Montgomery County Coroner’s Office / Miami Valley Regional Crime Laboratory - Toxicology

Confirmation and Quantitation of Cannabinoids by LC/MS/MS

July 2020

Summary of Validation

The toxicology laboratory validated a LC/MS/MS method for confirming and quantitating 4 cannabinoids in blood, serum, and urine. This method uses a solid phase extraction method and the analytes are analyzed using a LCMSMS instrument in MRM mode. All calibrators, controls and interference studies were prepared from NIST-Traceable reference standards with certificates of analysis. The drugs included are D9-Tetrahydrocannabinol (THC), 11-Nor-delta9-THC-carboxylic acid (THC-COOH), cannabidiol (CBD), and 11-Hydroxy-D9-THC (11OH).

The method was validated using blood calibrators and controls. Analytes are qualitative in postmortem urine and quantitative in blood, serum, and OVI urine. The analytical work was done by Treena Wiebe, Kialee Bowles, Elizabeth Kiely, Quinton Carter, and Brian Simons and reviewed by Matthew Juhascik and Heather Antonides.

This validation started on July 7, 2020 and ended on July 23, 2020. LCMSMS1, 2, and 3 were used during the method validation and all data was combined.

The method was determined to be acceptable for the qualitative and quantitative determination of THC, THC-COOH, 11-OH, and CBD.

The validated parameters are shown below:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acceptance Criteria</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bias –</td>
<td>Maximum of +/- 20%</td>
<td>Between day biases were less than 14%. Several low controls were not accurate for 11-OH or THC-COOH; LOQ will be calibrator 2 for those two compounds (4 ng/mL).</td>
</tr>
<tr>
<td>Carryover –</td>
<td>A negative specimen following the highest calibrator is a true negative</td>
<td>No carryover was seen in negative specimens following the highest calibrator or in a specimen following a case with a large amount of drug present. Injection loop wash method was added to the acquisition method.</td>
</tr>
<tr>
<td>Interference – for all analytes.</td>
<td>No interfering signal from matrix or drugs used in assay</td>
<td>Delta8-THC in high concentration is distinguishable from delta9-THC. In lower concentrations, delta8-THC will appear as a shoulder to the right of delta9-THC. In the chance that the integration cannot account for this, quantitation is reported as THC only for this</td>
</tr>
<tr>
<td><strong>Limit of Detection – for all analytes</strong></td>
<td>At least 1 ng/mL for THC, THC-COOH, 11-OH, and CBD</td>
<td>All curves are set to a quadratic regression. The LOD will be set as the lowest acceptable calibrator which is 1 ng/mL.</td>
</tr>
<tr>
<td><strong>Precision</strong></td>
<td>The % coefficient of variation for controls (within and between runs) not to exceed 15%</td>
<td>The % coefficient of variation for all controls (between runs and within run) was less than 15% for THC-COOH, CBD, and THC. The within run % coefficient of variation for the low control on one day for 11-OH reached 20%. LOD for 11-OH will be 1ng/mL but the LOQ will be 4ng/mL.</td>
</tr>
<tr>
<td><strong>Stability on the autosampler</strong></td>
<td>Samples analyzed against a curve from day 0 should calculate within 20%.</td>
<td>Samples were stable on the autosampler (&lt; 20% quantitative change) for 4 days after the initial extraction.</td>
</tr>
<tr>
<td><strong>Recovery –</strong></td>
<td>Greater than 80%</td>
<td>Recoveries were calculated for all analytes. None of the analytes had a recovery greater than 80%. The deuterated internal standards for each analyte had the same recovery, therefore, making the recovery acceptable.</td>
</tr>
<tr>
<td><strong>Matrix Effect –</strong></td>
<td>Between 80 – 120 %</td>
<td>Matrix effects for blood varied from 30%-97%; however, the internal standard chosen for a drug behaved in a similar manner as the drug. Matrix effects for urine varied from 43% -114%; however, the internal standard chosen for a drug behaved in a similar manner as the drug.</td>
</tr>
<tr>
<td><strong>Uncertainty of Measurement –</strong></td>
<td>To be calculated</td>
<td>The UOM for each of the drugs was determined by using the toxicology laboratories standard uncertainty budget. The UOM’s are listed below: THC – 15% THC-COOH – 17% CBD – 15% 11-OH – 11%</td>
</tr>
<tr>
<td><strong>Dilution Accuracy</strong></td>
<td>Within 20% of target</td>
<td>Dilutions were within 12% of target concentrations; therefore, dilutions may be performed for this assay. Any dilution must be made up with matching blank matrix volume.</td>
</tr>
<tr>
<td>----------------------</td>
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</tr>
<tr>
<td><strong>Previous Casework</strong></td>
<td>Cases will be analyzed using the new method and quantitations evaluated against previous results.</td>
<td>Thirty-Eight cases were analyzed with the new LCMSMS method and compared against the GCMS method. 37 results agreed within +/-20%. 1 result fell outside of the +/-20% range (33%). In all, the results were considered acceptable and the discrepancies toxicologically insignificant.</td>
</tr>
</tbody>
</table>
Addition of delta 8 THC and THCCOOH to THC by LC/MS/MS

Summary of Validation

The toxicology laboratory validated the addition of delta 8 THC and THCCOOH to our currently validated THC method by LC-MS/MS. All calibrators, available controls and interference studies were prepared from NIST-Traceable reference standards with certificates of analysis. The analytical work was done by Philip Carter, Kialee Bowles, and Brian Simons and reviewed by Matthew Juhascik.

This validation was begun and completed in May of 2022. LCMSMS instrument #2 was used during the method validation.

The method was determined to be acceptable for the qualitative confirmation of delta 8 THC and delta 8 THCCOOH. Currently, this analysis will only be done using LCMSMS 2. Additional instruments will be validated as necessary.

The method is fit for its intended purpose.

The validated parameters are shown below:

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interferences</td>
<td>No interfering signal from matrix or drugs used in assay</td>
<td>No interfering signals were seen in the matrices or with related and unrelated drugs commonly seen in this laboratory. The internal standard does not interfere with the analytes and the analytes do not interfere with any of the internal standards.</td>
</tr>
<tr>
<td>Carryover</td>
<td>A negative specimen following the highest calibrator is a true negative</td>
<td>No carryover was seen in negative specimens following the highest calibrator</td>
</tr>
<tr>
<td>Limit of Detection</td>
<td>At least 1 ng/mL.</td>
<td>Both analytes can be detected at 1 ng/mL.</td>
</tr>
<tr>
<td>Recovery</td>
<td>N/A</td>
<td>Recoveries varied from ~26% to ~109%. Target analyte had similar recovery to its specific internal standard which was determined to be acceptable.</td>
</tr>
<tr>
<td>Ion Suppression / Enhancement</td>
<td>75% - 125%, CV &lt; 20%</td>
<td>Suppression/enhancement ranged from 86% - 180%. Some CVs were over 20%. Target analytes agreed with their internal standard. Delta 8 THC in urine did not agree;</td>
</tr>
<tr>
<td>Previous casework</td>
<td>Casework previously suspected of being positive for delta 8 THC/THCCOOH was confirmed as positive by this method.</td>
<td>however, delta 8 THC will not be seen in urine.</td>
</tr>
</tbody>
</table>