Detection of Marijuana Use by Oral Fluid and Urine Analysis Following Single-Dose Administration of Smoked and Oral Marijuana

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Abstract

We compared oral fluid testing to urine testing in subjects who were administered single doses of marijuana by smoked and oral routes. Oral fluid specimens were collected with the Intercept™ DOA Oral Specimen Collection Device, screened for THC with the Cannabinoids Intercept MICRO-PLATE Enzyme Immunoassay (EIA) utilizing a 1.0-ng/mL cutoff concentration, and confirmed for THC by gas chromatography–tandem mass spectrometry (GC–MS–MS) with a 0.5-ng/mL cutoff concentration. Urine specimens were screened for 11-nor-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH) by immunoassay utilizing a 50-ng/mL cutoff concentration and confirmed for THCCOOH by GC–MS with a 15-ng/mL cutoff concentration. Oral fluid specimens tested positive following smoked marijuana (N = 10) consecutively for average periods (± SEM; range) of 15 (± 2; 1–24) and 13 h (± 3; 1–24) by EIA and GC–MS–MS, respectively. The average THC detection times of the last oral fluid positive specimen following smoked marijuana by EIA and GC–MS–MS were 31 (± 9; 1–72) and 34 h (± 11; 1–72), respectively. In comparison to oral fluid, urine specimens generally tested negative for THCCOOH immediately after marijuana use. The average times to detection of the first urine specimen positive for THCCOOH by EIA and GC–MS were 6 (± 2; 1–16) and 4 h (± 1; 2–8), respectively. Urine specimens tested positive consecutively for average periods of 26 (± 9; 2–72) and 33 h (± 10; 4–72) for EIA and GC–MS, respectively. The average THCCOOH detection times of the last specimen by EIA and GC–MS were 42 (± 10; 2–72) and 58 h (± 6; 16–72), respectively. Considering the non-invasive nature of oral fluid collection and improved detection of recent marijuana use compared to urine testing, it was concluded that oral fluid testing for THC offers specific advantages over other means of marijuana testing when used in safety-sensitive testing programs.

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Introduction

Drug testing provides objective information regarding an individual's recent use or exposure to illicit drugs. Although urine is the body fluid most commonly collected and tested in workplace drug-testing programs, advances in the use of other types of biological specimens has accelerated over the last decade. Progress in the development of alternate matrices for drug testing prompted the Substance Abuse and Mental Health Services Administration (SAMHSA) in the United States to draft guidelines for the use of oral fluid, sweat, and hair in federally regulated workplace drug-testing programs (1). Presumably, adoption of these guidelines will lead to implementation of their use in regulated workplace drug-testing programs, and their use in non-regulated drug-testing programs will likely expand.

Drug testing of oral fluid offers the advantage over urine in being a less-invasive collection process. Also, compared with urine, sweat, or hair, a positive result from an oral fluid test can be interpreted with greater confidence as being attributable to very recent drug use, that is, generally within 24 h, and not the consequence of drug use that occurred days to weeks earlier. These properties of oral fluid make it an attractive alternative to urine testing for use in workplace drug-testing programs and for detection of drugged drivers.

Oral fluid is a mixture of saliva, gingival crevicular fluid, cellular debris, and other components (2). Drugs may appear in oral fluid via multiple pathways. The predominant modes for entry into oral fluid for most drugs are excretion via saliva from blood and direct deposition in the oral cavity during oral, intranasal, and smoked administration. For marijuana, the primary route of drug entry into oral fluid appears to be direct deposition during use. Commonly, marijuana is smoked, but it may also be consumed orally, usually mixed with food products (e.g., brownies). Residues of Δ^9-tetrahydrocannabinol (THC) are sequestered in oral tissue and appear in oral fluid (3–7). The

mechanism of THC deposition would appear to be direct sequestration of THC in shallow tissues of the oral mucosa during drug use. Contribution of THC to oral fluid from blood is minimal. A preliminary study of the disposition of radiolabeled THC administered by the intravenous route to humans revealed no evidence of THC excretion in saliva (8); however, there is evidence of transport of THC into oral fluid from animal studies. Autoradiograhic studies of radiolabeled THC in the monkey (*Hapale jacchus* species) indicated accumulation in the parotid and submandibular glands 30 min after intravenous injection (7). Unchanged THC persisted in the parotid gland for 6 h. In addition, THC was identified by thin-layer chromatography in oral fluid 30 min after injection.

There appears to be only a single report of detection of marijuana metabolites in oral fluids. Schramm et al. (9) reported identification by mass spectrometry of 11-nor-9-carboxy-Δ^9-THC (THCCOOH), 11-hydroxy-THC, cannabidiol, together with THC in a single saliva specimen after smoked marijuana. However, a study of oral fluid specimens in human subjects following smoking of marijuana cigarettes revealed no evidence by gas chromatography–mass spectrometry (GC–MS, detection limit = 0.5 ng/mL) of 11-hydroxy-THC or THCCOOH over a period of 7 days following smoked marijuana (10).

The usefulness of oral fluid testing for THC is predicated upon its ability to serve as a diagnostic indicator of recent marijuana use. This report describes the timecourse of detection of THC in oral fluid following smoked and orally ingested marijuana by human subjects under controlled supervision. The studies were designed to allow collection of biological fluids from subjects using marijuana in a social setting. Consequently, subjects were allowed to interact socially, drink soda and alcoholic beverages (beer), and eat food (pizza) at times that did not interfere with drug administration and collection of specimens. Oral fluid specimens were screened with the Cannabinoids Intercept MICROPLATE Enzyme Immunoassay (EIA). Confirmation of THC in oral fluid was accomplished by GC–MS–MS. Urine specimens were collected simultaneously with oral fluid specimens and analyzed by immunoassay and GC–MS. Comparison of the timecourse of THC in oral fluid was made to the excretion pattern of THCCOOH in urine.

Methods

Participants

The participants were 18 healthy subjects who reported a history of recent marijuana use and two subjects with no reported lifetime use of marijuana that served as passive control subjects. The 18 marijuana subjects were non-obese Caucasian males with an average age (± SD) of 22 years (± 2) (range 19–25). The two control subjects were Caucasian males aged 32 and 39 years. All volunteers gave written, informed consent prior to study participation and were not paid for their participation.

Marijuana doses

Marijuana cigarettes were purchased from a commercial "coffee-house" in Amsterdam, The Netherlands. Analysis of THC content was performed by GC–MS. The cigarettes contained an average THC content in the range of 20–25 mg.

Study procedures and specimen collection

Three separate outpatient studies involving marijuana administration were performed on single occasions in Leiden, The Netherlands. The studies were designed to simulate a typical social setting in which marijuana would be smoked or ingested. The studies began with the subjects reporting to the study site for orientation and informed consent. Marijuana was administered to all subjects (with the exception of the two passive control subjects) at approximately the same time with instruction that each subject was to smoke (or ingest) only his dose of marijuana and that no exchange of marijuana was permitted. Subjects were monitored throughout each drug administration period to ensure enforcement of this restriction. During each smoking study, all subjects smoked marijuana over a period of 20–30 min, according to their accustomed manner and with no external control over their smoking pattern. The marijuana cigarettes were smoked to a small butt size. The dimensions of the study room were approximately $20 \times 20 \times 12$ ft. There was no central ventilation system, but windows were opened occasionally to relieve the smoky atmosphere. During the marijuana administration period, subjects were allowed to interact socially and drink beer or soda. Food (pizza) was available after the first 2 h of specimen collection. Collection of oral fluid specimens was preceded by a 10-min "time-out" period during which eating and drinking was not allowed. Following marijuana administration, a 2–4-h period of intensive specimen collection ensued; during this time all subjects were under constant supervision. If further specimen collections were scheduled (Studies 1 and 3), subjects were allowed to leave and return at designated collection times. Subjects were admonished to refrain from smoking while absent from the clinic.

In the first study (Study 1), 10 subjects smoked a single marijuana cigarette that contained a 20–25-mg dose of THC; two drug-free control subjects remained in the study room while subjects were smoking, but abstained from smoking. The first 5 subjects (#1–#5) were self-reported chronic marijuana smokers (defined as daily use over a period of > 1 month). The next 5 subjects (#6–#10) were self-reported casual users who smoked marijuana on a less than daily frequency. Based on self-report information, all 10 subjects refrained from smoking marijuana for at least 12 h prior to the start of the study and during the experimentation period. The two passive control subjects (#11, #12) reported no history of marijuana use. Oral fluid and urine specimens were collected from all subjects prior to smoking ("zero time") and at 1, 2, 4, 8, 16, 24, 48, and 72 h following marijuana administration.

In the second study (Study 2), five casual marijuana users smoked a single marijuana cigarette that contained a 20–25-mg dose of THC. Oral fluid specimens were collected more frequently over a shorter period at 0, 15, 30, 45, 60, 75, 90, and 105 min after smoking. Matching urine specimens were collected at the same times whenever possible.

In the third study (Study 3), three casual marijuana users consumed marijuana-laced brownies containing marijuana plant material equivalent to a single cigarette (20–25 mg THC). The

brownies were prepared according to the package instructions.

Specimen collection

Oral fluid specimens were collected by staff utilizing the Intercept DOA Oral Specimen Collection Device (OraSure Technologies, Bethlehem, PA) according to manufacturer's instructions. The collection device consists of a treated absorbent-cotton-fiber pad affixed to a nylon stick and a preservative solution (0.8 mL) in a plastic container. With this device, an average of 0.4 mL of oral fluid is collected with each device. (A study of 83 healthy adult volunteers indicated that a mean volume of oral fluid collected by the device was 0.38 ± 0.19 (SD) with a range of 0.05 to 0.8 mL). The collection device pad was placed between the lower gum and cheek for 2–5 min, then placed in the preservative solution. The resulting total volume was approximately 1.2 mL (0.4 mL specimen and 0.8 mL preservative solution). Consequently, the oral fluid specimen was diluted by a factor of 3. All testing was performed on exact measurements of the dilute specimen, and concentrations are reported based on the final diluted specimen. Approximate concentrations of THC in oral fluid are obtained by multiplying the reported result by a factor of 3.

Two nearly simultaneous, separate oral fluid collections (left and right side of the mouth) were made at each specified time. All specimens were shipped to the testing laboratory and refrigerated until analyzed. Immediately following oral fluid collection, a urine specimen was collected from each subject at the specified intervals. A minimum of 10 mL of urine was collected in a plastic urine collection bottle (Capitol Vial Companies, Auburn, AL) which was refrigerated until analyzed.

Immunoassay

Oral fluid specimens were analyzed with the Cannabinoids Intercept MICRO-PLATE EIA by OraSure Technologies following manufacturer's procedures. Briefly, the EIA procedure consisted of addition of 25 µL of specimen, calibrator, or control to each well of an anti-THC coated plate (immobilized sheep anti-cannabinoids polyclonal antibody), addition of 25 µL of buffer, and incubation for 60 min at room temperature (RT). After incubation, 50 µL of THC enzyme conjugate (horseradish peroxidase labeled with THC derivative) was added, and the plate was incubated for an additional 30 min at RT. The plate was then washed six times with 0.3 mL of distilled water, followed by the addition of 0.1 mL substrate reagent (tetramethylbenzidine), and incubation for 30 min at RT in the dark. After incubation, 0.1 mL of stopping reagent (2N sulfuric acid) was added. Absorbance was measured at 450 nm and 630 nm within 15 min of stopping the reaction. Each specimen was analyzed in duplicate. Duplicate specimens with responses that differed by 10% or more were repeated. Quality-control samples (below cutoff control containing 0.5 ng/mL THC; above cutoff control containing 2.0 ng/mL THC) were required to test appropriately. Mean responses of specimens were compared to the mean responses of the calibrator (1 ng/mL, $N = 4$). Specimens with absorbance less than or equal to the calibrator were considered positive, and specimens with responses greater than the calibrator were considered negative.

The limit of detection (LOD) of the oral fluid EIA was determined by obtaining the average absorbance value for 64 readings of Oral Fluid Diluent (buffered matrix of protein and salts) and defined as the concentration obtained by extrapolation of the mean zero absorbance minus three standard deviations. Precision measurements of THC were determined in diluent buffer at a concentration of 2.0 ng/mL. Cross-reactivity of the antibody for marijuana metabolites and related constituents was determined in diluent buffer at concentrations in the range 0.5–500 ng/mL. Cross-reactivity with other commonly encountered drugs was determined at concentrations of 10,000 ng/mL in diluent buffer. Interferant studies and effect of pH were conducted in artificial saliva (buffered matrix) in the presence of THC at concentrations of 0.5–1.5 ng/mL. Additional interferant studies in the absence of THC were conducted by addition of the interferant (0.1 mL) plus 0.3 mL of artificial saliva to the Intercept DOA Oral Specimen Collection Device followed by analysis by EIA.

Oral fluid specimens were also analyzed for IgG content to ensure that a valid specimen was collected. IgG was measured with the IgG Intercept MICRO-PLATE EIA by OraSure Technologies according to manufacturer's instructions. Mean responses of specimens were compared to the mean responses of the IgG calibrator (0.5 µg/mL). Specimens with IgG concentration equal to or greater than the calibrator were considered to be valid specimens for testing.

Urine specimens were analyzed on an Olympus 5200 Analyzer (Melville, NY) following manufacturer's procedures with the Abuscreen OnLine™ Kit (OnLine) for Cannabinoids by Roche Diagnostic Corp. (Indianapolis, IN). The Abuscreen THC immunoassay had the following cross-reactivity to THC and related compounds: 8-α-hydroxy-Δ^9-THC, 22%; 11-hydroxy-Δ^9-THC, 18%; Δ^9-THC, 11%; 8-β-11-dihydroxy-Δ^9-THC, 10%; 11-hydroxycannabinol, 5%; cannabinol, 2%; and cannabidiol, < 0.01%. A modified Jaffé method was used to measure urine creatinine concentrations.

Confirmation methods

Quantitative analysis of THC in oral fluid specimens was performed by GC–MS–MS on a Finnegan TSQ 7000 Triple Stage Quadrupole (ThermoQuest, San Jose, CA) equipped with a 5% phenyl methyl silicone capillary column (15 m × 0.25-mm i.d.). The capillary inlet system was operated in the splitless mode. Instrumental conditions were as follows: injection port, 275°C; GC temperature program, 100°C for 0.5 min, ramp to 235°C at 45°C/min, hold 1.5 min, ramp to 310°C at 45°C/min, and hold 1.5 min; transfer line, 250°C; source, 200°C; and manifold, 90°C. A total of 200 µL of each oral fluid specimen was used in the extraction procedure. Initially, internal standard (THC-d_3) at a concentration of 0.5 ng/mL was added to each specimen, calibrator, and control sample. Each sample was treated with 2 mL of 0.2M NaOH and 3 mL of hexane/ethyl acetate (9:1, v/v). The tubes were rocked for 30 min and then centrifuged. The upper organic layer was removed, acidified with 3 mL of 0.1M HCl, and rocked an additional 15 min. Following centrifugation, the upper organic layer was removed and evaporated to dryness at 40°C. The residue was derivatized with 30 µL of BSTFA (1% TMCS) and 30 µL of ethyl acetate at 70°C for 30 min. A calibration standard of THC was prepared for each batch at 0.5 ng/mL

concentration in artificial saliva (certified blank matrix). Calibration was performed as a linear fit through zero. Control samples containing 0, 0.25, and 1 ng/mL THC in oral fluid diluent were included in each batch. The following parent ions were selected for each compound to form product ions: THC, *m/z* 386 and THC-d_3, *m/z* 389. The following product ions were selected for quantitation: THC, *m/z* 371 and THC-d_3, *m/z* 374. For a specimen to be considered positive for THC, both the parent and product ions had to be present and within 2% of the retention time of the calibrator. In addition, the area of each ion had to be greater than the corresponding area of the ion in the calibration standard. The assay exhibited a between-run precision ($N = 3$ runs, 5 samples/run) for THC in oral fluid specimens of 4.3% at 0.25 ng/mL and 9.5% at 1 ng/mL. The assay LOQ/LOD for THC was 0.2 ng/mL for a 0.2-mL extracted specimen.

Quantitative analysis of THCCOOH in urine specimens was performed by GC–MS on a Finnegan Voyager (ThermoQuest) equipped with a 5% phenyl methyl silicone capillary column (15 m × 0.25-mm i.d.). The capillary inlet system was operated in the splitless mode. Instrumental conditions were as follows: injection port, 255°C; GC temperature program; 180°C for 1 min, ramp to 280°C at 30°C/min; and transfer line and source temperature, 200°C. A total of 2 mL of each urine specimen was used for the extraction. Initially, internal standard (THCCOOH-d_3) at a concentration of 15 ng/mL was added to each specimen, calibrator, and control sample. Each sample was treated with 80 µL of 10M NaOH. The tubes were hydrolyzed 20 min at room temperature. The samples were acidified with 800 µL of glacial acetic acid and vortex mixed. The samples were added to solid-phase extraction columns (type CSDAUA83, Worldwide Monitoring, Horsham, PA) that had been prewashed with methanol, deionized water, and 0.1M HCl. Following sample addition, the columns were washed with deionized water and 0.1M HCl/acetonitrile (7:3, v/v). The columns were dried for 10 min and eluted with 3 mL of hexane/ethyl acetate (85:15, v/v). The eluent was evaporated to dryness at 40°C and derivatized with 100 µL of pentafluoropropionic anhydride and 50 µL of pentafluoroisopropanol at 70°C for 20 min. The derivatized extracts were evaporated to dryness at 40°C and reconstituted with 100 µL of ethyl acetate. A calibration standard of THCCOOH was prepared for each batch at 15 ng/mL concentration in certified blank urine. Calibration was performed as a linear fit through zero. Control samples containing THCCOOH in urine at concentrations of 0, 6, and 19 ng/mL (ElSohly Laboratories, Oxford, MS) were included in each batch. The derivatized extracts were analyzed by GC–MS in the selected ion monitoring mode. The following ions were monitored for each compound (quantitative ion underlined): THCCOOH, *m/z* 459, 607, 622 and THCCOOH-d_3, *m/z* 462, 610. For a specimen to be considered positive for THCCOOH, both the quantitative and qualifier ions had to be present and within 2% of the retention time of the calibrator. In addition, qualifier ion ratios had to be within ± 20% of the calibration standard. The assay LOQ/LOD for THCCOOH was 5 ng/mL for a 2-mL extracted specimen.

Concordance analysis

The results of oral fluid analysis for THC by EIA were compared to oral fluid analysis by GC–MS–MS. For the purpose of this comparison, a true positive (TP) was assigned if the THC response by EIA was ≥ 1.0 ng/mL and the GC–MS–MS concentration was ≥ 0.5 ng/mL; a false positive (FP) was assigned if the THC response by EIA was ≥ 1.0 ng/mL and the GC–MS–MS concentration was < 0.5 ng/mL; a true negative (TN) was assigned if both EIA and GC–MS–MS were negative; and a false negative (FN) was assigned if the EIA response was negative and GC–MS–MS was positive.

Results

Oral fluid EIA performance

All oral fluid specimens were tested by immunoassay for the presence of IgG and cannabinoids. IgG is excreted in oral fluid by transudation from blood capillaries and serves as a convenient marker for validation of oral fluid specimens as being of human origin (i.e., antibody does not cross-react with IgG in animal urine) and as an indication that an adequate volume of oral fluid has been collected (11,12). IgG was measured in oral fluid specimens with the IgG Intercept MICRO-PLATE EIA (IgG cutoff concentration = 0.5 µg/mL). All specimens collected in the study tested positive for IgG, indicating that the specimens were of human origin and contained sufficient volume for cannabinoid testing. Cannabinoids were tested in oral fluid specimens by the Cannabinoids Intercept MICRO-PLATE EIA. This EIA is a competitive micro-plate immunoassay. In the EIA well, there is a competition between THC and the enzyme-labeled hapten to bind the antibody fixed onto the EIA well. After incubation and washing, substrate is added and color is produced. The absorbance measured at 450 nm is inversely proportional to the amount of THC present in the specimen. Responses are compared to responses of a calibrator (cutoff concentration = 1.0 ng/mL) run with each micro-plate. Performance characteristics of the EIA assay were as follows: LOD = 0.37 ng/mL; intra-assay precision, $N = 64$, 4 runs, 4.5–5.5%; and interassay precision, $N = 80$, 40 runs, 9.3–11.6%. Cross-reactivity for marijuana constituents were as follows: THC, 100%; cannabindiol, 0.3%; cannabinol, 15.1%; Δ^8-THC, 105.5%; 11-hydroxy-Δ^9-THC, 174.1%; and THCCOOH, 279.4%. In addition, the cross-reactivity of 54 representative drugs (over-the-counter, prescription, and illicit) were examined at concentrations of 10,000 ng/mL (Table I). No measurable cross-reactivity was found with any of these compounds.

The effect of potential interferants (dissolved when necessary in deionized water) on the oral fluid EIA were evaluated by addition to artificial saliva and assay by EIA. Table II lists potential interferants and concentrations that were tested. Interferant effects were measured in the presence (0.5 ng/mL and 1.5 ng/mL) and absence of THC. In the presence of THC at a concentration of 0.5 ng/mL, all adulterated samples tested negative (THC cutoff concentration = 1.0 ng/mL), whereas, in the presence of 1.5 ng/mL, all adulterated samples tested positive, indicating that these agents do not interfere with the performance of the oral fluid EIA. Adulterated samples also were tested by addition of the potential interferant to the oral fluid collection device (absence of THC). These samples tested negative, indicating that

Table I. Compounds that Exhibited Negligible Cross-Reactivity at a Concentration of 10,000 ng/mL in the Cannabinoids Intercept MICRO-PLATE Enzyme Immunoassay

Acetylsalicylic acid	D-Methamphetamine	Morphine
Alprazolam	Dextromethorphan	Nalorphine
Amobarbital	Diacetylmorphine	Naproxen
Ampicillin	Fenoprofen	Niacinamide
β-Phenethylamine	Gemfibrozil	Norchlordiazepoxide
Benzoylecgonine	Gentisic Acid	Penicillin
Butabarbital	Glipizide	Pentobarbital
Butalbital	Hydrocodone	Phencyclidine
Caffeine	Hydromorphone	Phenobarbital
Chlordiazepoxide	Ibuprofen	Phenylephrine
Chlorpromazine	Imipramine	Phenylpropanolamine
Clonazepam	L-Ephedrine	Procainamide
Clorazepate	L-Methamphetamine	Procaine
Cocaethylene	Lidocaine	Pseudoephedrine
Cocaine	Medazepam	Quinidine
Codeine	Meperidine	Temazepam
Cotinine	Methadone	Theophylline
D-Amphetamine	Metoprolol	Zomepirac

Table II. Tests for Interferants in the Cannabinoids Intercept MICRO-PLATE Enzyme Immunoassay

Interferant	Manufacturer	Aqueous concentration
Sugar water	Domino Pure Cane Sugar	580 mg/mL
Toothpaste	Procter & Gamble Crest (Original)	100 mg/mL
Cranberry juice	Ocean Spray	Undiluted
Baking soda	Arm & Hammer	100 mg/mL
Orange juice	Tropicana 100% Pure	Undiluted
Cola	Coca Cola	Undiluted
Cough syrup	NyQuil Adult Nighttime	0.1975 g/mL
Antiseptic	Listerine	Undiluted
Water	Deionized water	Undiluted
Hemoglobin	Sigma-Lyophilized Human	5 & 10 mg/dL

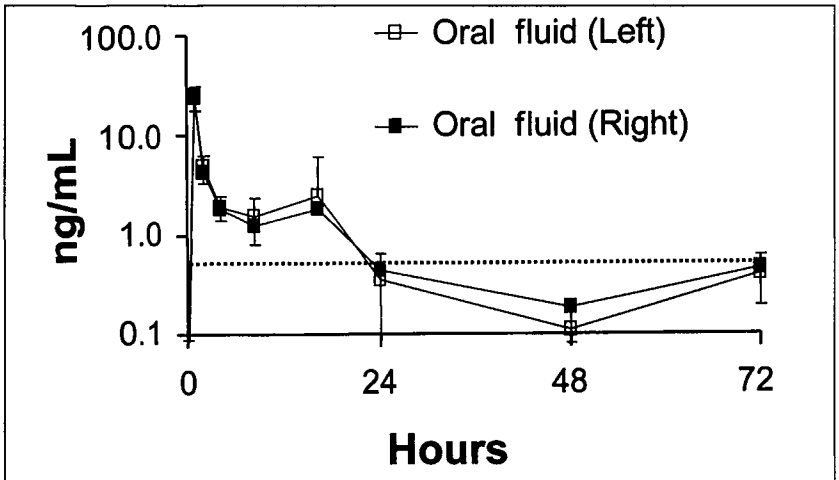

Figure 1. Mean THC concentrations by GC–MS–MS in oral fluid specimens collected simultaneously from the left and right sides of the oral cavity of 10 subjects following smoking of a single marijuana cigarette (Study 1). Error bars represent standard error of the mean. The dotted line indicates the cutoff concentration (0.5 ng/mL) used to designate positive specimens in this assay.

the agents do not produce false-positive results in the EIA. False-positive results were observed when the pH of samples was adjusted with hydrochloric acid (7.4%) to acidity levels below the normal physiological range, that is, at pH levels between 3.0 and 5.0. In this pH range, the effect of the acid was to significantly suppress optical density measurements, leading to false-positive results.

Timecourse of THC in oral fluid after smoked and consumed marijuana

THC concentrations were monitored by EIA (1.0-ng/mL cutoff concentration) and GC–MS–MS (0.5 ng/mL) in oral fluid of casual and chronic marijuana users following administration of a single dose of marijuana (20–25 mg THC) by the smoked and oral routes (Table III). Simultaneous oral fluid collections (left and right side of mouth) were made with each subject at each specified collection time. All oral fluid specimens collected prior to marijuana dosing tested negative by EIA and GC–MS–MS with the exception of Subject #17 (right side specimen collection was positive by EIA and negative by GC–MS–MS, left specimen was negative by EIA and GC–MS–MS) and Subject #20 (right and left specimens were positive by EIA and by GC–MS–MS).

Study 1 consisted of chronic and casual marijuana users (N = 5, each group) smoking a single marijuana cigarette. Oral fluid specimens were collected periodically over 3 days; mean peak (± SEM) THC concentrations for chronic users (Subjects #1–#5) by GC–MS–MS in the first specimen collected (1 h) following marijuana smoking were 27.8 ng/mL (± 6.2) and 22.6 ng/mL (± 6.2) from the left and right sides of the oral cavity, respectively. For the casual users (Subjects #6–#10), mean peak (± SEM) THC concentrations at 1 h were 23.3 ng/mL (± 7.9) and 25.3 ng/mL (± 6.5) from the left and right sides, respectively. Figure 1 illustrates mean THC concentrations (left side collection versus right side collection) by GC–MS–MS for all 10 subjects in Study 1 over 72 h following smoking of a single marijuana cigarette.

Two subjects (Subject #11 and #12) participated in Study 1 as passive controls. These subjects remained in the experimental rooms and experienced the same conditions as the 10 active marijuana smokers, but they did not smoke marijuana. Their specimens were uniformly negative with the exception of the first oral fluid specimen collected at 1 h (both left and right collections), which tested positive by EIA for both subjects, and a single specimen (left side) collected for Subject #12 at 4 h, which tested positive by EIA. None of the five EIA-positive specimens were confirmed for THC by GC–MS–MS. In addition, there was no evidence found of marijuana metabolite in the urine specimens of these subjects.

Study 2 employed more frequent oral fluid collections over a shorter period (1.75 h) for Subjects #14–#18 (N = 5) following smoked marijuana administration. Mean peak (± SEM) THC concentrations occurred at the first specimen collection (0.25 h) and were 80.6 ng/mL (± 37.7) and 59.4 ng/mL (± 21.6) from the left and right sides, respectively. Thereafter, THC concentrations

Table III. Oral Fluid Analysis for THC by EIA and GC–MS–MS and Urinalysis for THCCOOH by EIA and GC–MS Following a Single Smoked Dose and a Single Oral Dose of Marijuana*

				Left Oral Fluid, THC†		Right Oral Fluid, THC†		Urine, THCCOOH		
Study	Subject	Route	Time (h)	GC–MS–MS (0.5 ng/mL)	EIA (1.0 ng/mL)	GC–MS–MS (0.5 ng/mL)	EIA (1.0 ng/mL)	GC–MS (15 ng/mL)	EIA (50 ng/mL)	Creatinine (mg/dL)
1	1	Sm	0	0	–	0	–	0	1	172
1	1	Sm	1	19	+	14	+	0	4	34
1	1	Sm	2	10	+	7	+	9	16	36
1	1	Sm	4	3	+	3	+	13	19	24
1	1	Sm	8	5	+	3	+	45	79	82
1	1	Sm	16	2	+	2	+	71	102	191
1	1	Sm	24	0	–	0	+	29	43	106
1	1	Sm	48	0	–	0	–	28	36	206
1	1	Sm	72	0	–	0	–	7	11	159
1	2	Sm	0	0	–	0	–	61	101	189
1	2	Sm	1	40	+	29	+	10	20	26
1	2	Sm	2	6	+	5	+	26	49	24
1	2	Sm	4	4	+	2	+	28	45	11
1	2	Sm	8	2	+	2	+	79	100	37
1	2	Sm	16	0.8	+	0.8	+	190	101	171
1	2	Sm	24	0.6	–	2	–	72	97	51
1	2	Sm	48	0.4	–	0.5	–	63	92	93
1	2	Sm	72	1	+	1.3	+	152	100	117
1	3	Sm	0	0.2	–	0	–	8	15	143
1	3	Sm	1	30	+	15	+	2‡	8	25
1	3	Sm	2	3	+	2.8	+	4‡	10	10
1	3	Sm	4	1.3	+	1.3	+	20	30	27
1	3	Sm	8	1.5	+	0.9	+	17	31	49
1	3	Sm	16	0.9	+	0.47	–	30	54	140
1	3	Sm	24	0.3	–	0.4	–	43	49	140
1	3	Sm	48	0.3	–	0	–	27	40	253
1	3	Sm	72	0.48	–	0.7	–	91	101	137
1	4	Sm	0	0.2	–	0.2	–	364	102	267
1	4	Sm	1	41	+	44	+	344	100	249
1	4	Sm	2	4.7	+	5.7	+	765	103	335
1	4	Sm	4	2.7	+	2.9	+	755	99	164
1	4	Sm	8	1.7	+	1	+	283	102	115
1	4	Sm	16	19	+	12	+	306	101	189
1	4	Sm	24	1.6	+	0.7	+	313	102	144
1	4	Sm	48	0.2	–	0.2	–	198	101	219
1	4	Sm	72	1.2	+	1.1	+	218	102	133
1	5	Sm	0	0	–	0	–	6	12	91
1	5	Sm	1	9	+	11	+	13	33	49
1	5	Sm	2	1.3	+	1.8	+	191	103	227
1	5	Sm	4	0.8	+	0.9	+	84	102	43
1	5	Sm	8	0	+	0.7	+	23	34	17
1	5	Sm	16	0	–	0	–	10	103	133
1	5	Sm	24	0.2	–	0	–	31	40	39
1	5	Sm	48	0	–	0	–	42	40	38
1	5	Sm	72	0.3	–	0	–	21	24	44
1	6	Sm	0	0	–	0	–	0	0	108
1	6	Sm	1	2	+	2	+	0	2	50
1	6	Sm	2	0.3	–	0.4	–	0	1	13
1	6	Sm	4	0.4	–	0.2	–	0	3	72
1	6	Sm	8	0	–	0	–	0	1	10
1	6	Sm	16	0	–	0	–	0	5	67

* Abbreviations: Sm, smoked; Pass, passive; NS, no sample; NA, no test result.
† Concentrations of oral fluid are expressed as ng/mL of THC in the diluted specimen (See Methods). Approximate concentrations of THC in oral fluid can be obtained by multiplying the reported value by a factor of three.
‡ Some THC and THCCOOH concentrations are reported below the experimentally determined LOQ.

Table III (continued). Oral Fluid Analysis for THC by EIA and GC–MS–MS and Urinalysis for THCCOOH by EIA and GC–MS Following a Single Smoked Dose and a Single Oral Dose of Marijuana*

				Left Oral Fluid, THC†		Right Oral Fluid, THC†		Urine, THCCOOH		
Study	Subject	Route	Time (h)	GC–MS–MS (0.5 ng/mL)	EIA (1.0 ng/mL)	GC–MS–MS (0.5 ng/mL)	EIA (1.0 ng/mL)	GC–MS (15 ng/mL)	EIA (50 ng/mL)	Creatinine (mg/dL)
1	6	Sm	24	0	–	0.4	–	0	3	128
1	6	Sm	48	0	–	0.4	–	0	6	203
1	6	Sm	72	0	–	0	–	0	0	74
1	7	Sm	0	0	–	0	–	0	0	129
1	7	Sm	1	38	+	37	+	4‡	13	83
1	7	Sm	2	7.8	+	4.6	+	7	14	19
1	7	Sm	4	1.3	+	1.5	+	47	83	53
1	7	Sm	8	2.1	+	1.5	+	5	104	200
1	7	Sm	16	0.8	+	1.2	+	115	102	199
1	7	Sm	24	0.4	–	0.5	–	55	102	177
1	7	Sm	48	0.2	–	0.3	–	36	42	165
1	7	Sm	72	0.9	+	1.1	+	53	69	127
1	8	Sm	0	0	–	0	–	12	18	150
1	8	Sm	1	14.3	+	29.5	+	NS	NS	NS
1	8	Sm	2	3.4	+	3.9	+	97	102	188
1	8	Sm	4	0.8	+	1.8	+	35	46	31
1	8	Sm	8	0.5	+	0.8	+	0	39	37
1	8	Sm	16	0.3	+	0.2	+	62	103	134
1	8	Sm	24	0.1‡	–	0	–	6	9	11
1	8	Sm	48	0	–	0.2	–	41	45	128
1	8	Sm	72	0	–	0.2	–	6	8	44
1	9	Sm	0	0	–	0	–	0	5	155
1	9	Sm	1	17	+	37	+	9	85	183
1	9	Sm	2	5	+	6.8	+	28	91	103
1	9	Sm	4	1.3	+	1.3	+	18	40	37
1	9	Sm	8	0.4	+	0.7	+	6	14	17
1	9	Sm	16	0.3	+	0.5	+	14	37	92
1	9	Sm	24	0.3	–	0.3	–	11	16	13
1	9	Sm	48	0	–	0.2	–	21	32	154
1	9	Sm	72	0.1‡	–	0.2	–	11	19	163
1	10	Sm	0	0	–	0	–	0	1	25
1	10	Sm	1	45	+	21	+	0	6	51
1	10	Sm	2	7.1	+	4.8	+	6	15	38
1	10	Sm	4	3.4	+	3.3	+	3‡	7	12
1	10	Sm	8	1.9	+	1.6	+	27	40	177
1	10	Sm	16	0.7	+	0.8	+	169	30	143
1	10	Sm	24	0	–	0	–	0	6	31
1	10	Sm	48	0	–	0	–	4‡	8	88
1	10	Sm	72	0	–	0	–	1‡	3	44
1	11	Pass	0	0	–	0	–	0	0	80
1	11	Pass	1	0	+	0	+	0	3	30
1	11	Pass	2	0	–	0	–	0	0	30
1	11	Pass	4	NA	–	0	–	0	2	25
1	11	Pass	8	0	–	0	–	0	3	155
1	11	Pass	16	0	–	0	–	0	8	264
1	11	Pass	24	0	–	0	–	0	5	127
1	11	Pass	48	0	–	0	–	0	2	109
1	11	Pass	72	0	–	0	–	0	2	169
1	12	Pass	0	0	–	0	–	0	0	168
1	12	Pass	1	0	+	0	+	0	1	60
1	12	Pass	2	0	–	0	–	0	1	40
1	12	Pass	4	0	+	0	–	0	3	29

* Abbreviations: Sm, smoked; Pass, passive; NS, no sample; NA, no test result.
† Concentrations of oral fluid are expressed as ng/mL of THC in the diluted specimen (See Methods). Approximate concentrations of THC in oral fluid can be obtained by multiplying the reported value by a factor of three.
‡ Some THC and THCCOOH concentrations are reported below the experimentally determined LOQ.

Table III (continued). Oral Fluid Analysis for THC by EIA and GC–MS–MS and Urinalysis for THCCOOH by EIA and GC–MS Following a Single Smoked Dose and a Single Oral Dose of Marijuana*

				Left Oral Fluid, THC†		Right Oral Fluid, THC†		Urine, THCCOOH		
Study	Subject	Route	Time (h)	GC–MS–MS (0.5 ng/mL)	EIA (1.0 ng/mL)	GC–MS–MS (0.5 ng/mL)	EIA (1.0 ng/mL)	GC–MS (15 ng/mL)	EIA (50 ng/mL)	Creatinine (mg/dL)
1	12	Pass	8	NS	NS	NS	NS	NS	NS	NS
1	12	Pass	16	0	–	0	–	0	4	119
1	12	Pass	24	0	–	0	–	0	4	119
1	12	Pass	48	0	–	0	–	0	6	130
1	12	Pass	72	0	–	0	–	0	8	44
2	14	Sm	0	0	–	0	–	0	2	180
2	14	Sm	0.25	49	+	45	+	NS	NS	NS
2	14	Sm	0.5	14	+	14	+	0	13	220
2	14	Sm	0.75	10	+	10	+	NS	NS	NS
2	14	Sm	1	11	+	17	+	21	69	311
2	14	Sm	1.25	6	+	1	+	NS	NS	NS
2	14	Sm	1.5	4	+	3	+	43	97	284
2	14	Sm	1.75	3	+	3	+	NS	NS	NS
2	15	Sm	0	0	–	0	–	7	14	219
2	15	Sm	0.25	15	+	8.6	+	6	13	200
2	15	Sm	0.5	10	+	5	+	7	15	202
2	15	Sm	0.75	2	+	2	+	NS	NS	NS
2	15	Sm	1	5	+	5	+	17	41	139
2	15	Sm	1.25	5	+	6	+	44	90	249
2	15	Sm	1.5	3	+	2	+	88	102	367
2	15	Sm	1.75	3	+	3	+	64	100	296
2	16	Sm	0	0	–	0.3	–	336	102	262
2	16	Sm	0.25	228.2	+	136.4	+	NS	NS	NS
2	16	Sm	0.5	36.8	+	46.9	+	NS	NS	NS
2	16	Sm	0.75	22.5	+	34.9	+	473	100	297
2	16	Sm	1	16.9	+	28.2	+	NS	NS	NS
2	16	Sm	1.25	20.7	+	22	+	NS	NS	NS
2	16	Sm	1.5	6.6	+	10.8	+	NS	NS	NS
2	16	Sm	1.75	23.6	+	20.2	+	548	102	278
2	17	Sm	0	0	–	0.2	+	112	43	20
2	17	Sm	0.25	48	+	37	+	20	26	13
2	17	Sm	0.5	24	+	23.1	+	33	44	21
2	17	Sm	0.75	11	+	10	+	39	49	22
2	17	Sm	1	4	+	4	+	39	47	21
2	17	Sm	1.25	5	+	6	+	56	81	31
2	17	Sm	1.5	4	+	4	+	64	96	36
2	17	Sm	1.75	2	+	4	+	52	69	23
2	18	Sm	0	0	–	0	–	5	15	112
2	18	Sm	0.25	63	+	70	+	NS	NS	NS
2	18	Sm	0.5	35	+	25	+	NS	NS	NS
2	18	Sm	0.75	16	+	29	+	NS	NS	NS
2	18	Sm	1	16	+	31	+	26	64	94
2	18	Sm	1.25	12	+	24	+	NS	NS	NS
2	18	Sm	1.5	13	+	20	+	16	34	26
2	18	Sm	1.75	8	+	18	+	31	49	33
3	19	Oral	0	0	–	0	–	2‡	8	21
3	19	Oral	1	1.2	+	2.2	+	15	40	101
3	19	Oral	2	0.7	+	0.6	+	11	34	15
3	19	Oral	4	0.7	+	0.2	+	31	48	12
3	19	Oral	16	0	–	0	–	222	100	224
3	19	Oral	24	0	–	0	–	131	101	148

* Abbreviations: Sm, smoked; Pass, passive; NS, no sample; NA, no test result.
† Concentrations of oral fluid are expressed as ng/mL of THC in the diluted specimen (See Methods). Approximate concentrations of THC in oral fluid can be obtained by multiplying the reported value by a factor of three.
‡ Some THC and THCCOOH concentrations are reported below the experimentally determined LOQ.

Table III (continued). Oral Fluid Analysis for THC by EIA and GC–MS–MS and Urinalysis for THCCOOH by EIA and GC–MS Following a Single Smoked Dose and a Single Oral Dose of Marijuana*

				Left Oral Fluid, THC†		Right Oral Fluid, THC†		Urine, THCCOOH		
Study	Subject	Route	Time (h)	GC–MS–MS (0.5 ng/mL)	EIA (1.0 ng/mL)	GC–MS–MS (0.5 ng/mL)	EIA (1.0 ng/mL)	GC–MS (15 ng/mL)	EIA (50 ng/mL)	Creatinine (mg/dL)
3	19	Oral	48	0	–	0	–	43	71	140
3	19	Oral	72	0	–	0	–	19	30	118
3	20	Oral	0	2.6	+	3.7	+	99	103	296
3	20	Oral	1	6.9	+	7.1	+	82	101	209
3	20	Oral	2	6.2	+	5.6	+	86	100	186
3	20	Oral	4	3	+	2.7	+	46	NA	NA
3	20	Oral	16	1.2	+	0.5	+	NS	NS	NS
3	20	Oral	24	0.4	+	0.5	+	20	39	26
3	20	Oral	48	0.7	+	0.6	+	51	91	101
3	20	Oral	72	1.4	+	0.7	+	117	102	256
3	21	Oral	0	0	–	0	–	6	14	207
3	21	Oral	1	2	+	5.2	+	7	20	209
3	21	Oral	2	2.2	+	5.6	+	11	41	35
3	21	Oral	4	0.4	+	0	+	78	102	46
3	21	Oral	16	0	–	0	–	137	101	180
3	21	Oral	24	0	–	0	–	167	94	215
3	21	Oral	48	NS	NS	NS	NS	NS	NS	NS
3	21	Oral	72	0	–	0	–	62	101	231

* Abbreviations: Sm, smoked; Pass, passive; NS, no sample; NA, no test result.
† Concentrations of oral fluid are expressed as ng/mL of THC in the diluted specimen (See Methods). Approximate concentrations of THC in oral fluid can be obtained by multiplying the reported value by a factor of three.
‡ Some THC and THCCOOH concentrations are reported below the experimentally determined LOQ.

declined steadily to mean concentrations of 7.9 ng/mL (± 4.1) and 9.6 ng/mL (± 3.0) at 1.75 h from the left and right sides, respectively (Figure 2).

Following oral marijuana administration (Study 3, $N = 3$, Subjects #19–#21), mean peak (± SEM) THC concentrations in oral fluid were 3.4 (± 1.8) ng/mL and 4.8 (± 1.4) ng/mL from the left and right side, respectively. Peak concentrations occurred at 1 h (first collection) for 2 subjects and at 2 h for the third subject. Concentrations declined rapidly for two subjects and were below the LOD of the GC–MS–MS by 16 h. Oral fluid specimens from the second subject (Subject #20) contained THC at baseline (0 h). His specimens remained positive for THC throughout the 72 h time of the study. The individual data for THC in oral fluid (both left and right collections) are illustrated in Figure 3.

In general, THC concentrations in oral fluid generally declined rapidly over the first 2–4 h following smoked or orally ingested marijuana, but substantial individual variation was observed in the pattern of elimination. For example, THC concentration in oral fluid specimens for Subject #6 (casual user, smoked marijuana) dropped below the LOD for the GC–MS–MS assay in 2 h, whereas 5 subjects (#2, #3, #4, chronic users, smoked marijuana; #7, casual user, smoked marijuana; #20, oral marijuana) had positive specimens at the last collection (72 h).

Concordance of simultaneously collected oral fluid specimens

Dual oral fluid specimens were collected simultaneously from the left and right sides of the oral cavity for each subject and analyzed by EIA and GC–MS–MS (Table III). Results were evaluated qualitatively and quantitatively to determine if differences existed between the left- and right-side collections. For EIA (1.0-ng/mL cutoff concentration), 165 of 169 specimen results were in agreement (97.6%; 99 positives and 66 negatives). The four discordant results were equally divided between the left and right sides. Two specimens displayed negative results on the left side and positive results on the right side (two negative/positive results), and two specimens displayed the opposite pattern (two positive/negative results). By GC–MS–MS analysis (0.5-ng/mL cutoff concentration), 162 of 169 specimen results were in qualitative (positive/negative) agreement (95.9%; 93 positives and 69 negatives). The seven discordant results were unevenly divided between the left and right sides (six negative/positive results and one positive/negative result). The average THC (± SEM) concentrations for the seven discordant samples collected from the left and right sides of the oral cavity were 0.4 (± 0.1) and 0.5 (± 0.1) ng/mL, respectively. Paired t-tests of the sample mean concentrations indicated no significant difference ($p > 0.05$) in these specimens.

Figure 1 illustrates the concordance over time of THC concentrations by GC–MS–MS collected from the left and right side of the oral cavity for the 10 subjects who participated in Study 1. Quantitative comparisons were also made by correlation analysis of left versus right side specimen results by GC–MS–MS for all 20 subjects who participated in Studies 1–3. Although there was considerable variance in magnitude in some specimens, correlation of THC concentrations by GC–MS–MS was high. The correlation coefficient (r^2) of linear regression across all samples ($N =$ 20 subjects; 169 specimen pairs) collected from the three studies was 0.868. Correlations from individual studies were as follows: Study 1 ($N = 12$ subjects; 106 specimen pairs), $r^2 = 0.793$; Study 2 ($N = 5$ subjects; 40 specimen pairs), $r^2 = 0.887$; and Study 3

Table IV. Concordance of Oral Fluid Assay by EIA with Oral Fluid Assay by GC–MS–MS and Urine EIA with Urine GC–MS.

EIA (1.0 ng/mL)		GC–MS–MS (0.5 ng/mL) Positive	Negative
Oral fluid left side (N = 169)	Positive	92	9
	Negative	2	66
Oral fluid right side (N = 170)	Positive	94	7
	Negative	5	64
Oral fluid combined (N = 339)	Positive	186	16
	Negative	7	130
EIA (50 ng/mL)		**GC–MS–MS (0.5 ng/mL) Positive**	**Negative**
Urine	Positive	53	3
	Negative	31	67

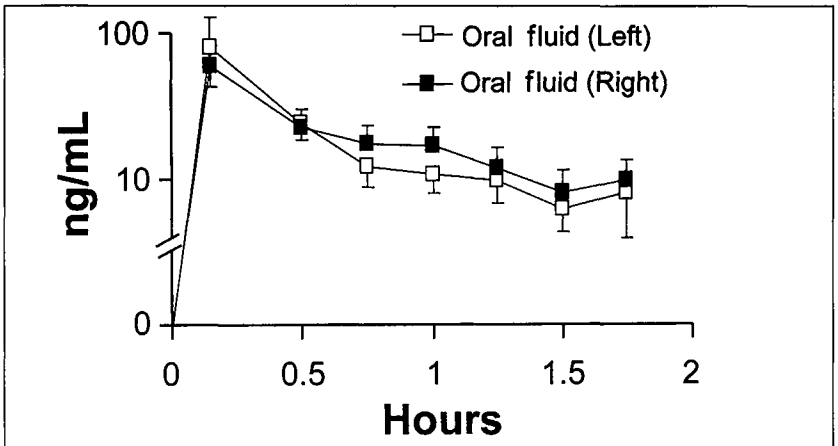

Figure 2. Mean THC concentrations by GC–MS–MS in oral fluid specimens collected simultaneously from the left and right sides of the oral cavity of five subjects following smoking of a single marijuana cigarette (Study 2). Error bars represent standard error of the mean.

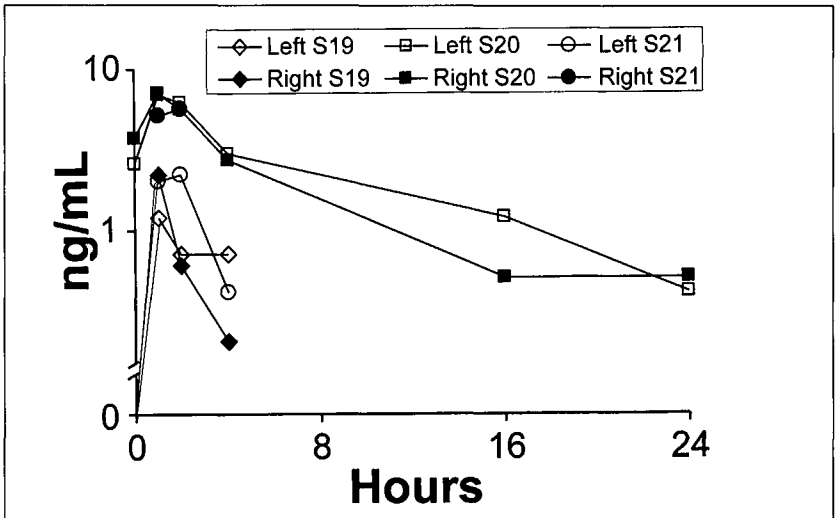

Figure 3. Individual THC concentrations by GC–MS–MS in oral fluid specimens collected simultaneously from the left and right sides of the oral cavity of three subjects following oral ingestion of a brownie containing plant content equivalent to a single marijuana cigarette (Study 3).

(N = 3 subjects; 23 specimen pairs), r^2 = 0.787. The mean (± SEM) THC concentrations (GC–MS–MS) across all left- and right-side collections (N = 339) were 6.9 ng/mL (± 1.6) and 6.6 ng/mL (± 1.2), respectively. Paired t-tests of the sample means indicated that there was no significant difference ($p > 0.05$) between left- and right-side oral fluid specimens.

Sensitivity and specificity of EIA compared with GC–MS–MS for oral fluid and urine

Comparison of test results of oral fluid EIA (1.0 ng/mL) to oral fluid GC–MS–MS (0.5 ng/mL) was made for left- and right-side specimen collections across all 20 subjects (Table IV). The sensitivity, specificity, and efficiency for detection of THC by EIA (compared to detection by GC–MS–MS) for the left- and right-side collected specimens were 97.9%, 88.0%, and 93.5% and 95.0%, 90.1%, and 92.9%, respectively. The combined results for all oral fluid EIA determinations (N = 339) provided sensitivity, specificity, and efficiency of 96.4%, 89.0%, and 93.2%, respectively. There were combined totals of 16 FP (4.7%) and 7 FN (2.1%) results. The 16 FP results came from 9 subjects and had a mean (± SEM) THC concentration by GC–MS–MS of 0.2 ng/mL (± 0.04) (range 0–0.4 ng/mL). The seven FN specimens came from three subjects and had a mean (± SEM) THC concentration by GC–MS–MS of 0.8 ng/mL (± 0.21) (range 0.5–2.0 ng/mL). The occurrence of FP specimens tended to appear at earlier collection times (mean collection time = 8.2 h, range = 0–24 h) than FN specimens (mean collection time = 40 h, range = 16–72 h).

Comparison of test results of urine EIA (50 ng/mL) to urine GC–MS (15 ng/mL) was also made across all 20 subjects (Table IV). The sensitivity, specificity, and efficiency for detection of THCCOOH by EIA (compared to detection by GC–MS) for the 20 subjects were 62.1%, 95.7%, and 77.9%, respectively. There were totals of 3 FP (1.9%) and 31 FN (20.1%). The 3 FP results came from 3 subjects and had a mean (± SEM) THCCOOH concentration by GC–MS of 8.0 ng/mL (± 1.5) (range 5–10 ng/mL). The 31 FN specimens came from 12 subjects and had a mean (± SEM) THCCOOH concentration by GC–MS of 34.7 ng/mL (± 5.5) (range 15–169 ng/mL).

Comparison of oral fluid with urine

The timecourse of detection of THC in oral fluid was compared to detection of THCCOOH in urine (Figure 4). The left-side oral fluid specimens were arbitrarily selected for the comparison because results from the simultaneously collected specimens (left- and right-side collections) were equivalent. In Study 1, the first oral fluid specimen collected at 1 h after smoked marijuana contained the highest concentration of THC; these specimens consistently tested pos-

itive for THC by EIA (1-ng/mL cutoff concentration) and GC–MS–MS (0.5-ng/mL cutoff concentration). Thereafter, THC concentrations declined and began to test negative by EIA and GC–MS–MS over a range of 2 to 24 h. Oral fluid specimens tested positive consecutively for average periods (± SEM; range) of 15 (± 2; 1–24) and 13 h (± 3; 1–24) by EIA and GC–MS–MS, respectively. Thereafter, specimens tested negative, followed by the occasional reappearance of positive specimens. The average THC detection times of the last oral fluid positive specimen by EIA and GC–MS–MS were 31 (± 9; 1–72) and 34 h (± 11; 1–72), respectively. A similar pattern was noted in Study 3 for the three subjects who consumed marijuana brownies (see Table III). In comparison, urine specimens collected in Study 1 immediately after smoked marijuana generally tested negative. The average times to detection of the first urine specimen positive for THC-COOH by EIA (50 ng/mL cutoff concentration) and GC–MS (15-ng/mL cutoff concentration) were 6 (± 2; 1–16) and 4 h (± 1; 2–8), respectively. Urine specimens tested positive consecutively for average periods of 26 (± 9; 2–72) and 33 (± 10; 4–72) h for EIA and GC–MS, respectively. Thereafter, specimens tested negative, followed by the occasional reappearance of positive specimens. The average THCCOOH detection times of the last specimen by EIA and GC–MS were 42 (± 10; 2–72) and 58 h (± 6; 16–72), respectively.

Two subjects in Study 1 displayed somewhat unusual test patterns in urine and oral fluid. One subject's pre-test urine specimen (Subject #4, self-reported chronic user) was highly positive by EIA and GC–MS. The baseline THCCOOH concentration by GC–MS was 364 ng/mL. This subject's urine specimens remained highly positive for marijuana metabolite throughout the 72 h test period. However, his pre-test oral fluid specimens (left and right collections) were negative for THC by EIA and GC–MS–MS, but were consecutively positive after smoked marijuana by EIA and GC–MS–MS for 24 h, negative at 48 h, and positive at 72 h. A different pattern was observed for Subject #6 (self-reported casual user). His urine specimens collected following smoked marijuana tested negative by EIA and GC–MS throughout the 72-h test period. However, oral fluid specimens (both left and right side) collected at 1 h after smoking were positive by both assays; thereafter, the remainder of oral fluid specimens tested negative.

The likelihood of testing positive by oral fluid and urine was evaluated by determining the percent-positive rate by EIA and GC–MS–MS for the 10 subjects in Study 1 at each collection time (Figure 5). All subjects initially tested negative for THC in oral fluid by EIA and GC–MS–MS prior to smoking marijuana. At 1 h after smoked marijuana, all subjects tested positive (100%) for THC in oral fluid by both methods. Positive rates remained at 90% for both methods through 4 h, then dropped slowly to 80% and 60% at 16 h for EIA and GC–MS–MS, respectively. Beyond 16 h, positive rates for oral fluid dropped sharply to the 10–20% range by 24 h. All oral fluid specimens were negative at 48 h; however, at 72 h three positive specimens (30%) were detected by EIA and confirmed by GC–MS–MS (data not illustrated in Figure 5).

Positive rates by urine testing were generally lower than by oral fluid testing over the first 16 h. In addition, the positive rate for detection of THCCOOH by GC–MS was higher than by EIA. The average percent-positive rate for urine specimens at 1 h was 22% by EIA. Thereafter, positive rates by EIA rose slowly to a peak positive rate of 70% at 16 h and then declined to 30% at 24 h. EIA positive rates at 48 h and 72 h were 20% and 40%, respectively (data not illustrated in Figure 5). The average percent-positive rate for THCCOOH by GC–MS was 11% at 1 h. The positive rate rose to 50% at 2 h and was 70% at 4 h; the rate remained in this range through 48 h and declined to 50% at 72 h (data not illustrated in Figure 5). Of the 10 subjects who participated in Study 1, there was evidence of recent use of marijuana by two subjects (Subjects #2 and #4) who initially tested positive for THCCOOH in urine by EIA and GC–MS prior to smoking marijuana (0 h).

Notably, two subjects (Subject #6 and Subject #10) tested negative (0% positives) by urine EIA throughout the three-day monitoring period. Subject #6 also tested negative by urine GC–MS; Subject #10 produced two urine specimens at 8 h and 16 h that tested positive by GC–MS, and the remainder tested negative. By oral fluid assay, Subject #6 tested positive (both left and right side) for THC by EIA at 1 h (also confirmed by GC–MS–MS) with the remaining specimens testing negative. Subject #10 tested positive (left and right side) from 1 to 16 h by EIA and by GC–MS–MS.

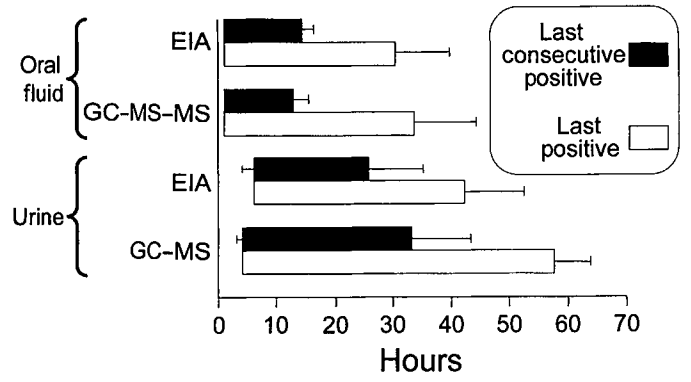

Figure 4. Mean detection times of THC in oral fluid (left collection) and THCCOOH in urine of 10 marijuana smokers following smoking a single marijuana cigarette (20–25 mg THC dose). Solid bars represent detection of first positive specimen and last consecutive positive specimen; open bars represent detection time from first positive specimen to last positive specimen. Error bars represent standard error of the mean. $N = 10$ for oral fluid EIA and GC–MS–MS measures; $N = 8$ for EIA and GC–MS detection of first positive urine specimen; and $N = 9$ for other urine detection times. Note that the detection period started with detection of the first positive specimen.

Discussion

Cannabis is the most commonly used illegal drug in most countries. In 1999, there were an estimated 14.8 million current illicit drug users (defined as use within 30 days of survey) in the United States, of which 25% (3.7 million) used an illicit drug other than marijuana, 57% (8.4 million) consumed only marijuana, and an addi-

tional 18% (2.7 million) used marijuana and another illicit drug (13). Lifetime prevalence was estimated to be 34.6% for persons aged 12 or older. Similar lifetime prevalence rates for marijuana use are found in the European Union. Lifetime experience of cannabis among adults was reported to range from 10% in Finland to 20–30% in Denmark, Spain, and the United Kingdom (14). Given that marijuana is known to impair sensory-perceptual abilities, gross motor coordination, learning, and complex divided attention tests (e.g., driving) (15), this widespread prevalence of marijuana use is of special concern for individuals involved in safety-sensitive activities, especially the workplace and in driving. Many national and international organizations have expressed interest in the development of reliable testing methodology applicable for detection of recent marijuana use (16).

Oral fluid testing for cannabinoids offers some distinct advantages over urine testing. The parent compound, THC, the psychoactive component of marijuana, is the analyte measured in oral fluid. The presence of THC in oral fluids can be generally regarded as evidence of recent cannabis use, whereas detection of THCCOOH in urine may be due to either recent use or to an accumulation from long-term use. Basically, there are fundamental dispositional and kinetic differences between oral fluid and urine that account for the differences observed in their timecourse of detection. Foremost in consideration are the different mechanisms involved in the disposition of THC and THCCOOH in oral fluid and urine, respectively. The timecourse of THCCOOH's appearance in urine (as free and conjugated metabolite) is determined by a host of pharmacologic and physiologic factors including marijuana strength (THC content), amount consumed (dose), time of sampling relative to use, and metabolic and renal characteristics of the individual undergoing testing. With smoked marijuana, THC appears immediately in blood after the first puff of marijuana smoke and reaches peak concentrations rapidly. Huestis et al. (17) reported finding means of 7.0 and 18.1 ng/mL of THC in plasma specimens from six subjects after their first puff from low- (1.75% THC) and high-dose (3.55% THC) marijuana cigarettes, respectively. Average peak THC plasma concentrations were reached in 8 min after the beginning of marijuana administration and prior to the end of the scheduled smoking protocol. Upon entry into the bloodstream, THC undergoes broad distribution to body tissues. Hepatic uptake is rapid, and subsequent biotransformation occurs with the formation of many cannabinoid metabolites (18). THCCOOH (free and conjugated) appears in blood slowly relative to THC (17). Time to peak concentrations of THCCOOH after smoked marijuana ranged from 1.4 to 2.4 h. Urinary excretion of THCCOOH occurs quickly, but initial specimens after marijuana use do not always test positive for THCCOOH. The detection times by various immunoassays for THCCOOH in urine following smoking of a single marijuana cigarette for an occasional user is generally 1–3 days, but considerable intersubject variability is often encountered (19). On the other hand, the urinary detection time for chronic marijuana users during cessation of use may be extremely long, that is, weeks to months (20). In contrast, the majority of THC appears in oral fluid as a result of the direct contact of cannabis with the oral mucosa during marijuana use. The passive diffusion of THC from blood back to oral fluid remains a possible contributory pathway, but the amount contributed by this route is likely to be minor (8).

Despite the apparent lack of contribution from blood THC to oral fluid concentrations, the timecourse of this psychoactive substance in these biological fluids following smoked marijuana is remarkably similar. Figure 6 illustrates THC concentrations in oral fluid (present study) compared to plasma THC concentrations reported by Huestis et al. (17). Figures 6A and 6B illustrate the timecourse of THC in plasma and oral fluid over 2 and 24 h, respectively. Whereas THC plasma concentrations dropped below 1 ng/mL by 6 h and were no longer detectable for any subject at 24 h by GC–MS (0.5-ng/mL cutoff concentration) following low or high doses, THC oral fluid concentrations (present study) dropped to a plateau in the low nanogram-per-milliliter concentration range over a period of 4–16 h. This plateau was followed by a slow, erratic decline to non-detectable concentrations over 24–72 h (48–72-h data not illustrated in Figure 6). The slower decline of THC in oral fluid compared to plasma THC resulted in a slightly longer detection time for THC in oral fluid than in plasma. Huestis et al. (17) reported that the mean detection times (range) of THC in plasma by GC–MS (0.5-ng/mL cutoff concentration) of six marijuana users under controlled conditions were 7.2 (3–12) and 12.5 h (6–27) following smoking of single marijuana cigarettes containing approximately 15.8 mg and 33.8 mg of THC. In the present study, following the smoking of a single marijuana cigarette containing the equivalent of 20–25 mg of THC, THC in oral fluid of 10 subjects was detected by GC–MS–MS (0.5-ng/mL cutoff concentration) for an average of 13 h (1–24) for consecutive positive specimens and 34 h (1–72) for detection of the last positive specimen.

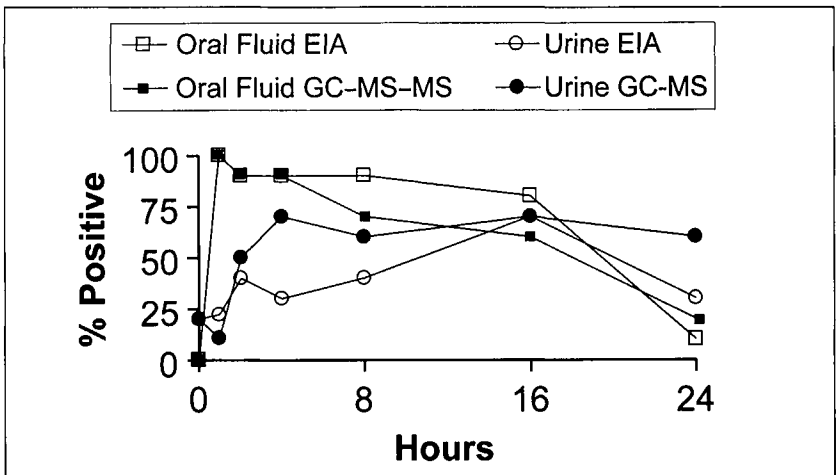

Figure 5. Percent of specimens testing positive over time in oral fluid and urine by EIA and GC–MS–MS (oral fluid) or GC–MS (urine) following smoking of a single marijuana cigarette by 10 subjects.

The surprising similarity in THC oral fluid and plasma curves merits further examination. Although THC in blood does not seem to contribute substantively to oral fluid content, the reverse contribution of oral mucosal THC to blood has not been thoroughly evaluated. It seems entirely plausible that a portion of the

THC "depot" retained in the oral cavity is absorbed transmucosally into the bloodstream over a period of several hours after marijuana use, thereby enhancing and prolonging its pharmacologic effects. Transmucosal drug absorption is known to occur for many types of drugs and is favored by low-molecular-weight compounds that have lipophilic properties (21). For example, buprenorphine, a lipophilic opioid compound can be administered effectively by the transmucosal route with a bioavailability of approximately 30% (22). Indeed, Mattes et al. (23) found that placing 10–15 mg of THC in sesame oil (Marinol®) under the tongue of drug-free marijuana users resulted in peak plasma levels of approximately 2 ng/mL of THC at 4 h and approximately 34 ng/mL of THCCOOH at 6 h. Additional measures at 2, 6, and 8 h indicated a slow, shallow decline in plasma levels of THC and THCCOOH. Although the possibility that the plasma levels of THC and metabolite observed after sublingual administration were the result of swallowed drug was not ruled out in that study, the findings strongly support the contention that sequestered THC in the oral cavity can be absorbed by the sublingual route. Thus, it appears likely that THC sequestered in the oral cavity after marijuana use could be released bi-directionally to oral fluid and to the highly vascular tissue underlying the oral mucosa, thereby enhancing THC concentrations in blood.

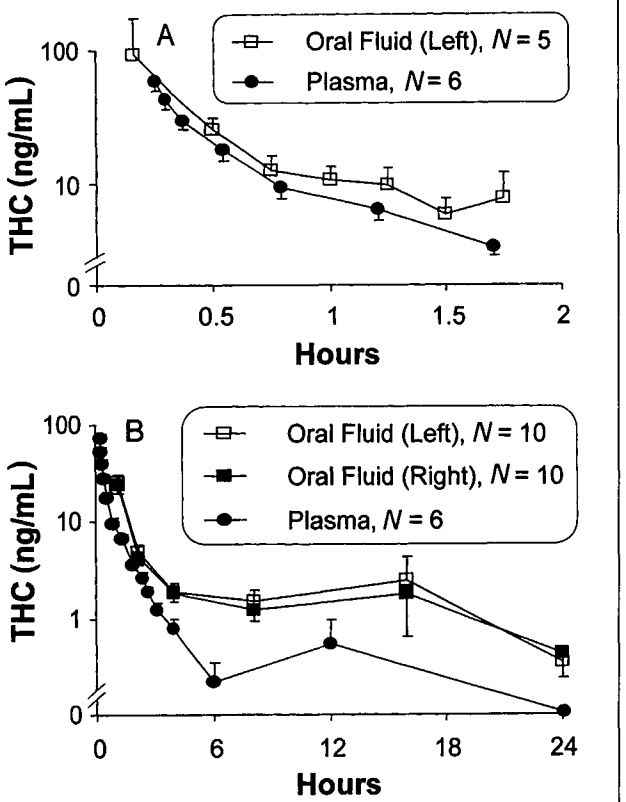

Figure 6. Comparison of THC in oral fluid and plasma (17) over short (A) and long (B) timecourses following marijuana smoking. Panel A illustrates THC in oral fluid of Study 2 (current study) following smoking of a single marijuana cigarette (20–25 mg of THC) by 5 male subjects compared to THC in plasma (17) following smoking of a single marijuana cigarette (15.8 mg of THC) by 6 male subjects. Panel B illustrates THC in oral fluid of Study 1 (current study) following smoking of a single marijuana cigarette (20–25 mg of THC) by 10 male subjects compared to THC in plasma following smoking of a single marijuana cigarette (15.8 mg of THC) by 6 male subjects. Error bars represent standard error of the mean (SEM).

In comparison to urine testing, oral fluid testing was more effective at detection of very recent marijuana use. This unique characteristic for oral fluid is of some importance when one considers the short timecourse of marijuana's effect on performance. For example, Kelly et al. (24) reported that subjects smoking a 1-g marijuana cigarette (3.5% THC) demonstrated significant impairment effects on laboratory tests only for 3–4 h after use. In the current study, it was noted that THC was immediately detected in the oral cavity during smoking with the highest concentrations of THC being present in the first collection. However, a lag period was observed for urine between the time marijuana was used and the time subjects produced positive urine specimens. This lag time occurred because of the time required for THC to be absorbed, distributed, metabolized, and excreted in urine in sufficient quantity for urine specimens to test positive at established cutoff concentrations. For example, the mean time (range) to detection of the first positive urine specimen by EIA (50 ng/mL) and GC–MS (15 ng/mL) for the 10 subjects in Study 1 were 6 (1–16) and 4 (2–8) h, respectively (Figure 4). A similar lag period was observed for some subjects by Huestis et al. (25). Overall, there was a substantially higher rate of detection over the first eight hours of positive specimens by oral fluid testing compared to urine testing (see Figure 5). During the 8-h interval following marijuana smoking, oral fluid testing by EIA was 2–4 times more likely to detect marijuana use than by urine EIA. By 16 h, positive EIA urine test results rose to comparable rates as oral fluid; thereafter, positive urine rates tended to be somewhat higher than oral fluid testing.

The success of oral fluid testing for marijuana is intimately linked to the efficacy of the entire test system, that is, the collection device, the screening procedure, and the confirmation assay. The first component in the oral fluid testing system, the collection device, must be capable of reproducible collection of adequate volumes of biological specimen. In the present study, oral fluid was collected by means of the Intercept DOA Oral Specimen Collection Device, a system that contains an absorbent-cotton-fiber pad affixed to a nylon stick. The device collects an average of 0.4 mL of oral fluid on the absorbant pad; the pad is then placed in a vial containing 0.8 mL of preservative solution. Aliquots of the diluted specimen were withdrawn and measured. The data are expressed as nanograms per milliliter of diluted specimen. The exact volume of specimen collected per each device was not determined. Consequently, these data are limited by the assumption that 0.4 mL of oral fluid was collected. An approximate concentration of THC in oral fluid for the data in Table III is obtained by multiplying the reported result by a factor of three.

Exact volume measurements are more difficult to obtain for oral fluid than for urine. This is a property inherent to most alternate matrices that is different from urinalysis and is worthy of comment. Collection devices that absorb specimen onto a matrix such as used in this study makes it difficult to measure exact volumes. Consequently, there is greater variability in the accuracy of reporting exact concentrations with alternate matrices than with urine testing. The problem with determining exact drug concentrations for oral fluid is similar to the problem faced with sweat collection (absorption onto a pad) and is not

unlike the problem associated with reporting exact drug concentrations in hair testing. In hair analysis, one is faced with reporting drug concentration based on weight of the specimen. However, there are numerous sources of variability associated with this process that may substantially alter the actual reported drug concentration. For example, different washing procedures may alter the final concentration. More importantly, drug concentration in hair strands is not uniform along the entire strand and may vary from zero to very high drug concentrations. Consequently, drug concentration expressed in ng/mg of hair represents an overall average concentration for the specimen and not an exact concentration. In contrast to hair, one may reasonably assume that drug concentrations in oral fluid and sweat specimens are constant throughout the specimen. This assumption for oral fluid is supported by the similarity in results of dual specimen collections in this report (Table III). Similarity in drug concentrations for dual sweat patch collections also supports this supposition (26). It should also be noted that even when exact volume measurements of oral fluid are attempted, they tend not to be as accurate as urine measurements. Pipettes are calibrated for aqueous solutions, but oral fluid may contain solid matter or bubbles and is more viscous than urine. Consequently, even measurements with calibrated pipettes are not as likely to be accurate for oral fluid as urine measures. Clearly, additional technical improvements are needed for collection devices and for laboratory measurements of alternate matrices. These needed improvements are likely to occur rapidly as interest in the use of alternate matrices continues to increase. Further, there are many known weaknesses of urine drug testing (e.g., adulteration, dilution, etc.) that have proven to be difficult to overcome. These weaknesses in urine testing provide impetus to further exploration and development of alternate matrices testing.

As mentioned, one collection aspect that was considered worthy of evaluation was the similarity in drug content between simultaneously collected specimens. Duplicate collection devices were employed (left and right side collections) in the study to determine if simultaneously collected specimens produced equivalent results in testing. Results were in agreement > 95% for all paired collections by EIA and GC–MS–MS. Statistical analysis of the sample means (GC–MS–MS) showed no significant difference between the simultaneously collected specimens. Consequently, these results indicated that two oral fluid specimens near simultaneously collected by means of two Intercept Collection Devices produced the same result and would meet criteria to serve as a "split specimen" as defined in the current proposed DHHS Guidelines for Federal Workplace Drug Testing Programs (1).

The second component in the oral fluid testing system, the screening assay, must be able to differentiate positive and negative specimens. The sensitivity and specificity of the oral fluid EIA (compared with GC–MS–MS) were 96.4% and 89.0%, respectively, for the combined left and right oral fluid collections thus demonstrating that the oral fluid EIA performed comparably to existing urine screening assays (19,27). In addition, the screening assay should be free from interference from common substances and resistant to adulteration. Experiments designed to challenge the accuracy of the EIA in the presence of common interferants (Tables I and II) indicated that the assay provided the correct results in the presence of the tested interferants.

The third component in the oral fluid testing system, the confirmation assay, must be able to identify the presence of a specific drug (i.e., THC) and eliminate screening false positives. In the present study, two passive control subjects who remained in the room with the active smokers, but did not smoke marijuana, produced a total of five EIA positive specimens, four of which represented the first oral fluid collection (left and right) after exposure to marijuana smoke. All five EIA positive specimens tested uniformly negative by GC–MS–MS. These results indicate that passive exposure to marijuana smoke can result in false positives by EIA and may be caused by the fact that immunoassays cross-react with a number of THC/THC metabolites, but such results can readily be differentiated from true positive specimens by GC–MS–MS. For the active marijuana users in this study, THC was monitored in oral fluid following administration of a single dose of marijuana by the smoked and oral routes. The timecourse of THC in oral fluid was surprisingly similar to THC in plasma over the course of the first several hours (Figure 6). Concentrations of THC in oral fluid declined in a multi-phasic manner suggestive of the presence of fast- and slow-release components of sequestered THC in the oral cavity. Compared to urine, the probability of a positive test was higher over the first 6 h for oral fluid than for urine. Thereafter, urine specimen positivity rose and generally exceeded that of oral fluid generally after 16 h.

Overall, the oral fluid testing system investigated in this report was highly successful in the early identification of marijuana use compared to urine testing. The doses employed in this study were commercial doses of marijuana obtained from a coffeehouse in Amsterdam and could be considered typical of what the regular marijuana user might consume. Simultaneously collected oral fluid specimens produced equivalent results demonstrating the reproducibility of oral fluid testing results. Oral fluid THC concentrations were shown to follow a similar timecourse as plasma THC following smoked marijuana. The remarkable similarity of THC in oral fluid compared to plasma suggests that a more direct link exists between THC oral fluid concentrations and plasma than has been previously recognized.

In conclusion, drug testing is intended to identify individuals in numerous situations that have ingested or smoked THC. These situations may include police actions or pre-employment testing. The actual number of positives identified and confirmed as positive in most situations may actually be small (28) as compared to the population at large. Therefore, drug testing will function mostly as a deterrent to drug use. Oral fluid, given its window of detection and convenience of use, offers an easy means of collection with reliable testing. Furthermore, the correlation between plasma and oral fluid shown in this study suggests that oral fluid is a better indicator than urine for active drug use. Future studies are needed to confirm these preliminary toxicological studies, but the utility of oral fluid as a deterrent is compelling.

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