Rapid and Robust Detection of THC and Its Metabolites in Blood

Application Note

Forensics/Doping Control

Author

Stephan Baumann

Agilent Technologies, Inc.

Santa Clara CA 95051

USA

Abstract

A robust method for detection of THC and its metabolites in blood has been developed using SPE extraction and GC/MS/MS with backflushing. The dynamic range of quantification was 0.1 to 50 ng/mL for THC and 11-OH-THC, and 1 to 100 ng/mL for THCA, with a run time of 6 minutes and a cycle time of 8 minutes.

Introduction

In the past decade, a great deal of research concerning the impact of cannabis use on road safety has been conducted. More specifically, studies on effects of cannabis smoking on driving performance, as well as epidemiological studies and cannabis-detection techniques have been published. As a result, several countries have adopted driving under the influence of drugs (DUID) legislation, with varying approaches worldwide. While a wide variety of bodily fluids have been used to determine the presence of cannabis, blood testing is considered the most reliable indicator of impairment. Blood testing for active tetrahydrocannabinol (THC) may also be considered by employers who wish to identify employees whose performance may be impaired by their cannabis use. Gas chromatography/mass spectrometry (GC/MS) is a standard method for detection and quantification of THC and its metabolites in blood.

One key to reliable THC testing in blood is an efficient extraction method. The use of tandem MS (MS/MS) also increases the sensitivity and reliability of quantification of THC and its metabolites in blood, due to the elimination of interferences. This application note describes a method using the High Flow Bond Elut Certify II SPE cartridge to rapidly and efficiently extract THC and its metabolites from blood. The extracts were derivatized to improve volatility and analyzed on the Agilent 7890A Triple Quadrupole GC/MS system equipped with a Low Thermal Mass Module (LTM)

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oven and backflushing. It was in turn coupled with an Agilent 7000B Triple Quadrupole GC/MS system, using MS/MS in the multiple reaction monitoring (MRM) mode to provide rapid and sensitive detection of THC and its metabolites, 11-OH-THC (11-hydoxy-Δ9-tetrahydrocannbinol) and THCA (11-nor-Δ9-Tetrahydrocannabinol-9-Carboxylic Acid). Backflushing was used to increase robustness and speed, enabling a run time of 6 minutes and a cycle time of 8 minutes. MRM MS/MS analysis on the Triple Quadrupole GC/MS system delivers excellent results, with a dynamic range of 0.1 to 50 ng/mL.

Experimental

Standards and Reagents

Tri-deuterated THC, 11-OH-THC and THCA, which were used as internal standards (100 µg/mL in methanol), and unlabelled THC, 11-OH-THC and THCA (100 µg/mL in methanol) were obtained from Cerilliant (Round Rock, TX). The internal standard concentrations in the method were both 10 µg/mL.

Methanol, acetonitrile, toluene, ethyl acetate, hexanes, glacial acetic acid, and methylene chloride were obtained from Sigma Aldrich (St. Louis, MO). All solvents were high-performance liquid chromatography (HPLC) grade or better, and all chemicals were ACS grade. Agilent High Flow Bond Elut Certify II solid-phase extraction columns were used for the method. The derivatizing agents, BSTFA (N,O-bis(trimethylsilyl) trifluoroacetamide) and TMCS (trimethylchlorosilane) were purchased from Cerilliant. Normal human whole blood stabilized with potassium oxalate and sodium fluoride was obtained from Bioreclamation (Hicksville, NY). Standards were prepared in this drug-free matrix to construct the calibration curves.

Instruments

The experiments were performed on an Agilent 7890N gas chromatograph equipped with a multimode inlet (MMI) and an LTM oven, coupled to a 7000B Triple Quadrupole GC/MS. Chromatography was performed using a pre-column for backflushing, and a Low Thermal Mass (LTM) column connected by a Purged Ultimate Union (Figure 1). The instrument conditions are listed in Table 1.

a.

Loading the sample on the pre-column

b.

Backflushing the pre-column and separation of THC and its metabolites on the primary column

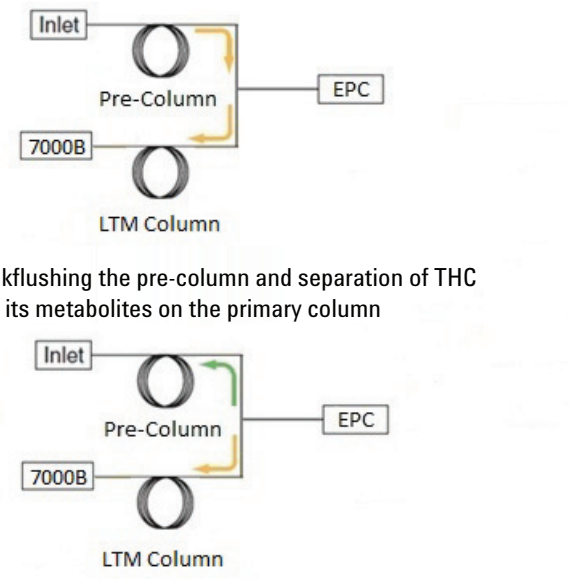


Figure 1. Schematic representation of the backflush system used to develop the method. EPC: Electronic Pneumatic Control module; 7000B: Agilent Triple Quadrupole GC/MS system

Table 1. Agilent 7890N/7000B Gas Chromatograph and Triple Quadrupole Mass Spectrometer Conditions

GC Run Conditions	
Pre-column	1 m section from a 15 m × 0.25 mm, 0.25 µm HP-5 ms Ultra Inert column (p/n 19091S-431UI)
Analytical column	15 m × 0.25 mm, 0.25 µm DB-17 ms LTM Column Module (p/n 122-4712LTM)
Injection volume	1 µL
Inlet temperature	Isothermal at 280 °C
Injection mode	0.5 min pulsed splitless at 35 psi
Oven temperatures	GC oven: 6 min hold at 280 °C (isothermal)
LTM module:	50 second hold at 100 °C 100 °C to 230 °C at 200 °C/min 230 °C to 280 °C at 10 °C/min Hold at 280 °C for 1 min
Carrier gas	Helium in constant pressure mode. Pre-column: 1 psi; Column 1: 5 psi; Column 2: 9.6 psi
Transfer line temp	300 °C
MS Conditions	
Tune	Autotune
Gain	20
Acquisition parameters	EI mode; multiple reaction monitoring (MRM)
Collision gas	Nitrogen constant flow, 1.5 mL/min
Quench gas	Helium, constant flow, 2.25 mL/min
Solvent delay	3.0 min
MS temperatures	Source 230 °C; Quadrupole 150 °C

Sample Preparation

A 2 mL blood sample containing 10 µg/mL of each internal standard (ISTD) and spiked with THC, 11-OH-THC and THCA was pipetted into a clean tube, and 4 mL of acetonitrile was added. After centrifugation at 2500 rpm for 5 minutes, the supernatant was transferred and evaporated to about 3 mL with nitrogen at 35-40 °C, and 7 mL of 0.1 M sodium acetate (pH 6.0) was added.

High Flow Bond Elut Certify II SPE columns were conditioned with 2 mL of methanol, then 2 mL 0.1 M sodium acetate buffer, pH 6.0 with 5% methanol. Cartridges were not be allowed to go to dryness prior to sample addition. The sample was drawn through the column slowly, at 1 to 2 mL/min. The column was then washed 2 mL sodium acetate buffer, pH 6.0, dried under maximum vacuum for approximately 5 minutes, then washed with 1 mL hexanes. THC was eluted under neutral conditions with 2 mL of 95:5 hexane: ethyl acetate. This was followed by a 5 mL 1:1 methanol:deionized water wash. The column was again dried under maximum vacuum for approximately 5 minutes and washed again with 1 mL hexanes. Elution of 11-OH-THC and THCA was performed with 2 mL 1% acetic acid in 75:25 hexane:ethyl acetate. The THC and the metabolite fractions were combined and dried before derivatization.

The eluent was evaporated under nitrogen at a temperature no higher than 40 °C, then reconstituted in 60 µL of toluene and 40 µL of BSTFA, 1% TMCS for derivatization. The sample tubes were capped and heated 20 minutes at 70 °C before injection into the tandem quadrupole GC/MS system.

Analysis Parameters

The Agilent Triple Quadrupole GC/MS system parameters used are shown in Table 2.

Table 2. Agilent 7000B Triple Quadrupole GC/MS System Analysis Parameters

Compound	RT (min)	MRM	Dwell time (ms)	Collision energy (EV)
THC (Δ9-Tetrahydrocannabinol)	3.5	386→303*	25	20
		386→330	27	10
		386→289	30	25
THC-d3	3.5	389→306*	10	20
		389→330	11	10
		389→292	15	25
11-OH-THC (11-hydoxy-Δ9-tetrahydrocannabinol)	4.5	371→289*	24	20
		371→305	26	15
		371→265	27	15
11-OH-THC-d3	4.5	374→292*	10	20
		374→308	12	15
		374→268	12	15
THCA (11-nor-Δ9-Tetrahydrocannabinol-9-Carboxylic Acid)	5.6	371→289*	23	15
		488→297	44	20
		488→371	29	20
THCA-d9	5.5	380→292*	15	15
		497→306	30	20
		497→380	22	20

*Target transition. All other transitions are qualifier transitions.

Results

SPE Sample Preparation with High Flow Bond Elut Certify II Columns

Screening for drugs of abuse in biological fluids requires rugged methods that provide high purification and recovery. The Bond Elut Certify was developed specifically for the rapid and effective extraction of compounds that possess both non-polar and anionic characteristics from urine and other biological matrices [1]. The mixed mode (non-polar C8 and strong anion exchange) sorbent takes advantage of non-polar, polar, and ion exchange properties to ensure rapid, reproducible, simple, and clean extraction of many drug classes. These columns enable the rapid and high recovery of THC, 11-OH-THC and THCA from whole blood.

Backflushing

Backflushing makes this a very robust and rapid method, preventing build-up of high-boiling compounds on the column and thus reducing retention time shifts, peak distortion, and chemical noise, while improving quantification. Contamination of the MS source and the resultant need for cleaning are also reduced, while the run time is shortened. The end result is a robust method that provides excellent dynamic range with 6 minute run times (not including sample prep) and 8 minute cycle times.

The suite of Agilent Capillary Flow Technology modules enables easy and rapid backflushing with minimal dead volumes for maintaining chromatographic resolution. During injection, the inlet Pneumatic Control Module (PCM) is held at an elevated pressure long enough to transfer the target analytes from the pre-column to the analytical column (Figure 1a). When backflushing, the inlet pressure is dropped to 1 psi, forcing the flow to reverse through the pre-column and out the split vent (Figure 1b). In this way, THC, 11-OH-THC and THCA are passed on to the primary column for further separation, while high-boiling compounds are swept back though the split vent.

Low Thermal Mass Modules

This method also employs a Low Thermal Mass (LTM) column module external to the GC oven that enables independent and optimal temperature control of the analytical column (Figure 1). The unique design of these modules makes it possible to employ very fast temperature ramping and rapid cooling. The LTM column modules can be added to an Agilent GC without requiring any changes in the injectors, autosamplers, or detectors.

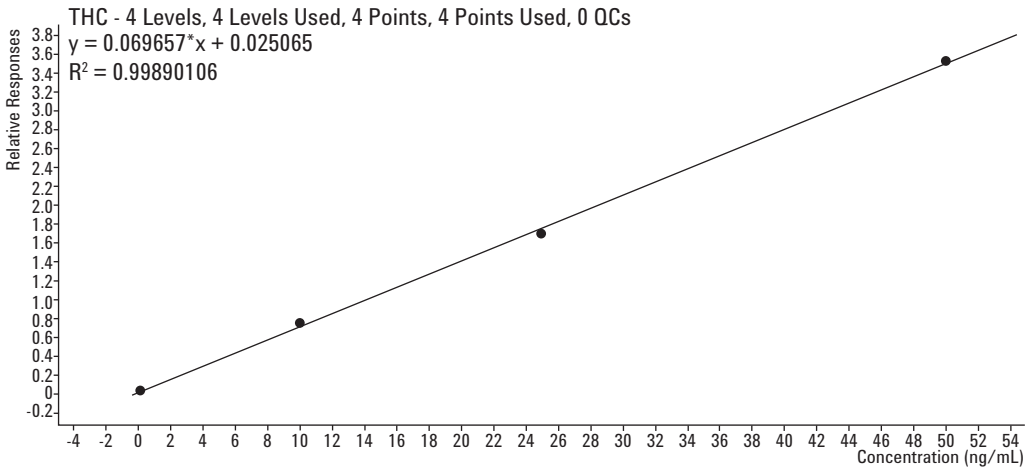
Dynamic Range

This method has a dynamic range of 0.1 to 50 ng/mL for THC and 11-OH-THC, and 1 to 100 ng/mL for THCA (Figure 2), which match industry norms. The accuracy of quantification is also quite good, with an R^2 of 0.999 for all three analytes.

MRM Results

Using a MassHunter forensic report template, Quantitative Analysis Sample Reports were quickly and easily prepared for THC and its two analytes (Figures 3-5), featuring a Total Ion Current (TIC) chromatogram and spectra for all of the transitions, including the internal standard. Note the lack of interference in all of the transitions, even at the lowest end of the dynamic range for each analyte.

a.



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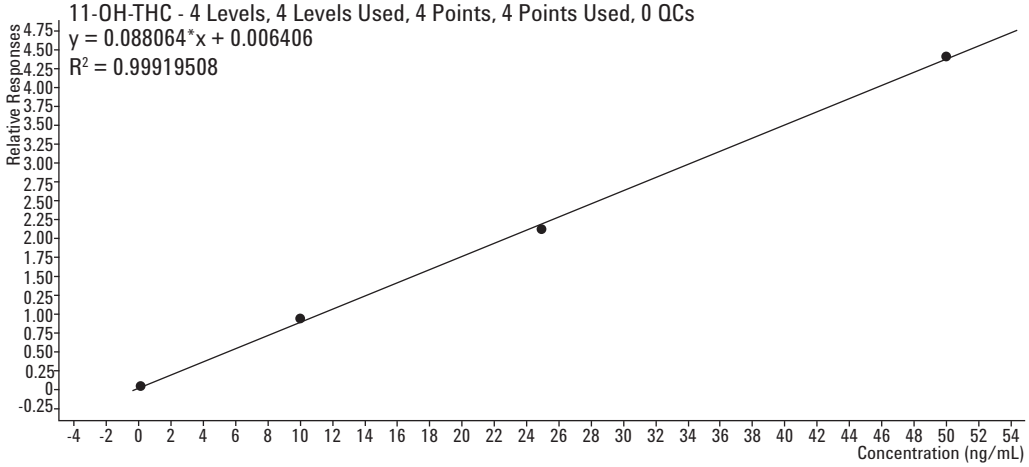

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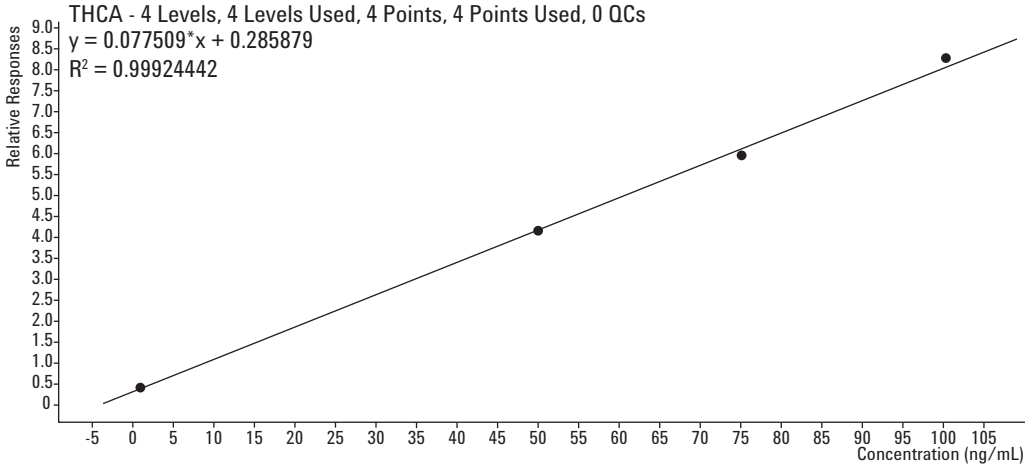
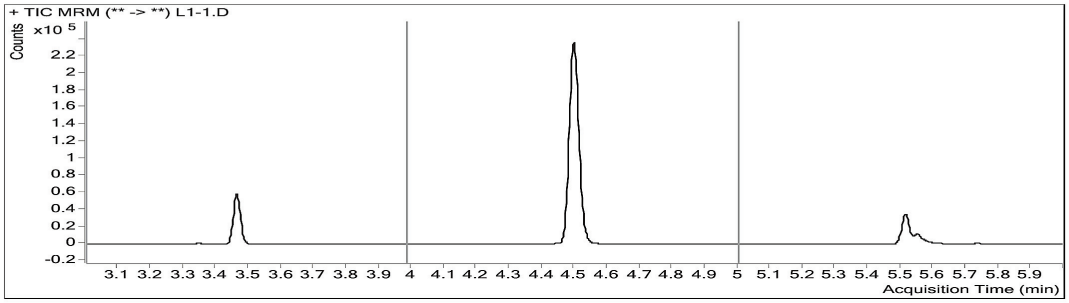


Figure 2. Calibration curves for THC (a), 11-OH-THC (b) and THCA (c) in blood. Data points were taken at 0.1, 10, 25, and 50 ng/mL for THC and 11-OH-THC, and at 1, 50, 75, and 100 ng/mL for THCA.

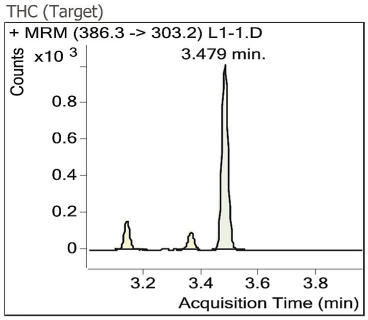
Quantitative Analysis Sample Report

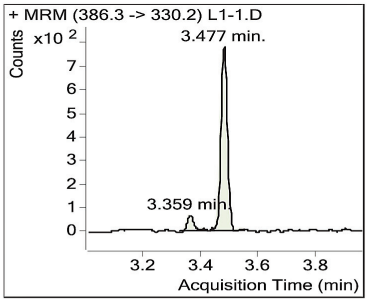
Data File	:	L1-1.D
Operator	:	DATASYSTEM01\Admin
Acq Method Name	:	LTM.THC.OMRM.7000.eiex.m
Acquisition date	:	2011-04-28 18:19
Sample Name & Path	:	L1-1, D:\MassHunter\GCMS\1\data\04-28-2011-redo\
Vial	:	3
Dilution	:	0.0
Sample Info	:	
Last Calib Update	:	2011-05-05 13:57

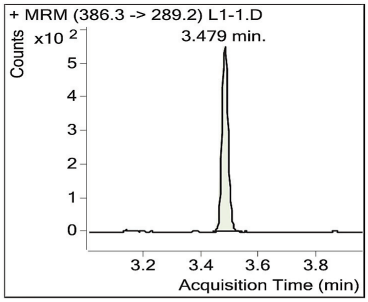
Cmpnd	Signal	RT	Limits	Response	QRatio	Limits	FinalConc
D3-THC	389.3 -> 306.2	3.46		37956			
	389.3 -> 330.2			32853	86.6	64.3-104.3	
	389.3 -> 292.2			18032	47.5	22.8-62.8	
THC	386.3 -> 303.2	3.48	3.29 - 3.64	1554			0.100
	386.3 -> 330.2			1237	79.7	62.4-102.4	
	386.3 -> 289.2			848	54.6	23.6-63.6	



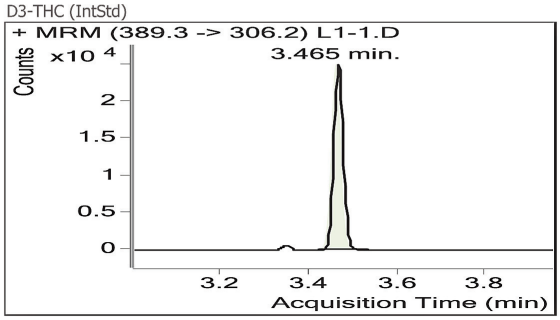
THC (Target)







D3-THC (IntStd)



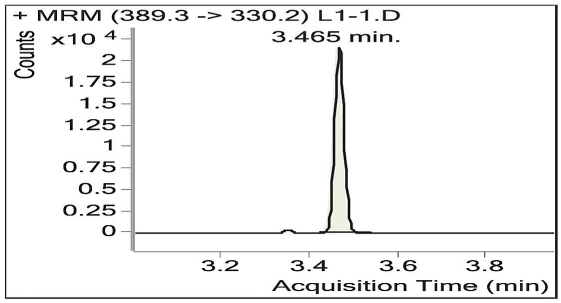
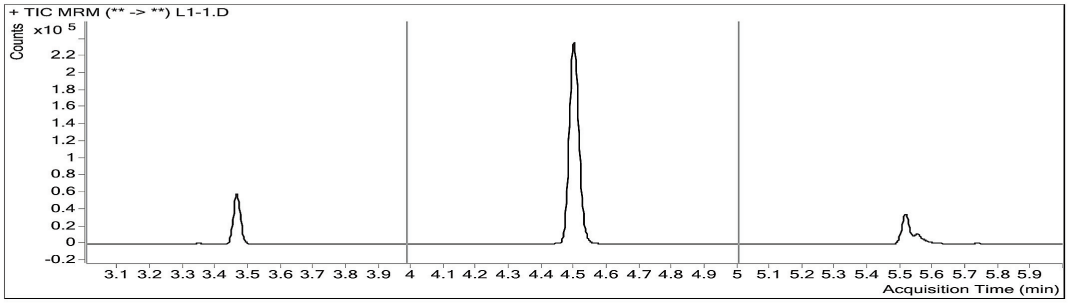


Figure 3. Quantitative Analysis Sample Report for 0.1 ng/mL of THC in blood. The RMS signal-to-noise is 175:1 with a noise region of 3.6 to 3.9 min.

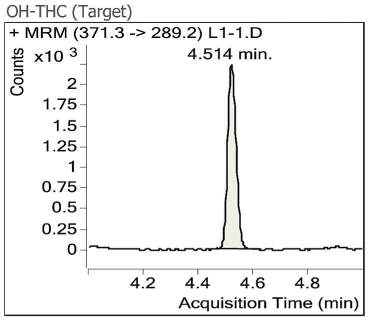
Quantitative Analysis Sample Report

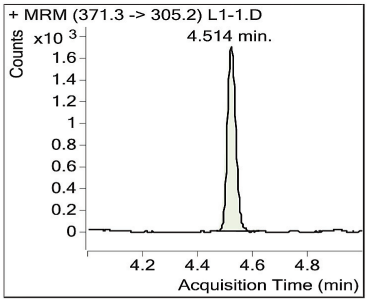
Data File	:	L1-1.D
Operator	:	DATASYSTEM01\Admin
Acq Method Name	:	LTM.THC.OMRM.7000.eiex.m
Acquisition date	:	2011-04-28 18:19
Sample Name & Path	:	L1-1, D:\MassHunter\GCMS\1\data\04-28-2011-redo\
Vial	:	3
Dilution	:	0.0
Sample Info	:	
Last Calib Update	:	2011-05-05 13:57

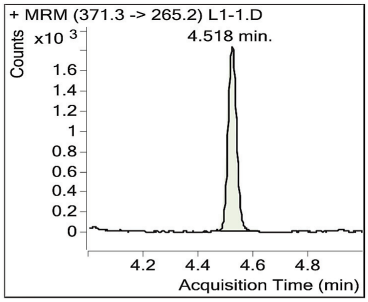
Cmpnd	Signal	RT	Limits	Response	QRatio	Limits	FinalConc
d3-OH-THC	374.3 -> 292.2	4.5		175705			
	374.3 -> 308.2			157882	89.9	68.8-108.8	
	374.3 -> 268.3			148545	84.5	59.4-99.4	
OH-THC	371.3 -> 289.2	4.51	4.27 - 4.72	4924			0.099
	371.3 -> 305.2			3707	75.3	67.4-107.4	
	371.3 -> 265.2			4050	82.3	58.2-98.2	



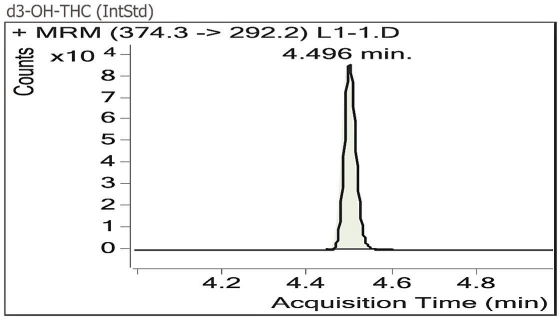
OH-THC (Target)







d3-OH-THC (IntStd)



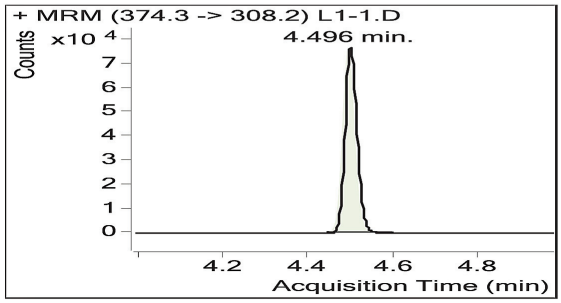
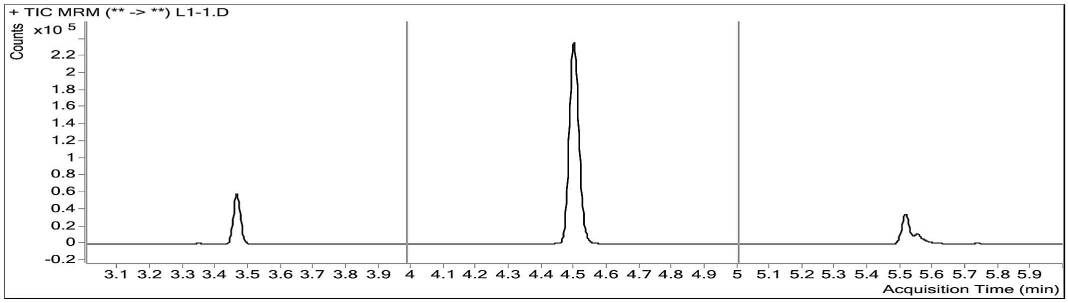


Figure 4. Quantitative Analysis Sample Report for 0.1 ng/mL of 11-OH-THC in blood. The RMS signal-to-noise is 46:1 with a noise region of 4.6 to 4.9 min.

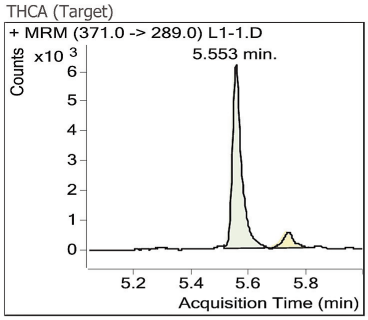
Quantitative Analysis Sample Report

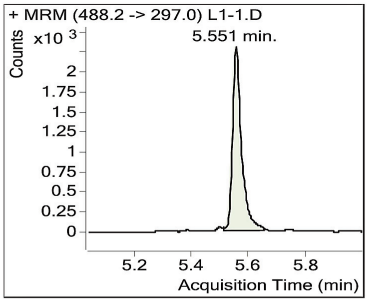
Data File	:	L1-1.D
Operator	:	DATASYSTEM01\Admin
Acq Method Name	:	LTM.THC.OMRM.7000.eiex.m
Acquisition date	:	2011-04-28 18:19
Sample Name & Path	:	L1-1, D:\MassHunter\GCMS\1\data\04-28-2011-redo\
Vial	:	3
Dilution	:	0.0
Sample Info	:	
Last Calib Update	:	2011-05-05 13:57

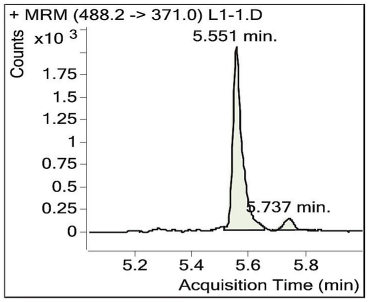
Cmpnd	Signal	RT	Limits	Response	QRatio	Limits	FinalConc
d9-THCA	380.0 -> 292.2	5.51		38358			
	497.0 -> 306.1			9800	25.5	5.6-45.6	
	497.0 -> 380.1			11765	30.7	12.5-52.5	
THCA	371.0 -> 289.0	5.55	5.26 - 5.82	13985			1.015
	488.2 -> 297.0			5124	36.6	9.1-49.1	
	488.2 -> 371.0			4758	34	14.4-54.4	



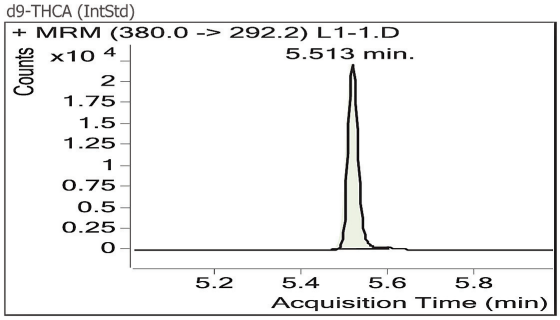
THCA (Target)







d9-THCA (IntStd)



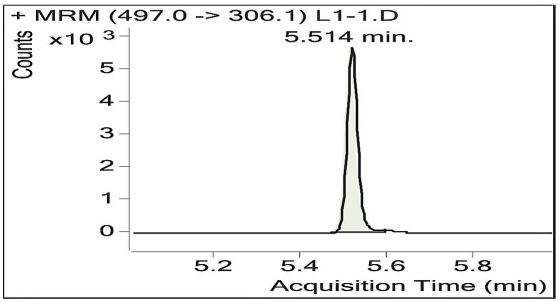


Figure 5. Quantitative Analysis Sample Report for 1 ng/mL of THCA in blood. The RMS signal-to-noise is 39:1 with a noise region of 5.1 to 5.3 min.

Conclusion

Coupling the Agilent 7890N gas chromatograph utilizing an LTM system with the Agilent 7000B Triple Quadrupole GC/MS system enables a rapid and robust method for the analysis of THC and its metabolites in blood. Using the High Flow Bond Elut Certify II SPE cartridge , backflushing of the GC column, and MRM eliminate all interferences, with a resulting dynamic range of quantification of 0.1 to 50 ng/mL for THC and 11-OH-THC, and 1 to 100 ng/mL for THCA. The LTM module and backflushing facilitate rapid analysis, with a run time of 6 minutes and a cycle time of 8 minutes.

References

1. R.M Sears, Solid Phase Extraction of THD, THC-COOH and 11-OH-THC from Whole Blood, Agilent Technologies Application Note 00315.

For More Information

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