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Quantification of 11-Nor-9-Carboxy- 9-Tetrahydrocannabinol in Human Oral Fluid by Gas Chromatography–Tandem Mass Spectrometry

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Abstract

A sensitive and specific method for the quantification of 11-nor-9-carboxy- 9-tetrahydrocannabinol (THCCOOH) in oral fluid collected with the Quantisal and Oral-Eze devices was developed and fully validated. Extracted analytes were derivatized with hexafluoroisopropanol and trifluoroacetic anhydride and quantified by gas chromatography–tandem mass spectrometry with negative chemical ionization. Standard curves, using linear least-squares regression with $1/x^2$ weighting were linear from 10 to 1000 ng/L with coefficients of determination >0.998 for both collection devices. Bias was 89.2%–112.6%, total imprecision 4.0%–5.1% coefficient of variation, and extraction efficiency $>79.8\%$ across the linear range for Quantisal-collected specimens. Bias was 84.6%–109.3%, total imprecision 3.6%–7.3% coefficient of variation, and extraction efficiency $>92.6\%$ for specimens collected with the Oral-Eze device at all 3 quality control concentrations (10, 120, and 750 ng/L). This effective high-throughput method reduces analysis time by 9 minutes per sample compared with our current 2-dimensional gas chromatography–mass spectrometry method and extends the capability of quantifying this important oral fluid analyte to gas chromatography–tandem mass spectrometry. This method was applied to the analysis of oral fluid specimens collected from individuals participating in controlled cannabis studies and will be effective for distinguishing passive environmental contamination from active cannabis smoking.

Keywords

cannabinoids; THCCOOH; oral fluid; GC-MS/MS

INTRODUCTION

According to recent reports, cannabis is the most widely used illicit drug.^{1,2} Almost 1 in 10 individuals who smoke cannabis will develop dependence.³ Cannabis is the most prevalent illicit drug in motor vehicle accidents and fatalities, and is monitored in forensic, pain

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management, driving under the influence of drugs, workplace, and drug treatment programs. Traditionally, drug monitoring programs relied on urine to monitor illicit drug use, but many programs are expanding the role of oral fluid as an important alternative matrix. Oral fluid collection is noninvasive and occurs under gender-neutral direct observation reducing the possibility of adulteration, substitution, and dilution.^{1,4,5} There also is evidence that oral fluid drug concentrations more closely correlate to blood concentrations after oral mucosal contamination from cannabis smoke dissipates than those of urine, but intersubject variability suggests that predicting blood concentrations from oral fluid concentrations is inaccurate.^{6–9}

The Substance Abuse and Mental Health Services Administration (SAMHSA) proposed oral fluid testing guidelines for federally mandated workplace drug testing.¹⁰ Although these guidelines have yet to be approved, oral fluid testing in the United States has greatly increased. Similar guidelines were established by the European initiative Driving Under the Influence of Drugs, Alcohol and Medicines (DRUID)¹¹ and throughout Europe and Australia.^{4,12} SAMHSA and DRUID only list 9-tetrahydrocannabinol (THC) as the target analyte for detection of cannabis use in oral fluid at confirmation cutoffs of 2 and 1 mcg/L, respectively.

THC is the primary analyte present in oral fluid after smoking and has been detected for up to 29 days during sustained abstinence in chronic frequent cannabis smokers.¹³ However, THC also was detected in oral fluid of nonsmoking volunteers during passive cannabis exposure studies,^{14–16} potentially leading to false-positive cannabinoid results. Monitoring 11-nor-9-carboxy-9-tetrahydrocannabinol (THCCOOH) concentrations in oral fluid was proposed to minimize potential false-positive results due to passive environmental exposure, as the metabolite (THCCOOH) is not present in cannabis smoke.^{16,17} After a single smoked cannabis cigarette, Lee et al¹⁸ reported THCCOOH concentrations up to 320 ng/L in Quantisal-collected oral fluid, whereas Milman et al¹⁹ reported concentrations of 561 ng/L in expectorated oral fluid. After around-the-clock high-dose oral THC administration, THCCOOH concentrations in Quantisal samples were as high as 1118 ng/L.⁷ These elevated THCCOOH concentrations also provided longer detection windows that are useful as a deterrent to drug use in workplace drug testing.¹⁸ Quantification of THCCOOH requires highly sensitive analytical methods as it is present in oral fluid in low nanogram per liter concentrations. Methods using 2-dimensional gas chromatography–mass spectrometry (GC-GC/MS),^{20,21} gas chromatography–tandem mass spectrometry (GC-MS/MS),¹⁷ and liquid chromatography–tandem mass spectrometry^{22–24} were successfully developed with low limits of quantification (2–10 ng/L).

Our aim was to develop and fully validate a high-throughput method using GC-MS/MS that would be capable of measuring THCCOOH nanogram per liter concentrations in oral fluid collected with the Quantisal and Oral-Eze devices. In our current GC-GC/MS laboratory method for quantification of 5 cannabinoids in oral fluid, 2 different elutions from 1 oral fluid sample are injected on 2 separate GC-GC/MS systems. Negative chemical ionization is used for quantifying THCCOOH and electron impact for THC, 11-hydroxy-THC (11-OH-THC), cannabidiol (CBD), and cannabinol (CBN).²¹ As solid phase extraction (SPE) and elution parameters were previously optimized, we focused on best using the fluorinated

derivatives on GC-MS/MS with negative chemical ionization to reduce analysis time and enhance THCCOOH sensitivity and linearity. Although 2-dimensional chromatography can improve sensitivity, it often suffers from longer retention times as analytes of interest must travel through 2 columns. Additionally, routine column maintenance can be complex and time consuming as new column lengths and corresponding pressures must be balanced for optimum performance. The use of tandem mass spectrometry (MS/MS) can reduce analysis time and enhance linearity without sacrificing the required sensitivity for detecting THCCOOH in oral fluid.

MATERIALS AND METHODS

Reagents and Supplies

THCCOOH (1 mg/mL) and THCCOOH-d3 (100 mcg/mL) were purchased from Cerilliant Corporation (Round Rock, TX). Trifluoroacetic anhydride (TFAA) and hexafluoroisopropanol (HFIP) were supplied by Campbell Science (Rockton, IL). Acetone, acetonitrile, ethyl acetate, and hexane were purchased from Sigma-Aldrich (Milwaukee, WI), and ammonium hydroxide (28%–30%), glacial acetic acid, and methanol were from Mallinckrodt Baker (Phillipsburg, NJ). All chemicals and organic solvents were ACS reagent grade and high pressure liquid chromatography grade, respectively. A CEREX System 48 positive pressure manifold and CEREX Polychrom THC (3 mL/35 mg) extraction columns from SPEware Corporation (Baldwin Park, CA) were used for specimen extraction. Quantisal devices for the collection of oral fluid specimens and Quantisal transport buffer for diluting calibrator standards were obtained from Immunalysis Corporation (Pomona, CA). Oral-Eze devices and Oral-Eze transport buffer were obtained from Capital Vial, Inc (Auburn, AL).

Oral Fluid Collection Procedure

The Quantisal collection device consists of an absorptive cellulose pad with a polypropylene stem and plastic tube containing a transport buffer solution. The collection pad has a volume adequacy indicator that turns blue when 1.0 ± 0.1 mL oral fluid is collected. During specimen collection, the collection pad is placed into the mouth; the pad is removed when the indicator window turns blue and is placed into the collection/transport tube containing 3 mL buffer. Similarly, the collection pad on the Oral-Eze device consists of cotton fiber filter paper attached to a plastic handle. The donor inserts the collection device between the cheek and gum, and when the sample adequacy indicator turns blue after 1.0 mL oral fluid is collected, the collection device is removed and the pad is detached into the transport tube containing 2 mL buffer. Buffer solutions in these collection devices act as a preservative to inhibit bacterial growth, help prevent drug and metabolite deterioration and adsorption during shipment or storage, and improve efficiency of removal of the drug from the collection pad. A total specimen volume of 4 mL (1 mL oral fluid +3 mL buffer) is available for analysis with the Quantisal device and a total volume of 3 mL (1 mL oral fluid +2 mL buffer) with the Oral-Eze device. This increased specimen volume allows for confirmation of multiple drug classes from a single specimen.

Preparation of Standard Solutions

Working THCCOOH standard solutions (0.5–5 mcg/L) were prepared by diluting 1.0 mg/mL stock solution with methanol. Working oral fluid calibrators (10, 20, 50, 100, 250, 500, and 1000 ng/L) were prepared daily by fortifying appropriate amounts of working standard into 0.25 mL blank authentic oral fluid and the appropriate amount of device buffer (Quantisal 750 μ L and Oral-Eze 500 μ L). Quality control (QC) solutions were prepared from different lots of stock solutions than those for preparing standards. Low, medium, and high QC samples across the dynamic range of the assay were prepared daily in blank oral fluid to achieve final THCCOOH concentrations of 30, 120, and 750 ng/L. Deuterated stock internal standard (THCCOOH-d3) was diluted with methanol to achieve a working internal standard concentration of 500 ng/L. Twenty-five microliters working internal standard was added to each sample before extraction yielding a final internal standard concentration of 50 mcg/L. All standards, QC, and internal standards solutions were stored in amber vials at -20°C .

Extraction and Derivatization

We analyzed oral fluid collected with the Quantisal and Oral-Eze devices with modifications to a published SPE GC/MS procedure.²¹ Modifications were necessary to streamline the analytical procedure for the determination of only THCCOOH. Briefly, 250 μ L blank oral fluid and 750 μ L Quantisal buffer (or 500 μ L Oral-Eze buffer) were added to 10 mL Sarstedt tubes. Samples were fortified with calibrator, control, and/or deuterated internal standard solutions before vortexing. One milliliter of ice-cold acetonitrile was added, and the tubes were centrifuged for 7 minutes at 4000g to pellet proteins. Supernatants were applied to SPE columns preconditioned with methanol (1 mL) and washed with water/acetonitrile/ammonium hydroxide (84:15:1, vol/vol/vol). Columns were dried under full vacuum for 15 minutes before column beds were wetted with 0.4 mL hexane. SPE columns were washed with a 3 mL hexane/acetone/ethyl acetate solution (60:30:20, vol/vol/vol) that will elute THC, 11-OH-THC, CBD, and CBN. THCCOOH was eluted into glass centrifuge tubes with 3 mL hexane/acetone/ethyl acetate (75:25:2.5, vol/vol/vol). Eluates were evaporated to dryness under a stream of nitrogen at 35°C , reconstituted with 20 μ L HFIP and 40 μ L TFAA, and incubated for 40 minutes at 65°C . Fluorinated derivatives were cooled, evaporated to dryness at 35°C , and reconstituted in 20 μ L of toluene before transfer to glass autosampler vials containing glass inserts.

Instrumentation and Conditions

Analyses were performed on an Agilent 7890A gas chromatograph equipped with a 7963 autosampler and 7000A triple quadrupole mass spectrometer (Palo Alto, CA). Data acquisition and analysis were performed using MassHunter Workstation software. Analytes were separated using 2 HP 5-MS capillary columns (15 m $\times$ 0.25 mm internal diameter, 0.25- μ m film thickness) connected with a metal union. This dual column setup, operated in constant flow, allowed for a more efficient column backflush. High purity helium was the carrier gas with a constant flow of 1.2 mL/min for the first column and a constant flow of 1.4 mL/min for the second. Pulsed splitless injection (5 μ L) at 40 psi for 1 minute was performed using a multi-mode inlet maintained at 275°C . The initial oven temperature of 175°C was held for 1 minute, followed by a $30^{\circ}\text{C}/\text{min}$ increase to 275°C and held for 0.9

minutes, for a total analysis time of 5.2 minutes. An auto-tune was initiated before each assay, and a gain factor of 40 was added to optimize instrument response. Collision cell gases were set at 2.25 and 1.5 mL/min for nitrogen and helium, respectively. The mass spectrometer was operated in the negative ion chemical ionization mode with ammonia as the reagent gas. Transfer line and ion source temperatures were 280°C and 275°C, respectively. Initial MS/MS experiments were performed to determine abundant and specific precursor and product ions and optimal collision energies. MS/MS method parameters are listed in Table 1.

Method Validation and Acceptance Criteria

Both analytical methods (Quantisal and Oral-Eze) were validated by determining specificity, sensitivity, linearity, carryover, accuracy, imprecision (inter- and intra-assay), extraction efficiency, dilution integrity, and stability. All validation experiments were conducted with authentic blank oral fluid–buffer mixture.

Specificity was defined as the ability to identify and quantify an analyte with and without endogenous or exogenous potential interferences. Endogenous interferences were evaluated with oral fluid specimens from 10 different donors and fortified with internal standard only (negative) to demonstrate the absence of common interferences from the matrix and allow us to assess if the mass spectra of the deuterated internal standard contained fragment ions with the same mass to charge ratio as the target analyte. Exogenous interferences were evaluated by fortifying 80 compounds, including drugs of abuse, metabolites, over-the-counter medications, and structurally similar compounds into low QC samples (30 ng/L). The fortified low QC was required to quantify within $\pm 20\%$ of target, have acceptable retention time, symmetrical peak shape, and qualifier ion ratios within $\pm 20\%$ of mean calibrator ratios.

Sensitivity was evaluated by determining the limits of detection (LOD) and quantification (LOQ) with a series of decreasing concentrations of drug-fortified oral fluid–buffered specimens. LOD was defined as the lowest concentration with correct retention time, symmetrical peak shape, a signal-to-noise ratio of at least 3:1 for all ions and a qualifier ion ratio within $\pm 20\%$ of averaged calibrators. LOQ was defined as the lowest calibrator that met the LOD criteria and yielded analyte concentrations within $\pm 20\%$ of target.

Analyte linearity was determined with 7 calibrators (10–1000 ng/L) that were prepared daily. Calibration curves were generated by plotting the ratio of analyte and internal standard responses versus concentration. A least-squares regression line with $1/x^2$ weighting factor was used to determine slope and intercept. Calibrators were required to satisfy all identification criteria and quantify within 15% of target concentration, except at the LOQ where 20% variation was permitted. Chromatographic carryover was investigated in triplicate by injecting extracted negative specimens (blank oral fluid–buffered samples containing internal standards) immediately after samples containing 2000 ng/L THCCOOH.

Analytical recovery (bias) and imprecision were determined with 4 replicates at each QC concentration (30, 120, and 750 ng/L) over 5 days. Intra-assay data was collected within 1 analytical run ($N = 4$), whereas inter-assay data were determined from the 4 replicates in 5

runs ($N = 20$). Bias was determined by comparing mean measured concentrations to target and expressed as percent of target concentration. Imprecision was expressed as percent coefficient of variation (%CV). Additional calculations of pooled intraday, interday, and total imprecision were performed according to Krouwer and Rabinowitz.²⁵

Extraction efficiency was determined at low and high control concentrations (30 and 750 ng/L) by adding standard solutions to blank oral fluid–buffered samples ($n = 10$) before SPE and to a second set after extraction, but before evaporation and derivatization. Extraction efficiency (expressed as a percentage) was calculated by comparing mean analyte areas of the set fortified before SPE with the corresponding mean analyte areas in the set fortified after SPE.

Dilution integrity was investigated by diluting the high QC sample (750 ng/L) with blank oral fluid–buffer mixtures. To confirm dilution integrity of Quantisal specimens, 250 μ L of the high control sample was diluted with 500 μ L of a blank oral fluid–Quantisal buffer mixture. For Oral-Eze dilution integrity, 250 μ L of the high control sample was diluted with 750 μ L of a blank oral fluid–Oral-Eze buffer mixture. Triplicates of each dilution were evaluated. Assayed concentrations of diluted samples were corrected by a multiplication factor and compared with mean undiluted QC concentrations ($n = 3$).

Analyte stability may be influenced by storage and handling conditions. Stabilities of stored samples were investigated by fortifying blank oral fluid–buffered samples ($n = 4$) at 2 QC concentrations (30 and 750 ng/L) and storing them at room temperature (RT) for 16 hours, 4°C for 24 hours, and after 3 freeze/thaw cycles at -20°C . On completion of these storage conditions, internal standard was added and the samples were extracted and analyzed as described above. Stability of derivatized extracts also was evaluated. Autosampler vials were stored at RT and reinjected 48 hours after initial analysis. All samples were quantified against the initial calibration curve and were required to be within $\pm 20\%$ of initial results.

RESULTS

Specificity

Blank oral fluid from 10 drug-free volunteers was analyzed to verify the absence of potential endogenous interferences. There were no interfering peaks in chromatograms from donated oral fluid combined with Quantisal or Oral-Eze buffers. In addition, the method was challenged with 80 potential interferences from structurally similar or commonly coadministered compounds, metabolites, and over-the-counter medications. The following compounds were fortified at 1000 mcg/L into low QC samples prepared along with calibrators in neat solutions: Δ^9 -tetrahydrocannabinol, 11-hydroxy- Δ^9 -tetrahydrocannabinol, cannabidiol, cannabinol, nicotine, cotinine, norcotinine, hydroxycotinine, ephedrine, pseudoephedrine, methamphetamine, amphetamine, *p*-hydroxymethamphetamine, *p*-hydroxyamphetamine, *p*-methoxymethamphetamine, *p*-methoxyamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxyethylamphetamine, 4-hydroxy-3-methoxymethamphetamine, 4-hydroxy-3-methoxyamphetamine, cocaine, benzoylecgonine, cocaethylene, norcocaethylene, *m*-hydroxycocaine, *m*-hydroxybenzoylecgonine, *p*-hydroxycocaine, *p*-

hydroxybenzoylecgonine, ecgonine ethyl ester, ecgonine methyl ester, anhydroecgonine methyl ester, ecgonine, buprenorphine, norbuprenorphine, morphine, codeine, normorphine, norcodeine, hydromorphone, hydrocodone, oxymorphone, oxycodone, noroxymorphone, noroxycodone, 6-acetylmorphine, 6-acetylcodeine, morphine-3-glucuronide, morphine-6-glucuronide, methadone, phencyclidine, ketamine, propoxyphene, diazepam, nordiazepam, lorazepam, oxazepam, alprazolam, fluoxetine, norfluoxetine, nitrazepam, 7-aminonitrazepam, flunitrazepam, 7-aminoflunitrazepam, 7-aminoclonazepam, temazepam, paroxetine, imipramine, clomipramine, clonidine, pentazocine, acetaminophen, ibuprofen, acetylsalicylic acid, caffeine, dextromethorphan, phentermine, chlorpheniramine, brompheniramine, and diphenhydramine. No exogenous interferences were noted because all fortified low QC samples quantified within $\pm 20\%$ of target, with acceptable retention time, peak shape, and qualifier ion ratios.

Sensitivity and Linearity

Linear ranges were determined with 7 calibrators over 5 assays. Performance criteria and calibration data for both devices are presented in Table 2. LOD were evaluated on 3 different assays by extracting duplicate oral fluid specimens from 3 different sources. Acceptable ion ratios and chromatographic conditions were achieved at 7.5 ng/L in both collection devices. Similarly, quantification limits also were evaluated in triplicate experiments with duplicates from 3 different oral fluid sources. Assays were linear from 10 to 1000 ng/L. An LOQ specimen was included in each analytical batch and defined as the lowest concentration with correct retention time, symmetrical peak shape, qualifier ion ratios within $\pm 20\%$ of averaged calibrators and analyte concentrations within $\pm 20\%$ of target. The 10 ng/L calibrator (LOQ) was within 9.5% and 13.0% of target concentrations for Quantisal and Oral-Eze devices, respectively, whereas the remaining calibrators were always $<10.6\%$ of target concentrations. The coefficient of determination (R^2) exceeded 0.998 for both oral fluid collection devices.

Carryover

Triplicate extracted blank oral fluid specimens containing internal standards were injected after samples containing target analytes at twice the upper LOQ. No peaks in oral fluid from either collection device fulfilled LOD criteria after injections of 2000 ng/L THCCOOH.

Analytical Recovery and Imprecision

Intra- and inter-day analytical recovery (bias) and method imprecision were evaluated at 3 QC concentrations across the linear range (Table 3). Analytical recovery was expressed as %bias of expected concentrations and imprecision as %CV. Intraday ($N = 4$) and interday ($N = 20$) analytical recoveries from Quantisal oral fluid ranged from 89.2% to 112.6%. Oral-Eze analytical recoveries were between 84.6% and 109.3% of target concentrations. Total imprecision ($n = 20$) evaluated at each QC concentration was 4.0%–5.1% and 4.0%–7.3% CV for Quantisal and Oral-Eze oral fluid specimens, respectively.

Extraction Efficiency

Extraction efficiency was evaluated for Quantisal and Oral-Eze at low and high QC concentrations with oral fluid from 10 drug-free volunteers. In the first investigation, oral fluid samples (n = 10) were fortified with analyte and internal standard before SPE. In the second investigation, oral fluid samples (n = 10) were fortified after SPE. Mean peak areas of low and high controls were compared, and extraction efficiency were expressed as a percentage. Quantisal mean extraction efficiencies at 30 and 750 ng/L were 89.7% and 79.8%, respectively. Mean extraction efficiencies at low (98.1%) and high (92.6%) QC concentrations for Oral-Eze samples were slightly higher.

Dilution Integrity

To account for specimens exceeding the upper LOQ, the dilution integrity was investigated by diluting the high QC sample (750 ng/L) with blank oral fluid–buffer mixtures. For consistency, 250 µL of the high control sample was removed and diluted to the required sample volume with the appropriate buffer. Triplicates of each dilution were evaluated. Assayed concentrations of Quantisal-diluted samples were corrected by a multiplication factor ($\times 4$) and compared with mean undiluted QC concentrations (n = 3). Mean measured concentrations were 96.0%–97.2% of the target concentrations. Similarly, for Oral-Eze diluted specimens ($\times 3$), mean measured concentrations were 89.3%–95.8% of the target concentrations (n = 3).

Stability

Mean percent differences between freshly prepared low and high QC specimens (n = 4) and stability specimens (n = 4) at various temperature storage conditions are presented in Table 4. Analyte concentrations in both devices were within 20% of initial target concentrations after 16 hours at RT ($\pm 8.0\%$), 24 hours at 4°C refrigeration ($\pm 4.5\%$), or after 3 freeze and thaw cycles at -20°C ($\pm 5.4\%$). Derivatized extracts were stable for up to 48 hours at RT.

Application of Method

This method was used to quantify THCCOOH in authentic oral fluid specimens collected with Quantisal (n = 10) and Oral-Eze (n = 14) devices from the 2 approved protocols. Volunteers provided written informed consent to participate in protocols approved by the Johns Hopkins and National Institute on Drug Abuse Institutional Review Boards. Quantisal specimens reflect THCCOOH concentrations after administration of an Oromucosal Sativex Spray. Cannabis smokers were randomly assigned to receive placebo, 5 and 15 mg oral synthetic THC, and low (5.4 mg THC and 5.0 mg CBD) and high (16.2 mg THC and 15.0 mg CBD) doses of Sativex. THCCOOH concentrations in Oral-Eze specimens were obtained from a cannabis user who smoked a single 6.8% THC cigarette ad libitum. Results are presented in Table 5 and Figures 1C and 2C.

DISCUSSION

Our objective was to develop and validate an efficient sensitive method for THCCOOH quantification in oral fluid collected with the Quantisal and Oral-Eze devices. The method requires adequate sensitivity to monitor nanogram per liter concentration of THCCOOH to

distinguish passive environmental exposure from active cannabis smoking and have a large dynamic range as THCCOOH is elevated in chronic smokers. Despite a larger volume of transport buffer in the Quantisal device (3 mL) compared with the Oral-Eze device (2 mL), LOQ (10 ng/L) were identical for both devices, and an upper limit of linearity of 1000 ng/L should be sufficient for quantification of most THCCOOH concentrations in routine oral fluid specimens. Method validation parameters were similar for both collection devices. Intra- and inter-day analytical recovery, expressed as a percentage difference between mean and target concentrations, ranged from 89.2% to 112.6% for low and high QC samples prepared with the Quantisal collection device. Similarly, Oral-Eze SPE intra- and inter-day recoveries were between 91.1%–101.3% and 84.6%–109.3%, respectively. Total imprecision also was less than 5.1% (Quantisal) and 7.3% (Oral-Eze). Quantisal and Oral-Eze stability was within $\pm 9\%$, at all evaluated conditions.

In this manuscript, the extraction procedure described by Milman et al.²¹ was used without modification. Only minor changes in reconstitution (25 versus 20 μ L) and injection volumes (5 versus 4 μ L) were adapted to achieve similar sensitivities (7.5 versus 10 ng/L) and improve linearity from 500 to 1000 ng/L.

GC-GC/MS is a relatively inexpensive upgrade that can improve sensitivity over traditional single quadrupole mass spectrometers. Although GC-GC/MS with negative chemical ionization provided low detection limits,^{20,21} this technique does not generate adequate fragment ions for many forensic applications. Monitoring a primary target ion, 1 qualifier ion, and their ratio has become accepted but is not ideal. It also can be challenging to set up and maintain these systems where lengthy analysis times are common. In addition, developing additional assays is highly dependent on predefined column configurations in analytical systems that can be less flexible. For these reasons, we investigated the possibility of GC-MS/MS analysis of THCCOOH in oral fluid.

In a typical GC-MS/MS analysis, precursor ions are isolated from matrix and selected by the first quadrupole. Fragmentation of these precursor ions occur in a collision cell (quadrupole 2) as selected ions collide with nitrogen gas under optimized collision energy to create product ions. Finally, these product ions are filtered in the third quadrupole and measured by the detector. The main benefit of MS/MS is improved specificity and reduction of interferences and background that improve the signal-to-noise ratio for analytes of interest, especially in complex matrices. The increased selectivity of our GC-MS/MS method, applicable for forensic challenges, was achieved by monitoring 2 transitions for each compound. Analyte identification was confirmed by both retention time and the ratio of quantification product ion and confirmation product ion.

Quantifying transitions for each analyte are listed in bold: THCCOOH (**590.1** $\rightarrow$ **422.4** and 590.1 $\rightarrow$ 402.4) and THCCOOH-d3 (**593.1** $\rightarrow$ **425.4** and 593.1 $\rightarrow$ 405.4). An initial analysis time of 15.1 minutes was required for GC-GC/MS with negative chemical ionization compared with only 5.2 minutes with the triple quadrupole method. A post-run backflush of 0.8 minutes reduced the need for elevated temperature ramps at the end of the analytical run to remove later eluting compounds from the column. The negligible loss of sensitivity and increased linearity coupled with reduced analysis time increases specimen throughput.

Evaluating cannabinoids in oral fluid collected with Quantisal and Oral-Eze devices can require expensive, hyphenated analytical techniques to achieve low THCCOOH concentrations. In addition, oral fluid specimen volumes are usually lower than traditional matrices and can also require confirmation of multiple drug classes.²⁶ Regardless of these analytical challenges, it is important to monitor THCCOOH oral fluid concentrations, and failure to do so may complicate interpretation of forensic cannabinoid testing.

After 3 hours of passive exposure to cannabis smoke in 2 Dutch coffee shops, Moore et al¹⁶ reported THC oral fluid concentrations up to 17 ng/L, whereas THCCOOH was not detected (<2 ng/L) under these realistic conditions. Moore et al also collected oral fluid specimens from a frequent cannabis smoker for up to 8 hours after smoking. THCCOOH concentrations were >51 ng/L after the first 2 hours, increasing to 134 ng/L after 8 hours, suggesting that monitoring THCCOOH is important in reducing claims of passive environmental contamination of the oral cavity.²⁰ Lee et al¹³ evaluated cannabinoid oral fluid cutoffs in chronic daily cannabis smokers and found that including a metabolite (THCCOOH, CBD, or CBN) with a THC cutoff would help to detect recent exposure and rule out residual THC excretion. Lee et al¹⁸ also determined that including THCCOOH, with or without THC, minimized the issue of passive exposure.

In our laboratory, maintaining our existing extraction and derivatization procedure for cannabinoids in oral fluid allowed quantification of THC, CBD, CBN, and 11-OH-THC by GC-GC/MS with electron ionization and allows rapid trace-level quantification of THCCOOH by GC-MS/MS. This procedure reduces THCCOOH analysis time by 9 minutes per injection, with similar LOQ and increased linearity. This method was applied to the analysis of oral fluid specimens collected from individuals participating in controlled cannabis studies, and may be a useful in workplace, pain management, drug treatment, and forensic drug testing programs where distinguishing passive environmental exposure from active cannabis smoking is critical.

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FIGURE 1. GC-MS/MS chromatograms showing target and qualifier ions for specimens collected with the Quantisal collection device. Representative chromatography includes blank oral fluid (A), a 10 ng/L LOQ (B), and an authentic specimen (C) containing 167.5 ng/L of THCCOOH.

FIGURE 2. GC-MS/MS chromatograms showing target and qualifier ions for specimens collected with the oral-Eze collection device. Representative chromatography includes blank oral fluid (A), a 10 ng/L LOQ (B), and an authentic specimen (C) containing 632.8 ng/L of THCCOOH.

TABLE 1

GC-MS/MS Parameters for THCCOOH in Oral Fluid

Analyte	<u>Molecular Weight (g/mol)</u>		Precursor Ion	Product Ion *	Collision Energy (eV)	Dwell Time (ms)
	Compound	Derivative				
THCCOOH	344	590	590.1	<u>422.4</u>	3	75
				402.4	9	75
THCCOOH-d ₃	347	593	593.1	<u>425.4</u>	3	75
				405.4	9	75

* Quantification ion is underlined.

TABLE 2

Calibration Curve Characteristics of THCCOOH in Oral Fluid by GC-MS/MS (n = 5)

Analyte	Internal Standard	LOD (ng/L)	LOQ (ng/L)	Linear Range (ng/L)	Slope, Mean $\pm$ SD	Y-Intercept, Mean $\pm$ SD	R ² * (Range)
Quantisal							
THCCOOH	THCCOOH-d ₃	7.5	10	10–1000	0.0192 $\pm$ 0.0010	0.0305 $\pm$ 0.0323	0.9992–0.9999
Oral-Eze							
THCCOOH	THCCOOH-d ₃	7.5	10	10–1000	0.0174 $\pm$ 0.0046	0.0541 $\pm$ 0.0408	0.9985–0.9997

* Coefficient of determination.

TABLE 3

Analytical Recovery and Imprecision Data for THCCOOH in Oral Fluid by GC-MS/MS

Concentration (ng/L)		Analytical Recovery (Percent of Expected Concentration)				Imprecision (Percent of CV, N = 20)		
		Intraday (N = 4)		Interday (N = 20)		Pooled Intraday	Interday	Total
		Mean	Range	Mean	Range			
Quantisal								
THCCOOH	30	95.7	91.7–101.3	96.5	90.7–105.7	3.2	2.8	4.2
	120	96.5	94.6–99.9	98.2	89.2–106.3	2.2	4.6	5.1
	750	99.4	98.4–101.2	103.2	97.8–112.6	2.2	3.3	4.0
Oral-Eze								
THCCOOH	30	96.2	91.1–98.7	94.8	84.6–109.0	4.6	5.7	7.3
	120	98.1	95.6–99.4	99.0	92.5–109.3	2.8	2.2	3.6
	750	99.0	95.4–101.3	97.6	89.6–103.5	1.5	3.7	4.0

TABLE 4**THCCOOH Stability in Oral Fluid**

Analyte	48 h Autosampler (Percent Difference, N = 4)		16 h Room Temperature (Percent Difference, N = 4)		24 h 4°C (Percent Difference, N = 4)		3 Freeze/Thaw Cycles (Percent Difference, n = 4)	
	Low	High	Low	High	Low	High	Low	High
Quantisal								
THCCOOH	2.0	9.0	-8.0	-1.0	-3.5	-0.3	-5.4	-0.6
Oral-Eze								
THCCOOH	-8.0	2.6	-7.2	-3.8	-4.5	-1.9	-1.4	-1.8

THCCOOH low and high QC concentrations were 30 and 750 ng/L, respectively.

TABLE 5

THCCOOH Concentrations in Authentic Oral Fluid Specimens Collected With the Quantisal and Oral-Eze Device (n = 24)

Device	Specimen ID	Time After Smoking, h	THCCOOH, ng/L
Quantisal	001	−0.5 (Admission)	149.5
	002	0.25	87.5
	003	1.0	81.6
	004	4.5	89.6
	005	7.5	167.5
	006	10.5	147.5
	007	−0.5	141.6
	008	0.25	124.0
	009	1.0	110.0
	010	4.5	55.5
Oral-Eze	001	−19.0 (Admission)	632.8
	002	−1.0	288.0
	003	0.5	347.2
	004	1.0	451.3
	005	2.0	528.9
	006	3.0	276.5
	007	4.0	254.6
	008	5.0	356.0
	009	6.0	130.5
	010	8.0	171.0
	011	10.5	139.8
	012	13.5	98.4
	013	21.0	284.3
	014	24.0	75.3