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

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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-carboxy-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. The samples were then derivatized with bis (trimethylsilyl) trifluoroacetamide (BSTFA) with 1% TMCS. This final reaction product was then injected onto a Thermo Scientific DSQ™ GC/MS. 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. 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-carboxy-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. 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.

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).
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. 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 $3 \times 10^5$.

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

Acquisition Time: GC Run Time
Source Temp: 250 °C
Start Time: 1.00 minutes
Detector gain: $3 \times 10^5$ (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 3: TRACE GC Ultra instrument method. This information is saved with every datafile and is accessed from Xcalibur’s™ Qual Browser.

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.

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**Average** 39.762 Average 48.790

+20 Percent 47.714 +20 Percent 58.548

-20 Percent 31.810 -20 Percent 39.032
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 coefficient 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, 19007-6891
2. DOA GC/MS Liquid Assayed Drugs of Abuse Control, Medical Analysis Systems, Inc. (MAS), 5300 Adolfo Road, Camarillo, CA 93012
5. Rtx-SMS, Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823

Acknowledgement

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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%.