Rapid, Robust and Sensitive Detection of 11-nor-Δ^9-Tetrahydrocannabinol-9-Carboxylic Acid in Hair

Application Note

Forensics/Doping Control

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Abstract

A robust method for the detection of the THCA marijuana metabolite in hair was developed with a run time of 7 min and a cycle time of 9 minutes using column switching and backflushing. The method LOD is 0.002 pg/mg and the LOQ is 0.01 pg/mg.

Introduction

Testing hair for drugs of abuse has been practiced for over 50 years, due in large part to the ability to detect drug use over a longer period of time, as compared to other biological matrices, because many drugs are well-preserved in hair. Hair testing is widely used in criminal investigations such as monitoring abstinence of parolees, verifying drug use history, and identifying drug facilitated sexual assault. It is also commonly used to screen and monitor drug use in employees, drug treatment participants, and parties involved in child custody cases. Workplace programs include hair testing due to the ease of collection, difficulty of adulteration and longer detection times.

Marijuana is one of the drugs tested most often in forensic and drug screening applications. The parent compound, tetrahydrocannabinol (THC), is found in higher concentration in hair samples, but detection of the acid metabolite THCA (11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid) is preferred, in order to eliminate the possibility of potential environmental contamination from marijuana smoke. While guidelines for workplace hair testing have not yet been adopted by the Substance Abuse Mental Health Services Administration (SAMHSA) in the United States, a cutoff concentration for nor-9-carboxy-Δ^9-tetrahydrocannbinol as low as 0.05 pg/mg hair has been suggested, and such guidelines are a topic of additional study and analysis by this regulatory body. The Society of Hair Testing recommends a limit of quantification (LOQ) of $\leq$ 0.2 pg/mg for THCA.

Agilent Technologies

This application note describes a method developed on the Agilent 7890A GC System coupled with an Agilent 7000B Triple Quadrupole GC/MS System that provides rapid and sensitive detection of a THC metabolite in hair, using 2-D GC and negative ion chemical ionization (CI) MS/MS in the multiple reaction monitoring (MRM) mode (also called SRM, Selected Reaction Monitoring). The method is modified from a previous GC/MSD method [1] to take advantage of the lower chemical background and higher sensitivity provided by triple quadrupole MS/MS analysis. Backflush is used to increase robustness, and low thermal mass (LTM) column modules speed the chromatography process, enabling a run time of 7 min and a cycle time of 9 min. MRM MS/MS analysis on the Triple Quadrupole GC/MS System delivers excellent sensitivity, with an LOD of 0.002 pg/mg and an LOQ of 0.01 pg/mg.

Experimental

Standards and Reagents

Tri-deuterated THCA, which was used as the internal standard (100 µg/mL in methanol), and unlabelled THCA (100 µg/mL in methanol) were obtained from Cerilliant, (Round Rock, TX). The internal standard concentration in the method was 0.05 pg/mg of hair.

Methanol, acetonitrile, toluene, ethyl acetate, hexane, glacial acetic acid, and methylene chloride were obtained from Spectrum Chemicals (Gardena, CA). All solvents were high-performance liquid chromatography (HPLC) grade or better, and all chemicals were ACS grade. Bond Elut Certify I solid-phase extraction columns (130 mg) from Agilent, Inc. (Walnut Creek, CA), or Clean Screen ZSTHC020 extraction columns (200 mg) from United Chemical Technologies, Inc. (Bristol, PA) were interchangeable for the assay. The derivatizing agents, pentafluoropropionic anhydride (PFPA) and 1,1,1, 3, 3, 3-hexafluoro-2-propanol (HFIP), were purchased from Sigma –Aldrich (St. Louis, MO) and Campbell Science (Rockton, IL), respectively.

Instruments

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

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

GC Run Conditions	
Pre-column	1 m × 0.15 mm × 1.2 µm DB-1 (p/n 12A-1015)
Analytical columns	
Column 1	15 m × 0.25 mm × 0.25 µm DB-1ms LTM Column Module (p/n 122-0112LTM)
Column 2	15 m × 0.25 mm × 0.25 µm DB-17ms LTM Column Module (p/n 122-4712LTM)
Injection volume	2 µL
Inlet temperature	Isothermal at 250 °C
Injection mode	0.75 minute pulsed splitless at 35 psi
Oven temperatures	
GC oven	7 minute hold at 250 °C (isothermal)
1st LTM module	50 sec hold at 100 °C
	100 °C to 210 °C at 200 °C/min
	210 °C to 267 °C at 10 °C/min
	Hold at 267 °C for 2 min
2nd LTM module	324 sec hold at 100 °C
	100 °C to 230 °C at 200 °C/min
	230 °C to 240 °C at 10 °C/min
	Hold at 240 °C for 2 min
Carrier gas	Helium in constant pressure mode. Pre-column: 1 psi; Column 1: 26.6 psi; Column 2: 19.6 psi
Transfer line temp	300 °C
MS conditions	
Tune	Autotune
EMV Delta	1200 V
Acquisition parameters	NCI mode; multiple reaction monitoring (MRM)
Reagent gas	Ammonia, 35% flow
Collision gas	Argon, constant flow, 0.9 mL/min
Quench gas	Helium, constant flow, 0.5 mL/min
Solvent delay	6.2 min
MS temperatures	Source 150 °C; Quadrupole 150 °C

Sample Preparation

Samples were prepared as previously described [2]. Calibrators, controls or hair specimens (20 mg) were weighed into silanized glass tubes and washed with methylene chloride (1.5 mL). The solvent was decanted and the hair samples were allowed to dry. The internal standard, THCA-d3 (0.05 pg/mg), was added to each hair specimen. For the calibration curve, unlabelled THCA was added to the hair at concentrations of 0.002, 0.01, 0.02, 0.05, 0.1, and 0.5 pg/mg of hair.

Deionized water (0.5 mL) and 2N sodium hydroxide (0.5 mL) were added, and the hair was heated at 75 °C for 15 min. The sample was allowed to cool and then centrifuged (2500 rpm, 15 min). The supernatant was poured into glass tubes already containing acetic acid (1 mL), 1 M acetic acid (3 mL), and 0.1 M sodium acetate buffer (pH 4, 2 mL). The tubes were capped and mixed.

SPE columns were conditioned with hexane/ethyl acetate (75:25, v/v; 2 mL), methanol (3 mL), deionized water (3 mL), and 0.1 M hydrochloric acid (1 mL). The acidified samples were loaded onto the SPE columns and allowed to dry. The SPE columns were washed with deionized water (2 to 3 mL) and allowed to dry for 5 min. The SPE columns were washed with 0.1 M hydrochloric acid/acetonitrile (70:30 v/v; 3 mL) and allowed to dry at 30 psi for 10 min. The SPE columns were finally rinsed with hexane/ethyl acetate (75:25 v/v; 3 mL) in order to elute the THCA into silanized glass tubes.

The eluent was evaporated to dryness under nitrogen at 40 °C and reconstituted in PFPA (70 µL) and HFIP (30 µL) for derivatization. The mixture was transferred to autosampler vials with glass inserts and capped. The vials were heated at 80 °C for 20 min, then left at room temperature for 10 min. The extracts were evaporated to dryness in a vacuum oven. The samples were finally reconstituted in toluene (50 µL), for injection into the 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)
THCA*	6.714	620→492	50	5
		620→383	50	5
THCA-d3	6.710	623→495	20	5
		623→386	20	5

*11-nor-Δ^9-Tetrahydrocannabinol-9-Carboxylic Acid

Results

Two Dimensional Gas Chromatography with Heart-Cutting

The use of two serial GC columns to separate background from the required peak is a well-established technology that is widely used to provide excellent separation of the analyte from matrix interferences. Once the analyte retention time on the first column has been determined, the pneumatic switch (Deans Switch) is turned on at that time to divert the flow to the second column, and turned off a short time later. This diverts a narrow, heart-cut "window" of the effluent from the first column that contains the analyte and minimal background, for further separation on the second column (Figure 1). The two columns function optimally when the stationary phases are as different as possible.

Exceptional Robustness and Speed

The unique combination of backflushing and low thermal mass (LTM) column modules make this a very robust and rapid method, compared to the traditional single column approach. Three independently programmed pressure zones are used in conjunction with three independently heated zones (Figure 1). The pre-column and the first LTM column are coated with relatively non-polar DB-1ms phase, and the second LTM column is coated with a more polar DB-17ms phase. The heart-cut window is only 0.2 min (5.5 to 5.7 min) wide.

A unique system for rapid and robust detection of THCA in hair

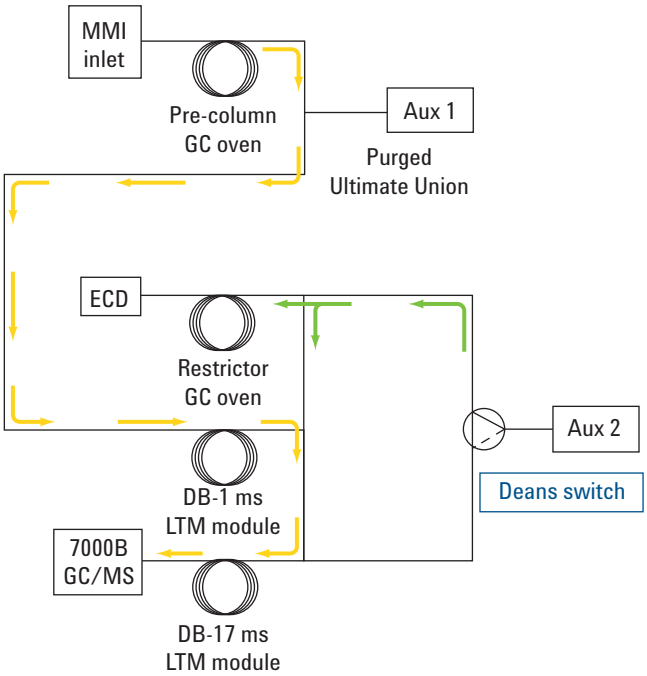

Figure 1. Schematic representation of the system used to develop the THCA method.

The precolumn and auxiliary pressure control module (AUX EPC) provides backflushing capability to protect and preserve the LTM analytical columns. The precolumn was in backflush mode with a constant pressure of 1 psi during the run. The inlet pressure pulse overrides the backflush for the initial 0.75 min. The use of backflushing prevents build-up of high-boiling compounds on the column, 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.

This method also employs LTM column modules external to the GC oven that enable independent and optimal temperature control of the two analytical columns (Figure 2). 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, and they can be controlled from the GC software.

The end result of this unique backflushing and LTM approach is a robust method that provides excellent quantification and sensitivity (see next section) with 7 min run times and 9 min cycle times.

Unique LTM Column Modules enable rapid temperature ramping and cooling

Figure 2. Low thermal mass (LTM) column modules interfaced with the Agilent 7890A GC.

Sensitivity and Quantification

This method has a limit of detection (LOD) of 0.002 pg/mg, demonstrating excellent sensitivity that is far below the suggested cutoff of 0.05 pg/mg (Figure 3). The accuracy of quantification is also quite good, with an R^2 of 0.995, from 0.002 to 0.5 pg/mg of hair (Figure 4). The limit of quantification (LOQ) is 0.01 pg/mg, which again is more than an order of magnitude below the 0.2 pg/mg LOQ suggested guideline established by the Society of Hair Testing (Figure 5). This method also provides a compliant quantitative analysis report that includes the retention times (with limits), response level, qualifier ion ratio (with limits), and the calculated concentration. The total ion current (TIC) trace and the quantifier and qualifier MRM traces are also displayed on the report, for both the sample and the THCA-d3 internal standard (Figure 6).

LOD of 0.002 pg/mg

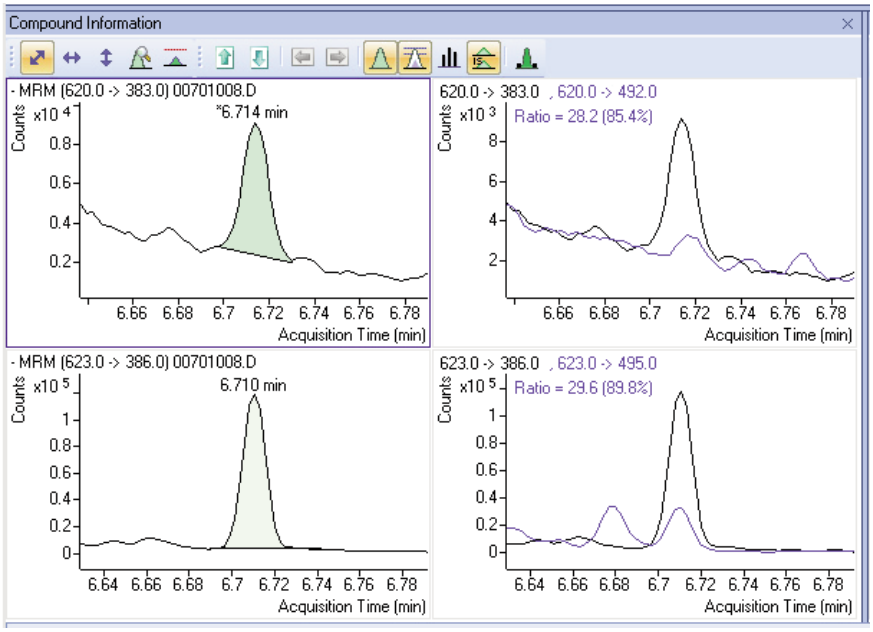

Figure 3. MRM traces for the quantifying transition (left) and both the quantifying and qualifying transitions (right) for the 0.002 pg/mg LOD of THCA (upper panel) and the deuterated standard (lower panel) spiked into a hair sample.

Reliable calibration

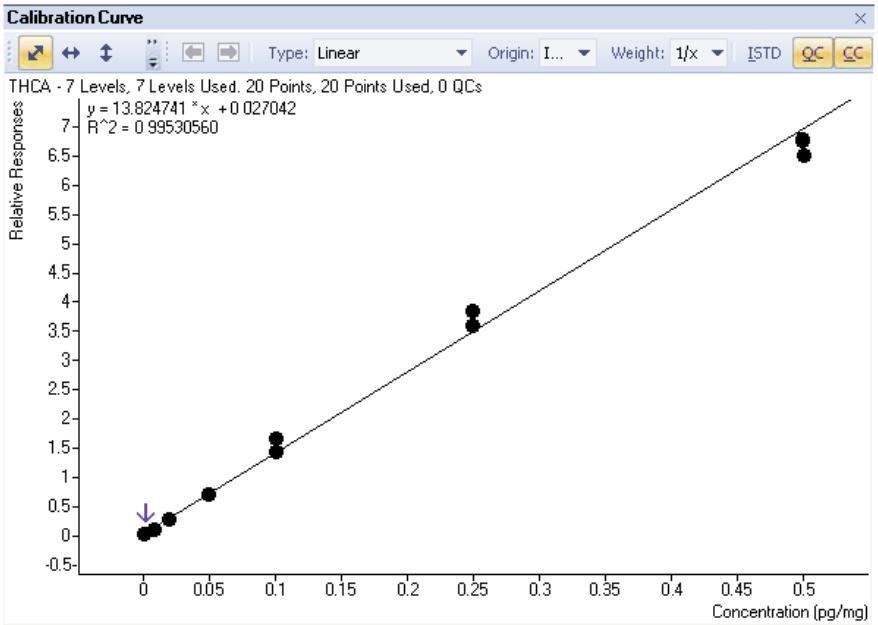

Figure 4. Calibration curve for THCA spiked into hair samples at 0.002, 0.01, 0.02, 0.05, 0.1, and 0.5 pg/mg of hair.

0.01 pg/mg LOQ

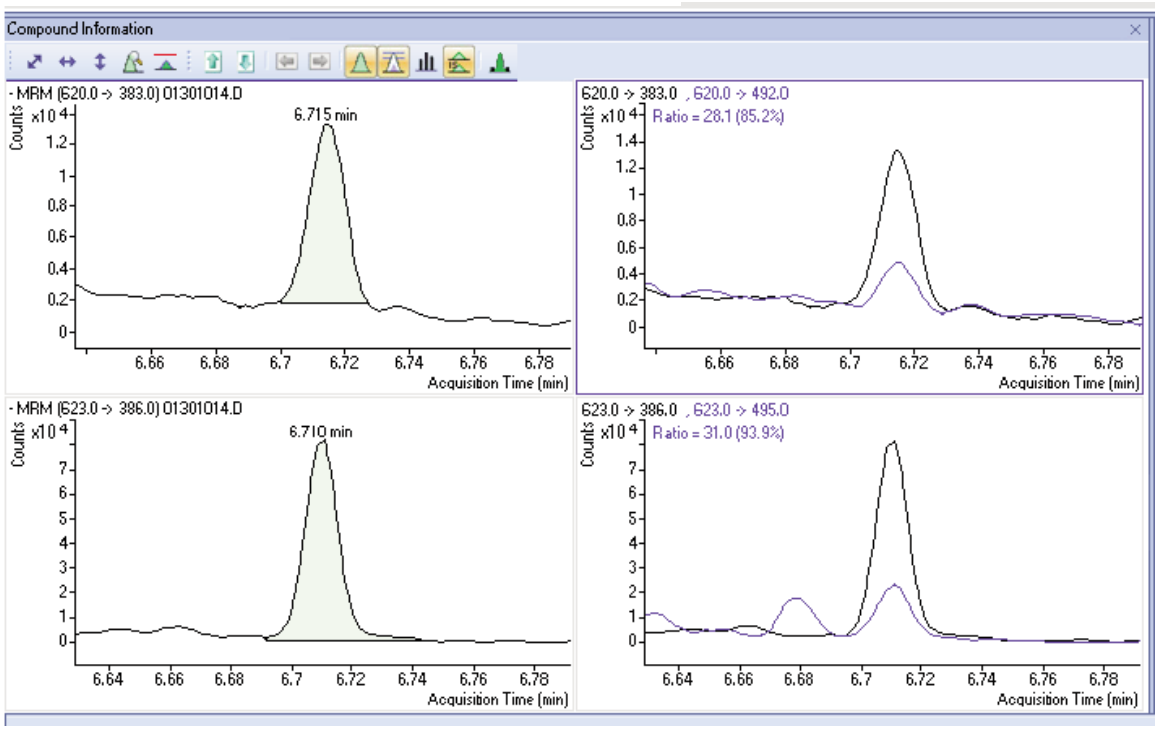

Figure 5. MRM traces for the quantifying transition (left) and both the quantifying and qualifying transitions (right) for the 0.01 pg/mg LOQ of THCA (upper panel) and the deuterated standard (lower panel) spiked into a hair sample.

Data File	01401015.D	Vial	14
Operator	DATASYSTEM01/Admin	Dillution	0.0
Acq method name		Sample information	
Acquisition date	2010-10-08 16:24	Last calib update	2010-11-28 09:34
Sample name and path	0.01 pg/mg, D:/MassHunter/GCMS/1/data/PFAA Curve Extracted/		

Compound	Signal	RT	Limits	Response	QRatio	Limits	Final conc
THCA-d3	623.0 -> 386.0	6.71		82558		35770 - 143081	
	623.0 -> 495.0			24962	30.2	23.1 - 42.9	
THCA	620.0 -> 383.0	6.71	6.38 - 7.05	10999			0.008
	620.0 -> 492.0			3908	35.5	23.1 - 42.9	

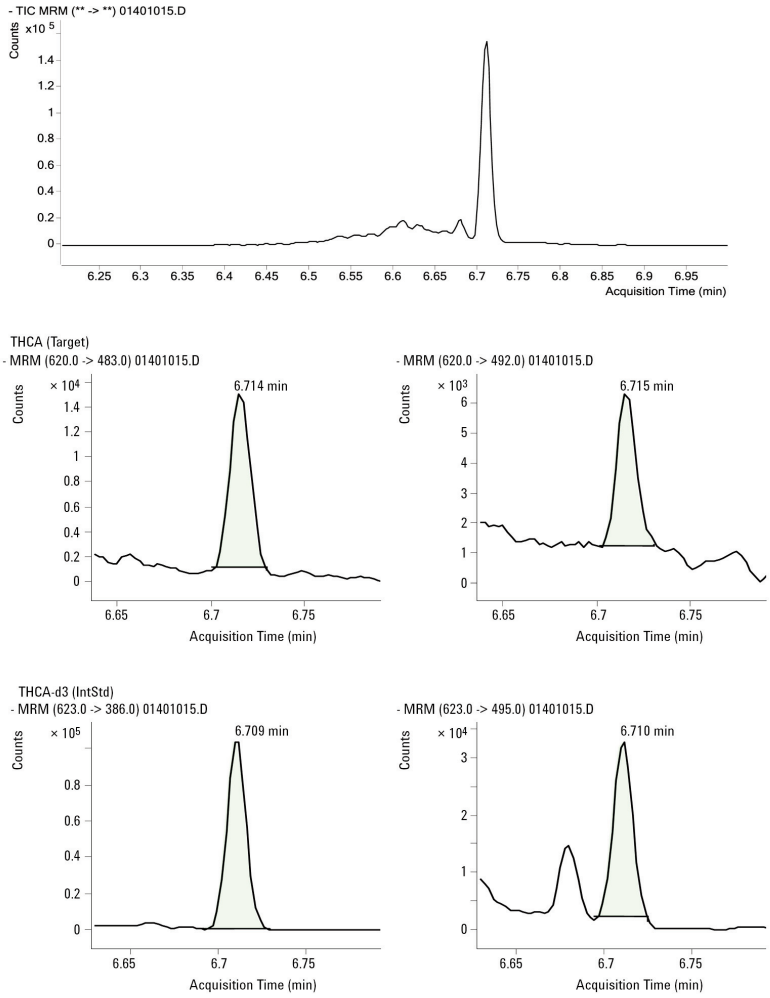

Figure 6. Quantitative Analysis Sample Report for a 0.01 pg/mg (the LOQ) sample spiked into hair.

Conclusion

The time-proven technique of heart-cutting to improve chromatographic separation is given new life in this unique method which utilizes state-of-the-art microfluidics-aided backflushing and low thermal mass column temperature ramping modules to deliver sensitive and robust detection and quantification of THCA in hair (LOD 0.002 pg/mg; LOQ 0.01 pg/mg) with run times of only 7 minutes, and cycle times of 9 minutes.

References

1. F. Feyerherm, R. Lowe, J. Stuff, D. Singer, "Rapid Multidimensional GC Analysis of Trace Drugs in Complex Matrices", Gerstel publication AN-2007-8.
2. C. Moore, S. Rana, C. Coulter, F. Feyerherm, H. Prest, "Application of Two-dimensional Gas Chromatography with Electron Capture Chemical Ionization Mass Spectrometry to the Detection of 11-nor-D9-Tetrahydrocannabinol-9-carboxylic acid (THCA) in Hair", J. Anal. Toxicol. 30, 171–177 (2006).

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