

Analytical Procedure for the Determination of the Marijuana Metabolite 11-nor- Δ^9 -Tetra-hydrocannabinol-9-carboxylic acid (THCA) in Oral Fluid Specimens

Christine Moore*, Cynthia Coulter, Sumandeep Rana, Michael Vincent, and James Soares

Immunalysis Corporation, 829 Towne Center Drive, Pomona, California 91767

Abstract

The determination of the marijuana metabolite 11-nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid (THCA) in oral fluid specimens is described for the first time using a Quantisal™ oral fluid collection device and gas chromatography with single-quadrupole mass spectrometric detection. Oral fluid specimens were confirmed for the presence of THCA using two-dimensional gas chromatography–mass spectrometry in order to achieve the low concentration levels previously reported to be present in oral fluid. The extraction efficiency for THCA from the oral fluid collection pad was determined to be 80% at a concentration of 10 pg/mL with a coefficient of variation of 8.23%. The intraday precision of the assay ranged from 3.4% to 7.9% over four concentrations; the interday precision ranged from 8.3 to 17%. The limit of quantitation was 2 pg/mL. The method was applied to oral fluid specimens collected from a frequent user of marijuana. Samples were collected almost immediately after the subject smoked and then at intervals of 15 and 45 min and 1, 2, and 8 h after smoking. THCA was present in all the specimens, even the initial specimen taken almost immediately. The presence of THCA minimizes the argument for passive exposure to marijuana in drug-testing cases.

Introduction

Tetrahydrocannabinol (THC) is the active ingredient in marijuana and is generally administered orally or by smoking, resulting in euphoria and hallucinations, and is the main drug detected in oral fluid following marijuana intake (1). In the smoking process, the THC is deposited into the oral cavity, and it appears that fluid from this depository is the main source of the THC collected and measured in oral fluid analysis, rather than drug which has circulated through the body. There are several studies addressing the concentration of THC in the oral fluid of passive smokers (2,3), and even though high concen-

trations of drug from passive situations appear to drop rapidly, the detection of a metabolite such as 11-nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid (THCA) in oral fluid would minimize this defense because its presence is difficult to explain as contamination.

To date, the detection of THCA in oral fluid has been extremely problematic due to the very low levels present. In 2005, Day et al. (4) presented data on the detection of THCA in oral fluid using gas chromatography with tandem mass spectrometry (GC–MS–MS) to achieve the sensitivity required for the detection of THCA in oral fluid. Their procedure was more sensitive than several methods currently in use, providing a better tool for THCA detection. They reported a quantitation limit of 10 pg/mL and concentrations up to 240 pg/mL present in the oral fluid specimens. However, in order to approach the required concentration using single quadrupole mass spectrometric system, in this paper, we employed a micro-fluidic Deans switch two-dimensional GC–GC instrument. The application of two-dimensional (2d) chromatography to forensic toxicological problems was first described in 2003 (5), and coupling of 2d chromatography to MS for the detection of drugs of abuse was reported for the first time in 2004 (6). The authors previously reported the use of this technology for the detection of low amounts of THCA in hair specimens (7) and have currently adapted the assay to the quantitation of THCA in oral fluid.

Materials and Methods

Reagents and consumables

Methanol, toluene, ethyl acetate, hexane, and glacial acetic acid were obtained from Spectrum Chemicals (Gardena, CA). All solvents were high-performance liquid chromatography grade or better and all chemicals were ACS grade. Trace-N (TN-315) solid-phase extraction columns and the positive pressure extraction manifold were purchased from SPEWare (San Pedro, CA). The derivatizing agents, trifluoroacetic anhydride (TFAA) was purchased from Pierce Chemical Company (Rockford, IL),

* Author to whom correspondence should be addressed: Christine Moore, Ph.D. E-mail: cmmuk@yahoo.com.

and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) was purchased from Campbell Science (Rockton, IL). GC columns were obtained from J&W Scientific (Palo Alto, CA).

Standards

Tri-deuterated THCA, which was used as the internal standard (100 mg/mL in methanol), as well as unlabelled drug (1 mg/mL in methanol) were obtained from Cerilliant, (Round Rock, TX). The internal standard concentration for THCA-d₃ was 50 pg/mL of oral fluid.

Extraction efficiency from the Quantisal device

The Quantisal collection device consists of a pad that is placed into the mouth, and a blue adequacy line becomes visible when 1 mL (\pm 10%) of oral fluid has been collected (Figure 1A). The cellulose pad affixed to a polypropylene stem is placed under the tongue of an individual until a defined volume is collected. The pad is then placed in a non-azide preservative stabilization buffer (3 mL) and capped; the specimen is then sent to the laboratory for analysis (Figure 1B). The buffer causes the amount of oral fluid in the testing sample to be diluted (1 mL oral fluid + 3 mL buffer).

At the laboratory, the buffer is directly assayed using ELISA screening technology according to the manufacturer's instructions (Immunoanalysis Corporation, Pomona, CA).

Previously, the authors reported the extraction efficiency of THC from the collection pad into the oral fluid transportation buffer and the stability of the drug. THC was extracted from the collection pad and buffer with an average efficiency over 80% and was stable in Quantisal buffer when stored at refrigerated temperatures. Fluorescent lighting caused losses of over 50% and when stored with the serum separators in place more than 60% of THC was lost over a period of 14 days (8).

In a more recent study, the extraction efficiency of the Quantisal collection system was determined for several drugs including THC by an independent research group. The extraction efficiency of the buffer was reported to be between 79.6 and 91.4% for various concentrations of THC (9).

In order to assess the extraction efficiency of the metabolite THCA from the pad, six solutions of THCA at a concentration of 40 pg (equivalent to 10 pg/mL) in Quantisal buffer were prepared. Collection pads were placed into the buffer, sealed, and allowed to remain overnight at room temperature. Two control devices containing only THCA at a concentration of 10 pg/mL

with no pad were also set up in order to assess losses due to absorption on the pad. The following day, 1 mL of oral fluid + buffer was extracted according to the protocol described.

Precision of the assay

The precision of the assay was determined by analyzing five oral fluid specimens containing THCA at a concentration of 2, 5, 10, and 20 pg/mL on the same day (intraday precision) and on different days (interday precision; $n = 5$). The variable of potential THCA loss on the pad was removed by adding THCA directly to the Quantisal buffer prior to extraction.

Extraction procedure

A calibration curve, oral fluid specimens and drug free negative controls were included in every batch. The drug-free controls were prepared using synthetic oral fluid and diluted in the Quantisal buffer to simulate extraction matrix effects. The internal standard, THCA-d₃ (50 pg/mL), was added to each specimen (1 mL). For the calibration curve, unlabelled THCA was added to the oral fluid at concentrations of 2, 5, 10, 20, 40, and 80 and 160 pg/mL of oral fluid respectively. Trace-N (TN-315, SPEWare) were conditioned with methanol (0.5 mL) and 0.1M acetic acid (0.1 mL). The acidified samples were loaded onto the respective columns and allowed to dry. The columns were washed with deionized water/acetic acid (80:20 v/v; 1 mL) then deionized water/methanol (40:60 v/v 1 mL). The columns were allowed to dry for 5 min. The THCA was eluted with hexane/acetic acid (98:2, 1 mL) into silanized glass tubes. The entire extraction procedure was carried out using a positive pressure manifold, which allows the flowrate through the columns to be highly uniform. The eluent was evaporated to dryness under nitrogen at 40°C, and reconstituted in TFAA (50 mL), and HFIP (30 mL). The mixture was transferred to autosampler vials with glass inserts and capped. The vials were heated at 70°C for 15 min, then left at room temperature for 10 min. Finally, the extracts were evaporated to dryness in a vacuum oven. The samples were finally reconstituted in toluene (25 mL), for injection into the GC-MS system.

Confirmation assay

The use of two serial GC columns to separate background from the required peak is established technology. The two columns function optimally when the stationary phases are as different as possible. Once the analyte retention time on the first column had been determined, the pressure switch (Deans switch) was turned on at that time to divert the flow, and turned off 0.4 min later, diverting a narrow "window" of the effluent from the first column that contains the analyte and minimal background. A cryogenic focuser was incorporated around the analytical column to allow for peak sharpening and improved detection limits. Details of the development of this assay were published previously (7).

Briefly, the primary column was a DB-35 MS column (30 m \times 0.25-mm i.d., 0.25- μ m film thickness), the inlet pressure was 32.63 psi for 6.6 min and the pressure was lowered to 0.5 psi allowing an average velocity of 64 cm/s. The secondary column was a DB-1 stationary phase (15 m \times 0.25 mm i.d., 0.25- μ m film thickness) operating at a flowrate of 2 mL/min. The

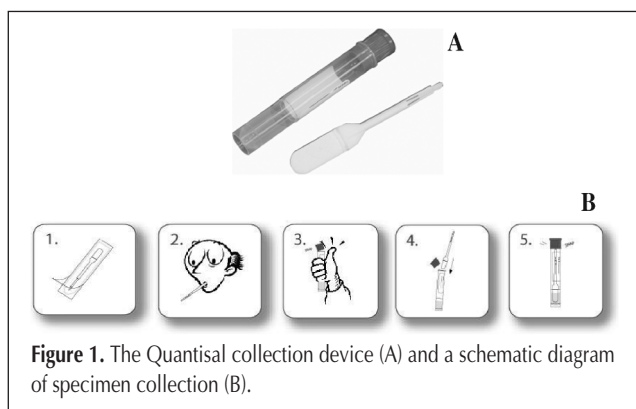


Figure 1. The Quantisal collection device (A) and a schematic diagram of specimen collection (B).

Deans Switch started at a pressure of 15.2 psi, and was lowered to 10 psi for 5 min. The pressure was then raised to 19.17 psi at a rate of 20 psi/min where it remained for 1.1 min. The pressure was lowered to 2 psi after the flow to the analytical column had occurred. The valve operating the Deans switch was turned on after 6.25 min for 0.25 min. At 6.5 min, the flow was returned to the FID vent. The cryofocuser was cooled from the oven temperature of 280°C to 100°C after 5.5 min where it was held at 100°C for 1.5 min. At 7.0 min, the focuser was heated to a final temperature of 280°C. The front inlet was operated in pulsed splitless mode at an initial temperature of 220°C. The pressure was 32.63 psi and the pulse time was 1 min. The oven was programmed from 150°C for 0.5 min; ramped at 50°C/min to 230°C; ramped at 10°C to 275°C, then cooled at 120°C/min to 200°C where it was held for 1 min. The oven was then heated at 10°C/min to 275°C, lowered to 200°C at 120°C/min where it was held for 1 min and finally ramped to 240°C at 10°C/min. The postrun temperature was raised to 320°C for a total run time of 12.23 min. The MS was operated in the electron capture

chemical ionization (ECCI) mode using ammonia as the reagent gas. The MS ion source was held at 150°C, the quadrupole at 106°C, the transfer line at 280°C, operated at 800eV over tune. The flow of the ammonia into the source was maintained between 7.0×10^{-5} and 1.0×10^{-4} Torr. The MSD was operated in selected ion monitoring mode with 4 ions in a single group. Ions 593.1 and 425.1 were monitored for THCA-d₃ and 590.1 and 422.1 for THCA with a dwell time of 50 ms for each ion. The retention time of THCA was 11.33 min. A chromatogram from an oral fluid extract at a concentration level of 5 pg/mL is shown in Figure 2.

Results and Discussion

Method validation

The analytical procedure described details the determination of THCA in oral fluid specimens with a limit of quantitation of 2 pg/mL. The extraction efficiency from the pad was 80% ($n = 6$), and the intraday and interday precisions of the assay at 10 pg/mL were 3.7% and 15.7%, respectively. The intraday precision did not exceed 10% at any point of the assay; the interday did not exceed 20%. The precision improved as the concentration increased. The validation data are shown in Tables I and II.

Interference studies revealed that cocaine, norcocaine, co-cathylene, benzoylecgonine, methamphetamine, amphetamine, MDMA, MDA, morphine, codeine, 6-acetylmorphine, hydrocodone, hydromorphone, phencyclidine, cannabiol, THC, 11-hydroxy-THC, and cannabidiol did not interfere with the assay when injected at concentrations higher than 10 ng/mL. The correlation coefficient was 0.999 for the calibration curve, and the upper limit of linearity was 160 pg/mL.

Authentic specimens

The method was applied to oral fluid specimens collected from a frequent user of marijuana. The subject, a 46-year-old male (210 lbs) who has been a marijuana smoker for over 20 years and smokes at least every other day, willingly consented to sample collection. In the presence of one of the authors, samples were collected almost immediately after the subject smoked and then at intervals of 15 and 45 min and 1, 2, and 8 h after smoking. THCA was present in all the specimens, even the ini-

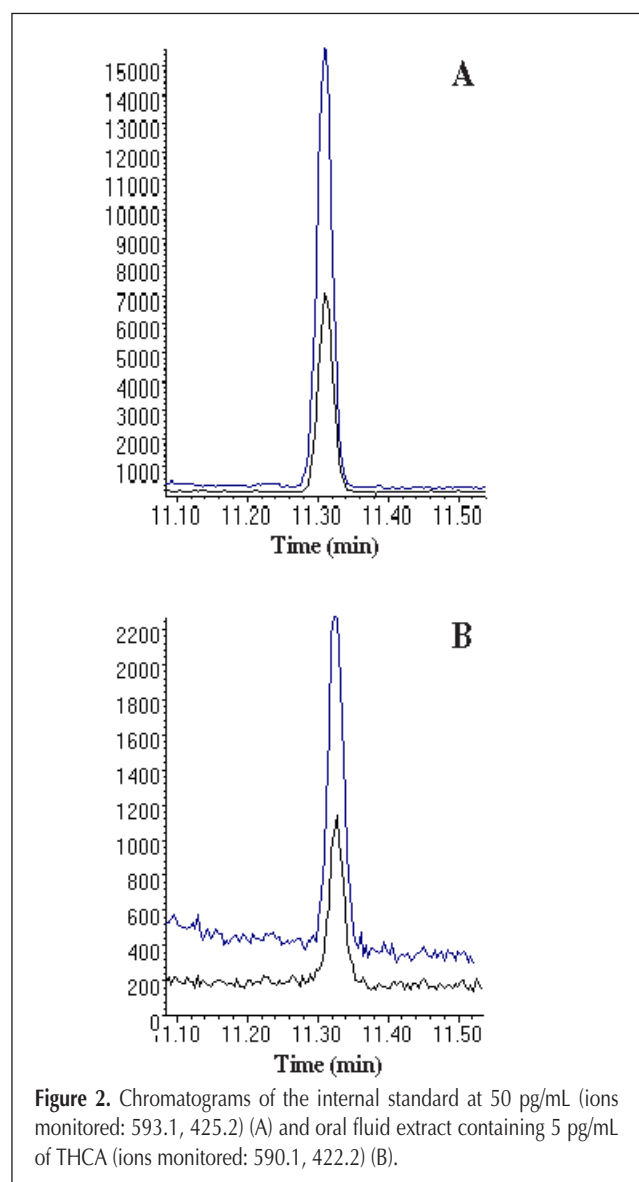


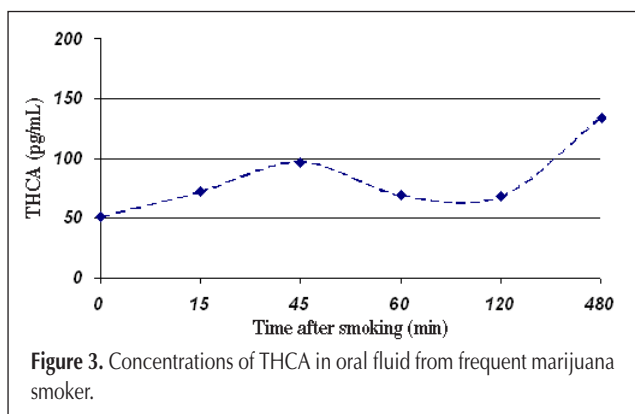
Figure 2. Chromatograms of the internal standard at 50 pg/mL (ions monitored: 593.1, 425.2) (A) and oral fluid extract containing 5 pg/mL of THCA (ions monitored: 590.1, 422.2) (B).

Table I. Extraction Efficiency from the Pad

Pad	Target Concentration (pg)	Recovery (%)
	40 (10 pg/mL)	100
1	8.3	83
2	7.65	76.5
3	6.96	69.6
4	8.92	89.2
5	8.22	82.2
6	8.27	82.7
	Mean	80.0
	SD	6.59
	CV (%)	8.23

Table II. Intraday and Interday Precision

Replicate/Day	Target Concentration							
	2 pg/mL		5 pg/mL		10 pg/mL		20 pg/mL	
	Intra	Inter	Intra	Inter	Intra	Inter	Intra	Inter
1	1.9	2.2	5.1	5.6	9.4	9.8	23.6	23.6
2	2.2	1.4	5.7	3.5	10	7.3	22.9	24.1
3	2.2	1.8	5.8	4.1	9.2	7.4	23.9	21.6
4	2.0	1.9	5.6	4.5	9.8	9.7	22.7	20.1
5	2.1	1.6	4.8	3.9	10	7.4	21.9	20.4
Mean	2.0	1.7	5.4	4.3	9.6	8.3	23	21.9
SD	0.13	0.3	0.43	0.8	0.36	1.3	0.78	1.8
CV (%)	6.2	17.0	7.9	18.5	3.7	15.7	3.4	8.3

**Figure 3.** Concentrations of THCA in oral fluid from frequent marijuana smoker.

tial specimen taken almost immediately after smoking (51 pg/mL). After 15 min, the concentration was 72 pg/mL; after 45 min, it was 97 pg/mL. After 1 h, the level declined slightly to 69 pg/mL and remained there for the next hour (68 pg/mL after 120 min). Interestingly, after 8 h, the THCA concentration detected was 134 pg/mL (Figure 3). The subject did not smoke in the interim 8 h, suggesting that the increase may be reflective of deposition of THCA in the blood. This observation further suggests that THCA is a potential long-term marker of marijuana use because it is unlikely to come from oral contamination as is often suggested for parent THC. The subject reported smoking marijuana the day before the specimens were taken.

Conclusions

The detection of the marijuana metabolite THCA in oral fluid

is described. The collection pad releases 80% of THCA into the transportation buffer, and the procedure allows the identification of very low amounts of THCA in oral fluid using 2d chromatography and single-quadrupole MS. The method was applied to the analysis of specimens collected from a frequent marijuana user. The inclusion of THCA in the confirmation profile minimizes the argument for passive contamination of the oral cavity, and enhances long-term detection of marijuana use. The procedure can be applied in controlled studies for the determination of marijuana pharmacokinetic profiles in oral fluid.

References

1. M.A. Huestis and E.J. Cone. Relationship of Δ^9 -tetrahydrocannabinol concentrations in oral fluid and plasma after controlled administration of smoked cannabis. *J. Anal. Toxicol.* **28(6)**: 394–399 (2004).
2. S. Niedbala, K. Kardos, S. Salamone, D. Fritch, M. Bronsgeest, and E.J. Cone. Passive cannabis smoke exposure and oral fluid testing. *J. Anal. Toxicol.* **28(7)**: 546–552 (2004).
3. R.S. Niedbala, K.W. Kardos, D.F. Fritch, K.P. Kunsman, K.A. Blum, G.A. Newland, J. Waga, L. Kurtz, M. Bronsgeest, and E.J. Cone. Passive cannabis smoke exposure and oral fluid testing. II. Two studies of extreme cannabis smoke exposure in a motor vehicle. *J. Anal. Toxicol.* **29(7)**: 607–615 (2005).
4. D. Day, D. Kuntz, and M. Feldman. THCA detection in oral fluid down to 10 pg/mL. Presented at Society of Forensic Toxicologists Annual Meeting, Nashville, TN, 2005.
5. A.J. Kueh, P.J. Marriott, P.M. Wynne, and J.H. Vine. Application of comprehensive two-dimensional gas chromatography to drugs analysis in doping control. *J. Chromatogr. A* **1000(1-2)**: 109–124 (2003).
6. S.M. Song, P. Marriott, and P. Wynne. Comprehensive two-dimensional gas chromatography-quadrupole mass spectrometric analysis of drugs. *J. Chromatogr. A* **1058(1-2)**: 223–232 (2004).
7. C. Moore, S. Rana, C. Coulter, F. Feyerherm, and H. Prest. Application of two-dimensional gas chromatography with electron capture chemical ionization mass spectrometry to the detection of 11-nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid (THC-COOH) in hair. *J. Anal. Toxicol.* **30(3)**: 171–177 (2006).
8. C. Moore, M. Vincent, S. Rana, C. Coulter, A. Agrawal, and J. Soares. Stability of Δ^9 -tetrahydrocannabinol (THC) in oral fluid using the Quantisal™ collection device. *Forensic Sci. Int.*, in press.
9. O. Quintela and D. Crouch. Recovery of drugs of abuse from the Immunalysis Quantisal™ oral fluid collection device. *J. Anal. Toxicol.*, in press.

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