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Simultaneous Determination of Δ^9-Tetrahydrocannabinol and 11-nor-9-Carboxy-Δ^9-Tetrahydrocannabinol in Human Plasma by Solid-Phase Extraction and Gas Chromatography–Negative Ion Chemical Ionization-Mass Spectrometry

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Abstract

Δ^9-Tetrahydrocannabinol (THC) and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCA) in human plasma can be simultaneously detected using solid-phase extraction with gas chromatography and negative ion chemical ionization mass spectrometry. THC-d_3 and THCA-d_3 are added as internal standards; protein is precipitated with acetonitrile and the resulting supernatants diluted with 0.1M sodium acetate (pH 7.0) prior to application to the solid-phase extraction columns. THC and THCA were eluted separately and then pooled, dried under air, and derivatized with trifluoroacetic anhydride and hexafluoroisopropanol. The derivatized THC-d_0 gives abundant molecular anions (*m/z* 410), and the derivatized THCA-d_0 gives abundant fragment ions (*m/z* 422) formed by loss of $(CF_3)_2CHOH$ from its molecular anion. The recoveries of THC and THCA were 74% and 17%, respectively. The lower and upper limits of quantitation were 0.5 and 100 ng/mL for THC and 2.5 ng/mL and 100 ng/mL for THCA. The within-run accuracy and precision for THC (measured at 0.5, 1, 10 and 75 ng/mL) ranged from 98 to 106% (% target) and 4.1 to 9.5 (%CV), respectively. For THCA, the within-run accuracy and precision (measured at 2.5, 5, 10, and 75 ng/mL) ranged from 89 to 101% and 4.3 to 7.5%, respectively. The between-run accuracy and precision for THC ranged from 92 to 110% and 0.4 to 12.4%, respectively. The between-run accuracy and precision for THCA ranged from 97 to 103% and 6.5 to 12.3%, respectively. In processed samples stored in reconstituted form at −20°C, THC and THCA were stable for at least three days. THC and THCA stored in plasma were stable following three freeze/thaw cycles. THC and THCA in whole blood at room temperature for 6 h, or in plasma stored at room temperature for 24 h, did not show significant change. Storage in polypropylene containers for 7 days at −20°C and the presence of 1% sodium fluoride or the cannabinoid receptor antagonist, SR141716, at 1 µg/mL did not interfere with the quantitation of THC and THCA. In three individuals who smoked marijuana under controlled dosing conditions, peak THC concentrations of 151, 266, and 99 ng/mL were seen in the first plasma samples drawn immediately after the end of smoking, and corresponding peak THCA concentrations of 41, 52, and 17 ng/mL occurred at 0.33 to 1 h after cessation of smoking.

Introduction

Cannabis (marijuana, hashish, hashish oil, etc.) is a widely used drug that is illicit in many countries. The primary psychoactive component of cannabis is Δ^9-tetrahydrocannabinol (THC) (1). The determination of THC in biological fluids has proven invaluable in determining the pharmacokinetics of THC, the relationship of THC blood concentrations to its impairing effects, and in forensic investigations. These studies are further enhanced by determination of one or more metabolites of THC to determine metabolic patterns and in some cases to assist in

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estimates of time since exposure. Although there are many metabolites of THC (2), 11-nor-9-carboxy-THC (THCA) is often studied because it is quantitatively significant, it has a relatively long half-life, and THCA and its glucuronide conjugate are the primary analytes measured in urine.

Studies on the pharmacologic action of THC have been enhanced in recent years by discovery of central (3) and peripheral (4) cannabinoid receptors, and their respective endogenous agonists, anandamide (5) and sn-2-arachidonylglycerol (6). It has recently been hypothesized that some forms of schizophrenia act through the cannabinoid system (7), and a selective central cannabinoid receptor antagonist, SR141716A (8–10), has been found to ameliorate pharmacologic models of schizophrenia in experimental animals (11). During the initial clinical development of receptor antagonists such as SR141716, it is often desirable to evaluate the effect of the antagonist against an agonist (THC) challenge. Though the effects of the antagonist can be dynamically evaluated, it is important to document the relative concentrations of the agonist (THC) in these challenge experiments in an attempt to correlate plasma concentration of the agonist with effect. Therefore, the monitoring of THC and metabolites during clinical trials of cannabinoid receptor antagonists is providing another use for THC and THC metabolite analyses.

Gas chromatography–mass spectrometry (GC–MS) has been the major analytical tool for determination of THC and THCA in blood, plasma and serum. Our laboratory developed a sensitive GC–MS method that used negative ion chemical ionization (NCI) for determination of THC and THCA (12). This method has been used extensively for a number of pharmacokinetic (13–16) and forensic investigations (17–19). This GC–MS method, however, has an arduous extraction procedure that requires separation of the extract into neutral and acidic fractions with separate derivatizations and separate injections into the GC–MS. Moeller et al. (20) first demonstrated that solid-phase extraction (SPE) combined with electron-impact GC–MS could be employed to extract and detect THC and THCA from serum. Subsequently, Nelson et al. (21) using GC–MS–MS, and more recently Felgate and Dinan (22) and D'Asaro (23), who also used electron-impact GC–MS, have incorporated SPE into analysis of THC and THCA in blood and plasma. We have now developed an SPE method that allows collective elution of THC and THCA. This method, thereby, allows the derivatization of THC and THCA in a single step and analysis with a single injection into the GC–MS. To the best of our knowledge, this is the first combination of SPE with NCI-GC–MS for determination of THC and THCA in plasma.

Materials and Methods

Chemicals and materials

The drug standards were obtained from the following sources: THC-d_0, THCA-d_0, and THCA-d_3 from Radian (now Cerilliant, Austin, TX) and THC-d_3 from RTI (Research Triangle Park, NC). Human plasma containing sodium heparin as anticoagulant was obtained from the University of Utah Blood Bank (Salt Lake City, UT). Trifluoroacetic anhydride (TFAA) and hexafluoro-2-propanol (HFIP) were purchased from Pierce Chemical Co. (Rockford, IL) and Aldrich Chemical Co. (Milwaukee, WI), respectively. All organic solvents were high-performance liquid chromatographic grade from Burdick & Jackson Chemical Co. (Muskegon, MI). CleanScreen SPE columns (ZSTHC020) were purchased from United Chemical Technologies (Bristol, PA). All other chemicals were reagent grade.

Preparation of stock solutions and standard curves

A THC reference solution (1 mg/mL) and a THCA reference solution (100 µg/mL) were diluted 10-fold separately in methanol to prepare the stock solutions (100 µg/mL for THC and 10 µg/mL for THCA). From dilution of the two stock solutions, a working solution containing 1.0 ng/µL of both THC and THCA was obtained. Subsequent dilutions provided working solutions of 0.1 and 0.01 ng/µL. Stock and working solutions were stored at –20°C when they were not in use. Daily calibration curves were prepared by fortifying drug-free human plasma with known amounts of working solutions to the following concentrations: 0.5, 0.8, 1.0, 2.5, 5, 10, 25, 50, 75, and 100 ng/mL of both THC and THCA. The three lowest concentrations were not used for the THCA calibration curve.

Extraction

Internal standard solution (100 µL of 50 ng/mL THC-d_3 and THCA-d_3) was added to 1 mL of freshly prepared calibrators, quality-control samples, and study samples that were prepared in separate 16 × 100-mm culture tubes. Samples were vortex mixed and allowed to stand at room temperature for 1 h. One milliliter of acetonitrile was added to each sample and then vortex mixed and centrifuged. Supernatants were transferred to separate clean tubes, and 4 mL of 0.1M acetate buffer (pH 7.0) was added. SPE columns were conditioned by sequentially adding 3 mL methanol, 3 mL Milli-Q water, and 3 mL of 0.1M acetate buffer (pH 7.0). The supernatants were poured onto the conditioned columns and allowed to drain. Each column was then washed with 4 mL 0.1M acetate buffer (pH 7.0), and dried under maximum vacuum for at least 5 min. THC was eluted into clean collecting tubes with 3 mL hexane/ethyl acetate/ammonia hydroxide (93:5:2, v/v/v). After the THC elution, each column was rinsed with 4 mL Milli-Q water, dried, and rinsed with 4 mL of acetonitrile/0.1M acetic acid (40:60, v/v). The columns were dried under maximum vacuum for at least 5 min, and 400 µL of hexane was added to each column. THCA was eluted into the same collecting tube as THC with 3 mL hexane/ethyl acetate (70:30, v/v). The elution liquid containing both compounds were evaporated to dryness at 40°C under air.

Derivatization

The residues in the collecting tubes were derivatized by adding reagents in the following order: 150 µL of chloroform, 150 µL TFAA, and 75 µL of HFIP. The contents in the tubes were mixed well, heated at 70°C for 25 min, and then dried at 40°C under air. The derivatized residues were reconstitued with 50 µL of heptane, transferred into autosampler vials, and 1.2 µL were injected onto the GC–MS system.

GC–MS analysis

Analyses were performed on a Finnigan-MAT™ 4500 GC–MS equipped with INCOS® software (Finnigan MAT, San Jose, CA).

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The GC column was a Hewlett-Packard DB-1 capillary column (30 m × 0.32-mm i.d., 0.25 µm). The initial column temperature of 160°C was held for 0.2 min, then programmed to 280°C at the rate of 20°C/min, and held for 8 min at 280°C. The carrier gas was helium with a head pressure of approximately 0.8 psi. Temperatures of the injector port, interface, transfer line, and ionizer were 250, 250, 275, and 140°C, respectively. The reagent gas was methane adjusted to a source pressure of 0.60 Torr. The MS was operated in the NCI detection mode. Splitless injection was used. The MS was programmed for selected ion monitoring (SIM) detection of *m/z* 410 (THC-d_0), *m/z* 413 (THC-d_3), *m/z* 422 (THCA-d_0), and *m/z* 425(THCA-d_3). The ratios of peak height of each analyte to the corresponding internal standard were calculated. The concentration of THC or THCA for each sample was determinated from least-squares equations for curves of peak-height ratios versus concentration of calibrators.

Subjects

Participants were 21- to 45-year-old males with a history of self-reported cannabis use of one or more years without current intoxication and without current substantial intoxication or physical dependence on any other substance. In addition, cannabis use was objectively documented with a positive urine cannabinoid test (50-ng/mL cutoff). Participants provided written informed consent, were paid for their participation, and resided throughout the study on the closed research unit of the Intramural Research Program (IRP), National Institute on Drug Abuse (NIDA), NIH. On the basis of physical examination, history, routine laboratory chemistries, TB tests, and psychological screens, participants were found to be in good health and without significant psychiatric disturbance other than their drug abuse. The controlled marijuana administration was part of a larger clinical study to evaluate the ability of oral SR141716, the first CB1-specific cannabinoid receptor antagonist developed by Sanofi-Synthelabo, Inc., to antagonize the effects of smoked marijuana (24). This protocol was approved by the NIDA IRP Institutional Review Board.

Marijuana smoking

Placebo (0% THC) and active (2.64% THC) marijuana cigarettes were obtained from NIDA. Cigarettes were humidified for a minimum of 12 h prior to drug administration. The participant and medical and technical staff monitoring the session and collecting data were blind to the placement of placebo and active doses. Marijuana cigarettes were smoked according to the following procedure: total of 8 puffs per cigarette, ad libitum puff duration and retention of smoke in the lungs, and 60-s interpuff interval. This procedure resulted in nearly complete pyrolysis of each cigarette.

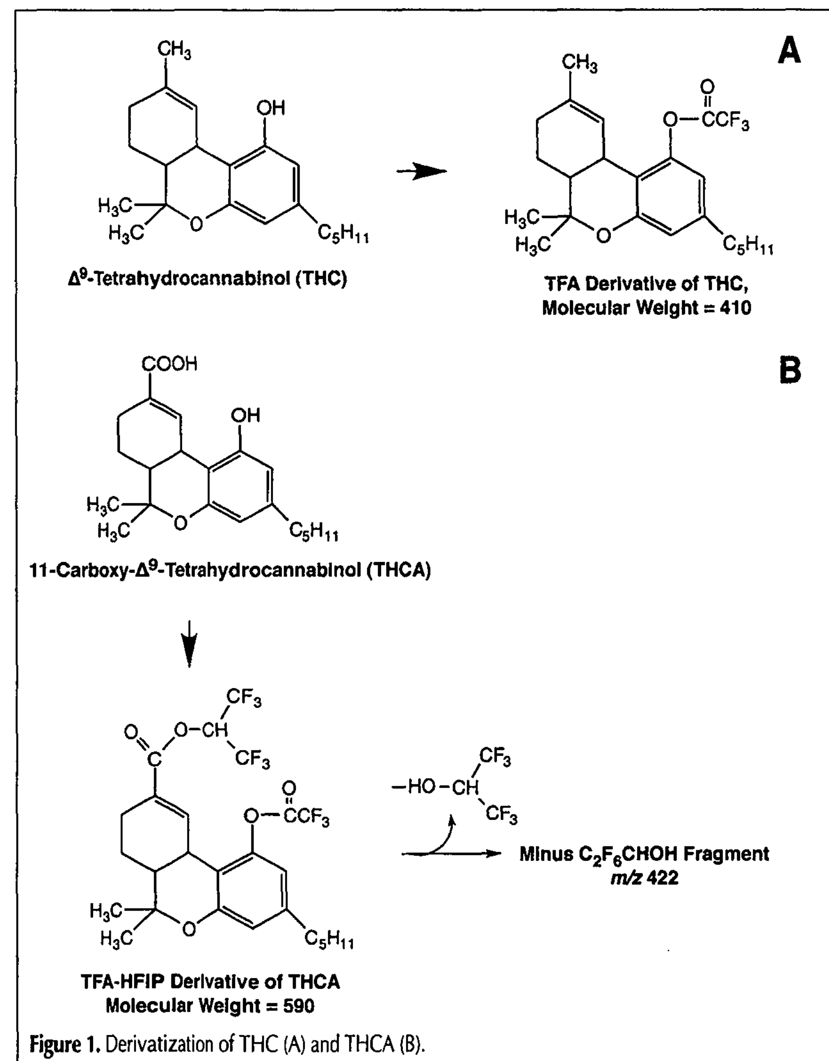

Figure 1. Derivatization of THC (A) and THCA (B).

Plasma specimen collection

At each time point, a 6-mL blood sample was collected through a catheter in a peripheral vein using a sodium heparin Vacutainer® tube. Samples were collected 15 min prior to the start of marijuana smoking, immediately following smoking completion, and 2, 5, 10, 15, 20, 40, 60, 80, 100, and 225 min after the end of smoking. Samples were immediately placed on ice, centrifuged within 2 h, and the plasma stored at –20°C until analysis.

Results and Discussion

Chromatography and spectra

The structures of THC and THCA are shown in Figures 1A and 1B, respectively. The NCI mass spectrum of derivatized THC (Figure 2A) has an intensive molecular anion at *m/z* 410 that is consistent with the trifluoroacetyl derivative. The NCI mass spectrum of derivatized THCA (Figure 2B) shows two major ions, one at *m/z* 422 and another smaller one at *m/z* 590. The ion at *m/z* 590 corresponds to the trifluoroacetyl-hexafluoroisopropyl derivative of THCA. The ion at *m/z* 422 is obtained through loss of the hexafluoroisopropanol group (C_2F_6CHOH) from the trifluoroacetyl-hexafluoroisopropyl derivative. The major ions at *m/z* 410 and *m/z* 422 were used to quantitate THC and THCA, respectively.

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Ion current profiles resulting from analysis of a plasma sample containing THC-d_0 at the lower limit of quantitation (LLOQ) of 0.5 ng/mL with deuterated internal standard THC-d_3 at 5 ng/mL (Figure 3) and those from analysis of a plasma sample of THCA-d_0 at the LLOQ of 2.5 ng/mL with deuterated internal standard THCA-d_3 at 5 ng/mL (Figure 4) show strong peaks with no interferences.

Selectivity from matrix was investigated, using human plasma prepared from six drug-free individuals. No significant peaks co-chromatographed with THC-d_0, THC-d_3, or THCA-d_3. A small peak co-eluting with THCA-d_0 in some drug-free sources contributed to setting the LLOQ for THCA at 2.5 ng/mL (i.e., with the LLOQ of 2.5 ng/mL, the peak-area ratio in blank plasma was 23% of that for LLOQ).

Recovery

Extraction efficiency (recovery) of the analytes was determined from human plasma at 1.0, 10, and 75 ng/mL. Two sets of blank plasma for each concentration (A and B, n = 6) were prepared. The deuterated internal standard was added to all samples before SPE; the nondeuterated standards of THC and THCA were added to set A before extraction and to set B after extraction. Recovery was calculated by dividing the mean peak-height ratio of set A by the corresponding peak-height ratio of set B and then multiplying by 100%. The recoveries of THC and THCA are 74% and 17%, respectively. It should be noted that during some preliminary recovery studies performed with smaller Ns, we saw higher recovery of THCA. Recovery appeared to vary with the lot of SPE columns. The data reported here represent the worst case scenario. Even though the recovery of THCA was low, this was compensated for by the electron capture properties of the three fluorinated groups in THCA derivatives with resultant strong signals observed in NCI.

A preliminary test of inclusion of 11-hydroxy-THC in the procedure resulted in the analyte displaying poor chromatography and sensitivity. No further experiments were performed to see if changes in the SPE or derivatization procedures would improve conditions for detection of this metaboite of THC.

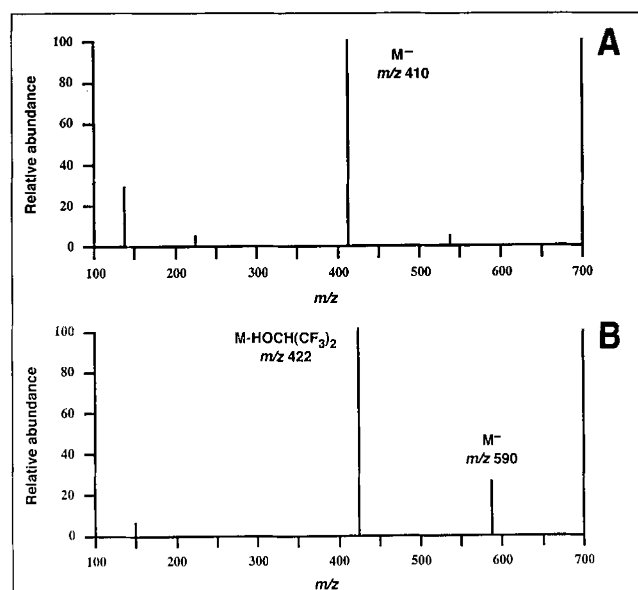

Figure 2. Spectrum of derivatized THC (A) and THCA (B). Note: because of the large scan window, the minor isotope fragments were not resolved from the main fragments and do not appear in this reconstruction.

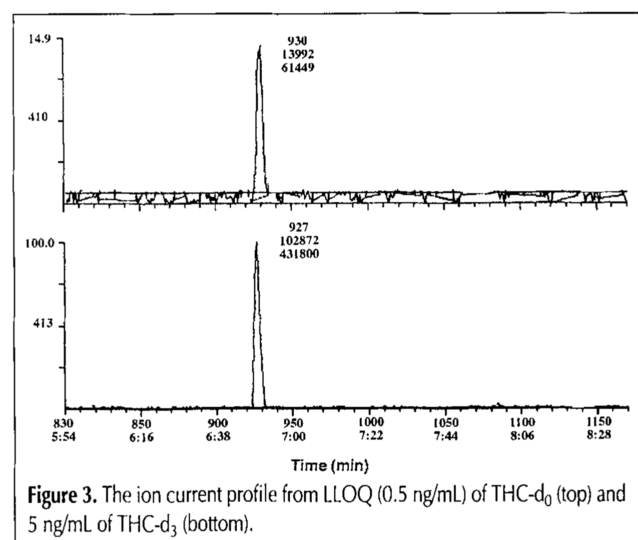

Figure 3. The ion current profile from LLOQ (0.5 ng/mL) of THC-d_0 (top) and 5 ng/mL of THC-d_3 (bottom).

Calibration curve

During validation, the correlation coefficients (r^2) (0–100 ng/mL) for all curves were greater than 0.99. The linear range of the assay for THC and THCA was established in human plasma calibrators from 0.5 to 100 ng/mL and 2.5 to 100 ng/mL, respectively.

Precision and accuracy

Intra-assay accuracy for the analysis of THC was determined at concentrations of 0.5, 1.0, 10, and 75 ng/mL (N = 6 per concentration) and ranged from 94 to 106% of targets with precision (%CV) ranging from 4.1 to 9.5% (Table I). Intra-assay accuracy for the analysis of THCA was determined at 2.5, 5.0, 10, and 75 ng/mL and ranged from 89 to 101% with precision ranging from 4.3 to 7.5% (Table I). For interassay studies, batches were run on six separate days, using the same concentrations as in the intra-assay studies. Results were determined from the average and standard variation of mean batch results. The accuracy of THC ranged from 92 to 111% with precision ranging from 4.1 to 10.7%; the accuracy of THCA ranged from 97 to 104% with precision from 4.2 and 12.6% (Table I).

Stability

A number of studies on the short and long-term stability of THC have been published (25–28). Some do (26,28), and some do not (25,27), include studies on THCA. During the course of this validation, it was necessary to address stability questions having a direct impact on the collection and processing of samples for analysis. These include some novel observations on the stability of THC and THCA.

To determine the limits of storage of whole blood before plasma was prepared, THC and THCA were spiked into whole blood and aliquots taken at timed intervals prior to plasma preparation. Samples were then stored at –20°C until time of analysis. The concentrations of THC and THCA quantitated in plasma were 170 to 179% and 201 to 208% of the amount added

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to whole blood, respectively (Table II). This is consistent with the findings of Owens et al. (29) and Mason and McBay (30): plasma levels of THC and THCA are approximately twice those found in whole blood. We found that whole blood could be stored for 6 h at room temperature before preparation of plasma with no changes in concentration of THC and THCA. Our study differed from others on stability in whole blood in that final analysis was made of an extract of plasma after storage of whole blood. Wong et al. (25) reported that THC is stable in whole blood at room temperature for a similar short time period (25 h), but how long room temperature stability can be extended is uncertain, as Johnson et al. (26) reported degradative loss of both THC and THCA in whole blood stored at room temperature for six months.

When THC and THCA in human plasma with nominal concentrations of 1.0 ng/mL THC and THCA and 75 ng/mL of THC and THCA were subjected to three freeze/thaw cycles, the precision and accuracy of quantitation were acceptable for both compounds (Table III). Wong et al. (25) showed that THC in whole blood was stable under similar freeze/thaw conditions, but this appears to be the first study of both THC and THCA, and the plasma matrix. Consistent with findings of Johnson et al. (26), control samples (1.0 and 75 ng/mL in plasma) stored at room temperature for 24 h had no signifigant change in concentrations of THC and THCA.

The stability of THC and THCA in processed samples has not been previously described. Nominal concentrations of 1.0 and 75 (ng/mL) were processed to the step of transferring into autosampler vials in the reconstituted state and the vials then stored at –20°C for 3, 5, and 7 days prior to being analyzed with freshly prepared calibrators. Both THC and THCA failed to meet acceptance criteria of ± 15% of target at 5 and 7 days but succeeded at 3 days (Table III).

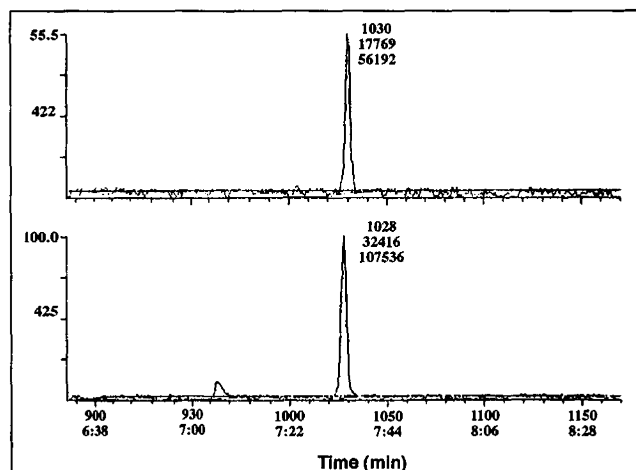

Figure 4. The ion current profile from LLOQ (2.5 ng/mL) of THCA-d_0 (top) and 5 ng/mL of THCA-d_3 (bottom)

Interference

The effect of storage in polypropylene containers on THC and THCA was investigated by aliquotting the validation human plasma samples at 1.0 and 75 ng/mL (n = 6) into polypropylene tubes and storing at –20°C for 7 days. Samples were thawed, processed, and analyzed with freshly prepared calibrators. No significant impact was observed for quantitation of either THC or THCA (Table III). Christophersen (27) found a significant loss of THC in whole blood stored in polystyrene tubes for as short as four days at –20°C. THCA values were not reported. Whether the discrepancy in findings for THC arose from use of a different type of plastic container and/or the different matrix is not known.

Measurement of THC and THCA in the presence of SR141716 will be required in forthcoming clinical studies. Quality-control samples fortified with 1 µg/mL of the cannabinoid receptor antagonist, SR141716, had acceptable precision and accuracy (Table III).

The validation samples in this study were prepared in plasma containing 1% sodium fluoride, but many study samples will not contain this additive. The precision and accuracy for determination of THC and THCA were still acceptable when sodium fluoride was omitted (Table III).

Clinical results

THC concentrations increased rapidly, with peak concentrations occurring prior to the first blood specimen collected immediately after the end of smoking (Figure 5). Observed THC Cmax concentrations for subjects A, B, and C were 151, 266, and 99 ng/mL, respectively. Plasma THC concentrations decrease quickly after smoking, primarily because of rapid distribution of the lipophilic drug throughout the body. Metabolism to the active 11-hydroxy-THC metabolite and the inactive THCA metabolite also contribute to the precipitous decrease in THC concentrations. Following initial drug distribution, a much slower elimination phase begins that is characterized by low THC concentrations. THCA Cmax were 41.1, 52.0, and 17.3 ng/mL for subjects A, B, and C, respectively. THCA Cmax occurred between 0.5 and 1.2 h after completion of smoking. A much slower elimination half-life is apparent for the inactive metabolite.

Marijuana is the most commonly abused illicit drug in the world, and its use is increasing in young people. It is essential that subjective and performance responses to drugs be evaluated in humans; studies of these parameters in animals, although very useful, cannot substitute for human abuse liability and performance impairment investigations. Integration of physiological, behavioral, and biochemical effects with simultaneously obtained drug concentrations is important for determining drug bioavailability, rate of

Table I. Precision and Accuracy for the Determination of THC and THCA in Human Plasma*

	THC			THCA		
Sample	Target (ng/mL)	Intra-assay (%Target ± CV)	Interassay (%Target ± CV)	Target (ng/mL)	Intra-assay (%Target ± CV)	Interassay (%Target ± CV)
LLOQ	0.5	106 ± 7.5	111 ± 10.7	2.5	89 ± 7.5	98 ± 4.2
QC1	1.0	98 ± 4.1	98 ± 7.6	5.0	89 ± 6.3	104 ± 12.6
QC2	10.0	94 ± 9.5	97 ± 4.1	10.0	101 ± 4.9	102 ± 12.4
QC3	75.0	99 ± 6.6	92 ± 6.2	75.0	100 ± 4.3	97 ± 6.6

* For intra-assay values, n = 6 samples analyzed in a single run. For interassay values, n = 6 batch means.

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drug delivery to the brain, and differences in abuse liability; explaining intersubject variability, potential toxicities, variations in metabolism and production of active or inactive metabolites; and defining concentration-effect curves (where hysteresis rather than linear relationships may be found).

Smoked drugs are highly abused, partly because of the speed of delivery of drug to the brain. Effects of smoked marijuana must be evaluated in terms of concurrent drug levels because of the inability to deliver a specified dose by the smoking route. THC is rapidly absorbed and quickly penetrates the CNS to produce psychoactive effects. Despite the use of uniform potency marijuana cigarettes and a standardized 8-puff, 60-s puff interval in this smoking study, significant interindividual variability in peak-plasma concentrations of THC and THCA was noted (Figure 5). This finding is in agreement with previously described THC and THCA pharmacokinetics data (15) and is believed to be due to subject-controlled dose titration based on concurrent physiological and subjective effects and marijuana smoking experience. Concurrent plasma THC concentrations are important parameters for understanding the mechanisms underlying the physiological and behavioral effects of marijuana, as well as aiding intelligent interpretation of cannabinoid blood levels in drug research, driving under the influence cases, and other forensic investigations. In addition, there is renewed interest in the therapeutic potential of marijuana and other cannabinoids (31) and the need to follow THC as an agonist in clinical trials for cannabinoid receptor antagonists. All of these areas of research and investigation require simplified sensitive, accurate, and precise analytic methods to detect plasma THC and its metabolites. The method described in this study meets those needs.

Table II. Stability of THC and THCA in Whole Blood at Room Temperature*

Sample	N	THC (ng/mL)	THCA (ng/mL)
0-h	6	3.42 ± 0.32	20.6 ± 1.6
2-h	6	3.50 ± 0.20	20.1 ± 1.4
6-h	6	3.58 ± 0.18	20.8 ± 1.4

* Blood was fortified with THC at 2 ng/mL and THCA at 10 ng/mL while being mixed in an ice bath. Six 1-mL aliquots were removed at intervals and stored at room temperature for the time indicated. Plasma was then prepared and stored at −20°C until the time of analysis.

Table III. Stability and Interference Studies in Plasma or Processed Samples

		% Target Concentration ± % CV			
Study	N	THC 1 ng/mL	THC 75 ng/mL	THCA 1 or 5 ng/mL	THCA 75 ng/mL
Plasma stored at room temperature for 24 h*	6	86.0 ± 4.7	96.3 ± 1.9	87.0 ± 5.8	98.2 ± 0.8
Plasma undergoing three freeze-thaw cycles*	6	87.0 ± 8.9	95.9 ± 3.1	87.0 ± 5.7	100.2± 3.1
Processed samples stored for three days at −20°C*	6	101.0 ± 8.9	104.6 ± 7.3	108.0± 8.3	103.7 ± 7.2
Plasma stored in polypropylene containers for seven days at −20°C*	6	94.0 ± 6.4	90.5 ± 4.7	105.0 ± 7.6	89.1 ± 3.3
Plasma containing SR141716 at 1 µg/mL†	6	92.0 ± 10.9	96.9 ± 5.5	88.4 ± 7.5	102.3 ± 7.6
Plasma without NaF†	6	103.0 ± 7.8	100.2 ± 8.6	95.4 ± 6.3	90.7 ± 7.4

* The low THCA control was at 1 ng/mL.
† The low THCA control was at 5 ng/mL.

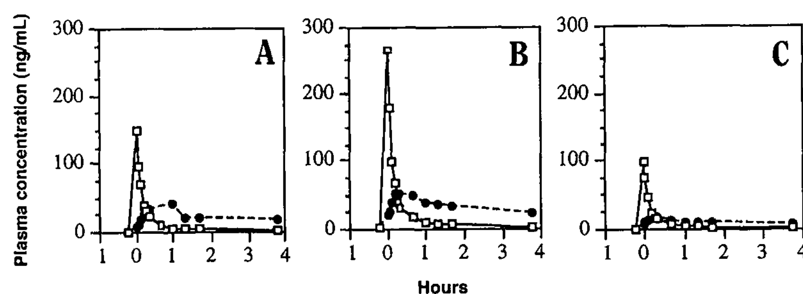

Figure 5. Plasma THC (□) and THCA (•) concentrations in subjects A, B, and C who smoked a marijuana cigarette containing 2.64% THC.

Conclusions

This paper describes a GC–NCI-MS procedure for quantitative analysis of THC and THCA in human plasma. The method was reliable, selective and accurate. The method has been fully validated from 0.5 to 100 ng/mL for THC and from 2.5 to 100 ng/mL for THCA. THC and THCA were stable during three freeze/thaw cycles, and processed samples were stable for three days when stored in the reconstituted state at −20°C. Use of polypropylene containers for storage of plasma samples and addition of NaF as preservative or up to 1 µg/mL SR141716 does not interfere with the accurate and precise quantitation of THC and THCA. Application of the method to plasma collected from individuals smoking marijuana achieved results similar to those found with other analytical methods.

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