Published in final edited form as:
J Anal Toxicol. 2009 May ; 33(4): 185–189.

Identifying New Cannabis Use with Urine Creatinine-Normalized THCCOOH Concentrations and Time Intervals Between Specimen Collections*

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Abstract

A previously recommended a method for detecting new cannabis use with creatinine-normalized 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH) urine concentrations in periodically collected specimens for treatment, workplace and judicial drug testing applications is refined by considering the time interval between urine collections. All urine specimens were collected from six less-than-daily cannabis users who smoked placebo, 1.75%, and 3.55% THC cigarettes in randomized order, each separated by one week. Ratios (n = 24,322) were calculated by dividing each creatinine-normalized THCCOOH concentration (U2) by that of a previously collected specimen (U1). Maximum, 95% limit, and median U2/U1 ratios with 15 and 6 ng THCCOOH/mL cutoff concentrations, with and without new use between specimens, were calculated for each 24-h interval after smoking up to 168 h and are included in tables. These ratios decreased with increasing interval between collections providing improved decision values for determining new cannabis use. For example, with a 15 ng THCCOOH/mL cutoff concentration and no new use between specimens, the maximum, 95% limit, and median U2/U1 ratios were 3.05, 1.59, and 0.686, respectively, when the collection interval was ≤ 24 h and 0.215, 0.135, and 0.085 when it was 96–119.9 h.

Introduction

There are a number of venues wherein urine drug testing identifies new cannabis use. Examples are drug treatment and parole programs in which individuals are required to be abstinent and are monitored by periodic urine drug testing. Officials detect new use by testing urine specimens collected at known intervals for the Δ^9-tetrahydrocannabinol (THC) metabolite, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH). A similar approach is taken in legal settings where authorities may ask if an accused with two positive urine specimens can be charged with more than one cannabis use.

Interpretation of results in monitoring or evidence scenarios is not simple. Part of the complexity is that individuals continue to excrete THCCOOH for days after abstinence and although concentrations generally decrease with time concentrations fluctuate with levels of

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hydration. Huestis and Cone (1) conducted a controlled THC administration study in occasional users and made recommendations to aid interpretation. They recommended that THCCOOH concentrations be normalized by dividing each by the urine creatinine concentration (1). This technique reduces the variability in concentration due to hydration effects. Using these creatinine-normalized THCCOOH concentrations, a ratio is calculated that is the concentration of any urine specimen (U2) divided by the concentration in a previously collected urine specimen (U1). With the restrictions of THCCOOH ≥ 15 ng/mL, separation in collection time ≥ 24 h, and evaluation of less than daily cannabis users, new use is indicated by U2/U1 ≥ 0.5. This decision ratio gave maximum accuracy (85.4%) in their study with 5.6% false-positive and 7.4% false-negative rates (1). The ratio seemed well suited for clinical programs where new cannabis use did not involve serious consequences, such as incarceration or loss of child custody. When the consequence of a false-positive result was great, the authors recommended a more conservative decision ratio, previously suggested by Manno et al. (2), of 1.5. Using 1.5 as a decision ratio reduced the false-positive rate to 0.1% and increased the false negative rate to 24% (1).

The ratio method was successfully applied in a number of programs (3–5). Fraser and Worth (5–7) reported that the method predicted new use for chronic cannabis smokers in treatment. They noted that results were better when the time separation in urine collections was greater, recommending at least 48 h in one study and 96 h in another (6,7). Huestis and Cone (1) also discussed the fact that the expected U2/U1 ratio was a function of time, but did not include the time between collections in the decision matrix. The importance of time between collections can be demonstrated by a hypothetical, and typical, military court case. The accused has two different confirmed positive urine drug testing results with the second specimen collected after program personnel received results of the first test. Collections are separated by 70 h. Authorities charge the individual with more than one use of cannabis, which carries a much greater penalty than single use and also precludes a one-time unknowing ingestion defense. The accused claims single use at a party on a weekend 30 h prior to the first collection. U2/U1 for the specimens is 1.3; the defense proffers that his ratio does not exceed the conservative decision value of 1.5 and new use cannot be proved beyond reasonable doubt. A review of the original urinary excretion results from Huestis et al. (8) demonstrates that the accused's profile of urine THCCOOH concentrations is not consistent with any of the subjects who only smoked cannabis once in this interval and is more consistent with new cannabis use. Consideration of the time between specimens would be helpful in this and similar cases. The current study re-evaluates the urine ratio approach for determining new cannabis use as a function of time between specimen collections.

Methods

Subjects, experimental design, urine testing methods, and human use approval were reported previously (8). The original urine cannabinoid and creatinine data were reanalyzed to include intervals between specimens for this study (8). Briefly, in a National Institute on Drug Abuse Institutional Review Board-approved study, six healthy male subjects who smoked cannabis less than daily provided informed consent and resided on a closed research unit. After a washout period that was at least one week, subjects smoked a single cannabis cigarette (placebo, 1.75%, or 3.55% THC) once a week for three consecutive weeks. Random selection yielded the following administration sequences for two subjects each: placebo, 1.75% THC, 3.55% THC; 1.75% THC, 3.55% THC, placebo; and 3.55% THC, placebo, 1.75% THC. Every individual urine specimen was collected throughout the three-week study. Specimens (n = 955) were stored frozen at −20°C until analyzed for THCCOOH by gas chromatography–mass spectrometry (GC–MS). The GC–MS method included base hydrolysis, ion exchange extraction, and methylation for derivatization. The

limit of quantification was 0.5 ng/mL. Urine creatinine was quantified using a modified Jaffe method on an Hitachi 704 automated clinical analyzer (8).

Creatinine-normalized THCCOOH concentrations were determined by dividing the THCCOOH concentration in nanograms per milliliter by the creatinine concentration in milligrams per deciliter, then multiplying by 100 in order to report results in nanograms THCCOOH per milligram of creatinine. For each subject, U2/U1 ratios were calculated by dividing the normalized THCCOOH concentration for each urine specimen by the normalized THCCOOH concentration of each previous specimen. Data were analyzed to address new cannabis use with two methods.

Method 1

U2/U1 was calculated and all urine pairs were considered. Ratios were segregated by individual and into groups with and without intervening cannabis smoking. Statistical examination of the data by individual demonstrated that there were no significant differences in the measures reported here between the 1.75% and 3.55% THC cigarette doses (paired Student's *t* test, $p > 0.05$); therefore, these data were combined for all subjects. THCCOOH cutoff concentrations of 6 and 15 ng/mL were both considered. The latter cutoff concentration is the most commonly employed and is required for United States federal drug testing programs. The former cutoff concentration is common for retests and in laboratories supporting criminal investigations. Normalized ratios were compartmentalized by the time between collections of urine pairs: 0–23.9, 24–47.9, 48–71.9, 72–95.9, 96–119.90, 120–143.9, and 144–167.9 h. Results of this analysis can be applied to determine new cannabis use in workplace, treatment, and criminal justice drug testing programs and forensic investigations.

Method 2

Method 2 was the same as Method 1 with no drug use between collections but omitting urine specimens collected in the first 24 h after drug administration. Most individuals reach peak normalized THCCOOH concentrations within this timeframe. For example, in our original experimental cannabis administration data, five of six subjects had peak creatinine-normalized THCCOOH concentrations within 12 h of smoking, with one peaking at 28 h, 4 h after the selected time restriction. Two urine specimens from this latter subject collected prior to peak urine THCCOOH concentration, that is, during the absorptive phase, were included in our study. For most individuals, one would expect THCCOOH concentrations to decrease and U2/U1 to be less than 1. Method 2 results are relevant when there is information that peak urine concentrations occurred prior to specimen collections or there is evidence that the individual being evaluated smoked cannabis more than 24 h prior to collection of the first urine specimen. This analysis could be used for urine specimens collected from individuals who state that their last drug use was more than 24 h prior to urine collection or results of additional specimens indicate that subsequent collections are after peak urine concentrations.

Results and Discussion

This study evaluated 24,322 urine specimen pairs collected from six subjects who each smoked a placebo and two cannabis cigarettes (1.75% and 3.55% THC) each separated by one week. It is the only study to date that examines the relationship between creatinine-normalized THCCOOH concentrations in urine following smoking as a function of time between specimens. Considering only pairs with no new cannabis use between urine collections and applying a 6 ng THCCOOH/mL cutoff concentration, the number of urine pairs was 2801 (Method 1). The median U2/U1 ratio decreased as expected with each

additional day between urine collections (Table I). This was not always true for minimum and maximum values. For example, the maximum ratio for the 72–95.9 h time interval (U2/U1 = 1.63) was greater than for the 48–71.9 h period (U2/U1 = 1.47). This resulted from one ratio, 1.63, with the next closest ratio of 1.40 in the 72–95.9 interval being lower, as expected. The range of ratios in each time category is broad. Both the data range and occasional unusual specimen concentration support past observations (9) that THCCOOH concentrations in the low concentration range, even when creatinine-normalized, are much more variable than those above 15 ng/mL. If a 95% limit is imposed, that is, the highest 5% of ratios are removed from the data set, outliers are also removed and the ratios decrease as expected with time between specimen collections. The 95% limit ratios decrease in each successive time interval from 1.42 in the first 24 h period to 0.073 in the last interval of the 168 h post-dose timeframe (Table I).

The medians reported in Table I are useful in understanding the decrease in expected ratios as the collection interval increases, but the maximum and 95% limits are more helpful in applications. Practitioners evaluating the ratio of an individual in their program can use these maximum and 95% ratio limits to identify new use, dependent on the level of certainty needed. For this reason, we will refer to them as decision ratios. If the ratio under consideration exceeds the maximum, the practitioner could tell the donor that, beyond a reasonable doubt, he or she had used cannabis between the urine collections. For ratios less than the maximum but greater than the 95% decision ratio, new use would be inferred with reasonable certainty.

There were 754 urine pairs with both THCCOOH concentrations equal to or greater than the more common cutoff concentration of 15 ng THCCOOH/mL. No urine specimens collected more than 123 h after smoking were positive resulting in no collection intervals greater than 96–119.9 h. For collection intervals greater than 24 h, the median, maximum, and 95% decision ratios were all lower than those determined for the 6 ng/mL cutoff concentration (Table I). One example of applying the 95% decision ratio might be in a treatment program that sends urine specimens to a laboratory reporting with the usual 15 ng/mL cutoff concentration. Consider a patient who donated two urine specimens separated by 60 h with a U2/U1 ratio of 0.51. A counselor using Table I would observe that 95% of subjects who did not use cannabis after the first specimen was collected had ratios less than 0.508. The patient could be challenged with the evidence that 95% of individuals who abstained from cannabis use for 48–71.9 h had a ratio lower than his or her result, suggesting new cannabis use between collections.

In Method 2, specimens in the original urine excretion data were not considered if collected less than 24 h after cannabis administration (Table II). This restriction removed most specimens collected during the absorption phase. The U2/U1 ratios are similar to those for Method 1 with median and 95% decision limits decreasing with increasing time intervals. Maximum U2/U1 ratios for the 48–71.9 and 72–95.9 h collection intervals were the same as for Method 1 and were lower for other timeframes. Using a 15 ng THCCOOH/mL cutoff concentration, all maxima were equal to or lower than those for Method 1. For the two common collection intervals of 24–47.9 and 48–71.9 h, maximum U2/U1 ratios were 0.841 and 0.529, respectively. Officials applying these in a program could infer new drug use by donors with ratios exceeding these decision ratios and have a high level of confidence in their conclusions. One application of these results is the hypothetical court case described in the Introduction. In that case, specimen collections were separated by 70 h with U2/U1 = 1.3, and the accused admitted drug use 30 h prior to the first collection. One can see from results in Table II that no ratios for urine pairs separated by 48–71.9 h in this study exceeded 0.529 and that the 1.3 ratio is greater than the maximum value. This information could be used as evidence that the accused smoked cannabis between urine collections. Therefore, the

charge of more than one cannabis use would be supported by the scientific evidence. The previously recommended conservative decision ratio of 1.5 was inappropriately high for this collection time interval.

Another way to express findings is to determine the percent false-positive results and true-negative rates for selected decision values of U2/U1. Any calculated U2/U1 in question equal to or greater than the decision value is indicative of new cannabis use. Decision values are different for each 24-h collection interval. Choosing the maxima shown in Table I as decision ratios would yield no false-positive results and a 100% true-negative rate, regardless of the THCCOOH cutoff concentration selected. If one were to select the highest ratio for 95% of subjects as a decision value, there would be 5% false-positive and 95% true-negative rates.

The experimental design of our study did not allow evaluation of false-negative and true-positive results by collection interval for general applications. Following a washout period, subjects smoked cannabis cigarettes one week apart, which created at least a one-week washout period prior to each smoking session. In most applications, individuals who smoke between urine collections do so at random times. For this reason, our results for expected ratios when subjects smoked cannabis between urine collections are only applicable to the post-washout circumstance and are offered to be helpful in understanding the general nature of expected ratios following new cannabis use. When there was drug use between urine collections, U2/U1 ratios were much higher than without intervening drug use (Table III). The values in Table III were determined by Method 1 in cases when there was known drug use between specimen collections. Median ratios decrease with increasing interval between collections but remain higher than corresponding ratios from specimens collected without intervening drug use (compare Tables I and III). For example, the range of U2/U1 when cannabis was smoked between specimens collected 24–47.9 h apart was 8.69 to 30.6 (data > 6 ng THCCOOH/mL cutoff concentration, Table III). The corresponding range of ratios when no new use occurred was 0.073 to 2.27 (Table I). There was no overlap of U2/U1 ratios, and this was true for up to 71.9 h between collections. As stated, these results are helpful in understanding the general nature of expected ratios when there is drug use between specimen collections, but minimum ratios should not be used as lower limit decision ratios. The upper limit decision ratios in Tables I and II should be used to identify new cannabis use.

Most urine drug testing laboratories use a cutoff concentration of 15 ng THCCOOH/mL. Our results with this cutoff concentration follow the same pattern as observed with a 6 ng/mL cutoff concentration but there are fewer data. No positive results were observed greater than 123 h after smoking for these less than daily cannabis users. This eliminated most results during the washout period and immediately preceding the next drug use. With an extended washout period following the last positive urine test in our study design, the only collection intervals for urine pairs with intervening drug use and THC-COOH concentrations ≥ 15 ng/mL were greater than 96 h. Despite this limitation, the results reflect the higher U2/U1 ratios expected when new use occurs between collections. Median ratios with no new use between collections separated by more than 24 h were less than 0.346 and those for new use exceeded 1.08.

This refinement of the previously published creatinine-normalized urinary THCCOOH new use prediction guidelines takes into consideration specific intervals after cannabis smoking rather than grouping all data into a single week after drug use. This improves the accuracy of prediction within each 24-h period.

In conclusion, our study results can be applied in treatment, workplace, and judicial cases to determine cannabis use between urine specimen collections from less than daily cannabis users. There must be THCCOOH and creatinine concentrations for at least two urine specimens with a known time interval between collections. The most conservative method for reporting new cannabis use between collections would apply a U2/U1 decision ratio equal to the maxima listed in Table I. A more realistic decision ratio with reasonable certainty would be the 95% limits in the same table. In cases where there is evidence that the donor's last cannabis use was greater than 24 h before the first collection, the same approach could be used but with the limits provided in Table II. These new cannabis use decision criteria update previously published approaches and refine criteria to specific 24-h intervals between urine specimen collections in less than daily cannabis users. These specific decision ratios will improve detection of new cannabis use and support claims of abstinence and residual urinary drug excretion by those participating in treatment, workplace, and judicial programs.

Acknowledgments

The authors thank the American Registry of Pathology and the National Institutes of Health, Intramural Research Program, National Institute on Drug Abuse for funding of this study.

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J Anal Toxicol. Author manuscript; available in PMC 2011 August 22.

Table I

U2/U1* Ratios by Specimen Collection Interval for Six Subjects with No Cannabis Use Between Collections

	Cutoff Concentration 6 ng THCCOOH/mL					Cutoff Concentration 15 ng THCCOOH/mL				
ΔT (h)	***N***	**Minimum**	**Maximum**	**Median**	**95% below**	***N***	**Minimum**	**Maximum**	**Median**	**95% below**
0–23.9	1063	0.217	6.29	0.734	1.42	399	0.217	3.05	0.686	1.59
24–47.9	791	0.073	2.27	0.421	1.01	235	0.073	1.74	0.346	0.910
48–71.9	469	0.023	1.47	0.255	0.853	85	0.038	1.45	0.180	0.508
72–95.9	250	0.024	1.63	0.165	0.595	23	0.038	0.250	0.161	0.242
96–119.9	140	0.026	0.555	0.102	0.347	12	0.055	0.215	0.085	0.135
120–143.9	64	0.026	0.197	0.060	0.146					
144–167.9	24	0.020	0.080	0.037	0.073					

*U2 = creatinine normalized THCCOOH concentration in a selected urine specimen, and U1 = creatinine normalized THCCOOH concentration in a previously collected urine specimen.

Table II

U2/U1* Ratios by Specimen Collection Interval for Six Subjects with No Cannabis Use Between Collections and Data Omitted for the First 24 h after Smoking

	Cutoff Concentration 6 ng THCCOOH/mL					Cutoff Concentration 15 ng THCCOOH/mL				
ΔT (h)	*N*	Minimum	Maximum	Median	95% below	*N*	Minimum	Maximum	Median	95% below
0–23.9	665	0.223	2.04	0.744	1.28	193	0.223	1.82	0.712	1.25
24–47.9	454	0.107	1.60	0.478	0.969	66	0.144	0.841	0.391	0.626
48–71.9	263	0.085	1.47	0.337	0.888	20	0.106	0.529	0.271	0.471
72–95.9	132	0.082	1.63	0.228	0.684	9	0.137	0.250	0.201	0.242
96–119.9	53	0.090	0.480	0.141	0.361					
120–143.9	11	0.081	0.175	0.123	0.169					

*U2 = creatinine normalized THCCOOH concentration in a selected urine specimen, and U1 = creatinine normalized THCCOOH concentration in a previously collected urine specimen.

Table III

U2/U1[*] Ratios by Specimen Collection Interval for Six Subjects Who Smoked THC Cigarettes[†] Between Collections

	Cutoff Concentration 6 ng THCCOOH/mL					Cutoff Concentration 15 ng THCCOOH/mL				
ΔT (h)	*N*	Minimum	Maximum	Median	95% above	*N*	Minimum	Maximum	Median	95% above
0–23.9	1	21.3	21.3	21.3						
24–47.9	14	8.69	30.6	24.1	8.86					
48–71.9	40	2.22	44.4	19.0	3.11					
72–95.9	66	0.750	34.2	9.50	1.29					
96–119.9	117	0.724	49.5	8.75	1.87	6	4.70	16.9	11.2	4.69
120–143.9	188	0.795	36.5	5.13	1.33	43	2.00	11.3	5.48	2.31
144–167.9	301	0.599	20.9	2.70	0.872	126	0.632	10.0	2.61	1.08
> 168	1061	0.500	7.43	1.04	0.551	276	0.502	5.90	1.08	0.566

[*] U2 = creatinine normalized THCCOOH concentration in a selected urine specimen, and U1 = creatinine normalized THCCOOH concentration in a previously collected urine specimen.

[†] Each subject smoked a placebo, 1.75% THC, and 3.55% THC cigarette one week apart.

J Anal Toxicol. Author manuscript; available in PMC 2011 August 22.