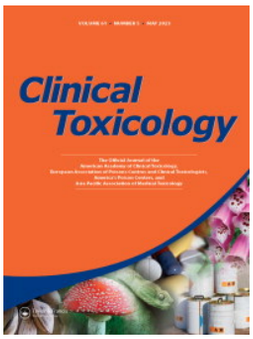

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CLINICAL RESEARCH

Blood cannabinoid molar metabolite ratios are superior to blood THC as an indicator of recent cannabis smoking

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ABSTRACT

Introduction: Cannabis use is a growing concern in transportation and workplace incidents. Because Δ9-tetrahydrocannabinol is detectable after acute psychoactive effects have resolved, it has limitations as an indicator of recent usage or potential impairment.

Methods: In an observational study of driving and psychomotor performance, we measured whole blood Δ9-tetrahydrocannabinol plus its metabolites 11-hydroxy-Δ9-tetrahydrocannabinol and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol by liquid chromatography with tandem mass spectrometry at baseline and 30 min after starting a 15-minute interval of smoking cannabis in 24 occasional and 32 daily cannabis smokers. We calculated two blood cannabinoid molar metabolite ratios: 1) [Δ9-tetrahydrocannabinol] to [11-nor-9-carboxy-Δ9-tetrahydrocannabinol] and 2) ([Δ9-tetrahydrocannabinol] + [11-hydroxy-Δ9-tetrahydrocannabinol]) to [11-nor-9-carboxy-Δ9-tetrahydrocannabinol]. We compared these to blood [Δ9-tetrahydrocannabinol] alone as indicators of recent cannabis smoking.

Results: Median Δ9-tetrahydrocannabinol concentrations increased from 0 (<limit of detection 0.2 μg/L) at baseline to 5.6 μg/L post-smoking in occasional users. Among daily users, these were 2.7 μg/L at baseline and 21.3 μg/L post-smoking. Median molar metabolite ratio 1 increased from 0 at baseline to 0.62 post-smoking in occasional users and from 0.08 at baseline to 0.44 post-smoking in daily users. The median molar metabolite ratio 2 increased from 0 to 0.76 in occasional users and from 0.12 to 0.54 among daily users. A molar metabolite ratio 1 cut-point of 0.18 yielded 98% specificity, 93% sensitivity, and 96% accuracy for identifying recent cannabis smoking. A molar metabolite ratio 2 cut-point of 0.27 yielded 98% specificity, 91% sensitivity, and 95% accuracy. The receiver operating characteristic curves for molar metabolite ratio 1 and molar metabolite ratio 2 were not statistically different ($P > 0.38$). By comparison, a cut-point for Δ9-tetrahydrocannabinol of 5.3 μg/L yielded 88% specificity, 73% sensitivity, and 80% accuracy.

Conclusions: In occasional and daily users, the blood cannabinoid molar metabolite ratios were superior to whole blood Δ9-tetrahydrocannabinol as indicators of recent cannabis smoking. We recommend measurement and reporting of Δ9-tetrahydrocannabinol, 11-hydroxy-Δ9-tetrahydrocannabinol, and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol, and their molar metabolite ratios in forensic and safety investigations.

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Blood cannabinoids; cannabis; Δ9-tetrahydrocannabinol; THC; Molar metabolite ratio; cannabis kinetics

Introduction

The role of recent cannabis use in the occurrence of a transportation crash or workplace mishap is of considerable interest to forensic toxicology and transportation and workplace safety. In occupational settings, it is common practice for employers to require that employees undergo post-incident urine drug tests [1]. If driving under the influence of cannabis is suspected, many law enforcement agencies require that drivers undergo measurement of whole blood Δ9-tetrahydrocannabinol (hereafter THC), the main psychoactive component of cannabis. In some jurisdictions, concentrations of THC in whole blood that exceed certain thresholds establish a *per se* determination that a driver was "under the influence" of cannabis [2].

These post-incident testing approaches have well-established limitations in their ability to accurately assess either recent cannabis use or cannabis-induced impairment. Acute cannabis-induced decrements in psychomotor or neurocognitive performance, which may occur in some but not all users,

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typically resolve within 6 h of cannabis smoking or vaping or within 8 h of cannabis ingestion [3]. By comparison, urine drug tests, which rely on the detection of the inactive THC metabolite, 11-nor-9-carboxy-Δ9-tetrahydrocannabinol, may be positive for days to weeks after last cannabis use, particularly in frequent users [4]. Blood concentrations of THC, a highly lipophilic drug that partitions into adipose tissue and equilibrates with the bloodstream, may also remain elevated for days to weeks after the last consumption in daily or near-daily users [5].

Research involving relatively small numbers of subjects has suggested that the ratio of the sum of the molar concentration in the blood of THC and its psychoactive metabolite 11-hydroxy-Δ9-tetrahydrocannabinol to the inactive metabolite 11-nor-9-carboxy–Δ9-tetrahydrocannabinol >0.30 may be useful in assessing cannabis smoking within the prior two hours [6]. Daldrup and Meininger [7] proposed that a version of this ratio (plasma or whole blood molar ratios multiplied by 100) be known as the "cannabis influence factor" based on a positive correlation between numerical values of the ratio and police reports of abnormal driving (e.g., swerving) or aggressive behaviour in apprehended drivers. In light of research demonstrating acquired tolerance to the acute psychomotor and neurocognitive effects of cannabis with daily or near-daily cannabis use [8], the use of the term "cannabis influence factor" may be inadvisable because it risks conflating pharmacokinetic findings (a temporal elevation in THC and 11-hydroxy-Δ9-tetrahydrocannabinol relative to 11-nor-9-carboxy-Δ9-tetrahydrocannabinol) with pharmacodynamic impacts (i.e., impaired performance) that may or may not be present.

We report the comparative reliability of the molar ratio of THC/11-nor-9-carboxy-Δ9-tetrahydrocannabinol, which we term the molar metabolite ratio 1, and the molar ratio of (THC + 11-hydroxy-Δ9-tetrahydrocannabinol)/11-nor-9-carboxy-Δ9-tetrahydrocannabinol, which we term molar metabolite ratio 2 to THC concentration alone as an indicator of recent cannabis smoking in this group with a mixed cannabis use history.

Methods

Study design and subjects

This project was part of a larger study that prospectively examined the within-subject change in performance on driving simulator-based measures of vehicle control and safety and on tablet-based psychomotor tests in occasional and daily cannabis users and nonusers assessed at a pre-smoking baseline and again 30 min after the end of a 15-minute interval of acute cannabis smoking or rest [9]. Healthy adults (aged 25–45 years) were recruited in the Denver, Colorado area between October 2018 and February 2020. Since a key objective of our larger study was to investigate the role of cannabis use history on acute changes in performance, subjects were recruited, within age and gender quotas, whose cannabis use pattern consisted of either (1) daily cannabis use, defined as smoking or vaping cannabis flower product at least once per day, every day of the week for 30 days prior to enrollment; or (2) occasional cannabis use, defined as smoking or vaping cannabis flower product on at least one day but no more than two days per week in the 30 days prior to enrollment. Enrollment criteria pertinent to the present report included the exclusion of individuals with a past or current history of significant medical illness, those who would not agree to refrain from the use of nonprescription psychotropic drugs, opioids, or sedative hypnotics during the study, individuals with a body mass index >35 kg/m^2, and those who were pregnant or nursing an infant.

Participants attended an in-person screening visit to review and confirm the criteria. Cannabis use history was confirmed by the completion of a 30-day timeline follow-back calendar reporting all cannabis use. Participants completed an alcohol breath test (Lifeloc FC10^{TM}) to screen for acute alcohol use and provided a urine sample to test for illicit drug use or use of prescription drugs not prescribed (30 mL Alere brand 13-panel iCup). A positive 11-nor-9-carboxy-Δ9-tetrahydrocannabinol was not exclusionary for occasional users and was requisite for daily users. A data collection visit was then scheduled within 10 days and usually less than a week from the screening visit. For the data collection visit, participants were instructed not to use inhaled cannabis for at least 8 h and not to consume edible cannabis for at least 12 h before the appointment. Their cannabis use pattern between the screening visit and data collection visit was also verified by a review of a diary they completed detailing the time and amount of all cannabis use, other medication and drug use, and sleep duration. Participants again completed an alcohol breath test and provided a urine sample to screen for acute alcohol or other exclusionary drug use. Written informed consent was obtained from all participants. The study was approved by the Colorado Multiple Institutional Review Board.

Measurements: cannabis consumption and blood cannabinoids

Cannabis use occurred within an observational, naturalistic design in which subjects obtained their own cannabis flower (bud) from a state-licensed Colorado dispensary that was brought to the study site in its original labelled packaging. The labelling listed the total percent THC (tetrahydrocannabinolic acid + Δ9-tetrahydrocannabinol), which for this study was required to be between 15% and 30% by weight and less than 2% cannabidiol by weight. During a 15-minute interval, participants in the user groups were instructed to smoke *ad libitum* "the amount you most commonly use for the effect you most commonly desire." Smoking occurred via a pipe, joint (rolled cigarette), bong or vaporizer according to the participant's choice. Only one subject, an occasional user, used a vaporizer. The weight of the product combusted during the smoking period was determined using a milligram scale. The number of inhalations was recorded by a member of the research team. The smoking occurred in a ventilated room with the subjects seated in a recliner. Subjective drug effect was measured with a visual analogue scale on which participants were asked to mark the point on the line

indicating "how high you are feeling right now" ranging from "not high at all (0 cm)" to "most high ever (100 cm)."

Prior to use, and 30 min after the start of smoking (15 min after the end of the smoking period), a certified phlebotomist collected approximately 10 mL of whole venous blood into grey-top tubes (BD brand vacutainer tubes containing 100 mg sodium fluoride and 20 mg potassium oxalate additive). Blood was stored at approximately 4 °C (39.2 °F) for analysis within 30 days. Whole blood samples were shipped on cold packs to the Colorado State University Analytical Toxicology Laboratory for analysis.

Whole blood samples were prepared for liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis by using solid phase extraction following a published methodology by Schwope and colleagues [10]. Prepared calibrators, controls, and samples were analyzed with an Agilent 1290 Ultra High-Performance Liquid chromatography (UHPLC) system coupled to an Agilent 6460 triple quadruple mass spectrometer equipped with an Agilent Jet Stream electrospray ionization source (Agilent, Santa Clara, CA). Cannabinoids were first chromatographically separated on a Restek Raptor Biphenyl column (2.1 × 100 mm, 5 μm) held at 40 °C. A sample volume of 10 μL was injected in a mixture of water with 5 mM ammonium acetate/0.1% acetic acid (A) and 15% methanol in acetonitrile (B) at a flow rate of 0.4 mL/min. The gradient elution used was 30% B for 1 min, increasing to 100% B at 7 min, and held at 100% B for 3 min. The ionization source conditions used were as follows: nebulizer 45 psi; gas flow of 12 L/min at 330 °C; sheath gas flow of 12 L/min at 390 °C. The electrospray ionization polarity was set to positive for THC. Negative ionization was used for 11-nor-9-carboxy-Δ9-tetrahydrocannabinol. Two ion transitions (m/z) were monitored for each analyte and the corresponding deuterium-labelled internal standard. The data collection and processing were performed by using Agilent MassHunter Quantitative software (B.08.01). Quantitation was performed with linear regression using 6-point calibration curves. Limits of quantitation (LOQ) were 0.5 μg/L for THC, 0.5 μg/L for 11-hydroxy-Δ9-tetrahydrocannabinol, and 2.5 μg/L for 11-nor-9-carboxy-Δ9-tetrahydrocannabinol. Limits of detection (LOD) were 0.2 μg/L for THC, 0.2 μg/L for 11-hydroxy-Δ9-tetrahydrocannabinol and 1 μg/L for 11-nor-9-carboxy-Δ9-tetrahydrocannabinol.

Data analysis

Baseline and post-smoking blood cannabinoids were obtained from 34 subjects in the occasional user group and 32 subjects in the daily user group. As described elsewhere [9], cannabinoid results from four of the subjects recruited into the occasional use group were excluded from further analysis because baseline blood 11-nor-9-carboxy-Δ9-tetrahydrocannabinol concentrations were >68 μg/L. This was higher than all but nine participants in the daily user group and was therefore considered to be inconsistent with occasional cannabis use. Cannabinoid values from an additional six subjects in the occasional user group were excluded from further data analysis because of post-smoking blood THC concentrations of less than 1.0 μg/L. It is possible that these participants did not sufficiently inhale the cannabis they smoked or vaped, or that the actual concentration of THC in the cannabis they used was much lower than the concentration stated on the product label. As these participants would be considered nonusers based on the limit of detection of THC of 1 μg/L used in many forensic drug assays, their values were not included in further data analysis. The decision to exclude participants based on THC or 11-nor-9-carboxy-Δ9-tetrahydrocannabinol concentrations was made prior to further data analysis. This yielded a final participant count of 56, consisting of 24 occasional users and 32 daily users.

The two molar metabolite ratios, both unitless values, were calculated as the sum of the nanomolar equivalents of THC alone, or of THC plus 11-hydroxy-Δ9-tetrahydrocannabinol, divided by that of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol, according to the equations:

$$\text{Molar metabolite ratio 1} = \frac{\frac{[\Delta 9\text{-tetrahydrocannabinol}]}{314.5}}{\frac{[11\text{-nor-9-carboxy-}\Delta 9\text{-tetrahydrocannabinol}]}{344.5}}$$

$$\text{Molar metabolite ratio 2} = \frac{\frac{[\Delta 9\text{-tetrahydrocannabinol}]}{314.5} + \frac{[11\text{-hydroxy-}\Delta 9\text{-tetrahydrocannabinol}]}{330.5}}{\frac{[11\text{-nor-9-carboxy-}\Delta 9\text{-tetrahydrocannabinol}]}{344.5}}$$

in which the bracketed values correspond to each the whole blood concentration of each cannabinoid in μg/L and the numerical values correspond to their respective molecular weight. As a memory aid, it may be noted that molar metabolite ratio 1 and molar metabolite ratio 2 have one and two cannabinoids in their numerator, respectively. Unlike the calculation of "cannabis influence factor" proposed by Daldrup and Meininger [7], the molar ratios shown here were not multiplied by 100. In calculating molar metabolite ratio 1 and molar metabolite ratio 2, blood cannabinoid values less than their respective limit of quantitation were assigned a value of zero. Molar metabolite ratio 1 and molar metabolite ratio 2 were assigned a value of zero when 11-nor-9-carboxy-Δ9-tetrahydrocannabinol was less than the LOQ.

Receiver operating characteristic (ROC) curves were constructed based on the sensitivity and specificity that corresponded to a range of possible thresholds of THC or molar metabolite ratio 1 and molar metabolite ratio 2 predicting the dichotomous outcome of whether or not the value was obtained from the baseline or post smoking blood collection. Candidate cut-points were examined in a way to warrant the best combination of sensitivity, specificity, Youden's J statistic (J = sensitivity + specificity − 1), and the distance to the upper left-hand corner of the ROC curve (coordinate 0,1). The area under the curve (AUC) for ROC curves pertaining to THC and the molar metabolite ratios were compared by the nonparametric approach [11]. All the statistical analysis was performed using SAS version 9.4 (SAS Institute, Cary NC)

Results

Cannabis use and blood cannabinoid concentrations of subjects

Table 1 presents the demographic features and cannabis use history of subjects recruited as daily users or occasional users. Based on the date and time of cannabis use recorded on a study-supplied diary, subjects in both groups reported adherence to the request for short-term abstention from cannabis use prior to data collection. The exact time of the last use was missing for five subjects. Three subjects, each daily user, were on stable doses of venlafaxine, sertraline, and nortriptyline, respectively. Although the metabolism of these drugs involves certain cytochrome P450 family enzymes that exert a minor contribution to the metabolism of THC, they lack a moderate or strong inhibition of the CYP enzymes involved in THC metabolism. These subjects were therefore included in the analysis.

Table 2 reports the THC content and quantity of cannabis consumed *ad libitum* by the subjects. Both groups self-supplied cannabis flowers with similar THC content (overall mean total THC concentration of 21.7 percent by weight). The daily users smoked more cannabis, for a longer interval of time, than the occasional users.

Whole blood concentrations of THC, 11-hydroxy-Δ9-tetrahydrocannabinol, and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol measured at baseline and again in blood collected approximately 30 min after the start of the 15 min allotted to *ad libitum* smoking is shown in Table 3. As expected, no THC or 11-hydroxy-Δ9-tetrahydrocannabinol was detectable at baseline in the blood of the occasional users. By comparison, the median blood THC concentration at baseline of the daily users was 2.7 μg/L, range < LOD − 26.0 μg/L. Eleven of the 32 daily users had a baseline whole blood THC concentration ≥ 5 μg/L. After smoking, the median whole blood THC concentration of the daily users was 21.3 μg/L, approximately 4-fold higher than the respective values in occasional users of 5.6 μg/L. The median visual analogue scale score of subjective "high" was rated zero on a 0 to 100 cm scale at baseline in both groups and increased post-smoking to 55.8 in the occasional users and 45.5 in the daily users.

Optimal cut-points for molar metabolite ratios and THC to assess recent cannabis smoking

The baseline and post-smoking values of molar metabolite ratio 1 and molar metabolite ratio 2 are reported in Table 3 and displayed in box and whisker plots in Figure 1. Molar metabolite ratio 1 and molar metabolite ratio 2 each increased significantly from baseline to post-smoking in the entire cohort, and in the occasional user group and the daily user group separately ($P < 0.0001$). The relative increase in molar metabolite ratio 1 and in molar metabolite ratio 2 in the daily users from baseline to post-smoking was smaller than that found in the occasional users ($P = 0.004$ and $P < 0.0001$, for molar metabolite ratio 1 and molar metabolite ratio 2, respectively).

Receiver operating characteristic curves of sensitivity versus (1–specificity) presented in Figure 2 compared the utility of relying on blood THC or molar metabolite ratio 1 or molar metabolite ratio 2 as an accurate indicator of whether a subject had recently smoked cannabis. Molar metabolite ratio 1 and molar metabolite ratio 2 yielded superior performance compared to THC, with both molar metabolite ratio 1 and molar metabolite ratio 2 each having an area under the curve (AUC) of 0.99 compared to 0.89 for THC. In the case of

Table 1. Participant demographic characteristics and cannabis use experience.

Demographic	Daily use $n = 32$	Occasional use $n = 24$
Gender	*n* (%)	*n* (%)
Male	18 (56.3)	14 (58.3)
Female	14 (43.8)	10 (41.7)
Age (years)		
Median	32.7	30.1
Range	(25.4, 45.3)	(25.1, 41.3)
Interquartile Range (IQR)	(28.7, 37.4)	(28.0, 34.7)
Cannabis use	Mean (±SD)	Mean (±SD)
Age in years at first use	17.2 (±5.7)	17.6 (±4.7)
Number of days used, in the past 30 days	29.7 (±1.2)	5.7 (±2.6)
Number of days of use per week, in the past 30 days	7.0 (±0.0)	1.5 (±0.5)
Times used per day on average, in the past 30 days	5.1 (±4.6)	1.4 (±0.9)
Time in hours of abstinence prior to baseline blood collection*	13.0 (±2.6)	39.1 (±30.6)

*Due to missing values, the mean and standard deviation (SD) were calculated based on 29 daily users and 22 occasional users.

Table 2. Characteristics of cannabis use during observed smoking.

	Daily use $n = 32$			Occasional use $n = 24$		
	Median	Range	IQR*	Median	Range	IQR*
THC concentration (% by weight)	22.4	(15.0, 27.5)	(19.8, 24.0)	20.1	(15.3, 29.7)	(18.7, 22.4)
Weight of cannabis combusted mg	334.0	(29.0, 1,101.0)	(173.5, 596.5)	113.0	(6.0, 463.0)	(53, 235.5)
Number of inhalations	17.0	(2.0, 49.0)	(9.5, 33.5)	8.0	(2.0, 21.0)	(5.0, 11.0)
Total time smoked minutes	11.5	(0, 15.0)	(8, 13.5)	5.0	(1.0, 13.0)	(3.0, 8.5)

*IQR = interquartile range (25^{th} and 75^{th} percentiles); THC = Δ9-tetrahydrocannabinol.

Table 3. Whole blood cannabinoid concentrations, molar metabolite ratios and perceived drug effect before and after observed cannabis smoking.

	Daily users ($n = 32$)						Occasional users ($n = 24$)					
	Baseline			Post-use			Baseline			Post-use		
	Median	Range	IQR	Median	Range	IQR	Median	Range	IQR	Median	Range	IQR
Whole blood concentrations												
Δ9-tetrahydrocannabinol concentration μg/L*	2.7	(<LOD, 26.0)	(1.2, 5.1)	21.3	(1.3, 146.7)	(8.7, 42.5)	<LOD	–	–	5.6	(1.0, 29.6)	(3.5, 7.1)
11-nor-9-carboxy-Δ9-tetrahydrocannabinol μg/L*	34.8	(3.6, 178.4)	(19.4, 86.4)	55.5	(8.2, 341.7)	(33.5, 139.3)	<LOD	(<LOD, 11.2)	(<LOD, 1.5)	8.2	(3.2, 46.0)	(5.3, 12.6)
11-hydroxy-Δ9-tetrahydrocannabinol μg/L *	1.4	(<LOD, 10.7)	(<LOD, 2.3)	5.1	(0.7, 30.1)	(2, 9.9)	<LOD	–	–	1.2	(<LOD, 5.6)	(0.9, 1.6)
Molar metabolite ratio 1*	0.08	(0, 0.25)	(0.05, 0.12)	0.44	(0.06, 0.69)	(0.28, 0.50)	0	0	0	0.62	(0.12, 1.19)	(0.53, 0.88)
Molar metabolite ratio 2*	0.12	(0, 0.34)	(0.07, 0.16)	0.54	(0.10, 0.80)	(0.36, 0.62)	0	0	0	0.76	(0.17, 1.44)	(0.62, 1.06)
Self-reported drug effect												
Visual analog score of high cm	0.0	(0, 8.0)	(0, 0.5)	45.5	(13.0, 81.0)	(37.5, 57.5)	0.0	(0, 8.0)	(0, 0.5)	55.8	(16.0, 79.0)	(41.5, 63)

LOD: limit of detection Visual analog score ranged from 0-100 cm. IQR = interquartile range (25th and 75th percentiles). *Denotes statistically significant two sample t-test with unequal variances, comparing pre-post differences in daily users versus occasional users at alpha = 0.05. The *P* values were: Δ9-tetrahydrocannabinol concentration ($P = 0.0005$), 11-nor-9-carboxy-Δ9-tetrahydrocannabinol concentration ($P = 0.0023$), 11-hydroxy-Δ9-tetrahydrocannabinol concentration ($P = 0.001$), molar metabolite ratio 1 ($P = 0.0041$), and molar metabolite ratio 2 ($P < 0.0001$).

molar metabolite ratio 1, the cut-point of 0.18 was associated with the lowest Euclidean distance from the upper-left-hand corner of the ROC curve (coordinate 0,1) of 0.07. This cut-point also yielded the highest value for Youden's J index (sensitivity + specificity − 1) of 0.91. Accuracy, an overall ability to distinguish recent cannabis smoking from an abstinent baseline, was defined as (true positive + true negative)/(true positive + true negative + false positive + false negative). For molar metabolite ratio 1, the cut-point of 0.18 also yielded the highest accuracy, equal to 96%.

Assessment of candidate cut-points of molar metabolite ratio 2 found that values of 0.22 and 0.27 both yielded values for Youden's J index of 0.89 and for accuracy of 0.95 (i.e., 95%). The molar metabolite ratio 2 cut-points of 0.22 and 0.27 were associated with respective Euclidean distances to the upper left-hand corner of the ROC curve (coordinate 0,1) of 0.08 and 0.09 respectively, a metric that would favour the selection of the molar metabolite ratio 2 cut-point of 0.22. However, the specificity associated with the molar metabolite ratio 2 cut-point of 0.27 was 98%, slightly higher than the specificity of 96% associated with 0.22. The sensitivity of the molar metabolite ratio 2 cut-point of 0.27 was 91%. When comparing cut-points of equivalent accuracy in a forensic determination, it may be advisable to favour the value with the higher specificity, and hence lower false positive rate.

When assessing only daily users, the optimal cut-point for molar metabolite ratio 1 remained 0.18, yielding an accuracy of 94%, a specificity of 97% and a sensitivity of 91%. In like manner, restricting the analysis to only the daily users, the optimal cut-point for molar metabolite ratio 2 remained 0.27 yielding an accuracy of 92%, a specificity of 97%, and a sensitivity of 88%. For both molar metabolite ratio 1 and molar metabolite ratio 2, there were no statistically significant differences in area under the ROC curve when the curves were calculated using either the entire subject population or just the daily users.

Among THC cut-points with low Euclidean distance to (0,1), the value of 5.3 μg/L yielded the highest specificity, 88%, as well as a sensitivity of 73%, an accuracy of 80%, and a Youden index of 0.61 when calculated for the entire study cohort ($n = 56$). Among just the 32 daily users, a THC cut-point of 6.4 μg/L yielded a specificity of 84%, a sensitivity of 84%, an accuracy of 84%, and a Youden index of 0.69. Table 4 presents 2×2 confusion matrices for the molar metabolite ratio1 cut-point of 0.18, the molar metabolite ratio 2 cut-point of 0.27 and the THC cut-point of 5.3 μg/L for the entire study cohort.

Discussion

This study assessed the relative utility of whole blood THC and two molar metabolite ratios as indicators of cannabis use within the previous 30 min in a group of subjects with occasional or daily cannabis use. Molar metabolite ratio 1 and molar metabolite ratio 2 were each found to be superior to THC alone as an indicator of very recent cannabis smoking. If the findings of this study were to be generalized to an individual who had blood collected for forensic cannabinoid

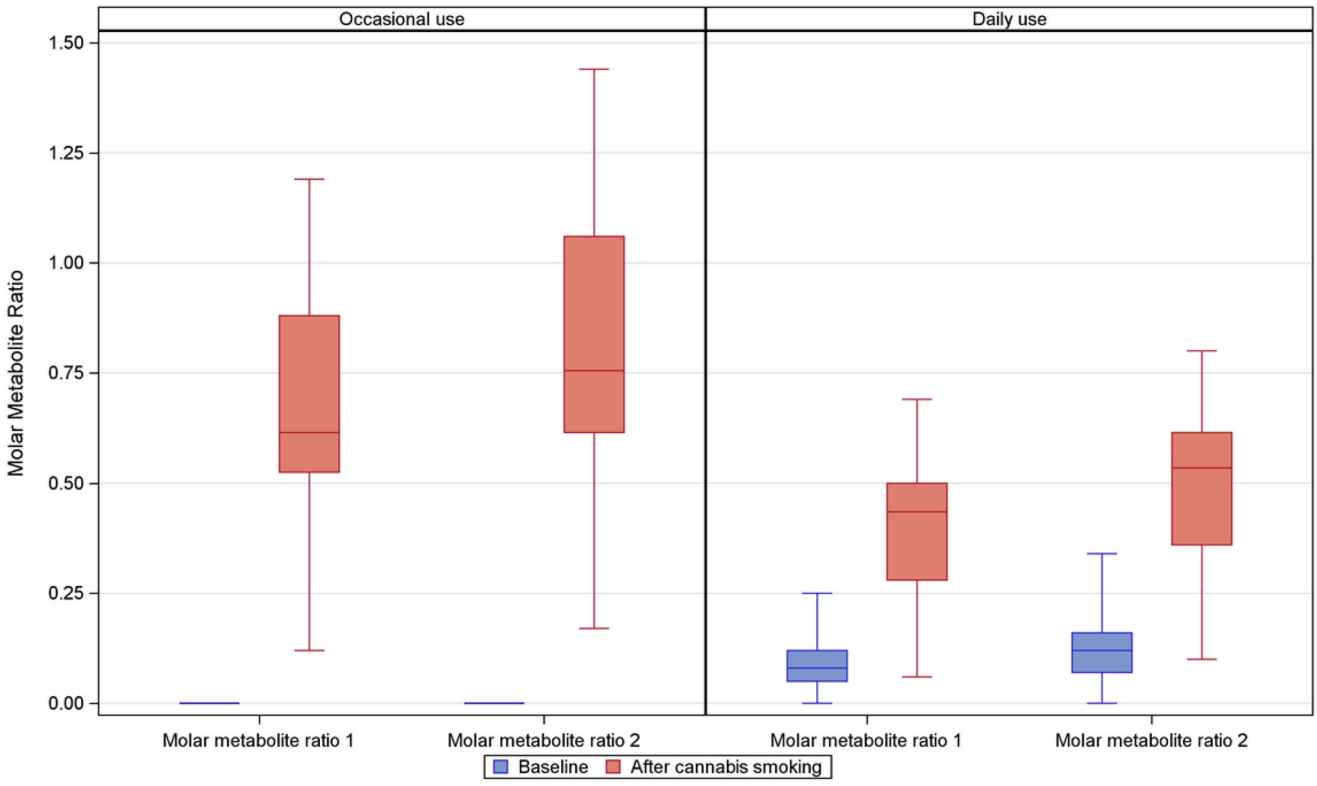

Figure 1. Molar metabolite ratio distributions among occasional and daily cannabis users. Boxes represent interquartile range (25–75th percentile); Horizontal line represents median. Whiskers are drawn to the minimal and maximal values.

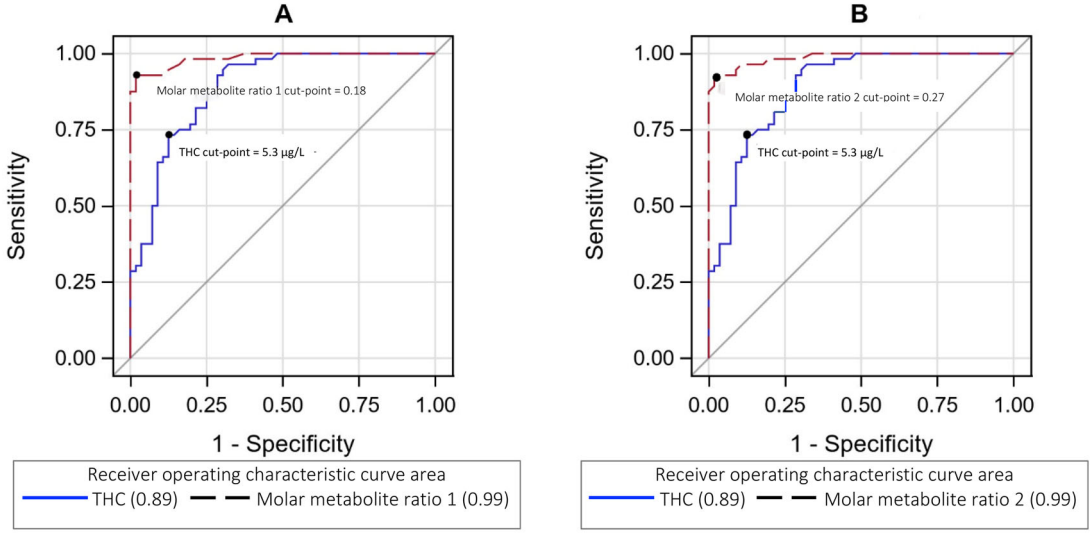

Figure 2. Receiver operating characteristic curves for molar metabolite ratios 1 (panel A) and 2 (panel B).

analysis soon after a transportation crash or a workplace mishap, a molar metabolite ratio 1 $\geq$ 0.18 or a molar metabolite ratio 2 $\geq$ 0.27 would each be $\geq$98% specific for inferring that the donor had very recently smoked cannabis (i.e., within the past 30 min). This inference would be valid regardless of whether the individual was an occasional or a daily cannabis user.

Δ9-Tetrahydrocannabinol has less reliability than molar metabolite ratio 1 or molar metabolite ratio 2 as an indicator of recent cannabis smoking because whole blood THC may remain detectable in daily or near daily cannabis users for many hours after smoking ends, frequently beyond the four to six hours post-smoking interval typically associated with acute psychoactive effects [5]. In Colorado, where medical use of cannabis was legalized in 2000 and recreational cannabis was permitted beginning in 2014, a whole blood THC concentration of $\geq$5 µg/L in a driver's blood serves as "a permissible inference of intoxication" in legal proceedings.

However, in the present study, approximately one-third (11 of 32) of daily users had whole blood concentrations of

Table 4. Confusion matrices and cut-point characteristics of molar metabolite ratios and Δ9-tetrahydrocannabinol.

	Actual cannabis consumption status				
	Baseline	Post smoking	Sensitivity	Specificity	Accuracy
Molar metabolite ratio 1 < 0.18	55 (true negative)	4 (false negative)	93%	98%	96%
Molar metabolite ratio 1 ≥ 0.18	1 (false positive)	52 (true positive)			
Molar metabolite ratio 2 < 0.27	55 (true negative)	5 (false negative)	91%	98%	95%
Molar metabolite ratio 2 ≥ 0.27	1 (false positive)	51 (true positive)			
Blood Δ9-tetrahydrocannabinol concentration <5.3 μg/L	49 (true negative)	15 (false negative)	73%	88%	80%
Blood Δ9-tetrahydrocannabinol concentration ≥5.3 μg/L	7 (false positive)	41 (true positive)			

THC ≥5 μg/L after a minimum of 8 h of short-term abstinence, by which time any acute psychoactive effects would likely have resolved [3]. Some chronic daily cannabis users may have whole blood THC ≥5 μg/L after ≥8 h of supervised abstinence [12,13]. Daily cannabis users constitute a considerable proportion of all cannabis users. In the 2020 Colorado Behavioral Risk Factor Surveillance System survey, among adult respondents using cannabis within the past 30 days, 48% reported use on a daily or near-daily basis (20 to 30 days/month) [14]. In the 2021 Canadian Cannabis Survey, of those using cannabis at least once in the past 12 months, 19% reported daily use [15].

Data from other studies that calculated molar metabolite ratios as a continuous variable after acute cannabis smoking are sparse. In a study of ten adult daily or near-daily heavy cannabis smokers (mean [± SD] of daily joints consumed = 4.9 ± 3.2), the median whole blood THC was approximately 25 μg/L, and median molar metabolite ratio 2 was approximately 0.75, 30 min after the onset of a 10 min interval of *ad libitum* cannabis smoking [6], (data interpolated from figures). Those values were slightly higher than the respective values of 21.3 μg/L and 0.5 found in the present study for molar metabolite ratio 2. Both parameters declined steeply during the first hour after the start of smoking, and at 60 min the molar metabolite ratio 2 value observed by Schwope and colleagues [6] was approximately 0.5. Molar metabolite ratio 2 did not correlate with subjective symptoms or psychomotor performance in that study.

The molar metabolite ratios as indicators of recent cannabis smoking may be particularly useful in the forensic evaluation of heavy cannabis users, who typically have detectable whole-blood THC even after many hours of abstinence. In this study, for both the entire cohort and for the subset of daily users, the optimal cut-point for molar metabolite ratio 1 and molar metabolite ratio 2 had the same specificity and nearly the same accuracy as indicators of recent cannabis smoking. At these cut-points molar metabolite ratio 1 offered slightly higher sensitivity, but the ROC curves for each ratio did not differ to a statistically significant extent. Molar metabolite ratio 1 may offer logistical advantages in settings where quantitation of 11-hydroxy-Δ9-tetrahydrocannabinol in blood by forensic toxicology laboratories may be less available than analysis of THC and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol [16]. When cannabis is ingested rather than inhaled, first-pass metabolism of THC in the liver diminishes the extent to which peak THC exceeds peak 11-hydroxy-Δ9-tetrahydrocannabinol [12], and it is conceivable that in such instances a different comparative utility of molar metabolite ratio 1 and molar metabolite ratio 2 might emerge.

A strength of the present investigation was its naturalistic observational design in which 56 subjects with either occasional or daily cannabis use patterns smoked self-supplied high-potency cannabis flower *ad libitum*. The collection of post-smoking blood at only one-time point 30 min after the onset of smoking was a logistical limitation, as was the inability to confirm by direct observation the period of abstinence prior to baseline blood collection. Δ9-Tetrahydrocannabinol and 11-hydroxy-Δ9-tetrahydrocannabinol [12], and molar metabolite ratio 2 [6] appear to decline steeply in the first hour after cannabis smoking. Because the parent study design only allowed for a single post-smoking blood collection, we selected 30 min after the start of smoking as a time point close to the occurrence of peak subjective effects when psychomotor performance deficits may be observed in some cannabis users [17,18]. Future studies that examine both molar metabolite ratios at multiple time points after consumption of smoked cannabis flower, edible cannabis products and smoked or vaped cannabis concentrates can further elucidate the relationship between molar metabolite ratios and recency of cannabis use.

Descriptive pharmacokinetic studies have found that after cannabis is smoked or vaped, the average peak blood concentration of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol appears within approximately 0.25 – 1 h [12,19,20]. Thereafter concentrations gradually decline as a majority of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol undergoes glucuronidation to form 11-nor-9-carboxy-Δ9-tetrahydrocannabinol-glucuronide. Many forensic and clinical laboratories that report THC, 11-hydroxy-Δ9-tetrahydrocannabinol and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol in whole blood or plasma are unable to quantitate 11-nor-9-carboxy-Δ9-tetrahydrocannabinol-glucuronide. When blood is stored at room temperature, partial enzymatic or spontaneous deconjugation of the 11-nor-9-carboxy-Δ9-tetrahydrocannabinol-glucuronide to 11-nor-9-carboxy-Δ9-tetrahydrocannabinol may occur over the course of days to weeks, a process that could potentially raise 11-nor-9-carboxy-Δ9-tetrahydrocannabinol concentration and lower the value of molar metabolite ratio 1 or molar metabolite ratio 2. 11-Nor-9-carboxy-Δ9-tetrahydrocannabinol-glucuronide has been found to be stable in whole blood stored for 4 weeks at 4 °C and for 12 weeks at −20 °C [21], confirming that appropriate storage of blood samples can minimize the potential for artifactual changes in either type of molar metabolite ratio.

Conclusions

In this study cohort, calculated molar metabolite ratio 1 and molar metabolite ratio 2 have superior accuracy compared to

THC alone as an indicator of recent (within 30 min) cannabis smoking. We recommend measurement of whole blood THC, 11-hydroxy-Δ9-tetrahydrocannabinol, and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol and calculation of molar metabolite ratio1 and molar metabolite ratio 2 in situations when ascertainment of recent cannabis consumption is of interest in forensic or safety investigations.

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