Ultrafast Analysis of THCCOOH in Urine Using the Agilent RapidFire High-Throughput Mass Spectrometry System

Application Note

Forensic Toxicology

Authors

Michelle Romm and Vaughn P. Miller
Agilent Technologies, Inc.
11 Audubon Rd,
Wakefield, MA 01880

Abstract

Forensic drug testing has traditionally used GC/MS as the primary analytical detection method. Steady increases in the need for greater analytical capacity and throughput have placed demands on traditional technologies. The Agilent RapidFire High-throughput Mass Spectrometry System is an ultrafast SPE/MS/MS system capable of analyzing samples with cycle times of less than 15 seconds. In this application note, we evaluated the ability of the Agilent RapidFire/MS system to analyze 11-nor-9-delta-9-tetrahydrocannabinol (THCCOOH), the major metabolite of marijuana, in urine with much faster sample cycle times and similar analytical results compared to GC/MS or LC/MS assays.

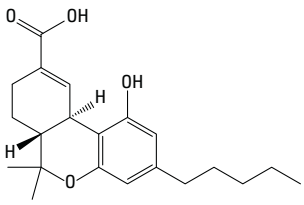

Figure 1. Structure of THCCOOH.

Agilent Technologies

Introduction

Traditional forensic drug testing has required extensive sample preparation prior to GC/MS analysis. Recently, LC/MS has been explored, but even LC/MS runs taking several minutes per sample require some offline sample cleanup due to the large amount of salt present following glucuronide hydrolysis. With an increasing demand for sample analysis, methods that require no sample preparation and analysis times that are seconds per sample are desired. The Agilent RapidFire High-Throughput Mass Spectrometry System is an ultrafast SPE/MS/MS system capable of analyzing samples with cycle times under 15 seconds and requires no sample cleanup prior to analysis. In this application note, a method to measure dilute and shoot urine for THCCOOH by ultrafast SPE/MS/MS was developed.

Experimental

RapidFire triple quadrupole conditions

The RapidFire/MS/MS system consisted of the following modules: an Agilent RapidFire 360, an Agilent 6490 Triple Quadrupole Mass Spectrometer, MassHunter Qualitative Analysis B.05.00, and MassHunter Quantitative Analysis B.05.00. Samples were analyzed at a rate of 12 seconds per sample (Figure 2), using the conditions shown in Table 1. Analyte and internal standard ions were monitored simultaneously in all experiments.

Chemicals and reagents

All analytes were purchased from Cerilliant, Round Rock, TX. All LC/MS grade solvents and reagents were purchased from Sigma-Aldrich, St. Louis, MO.

Table 1. RapidFire/MS/MS conditions.

RapidFire conditions						
Buffer A	Water with 10 mM ammonium acetate, 0.09 % formic acid, 0.01 % trifluoroacetic acid					
Buffer B	50 % methanol with 0.1 % formic acid					
Buffer C	85 % ethyl acetate 15 % isopropanol with 0.09 % formic acid, 0.01 % trifluoroacetic acid					
Injection volume	10 µL					
SPE cartridge	Agilent RapidFire cartridge E (reversed-phase C8, G9210C)					
RF state 1	sip sensor					
RF state 2	1,500 ms					
RF state 3	5,000 ms					
RF state 4	3,000 ms					
RF state 5	500 ms					
Triple quadrupole conditions						
Gas temperature	250 °C					
Gas flow	19 L/min					
Nebulizer	45 psi					
Sheath gas temperature	300 °C					
Sheath gas flow	11 L/min					
Mozzle voltage	2,000 V					
Capillary voltage	3,500 V					
Compound	**Q1**	**Q3**	**Dwell**	**Fragmentor**	**CE**	**CAV**
THCCOOH quantifier	345.2	299	50	380	20	2
THCOOH - d9	354.1	308.2	50	380	17	2
THCCOOH qualifier	345.2	193	50	380	25	2

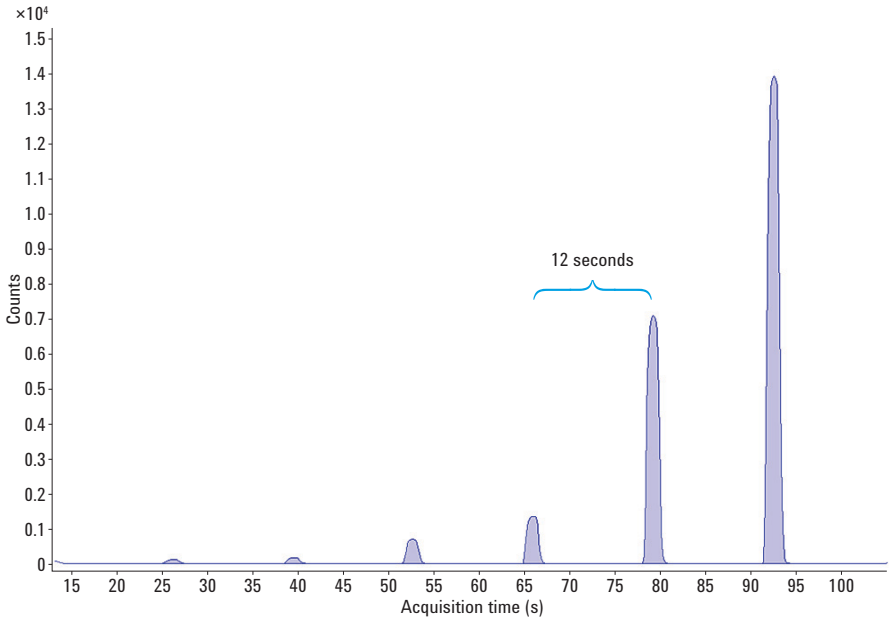

Figure 2. Standard curve showing injection cycle times of 12 seconds.

Sample preparation

Standard calibrators were prepared by spiking drug-free human urine with 1,000 ng/mL of THCCOOH. Serial dilutions were used to achieve the remaining standard calibrator concentrations. A set of standard calibrators within a concentration range of 5–1,000 ng/mL, as well as a negative matrix control were also spiked with 1,000 ng/mL of the same class of interfering drugs (various synthetic cannabanoids). All samples (135 µL) were hydrolyzed with 15 µL sodium hydroxide, mixed, incubated at 65 °C for 15 minutes, and diluted with 1350 µL of 50:50 methanol:water containing 0.1 % formic acid. Samples were transferred to 96-well plates, sealed with an Agilent PlateLoc Thermal Microplate Sealer, centrifuged, and injected onto the Agilent RapidFire/MS/MS system.

Data analysis

MassHunter Qualitative Analysis (B.05.00) and Quantitative Analysis (B.05.00) were used for data analysis. A $1/x^2$ weighting factor was applied during linear regression of the calibration curves. The quantitation using MassHunter Quantitative software was performed by spectral peak area ratio to a known concentration of the internal standards.

Results and Discussion

Samples were prepared by spiking THCCOOH into drug-free human urine, carrying out base hydrolysis, and then diluting samples 10-fold with water. Prepared calibration standards were run four times a day, over a series of four days to establish both intra and interday precision and accuracy. THCCOOH had intra and interday accuracies within 15 % and coefficient of variation values less than 10 % for all concentrations within the linear range (Table 2). This method had excellent linearity within the measured range of 5–1,000 ng/mL with an R^2 value of 0.9950 (Figure 3).

Matrix effects were investigated for THCCOOH by comparing standard curves prepared in PBS to those in urine. No significant differences in the standard curve results were observed. The negative matrix control was also tested and determined to be void of any interference when spiked with various synthetic cannabanoids.

Table 2. Intraday and interday precision and accuracy for RapidFire/MS/MS analysis of THCCOOH in urine.

THCCOOH (ng/mL)	Intraday % Accuracy (n=4)	Intraday % Precision (n=4)	Interday % Accuracy (n=4)	Interday % Precision (n=4)
5	96.3	7.9	95.7	1.6
10	110.2	7.2	107.9	1.6
50	92.3	2.5	89.7	2.2
100	95.1	3.9	94.5	1.9
500	100.2	4.1	100.7	2.4
1,000	110.2	2.3	111.4	1.5

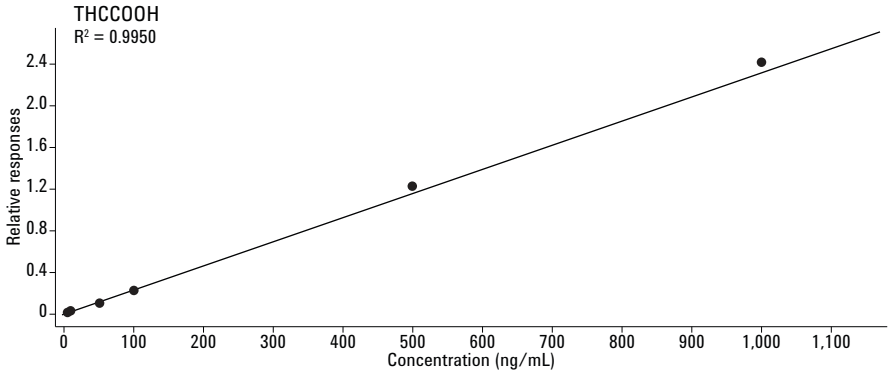

Figure 3. Representative standard curve for THCCOOH.

The reproducibility of the method was tested by measuring at least 2,000 sequential injections of THCCOOH spiked into urine at 20 ng/mL. The same cartridge was used for all injections without deviation in pump pressures or peak shape. The instrument response was stable for the analyte and had a coefficient of variation of less than 10 % and accuracy within 2 % (Figure 4).

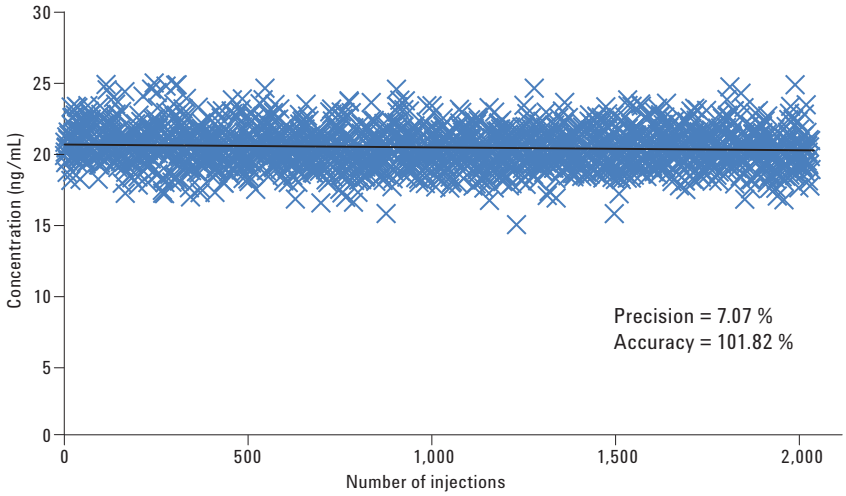

Figure 4. Repeatability evaluation: sequential injections of THCCOOH.

Conclusions

THCCOOH in urine was accurately and precisely measured using a simple hydrolysis, dilute, and shoot analytical method on the Agilent RapidFire/MS System. Analysis times of less than 15 seconds per sample provided a high-throughput detection method for this analyte. This forensic methodology is capable of throughputs greater than 240 samples per hour. As a result, this methodology provides comparable results to LC/MS/MS, but at > 10x the speed and efficiency of typical LC/MS/MS methods.

www.agilent.com/lifesciences/rapidfire

Forensic Use Only.
This information is subject to change without notice.

Published in the USA, August 7, 2014
5990-9763EN

Agilent Technologies