Detection of Cannabinoids in Oral Fluid Using Inert Source GC/MS

Application

Forensic Toxicology

Authors

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Abstract

Oral fluid is being considered as an alternative to urine in many forensic arenas. In general, the concen-tration of drugs in oral fluid is much lower than in urine, so sensitive extraction and analytical procedures are required. Tetrahydrocannabinol (THC) is the active ingre-dient in marijuana. Since it is generally smoked, the constituents of the plant material, as well as the active ingredient, may be present in oral fluid speci-mens collected for the purposes of drug testing. An ana-lytical procedure for the simultaneous determination of the pyrolytic precursor Δ^9-tetrahydrocannabinolic acid A (THCA-A, 2-carboxy-THC), tetrahydrocannabinol (THC), cannabinol (CBN), and cannabidiol (CBD) in human oral fluid specimens using an Agilent 5975 GC/MS with an inert source is presented. The method achieves the required sensitivity for the detection of tetrahydro-cannabinol (THC), cannabinol (CBN), cannabidiol (CBD), and the pyrolytic precursor 2-carboxy-THC in oral fluid specimens taken from a habitual marijuana smoker. While these drugs have been detected in other matrices, the increasing utility of saliva for drug analysis makes development of laboratory procedures necessary and timely.

Introduction

Tetrahydrocannabinol (THC) is the active ingredient in marijuana. Generally, it is administered via smoking. While THC is the main psychoactive ingredient in the marijuana plant, other reports have shown that some of the effects may be in combination with at least one other constituent of the plant, cannabidiol (CBD). Various cannabinoids have been analyzed in plasma, blood, and urine, but their detection in the more esoteric matrices, such as sweat, oral fluid, and hair, has only recently been addressed.

Oral fluid is becoming increasingly popular as a specimen for the detection of drugs at the roadside and in workplace testing. Several publications have reported the presence of THC in saliva using various collection devices. However, the presence of other cannabinoids, such as cannabinol (CBN) and cannabidiol (CBD) in the marijuana plant material, and therefore possibly in the oral fluid sample collected, has not been reported previously and may be of importance for screening and confirmatory assays. Further, Δ^9-tetrahydrocannabinolic acid A (THCA-A, 2-carboxy-THC) is the main pyrolytic precursor to tetrahydrocannabinol. The decarboxy-lation of 2-carboxy-THC to the active THC during smoking converts only approximately 70% of the precursor to the active form, so the potential presence of 2-carboxy-THC in oral fluid specimens was considered. While blood and urine are more commonly used for these test profiles, oral fluid is increasing in popularity as an alternative matrix

due to its ease of collection, difficulty of adulteration, and improving sensitivity of analytical techniques. One of the main issues with the quantitation of drugs in oral fluid is the difficulty of collection in terms of specimen volume. Many of the currently available devices do not give an indication of how much oral fluid is collected, thereby rendering any quantitative results meaningless without further manipulation in the laboratory. Further, devices incorporating a pad or material for the saliva collection do not always indicate how much of each drug is recovered from the pad before analysis, again calling into question any quantitative result. The drug concentration reported is dependent on the collection procedure used.

This work employed Immunalysis Corporation's QUANTISAL oral fluid collection device, which collects a known amount of neat oral fluid. The efficiency of recovery of the drugs from the collection pad into the transportation buffer was determined in order to increase confidence in the quantitative value. The extracts were analyzed using a standard single quadrupole Agilent GC/MS 6890-5975 instrument, with a limit of quantitation of 0.5 ng/mL.

Experimental

Oral Fluid Collection Devices

Quantisal devices for the collection of oral fluid specimens were obtained from Immunalysis Corporation (Pomona, CA). The devices contain a collection pad with a volume adequacy indicator, which turns blue when one milliliter of oral fluid (± 10%) has been collected. The pad is then placed into transport buffer (3 mL), allowing a total specimen volume available for analysis of 4 mL (3 mL buffer + 1 mL oral fluid). This is specifically advantageous in cases where the specimen is positive for more than one drug and the volume of specimen available for analysis may be an issue. The oral fluid concentration is diluted 1:3 when using Quantisal collection devices, and drug concentrations detected were adjusted accordingly. Since 4 mL of specimen is available for analysis, the single quadrupole Agilent GC/MS 6890-5975 instrument is sufficiently sensitive to meet the proposed regulations, using only 1 mL of the total specimen. However, it should be noted that if alternate collection devices that collect much smaller volumes of oral fluid are used, then a Deans switch microfluidic mechanism may need to be used to achieve the necessary sensitivity.

Standards and Reagents

- Tri-deuterated THC for use as an internal standard as well as unlabeled THC, CBN, and CBD were purchased from Cerilliant (Round Rock, TX). 2-carboxy-THC was purchased from Lipomed (Cambridge, MA).
- Trace N 315 solid phase extraction columns were purchased from SPEWare (San Pedro, CA).
- The derivatizing agent, N,O-Bis (trimethylsilyl) trifluoroacetamide + 1% trimethylchlorosilane (BSTFA + 1% TMCS), was from Pierce (Rockford, IL).

Internal Standard Concentration

THC 40 ng/mL

Sample Preparation for Chromatographic Analysis

- 1 mL Quantisal specimen (equivalent to 0.25 mL of oral fluid)
- Add internal standard (40 ng/mL)
- Add 0.1 M sodium acetate buffer (pH 4.5; 1 mL)
- Condition SPE columns: methanol (0.5 mL), 0.1 M acetic acid (0.1 mL)
- Add samples
- Wash columns:
  - Deionized water:0.1 M acetic acid (80:20; 1 mL)
  - Deionized water:methanol (40:60; 1 mL)
- Dry columns under nitrogen (30 psi; 2 min).
- Elute: hexane:glacial acetic acid (98:2; 0.8 mL)
- Evaporate to dryness under nitrogen

GC/MS Conditions

Instrument:	Agilent 6890 GC 5975 MSD; inert source; 220/240V oven
Detection mode:	Electron impact
Column:	DB-5 MS, 0.25 mm id, 0.25-µm film thickness, 15-m length
Injection temperature:	250 °C
Purge flow:	50 mL/min for 1 min
Carrier gas:	Helium
Injection mode:	Splitless
Injection volume:	2 µL
Mode of operation:	Constant flow at 1.5 mL/min
Transfer line:	280 °C
Quadrupole:	150 °C
Ion source:	230 °C
Dwell time:	50 ms
Oven program:	125 °C for 0.5 min; ramp at 40 °C/min to 250 °C; hold 1.3 min ramp at 70 °C/min to 300 °C
Retention times:	Deuterated THC: 4.27 min; THC 4.28 min; cannabidiol 3.88 min; cannabinol 4.61 min; 2-c-THC 5.66 min

Ions Monitored

Drug	Ions monitored
THC	Deuterated (d3) **374.3**, 389.3; Unlabeled THC **371.2**, 386.2, 303.1
CBN	**367.3**, 382.2, 310.1
CBD	**390.1**; 301.2
2-carboxy-THC	**487.3**, 488.2, 489.2

Quantitative ions in bold type

Derivatization

Reconstitute in ethyl acetate (30 µL); add BSTFA +1% TMCS (20 µL); transfer to autosampler vials; cap; incubate (60 °C/15 min).

Results and Discussion

One of the issues associated with oral fluid analysis is recovery of drug from a collection pad if a device is used. Extraction efficiency of the collection system for these drugs was determined. Six synthetic oral fluid specimens fortified with all the cannabinoids at a concentration of 4 ng/mL were prepared. The collection pad was placed into the samples until 1 mL had been collected, as evidenced by the blue volume adequacy indicator incorporated into the stem of the collector. The pad was then transferred to the Quantisal buffer, capped, and stored overnight to simulate transportation to the laboratory. The following day, the pads were removed with a serum separator, and an aliquot of the specimen was analyzed as described. The amount recovered from the pad was compared to an absolute concentration (100%) where drug was added to the buffer and left overnight at room temperature without the pad, then subjected to extraction and analysis.

	THC	CBD	CBN	2-carboxy-THC
Mean drug recovery (%)	89.2 ± 9.0	71.9 ± 19.1	79.7 ± 7.8	78.2 ± 11.8

GC/MS Method Verification

The analytical methods reproducibility were verified according to standard protocols, whereby the limit of quanti-tation, linearity range, correlation, and intra- and inter-day precision were determined via multiple replicates (n = 6) over a period of four days.

Analyte	LOQ (ng/mL)	Linear equation	Correlation r^2	Ion ratio range (%)
THC	0.5	y = 0.0266x + 0.00273	0.998	386/371:69.7–104.5 303/371:44.0–66.0
CBN	0.5	y = 0.138x + 0.0022	0.999	382/367:7.4–11.2 310/367:5.7–8.5
CBD	1	y = 0.0271x + 0.00178	0.998	301/390:17.1–25.7
2-carboxy-THC	1	y = 0.0571x + 0.0195	0.998	488/487:31.7–47.5 489/487:11.0–16.6

	THC CV (%)		CBN CV (%)		CBD CV (%)		2-c-THC CV (%)	
Concentration	**Intra**	**Inter**	**Intra**	**Inter**	**Intra**	**Inter**	**Intra**	**Inter**
1 ng/mL	0	4.8	5.26	15.3	7.07	6.08	5.73	15.2
2 ng/mL	0	2.53	2.21	2.41	2.82	3.12	10.3	8.3
4 ng/mL	1.39	1.46	5.96	4.20	4.08	4.52	7.03	8.5
8 ng/mL	0.68	1.77	4.66	5.58	1.66	6.84	2.99	2.25

Precision: Inter-day (n = 4) and intra-day (n = 6) precision for the determination of cannabinoids in oral fluid.

Specificity: Commonly encountered drugs were extracted and analyzed at high concentrations and found not to interfere with the assays.

Authentic Specimens

The method was applied to specimens taken from an authentic user. The subject, a 46-year-old male willingly consented to sample collection; he had been a marijuana smoker for over 20 years. For the purpose of this study, he remained marijuana free for five days before smoking. The initial specimen was negative for the four cannabinoids. Samples were collected almost immediately after the subject smoked (5 min), then at intervals of 30 minutes and 1, 2, 12, 24, 36, and 48 hours after smoking. Parent THC was detectable at concentrations well above over 2000 ng/mL in the 5-minute and 30-minute samples, apparently due to excessive oral cavity contamination by THC. The parent drug was detected for 24 hours, and 2-carboxy-THC was identified for up to 16 hours after intake. Cannabidiol was detected only in the specimens from 5 minutes and 30 minutes after smoking and at a concentration of 5 ng/mL. Cannabinol was measurable for only 2 hours (Figure 1).

An extracted ion chromatogram of the sample collected 1 hour after smoking is presented in Figure 2. The extracted ions for cannabidiol were not included since there was no CBD present in the specimen.

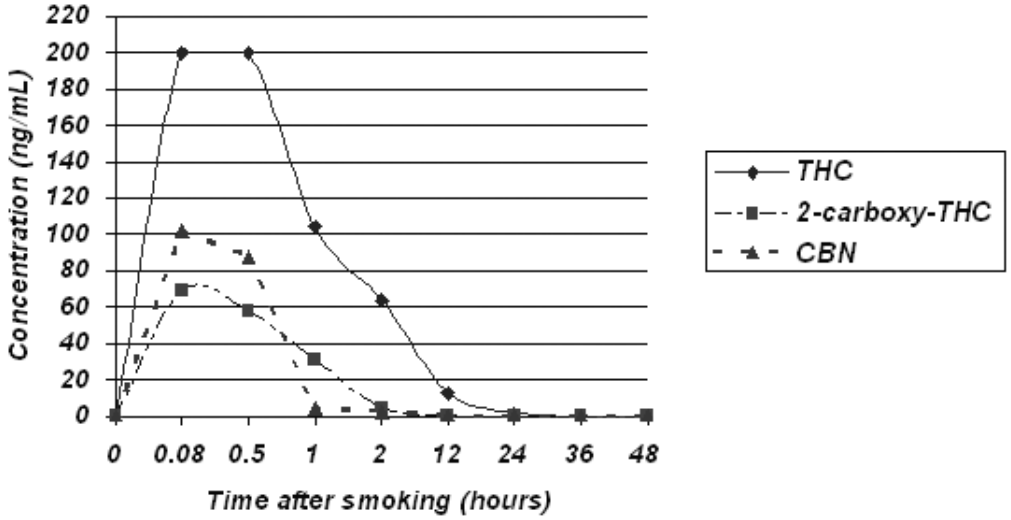

Figure 1. Cannabinoids in oral fluid following marijuana smoking.

Conclusions

The procedure described is suitable for the routine detection and confirmation of THC, CBN, and 2-carboxy-THC in oral fluid using the Quantisal oral fluid collection device and an Agilent single quadrupole GC/MSD.

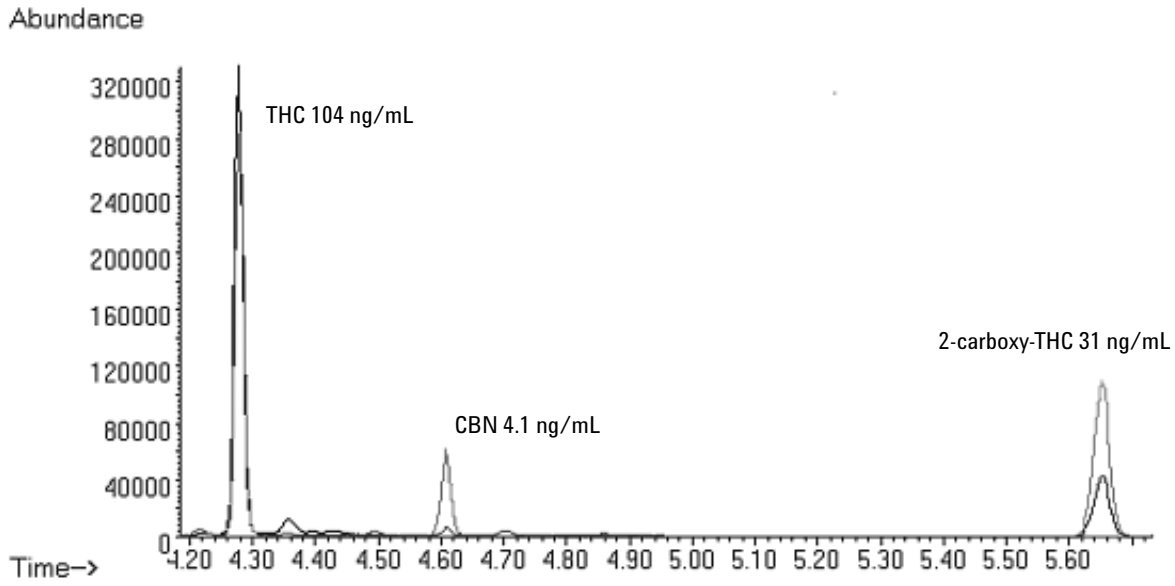

Figure 2. Oral fluid specimen collected 1 hour after marijuana smoking.

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