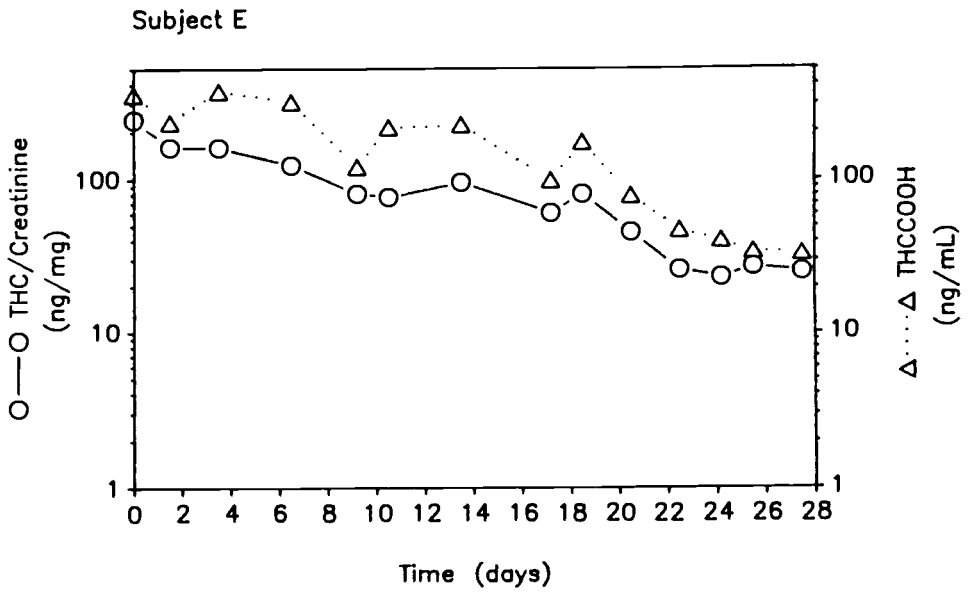

Figure 8. Subject E

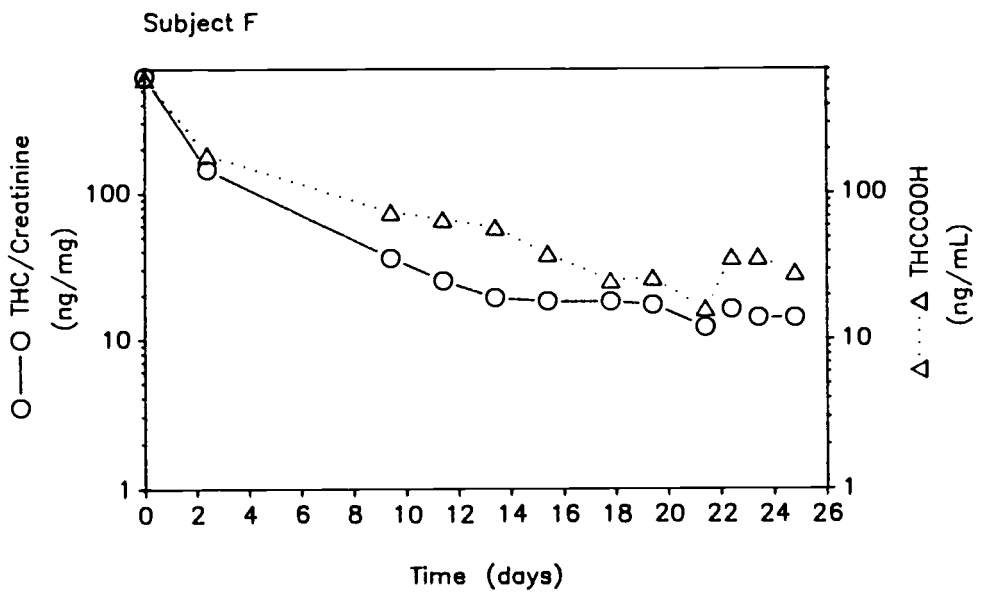

Figure 9. Subject F

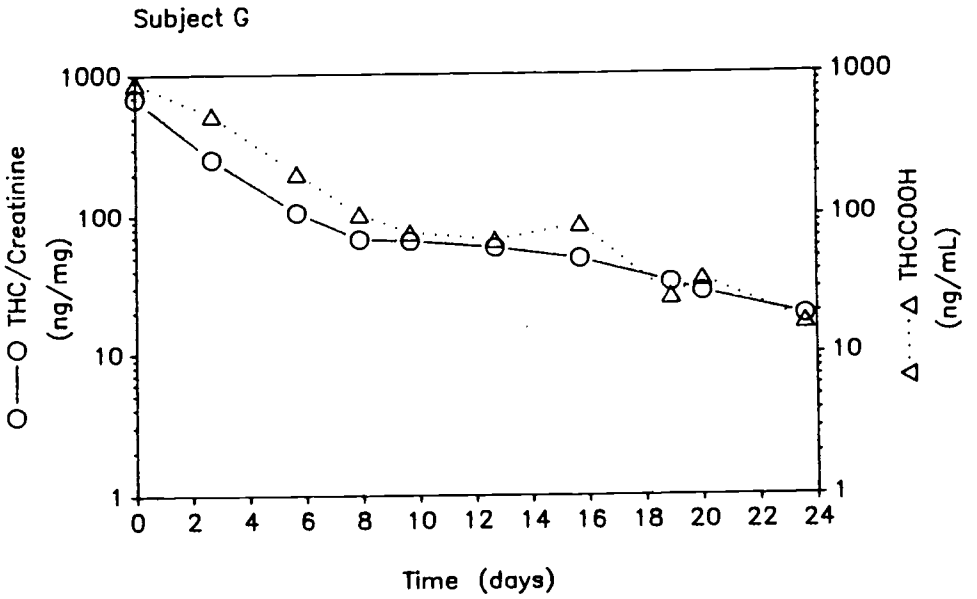

Figure 10. Subject G

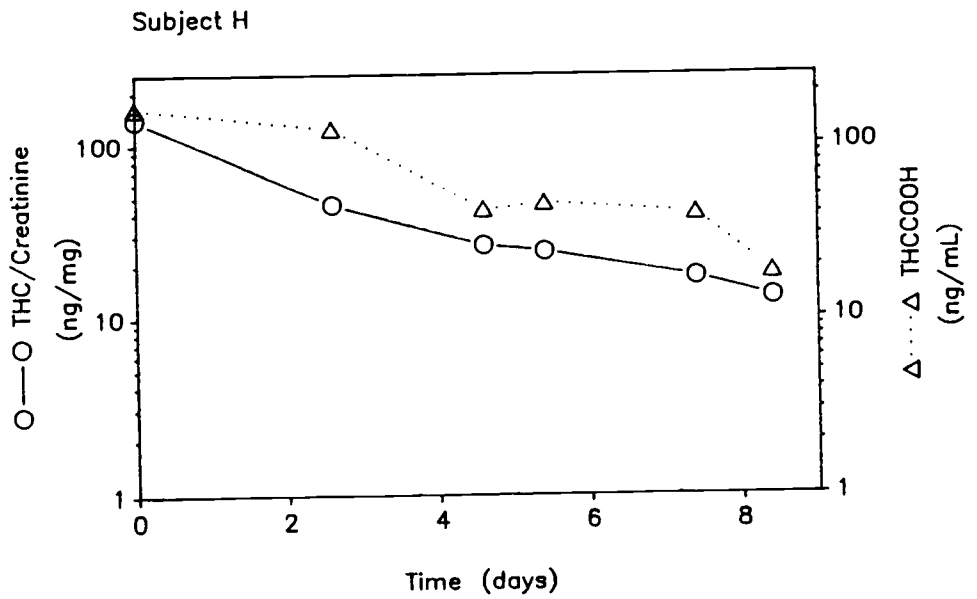

Figure 11. Subject H

Table 2 lists the kinetic parameters determined from THCCOOH/Cr ratio normalized data. The mean urinary elimination rate constant (K_1) at the terminal phase of the multiphasic urine concentration time curve from Subjects A through H was 0.08433 $days^{-1}$ when determined from nonlinear regression analysis. The elimination half-life ranged from 4.19 to 12.81 days with a harmonic mean of 8.22 days (jackknife variance +/- 1.176 days) (29). The initial elimination rate constant (K_2) determined from these urinary data was more rapid (0.60190 $days^{-1}$ for THCCOOH/Cr) than the terminal elimination rate constant (K_1) in each subject A through H (0.08433 $days^{-1}$ for THCCOOH/Cr), and the elimination half-life harmonic mean was 1.15 days (jackknife variance +/- 0.764 days). The THCCOOH/Cr mean value of the initial phase half-life [$(t_{1/2})_2$] suggests the initial phase of elimination is complete, and the terminal phase begins, in 3 to 4 days after cannabis administration.

Table 3 tabulates the kinetic parameters for THCCOOH when not normalized to urinary creatinine. The mean urinary elimination rate constant (K_1) of the terminal phase was 0.09192 $days^{-1}$, which is less rapid than the initial elimination rate constant (K_2) value (0.54304 $days^{-1}$) when determined from nonlinear regression analysis. The excretion elimination half-life for THCCOOH ranged from 3.15 to 22.01 days with a harmonic mean of 7.54 days (jackknife variance

+/- 2.775 days).

The area under the urine concentration versus time curve from day 0 to infinity (AUC) was 1350 ng*day/mg and 2255 ng*day/mL for THCCOOH/Cr and THCCOOH, respectively. The mean residence time (MRT) was 5.701 days and 6.144 days for THCCOOH/Cr and THCCOOH, respectively. The mean standard error for the two normalized and non-normalized fits showed there is significantly less variation in the THCCOOH/Cr data than the THCCOOH data.

Table 2. Pharmacokinetic Parameters Determined from Normalized THCCOOH:Creatinine Data in Adolescent Marijuana Users

Subj.	A_1	K_1	$(t_{1/2})_1$	A_2	K_2	$(t_{1/2})_2$	AUC	MRT
A	58.35	0.11825	5.86	453.03	0.63427	1.09	1165	2.837
B	35.71	0.05608	12.36	84.28	0.83267	0.83	738	8.447
C	29.79	0.06575	10.54	85.70	0.47957	1.45	632	5.078
D	33.71	0.06304	10.99	148.13	0.71357	0.97	742	6.735
E	423.85	0.08319	8.33	(NA)	0.08735	7.94	2591	9.115
F	46.62	0.05408	12.81	572.75	0.71065	0.98	1668	5.070
G	117.11	0.06880	10.07	572.68	0.50488	1.37	2837	5.674
H	54.45	0.16544	4.19	85.57	0.85222	0.81	430	2.654
Mean	99.32	0.08433	8.22	286.02	0.60190	1.15	1350	5.701
SD	134.02	0.03870	1.176	235.337	0.24796	0.764	925	2.347

Where A_1 and A_2 are y-axis intercepts (ng/mg) of the extrapolated lines for the urinary terminal elimination phase and initial elimination phase, respectively. K_1 and $(t_{1/2})_1$, and K_2 and $(t_{1/2})_2$ are the elimination rate constant ($days^{-1}$) and the half-life (days) for the urinary terminal elimination phase and initial elimination phase, respectively. AUC is area under the urine drug concentration vs. time curve (ng*day/mg). MRT is mean resonance time (days). NA = not available.

Table 3. Pharmacokinetic Parameters Determined from Non-Normalized THCCOOH Data in Adolescent Marijuana Users

Subj.	A_1	K_1	$(t_{1/2})_1$	A_2	K_2	$(t_{1/2})_2$	AUC	MRT
A	(NA)	0.14242	4.87	213.52	0.14957	4.63	1237	3.286
B	120.59	0.06198	11.18	235.31	0.90229	0.77	2206	8.425
C	9.22	0.03149	22.01	305.43	0.32550	2.13	1231	3.524
D	55.47	0.05118	13.54	490.28	0.97783	0.41	1585	6.435
E	353.22	0.07682	9.02	(NA)	(NA)	(NA)	4598	13.017
F	161.40	0.08668	8.00	439.58	0.89754	0.77	2351	6.625
G	100.28	0.06458	10.73	818.74	0.31736	2.18	4132	4.987
H	(NA)	0.22018	3.15	359.39	0.23119	3.00	699	2.855
Mean	65.66	0.09192	7.54	408.89	0.54304	1.28	2255	6.144
SD	163.47	0.06120	2.775	206.96	0.36380	0.749	1413	3.380

Where A_1 and A_2 are y-axis intercepts (ng/mL) of the extrapolated lines for the urinary terminal elimination phase and initial elimination phase, respectively. K_1 and $(t_{1/2})_1$, and K_2 and $(t_{1/2})_2$ are the elimination rate constant ($days^{-1}$) and the half-life (days) for the urinary terminal elimination phase and initial elimination phase, respectively. AUC is area under the urine drug concentration vs. time curve (ng*day/mL). MRT is mean resonance time (days). NA = not available.

Elimination data collected from subjects I and J are presented in Table 4. For subject I, a 16 year old male outpatient having a history of recent marijuana use, day 0 was the first specimen collected for this study. Over the next 10 days, he acknowledged smoking marijuana with self described dosages ("heavy" or "light"), on three separate occasions. Creatinine, THCCOOH and THCCOOH/Cr are listed for the specimens collected intermittently (mean = 2.48 days) during the course of his treatment. On day 6.1 after "lightly" smoking marijuana 4.1 days earlier, the THCCOOH/Cr increased (7%) from 150 ng/mg to 161 ng/mg while the THCCOOH concentration increased (99%) from 210 ng/mL to 418 ng/mL. On days 8 and 10 he allegedly smoked marijuana again, however the 13.7 day urine specimen showed decreases in both the THCCOOH and THCCOOH/Cr concentrations. It is noted that decrease in THCCOOH/Cr and THCCOOH values were compared to a specimen collected 7.6 days earlier, which should have allowed enough time to elapse after "heavy" cannabis smoking to miss the K_2 phase entirely. However this subject's data still suggested he was in the initial elimination phase. During the remaining period of continued cannabinoid elimination, subject I had a negative cannabinoid result by immunoassay (GC/MS quantitation was not performed) followed by a cannabinoid positive specimen result the next day. This "negative gap" specimen (day 18.7) was a relatively dilute urine having a creatinine concentration of 26 mg/dL. An

attempt may have been made by subject I to induce a negative result by *in vivo* dilution of the sample utilizing excessive hydration.

Subject J, an adult female, was found smoking marijuana with visitors 3.5 days after admission to inpatient detoxification, just 12 hours after her first urine specimen was collected for drug testing. The next specimen (day 10) had both increased THCCOOH and THCCOOH/Cr concentrations 6.5 days after using marijuana. Although the THCCOOH/Cr level tended to fall, the specimens collected after she was released to outpatient status had essentially unchanged THCCOOH/Cr levels and slightly increasing THCCOOH levels through day 41. Subject J left the outpatient treatment program on day 41 against her therapist's recommendation and no other urine specimens were collected after that time.

Table 4. THCCOOH:Creatinine Ratio Elimination Profile In Marijuana Reusers

Subject: I (16yr. Male)
(Outpatient, First Collection = Day 0)

Days	Creatinine (mg/dL)	THCCOOH (ng/mL)	THCCOOH/Cr (ng/mg)
0.0	140	210	150
2.0	---------- < Smoked "lightly" >[1] ----------		
6.1	259	418	161
8.0	---------- < Smoked "heavily" >[1] ----------		
10.0	---------- < Smoked "lightly" >[1] ----------		
13.7	128	126	98
14.0	262	122	47
18.7	26	Negative	(na)[2]
19.8	174	18	11

[1] Subject interview reported history.

[2] (na = not applicable). GC/MS quantitation was not performed.

Subject: J (Adult, Female)
(Admit 08/09/88 = Day 0)

Days	Creatinine (mg/dL)	THCCOOH (ng/mL)	THCCOOH/Cr (ng/mg)
0.0			
3.0	54	68	125
3.5	---------- < Smoked with visitors >[1] ----------		
10.0	66	135	204
12.0	19	26	136
13.0	---------- < Outpatient "Pass" >[2] ----------		
34.0	64	67	104
36.0	69	77	111
41.0	79	83	105

[1] Reported by inpatient facility staff.

[2] Treatment care provided during daytime hours only.

The elimination data from Table 4 are shown graphically using semilog plots of THCCOOH/Cr (left y-axis) and THCCOOH (right y-axis) concentrations versus time in Figures 12 and 13 for subjects I and J, respectively. Inspection of the excretion plots for subject I revealed an atypical pattern when compared to those of subjects A through H. A flat line plot was observed for THCCOOH/Cr and THCCOOH up through day 14 before a decreasing slope became apparent. The graph of subject J also demonstrated a flat THCCOOH/Cr semilog plot over the 41 day duration of testing. However, the THCCOOH plot illustrated a considerable decrease on day 12, only to return to parallel the THCCOOH/Cr pattern in the weeks that followed.

There was a significant difference between reuse subject I and subject J at the terminal slope urinary elimination half-life ($(t_{1/2})_1$) value for THCCOOH/Cr, as well as for THCCOOH (Table 5). For subject I, the ($(t_{1/2})_1$) of THCCOOH and THCCOOH/Cr were similar and consistent to the respective means from subjects A through H who had not reused marijuana during the elimination phase. Conversely, for subject J the ($(t_{1/2})_1$) for THCCOOH/Cr was 82.04 days and for THCCOOH was 3.44E+6 days, both of which did not compare to the abstaining subjects in this study.

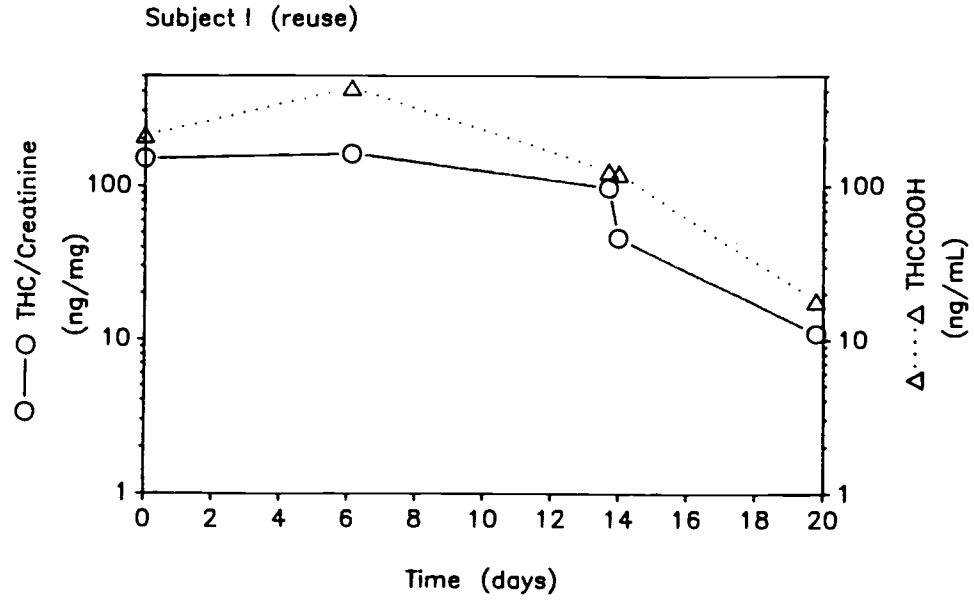

Figure 12. Subject I

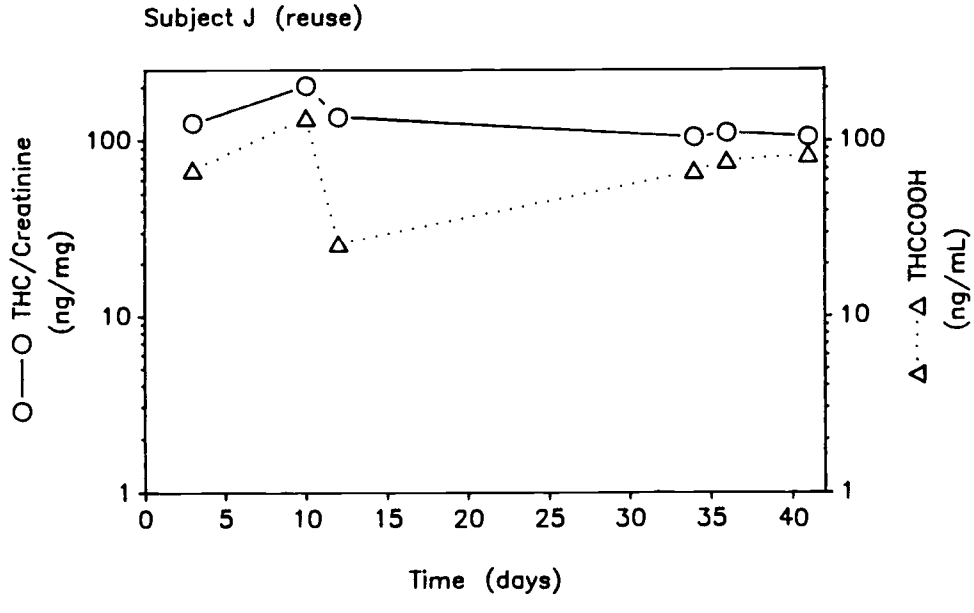

Figure 13. Subject J

Table 5. Elimination Rate Constants and Half-life Determined from Normalized THCCOOH:Creatinine and Non-normalized THCCOOH in Marijuana Reusers

THCCOOH/Cr

Subj.	A_1	K_1	$(t_{1/2})_1$	AUC	MRT
I	188.54	0.11014	6.29	1712	6.5581
J	151.03	0.00845	82.04	17877	19.3188

THCCOOH

Subj.	A_1	K_1	$(t_{1/2})_1$	AUC	MRT
I	286.81	0.09801	7.07	2926	6.8824
J	59.68	<0.00000	3.44E+6	2.9E+8	20.5000

Where A_1 is the y-axis intercept (ng/mg for THCCOOH/Cr, ng/mL for THCCOOH) of the extrapolated line for the urinary terminal elimination phase. K_1 is the urinary elimination rate constant ($days^{-1}$) and $(t_{1/2})_1$ is the half-life (days) for the urinary terminal elimination phase. AUC is area under the drug concentration vs. time curve (ng*day/mg for THCCOOH/Cr, ng*day/mL for THCCOOH). MRT is mean resonance time (days). Assumed that continued THC input did not occur when calculating the pharmacokinetic parameters.

DISCUSSION

Following the use of marijuana, the major psychoactive constituent, THC, is assimilated and distributed to the lipophilic tissues of the user. It is then slowly released back to the blood and metabolized in the liver yielding a metabolite with a relatively long terminal half-life (12,-14). In a study by Ellis et al., cannabinoid metabolites were detected by the EMIT-d.a.u. method (sensitivity, 20 ng/mL) in serial collections of urine after discontinuation of marijuana use in chronic users for up to 46 consecutive days before observing the first negative test, and it was found to take as many as 77 days to finally drop below the sensitivity threshold for 10 consecutive days (30). The long persistence of urinary cannabinoid metabolites coupled with varying urine concentration in an individual presents a unique problem when verification of abstinence is attempted, or when excessive hydration by the abuser is undertaken to escape detection (19,31,32). The determination of urine creatinine to adjust for concentration or dilution of urine has been attempted for the analysis of endogenous hormones (33) and exogenous agents such as nicotine (34). In this study, normalization of the primary urinary cannabinoid to urinary creatinine as a technique to corroborate marijuana reuse by measuring serial random samples was evaluated and

found to be a useful indicator in detecting marijuana reuse.

The urinary excretion profiles of experienced marijuana users (n = 13) was studied by Johansson and Halldin after a smoking dose of 56 mg of THC was administered within a two day period (35). In that study, urine samples taken at 15 minutes, 24 hours, and 3 days after smoking were found to contained THCCOOH/Cr levels that ranged over 14-219 ng/mg, 16-158 ng/mg, and 5-75 ng/mg, respectively. In comparison, the eight adolescent marijuana users presented in our study (subjects A - H) had even higher THCCOOH/Cr concentrations (range 114-685 ng/mg) on day 0 of admission and therefore, were considered to be recent users of marijuana.

In this study, the mean urinary excretion half-life of THCCOOH/Cr determined from subjects A through H was 8.22 days and compared favorably to two previous investigations. One case reported a half-life of 10.8 days, and in the other study (n = 13) a half-life up to 9.8 days was described (32,35). In this study, the correlation between the THC-COOH/Cr urinary excretion half-life and the number of days a subject (A through H) had detectable urinary THCCOOH was significant ($r = 0.7738$, $p = 0.024$). The mean residence time (MRT), which is the average time the molecules of an administered drug will reside in the body, is increased when the drug remains in the body for long periods of time (36). Interestingly, a stronger correlation was found between MRT and the number of days a subject (A through H) had detect-

able urinary THCCOOH concentration ($r = 0.8710$, $p = 0.005$) than was seen with half-life values. Therefore, MRT may be preferred as a predictor of the number of days an individual would remain positive after using marijuana than would the half-life. This finding is of interest in that the mean THC plasma distribution phase half-life of frequent users is significantly longer (89 +/- 22 minutes) when compared to infrequent users (45 +/- 13 minutes) in a recent report (37). Whether this discrepancy reflects reduced clearance of THC due to tissue and lipid saturation, or THCCOOH saturation of hepatic glucuronyl transferase, or both mechanisms occurring in frequent users has yet to be determined.

Evaluation of urine THCCOOH/Cr elimination profiles from serial urine collections in subjects A, C, D, E, and F demonstrated the occurrence of occasional increases in THCCOOH/Cr during terminal phase of the monitoring period, however no increase was greater than 38% of the previous sample measurement (mean = 21%, range 5-38%). These fluctuations may be due to the sum effects of exercise, diet, and random error in the analysis of the creatinine and THCCOOH. However the concentration of THCCOOH exhibited much greater increases (mean = 48%, range 4%-125%) in the same subject group. These increases in THCCOOH were proportionate to the increases in urine creatinine. To illustrate an example of correction using creatinine normalization it is noted that

subject A might have been misidentified as having reused marijuana between day 4.9 and 5.7 when the concentration of THCCOOH increased by 62%. However, the THCCOOH/Cr normalization shows that there does exist a fall in values confirming abstinence. Another example of where normalization to creatinine was necessary to interpret normal elimination of cannabinoid was in subject C, where THCCOOH values had increasing fluctuations following day 8.4 of inpatient admission but corrected after normalization. Subjects D, E and to a lesser degree, subject F illustrate how the THCCOOH/Cr concentration may fluctuate during the progression of cannabinoid elimination; and subject B may be the extreme example, if it is assumed that he did not reuse marijuana between day 13 and day 16. It is notable that the sporadic THCCOOH/Cr increases in the terminal phase returned to the lowest previous value within 4 days (mean = 1.95 days) in each of these subjects. Subjects G and H had consistently decreasing THCCOOH/Cr values that corrected for increases in THCCOOH during the study period of serial urine collections.

Examination of the elimination profiles from most of the adolescent marijuana users demonstrates that the cannabinoid metabolite pattern is representative of a multicompartment model with a very steep decreasing slope (K_2), which is followed by a less steep decreasing terminal slope (K_1). The half-life determined from the K_2 value suggests that after 3 to 4 days the levels of urinary cannabinoid

would be expected to be relatively low. If serial urine sample times are at longer intervals, the K_2 phase will pass undetected and indications of surreptitious reuse of marijuana will be obscured. Subject B most likely did not reuse marijuana between days 13 and 16 because there is no evidence of the higher THCCOOH/Cr values that would be expected in the K_2 phase.

In the study presented, the data show that the reuser of marijuana will exhibit dissimilar elimination characteristics relative to the abstaining marijuana user. Subject J had a urine elimination profile that confirmed reuse while she was an inpatient with significant increases in both THCCOOH (98%) and THCCOOH/Cr (63%) seven days after the previous urinalysis. After she entered outpatient care, little change was observed in the cannabinoid concentration over an extended period. For subject J, the half-life for THCCOOH/Cr was 82.04 days and for THCCOOH was approximately 9,424 years if it was erroneously assumed that reuse did not occur. Subject I also had indications from the elimination data of having reused marijuana. Both the THCCOOH and the THCCOOH/Cr concentrations were increased 6.1 days after the previous urine collection and substantiated his reuse history. However, the next collection taken 7.6 days later (day 13.7) provided cannabinoid levels that did not support his alleged reuse of marijuana during the sample collection interval. It was not determined if this discrepancy was due

to an inaccurate recall of the time of his use, or if a more frequent sampling interval was needed to identify reuse.

CONCLUSION

In this study, I have been able to demonstrate that the urinary excretion half-life of marijuana metabolite is long, that the absolute concentration of this metabolite is dependant on the degree of hydration of the individual, and that utilizing normalization to urinary creatinine will more accurately delineate the cannabinoid elimination profile.

Rigorous testing programs for identifying marijuana users and to discourage its abuse have been achieved in clinical and forensic settings. Individuals found to be using cannabinoids may be followed in medical treatment programs that will look for an encouraging decrease in urine cannabinoid level or to document failure of drug abstinence. The ramifications of reuse may be punitive consequences or may require additional treatment care with the associated costs. The use of quantitative urine cannabinoid results normalized to urinary creatinine, as presented in this study, should become the preferred, if not required, model in the medical drug treatment program environment. Prevention of any factor that might produce false interpretation, and therefore compromise patient management by the loss of both patient and treatment staff confidence in analytical evaluations, must be understood and utilized.

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APPENDIX

Appendix Figure 1: An example output chromatograph report for a positive THCCOOH result from subject C using selected ion monitoring gas chromatography/mass spectrometry.

Data file: DATA:1753THC.D
File type: GC / MS DATA FILE

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Misc Info:
Operator : KJN 4/10/89

Date : 10 Apr 89 12:19 pm
Instrment: MS_5970
Inlet : GC

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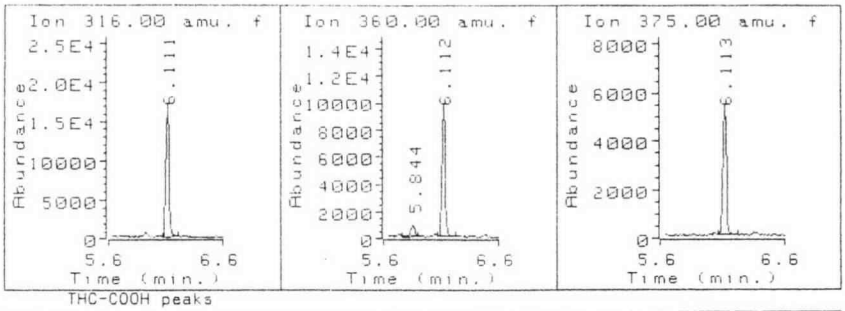
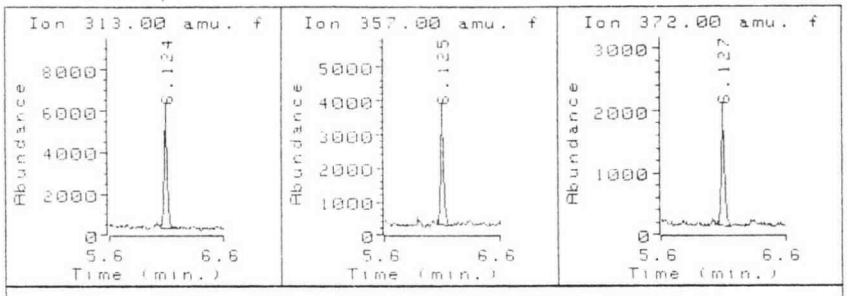
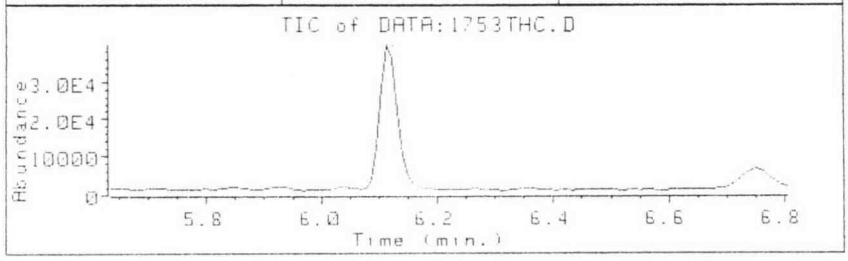
Als bottle num : 0
Replicate num : 1

d3 THC (ISTD) peaks



THC-COOH peaks





THC ISTD and Ratio Report

Cal Inj Date : 10 Apr 89 11:37 Cal Operator : KJN 4/10/89
Cal Date : 10 Apr 89 11:47 INSTRUMENT Cal File: DATA:THCCAL.D
Date : 10 Apr 89 12:19 Inst ID: HP GC/MS
Data file: DATA:1753THC.D Tune Date: 9 Apr 89 14:55
Name Info: 7/1753 [redacted] URINE THC
Misc Info:
Operator : KJN 4/10/89
Comment :

Retention Time Min. (Rel)	MASS	AREA	AMT(ng/mL)	TARGET RANGE	RATIO
* 1 d3 THC (ISTD)			CAS #:		
6.11(1.000)	316	311442			100.00
6.11	360	177941	20.00	39.45 - 59.17	57.13
6.11	375	98221		22.06 - 33.10	31.86
2 THC-COOH			CAS #:		
6.12(1.002)	313	113420	8.75		100.00
6.13	357	68118		47.62 - 71.42	60.06
6.13	372	38435		26.11 - 39.17	33.88

Appendix Figure 2: An example output chromatograph report for a positive THCCOOH result from subject A using selected ion monitoring gas chromatography/mass spectrometry.

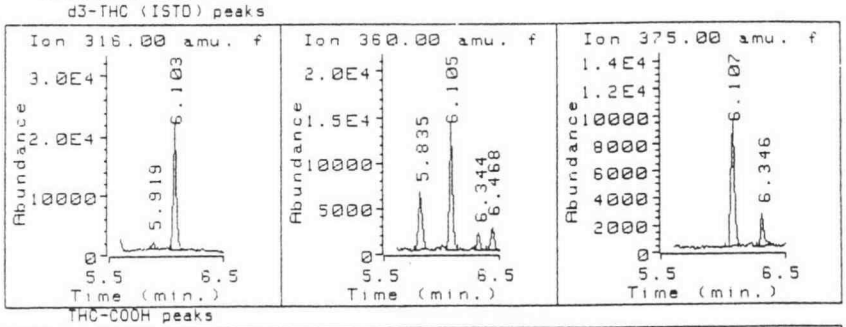
Data file: DATA:[redacted].D
File type: GC / MS DATA FILE

Name Info: 10/2895 [redacted] URINE THC
Misc Info:
Operator : GDB 5/11/89

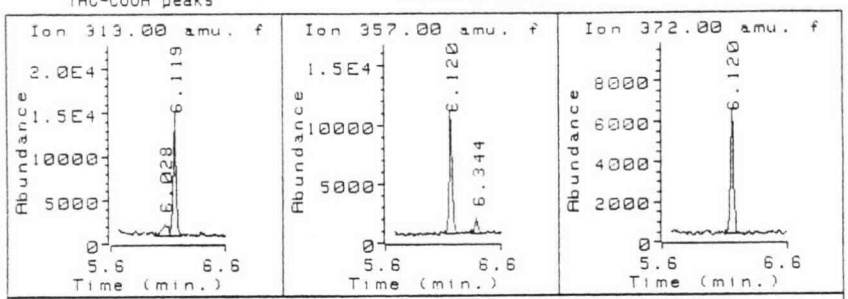
Date : 11 May 89 2:20 pm
Instrment: MS_5970
Inlet : GC

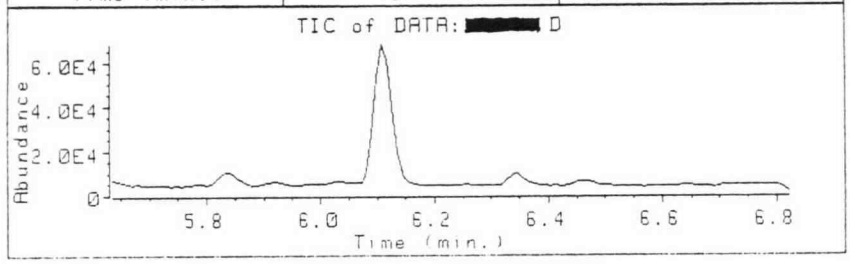
Sequence index : 0
Als bottle num : 0
Replicate num : 1

d3-THC (ISTD) peaks



THC-COOH peaks





THC ISTD and Ratio Report

Cal Inj Date : 11 May 89 13:41 Cal Operator : GDB 5/11/89
Cal Date : 11 May 89 13:50 INSTRUMENT Cal File: DATA:THCCAL.D
Date : 11 May 89 14:20 Inst ID: HP GC/MS
Data file: DATA:[redacted].D Tune Date: 11 May 89 10:06
Name Info: 10/2895 [redacted] URINE THC
Misc Info:
Operator : GDB 5/11/89
Comment :

Retention Time Min. (Rel)	MASS	AREA	AMT(ng/mL)	TARGET RANGE	RATIO
* 1 d3-THC (ISTD)			CAS #:		
6.10(1.000)	316	428374			100.00
6.11	360	269955	20.00	46.02 - 69.04	63.02
6.11	375	168317		29.27 - 43.91	39.29
2 THC-COOH			CAS #:		
6.12(1.002)	313	264604	17.99		100.00
6.12	357	195450		57.92 - 86.88	73.87
6.12	372	112234		34.57 - 51.85	42.42