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LC/MS

Application Note

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Analysis of (\pm)-11-Nor-9-Carboxy-Delta-9-THC in Urine by Negative Ion Electrospray LC/MS/MS

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Introduction

Marijuana is one of the most commonly used illegal recreational drugs in the United States. The compound that gives the "high" from smoking marijuana is tetrahydrocannabinol or THC. Toxicology, forensic, and clinical labs are often asked to analyze urine samples for the presence of one of the metabolites of THC, most frequently the carboxy form.

Traditionally, samples are screened for THC metabolites by immunoassay and confirmed using GC/MS. GC/MS, while the current standard for THC metabolite testing, requires time consuming sample derivatization prior to analysis. LC/MS provides the same specificity and sensitivity without the need for a derivatization process.

A simple, high throughput LC/MS/MS method is described here for the detection and quantitation of (\pm)-11-nor-9-carboxy-delta-9-THC in urine.

Instrumentation

- Varian ProStar 410 AutoSampler
- Varian ProStar 210 Solvent Delivery Modules
- Varian 1200L LC/MS/MS equipped with ESI source

Materials and Reagents

- Standard solution: 0.1 mg/mL (\pm)-11-Nor-9-Carboxy-Delta-9-THC (Catalog No. T-006), from Cerilliant Corp., Texas, USA.
- Internal standard (IS) solution: 500 ng/mL (\pm)-11-Nor-9-Carboxy-Delta-9-THC-d9 in methanol, a gift from Norchem Drug Testing, Flagstaff, AZ.
- Test samples: samples containing various amounts of (\pm)-11-Nor-9-Carboxy-Delta-9-THC, also gifts from Norchem Drug Testing, Flagstaff, AZ.
- All other chemicals are reagent grade or HPLC grade.

Sample Preparation

Serial dilutions of the standard solution of carboxy-THC (THCC) were prepared in deionized water. The concentrations of the samples ranged from 1 ng/mL (1 ppb) to 1000 ng/mL. A 50 μ L aliquot of the internal standard solution was added to 1 mL of each sample. A 20 μ L aliquot was injected directly onto the column for analysis.

Test samples from Norchem Drug Testing were prepared in 1:4 dilutions of pooled urine with deionized water.

HPLC Conditions

| | | | | |
|------------------|--|----|----|---------------|
| Column | Varian Pursuit Diphenyl 3 μ m, 50 x 2 mm (Varian Part No. A3041-050X020) | | | |
| Solvent A | deionized water | | | |
| Solvent B | methanol | | | |
| LC Program | Time (min:sec) | %A | %B | Flow (mL/min) |
| | 0:00 | 60 | 40 | 0.2 |
| | 0:30 | 60 | 40 | 0.2 |
| | 1:00 | 5 | 95 | 0.2 |
| | 3:00 | 5 | 95 | 0.2 |
| | 3:01 | 60 | 40 | 0.2 |
| | 6:30 | 60 | 40 | 0.2 |
| Injection Volume | 20 μ L | | | |

MS Parameters

| | |
|--------------------|----------------------------|
| Ionization Mode | ESI negative |
| Collision Gas | 2.0 mTorr Argon |
| API Drying Gas | 25 psi at 325 $^{\circ}$ C |
| API Nebulizing Gas | 51 psi |
| Scan Time | 0.5 sec |
| SIM Width | 0.7 amu |
| Needle | -4200V |
| Capillary | -30V |
| Detector | 1620V |

Scan Parameters

| Analyte | Precursor Ion (m/z) | Product Ion (m/z) | Collision Energy (V) |
|--------------------|---------------------|-------------------|----------------------|
| (±)-Carboxy-THC | 343.5 | 299 | 17.5 |
| (±)-Carboxy-THC-d9 | 352.5 | 308 | 20.5 |

Results and Discussion

The LC gradient for this analysis results in a retention time of 4.6 minutes for the THCC analyte and the IS peak. Chromatograms for the 1 ng/mL sample and 25 ng/mL IS (20 pg and 500 pg on column, respectively) are shown in Figure 1. This level is 50 times below the proposed drug cutoff level for the initial immunoassay screen published by the Substance Abuse and Mental Health Services Administration (SAMHSA)¹ and 15 times below the proposed cutoff level for the GC/MS confirmatory test.

The LC/MS/MS method described here is linear from 1 ppb to 1000 ppb as shown in Figure 2. Each calibration standard was run in triplicate and the three data points were averaged.

A series of test samples ranging from 1 ng/mL to 800 ng/mL were run on the 1200L LC/MS/MS. The calculated and actual values are shown in Table 1.

A representative chromatogram for the Norchem Drug Testing samples is shown in Figure 3. At the 1 ng/mL LOQ level, no interference is observed, demonstrating the specificity of the LC/MS/MS method.

Conclusion

The LC/MS/MS method presented in this application note is very simple and sensitive. The method eliminates the need for a time-consuming derivatization step which can take an hour or more. The Varian 1200L LC/MS/MS can be a powerful tool in forensic, clinical, and toxicology laboratories offering significant cost and time savings.

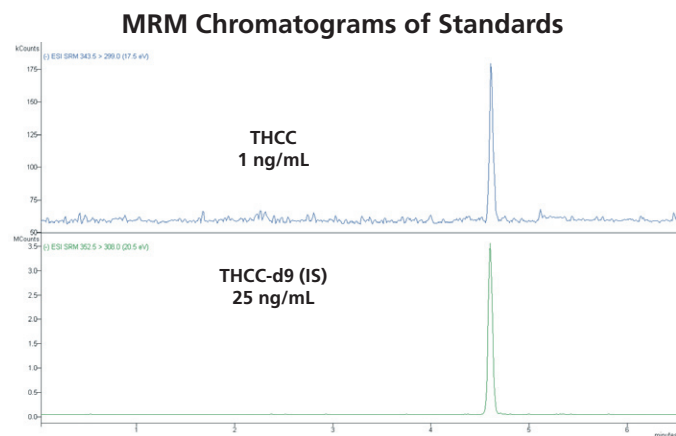


Figure 1. Signal-to-noise (300:1 RMS) is excellent at the lowest calibration level of 1 ng/mL for THCC.

Standard Calibration Curve for THCC

Curve Fit: Linear, Origin: Ignore, Weight: None
 Resp. Fact RSD: 14.72%, Coeff. Det.(r²): 0.999255
 y = +0.0229x - 2.4789e-4

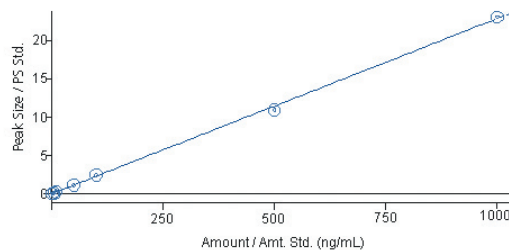


Figure 2. Seven calibration levels for THCC (1, 5, 10, 50, 100, 500, and 1000 ng/mL) with 25 ng/mL internal standard.

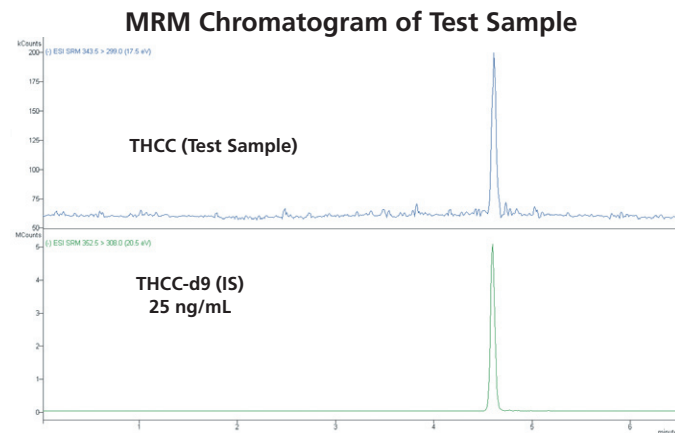


Figure 3. For the THCC test sample in diluted urine at 1 ng/mL, the calculated value based on the calibration curve is 1.1 ng/mL.

Results of LC/MS/MS Study of THCC

| Sample ID | Calculated Amount (ng/mL) | Actual Amount (ng/mL) |
|-----------|---------------------------|-----------------------|
| F | 1.1 | 1.0 |
| E | 5.4 | 5.5 |
| G | 10.9 | 11.0 |
| D | 15.3 | 15.0 |
| C | 106.0 | 100.0 |
| B | 411.0 | 400.0 |
| A | 802.0 | 800.0 |

Table 1. The calculated results correspond very well to the actual concentration of the spiked samples provided by Norchem Drug Testing.

Acknowledgement

The authors would like to thank Dr. A. Fischinger, Norchem Drug Testing, Flagstaff, AZ for kindly supplying technical advice and the THCC test samples for this study.

Reference

- <http://workplace.samhsa.gov/ResourceCenter/DT/FA/GuidelinesDraft4.htm>

These data represent typical results.

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