Application Note

Forensics,
Workplace Drug Testing

Detection of Cannabinoids in Oral Fluid with the Agilent 7010 GC-MS/MS System

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Abstract

A fast, simple, and robust method for the quantification of Δ^9-tetrahydrocannabinol (THC), metabolites of THC, and two other cannabinoids in small volumes of oral fluid was developed using the highly sensitive Agilent 7010 GC-MS/MS system. After SPE extraction, the cannabinoids were derivatized using a simple silylating reagent, and analyzed in less than 10 minutes. Limits of quantitation were determined as 0.20 ng/mL for THC, 11-hydroxy-tetrahydrocannabinol (OH-THC), cannabinol (CBN), and cannabidiol (CBD), and 0.015 ng/mL for 11-nor-Δ^9-carboxytetrahydrocannabinol (THCA). This method can easily be implemented and integrated into forensic and workplace drug testing laboratories.

Introduction

In forensics and workplace drug testing environments, the identification and quantification of cannabinoids introduced through the ingestion or pyrolysis of marijuana (*Cannabis sativa*) typically uses biological samples such as urine, blood, hair, umbilical cord blood, and meconium. Oral fluid has recently become a popular alternative to these due to its noninvasive, ease-of-collection nature. Δ^9-tetrahydrocannabinol (THC) is the major psychoactive component of marijuana and the primary target of these analyses. However, there are more than 60 cannabinoids synthesized by *C. sativa*, and THC undergoes phase I metabolism to form 11-hydroxy-tetrahydrocannabinol (OH-THC), which is further oxidized to 11-nor-Δ^9-carboxytetrahydrocannabinol (THCA). These metabolites, and two other minor metabolites, then undergo phase II conjugation to form glucuronides[1,2]. In addition to THC, common cannabinoid targets of these assays include cannabidiol (CBD), cannabinol (CBN), OH-THC, and THCA.

Low-level detection of cannabinoids in oral fluid typically requires chemical derivatization with alkylating and acylating reagents, and analysis using electron capture negative chemical ionization gas chromatography-tandem mass spectrometry (GC-NCI-MS/MS). A caveat to these methods is that exposure to acidic conditions can oxidize THC to CBN (Heustis, 2007), and potentially form tetrahydrocannabinolic acid (THC-COOH), an immediate precursor to THC that, under pyrolytic conditions, decarboxylates to THC. Although these methodologies have been greatly improved over the years and made much simpler to implement, many laboratories do not have access to this type of instrumentation or would rather use simpler derivatization chemistries with a GC/MS system in electron ionization (EI) mode. To address these concerns, we set out to develop a simple and robust silylization procedure and analytical method by which the resulting cannabinoid analogues can be analyzed in EI mode and still elicit very low levels of detection from small sample volumes of oral fluid.

Experimental

Chemicals and Reagents

All solvents and gasses used in this study were ultra-high purity grade. The target analytes were THC, CBD, CBN, OH-THC, and THCA (Cerilliant, Round Rock, TX). Stable isotopically labeled internal standards THC-d3, CBD-d3, CBN-d3, OH-THC-d3 (Cambridge Isotopes, Tewksbury, MA) and THCA-d9 (Cayman Chemicals, Ann Arbor, MI) were added at a constant concentration across all samples. The derivatization reagent was N-*tert*-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA Sigma-Aldrich, St. Louis, MO).

GC/MS Conditions

Parameter	Value
GC conditions	
Instrument	Agilent 7890B GC combined with an Agilent 7010 GC-MS/MS system equipped with a Multi-Mode Inlet (MMI) in solvent vent mode
MMI temperature program	50 °C for 0.5 minutes, then 600 °C/min to 250 °C
Purge flow to split vent	100 mL/min at 1.5 minutes
Vent flow	100 mL/min
Vent pressure	0.99999 psi until 0.2 minutes
Injection volume	5 µL
GC column	Agilent DB-5ms, 15 m × 250 µm, 0.25 µm (p/n 122-5512)
Carrier gas	Helium
Gas flow	1.2 mL/min
Oven program	80 °C for 0.5 minutes, then 40 °C/min to 250 °C (0 minutes), then 10 °C/min to 300 °C (0 minutes)
Transfer line temperature	280 °C
MS Conditions	
High-efficiency source mode	EI
Source temperature	280 °C
Quadrupole temperatures	150 °C each
Quench gas	Helium, 4.0 mL/min
Collision gas	Nitrogen, 1.5 mL/min
Quadrupole resolution	1.2 amu
Solvent delay	6 minutes

Sample Preparation

All samples were prepared in 200 µL of cannabinoid-free oral fluid. Calibrators and limit of quantitation (LOQ) standards were prepared through standard addition of the appropriate amounts of each cannabinoid and labeled internal standard over a concentration range of 0.2–50 ng/mL for THC, CBD, CBN, and OH-THC. A calibrator concentration range of 15–3,750 pg/mL was prepared for THCA. Samples were extracted using a modification of an extraction procedure[1,3].

A 50 µL volume of MTBSTFA derivatization reagent was added to each dry extract, resulting in a 4-fold concentrating factor of each sample. The vials were capped and heated to drive the reaction, as described elsewhere[4], then cooled prior to injection on the GC-MS/MS system.

Table 1 gives the MRM transitions and parameters for each measured cannabinoid. All data were collected and analyzed using Agilent MassHunter software.

Table 1. MRM Conditions.

Time segment	Start time (min)	Compound	ISTD	Precursor ion	Product ion	Dwell (ms)	Collision energy	Gain
1	6.00	THC-d3	True	431	416	50	20	1
1	6.00	THC-d3	True	431	374	50	20	1
1	6.00	THC	False	428	413	50	20	1
1	6.00	THC	False	428	371	50	20	1
1	6.00	THC	False	428	289	50	30	1
2	6.42	CBD-d3	True	477	420	50	10	1
2	6.42	CBD-d3	True	477	346	50	20	1
2	6.42	CBD	False	474	417	50	10	1
2	6.42	CBD	False	474	343	50	20	1
2	6.42	CBD	False	474	273	50	30	1
3	6.64	CBN-d3	True	412	396	50	45	1
3	6.64	CBN-d3	True	412	340	50	45	1
3	6.64	CBN	False	409	393	50	45	1
3	6.64	CBN	False	409	337	50	45	1
3	6.64	CBN	False	409	281	50	50	1
4	7.50	CH-THC-d3	True	416	350	50	30	1
4	7.50	CH-THC-d3	True	416	334	50	30	1
4	7.50	CH-THC	False	413	347	50	30	1
4	7.50	CH-THC	False	413	331	50	30	1
4	7.50	CH-THC	False	413	289	50	40	1
5	8.70	THCA	False	571	413	30	30	100
5	8.70	THCA	False	571	297	30	40	100
5	8.70	THCA-d9	True	524	406	30	30	100
5	8.70	THCA-d9	True	524	364	30	30	100
5	8.70	THCA	False	515	471	30	30	100
5	8.70	THCA	False	515	397	30	30	100
5	8.70	THCA	False	515	355	30	30	100

Results and Discussion

THC, CBD, CBN, OH-THC, and THCA were linear over 2.5 orders of magnitude in concentration and signal-to-noise ratios (S/N, peak-to-peak) of 261:1, 69:1, 9:1, and 547:1 at 0.20 ng/mL, respectively, and 30:1 for THCA at 0.015 ng/mL were determined. Table 2 illustrates the resulting quantitative linear parameters, LOQ, and the theoretical limit of detection (LOD) in pg/mL using an S/N of 3 for each cannabinoid. For comparison, using polyfluorinated alkylating and acylating derivatization reagents and GC-NCI-MS/MS with Deans Switch, an LOQ of 0.01 pg/mL for THCA can be achieved from 20 mg of hair and 50 pg/g THCA from 1 g of cord blood or meconium. Table 3 illustrates these results when normalized for extraction and concentrating factors in fg/µL injected on-column.

Table 2. Quantitative results determined in Agilent MassHunter Quantitative software revision B.08.00.

Analyte	Theoretical LOD (pg/mL)	LOQ (ng/mL)	Linear equation	R^2	Qualifier ion ratio range (%)
THC	2.30	0.20	y = 0.184x + 0.0131	0.999	15.8–29.4
CBD	8.70	0.20	y = 0.186x + 0.0239	0.992	37.2–55.8
CBN	66.67	0.20	y = 0.0757x + 0.0478	0.997	66.4–100.0
CH-THC	1.10	0.20	y = 0.0189x + 0.0103	0.999	55.4–83.0
THCA	1.50	0.015	y = 0.00327x + 0.00429	0.995	35.4–53.2

Table 3. LOQ normalized for extraction and concentrating factors.

Matrix	Sample size	Derivatization	GC/MS Method	Normalized LOQ (fg/µL)	Compound
Hair	20 mg	Pentafluoropropionic anhydride/hexafluoroisopropanol	GC-NCI-MS/MS	4	THCA only
Cord blood	1 g	Pentafluoropropionic anhydride/hexafluoroisopropanol	GC-NCI-MS/MS	1,000	THCA only
Meconium	1 g	Pentafluoropropionic anhydride/hexafluoroisopropanol	GC-NCI-MS/MS	1,000	THCA only
Oral fluid	200 µL	MTBSTFA	GC-EI-MS/MS	60	THCA only
Oral fluid	200 µL	MTBSTFA	GC-EI-MS/MS	800	THC, CBD, CBN, CH-THC

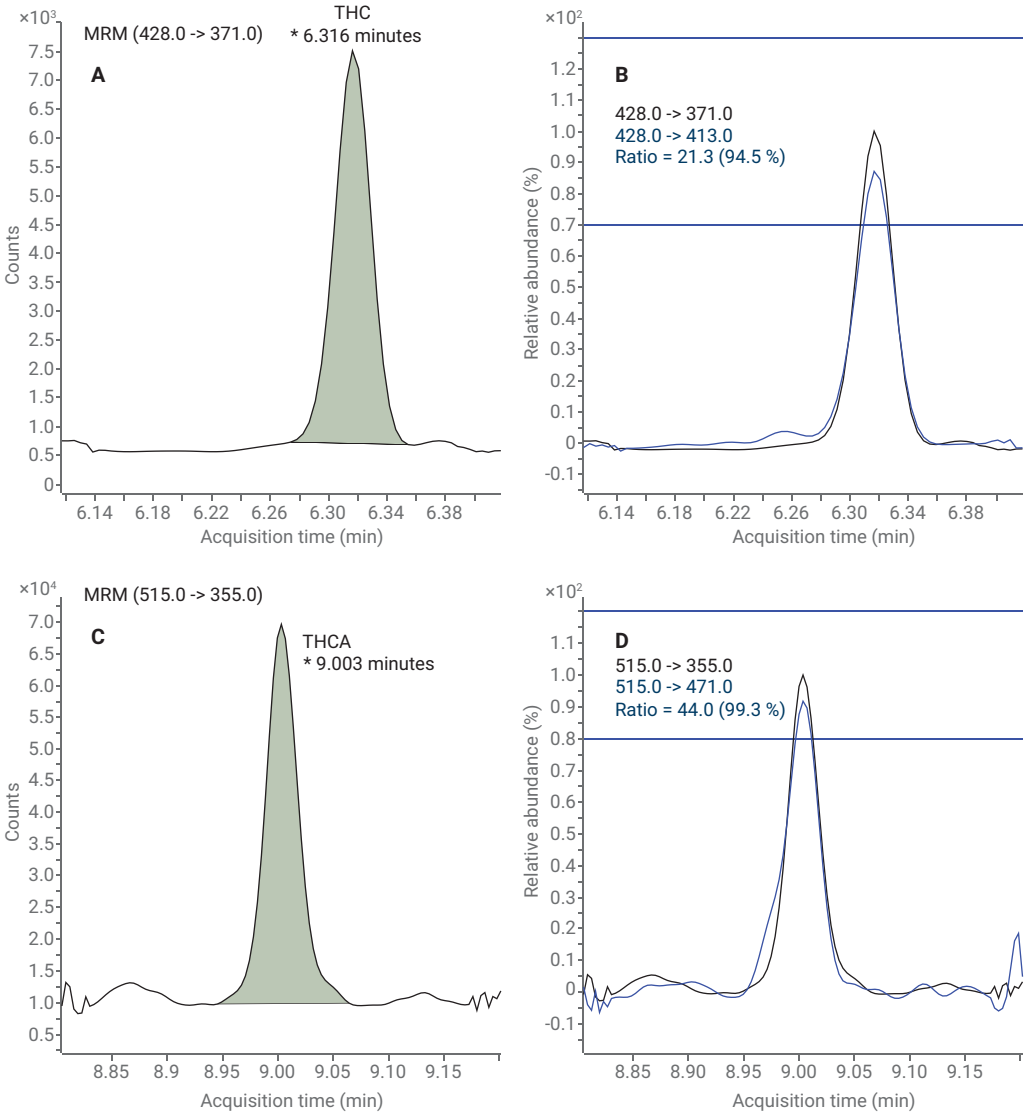

Figure 1. MRM chromatograms of: THC quantifying ion (A), THC qualifying ion (B), THCA quantifying ion (C), and 11-NOR-THCA qualifying ion (D).

Conclusion

Considering recent legislation at the state level in the United States legalizing the use of medicinal and recreational marijuana, there is an increasing need for robust analytical methods that can simultaneously measure psychoactive THC, THC metabolites, and other cannabinoids in easily collected biospecimens such as oral fluid. The LOD and LOQ coupled with the high specificity of MS/MS determined in this study offers excellent specificity and sensitivity for the detection of these cannabinoids in small volumes of oral fluid samples in less than 10 minutes. The simple derivatization procedure described above can be automated using the Agilent 7696 Sample Prep Workbench to increase laboratory productivity. This methodology can offer forensic and workplace drug testing laboratories a reliable procedure for high-throughput environments.

References

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Printed in the USA, October 12, 2017
5991-8553EN

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