Application Note: 10006

Analysis of Carboxy-THC in Urine Using Single Quadrupole GC/MS

Thermo Fisher Scientific, Austin, TX, USA

Key Words

- DSQ Single Quadrupole GC/MS
- Carboxy-THC
- Drugs of Abuse
- Forensics
- Toxicology

Abstract

Marijuana contains the substance tetrahydrocannabinol (THC), which has mind-altering properties. The plant and drug are both federally regulated. These regulations require testing for this drug and its metabolites, which can be found in urine. 11-nor-9-carboy-delta-9-THC, also known as carboxy-THC, is the primary urinary metabolite of THC. Carboxy-THC can be found in urine up to 8 days after using marijuana. For this analysis, 2 mL of urine were spiked with known amounts of c-THC and c-THC-D9, which is used as the deuterated internal standard. The samples were hydrolyzed and then extracted using THC Clean Screen® SPE columns from United Chemical Technologies, Inc.[1] After the samples were extracted, they were derivatized with bis (trimethylsilyl) trifluoroacetamide (BSTFA) with 1% TMCS. This final reaction product was then injected onto a Thermo Scientific DSQ™. The calibration curve that was generated ranged in concentration from 6 to 300 ng/mL, giving a correlation coefficient of 0.9974. A cutoff concentration of 15 ng/mL and a maximum limit of quantitation (LOQ) of 6 ng/mL were used for this method. Two commercial control samples, MAS DOA-GC/MS G2 and G3, were used to test this method.[2] The samples had a concentration range of 9.0 – 11.2 and 18.0 – 18.9 ng/mL, respectively. The calculated amounts were found to be 9.7 ng/mL for the G2 sample and 17.4 ng/mL for the G3 sample.

Introduction

Marijuana is a federally regulated drug that is tested for a variety of reasons, e.g. employment screening. Its use produces a euphoria and has a sedative effect. The use of marijuana has long term effects on the respiratory system and memory. Typical users of marijuana are not physiologically addicted, but they suffer from a compulsion to use it. THC can be smoked through the use of marijuana or hashish. It can also be ingested through food and drink. When THC enters the body, it is metabolized to, among others, 11-nor-9-carboy-delta-9-THC, also known as carboxy-THC. The presence of carboxy-THC in a sample of urine identifies that person as one who has smoked or ingested some form of THC. Carboxy-THC can be detected at appreciable levels for up to 8 days after the drug has entered the body.[3] The molecule of carboxy-THC contains a carboxyl functional group that does not lend itself well to being separated by gas chromatography. Therefore, it needs to be derivatized to take advantage of the speed and ease of use that is afforded by the GC/MS system. A typical derivatizing agent, BSTFA + 1% TMCS, caps the functional group with trimethylsilyl groups. The Thermo Scientific DSQ GC/MS (Figure 1), which uses a curved prefilter to minimize background noise derived from excited neutrals, was used for this analysis. This feature gives the instrument the ability to detect levels that have not been previously possible on a GC/MS single quad system. The United States Substance Abuse and Mental Health Services Administration (SAMHSA) mandates that the LOQ be 40% of the cutoff level. The value for the cutoff level of carboxy-THC is 15 ng/mL, and the LOQ is 6 ng/mL.[4]

Figure 1: Thermo Scientific DSQ single quadrupole GC/MS

Methods

Blank urine was collected and used for sample preparation. Two milliliter aliquots were then taken from this sample and placed in clean glass vials. Each vial was then spiked with amounts of carboxy-THC and carboxy-THC-D9 as the internal standard, from Cerilliant. These were used to generate a calibration curve with points at 6, 15, 75, 150, and 300 ng/mL. Prior to extraction the samples are hydrolyzed by adding 0.5 mL of a 1.0 M solution of KOH. This reaction takes place at 45 °C for 15 minutes. After the hydrolysis reaction, the pH of the samples was adjusted to 3 using glacial acetic acid. Each sample was extracted by solid phase extraction on THC Clean Screen columns (ZSTHC020).

Thermo SCIENTIFIC

These extraction columns were conditioned with sequential rinses of the following solutions: 3 mL of methanol, 3 mL of DI water, and 1 mL of 0.1 M HCl. Between each rinse, the columns were aspirated but not allowed to dry. The 2 mL sample of spiked urine was then loaded onto the column and was pulled through by vacuum at a rate of 1 mL/min. After the samples were loaded, the columns were washed once each with 2 mL of DI water and 2 mL of a 0.1 M HCl: acetonitrile solution (70:30). The columns were then vacuum dried. A final rinse of 200 µL of hexane was then pulled through the columns. The carboxy-THC was eluted from the column with 3 mL of a solution of hexane and ethyl acetate (50:50). The sample was eluted at 1 mL/min and collected into clean glass vials. The sample was evaporated to dryness at 40 °C with a flow of nitrogen to assist in the evaporation. The samples were derivatized with 50 µL of BSTFA with 1% TMCS at room temperature for 15 minutes, and then transferred to GC ALS vials with glass inserts and loaded onto the AS 3000 autosampler.

The DSQ that was used for this analysis was a 70 L/s turbo pump, EI only system and was configured with a standard split/splitless injector. A 5 mm silanized glass liner was used in the injector. A 2 µL injection on the AS 3000 autosampler was used for the injection. The analytical column was a 15 m x 0.25 mm i.d. x 0.25 µm Rtx®-5MS column from Restek Corporation.[5] This column is a fused silica column with a 5% diphenyl/95% dimethyl polysiloxane base that lends itself to this type of application. A hot needle injection technique was used with a 3-second pre-injection delay. The hot needle injection is performed by drawing at least 1 µL of air following the sample to clear the needle. The autosampler inserts the needle into the injector, and an injection delay of three seconds allows the needle to reach the injection port temperature. This technique delivers all of the sample into the injector at once, thus ensuring proper chromatographic peak shape.

The sample was injected in the splitless mode with a splitless time of 12 seconds. The split/splitless injector was set to 260 °C. The He carrier was set to a constant flow of 1.0 mL/min. The initial temperature on the TRACE GC Ultra was set to 190 °C. The high temperature at the beginning of the analytical run allowed the c-THC to elute from the column as quickly as possible. Due to the high boiling point of c-THC, it is not necessary to use the solvent to recondense the sample at the head of the column. The first temperature was held for one minute, and the GC was then ramped at 40 °C/min to a final temperature of 270 °C and held for 5 min. The source temperature was set to 250 °C and was tuned using the optimal sensitivity option to ensure the best sensitivity across the entire mass range. All settings on the instrument were taken from the autotune settings except for detector gain. The detector gain was set to 3 x 10^5.

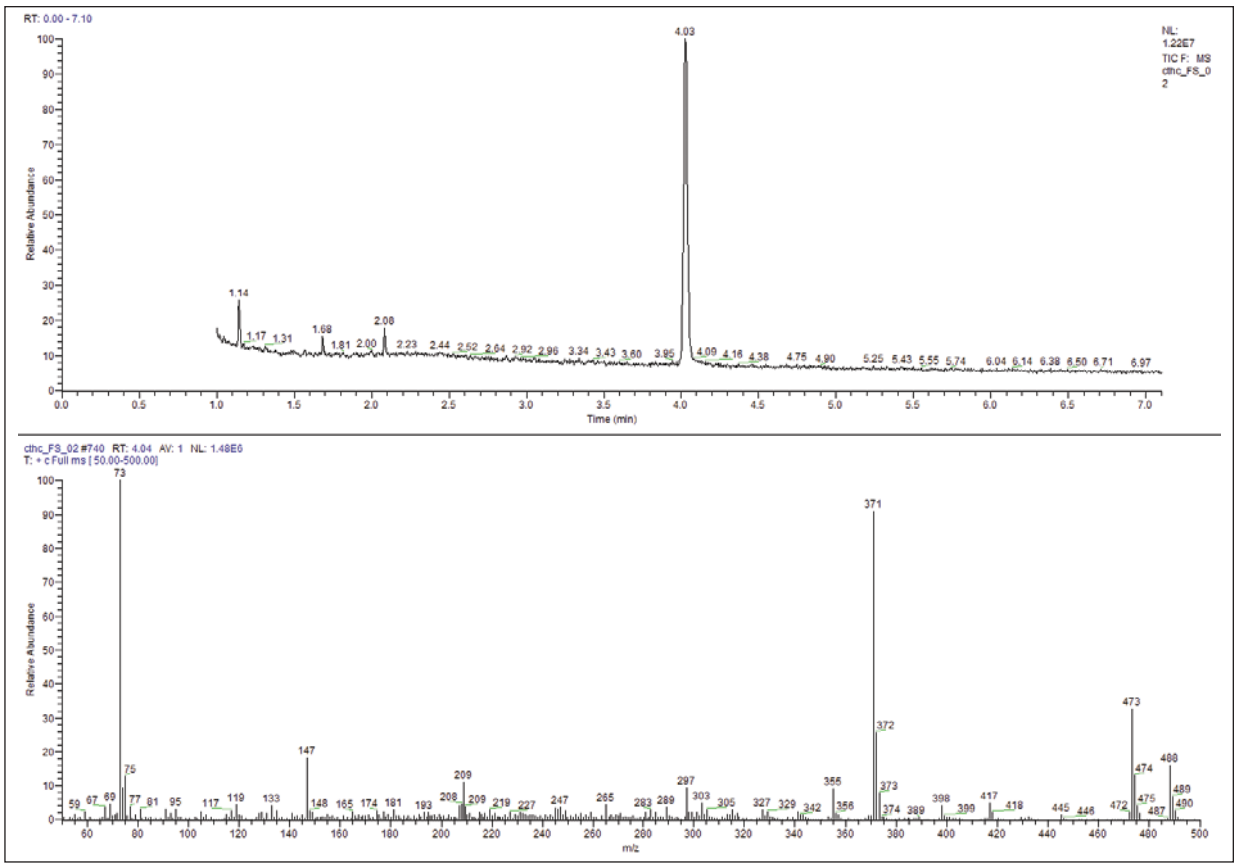

Figure 2: Full scan of carboxy-THC for the development of the SIM method. The same type of analysis was done on the internal standard, carboxy-THC-D9.

Carboxy-THC was injected at a high concentration and analyzed in full scan to determine masses for the SIM experiment (Figure 2). This was also done for the internal standard. The set of SIM masses and dwell times used to detect carboxy-THC and its deuterated internal standard are shown in Figure 4. Mass 371 was used as the quantitation mass for c-THC and mass 380 was the quantitation mass for internal standard c-THC-D9.

Oven Method

Initial Temperature (°C):	190
Initial Time (min):	1.00
Number of Ramps:	1
Rate #1 (deg/min):	40.0
Final Temperature #1 (° C):	270
Hold Time #1 (min):	5.00

Left SSL Method

Base Temperature (°C):	260
Mode:	Splitless
Splitless Time (min):	0.20

Left Carrier Method

Mode:	Constant Flow
Initial Value (ml/min):	1.00
Vacuum Compensation:	On

Figure 3: TRACE GC Ultra instrument method. This information is saved with every datafile and is accessed from Xcalibur's™ Qual Browser.

Acquisition Time:	GC Run Time
Source Temp:	250 °C
Start Time:	1.00 minutes
Detector gain:	3 x 10^5 (Multiplier voltage: 1401 V)

Scan Event 1

Scan Mode: SIM

Mass: 371.0	Width: 1.0	Dwell Time: 90.0
Mass: 372.0	Width: 1.0	Dwell Time: 90.0
Mass: 380.0	Width: 1.0	Dwell Time: 70.0
Mass: 473.0	Width: 1.0	Dwell Time: 90.0
Mass: 497.0	Width: 1.0	Dwell Time: 70.0

Figure 4: DSQ instrument method from Qual Browser.

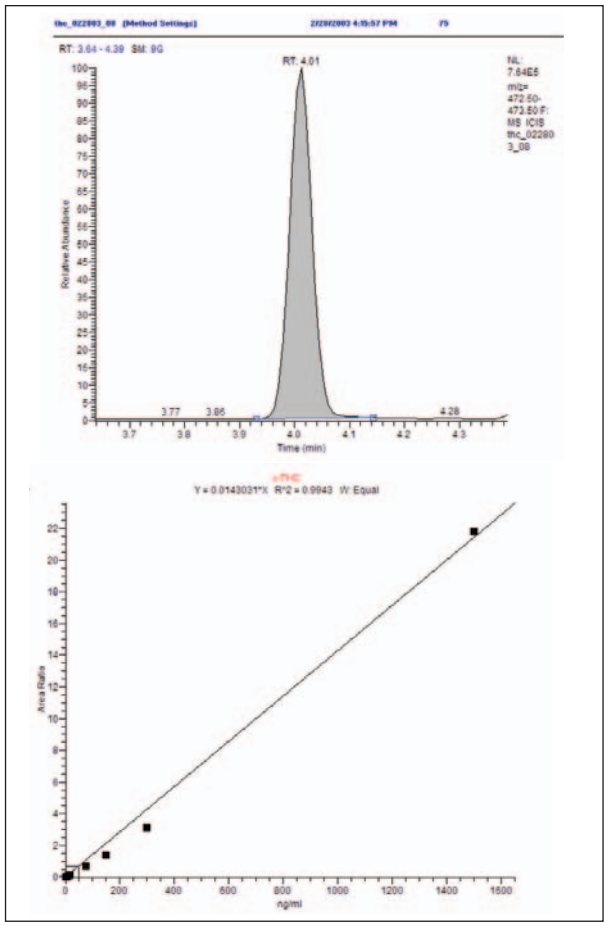

Figure 5: Calibration curve shows a correlation coefficient of 0.9974. The mass chromatogram shows the quant mass, 371, at the cutoff concentration of 15 ng/mL.

SAMPLE NAME	371	372	372/371	473	473/371
1.5	105178	50147	47.678	61501	58.473
3	189837	88285	46.506	86786	45.716
6	342247	145346	42.468	171083	49.988
15	1048349	410984	39.203	479763	45.764
75	4598469	1583740	34.441	2154442	46.851
150	9970663	3435445	34.456	4675968	46.897
300	22418423	7528900	33.584	10724726	47.839
		Average	39.762	**Average**	48.790
		+20 Percent	47.714	**+20 Percent**	58.548
		-20 Percent	31.810	**-20 Percent**	39.032

SAMPLE TYPE	SAMPLE NAME	AREA	LEVEL	AMOUNT	AMOUNT
Std Bracket	1.5	105178	1	1.500	1.551
Std Bracket	3	189837	2	3.000	2.783
Std Bracket	6	342247	3	6.000	5.084
Std Bracket	15	1048349	4	15.000	13.561
Std Bracket	75	4598469	5	75.000	67.893
Std Bracket	150	9970663	6	150.000	139.913
Std Bracket	300	22418423	7	300.000	306.912
Unknown	etoac blk			N/A	N/F
Unknown	nes	850859			13.095
Unknown	mb			N/A	N/F
Unknown	etoac blk			N/A	N/F
Unknown	neg			N/A	N/F
Unknown	G2	674461			9.706
Unknown	G3	1303708			17.421
Unknown	15x-1	1045646			13.263
Unknown	15x-2	962082			13.494
Unknown	15x-3	982051			13.555
Unknown	15x-4	923903			13.300
Unknown	15x-5	989367			12.999
Unknown	15x-6	967552			13.142

Figure 7. Integrated values for the quantitation ion for the sample set. The coefficient of variation of five injections of the cutoff is 1.58% with a % difference of the mean of 11.39%.

Results

The set of samples that were run included a solvent blank, a negative control, a matrix blank, a set of calibrators, two control samples, and a set of six samples to test reproducibility at the cutoff level. A linear calibration curve was used with a calculated correlation coeffienct of 0.9974 (Figure 5). The ions used for carboxy-THC confirmation were 372 and 473. The determination of ion ratio range was calculated based on a normal average of the calibration curve. The ion ratios were calculated and found to be within a range of ± 20% (Figure 6). Commercial control samples, designated as G2 and G3, were used to check calibration accuracy. G2 had a stated concentration range of 9.0 – 11.2 ng/mL. When analyzed and calculated against the curve, the result was 9.7 ng/mL. G3 had a stated concentration range of 18.0 – 18.9 ng/mL. When it was analyzed and calculated against the curve, the result was 17.4 ng/mL. The reproducibility at the cutoff level or 15 ng/mL gave a coefficient of variation of 1.58%, and a percent difference of the mean is 11.39% (Figure 7).

Conclusion

The analysis of carboxy-THC on the Thermo Scientific DSQ GC/MS system was completed in just over four minutes. It was also shown that this method is sufficiently sensitive, yielding a calibration curve from 1.5 to 300 ng/mL with a correlation coefficient of 0.9974. A series of replicate injections at the cutoff level of 15 ng/mL gave a coefficient of variation of 1.58% and a percent difference of the mean of 11.39%. This method was found to be both sensitive and very reproducible. Due to the method development in a urine matrix, it was also shown that the Thermo Scientific DSQ was able to handle the matrix contamination if a sufficient amount of sample preparation was done. This instrument has shown its ability to handle matrix contamination and still deliver superior results.

References

1. Clean Screen (registered trademark), United Chemical Technologies, Inc. 2731 Bartram Rd. Bristol, PA, 19007-6893
2. DOA GC/MS Liquid Assayed Drugs of Abuse Control, Medical Analysis Systems, Inc. (MAS), 5300 Adolfo Road, Camarillo, CA 93012
3. Marihuana Research Findings, 1976. NIDA Research Monograph 14. Peterson, R.C. (Ed.). http://www.drugabuse.gov/pdf/monographs/14.pdf
4. Guidance Document for Laboratories and Inspectors, November 2002 Edition. National Laboratory Certification Program, OMB No. 0930-0158.
5. Rtx-5MS, Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823

Acknowledgement

Authors: Eric Phillips and Trisa Robarge

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

Africa
+43 1 333 5034 127
Australia
+61 2 8844 9500
Austria
+43 1 333 50340
Belgium
+32 2 482 30 30
Canada
+1 800 530 8447
China
+86 10 5850 3588
Denmark
+45 70 23 62 60
Europe-Other
+43 1 333 5034 127
France
+33 1 60 92 48 00
Germany
+49 6103 408 1014
India
+91 22 6742 9434
Italy
+39 02 950 591
Japan
+81 45 453 9100
Latin America
+1 608 276 5659
Middle East
+43 1 333 5034 127
Netherlands
+31 76 587 98 88
South Africa
+27 11 570 1840
Spain
+34 914 845 965
Sweden/Norway/ Finland
+46 8 556 468 00
Switzerland
+41 61 48784 00
UK
+44 1442 233555
USA
+1 800 532 4752

www.thermo.com

AN10006_E 10/07M